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Quantification of aluminum in human bone with neutron activation analysis

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QUANTIFICATION OF ALUMINUM IN HUMAN BONE WITH NEUTRON ACTIVATION ANALYSIS

by

**KANAKAM DAVIS, M.Sc.
Mahatma Gandhi University, India, 2003**

A Thesis

Presented to Ryerson University

in Partial Fulfillment of the

requirements for the Degree of

Master of Science

in the program of

Biomedical Physics

Toronto, Ontario, Canada, 2008

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ABSTRACT

In Vivo Neutron Activation Analysis (IVNAA) has been investigated to measure aluminum levels in bone for several years. Aluminum, being a toxic element, should be routinely monitored in both clinical and occupational environments. The non-invasive IVNAA technique developed at the McMaster University Accelerator Lab is currently being improved for future use.

New sets of hand phantoms were prepared that closely resemble spectra that were collected from the hand of healthy subject. Following the IVNAA of aluminum phantoms, the technique was applied for the first time to measure bone Al levels in 18 healthy subjects. The mean hand bone Al concentration was determined as 28 $\mu\text{g Al/g Ca}$. The results were achieved with a dose equivalent of 17.6 mSv. Further investigations using an enhanced detection system and applying optimization of the irradiation protocol with radiation dose up to 50 mSv showed that minimum detectable limit was improved to 0.10 mg Al.

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Chapter I

Introduction

1.1 Importance of Aluminum

Aluminum (Al) is the third most naturally abundant and the most prevalent element in the earth's crust. Al is a toxic element to which all individuals are exposed through food, air, water, and soil. Since Al is ubiquitous, there is a continuous exposure to this element via the environment and diet. Al is a chemically reactive element and is found combined with other elements like oxygen, silicon, and fluorine. It is found to be dissolved in water and acid rain, due to its low pH level, increases the solubility of Al in rivers and lakes.

1.1.1 Biological importance

Even if this element is present only in trace amounts in biological matter, harmful biological effects of Al have been a topic of interest of research for several decades. There are no known physiological needs for Al in the human body (Garbossa *et al.*, 1998) and, in addition, its atomic size (0.051nm) and electric charge (3+) makes it an aggressive inhibitor of several essential elements of similar

characteristics, such as magnesium, calcium and iron. Al could interfere with homeostatic and metabolic activities of these essential human body elements and thus, inhibit bone mineralization (Alfrey *et al.*, 1984, 1994; Yokel, 2000; Campbell *et al.*, 2001; Ward *et al.*, 2001).

1.1.2 Aluminum Products

Al mixes with other metals to form alloys which are harder and stronger and are used to make many products. Al compounds are found in a number of engineering and end-user products, such as alums in water-treatment, alumina in abrasives, furnace linings, siding and roofing and airplanes (ASTDR). It is also found in antacids (Yokel *et al.*, 2004), astringents, buffered aspirins (Hem and White, 1989), antiperspirants (Laden *et al.*, 1988; Darbre *et al.*, 2003, 2005), dental rinses and tooth pastes (Yokel *et al.*, 2004), pots and pans as well as being used in explosives and fireworks (Gitelman, 1989). Furthermore, it is present in processed food, drinking water, soy based infant formula, intravenous fluids (Sedman *et al.*, 1985; Bishop *et al.*, 1997; Advenier *et al.*, 2003) and in vaccinations (Keith *et al.*, 2002).

1.2 Exposures to Aluminum

All living beings are exposed to Al through the skin, lungs and intestinal tract. It has been documented that exposure of fish to Al resulted in severe haematological disorders (Witters *et al.* 1990). Al may also be involved in the action of enzymes such as succinic dehydrase and δ -aminolevulinate dehydrase which are present in porphyrin synthesis. Increased erythrocyte protoporphyrin was reported in patients on chronic haemodialysis (Fontanellas *et al.* 1994). It was also found that Al retards bacterial growth and enhances the porphyrin formation (Scharf *et al.*, 1994).

1.2.1 Clinical Exposure

Al was present in hemodialysis fluid until the 1970s; as a consequence higher Al load in bones was found in dialysis patients which could result in death (Alfrey *et al.*, 1972, Alfrey, 1976). Studies have identified Al as a co-factor for the development of dementia and osteomalacic osteodystrophy (Parsons *et al.*, 1971; Ellis *et al.*, 1979; Fontanellas *et al.*, 1994). Presently, clinical exposure to trace levels of Al in the dialysis fluid and use of Al based phosphate binders in patients with kidney and renal failure diseases are associated with the dialysis encephalopathy syndrome (Alfrey 1993; Yokel, 2000; Ward *et al.*, 2001; El-

Rahman *et al.*, 2003) and bone related secondary ailments like osteodystrophy, osteoarthritis (Cassidy, 2003), and osteomalacia (Alfrey, 1976, 1993; Ott *et al.*, 1983). Another clinical exposure to Al is through intravenous solutions (Wilhelm *et al.*, 2001). Sedman and colleagues (1985) reported elevated levels of this element in the bone, urine and plasma of premature infants who were undergoing intravenous therapy (Sedman *et al.*, 1985; Bishop *et al.*, 1989). In addition, victims of Al exposure also include patients on total parenteral nutrition [TPN] (Klein and Coburn, 1994) and patients with severe burns (Klein *et al.*, 1994). The process of ceasing hemorrhage in the urinary bladder using alum irrigation (Murphy *et al.*, 1992), and patients undergoing cranial bone reconstruction with Al made bone cement (Hantson *et al.*, 1994; Renand *et al.*, 1994) leads to Al accumulation. These clinical exposures to Al are found to be fatal, according to the research by Nakamura and colleagues (Nakamura *et al.*, 2000).

1.2.2 Occupational Exposure

Exposure to Al in the occupational setting is of concern. For example, ground Al and Al oxide in 'McIntyre powder' was used as a prophylactic agent against silicosis (lung disease) between 1944 and 1979 in Ontario mines. A morbidity prevalence study conducted on the exposed miners showed they performed worse in cognitive tests than unexposed miners (Rifat *et al.*, 1990). It

is also accepted that workers in welding and Al smelting plants exposed to excessive Al show neuropsychiatric symptoms characterized by poor coordination, memory loss, and depression (Sjögren *et al.*, 1990; White *et al.*, 1992; Sim *et al.*, 1997; Polizzi *et al.*, 2002). There is a steady buildup and long-term retention of Al within the respiratory tract of workers who are repeatedly exposed in occupational surroundings (Schlesinger *et al.* 2000).

Furthermore, the occupational exposure to Al dust through the respiratory tract in welders resulted in raised levels of the element in serum and urine, as well as in bone biopsy samples (Elinder *et al.* 1991; Gitelman *et al.*, 1995; Gitelman, 1995; Bast-Pettersen *et al.*, 2000). Studies performed to investigate the impact of occupational exposure of Al in welders on cognitive and motor test showed an inferior performance of exposed group compared to that of control group (Buchta *et al.*, 2005; Meyer-Baron *et al.*, 2007). Al in serum and urinary excretion of highly exposed workers depends on both the level and duration of exposure but provides information only about the recent exposure levels of Al.

1.2.3 Dietary Exposure

Small amounts of Al are present in our food. Aluminum foils and cooking utensils add further Al to our food during cooking. Food additives such as baking

powder, cake mixes, pancake mixes, frozen dough and processed cheese all contain this element. Studies state that the average Al intake by an adult male is 10mg/day and by an adult female is 7 mg/day (Flarend, 2001). The concentration of Al in natural water (less than 0.1 mg/L generally) can vary significantly, in a range of 0.4-1 mg/L, depending on various physicochemical and mineralogical factors. Al levels in drinking water vary according to the levels found in the source and whether or not it is used during water treatment. Al salts are widely used in water treatment as coagulants to reduce organic matter, color, turbidity, and microorganism levels. Such use may lead to increased concentrations of Al in treated water, but generally there is very little consumption of the element from drinking water (Yokel *et al.*, 2001).

1.3 Intake, Distribution and Elimination of Aluminum in the body

As stated above, the main pathways of Al to the human body are through the skin, lungs and intestinal tract. One of the major routes of Al absorption is through the gastrointestinal tract (Ittel, 1993). Several proposals have been put forward to explain the Al absorption mechanism through the gut, but there is no exact and valid justification for it (Exley *et al.*, 1996). Cochran *et al.* (1993) proposed the presence of aluminum-specific proteins that bind this element with the intestinal mucosa and thereby regulate Al absorption.

In aluminum smelting plants the workers are exposed to Al through inhalation of dusts and aerosols. Once Al is inhaled, it accumulates in the brain through the olfactory system (Exley *et al.*, 1996). It has also been shown that Al dust and particle intake by the workers are uploaded through the lung epithelia (Gitelman *et al.*, 1995) as well as through the gut as particulates are swallowed (Rollin *et al.*, 1993).

The skin acts as a minor route of Al entry into the body. Application of deodorant containing aluminum chlorohydrate results in up to 0.012% absorption through the skin (Flarend *et al.*, 2001). A case study of a hyperaluminemia in a female using an antiperspirant for 4 years has recently been reported in literature (Guillard *et al.* 2004). On the other hand, some studies report that the skin applications of Al compounds in cosmetic and health care products generally do not induce harmful effects on skin or other organs (Sorrenson *et al.*, 1974; Brusewitz, 1984).

It is estimated that in healthy human subjects, the total body burden is 30-50 mg (ATSDR, 1999; U.S. Public Health Service, 1992, p. 26; Alfrey, 1984; Ganrot, 1986). In a normal population, about one-half of the total amount of Al stored in the body is in the skeleton (50%) and the rest is in the lungs (25%), kidneys and brain (Ganrot, 1986). Al is found to be retained in bone and kidneys

after an oral exposure as reported by Chan (Chan *et al* 1988). The highest concentration of Al is found to be stored in bone (Zafar *et al.*, 1997). It has also been documented that a higher Al concentration is found in trabecular bone in comparison with the cortical bone (Alfrey *et al.*, 1976). Therefore, bone represents a suitable organ to obtain information about the cumulative Al levels in a person.

The major route for the elimination of Al normally absorbed by the body is through kidney. If one is exposed to large amounts of this element, the kidneys may not be capable of excreting the excess Al resulting in its retention. Another pathway for Al elimination is through the bile. Almost 75-90% of an oral dose of Al in humans was reported to be excreted in the feces (Greger *et al.*, 1983). However, urinary excretion is the major excretory route once the element enters the blood stream (Skalsky & Carchman, 1983).

1.4 Aluminum Related Diseases

Dialysis encephalopathy and Alzheimer's disease are the main two diseases that are associated with Al toxicity. The first one is observed in dialysis patients through Al exposure from dialysis fluid and aluminum hydroxide phosphate binders. The predominant features of Al-related bone diseases are

defective mineralization and osteomalacia; they result from excessive deposits at the site of osteoid mineralization, where calcium would normally be placed. An excess of Al has been shown to induce microcytic anemia as daily injections of Al into rabbits produced severe anemia within 2-3 weeks (Yokel *et al.*, 1997).

The role of Al in Alzheimer's disease remains a controversial subject in research (Crapper *et al.*, 1980, 1994; McLachlan, 1986, 1995; Savory *et al.*, 2007; Zatta *et al.*, 1993, 2006; Munoz *et al.*, 1998; Exley, 2001; Exley and Korchaazhkina, 2001). A pathogenetic role of the element in Alzheimer's disease has been suggested in animal studies of Al-induced neurofibrillary degeneration (NFD). In this, an increased concentration of Al in brain is absorbed in the form of Al alkaloid and forms neurofibrillary tangles that affect the brain nerves. This also affects the blood brain barrier (BBB), thus allowing toxins to reach the central nervous system. Divergent results have been reported independently in literature (Klatzo *et al.*, 1965; Makjanic *et al.*, 1998; Kawahara *et al.*, 2001). These controversies have led to an overall understanding that, under normal circumstances, if the gut Al absorption and renal functions are not compromised, and the blood - brain barrier (BBB) is not damaged through surgery, then Al toxins are blocked from entering the brain and Al does not pose a health risk from neurotoxicity (Imray *et al.*, 1998).

Nausea, anorexia and skin problems are associated with severe Al toxicity. It has been documented that accumulation of Al has caused bone fragility in patients with Alzheimer's disease (Mjoberg *et al.*, 1997). Bone Al upload is associated with reduced amounts of unmineralized osteoid and low bone formation and is referred to as aplastic bone disease (Andress *et al.*, 1987). Osteopenia and fractures have been found in premature infants fed with parenteral nutrition (Popinska *et al.*, 1999). Even if Al toxicity is a well studied subject, its mechanism of action at cellular level is still not clear (Levesque *et al.*, 2000).

1.5 Detection of Aluminum

There are several *in vitro* techniques that are used for the detection of Al in human tissues such as atomic emission and absorption spectroscopy, electro thermal atomic absorption spectroscopy (Litov *et al.*, 1989; Kruger and Parsons, 2007), and neutron activation analysis (Sharif *et al.*, 2004). These analytical techniques have limited success in the detection of this element in human body and tissues due to several reasons like sensitivity, contamination and/or need to destroy the sample. Some of these *in-vitro* methods such as atomic absorption spectroscopy are now routinely available in a clinical environment along with staining and digestion techniques.

Iliac crest bone biopsy with specific staining is the routine clinical method of detection of cumulative toxic levels of Al in bone (Malluche *et al.*, 1999; Walton *et al.*, 2007); however the limitations of bone biopsy are that it is an invasive and painful method (Spasovski, 2004); consequently, it has a low patient acceptance. Bone biopsy cannot be routinely repeated over time and the mass of the biopsy sample being small may not be representative of the levels of Al in the total bone or body. For all these reasons, this method has limited value for routine monitoring of cumulative bone Al levels.

Another method of detection of Al in human body is the *in vivo* method called neutron activation analysis, which depends on the excitation of Al nuclei within the human body via neutron activation. This method is non invasive and painless, and thus believed to be more suitable than biopsy for monitoring the accumulation of this element in the human body.

1.5.1 *In Vivo* Neutron Activation Analysis

In Vivo Neutron Activation Analysis (IVNAA) is an analytical method for determining elements of interest in a sample when irradiated by neutrons (Scott and Chettle, 1986). Reactors, isotopes or accelerators can be used as sources of neutrons in IVNAA. Neutrons with energies in the range of kilo to mega electron

volt are utilized depending upon the type of neutrons used in this technique. For most IVNAA of trace elements in the human body, thermal neutrons are employed. Once neutrons hit the biological sample, they undergo elastic and inelastic scattering with a loss of energy to form thermal neutrons with energy in the range of milli electron volt. The energy of thermal neutrons results in a neutron cross section (σ) that is inversely proportional to the velocity of neutrons of the element. When the thermal neutrons strike a sample, high energy penetrating gamma rays are emitted. The gamma rays that are emitted during irradiation are called prompt gamma rays and those emitted after irradiation are called delayed gamma rays. These gamma rays are representative of the nuclei which emit them. The excited nucleus as a result of nuclear interaction of neutrons will descend to a stable state by the emission of gamma rays of energy characteristic to that particular nucleus or isotope.

The characteristic gamma rays are detected using energy sensitive detectors and they identify the emitting nucleus. The intensity of the gamma rays gives a measure of the abundance of the nucleus in the sample. The main advantage of IVNAA is the long attenuation mean free paths of neutrons and gamma rays in tissue, which makes it possible to detect elements in deep seated organs.

The main disadvantage of IVNAA is the production of the same final product by neutron activation of different elements present in the sample resulting in a direct interference. The other drawbacks include the energy dependence of the nuclear cross section and the complex spectra of gamma rays from the human body. The IVNAA not only depends on the cross section but also depends on the half-life of the element under consideration. In order to obtain maximum gamma ray intensity, it is ideal to set high efficiency detectors close to the target organ. The detectors should have high energy resolution and the efficiency should be independent of the size and shape of the target organ. There are different types of detectors used in the IVNAA, and the various types and sizes could affect the measurement sensitivity. Another factor that can affect measurement sensitivity is the non-uniform distribution of thermal neutrons. The IVNAA method has been successfully utilized to detect several minor and trace elements in human tissues *in vivo*, like manganese (Aslam *et al.*, 2008a), magnesium (Aslam *et al.*, 2008b) and Al using thermal neutron activation (Ellis *et al.*, 1988; Green *et al.*, 1993; Pejović-Milić *et al.*, 2005).

1.5.2 In Vivo Neutron Activation Analysis of Aluminum

Al can be measured using *in vivo* neutron activation analysis via thermal neutron activation of ^{27}Al using the reaction $^{27}\text{Al}(n, \gamma)^{28}\text{Al}$. Of the nine isotopes of

Al whose mass numbers range from 23 to 30, only ^{27}Al and ^{26}Al occur naturally. ^{27}Al is the stable isotope of Al, with 100% abundance. The thermal neutrons used in this IVNAA have energy 0.025 eV which results in a maximum cross-section of 0.23 barn. ^{28}Al produced is radioactive and unstable. It will decay with a half life of 2.25 min to form an excited state of ^{28}Si . The unstable ^{28}Si soon undergo nuclear de-excitation with the emission of gamma rays of energy 1.78 MeV (100%). If fast neutrons are present in IVNAA, interfering reactions of $^{31}\text{P}(\text{n}, \alpha)^{28}\text{Al}$ and $^{28}\text{Si}(\text{n}, \text{p})^{28}\text{Al}$ with thresholds of 1.95 MeV and 4 MeV respectively are also present. Since phosphorus is present in large amounts compared to Al in human body, a small proportion of the fast energetic neutrons can cause interference, and thus ambiguity in the result. Elimination of fast neutrons from the neutron beam avoids the activation of ^{31}P and ^{28}Si and makes the energy spectrum simpler to analyze. The half-life of ^{28}Al is short, 2.25 min; thus the transfer of the irradiated sample from the neutron beam area to the counting area should be rapid. The irradiation time should also be kept short in order for ^{28}Al not to decay too much during the irradiation. The other elements in the biological sample that typically undergo neutron activation simultaneously with Al are calcium, sodium and chlorine. The corresponding reactions are $^{48}\text{Ca}(\text{n}, \gamma)^{49}\text{Ca}$, $^{37}\text{Cl}(\text{n}, \gamma)^{38}\text{Cl}$, $^{23}\text{Na}(\text{n}, \gamma)^{24}\text{Na}$ and $^{26}\text{Mg}(\text{n}, \gamma)^{27}\text{Mg}$ respectively. The gamma rays emitted from these elements along with that emitted from Al form a spectrum typical of all these isotopes.

According to the chemical composition of Reference Man cited by ICRP 23(1975) the estimated amount of Al in the skeleton is 21 mg. This accounts for approximately 50% of the total body Al burden (Zafar *et al.*, 1997). The Al concentration in bone tissue (hand bone) was measured in the present work (further details are given in section 2.1). The radiation dose associated with IVNAA was estimated by employing a tissue equivalent proportional counter (TEPC). The radiation dose was kept as low as possible based on statistical precision requirements of the technique. The equivalent dose received by a subject during an irradiation procedure was estimated to be 17.6 mSv with an effective dose of 14.4 μ Sv.

A non-invasive, IVNAA measurement system of Al in human bone at McMaster Accelerator laboratory was previously reported (Pejović-Milić *et al.*, 1998, 2005); however, preceding this work there were several laboratories in different countries involved in the development and use of application of the technique of IVNAA of bone Al in medical and health fields. IVNAA of Al in human bone could be assessed by a minimal detectable limit (MDL) of the element under investigation and associated radiation dose delivered to a subject. The MDL of the system is obtained as an estimate of twice the uncertainty of zero Al phantoms over the slope of the calibration line (see section 2.5.3 for more information). The IVNAA of Al in human bone tissue and phantoms at

Brookhaven reported by Ellis and colleagues in 1988 made use of a research reactor as a thermal neutron source, achieving MDL of 0.4 mgAl in a hand (or 22 $\mu\text{gAl/gCa}$) with a radiation dose of less than 20 mSv. However, the techniques suffered from the interfering reactions of phosphorus $^{31}\text{P}(n, \alpha)^{28}\text{Al}$ due to higher energy neutron contamination in the thermal neutron beam. In following years, Wyatt *et al.*, 1993 at Swansea used a ^{252}Cf source-based system, while Green and Chettle (1992) and Green *et al.*, 1993 at Birmingham used a Dynamitron accelerator while Pejović-Milić and colleagues (1998, 2005) utilized a KN accelerator as neutron sources. For a 20 mSv hand dose, the detection limits achieved for these systems were between 0.7 and 2.5 mg.

The present work discusses the non-invasive *in vivo* measurement of Al in human body based on neutron activation analysis. This presents the first *in vivo* human measurements of Al stored in a hand bone in the population living in Southern Ontario, Canada. It aims at testing the already developed, non-invasive and painless clinical diagnostic tool for the determination of Al concentration in bone that could help monitoring the accumulation of the element in the human body, and predict the inception of bone disorder symptoms. The results obtained here open new directions for further refinement of bone Al detection system and set the stage for using this diagnostic tool in the clinical environment, especially in

monitoring the bone Al loading evident in dialysis patients and workers
occupationally exposed to the element.

Chapter II

In Vivo Neutron Activation Analysis of Aluminum in Human Hand Bone

In the technique of *in vivo* neutron activation analysis (IVNAA), neutrons of appropriate energies are produced from a neutron source (Tandatron Accelerator). These neutrons are used to irradiate the sample (phantom or hand bone) which is placed in an irradiation/shielding cavity. The sample contains ^{27}Al , which is the only stable isotope of Al. This causes the $^{27}\text{Al}(n, \gamma)^{28}\text{Al}$ reaction to occur with a half life of 2.25 minutes and ^{28}Al then decays to an excited state of ^{28}Si , which instantly de-excites with the emission of 1.78MeV γ -rays. The γ -ray spectrum is detected using an array of eight 4π NaI(Tl) detectors. There are also other elements like Ca, Cl, Na and Mg in the tissue that will react with thermalised neutrons resulting in γ -ray emission. After data analysis (Marquardt Method) and calibration of the energy spectrum and counts, the peak intensity ratio of Al emitted γ -rays to Ca emitted γ -rays gives the ratio of Al to Ca concentration in the sample.

2.1 Why Hand Bone?

The major parts of the human body that get affected by Al toxicity are bone, brain, kidneys and liver. The kidneys and liver are inconvenient target organs for IVNAA since they are surrounded by other critical organs. Moreover, these organs also contain many other toxic elements which would make the detection of Al more complex. The brain is not considered as a suitable organ for this diagnostic technique because of the complex central nervous system and proximity of other radiosensitive organs.

Zafar and colleagues reported in 1997 that the highest concentration of Al is found in bone. Bones of the human extremities, such as the hand, have a small amount of overlying tissue compared to bones present in other parts of the body. This allows for minimal signal attenuation and therefore a better detection of the elements present in the hand bone, including Al. Hands can be stretched out so that radiation dose can be restricted to the palm, thus sparing the rest of the body from the unnecessary radiation dose. The hands of a healthy individual contain 3% of the skeleton (ICRP 23, 1975). Because of all mentioned reasons the hand bone is considered to be an apt site for Al measurement.

Proper radiation shielding is placed around the extended arm to confine the radiation field only to the hand. In order to achieve a superior sensitivity of

the technique, the neutron irradiation should be uniform throughout the target and this target must contain homogeneously distributed Al. The attenuation of neutrons by tissue is negligible as the layer of overlying tissue around the hand bone is thin and the source is close to the target.

2.1.1 Distribution of Aluminum in Cortical and Trabecular Bone

The estimated amount of Al in skeleton is 21 mg according to the chemical composition of Reference Man cited by ICRP 23(1975). It has been documented that the trabecular bone contains a higher Al concentration when compared to the cortical bone (Alfrey et al., 1976). The human hand is composed of 95% of cortical bone and 5% of trabecular bone (ICRP 70, 1994). Based on the estimated measurements of intact cortical bones, the expected amount of Al in one hand of a healthy adult was found to be 0.3-0.4 mg. One of the primary reasons for taking the hand bone as the measurement site is the different distribution of the element in the cortical and trabecular bone. The choice of the subject's extremities like hands, feet and leg bones provides important information regarding the distribution and metabolism of the analyte element. Since the human hand consists of 95% cortical bone, Al dissolved hand bone phantoms have been prepared based on the ICRP 23 Reference Man cortical bone composition and used for the calibration throughout this work.

2.2 Neutron Source

The neutrons that are utilized for the IVNAA technique can be produced using reactors, radioisotopes like ^{252}Cf or accelerators. The type of neutron source and the production of neutrons play an important role in the system sensitivity. Different neutron sources generate neutrons with different energies, ranging from kilo-electron volt to mega-electron volt, as well as with different neutron flux. The sources which produce neutrons in the kilo electron volt range include reactors with filtered beams and radio isotope sources (γ, n). The Brookhaven Medical Research Reactor (Ellis *et al.*, 1988) and McMaster Research Reactor (Palermo *et al.*, 1993) are of the few neutron reactors that produce thermal/epithermal neutron beams used to measure bone Al *in vivo*. Reactors have high neutron flux, which is desirable for this technique, but they are expensive and are not typically clinically available. ^{252}Cf is one neutron source with a limited use to measure normal Al levels in a clinical environment due to the poor neutron beam quality (mega electron volt range) produced. The presence of fast neutrons in the beam of this source causes interference reactions from P and Si, and thus makes this source less sensitive to use for IVNAA of Al (see section 2.1.1).

Particle accelerators produce neutrons with energies in the mega-electron volt range, with a medium to high neutron flux, which are adequate for the

detection of both minor and trace elements. For the bone Al IVNAA, however, the energy of produced neutrons could be controlled to be below the threshold energy of interfering reactions of P and Si (Pejović-Milić *et al.*, 2005). This significantly simplifies the described technique by eliminating neutron activation of P and Si that are always present in a biological tissue. Therefore, in this work the portable accelerator-based source of neutrons is the preferred one due to its availability for clinical applications.

2.2.1 Tandetron Accelerator

The Tandetron Accelerator at McMaster Accelerator Laboratory (MAL), McMaster University, is a high current accelerator which can produce a mixed neutron-gamma field. The process of IVNAA using the Tandetron accelerator has been previously studied for elements like manganese (Mn) and magnesium (Mg) and reported in the literature (Aslam *et al.*, 2008a; Aslam *et al.*, 2008b). The high beam current of the Tandetron accelerator was utilized for the first time for Al measurements in phantoms and human hand in this work.

A thick ${}^7\text{Li}$ target was used to produce suitable energy neutrons by means of ${}^7\text{Li} (p, n) {}^7\text{Be}$ reaction. The target is mounted at the tip of the accelerator beam line. The intense heating produced by the reaction is cooled down by using water

cooling. The ^7Li target was bombarded with protons with energies in the range of 2.00-2.25 MeV and neutrons of energies in the range of 230-500 keV are produced (Aslam *et al.*, 2006). This high current accelerator can presently run with currents up to 600 μA with appropriate proton energy and irradiation time. The present work adopts proton energy of 2 MeV for the IVNAA technique.

One of the main advantages of this neutron source is that the energy of the neutrons produced is lower than the thresholds of 1.95 and 4.00 MeV needed to produce ^{28}Al from the interfering $^{31}\text{P} (n, \alpha) ^{28}\text{Al}$ and $^{28}\text{Si} (n, p) ^{28}\text{Al}$ reactions. A study of the ratio of fast and thermal fluence in one system has shown an interference of 1g of Al is present in every 7g of phosphorus (Wyatt *et al.*, 1993). Thus the use of neutrons with energies below these threshold energies eases the analysis of Al in a bone sample by eliminating the call for correction due to the substantial amount of phosphorus in the human body.

Alternatively ^{27}Al can be activated with the fast neutrons via the $^{27}\text{Al}(n, p)^{27}\text{Mg}$ reaction, with a threshold energy of 3MeV. ^{27}Mg decays with the emission of 0.84 MeV of gamma rays. Similarly this neutron reaction is not allowed since its energy threshold is higher than the maximum neutron energy generated by the particle accelerator utilized.

The fundamental requirement in this work is to have a beam of thermal neutrons for irradiation of the hand. The neutrons produced with up to 230 keV energy, with a proton energy of 2 MeV at the Tandetron Accelerator, are later moderated in the irradiation/shielding cavity (see section 2.3 for more information) to become thermal neutrons. The high energy neutrons produced in the Tandetron accelerator collide with atoms of a polyethylene moderator and transfer a part of their energy to the nuclei of the moderator. Thus the velocities of neutrons are reduced to the thermal velocities of the nuclei producing thermal neutrons.

2.3 Irradiation/Shielding Cavity

In the present research, all the phantoms and subjects were irradiated within an irradiation/shielding cavity. Pejović-Milić and colleagues in 2006 designed an irradiation/shielding cavity based on MCNP Monte Carlo simulations and further optimized it for clinical application (Pejović-Milić *et al.*, 2000; Pejović-Milić *et al.*, 2006). The design of the cavity is such that irradiation of the patient is confined to the hand. The radiation dose to the rest of the body is maintained as low as possible. The cavity allows maximum thermal neutron flux to activate the Al and minimizes the fast and epithermal neutron components. It also minimizes the production of photons from the interaction of neutrons inside

the cavity as well as photons emitted by the Li target. The irradiation/shielding cavity plays a key role in the improvement of sensitivity of the technique of IVNAA.

The *in-vivo* neutron irradiation facility at MAL for the measurement of Al and other trace elements consists of the cavity with a polyethylene moderator, a lead filter, a graphite reflector, borated plastic sheets, outer lead walls, an opening in the middle for the access of hand or phantoms, and proper shielding of the irradiated extremity. A layout of the irradiation facility is illustrated in Figure 1. The self enclosed type geometry of the cavity has a hand/phantom access port on one side. The dimension of the cavity is 50x60x50 cm³. The polythene moderates the neutron beam produced by the lithium target and changes the fast neutron energy towards lower energies (0.025 eV). The graphite reflector redirects some neutrons back to the irradiation site thus increasing Al activation. The lead filter was included in the design to reduce the unnecessary gamma dose to the hand from the target. A lead filter of thickness of 2.0 cm (~4 Half Value Layer (HVL)) was chosen to reduce the transmission probability to the hand to ~5% for 478 keV inelastic gamma rays produced in the target (Aslam *et al.*, 2002). The outer lead walls and the borated plastic sheets are an integral part of the irradiation/shielding cavity, which minimize the neutron and gamma dose to the subject's body. Majority neutrons in the reflector are absorbed by the

surrounding 1.4 cm thick borated plastic sheets through the $^{10}\text{B}(\text{n}, \alpha)$ reaction. This blocks the transmission of neutrons to the outside of the cavity with 99.8% efficiency. The irradiation/shielding cavity ensures a low radiation dose to the subject by filtering out the unnecessary gamma dose. The equivalent dose received outside of the irradiation/shielding cavity is approximately 1/2260 of that received by the hand thus ensuring the efficacy of the cavity (Byun *et al.*, 2007).

FIGURE 1. Layout of a hand/phantom irradiation facility for IVNAA.

2.4 Detection System

After the hand/phantom has been irradiated with neutrons, hand/phantoms are transported from the hand access port of the cavity to the detection system for analyzing the spectra produced from the neutron activation. One of the main goals of the present study is to produce a technique that is feasible to measure Al in subjects with a normal concentration of this element. Byun and colleagues (2006) developed and tested a 4π detection system at MAL with high efficiency gamma ray detection. The present research utilized this 4π detection system for the *in vivo* Al measurement.

The 4π detection system (Figure 2) consists of an array of eight NaI (Tl) detectors. The cross-sectional dimension of each detector is $102 \times 102 \times 406$ mm. An empty square shaped opening in the middle of the detector array is used to place an irradiated hand/phantom. Since the opening extends from one side of the array to the opposite side, the solid angle is 3.83π which provides a high efficiency of the detector system. Signals from all the eight detectors were summed up using a locally developed summing circuit which was connected to a preamplifier. This signal was amplified using a NIM spectroscopy amplifier for a PC-based Multi-channel analyzer (MCA). The gamma ray spectra were collected using the software MaestroTM. It is important to note that although it would be

beneficial to have the irradiation and counting rooms in close proximity to minimize a transfer time between the activation and detection, they are in fact located at a distance to avoid the activation of the detector.

FIGURE 2. Layout of a 4π NaI(Tl) detector array for IVNAA.

The detection system is usually calibrated before the start of the experiment using either ^{137}Cs or ^{60}Co . In order to calibrate the detection system, an isotope is placed in the opening of the detector array where the hand/phantoms are to be placed. ^{137}Cs is a single gamma emitter while ^{60}Co is a cascade gamma emitter. ^{60}Co emits gamma rays with energies 1.17 MeV and 1.33 MeV. If a ^{60}Co source is placed in the detector, the spectrum produced consists of 3 peaks.

The third peak is the sum peak of the two gamma rays with energy 2.505 MeV. The next step in the energy calibration is to fix the energies of the first two peaks of energies 1.17 MeV and 1.33 MeV, respectively.

2.4.1 Gamma rays: Prompt and Delayed Gamma Emission

When the hand/phantom is irradiated with the neutron beam, penetrating gamma rays are emitted and collected by the detector array. The emission of gamma rays during neutron activation is called prompt gamma ray emission. The emission of gamma rays after the neutron activation is called delayed gamma ray emission. These gamma rays are generated from the nuclei in the hand/phantom upon the absorption of energy from the impacting neutrons. In IVNAA of Al, the ^{27}Al is neutron activated to form ^{28}Al which decays to ^{28}Si by emission of gamma rays;

Thus, delayed gamma rays at 1.78 MeV are detected in this work.

2.4.2 Gamma Ray Spectrum

The gamma ray spectrum obtained consists of all gamma rays from the elements present in the biological sample plus background radiation. Figure 3

represents a spectrum acquired for an Al dissolved hand phantom using IVNAA. It can be seen that the gamma peaks from Al and Cl overlap each other. The regions of interest in this spectrum are those around the Al and Ca peaks, since we are looking for concentration of Al in the biological sample and Ca is used for the normalization procedure (see section 2.5.2).

FIGURE 3: Spectra acquired for an Al dissolved hand phantom using IVNAA.

2.5 Data Analysis

Gamma ray spectra were collected and analyzed to determine the areas under the Al 1.78 MeV and Ca 3.08 MeV peaks, using Marquardt analysis (Bevington, 1992) (a non-linear least squares function fitted to the data in the energy regions around the two principal peaks of interest). A mathematical model was fitted to the spectra and the reduced chi-squared space was searched for a minimum to determine the peak parameters. The spectra were analyzed from 1.20 – 2.56 MeV for the Al peak. The peak was fitted with a Gaussian function on a quadratic background; the fitting function also included two surrounding peaks of Na and Cl located at 1.37 and 2.17 MeV respectively. The position of the Al peak was coupled to that of the more prominent peak of Cl at 1.64 MeV. A separate fitting procedure using a Gaussian function with a quadratic background between 2.96-3.40 MeV was adopted to obtain the area under the Ca peak. Further details of the fitting procedures adopted in this study are available in the literature (Pejović-Milić *et al.*, 1998).

2.5.1 Marquardt Method

Marquardt analysis is a method of data analysis that uses a non-linear least squares function fitted to the data in the energy regions around the principal peaks

of interest. It is a mathematical model that replicates three Gaussian functions on a quadratic background that is fitted to the spectra. The function that was used for fitting the spectra in the present study is as follows

$$y = b_1 + b_2x + b_3x^2 + A_1/[s_1(2\pi)^{1/2}] \exp(-0.5(x-c_1)^2/s_1^2) + A_2/[s_2(2\pi)^{1/2}] \exp(-0.5(x-c_2)^2/s_2^2) + A_3/[s_3(2\pi)^{1/2}] \exp(-0.5(x-c_3)^2/s_3^2) \quad (\text{equation 2.2})$$

where $b_1 + b_2x + b_3x^2$ represents the quadratic background, $A_i/[s_i(2\pi)^{1/2}] \exp(-0.5(x-c_i)^2/s_i^2)$ represents the Gaussian function. A_1 represents the area under the first Cl peak; s_1 and c_1 represent the width and position of the first Cl peak, respectively. Similarly A_2 and A_3 represent the areas under the Al and second Cl peaks respectively. The corresponding width and position of the Al and second Cl peaks are s_2 & c_2 and s_3 & c_3 , respectively. An important step in the fitting is an iteration procedure that will reduce the number of parameters to be fitted by fixing the width of the Gaussian peaks. The peak parameters are determined with a least chi-square method in this analysis. The reduced chi-square of the analysis represents the effectiveness of the fitting. Thus, with the Marquardt analysis, the area under the peak of interest can be calculated. Since the Cl in the biological sample or human hand is activated with thermal neutrons simultaneously with Al, we fitted the overlapped region covering the Cl (1.64 MeV) and Al (1.78)

characteristic peaks with two Gaussian. There were also two separate fitting routines that were applied to Al and Ca characteristic peaks separately.

2.5.2 Calibration Line

The area under the Al peak in the case of phantom studies or the ratios of the Al/Ca in the case of human studies are used to develop the calibration curve. For phantom samples, the calibration line was obtained by plotting the area under the Al peak, determined from the Marquardt analysis, versus the Al mass in each phantom. The best fit line, regression line, was determined by a linear least-squares method.

For human studies, the calibration line was obtained from the plot of the ratio of the areas of the Al and Ca peaks versus Al mass. This Ca normalization is preferred to the direct use of Al, since it simplifies the technique utilized. In fact, the number of counts from Al in the hand depends upon various parameters like size of the hand, thickness of overlying tissue, neutron field profile and fluence along with the irradiation and counting geometry; thus, there is a need for extensive corrections for all these factors. To overcome all these necessary corrections, normalization with the Ca present has been performed as suggested in the literature (Ellis *et al.*, 1988). Since 98% of the Ca is stored in the skeleton, the

amount of irradiated bone mass can directly be estimated from ^{49}Ca which is simultaneously activated with Al in hand bone through the reaction $^{48}\text{Ca} (n, \gamma) ^{49}\text{Ca}$ ($\sigma = 1.09$ barn). The Al/Ca ratio provides an index of elevated Al level per unit bone mass.

2.5.3 Minimum Detectable Limit (MDL)

Once the calibration curve is generated, its slope provides information about the sensitivity of the IVNAA technique. Therefore, each different set of irradiation/counting parameters provides different detection limits. The minimum detectable limit (MDL) of the system is obtained as an estimate of twice the uncertainty of zero Al phantoms divided by the slope of the calibration line (Palermo *et al.*, 1993).

Another method of assessing the sensitivity and performance of the technique is by calculating a figure of merit (FOM), which depends on the MDL of the system. The FOM takes into account the MDL and the equivalent dose to hand which accompanies the IVNAA measurements. MDL, as defined earlier, is the ratio of the uncertainty in the area of the Al peak in a $0.0 \mu\text{g Al/g Ca}$ phantom to the sensitivity of the technique. The uncertainty in peak area is approximately proportional to the square root of the equivalent dose, $H^{1/2}$, and the sensitivity is

proportional to H . Thus the MDL is then proportional to $1/H^{1/2}$. The FOM is defined as the product of $H^{1/2}$ by MDL and compares the performance of techniques. FOM can be used to compare the performance of the technique developed here with various other IVNAA techniques reported in literature. A lower FOM will be indicative of better performance of a technique.

2.6 Dosimetry

The dosimetry measurements of the IVNAA technique must be performed prior to the application in human subjects. The dose delivered to a subject's hand during an Al measurement is total energy deposition coming from different kinds of radiation such as neutrons, gamma rays, recoil and decay of some atoms. It is also documented that the dose delivered to the hand is associated with many parameters such as the incident proton energy, irradiation time, irradiation cavity design, and neutron yield ((Aslam *et al*, 2003). The use of a tissue equivalent proportional counter (TEPC) measurement will take into account the neutron and gamma dose, and thus give a precise measurement of the absorbed dose. Neutron micro dosimetry measures the deposited energy, linear energy density and the quality factor (Arnold, 2000). The thermal neutron flux within the irradiation/shielding cavity was measured using an indium foil experiment

(Pejović-Milić *et al.*, 2006). This flux measurement gives an estimate of the thermal dose delivered to the subject's hand during the irradiation.

2.6.1 Neutron Dose

The neutron dose depends on the incident neutron energy since the radiation weighting factor for neutrons also depends on energy. The reactions in the hand by thermal neutrons are $^{27}\text{Al}(n, \gamma)^{28}\text{Al}$, $^{48}\text{Ca}(n, \gamma)^{49}\text{Ca}$, $^{37}\text{Cl}(n, \gamma)^{38}\text{Cl}$, $^{23}\text{Na}(n, \gamma)^{24}\text{Na}$, $^1\text{H}(n, \gamma)^2\text{H}$ and $^{14}\text{N}(n, p)^{14}\text{C}$. The dominant damaging effects result from the neutron activation of nitrogen atoms. The processes that contribute to the dose from this reaction are the production of protons, recoils and decay of the ^{14}C atoms.

2.6.2 Gamma Dose

The dose delivered by the gamma rays from the neutron reactions with hydrogen, chlorine and sodium is insignificant, because the hand has a thin layer of soft tissue and rather small and thin bones. This means almost all of the gamma rays will escape from the hand before making energy deposition. Gamma rays have the least radiation weighting factor with a value of 1. The dose from the decay of the carbon and sodium atoms is negligible compared with the dose

deposited by the protons. It should be noted that the dose from thermal neutrons comes from a mixture of low Linear Energy Transfer (LET) gamma and high LET protons and ^{14}C recoils LET types of radiation.

2.6.3 Dosimetry using Tissue Equivalent Proportional Counter (TEPC)

The total dose equivalent received from both neutrons and photons by humans during the irradiation procedure has been estimated by the trace element analysis group at McMaster University by employing a tissue equivalent proportional counter (TEPC) (Aslam *et al.*, 2003). Due to its severe limitations to provide accurate neutron quality factors at the low neutron energies employed in these measurements, a Monte Carlo radiation transport simulation code, MCNP5, was used to estimate the neutron quality factors (Aslam *et al.*, 2006). The calculated neutron quality factors and the TEPC measured neutron and gamma doses would give an estimate of the local and the average dose equivalent delivered to a human hand during this diagnostic irradiation procedure.

The dose equivalent received by a subject during an irradiation procedure is an important control for IVNAA measurements. The radiation dose was therefore kept as low as possible, consistent with the previously assessed

statistical precision requirements of the measurement. The dose equivalent to the hand during 3 min of irradiation at proton energy $E_p = 2.00$ MeV and with a beam current of 100 μA , measured using the TEPC was 17.6 mSv ((Aslam *et al.*, 2006)). A neutron quality factor of 13 was used for this purpose. The gamma dose contributes to approximately 13% of the local dose equivalent applied to the hand. Further details of dosimetric measurements are available in literature (Aslam *et al.*, 2003; Byun *et al.*, 2007).

Chapter III

Phantom Studies

3.1 Preparation of Hand Phantoms

Human hand bone consists primarily of cortical bone. Al dissolved hand bone phantoms were prepared using compounds of the elements Al, Ca, Na, Mg and Cl according to the ICRP 23 (1975) Reference Man cortical bone composition.

The elemental composition of the hand bone is presented in Table (1). Other elements like phosphorus and potassium are also present in the hand bone. The energy of the produced neutrons in IVNAA is not greater than 1.95 MeV to produce ^{28}Al from the interfering ^{31}P (n, α) ^{28}Al . Either because of the negligible cross-section at thermal neutron energies or negligible amount of the element in the biological sample, the induced activity of P and K is considered negligible when compared to those presented in Table (1). Such elements were not added to create the phantom.

	Elements			
	⁴⁸ Ca	²³ Na	³⁷ Cl	²⁶ Mg
Expected Amount (ICRP)	14.90 g	1.25 g	1.19 g	237 mg
Compound added	Ca(NO ₃) ₂ ·4H ₂ O	NaNO ₃	NH ₄ Cl	Mg(NO ₃) ₂ ·6H ₂ O
Amount of compound (g)	88.06±0.05	4.60±0.01	1.80±0.01	2.51±0.01

TABLE 1: Elemental composition of hand bone of a Reference man and hand bone phantom.

Calcium nitrate [Ca(NO₃)₂·4H₂O], sodium chloride (NaCl), ammonium chloride (NH₄Cl), magnesium nitrate [Mg(NO₃)₂·6H₂O] and varying amounts of aluminum nitrate [Al₂(NO₃)₃] were selected due to their excellent solubility in water. All these compounds were dissolved in distilled water in 250 cm³ cylindrical Nalgene bottles. The concentrations of Al in the phantoms ranged from 0 to 19.9 mg with 14.9 g of Ca in all of them, thus corresponding to concentration within the range of 0 to 1,335.6 µg Al per gram of Ca. The concentration of Al in the Reference man for human hand bone is estimated to be 0.3-0.4 mg [20-27 µg Al/g Ca].

The bone phantoms used in this study are cylindrical in shape and all the elements of interest including Al and Ca are homogenously distributed throughout the entire volume of each bone phantom. Its volume was chosen to represent the volume of a typical hand including palm and fingers. Thus the number density, n_T , of all the major and minor elements present in the phantom, with the exception

of Al, is the same as that in the cortical bone of Reference man. Recall that phantoms with different concentration of Al are needed to obtain the calibration line.

The rate of production of the corresponding radioisotopes as a result of thermal neutron activation of the phantom depends on the number density (n_T), neutron capture reaction cross-section (σ), and the incident neutron flux (ϕ). The neutron cross-section (σ) is independent of the shape or dimensions of the phantom as well as the number density. However, the neutrons flux (ϕ) is dependent on the dimensions of the phantom or hand.

The thermal neutron flux depends on neutron energy which occurs as a result of neutron interaction with tissue. A slight non-uniformity (<20%) in the spatial distribution of ϕ along the lateral direction may cause a difference in the activation of the elements of interest. This is expected to be compensated by the larger dimensions of the phantom in the transverse direction along the beam since ϕ decreases laterally from centre of the beam to the edges of the phantom/hand. The small difference introduced in the activation of the elements of interest due to different shapes, dimensions, and densities of the phantom/hand bone is taken into account by expressing the results relative to irradiated bone (*i.e.*, as a Al/Ca ratio) since it cancels out the dependence on ϕ .

FIGURE 4: Comparison of human hand and hand phantom spectra acquired under similar conditions of activation and counting.

A comparison of a human hand spectrum with that of an Al hand phantom under identical conditions of irradiation and counting is shown in figure (4). This figure demonstrates that the chemical composition of the hand phantom accounts for all significant radioactive nuclides, which are produced as a result of the neutron activation of the human hand.

3.2 Preliminary Phantom Studies using an Array of Eight 4π NaI(Tl) Detectors

An important step of this work was to determine the sensitivity of the developed bone Al IVNAA techniques using different running parameters and hand phantoms. The experimental set up used for the preliminary phantom studies included the following:

- High current Tandatron Accelerator as the neutron source;
- The new irradiation/shielding cavity inside where the phantoms are placed for neutron activation;
- The 4π detection system;
- Marquardt Method of analysis.

At first, the set of phantoms were neutron activated for 3 minutes using a proton beam of energy 2.00 MeV and proton beam current of 100 μ A. The accompanied radiation dose to the hand was 17.6 mSv as measured using the TEPC. The phantoms, after irradiation in the cavity, were transferred to the detection system within 35 seconds and counted for 10 minutes. The spectra were then analyzed using the Marquardt method and the calibration lines for Al were constructed. The slope of the calibration line represents the sensitivity of the technique to measure Al and the MDL values were then calculated as described

before. Phantom activation with the same running parameters was repeated three times, and, at each time, the sensitivity as well as the average MDL were determined to compare different experimental conditions.

The irradiation time of 3 minutes was found not to be appropriate for measuring Al, as its half life is 2.25 minutes. The amount of ^{28}Al that will remain after an irradiation of 3 minutes is about 60% of the total, and this amount will be greater if the irradiation time decreases. The expression for a neutron activation reaction in terms of radioactivity that remains after irradiation is given by,

$$A = n_T \phi \sigma (1 - e^{-\lambda t}) e^{-\lambda T}$$

(equation 3.1)

where,

- A is the radioactivity;
- n_T is the number density of neutrons;
- ϕ is the neutron flux;
- σ is the neutron cross-section;
- λ is the decay constant;
- t is the irradiation time;
- T is the transfer time.

Since neutron flux is directly proportional to the proton beam current, a reduction in irradiation time requires a corresponding increase in proton beam current. For instance, if the current is increased by a factor of 2 (from 100 to 200 μA) or if the flux is doubled, *i.e.*, 2ϕ , then the irradiation time has to be halved (*i.e.*, $t/2 = 90$ seconds) if the proton beam energy and number density remains the same.

This would enhance the radioactivity of ^{28}Al , and thus increase the sensitivity and performance of the system.

The phantoms were neutron activated with currents from 100 to 400 μA with a corresponding decrease in the irradiation time from 180 seconds to 45 seconds. The other irradiation parameters like proton beam energy, hand radiation dose, transfer time, and counting time were kept constant at 2 MeV, 17.6 mSv, 35 seconds and 10 minutes respectively.

3.3 Results of Preliminary Phantom Study

Figure (5) depicts the calibration curve obtained for the set of Al phantoms with the following irradiation parameters: 100 μA current, 180 s irradiation time, proton energy of 2 MeV, transfer time of 35 s, and 600 s of counting. The MDL achieved was 0.20 mg of Al. Two other different sets of phantoms with the same composition were also neutron activated under same irradiation parameters and the corresponding MDL values were estimated. The average MDL of the three sets of measurements was found to be 0.22 mg of Al, as shown in Table 2. The measured levels of Al in hand bone of a healthy person are in the range 0.3 – 0.4 mg Al (ICRP 23, 1975). Therefore the MDL obtained in these preliminary

phantom measurements was well below the ICRP range, and the application of the technique of IVNAA was considered feasible for *in vivo* human studies.

FIGURE 5: Calibration curve obtained from the measurements of Al hand phantoms. A hand phantom MDL of 0.20 mg of Al was achieved.

However, a possible improvement in the MDL value was investigated to further improve the sensitivity of the technique. Table (2) represents the values of average MDL achieved with an increase in proton beam current and corresponding decrease in irradiation time. A different protocol was also included, where a proton beam current of 400 μA with an irradiation time of 3

minutes and a proton beam energy of 1.92 MeV was tested. All other radiation parameters like transfer time and cooling time were kept constant. The radiation dose associated with this latter irradiation protocol was drastically reduced from 17.6 mSv to 5.1 mSv. The resulting MDL (Table 2) was comparable to that achieved for 100 μ A current, 2 MeV energy, 3 minutes of irradiation and a 17.6 mSv radiation dose.

Hand Dose	Proton Current	Proton Energy	Irradiation Time	Cooling/ Counting Time	MDL
<i>mSv</i>	μ A	MeV	min	Sec	mg
17.6	100	2	3	35/600	0.22 $\pm$ 0.05
17.6	200	2	1.5	35/600	0.18 $\pm$ 0.03
17.6	300	2	1	35/600	0.157 $\pm$ 0.03
5.1	400	1.92	3	35/600	0.235 $\pm$ 0.01

TABLE 2: Different MDLs achieved with *in vivo* neutron activation analysis of Al in hand phantoms.

One of the important results achieved in this preliminary phantom study is the improvement of MDL from 0.22 mg to 0.157 mg by increasing the proton beam current from 100 μ A to 300 μ A and decreasing irradiation time from 3 to 1 minute. Even the MDL of 0.22 mg of Al, achieved for an irradiation protocol of 100 μ A proton current, 2 MeV proton energy, 3 minutes irradiation time, 35 seconds of transfer time and 10 minutes of counting was better than the ones

obtained by Byun and colleagues (2006) and Ellis and colleagues (1988). The MDLs estimated from those previous studies were 0.24 mg and 0.4 mg of Al respectively.

Table (3) shows a comparison of MDLs and figures of merit (FOM) of IVNAA obtained by different research groups until today. As can be seen, the present research achieved a FOM of 0.92 which is lower than that reported by Byun *et al.*, 2006, and Ellis *et al.*, 1988. Further improvement in FOM could be achieved by placing the detection system closer to the irradiation cavity with a shield around it, which would decrease the transfer time to 35 s and thus reduce the FOM to below 1.

Publication	Source	Hand dose (mSv)	MDL (mg Al)	FOM
Ellis <i>et al.</i> , 1988	Research reactor	20.00	0.40	1.79
Wyatt <i>et al.</i> , 1993	²⁵² Cf	36.00	2.20	13.20
Green & Chettle, 1992	Dynamitron accelerator	50.00	2.00	14.14
Green <i>et al.</i> , 1993	Dynamitron accelerator	46.00	1.30	8.82
Palermo <i>et al.</i> , 1993	Nuclear reactor	4.00	1.50	3.00
Pejović-Milić <i>et al.</i> , 1998	KN accelerator	12.00	2.50	8.66
Comsa <i>et al.</i> , 2004	KN accelerator	20.00	0.70	3.13
Byun <i>et al.</i> , 2006	KN accelerator	20.00	0.24	1.07
This work, 2008	Tandetron accelerator	17.60	0.22	0.92

TABLE 3: Comparison of MDL and the FOM of IVNAA techniques developed by different research groups for measurement of Al in bone. The FOM is defined as the product of MDL achieved and the square root of hand dose equivalent.

All average MDL calculated from this preliminary work are well below the values presented in the reported studies in the literature and the estimated normal concentrations from ICRP 23. Therefore, it was concluded that the technique of IVNAA could be tested in an *in vivo* human study, especially on subjects with high bone Al concentration. In order to find the range of normal values of the amount of Al present in human bone, a pilot *in vivo* study was conducted involving population living in Southern Ontario. This *in vivo* study is discussed in the following chapter.

Chapter IV

In Vivo Human Studies

4.1 *In Vivo* Human Studies

The first human hand measurements designed to test the described bone Al IVNAA were conducted in 2007. Prior to these measurements, the *in vivo* study was approved by the research ethics boards of both Ryerson and McMaster Universities. The subjects were chosen from a local healthy population without exposure to Al through occupation or medical treatment. They were requested to sign a consent form which also gave them the option to withdraw from the studies at any time. Eighteen healthy male volunteers with a mean age of (51.8 ± 13.1) years in the range of 25 -74 years participated in the study.

During the measurements, each subject was provided a chair to sit on and was asked to insert their left arm into the opening of the irradiation/shielding cavity (Figure 6) for neutron activation by the Tandetron accelerator. The hand was positioned with the open palm facing the neutron source (i.e. the accelerator target). The preference of the open palm to fist position was to ensure a more homogeneous dose distribution. The fist position adds an air gap between the fingers and inside

of the palm, which can result in an inhomogeneous dose distribution. The facility is designed such that it irradiates neutrons to the entire hand which includes the fingers and the palm. The arm was tightly bound with a water sleeve to provide sufficient radiation protection to the rest of the body. It also restricts the motion of the arm. For comfort, the water sleeve could be adjusted around the different length and thickness of human arms. Each volunteer was given a direct-reading electronic dosimeter for personal monitoring of radiation dose to the rest of the body during the hand irradiation.

FIGURE 6. Human subject hand irradiation at MAL for IVNAA measurements.

The irradiation parameters used for this *in vivo* measurement of Al in human hands were chosen to be the same as one of those used in the IVNAA study with phantoms. They were: 3 minutes of irradiation at 2MeV proton energy and 100 μ A

proton current; counting time of 10 minutes. The average time taken by the subjects to transfer from the irradiation facility to the detection system was 105 seconds. Since this *in vivo* study is a side study of Mn study conducted at McMaster University, these parameters were approved by the ethics board.

The radiation dose used for the IVNAA technique was measured using the Tissue Equivalent Proportional Counter (TEPC) and was kept as low as possible according to the As Low As Reasonably Achievable (ALARA) principle. The equivalent dose to a subject's hand was measured to be 17.6 mSv with an estimated total effective dose to the body of 14.4 μ Sv.

4.2 Calibration Using Hand Phantoms

The calibration curves used for the *in vivo* human measurements of Al and Ca are illustrated in the Figures 7a and 7b. The only deviation from the previous (phantom) protocol for the *in vivo* measurements was for transfer time which here was taken to be 105 seconds. The irradiation and transfer time was corrected to obtain the ratio of amounts of Al. For Ca, phantoms with varying amounts of Ca in the range of 11.6 to 19.6 g and fixed amount of other elements taken from ICRP were irradiated and counted under similar conditions as used for Al. For Ca, it was suitable to use the same calibration line as the one used for the

manganese study. The slopes of the calibration lines were then used to find the amount of elements obtained from the counts under the respective peaks. The irradiation and transfer times were corrected to obtain the ratio of amounts of Al to that of Ca.

The MDL achieved was 0.29 mg, for a 100 μ A proton current set up with 3 minutes of irradiation at 2MeV proton energy, a counting time of 10 minutes and a transfer time of 105 seconds. Recall that the MDL is conventionally estimated as twice the uncertainty of zero Al content divided by the slope of the calibration line. The ratio of Al to Ca was taken as an index of elevated Al levels per unit bone mass. Moreover, the fluctuations in the amounts of Al activated due to variations of the thermal neutron flux, size/shape or positioning of hands are corrected by calcium normalization. The estimated level of Ca in hand bone was taken as 14.9 g according to the International Commission on Radiation Protection (ICRP) Reference Man (ICRP 23, 1975). Each Al dissolved hand phantom contained of 14.9 g of Ca. In phantom studies the ratio of Al/Ca was found by dividing the amount of detected Al by 14.9 g of Ca and the phantom MDL was determined to be 19.5 ± 1.5 μ g Al/g Ca. Since the expected levels of Al in the hand bone of a healthy person are in the range from 20 to 27 μ g Al/g Ca [0.3 – 0.4 mg Al] (ICRP 23, 1975), the technique yields the MDL within the range of the expected levels in hand bones.

FIGURE 7a: Calibration curve obtained from the measurements of Al dissolved hand phantoms. A hand phantom MDL of 0.29 mg of Al was achieved, for a 100 μA proton current set up with 3 minutes of irradiation at 2MeV proton energy, a counting time of 10 minutes and a transfer time of 105 s.

FIGURE 7b: Calibration curve obtained from the measurements of Ca dissolved hand phantoms (Aslam *et al* 2008a), for a 100 μ A proton current set up with 3 minutes of irradiation at 2MeV proton energy, a counting time of 10 minutes and a transfer time of 105 seconds.

4.3 Data Analysis and Results

Marquardt analysis was used for *in vivo* human measurements to determine the amount of Al and Ca present in the hand bone. Figure 8 illustrates the distribution of these element ratios present in the hands of the participants of

this study. The hand bone Al concentration ranged from -9.6 ± 11.6 to 60.3 ± 10.4 $\mu\text{g Al/g Ca}$. The error estimates have a confidence level of 68% (1σ).

In order to estimate a value for the population as a whole, the inverse variance weighted mean can be used. This gives a value of 27.9 ± 3.3 $\mu\text{g Al/g Ca}$. The values of the hand bone Al levels ranged from -0.14 ± 0.16 to 1.2 ± 0.20 mg with a mean of 0.47 ± 0.29 mg Al. The mean bone calcium level was 16.9 ± 3.20 g Ca, within the range from 10.9 ± 0.01 to 22.3 ± 0.00 g. The average relative standard error in Al/Ca was expected to be 50%. However, the relative error in three measurements was found to be larger than 50%, which was caused primarily because of gain shifts in the detectors during the measurements, and the data extraction methodology used.

As shown in figure 8, the distribution of Al/Ca ratio shows that the majority of data points are above the present phantom detection limit of the technique. The hand bone Al levels of almost all subjects are within the uncertainty and, close to the system's detectable limit. However, the subject numbered 18 not only had an Al/Ca ratio below the detection limit but also exhibited a negative value (which is zero within the uncertainty). Even though this negative value for the Al/Ca ratio is not physiologically possible, it is acceptable from the statistical point of view.

The presence of negative values for Al/Ca ratio are primarily due to the use of basic statistical processes of radiation detection and the fact that the achieved detection limit is close to zero. This phenomenon is often observed in other branches of analytical sciences (Analytical Methods Committee, 2001). McNeill and colleagues (1999) also reported negative values in the *in vivo* measurement of lead content for very low concentrations of lead in bone.

FIGURE 8. Distribution of the Al/Ca ratio in human subjects measured using IVNAA. The hand phantom minimum detectable limit (MDL) of $19.5 \pm 1.5 \mu\text{g Al/g Ca}$ is also included in this figure. The individual measurement uncertainties represent a confidence level of 68% (1σ).

Two of the volunteers participating in this study were not included in the data set displayed in Figure 8, due to problems during their hand activation. One volunteer had a very short arm and could not correctly place his hand inside the irradiation/shielding cavity. Therefore, only part of the palm (and bone) was activated. In this case the observed hand levels of Al and Ca were 0.70 ± 0.27 mg and only 7.29 ± 0.01 g respectively. The hand activation of the second volunteer was stopped before the completion of the 3 minutes of irradiation due to technical difficulties. The Al and Ca levels in this case were 0.15 ± 0.29 mg and only 7.41 ± 0.01 g, respectively. Once the calcium normalization was applied, the two measurements presented an Al/Ca ratio of 96.0 ± 36.4 and 20.5 ± 39.2 $\mu\text{g Al/g Ca}$. These measurements show significantly higher uncertainties when compared to the measurements of the other volunteers. Thus it was suggested to exclude these two cases from any further data analysis.

4.4 Comparison with Other Studies

It is difficult to directly compare the results for Al/Ca obtained in this study with other similar studies reported in the literature, due to the differences in the bone sites, bone types, and health conditions of the human subjects. However, it is interesting to note that the Al/Ca in bone measured in this study is within the

ranges of the concentration of Al/Ca in bones of human subjects reported in other studies (Ellis *et al.*, 1988; Wyatt *et al.*, 1993), using the IVNAA technique. Wyatt and colleagues have measured the amount of Al in 7 dialysis patients in the range of -42 to 518 $\mu\text{g Al/gCa}$. The comparable results of this *in vivo* study with reported studies demonstrates its potential in the monitoring of Al levels in the human bone of exposed population.

Contrary to two *in vivo* studies cited above, there are several *in vitro* studies available in literature that provide the bone aluminum values in autopsy and biopsy samples. For example, Scancar and colleagues (Scancar *et al.*, 2000) reported the amount of aluminum detected in iliac crest samples taken at the autopsy of 12 healthy subjects. The ratio of Al to Ca in these subjects ranged from 21 to 88 $\mu\text{gAl/gCa}$. In the same study, the authors reported the bone aluminum levels from the biopsies of 6 dialysis patients, ranging from 51 to 138 $\mu\text{gAl/gCa}$. Similarly, Hongve and colleagues (Hongve *et al.*, 1996) found Al concentrations in autopsy samples of a non-exposed population ranging from 0.5 - 5.8 mg Al/kg ash weight, while increased Al levels ranging from 16.8 to 18.0 mg Al/kg ash weight were found in the samples of a group of dialysis patients. If one takes bone ash to be 40% Ca by mass, these become 1.25 - 14.5 $\mu\text{gAl/gCa}$ for the non-exposed group and 42 to 45 $\mu\text{gAl/gCa}$ for the dialysis patients.

This shows that the IVNAA technique described here would allow the monitoring of normal Al levels in the human hand bone if the sensitivity of the technique can be improved. Furthermore, the measured values are comparable to both the *in vivo* and *in-vitro* values reported in the literature.

4.5 Conclusions of *In Vivo* Human Studies

The application of the IVNAA technique as a clinical diagnostic tool is possible only if the technique is able to measure the elevated levels of Al in clinically and occupationally exposed subjects as well as the levels of Al present in a non-exposed population. Since some of the human measurements obtained in this study are below the detection limit, the present technique has some limitation as how reliably it can estimate the hand bone Al reference values. The negative values and the values below the detection limit, thus stipulate a need to improve the sensitivity of the bone Al IVNAA technique which will be discussed in the following chapters of this work.

On the other hand, the majority of human Al/Ca levels are above or close to the detection limit, clearly presenting the potential of the described technique to be extended to a larger group of subjects, especially for the measurement of Al loads expected in the hand bone of dialysis patients.

Furthermore, according to ICRP 23 reference data, the values of aluminum and calcium result in an Al/Ca ratio of 20 to 27 $\mu\text{g Al/g Ca}$. The mean hand bone aluminum concentration of $(27.9 \pm 3.3) \mu\text{gAl/g Ca}$ obtained in this work is therefore comparable to the reference value of 20 - 27 $\mu\text{gAl/gCa}$ for human hand bone.

One of the objectives of the human studies is also to verify whether the composition of phantoms developed for *in vivo* studies is adequate by comparing the MDLs. The doubled median value of the uncertainties was used as a measure of the precision of the *in vivo* bone Al technique (Studinski *et al.*, 2006). This resulted in an *in vivo* detectable limit in the order of $(28.0 \pm 6.6) \mu\text{gAl/gCa}$ for the hand bone. The *in vivo* MDL calculated is then slightly greater than that of the hand phantoms $[(19.5 \pm 1.5) \mu\text{gAl/gCa}]$, which demonstrates that there is greater variability in the human measurements than there is with the phantoms and justifies the use of the new sets of hand phantoms developed for this study. Lastly, the *in vivo* MDL of the work presented here is promising and slightly improved when compared to the best previously reported detection limits obtained using IVNAA of the hand (see Table 3, Chapter 3).

Chapter V

Optimization of the Bone Aluminum IVNAA technique

5.1 Need to Optimize the Technique of IVNAA

In vivo measurements of Al in non-exposed subjects at MAL have reported some Al/ Ca values that are below or within the present detection limit, which demonstrated that the developed technique has some limitations to reliably estimate hand bone Al reference values. A means to improve the sensitivity of the bone Al IVNAA technique is therefore considered necessary (Chapter IV). Strategies to improve the detection limit of *in vivo* hand bone Al levels are considered in this chapter and contrasted with those measuring approaches previously described (Chapter III).

The first step to be taken is to improve the detection system (section 5.2) by replacing and/or adding new detectors, which was followed by the investigation of which irradiation parameters would improve the sensitivity of bone Al IVNAA technique. Two approaches to improve the detection limit were investigated in this study: 1) without change in the hand dose of less than 20 mSv and 2) with increase of the hand dose up to 50 mSv.

5.2 Enhanced Detection System

The two NaI detectors which were unstable and had poor resolution were replaced with new detectors of same size. A new, smaller detector has also been added to the rear end of the system to further approach the 4π geometry.

The improvement in resolution of the new system was tested using hand phantoms. A comparison of the resolution of the spectra using the original and enhanced detection system is shown in figures (9a) and (9b). Figure (9a) illustrates part of the spectrum of 19.9 mg Al phantom containing Na, Cl and Al peaks; generated using the upgraded detection system, while figure (9b) illustrates the same spectrum generated by the original detection system. Due to the improved system resolution and geometry, the Al and Cl peaks are better resolved using the enhanced detection system. A better resolution leads to a better system sensitivity as experimentally tested and presented in the next section.

FIGURE 9a: Al-Cl peak in the spectrum of 19.9 mg Al phantom detected with the enhanced detection system.

FIGURE 9b: Al-Cl peak in a 19.9 mg Al spectrum detected with the original detection system.

5.3. Phantom Studies with the Hand Dose Lower than 20 mSv

Since the Tandatron Accelerator is capable of delivering high proton beam currents, the irradiation time could be adjusted, while increasing proton currents accordingly to maintain a constant equivalent dose. This leads to an improved system sensitivity due to shorter activation time compared to the half-life of ^{28}Al nuclei. For example, if the irradiation time is reduced to 90 seconds and the

proton beam current increased to 200 μA , keeping constant the proton beam energy, then the number of ^{28}Al nuclei still present at the end of the irradiation period will increase. Similarly by reducing the length of irradiation from 180 seconds to 45 seconds and increasing the proton beam current from 100 μA to 400 μA at 2 MeV proton energy, the counts in the Al peak will increase by the factor of 2.25 resulting in an improvement of the MDL.

With the new enhanced detection system, the initial experiments utilized the same set of phantoms used in the previous studies (Section 3.1) with the original detection system. They were neutron activated for 3 minutes using a proton energy of 2.00 MeV and proton beam current of 100 μA to solely compare the improvement with the enhanced detection system. At this point, a transfer time of 30 s was used, followed by a counting time of 600 s.

Using the same method of data analysis (please refer to section 2.5), a linear regression line was constructed by plotting the area under Al peaks against the amount of Al in each phantom. The newly achieved MDL, 0.16 mg represents an improvement of approximately 20% when compared to the MDL of 0.22 mg obtained with the original detection system and similar protocols except for the transfer time of 30 seconds (Table 4).

Next, different irradiation parameters were tested. Thus the experiments were repeated for higher proton beam currents and corresponding decrease in irradiation times. Table (4) depicts the MDL obtained for different proton beam currents and irradiation times. For comparison, the last column of the table represents the MDL achieved for phantoms using the original detection system and the transfer time of 35 seconds.

Consistent improvement in the system's MDL was observed as the proton current was increased, reaching the MDL of 0.12 mg of Al accompanied with 17.6 mSv of the hand dose.

Hand Dose	Pr. Current / Pr. Energy	Irradiation Time	Counting Time	Current MDL (Transfer time = 30 sec)	Previous MDL (Transfer time = 35 sec)
mSv	μ A / MeV	Sec	Sec	mg Al	mg Al
17.6	100 / 2	180	600	0.16 $\pm$ 0.02	0.22 $\pm$ 0.05
17.6	200 / 2	90	600	0.15 $\pm$ 0.02	0.18 $\pm$ 0.03
17.6	300 / 2	60	600	0.14 $\pm$ 0.02	0.16 $\pm$ 0.03
17.6	400 / 2	45	600	0.12 $\pm$ 0.01	<i>n/a</i>
46.9	400 / 2	120	600	0.10 $\pm$ 0.01	<i>n/a</i>

TABLE 4: MDL achieved for different proton beam currents, irradiation times and irradiation doses.

5.4 Phantom Studies with the Hand Dose Lower than 50 mSv

Another irradiation protocol adopted for the optimization of the bone Al IVNAA technique was to increase the proton beam current to 400 μA and with an irradiation time of 2 minutes and proton beam energy of 2 MeV. The transfer time of 30 seconds and 600 seconds counting period was adopted as before. The irradiation dose associated with this protocol was calculated to be 46.9 mSv. Hand doses up to 50 mSv can be utilized for an IVNAA technique since it has been approved by the Ryerson (Approval # 2008 099) and McMaster (Approval # 08 255) Research Boards in anticipation of the next human study. The 50 mSv radiation dose for one measurement corresponds to an effective dose of 40 μSv . This can be compared to the annual natural background radiation dose in North America which is about 3000 $\mu\text{Sv}/\text{year}$. So, a bone aluminum measurement with a 50 mSv hand dose gives the same effective dose to an adult as about 5 days of natural background radiation.

Since the MDL is proportional to the square root of hand dose, the increase of hand dose from 17.6 mSv to 46.9 mSv (2.66 times) along with higher beam currents and reduced irradiation time yields a reduction of the detection limit of $\cong \sqrt{2.66}$. The experimentally achieved MDL for this protocol with an irradiation dose of 46.9 mSv was 0.103 mg of Al (Table 4).

5.5 Optimization of the Bone Aluminum IVNAA Technique: Discussion

This chapter suggests different procedures to optimize the technique of IVNAA to achieve better performance and sensitivity in terms of MDL. The *in vivo* measurements of Al in hand phantoms were carried out using the enhanced 4π detection system along with Tandetron accelerator, irradiation/shielding cavity and Marquardt data analysis.

The irradiation/counting protocol used for these experiments consisted at first of 180 s irradiation and 100 μ A proton beam current with 30 s transfer and 600 s counting time, for 2 MeV proton beam energy and 17.6 mSv hand dose. The MDL achieved with this protocol was 0.16 mg Al. Later the irradiation time was decreased to 90 s with an increase of beam current to 200 μ A with the other irradiation parameters remaining constant. The number of ^{28}Al nuclei that remained after 90 seconds of irradiation should be greater than that remained after 180 seconds of irradiation. From the neutron activation equation $A = N\phi\sigma(1-e^{-\lambda t})e^{-\lambda T}$ (eqn 3.1) it is clear that the detection limit should be increased by a factor of 1.11. The detection limit achieved in the experiment with the above protocol was 0.15 mg of Al which shows an improvement of MDL by a factor of 1.10. The experiment was repeated by increasing the proton beam currents to 300 and 400 μ A with decrease in the irradiation times to 60 and 45 s respectively. The MDLs

achieved were 0.14 and 0.12 mg of Al which correspond to improvement factors of 1.15 and 1.29 respectively.

The MDL achieved with the enhanced detection system was found to have improved when compared to that obtained with the original system. Consistent improvement in the MDL was experimentally achieved by increasing the proton current and simultaneously decreasing the irradiation time. This approach was followed by the dose optimization to achieve even better MDL. It was concluded, that the optimal parameters for the Tandetron accelerator are: 400 μ A proton beam current with 2 minutes irradiation time at 2 MeV proton beam energy, with a radiation hand dose of 46.9 mSv. As the MDL depends on the square root of the dose, the increase of the dose from 17.6 mSv to 46.9 mSv should reduce the detection limit by a factor of the square root of 2.66 ($46.9/17.6 = 2.66$). Thus, the detection limit should improve by a factor of 1.63. The experimental MDL achieved by applying these parameters was 0.10 mg of Al, an improvement by a factor of 1.56.

The MDL of 0.10 mg Al achieved is well below the predicted normal concentration of Al in the hand of 0.3-0.4 mg Al (ICRP 23, 1975). Therefore, it is concluded that the developed protocol is suitable for detecting concentration of Al in both healthy subjects as well as in patients with expected higher concentration

of this element. In conclusion, the protocol parameters of 2MeV proton energy with 400 μ A proton current, 120 seconds of irradiation time and 30 seconds of transfer time should be used in the future *in vivo* human studies.

Chapter VI

Quantification of Aluminum in Human Bone with Neutron Activation

Analysis

6.1 Summary and Discussion

The current medical field requires an acceptable diagnostic technique that can easily identify patients at increased risk of developing Al induced bone diseases. In the clinical environment the amount of Al in bone, which provides information about a cumulative exposure to the element, is currently estimated through biopsy of iliac crest bone. Limitations of bone biopsy include the fact that it is an invasive and painful technique. Bone biopsy cannot be repeated over time, and therefore cannot be used to follow a patient's Al loads. Furthermore, a biopsy is typically of a small mass and it therefore may not be representative of the bone Al levels. For all these reasons, bone biopsy is not a satisfactory method to measure bone Al levels, and interest has arisen to develop a method to measure this element *in vivo*, non invasively. An ideal method of this kind would be valuable in the determination of bone Al concentration over time. Furthermore, the knowledge of the bone Al concentration, incorporation and retention during dialysis treatment, for example, would also help to establish the onset of Al

induced bone disorders in this group of patients. The aim of this study is to further develop a technique using IVNAA for the measurement of bone Al that would be suitable for general use in hospitals.

The IVNAA facilities at McMaster University have been in use for the analysis of many trace elements. This readily available facility was successfully used for this application as well. The bone IVNAA technique has been under development for Al and Mn measurements in human hand bones at McMaster University for the last two decades. This work presents the first pilot *in vivo* human measurements of Al stored in the hand bone carried out on the population living in Southern Ontario, Canada. While it relies on previously reported work done at McMaster Accelerator Laboratory, it represents significant progress in developing a diagnostic tool suitable for monitoring bone Al in human skeleton.

A new generation of hand bone phantoms was built that closely resemble the human bone composition and spectra, and thus provide meaningful calibration of the technique. Furthermore, this study utilizes for the first time, a high current Tandetron accelerator and irradiation/shielding cavity designed for measurements of humans' extremities. Although the Tandetron has the ability to provide significantly higher proton currents (up to 1 mA), that was not presently exploited.

The enhanced 4π detection system was an added bonus for the improvement of the sensitivity of the technique that is reported in this work.

The ratio of Al and Ca masses in hand bone is used as a clinical indicator of bone aluminium levels. This is useful in comparing Al levels stored in the bone of healthy and exposed populations. For example, high Al levels in dialysis patients need to be regularly monitored to evaluate the side effects of Al intoxication that accompanies this treatment. Calcium normalization is usually adopted in the IVNAA technique because of the dependence of the technique's sensitivity on thermal neutron flux and the positioning of the hand upon irradiation. It reduces the uncertainties in the measurements of counting statistics, size and shape of the hand, transfer time, the data extraction methodology, and any gain shifts in the detector during the measurements.

Thus Ca normalisation improves sensitivity of the bone Al IVNAA technique. It also provides clinically relevant results suitable for regular monitoring of the Al loads, namely an index of elevated Al levels per unit bone mass.

This work presents the results of the first human bone Al measurements using the present developed IVNAA technique. The mean hand bone aluminum concentration of $(27.9 \pm 3.3) \mu\text{gAl/gCa}$, ranging from -9.6 to 60.3 $\mu\text{g Al/g Ca}$ was

measured in eighteen healthy volunteers participating in the *in vivo* human study. The variability in Al/Ca ratio in different subjects is not well established. This range of values is generally attributed to the differences in the bone sites, size and shape, age, lifestyle, dietary habits and demographic origin (Takata *et al.*, 2005). Despite these differences, the results were compared favourably to both *in vitro* and *in vivo* Al bone values available in the literature, demonstrating the effectiveness of the developed, painless, non-invasive diagnostic tool described here. However, the first human data also point in a direction of need to further decrease the detection limit of the technique.

The present study investigates, therefore, different approaches to improve the sensitivity of this diagnostic tool. The sensitivity of the technique was investigated by using a higher dose rate, optimization of the irradiation parameters and greater detection efficiency. After taking into consideration several different approaches, it is suggested that a desirable detection limit for non-exposed subjects can be achieved by increasing the proton current, and thus increasing the accompanied effective dose of each measurement. The running parameters of 2 MeV proton beam energy, proton current of 400 μ A, and 120 seconds of irradiation and 30 seconds transfer time provide the detection limit of 0.10 mg of Al accompanied with less than 50 mSv equivalent dose. The developed protocol seems sufficiently sensitive to detect the low levels of Al found in normal subjects

as well as screening patients with elevated levels of bone Al. However, it should be tested in *in vivo* human studies expected in the future.

The use of the spectral decomposition method (Comsa *et al.*, 2004) has been reported by the trace element analysis group at McMaster University as an advanced γ -ray spectrum analysis when compared to the non-linear fitting method used in this study. The spectral decomposition method provided an improvement in MDL when applied to the hand phantoms. It did not demonstrate similar improvement when applied to the human, *in vivo* spectra. One of the explanations might be that the human spectra have more noise than the phantom spectra, due to the shape/size of hand, presence of the overlying soft tissue and motion of volunteers.

6.2 Proposals for Future Research

Approval has been obtained from the Ryerson and McMaster Research Ethics Boards to perform the next human studies to measure Al in dialysis patients and occupationally exposed subjects. The goal of this study is to investigate whether Al levels in bone could serve as a biomarker of exposure and a means of monitoring exposed populations to this toxic element. This

anticipated study will also provide a platform to test a new protocol to lower the detection limit developed in this work.

More research should be done to find a possible correlation between bone Al values by measuring different types of bones and sites. The distribution of Al is not expected to be homogeneous throughout the human skeleton. Thus, measurements of other extremities with cortical and trabecular bone composition should be considered in further studies. These should be extended to other peripheral sites like foot bone, and accompanied with alterations in the irradiation/shielding cavity and detection facilities to accommodate measurement of this site. Another proposed future research is to find a way to correlate between the Al levels in the hand bone and human brain; this could answer many controversies regarding the relationship between Al and Alzheimer's disease.

Besides the use of an enhanced detection system that lowers the MDL, it is expected that future work should explore the construction of a detection room for human measurements close to the accelerator irradiation facility. This would reduce the time taken by the subjects or phantoms to move from the irradiation/shielding cavity to the detection room and thus improve the sensitivity of the presented technique. Clearly, the detection facility should have appropriate neutron/gamma shielding to avoid activation of detectors.

An Anticoincidence mode of detection for Al measurements should be also investigated in the near future. It has been reported that a reduction in the continuum level of the overlapping Al-CI occurs with anticoincidence techniques (Byun *et al.*, 2006). Even if the counting rate of the ^{28}Al peak is reduced compared to that in the direct mode detection used in this work, the ratio of the Al peak to the CI interfering peak would be improved considerably. This could result in a better MDL.

6.3 Final Remarks

For clinical *in vivo* measurements of Al detection in humans, it is necessary to test the suggested protocol in this work, which should lead to a lower *in vivo* minimum detectable limit (MDL), thus opening up a broad field of research that may improve our knowledge about Al health effects, its metabolism and besides the monitoring of the Al levels from occupational and clinical exposure.

REFERENCES AND BIBLIOGRAPHY

Advenier, E., Landry, C., Colomb, V., Cognon, C., Pradeau, D., Florent, M., Goulet, O., Ricour, C., and Corriol, O. (2003) Aluminum Contamination of Parenteral Nutrition and Aluminum Loading in Children on Long-Term Parenteral Nutrition. *J Pediatr Gastroentero& Nutr* **36** (4) 448-453.

Alfrey A. C. (1984) Aluminum intoxication. *New Engl J Med* **310** 1113-4.

Alfrey, A. C. (1976) The dialysis encephalopathy syndrome: Possible aluminum intoxication. *N Engl J Med.* **294** 184-188.

Alfrey, A. C. (1993) Aluminum and renal disease, In Moving points in nephrology, eds. E. Bourke, N. P. Mallick and V. E. Pollak, Basel, 110-124.

Alfrey, A. C. (1994) Aluminum intoxication in renal failure: a historic overview. *Life Chem Rep* **11** 197-205.

Alfrey, A. C., Mishell, M. M., Burks, J., Contiguglia, S. R., Rudolph, H., Lewin, E., and Holmes, J. H. (1972) Syndrome of dyspraxia and multifocal seizures associated with chronic hemodialysis. *Trans Am Soc Arti Intern Organs* **18** 257-261.

Andress, D. L., Maloney, N. A., Coburn, J. W., Endres, D. B., and Sherrard, D. J. (1987) Osteomalacia and aplastic bone disease in aluminum-related osteodystrophy. *J Clinic Endocrin Meta* **65** 11-16.

Aslam, Pejović-Milić, A., McNeill, F. E., Byun, S. H., Prestwich, W. V., and Chettle, D. R. (2008a) In-vivo assessment of magnesium status in human body using accelerator-based neutron activation measurement of hands: a feasibility study. *Med Phy* **35** 608-616.

Aslam, Pejović-Milić, A., Chettle, D. R., McNeill, F. E., Byun, S. H., and Prestwich, W. V. (2008b) Quantification of manganese in human hand bones: a feasibility study. *Phys Med Biol* **53** 4081-4092.

Aslam, Prestwich, W. V., McNeill, F. E., and Waker, A. J. (2003) Investigating the TEPC radiation quality factor response for low energy accelerator based clinical applications. *Appl Rad Isot* **103** 311-22.

Aslam, Prestwich, W. V., McNeill, F. E., and Waker, A. J. (2006) Monte Carlo simulation of neutron irradiation facility developed for accelerator based *in-vivo* neutron activation measurements in human hand bones. *Appl Rad Isot* **64** 63-84.

Aslam, Prestwich, W. V., and McNeill, F. E. (2002) Thin target $^7\text{Li}(p, p')^7\text{Li}$ inelastic gamma ray yield measurements. *J Radioanal Nucl Chem* **254** (3) 533-544.

ATSDR (Agency for Toxic Substances and Disease Registry) (1999) Toxicological Profile for Aluminum (Update). U.S. Department of Health and Human Services, Public Health Service.

Bast-Pettersen, R., Skaug, V., Ellingse, D., and Thomassen, Y. (2000) Neurobehavioral performance in aluminum welders. *Am J Ind Med* **37** 184-192.

Bishop, N. J., Morley, R., Chir, B., Day, J. P., and Lucas, A. (1997) Aluminium neurotoxicity in pre-term infants receiving intravenous feeding solutions. *N Engl J Med* **336** 1557-61.

Bishop, N. J., Robinson, M. J., Lenden, M., Hewitt, C. D., Day, J. P., and O'Hara, M. (1989) Increased concentration of aluminum in the brain of parenterally fed preterm infant. *Arch Dis Child* **64** 1316-1317.

Brusewitz, S. (1984). Aluminum. University of Stockholm, Stockholm, Sweden. **203**.

Buchta, M., Kiesswetter, E., Schaeper, M., Zschiesche, W., Schaller, K. H., Kuhlmann, A., and Letzel, S. (2005) Neurotoxicity of exposures to aluminium welding fumes in the truck trailer construction industry. *Environ Toxicol Pharmacol* **19** 677-85.

Byun, S. H., Pejovic-Milic, A., Prestwich, W. V., Chettle, D. R., and McNeill, F. E. (2006) Improvement of in vivo neutron activation analysis of Mn using a $^4\text{NaI(Tl)}$ detector array. *J Radioanal Nucl Chem* **269** 615-618.

Byun, S. H., Chin, K., Prestwich, W. V., Liu, Z., and Saxena, S. (2007) Development of a pulse processing system for real time analysis of microdosimetric spectra. *Nucl Instr Meth A* **579** 256-259.

Campbell, A., Hamai, D., and Bondy, S. C. (2001) Differential Toxicity of Aluminum Salts in Human Cell Lines of Neural Origin: Implications for Neurodegeneration. *Neurotoxicol* **22** 63-71.

Cassidy, M. J. D. (2003) Renal osteodystrophy, *Med* **31** 56-61.

Chan, J. C. M., Jacob, M., Brown, S., Savory, J., and Wills, M. R. (1988) Aluminum metabolism in rats; Effects of vitamin D dihydrocortisol, 1,25-dihydroxyvitamin D and phosphate binders. *Nephron* **48** 61-64.

Cochran, M., Goddard, G., Ramm, G., Ludwigson, N., Marshall, J., and Halliday, J. (1993) Absorbed aluminum is found with two cytosolic protein fractions, other than ferritin, in the rat duodenum. *Gut* **34** 643-646.

Committee, Analytical Methods, (2001) Measurement of near zero concentration: recording and reporting results that falls close to or below the detection limit. *The Analyst* **126** 256-259.

Comsa, D. C., Prestwich, W. V., McNeill, F. E., and Byun, S. H. (2004) Application of spectral decomposition analysis to in vivo quantification of aluminum by neutron activation analysis. *Appl Rad Isot* **61** 1353-1360.

Crapper McLachlan, D. R. (1994) Aluminum and Alzheimer's disease. Response. *Can Med Assoc J* **151** 268-9.

Crapper, D. R., Quittkat, S., Krishnan, S. S., Dalton, A. J. and De Boni, U. (1980) Intranuclear aluminum content in Alzheimer's disease, dialysis encephalopathy, and experimental aluminum encephalopathy. *Acta Neuropathol* **50** 19-24.

Darbre, P. D. (2003) Underarm cosmetics and breast cancer. *J Appl Toxicol* **23** 89-95.

Darbre, P. D. (2005) Aluminium, antiperspirants and breast cancer. *J Inorg Biochem* **99** 1912-1919.

Elinder, C. G., Ahrengart, L., Lidums, V., Pettersson, E., and Sjögren, B. (1991) Evidence of aluminum accumulation in aluminum welders. *Br J Ind Med* **48** 735-738.

Ellis, H. A., McCartney, J. H., and Harrington, J. (1979) Bone aluminum in haemodialysed patients and in rats injected with aluminum chloride: relationship to impaired bone mineralization. *J Clin Pathol* **32** 832-844.

Ellis, K. J., Kelleher, S., Raciti, A., Savory, J., and Wills, M. (1988) In-vivo monitoring of skeletal aluminum burden in patients with renal failure. *J Rad Nucl Chem* **124** 85-95.

El-Rahman, S. S. A. (2003) Neuropathology of aluminum toxicity in rats (glutamate and GABA impairment). *Pharmacol Res* **47** 189-194.

Exley, C. (2001) (Ed.) Aluminum and Alzheimer's disease: The Science that Describes the Link, Elsevier, London.

Exley, C., and Korchaazhkina, O. (2001) The association of aluminum and β amyloid in Alzheimer's disease in Aluminum and Alzheimer's disease, The Science that Describes the Link. edited by C. Exley (Elsevier Science), **22** 421-433.

Exley, C., Burgess, E., Day, J. P., Jeffery, E. H., Melethil, S., and Yokel, R. A. (1996) Aluminum toxicokinetics. *J Toxicol Environ Health* **48** 569-584.

Flarend, R. (2001) Absorption of aluminum from antiperspirants and vaccine adjuvants. In "Aluminum and Alzheimer's disease: The Science That Describes the Link" (C. Exley, Ed.), pp. 75-95. Elsevier, Amsterdam.

Flarend, R., Bin, T., Elmore, D., and Hem, S. L. (2001) A preliminary study of the dermal absorption of aluminium from antiperspirants using aluminium-26. *Food Chem Toxicol* **39** 163-168.

Fontanellas, A., Coronel, F., Santos, J. L., Herrero, J. A., Moran, M. J., Guerra, P., Tornero, F., and de Salamanca, R. E. (1994) Heme biosynthesis in uremic patients on CAPD or hemodialysis. *Kidney Int* **45**(1) 220-3.

Ganrot, P. O. (1986) Metabolism and possible health effects of aluminum. *Environ Health Perspect* **65** 363-441.

Garbossa, G., Gálvez, G., Pérez, G., Stripeikis, J., Tudino, M., and Nesse., A. (1998) Oral aluminum administration to rats with normal renal function. 2. Body distribution. *Hum Exp Toxicol* **17** 318-322.

Gitelman, H.J. (1989) Aluminum and Health: a critical review. MarcelDekker Inc, New York.

Gitelman, H. J. (1995a) Aluminum exposure and excretion. *Sci Total Environ* **163**, 129-135.

Gitelman, H. J., Alderman, F. R., Kurs-Lasky, M., and Rockette, H. E. (1995b) Serum and urinary aluminum levels of workers in the aluminum industry. *Ann Occup Hyg* **39** (2) 181-191.

Green, S., and Chettle, D. R. (1992) A feasibility study of the in vivo measurement of aluminum in peripheral bone. *Phys Med Biol* **37** (12) 2287-96.

Green, S., Bradley, D. A., Mountford, P. J., Morgan, W. D., Chettle, D. R., and Weaver, D. R. (1993) Characteristics of an Accelerator based system for In Vivo Aluminum Measurement in Peripheral Bone. *Basic Life Sci* **60** 289-292.

Greger, J. L., and Baier, M. J. (1983) Excretion and retention of low or moderate levels of aluminium by human subjects. *Food Chem Toxicol* **21** 473-477.

Guillard, O., Fauconneau, B., Olichon, D., Dedieu, G., and Deloncle, R. (2004) *Am J Med* **117** 956-959.

Hantson, P., Mahieu, P. Gersdorff, M., Sindic, C. J. M., and Lauwerys, R. (1994) Encephalopathy with seizures after use of aluminum-containing bone cement. *Lancet* **344** 1647.

Hem, S. L., and White, J. L. (1989) Pharmaceutical uses of aluminum. In: H. J. Gitelman, Editor, *Aluminum and Health*, Marcel Dekker, New York, pp. 257-282.

Hongve, D., Johansen, S., Andruchow, E., Bjertness, E., Becher, G., and Alexander J. (1996) Determination of aluminum in samples from bone and liver in elderly Norwegians. *J Trace Elem Med Biol* **10** 6-11.

ICRP(1975) Report of the Task Group on Reference Man. **Publication 23** Pergamon, Oxford.

ICRP(1994) Basic Anatomical and Physiological Data for use in Radiological Protection: The Skeleton, No. 70, Pergamon, Oxford.

Imray, P., Moore, M. R., Callan, P. W., and Lock, W., eds, (1998) Brisbane. Aluminium: Report of an international meeting 20-21 April 1995. *Nation Environ Health For Mono. Metal Series* 1 4.

Ittel, T. H. (1993) Determinants of gastrointestinal absorption and distribution of aluminum in health and uraemia. *Nephrol Dial Transplant* 8 17-24.

Kawahara, M., Kato, M., and Kuroda, Y. (2001) Effects of aluminum on the neurotoxicity of primary cultured neurons and on the aggregation of beta-amyloid protein. *Brain Res Bull* 55 211-217.

Keith, L. S., Jones, D. E., and Chou, C. H. S. J. (2002) Aluminum toxicokinetics regarding infant diet and vaccinations. *Agency for Toxic Subst Dis Reg* 20 S13-S17.

Klatzo, I., Wisniewski, H., and Streicher, E. (1965) Experimental production of neurofibrillary degeneration: I. Light microscopic observations. *J Neuropathol Exp Neurol* 24 187-199.

Klein, G. L., and Coburn, J. W. (1994) Total parenteral nutrition and its effects on bone disease of long term parenteral nutrition. *Crit Rev Clin Lab Sci* 31 135-167.

Klein, G. L., Herndon, D. N., Rutan, T. C., Barnett, J. R., Miller, N. L., and Alfrey, A. C. (1994) Risk of aluminum accumulation in patients with burns and ways to reduce it. *J Burn Care Rehab* 15 354-358.

Kruger, P. C., and Parsons, P. J. (2007) Determination of serum aluminum by electrothermal atomic absorption spectrometry: A comparison between Zeeman and continuum background correction systems. *Spectrochimica Acta. Part B. Atomi.Spec.* 62 (3) 288-296.

Laden K., and Felger C.B. (1988) Antiperspirants and Deodorants. *Cosmetic Science and Technology Series* Volume: 7, Marcel Dekker, New York.

Levesque, L., Mizzen, C. A., McLachlan, D. R., and Fraser, P. E. (2000) Ligand specific effects on aluminum incorporation and toxicity in neurons and astrocytes. *Brain Res* 877 191-202.

- Litov, R. E., Sickles, V. S., Chan, G. M., Springer, M. A., and Cordano, A. (1989) Plasma Aluminum Measurements in Term Infants Fed Human Milk or a Soy-Based Infant Formula. *Pediatrics* **84** 1105-1107.
- Makjanic, J., McDonald, B., Li-Hsian Chen C. P., and Watt, F. (1998) Absence of aluminium in neurofibrillary tangles in Alzheimer's disease. *Neurosci Lett* **240** 123-126.
- Malluche, M., Hartmut, H., Langub, C., Monier-Faugere, and Marie-Claude. (1999) The role of bone biopsy in clinical practice and research. *Kidney Int* **56** 20-25.
- McLachlan, D. R. C. (1986) Aluminum and Alzheimer's disease. *Neurobiol Ag* **7** 525-532.
- McLachlan, D. R. C. (1995) Aluminum and the risk for Alzheimer's disease. *Environmetrics* **6** 233-75.
- McNeill, F. E., Stokes, L., Chettle, D. R., and Kaye, W. E. (1999) Factors affecting *in vivo* measurement precision and accuracy of ^{109}Cd K x-ray fluorescence measurements. *Phys Med Biol* **44** 2263-2273.
- Meyer-Baron, M., Schäper, M., Knapp, Guido., and van Thriel, Christoph. (2007) Occupational aluminum exposure: Evidence in support of its neurobehavioral impact. *Neurotoxicol* **28** 1068-1078.
- Mjoberg, B., Hellquist, E., Mallmin, H., and Lindh, U. (1997) Aluminum, Alzheimer's disease and bone fragility. *Acta Orthop Scand* **68** 511-514.
- Munoz, D. G. (1998) Is exposure to aluminum a risk factor for the development of Alzheimer Disease?—No. *Arch Neurol* **55** (5) 737-739.
- Murphy, C. P., Cox, R. L., Harden, E. A., Stevens, D. A., Heye, M. M., and Herzig, R. H. (1992) Encephalopathy and seizures induced by intravesical alum irrigations. *Bone Marrow Trans* **10** 383-385.
- Nakamura, H., Rose, P. G., Blumer, J. L., and Reed, M. D. (2000) Acute encephalopathy due to aluminum toxicity successfully treated by combined intravenous deferoxamine and hemodialysis. *J Clin Pharmacol* **40** 296-300.

Ott, S. M., Maloney, N. A., Klein, G. L., Alfrey, A. C., Ament, M. E., Coburn, J. W., and Sherrard, D. J. (1983) Aluminum is associated with low bone formation in patients receiving chronic parenteral nutrition. *Ann Intern Med* **98** 910-914.

Palerme, S., Chettle, D. R., Kennett, T. J., Prestwich, W. V., and Webber, C. E. (1993) Pilot studies for *in-vivo* bone aluminum measurements. *Basic Life Sci* **60** 303-306.

Parsons, V., Davies, C., Goode, C., Ogg, C., and Siddiqui, J. (1971) Aluminum in bone from patients with renal failure. *Br Med J* **4** (782) 273-5.

Pejović-Milić, A., McNeill, F. E., Prestwich, W. V., Waker, A. J., and Chettle, D. R. (1998) Development of an Accelerator Based Determination of Aluminum Burden in Peripheral Bone by Neutron Activation Analysis. *Appl Rad Isot* **49** 717-719.

Pejović-Milić A., Arnold, M. L., McNeill, F. E., and Chettle, D. R. (2000) Monte Carlo design study for *in-vivo* bone aluminum measurement using a low energy accelerator beam. *Appl Rad Isot* **53** 657-664.

Pejović-Milić, A., Byun, S. H., Comsa, D. C., McNeill, F. E., Prestwich, W.V., and Chettle, D.R. (2005) In vivo measurement of bone aluminum: Recent developments. *J Inorg Biochem* **99** 1899-1903.

Pejović-Milić, A., Byun, S. H., Chettle, D. R., McNeill, F. E., and Prestwich, W. V. (2006) Development of an irradiation/ shielding cavity for in vivo neutron activation analysis of manganese in human bone. *J Rad analy Nucl Chem* **269** 417-420.

Polizzi, S., Pira, E., Ferrara, M., Bugiani, M., Papaleo, A., Albera, R., and Palmi, S. (2002) Neurotoxic Effects of Aluminium among Foundry Workers and Alzheimer's Disease. *Neurotoxicol* **23** 761-774.

Popinska, K., Kierkus, J., Lyszkowska, M., Socha, J., Pietraszek, E. (1999) Aluminum contamination of parenteral nutrition additives, amino acid solutions, and lipid emulsions. *Nutrition* **15** 683-686.

Renard, J. L., Felten, D., and Bequet, D. (1994) Post-otoneurosurgery aluminum encephalopathy. *Lancet* **344** 63-64.

Rifat, S. L., Eastwood, M. R., Crapper McLachlan, D. R. and Corey, P. N. (1990) Effect of exposure of miners to aluminium powder. *Lancet* **36** 1162-1165.

Rollin, H. B., Theodorou, P., and Kilroe-Smith, T. A. (1993) Changes in the concentration of copper and zinc in body fluids and tissues of rabbits following the inhalation of low concentrations of Al_2O_3 dust. *S African J Sci* **89** 246-249.

Savory, J., and Ghribi, O. (2007) Can Studies of Aluminum Toxicity In Vivo and In Vitro Provide Relevant Information on the Pathogenesis and Etiology of Alzheimer's Disease. *J Alzheimer's dis* **11** (4) 429-430.

Scancar, J., Milacic, R., Benedik, and M., Bukovec, P. (2000) Determination of trace elements and calcium in bone of the human iliac crest by atomic absorption spectrometry," *Clin Chim Acta* **293** 187-197.

Scharf, R., Mamet, R., Zimmels, Y., Kimchie, S., Schoenfeld, N. (1994) Evidence for the interference of aluminum with bacterial porphyrin biosynthesis. *Biometals : A Int J Metal Ion Biol Biochem Med* **7** 135-41.

Schlesinger, R. B., Snyder, C. A., Chen, L. C., Gorczynski, J. E., and Menache, M. (2000) Clearance and translocation of aluminum oxide (alumina) from the lungs. *Inhal Toxicol* **12** 927-939.

Scott, M. C., and Chettle, D. R. (1986) In vivo elemental analysis in occupational medicine. *Scand J Work Environ Health* **12** (2)81-96.

Sedman, A. B., Klein, G. L., Merritt, R. J., Miller, N. L., Weber, K. O., Gill, W. L., Anand, H., and Alfrey, A. C. (1985) Evidence of aluminum loading in infants receiving intravenous therapy. *N Engl J Med* **312** 1337-1343.

Sharif, A. A. M., Ghafourian, H., Ahmadiar, A., Waqif Husain, S., Saber-Tehrani M., and Ghods, H. (2004) Determination of aluminum levels in serum and red blood cells from long-term hemodialysis patients using instrumental neutron activation analysis. *J Radioanal Nucl Chem* **262** 473-477.

Sim, M., Dick, R., Russo, J., Bernard, B., Grubb, P., Krieg, E. Jr., Mueller, C., and McCammon, C. (1997) Are aluminum pot room workers at increased risk of neurological disorders? *Occup Environ Med* **54** 229-35.

Sjögren, B., Gustavsson, P., and Hogstedt, C. (1990) Neuropsychiatric symptoms among welders exposed to neurotoxic metals. *Br J Ind Med* **47** 704-7.

Skalsky, H.L., and Carchman, R. A. (1983) Aluminum homeostasis in man. *J Am Col Toxicol* **2** 405-423.

Sorrenson, J. R. J., Campbell, I. R., Tepper, L. B., and Lingg, R. D. (1974) Aluminum in the environment and human health. *Environ Health Perspect* **8** 3-95.

Spasovski, G. B. (2004) Bone biopsy as a diagnostic tool in the assessment of renal osteodystrophy. *Int J Artif Organs* **27** 918.

Studinski, R. C. N., McNeill, F. E., O'Meara, J. M., and Chettle, D. R. (2006) A method detection limit for potential *in vivo* arsenic measurements with a 50 W x-ray tube. *Phys Med Biol* **51** N381-N387.

Takata, M. K., Saiki, M., Sumita, N. M., Saldiva, P. H. N., and Pasqualucci, C. A. (2005) Trace element determinations in human cortical and trabecular bones. *J Radioanal Nucl Chem* **264** (1) 5-8.

U. S. Public Health Service. (1992) Toxicological Profile for Aluminum and Compounds. (Contact No. 205-88-0608). Prepared by Clement International Corp.

Walton, J. R., Diamond, T. H., Kumar, S., and Murrell, G. A. C. (2007) Sensitive stain for aluminum in undecalcified cancellous bone. *J Inorg Biochem* **101** 1285-1290.

Ward, R. J., Zhang Y., and Crichton, R. R. (2001) Aluminium toxicity and iron homeostasis. *J Inorg Biochem* **87** 9-14.

White, D. M., Longstreth, W. T. Jr., Rosenstock, L., Claypoole, K. H., Brodtkin, C. A., and Townes, B. D. (1992) Neurologic syndrome in 25 workers from an aluminum smelting plant. *Arch Intern Med* **152** (7) 1443-8.

Wilhelm, M., Hoelzer, J., Luebbbers, K., Stoeck, G., and Ohmann, C. (2001) Aluminum balances in intensive care patients. *J Trace Elem Med Biol* **14** 223-227.

Witters, H. E., Puymbroeck, S., Vangenechten, J. H. D., and Vanderborght, O. L. J. (1990b) The effect of humic substances on the toxicity of aluminum to adult rainbow trout. *Onchorhynchus mykiss (Walbaum)* *J Fish Biol* **37** 43-53.

Wyatt, R. M., Ryde, J. S., Morgan, W. D., McNeil, E. A., Hainsworth, I. R., and Williams, A. J. (1993a) The development of a technique to measure bone aluminum content using neutron activation analysis. *Physiol Meas* **14** 327-335.

Wyatt, R. M., Ryde, S. J. S., Williams, A. J., Evans, C. J., McNeil, E. A., and Morgan, W. D. (1993b) Development of an in vivo neutron activation analysis technique to measure bone aluminum. *Basic Life Sci* **60** 319-321

Yokel, R. A., Meurer, K. A., Hong, C. B., Dickey, K. M., Skinner, T. L., and Fredenburg, A. M. (1997) Short-Term Oral 3-Hydroxypyridin-4-one Dosing Increases Aluminum Excretion and Partially Reverses Aluminum-Induced Toxicity in the Rabbit Independent of Chelator Lipophilicity. *Drug Metab. Dispos* **25** 182-190

Yokel, R. A. (2000) The toxicology of aluminum in the brain: a review. *Neurotoxicol* **21** 813-828.

Yokel, R. A., and McNamara, P. J. (2001) Aluminum toxicokinetics: An updated minireview. *Pharmacol Toxicol* **88** 159-167.

Yokel R., Aluminum, Merian E., Anke M., Ihnat M., and Stoeppler M., (Ed.) (2004) *Elements and their Compounds in the Environment, Occurrence, Analysis and Biological Relevance*, Wiley-VCH, Weinheim. 635-658.

Zafar, T. W., Weaver, C. M., Martin, B. R., Flarend, R., and Elmore, D. (1997) Aluminum (^{26}Al) metabolism in rats. *Proc Soc Exp Biol Med* **216** 81-85.

Zatta, P., Bordin, C., and Favarato, M. (1993) The Inhibition of Trypsin and α -Chymotrypsin Proteolytic Activity by Aluminum (III). *Arch Biochem Biophys* **303** 407-411.

Zatta, P. (2006) Aluminum and Alzheimer's disease: A Vexata Questio between uncertain data and a lot of imagination. *J Alzheimers Dis* **10** 33-7.