

**FEASIBILITY OF MEASURING SELENIUM IN HUMANS USING *IN VIVO*
NEUTRON ACTIVATION ANALYSIS**

**FEASIBILITY OF MEASURING SELENIUM IN HUMANS USING *IN VIVO*
NEUTRON ACTIVATION ANALYSIS**

By

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**Feasibility of Measuring Selenium in Humans
Using *In-Vivo* Neutron Activation Analysis**

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ABSTRACT

Selenium (Se), an essential trace element, plays an important role in the normal function of a number of Se-dependent biological processes. Many studies have demonstrated that selenium deficiency in the body may contribute to an increased risk for certain neoplastic diseases (including colonic carcinoma, gastric carcinoma, pulmonary carcinoma and prostate carcinoma), as well as diseases of the cardiovascular, osseous, nervous systems and retardation of bone formation. However, at higher concentrations Se is cytotoxic. For these reasons it is desirable to have a means of monitoring selenium concentration in humans.

The feasibility of measuring selenium in humans using the *in vivo* neutron activation analysis (IVNAA) technique was studied. For this purpose human hand tissue equivalent phantoms were prepared with varying amounts of selenium and irradiated by a low energy neutron beam produced by the ${}^7\text{Li}(p,n){}^7\text{Be}$ reaction by employing the high beam current Tandetron accelerator. The counting data saved using the 4π NaI(Tl) detection system in anticoincidence, coincidence and singles modes of detection were analyzed. The selenium was detected via the neutron capture reaction, ${}^{76}\text{Se}(n,\gamma){}^{77\text{m}}\text{Se}$, whereas calcium was detected through the ${}^{48}\text{Ca}(n,\gamma){}^{49}\text{Ca}$ reaction.

The peak areas of Se and Ca were computed and the Se concentrations were normalized to the Ca concentrations for various time segments of detection. The calibration lines were drawn between Se/Ca concentration and Se/Ca counts ratio. The minimum detection limits (MDL) were obtained and the inverse variance weighted mean value of MDL was finally calculated for three time segments. During the analysis of counting data it was also found that ${}^{18}\text{O}$ is activated in water phantoms and becomes short lived radioactive ${}^{19}\text{O}$ having $T_{1/2}=26.9$ s.

To the author's best knowledge, this study for the first time presents the MDL value in terms of Se/Ca concentration for the human hand bone equivalent phantom obtained from *in vivo* neutron activation analysis and these results will provide a good basis for future investigations.

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Chapter 1

INTRODUCTION

Selenium (Se) is a naturally occurring, solid substance that is widely but unevenly distributed in the earth's crust. It is also commonly found in rocks and soil. Selenium, in its pure form of metallic gray to black crystals, is often referred to as elemental selenium or selenium dust. Selenium has an atomic number of 34, an atomic weight of 78.94 and occupies a position in Group VIA of the periodic table between the metal tellurium and the nonmetal sulfur (UKEGVM, 2002).

Selenium's chemical and physical properties are intermediate between those of metals and nonmetals. Selenium exists in multiple oxidation states (-2, +4, +6), i. e., it has a valence of -2 in combination with hydrogen or metals, and in oxygenated compounds it can exist as the +4 or the +6 oxidation states giving rise to an array of Se compounds (Haygarth, 1994). Six stable Se isotopes occur with varying degrees of abundance: ^{74}Se (0.87%), ^{76}Se (9.02%), ^{77}Se (7.58%), ^{78}Se (23.52%), ^{80}Se (49.82%), and ^{82}Se (9.19%) (Newland, 1982). The average Se concentration in the earth's crust is about 0.05 mg/kg to 0.09 mg/ kg (Lakin, 1972).

Selenium has unique electrical properties. Its conductivity, which is low in the dark, is increased several 100-fold on exposure to light which also generates a small electrical current in the element. It is, in addition, a semiconductor, possessing what is known as asymmetrical conductivity, able to conduct more easily in one direction than in

the other. These properties account for the element's exceptional usefulness to the electrical and electronic industries. Elemental selenium boils at the relatively low temperature of 684°C. As a consequence, atmospheric pollution can be caused by industrial processes that involve heating of the element or its compounds (Crystal, 1973). Elemental selenium itself is very stable and highly insoluble. These properties are important from an environmental point of view since, under reducing conditions, selenates and other soluble compounds of selenium that occur in certain soils can be converted into elemental selenium and thus become unavailable for absorption by plants. The process can also remove selenium from active recycling and thus reduce the possibility of environmental pollution (WHO, 1987).

The chemical properties of selenium are similar to sulfur. Selenium, like sulfur, has several allotropes. They include monoclinic or red selenium, an amorphous powder that exists in two forms, one of which is comparable to crystalline "flowers of sulfur." There is also a black amorphous form. A vitreous form that changes to gray selenium on heating also occurs. Gray, also known as metallic selenium is stable at ordinary temperatures and is the most common allotrope. Selenium combines with metals and many nonmetals directly or in aqueous solution. The selenides resemble sulfides in appearance, composition, and properties. Selenium may form halides by reacting vigorously with fluorine and chlorine, but the reactions with bromine and iodine are not rapid. Selenium does not react with hydrogen fluoride or hydrogen chloride directly, but decomposes hydrogen iodide to liberate iodine and yield hydrogen selenide. Selenium

reacts with oxygen to form a number of oxides, the most stable of which is selenium dioxide (Hoffmann and King, 1997).

Elemental selenium is commercially produced, primarily as a by-product of copper refining. Selenium is not often found in the environment in its elemental form, but is usually combined with other substances. Much of the selenium in rocks is combined with sulfide minerals or with silver, copper, lead, and nickel minerals (ATSDR, 2003). Selenium occurs naturally in water and some foods. While people only need a very small amount, selenium plays a key role in the metabolism.

Selenium as a trace element naturally exists in many foods, is added to others, and is available as a dietary supplement. Selenium, which is nutritionally essential for humans, is a constituent of more than two dozen selenoproteins that play critical roles in reproduction, thyroid hormone metabolism, DNA synthesis, and protection from oxidative damage and infection (Sunde et al., 2012). Selenium exists in two forms: inorganic (selenate and selenite) and organic (selenomethionine and seleno-cysteine). Both forms can be good dietary sources of selenium. Soils contain inorganic selenites and selenates that plants accumulate and convert to organic forms, mostly selenocysteine and selenomethionine and their methylated derivatives (Sunde et al., 2006).

Most selenium is in the form of selenomethionine in animal and human tissues, where it can be incorporated nonspecifically with the amino acid methionine in body proteins. Both selenocysteine and selenite are reduced to generate hydrogen selenide, which in turn is converted to selenophosphate for selenoprotein biosynthesis (Davis, 2012). The most commonly used measures of selenium status are plasma and serum

selenium concentrations (Sunde et al., 2012). Concentrations in blood and urine reflect recent selenium intake. Analyses of hair or nail selenium content can also be used to monitor longer-term intakes over months or years. Quantification of one or more selenoproteins (such as glutathione peroxidase and selenoprotein P) is used as a functional measure of selenium status. Plasma or serum selenium concentrations of 8 µg/dL or higher in healthy people typically meet needs for selenoprotein synthesis (Sunde et al., 2010).

1.1 Importance of selenium

Selenium is one of the rarest elements. It is about 70th in abundance among the 88 elements that naturally occur in the earth's crust. Yet, in spite of its scarcity, selenium plays a key role in all animal life. It is an essential component of the human diet, though only in minute amounts. If this intake is exceeded by relatively little, disastrous consequences can follow (Pieczyńska and Grajeta, 2015).

Selenium was first studied around 1930 after scientists noted that cows grazing on plants growing in high-selenium soil suffered from alkali disease. Studies linking nutritional diseases to selenium deficiency in sheep, cattle, and swine continued until 1973 when the biochemical function of the element was discovered. Researchers discovered that selenium was an essential component of the glutathione peroxidase enzyme system (Burk and Levander, 1999). The importance of selenium in the human diet was discovered in 1979 when Chinese scientists showed that children living in selenium-deficient areas were suffering from a cardiomyopathy disease. The symptoms of the disease were reversed when selenium was added to the diet.

Within biological systems, selenium is a constituent of the amino acids that comprise proteins. Studies have shown that as an essential element selenium is required for the formation of several selenoproteins with key roles in important metabolic processes. Consequently selenium deficiency may lead to various diseases. Being an essential element, the role of selenium in animal nutrition is well established. At least forty animal species have been shown to demonstrate selenium-responsive diseases. These include liver, kidney and heart necrosis, muscular dystrophy, growth depression and exudative diathesis (Behne et al., 2010).

In human nutrition, certain selenoenzymes and selenoproteins are considered to be of vital importance. The best known biochemical function of selenium is demonstrated as part of the enzyme glutathione peroxidase, which protects vital components of the cell against oxidative damage. Other selenium-dependent enzymes have been identified in bacterial systems, including glycine reductase, formate dehydrogenase, nicotinic acid hydrogenase and thiolase (McDowell et al., 1987). Studies have shown that selenium deficiency is one of the principal factors responsible for Keshan disease, a dilated cardiomyopathy that affects persons living in selenium deficient areas (Johnson et al., 1981).

Selenium also plays an important role in body antioxidation system; it is considered an individual antioxidant that can cooperate with other antioxidants, such as C and E vitamins and in processes protecting the cells from free radicals. In such manner selenium protects a body from development of cancer, cardiovascular diseases and masculine sterility (Mach, 2008). Selenium participates in thyroid hormone metabolism,

immune system, inhibits virulence, and slows down the development of AIDS through reducing the speed of HIV development. Furthermore, it can reduce the risk of spontaneous abortions as well (Rayman, 2000).

Balanced content of selenium in human food helps in the case of complications connected with diabetes and affects also the prevention of asthma. Through free radical inhibition selenium moderates harmful effects of radiation (Surai, 2006). Selenium is important for proper function of cerebral neurotransmitters and reduces epileptic waves in children.

1.2 Selenium kinetics and metabolism

Several forms of selenium enter the body as part of amino acids within proteins. The two most common forms of the element that enter the body are selenomethionine and selenocysteine which are found mainly in plants and animals respectively (Burk and Levander, 1999). The primary sites of absorption are from throughout the duodenum. Virtually no absorption occurs in the stomach and very little takes place in the remaining two segments of the small intestine (Groof et al., 1995). Selenomethionine is absorbed from the duodenum at a rate close to 100%.

Other forms of selenium have been shown to also be generally well absorbed in the body. However, absorption of inorganic forms of the element varies widely due to luminal factors (Burk and Levander, 1999). This variation in absorption reduces total absorption of all forms to somewhere between 50 and 100%. Selenium absorption is not affected by body selenium status. Absorption of selenium is closely related to multiple nutritional factors that inhibit or promote absorption. Vitamins A, C, and E along with

reduced glutathione enhance absorption of the element. In contrast, heavy metals (i.e., Hg) decrease absorption via precipitation and chelation (Groof et al., 1995).

The human body easily absorbs the organic selenium compounds (for example, selenoamino acids) when eaten, and makes them available where needed in the body. The selenium in drinking water is usually in the form of inorganic sodium selenate and sodium selenite; these forms of selenium are also easily absorbed from the digestive tract. The human body can change these inorganic selenium compounds into forms that it can use (ATSDR, 2003).

Most water-soluble inorganic and organic selenium compounds in foods are relatively efficiently absorbed across the gastrointestinal tract (80–95%) (Bopp et al., 1982), although elemental selenium (Medinsky, 1981) and selenium sulfide (Cummins and Kimura, 1971) are poorly absorbed. After absorption, selenium is cleared by the liver and then transported to peripheral tissues by a specific transporter, selenoprotein P. In this way, selenium is distributed to all organs, with the highest concentrations occurring in kidney, liver, spleen, testes and skeletal muscle (Thomassen and Aaseth, 1986).

Selenium that is absorbed becomes a part of both transport and storage proteins. The amount that builds up in the body depends on the chemical form of the selenium. Selenium is believed to influence the formation of the proteins. The uptake of selenium is a complex process that involves numerous factors. The heart, kidney, lung, liver, pancreas, and muscle contain very high levels of selenium as a component of glutathione (Groof et al., 1995). The liver is a major supplier of circulating reduced glutathione,

which is reflected in the amount of its reserves. Selenium can also build up in the nails and in hair, depending on time and amount of exposure (Powers and Ji, 1999).

Most of the selenium that enters the body quickly leaves the body, usually within 24 hours. However the biological half-life of selenium in the human body has been estimated to be approximately 100 days (Griffith et al., 1976). Actual retention times will depend on a number of factors, including present selenium status, the specific form in which the element is ingested, as well as the state of health of the subject. It has been shown in rats that the apparent whole-body retention of selenium is an average of several discrete processes, as each internal organ probably has its own rate of selenium turnover. Beyond what the body needs, selenium leaves mainly in the urine (Yang et al., 1989), but also in feces, breath and to limited extent in hair and nail. Selenium in the urine increases as the amount of the exposure goes up (Chen et al., 1980).

The metabolic pathways of selenium as an essential element are shown in Figure 1.1 (Combs, 2004) and molecular mechanisms of selenium are explained in Figure 1.2 (Zeng et al., 2013). Inorganic selenium is reduced stepwise to the hydrogen selenide and it is either incorporated into selenoproteins after being transformed to selenophosphate and selenocysteinyl or excreted into urine after being transformed into methylated metabolites of selenide (Lobinski et al., 2000). Selenium compounds are metabolized in three ways, i.e., to specific selenoproteins, to non-specific proteins and to excretory products. The nutritionally essential functions of selenium appear to be discharged by some 25 selenoproteins, each of which contains selenium in the form of selenocysteine.

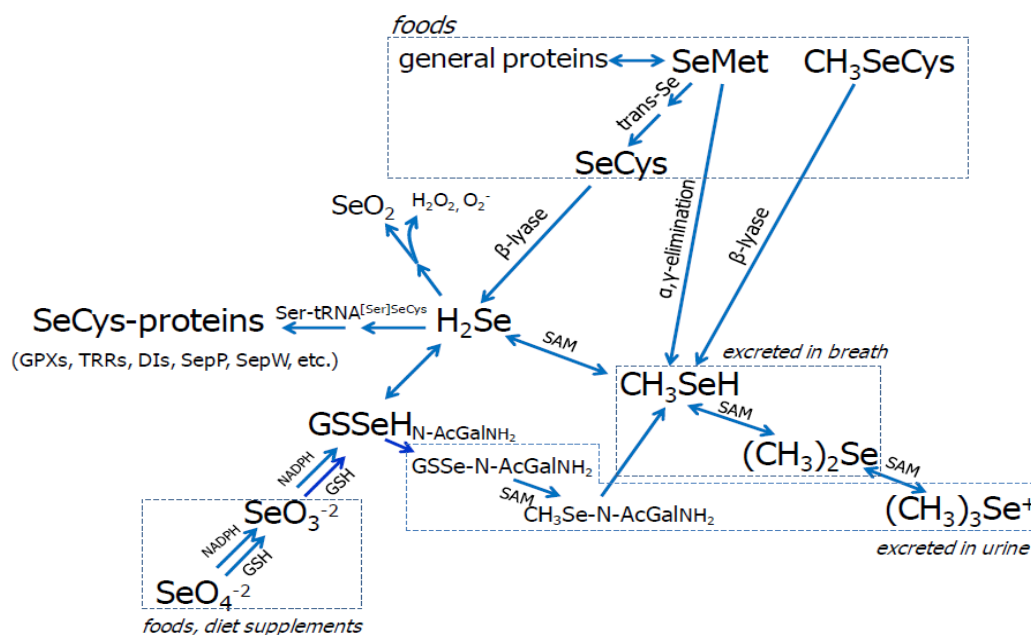


Figure 1.1: Metabolic pathways for selenium (Combs, 2004)

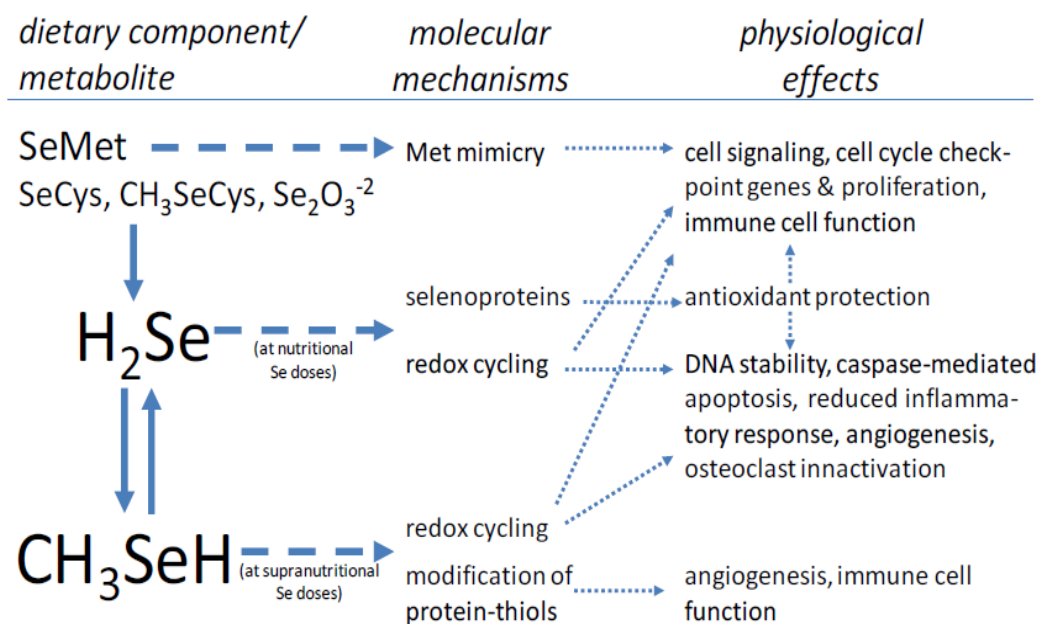


Figure 1.2: Molecular mechanism for selenium (Zeng et al., 2013)

This form is not found in any other protein, being produced by a unique co-translational modification of those specific proteins (Arthur, 2003).

The specific selenoproteins include glutathione peroxidases, thioredoxin reductases, 5-iodothyronine deiodinases, selenoprotein P and others. If selenomethionine is consumed, then that form of selenium can also be incorporated nonspecifically into proteins, as it can mimic methionine in protein synthesis. Many forms of selenium (including selenite, selenate, selenocysteine and selenomethionine) are metabolized to hydrogen selenide. While the latter metabolite is the obligate precursor to the formation of selenocysteine in the specific selenoproteins, it can also be serially methylated (to methyl selenol, dimethylselenide and trimethylselenonium ion) or converted to a selenosugar and excreted (WHO, 2011).

1.3 Sources and exposure to Selenium

Selenium content of food varies widely between regions throughout the world. Exposure pathways to selenium are air, water and food. Occupations in which humans may be exposed to selenium in the air are the metal industries, selenium-recovery processes, paint manufacturing, and special trades. Selenium is released into the air as hydrogen selenide, produced metabolically by plants, and as elemental selenium, selenites and selenates in particulate form. The level of selenium in most urban air ranges from 0.1 to 10 ng/m³, but higher levels may be found in certain areas, such as in the vicinity of copper smelters (WHO, 2011).

In surface water groundwater the levels of selenium range from 0.06 µg per liter to about 400 µg per liter (Lindberg, 1968). Concentrations increase at high and low pH as

a result of conversion into compounds of greater solubility in water. Levels of selenium in tap water samples from public water supplies around the world are usually much less than 10 µg per liter but may exceed 50 µg per liter (Gore et al., 2010). The amount of selenium in drinking water is not nutritionally significant in most geographic regions (IMFNB, 2010).

Most people obtain virtually all of their selenium from the foods they eat. In plant and animal tissues, selenium is found mostly bound to proteins. So sea foods and organ meats are the richest food sources of selenium. Other sources include muscle meats, cereals and other grains, and dairy products. In contrast, foods with relatively low protein levels, such as vegetables and fruits, tend to have relatively low selenium contents (<0.01 mg/kg) (Chun et al., 2010).

The amount of selenium in a given type of plant-based food depends on the amount of selenium in the soil and several other factors, such as soil pH, amount of organic matter in the soil, and whether the selenium is in a form that is amenable to plant uptake (Rayman, 2008). As a result, selenium concentrations in plant-based foods vary widely by geographic location. For example, according to the U.S. Department of Agriculture Food Composition Database, Brazil nuts have 19.2 µg selenium per g, but values from other analyses vary widely (USDA, 2012). The selenium content of soil affects the amounts of selenium in the plants that animals eat, so the quantities of selenium in animal products also vary. However, selenium concentration in soil has a smaller effect on selenium levels in animal products than in plant-based foods because animals maintain predictable tissue concentrations of selenium through homeostatic

mechanisms. Furthermore, formulated livestock feeds generally contain the same levels of selenium.

Selenium is available in multivitamin/multimineral supplements and as a stand-alone supplement, often in the forms of selenomethionine or of selenium-enriched yeast (grown in a high-selenium medium) or as sodium selenite or sodium selenate. The human body absorbs more than 90% of selenomethionine but only about 50% of selenium from selenite (IMFNB, 2010). Few studies have compared the relative absorption and bioavailability of different forms of selenium. In one investigation (Burk et al., 2006), 10 groups of selenium-replete subjects were randomly assigned to receive a placebo or either 200 or 600 µg/day selenium as seleno-methionine, sodium selenite, or high-selenium yeast (in which an estimated 75% of selenium was in the form of selenomethionine) for 16 weeks. Selenium bio-availability, based on urinary excretion, was greatest for selenomethionine and lowest for selenite. However, supplementation with any of these forms only affected plasma selenium levels and not glutathione peroxidase activity or selenoprotein concentration, confirming that study participants were selenium replete before they began taking selenium supplements.

1.4 Risks from excessive selenium

Selenium has an ambivalent behavior ranging from being essential to highly toxic, depending on the species, oxidation state and concentration. Selenium is an essential nutrient for humans and animals. However, selenium can be harmful when regularly taken in amounts higher than those needed for good health. About two orders of magnitude higher levels of Se are known to cause toxic effects (Zhang and Chatt, 2009).

People receive the majority of their daily intake of selenium from eating food. Excessive intakes of selenium causes selenium toxicity (selenium poisoning) which can result in a condition called selenosis. The average dietary intake that is associated with selenosis is in excess of 900 $\mu\text{g/day}$ (Yang et al., 1989).

Symptoms of selenosis include gastro-intestinal upsets (nausea, vomiting, abdominal pain, diarrhea), hair loss, white blotchy nails (Figure 1.3), garlic breath odor, fatigue, irritability, and mild nerve damage hyperreflexia. Extreme cases of selenosis can result in cirrhosis of the liver, pulmonary edema (an abnormal build up of fluid in the lungs, which leads to swelling), thrombocytopenia (low blood platelets), thyroid problems, and death (Motarjemi et al., 2014). According to Asrani et al., (2013), scarring of the liver may lead to kidney failure.



Figure 1.3 Deformation of nails at people intoxicated with selenium (Dhillon and Dhillon, 2003)

Chronically high intakes of the organic and inorganic forms of selenium have similar effects. Early indicators of excess intake are a garlic odor in the breath and a metallic taste in the mouth. The most common clinical signs of chronically high selenium intakes, or selenosis, are hair and nail loss or brittleness. Other symptoms include lesions of the skin and nervous system, nausea, diarrhea, skin rashes, mottled teeth, fatigue, irritability, and nervous system abnormalities. Acute selenium toxicity has resulted from the ingestion of mis-formulated over-the-counter products containing very large amounts of selenium (Sunde, 2006 & 2010).

Because of concern about the adverse effects resulting from exposure to excessive levels of selenium, various national and international organizations have established upper limits of exposure for selenium. The United States national academy of sciences panel on dietary oxidants and related compounds set an upper tolerable limit for selenium at 400 µg/day (NAS, 2000). This level has also been recommended by the Food and Agriculture Organization (a sister organization of the United Nations) to its member countries (FAO, 1998).

Studies have shown that high levels of selenium in the body show alterations in cell proliferation and differentiation. While these effects have been found to be strongest in cancer cells, they also occur in noncancerous cells (Zeng et al., 2012). Cells treated with high levels of selenite typically show arrest at the S/G2-M phases with an increase in cdk2 kinase activity and DNA damage-inducible *gadd* gene (Wang et al., 2002).

1.5 Risks from selenium inadequacy

Selenium deficiency in humans has been associated with a juvenile, multifocal myocarditis called Keshan disease (whose symptoms include dizziness, malaise, loss of appetite, and nausea in acute cases to restlessness, and a light dilation of the heart in the sub-acute type) and a chondrodystrophy called Kaschin-Beck disease. The symptoms of this disease include joint stiffness and pain, possible stunting, various degrees of disability, enlargement of joints and deformity of limbs in advanced cases (IPCS, 1987; FAO/WHO, 2004; Elis, 2008). A study by Beck et al. (2003) suggests a role of a cardiophilic virus, the virulence of which increases in selenium deficient hosts. Selenium deficiency is also connected with acceleration of senility and development of Alzheimer's disease (Kvíčala, 2003). Selenium inadequacy can also retard growth and change bone metabolism (Cao et al., 2012).

The study by Zeng et al. (2013) concludes that selenium deficiency can also result in increased levels of oxidative stress in the body at the cell level, particularly in individuals of low status with respect to other antioxidants (e.g., vitamins E and C). An increase in oxidative stress achieved experimentally either by elevating intracellular reactive oxygen species or adding exogenous H_2O_2 at micro-molar concentrations, has been shown to inhibit growth in a wide variety of mammalian cells (Day and Suzuki, 2006). It has been long established that the reactive oxygen species related oxidative stress induces cell cycle arrest, senescence, apoptosis and/or necrotic cell death (Stone and Yang, 2006; Day and Suzuki, 2006).

1.6 Analytical methods for selenium measurement

Analytical methods used for selenium determination are precise and accurate, largely because of refined instrumentation and technical expertise. The apparent large variations in selenium measurements in biological samples are because of wide differences in selenium status due to geographical differences in soil selenium. Literature survey reveals that four methods are commonly used for selenium analysis. Fluorometric determination of selenium requires exacting chemical separations, whereas neutron activation analysis (NAA) is used where facilities like particle accelerators and research reactors are available. Atomic absorption spectroscopy (AAS) using either hydride generation or graphite furnace is also a widely used method along with inductively coupled plasma-mass spectroscopy (ICPMS) for routine analysis of selenium. All these methods provide reliable analysis of selenium in biological and environmental samples (Sunde, 2006).

1.7 Rationale of the present study

As selenium is ubiquitous in the environment and has been detected in so many media, over exposure of population to selenium through a variety of ways such as inhalation, ingestion via foods, drinking water and selenium supplements; and occupational exposure may result in its retention in tissues of various organs of human body. Similarly selenium deficiency is also associated with certain health risks. Therefore analysis of selenium in biological tissues provide an index of exposure to selenium in humans for the purpose of monitoring its concentration and maintaining selenium level within the allowable limits (Subramanian and Meranger, 1982).

Some studies have been carried out in which selenium contents in various biological tissues were measured in-vitro from autopsy samples (Johnson and Lewin, 1976; Nicolaou et al., 1982; Milman et al., 1983; Milman et al., 1986, Aadland et al., 1987; Molokhia and Molokhia, 1990; ^aChen et al., 1999; ^bChen et al., 1999; Hac et al., 2003; Xing et al., 2006). In these studies, specimen samples were extracted by biopsy from living or deceased subjects and were analyzed using different analytical techniques. However, as biopsies are painful and are not practical for routine measurements and diagnostic procedures, therefore in this context there is a strong incentive to explore an alternate prospective non-invasive method for its measurement. Therefore at the first stage it is considered desirable to develop a non-invasive method to assess the feasibility of measuring selenium in humans.

Thus the main objective of the present study is to design a human hand tissue equivalent phantom for non-invasive quantification of selenium in humans using in vivo neutron activation technique (IVNAA). The IVNAA is a non-invasive and non-destructive technique which provides lower detection limit of an element (elements) of interest. This method provides a faster way of measuring selenium which utilizes the short-lived metastable ^{77m}Se radioisotope (half-life: 17.4 s), and the counting of gamma rays from the irradiated subjects can be initiated immediately after irradiation. (Bem, 1981; ATSDR, 2003).

The human hand bone equivalent phantom is chosen with an idea that in case it is found feasible to estimate selenium in the phantom, this technique may be extended to the real human hand for IVNAA. The importance of monitoring of selenium level in

human bone can be understood from the fact that several selenoproteins are expressed in bone and play important roles in bone metabolism (Ebert and Jakob, 2007; Martiniaková et al., 2013). Technically it is also convenient to measure a hand bone compared to complexity of using neutron activation method at other sites of body. The effective radiation dose to the subject is much lower when only the hand is exposed to a neutron beam as opposed to a whole body irradiation. During the exposure of the hand the rest of human body can be extended away from the hand to reduce radiation risk to the other more sensitive organs of the body. In some earlier studies various minor and trace elements have successfully been estimated in human hand bone using the IVNAA and 4π detection system at the McMaster Accelerator Lab. (Aslam et al., 2008; Davis et al., 2008; Chamberlain et al., 2012; Matysiak et al., 2013; Mostafaei et al., 2013; Bhatia et al., 2014).

1.8 Thesis layout

This thesis consists of six chapters. The rationale of the study is given in chapter one which also explains the importance of selenium element in terms of its chemical and physical properties, its kinetics and metabolism in body, various sources of exposure, health risks owing to excessive and deficient levels, and different techniques used for selenium measurement in biological specimens. Chapter two describes in detail the neutron activation analysis technique and 4π NaI(Tl) based γ -ray detection system. Chapter three shows the experimental part describing the materials and procedures used while pursuing the IVNAA method. Chapter four presents and discusses the results obtained from the experimental works performed for the detection of selenium and

calcium peaks in the gamma spectra. This chapter also reports the minimum detection limit (MDL) of selenium which was established after normalization of the selenium counts to the calcium counts. Chapter five reflects the contents of a draft paper submitted to the journal of “Applied Radiation and Isotopes” for publication. This is regarding the production of short lived radioactive ^{19}O during the irradiation of water phantoms. Conclusions drawn from the whole study are presented in chapter six besides adding suggestions for future work.

Chapter 2

NEUTRON ACTIVATION ANALYSIS AND 4π -DETECTION SYSTEM

In this chapter the *in vivo* neutron activation analysis (NAA) technique has been described including its origin, basic principle, types of NAA, sources of neutrons, factors affecting the detection limit of an element of interest; and advantages and disadvantages of this technique. Besides, the basic concepts of the gamma-ray spectrometry and the working of a 4π Na(Tl) based detection system are elaborated.

2.1 Origin of neutron activation analysis (NAA)

Following the discovery of the neutron by J. Chadwick in 1932 and the results of F. Joliot and I. Curie in 1934, neutron activation analysis was first developed by G. Hevesy and H. Levi in 1936. They used a neutron source ($^{226}\text{Ra} + \text{Be}$) and a radiation detector; and observed that the element Dy (dysprosium) in the sample became highly radioactive after exposure to the neutron source. They showed that the nuclear reaction may be used to determine the elements present in unknown samples by measuring the induced radioactivity (Hamidatou et al., 2013)

Later on the development of the nuclear reactors in the 1940s, the application of radiochemical techniques using low resolution scintillation detectors like NaI (Tl) in the 1950s' the development of germanium and silicon based semiconductor detectors and multichannel analyzer in the 1960s' and the advancement of computers and relevant software in the 1970s' the nuclear technique has advanced to become an important

analytical tool for determination of many elements at trace level. In spite of the developments in other chemical techniques, the simplicity and selectivity, the speed of operation, the sensitivity and accuracy of NAA have maintained its role as an accurate analytical technique (Greenberg et al., 2011).

Neutron activation analysis (NAA) is a powerful sensitive analytical technique to analyze the sample, i.e., to identify the elements present in the sample both qualitatively and quantitatively. Due to its accuracy and reliability, NAA is generally recognized as the "reference method" of choice when new procedures are being developed or when other methods yield results that do not agree. It is usually used as an important reference for other analysis methods. Worldwide application of NAA is so widespread, it is estimated that approximately 100,000 samples undergo analysis each year (Verma, 2007).

The NAA technique is based on the principle of converting various elements of the sample to radioactive isotopes by irradiating the sample with neutrons in a nuclear reactor or using any other neutron source. During irradiation the naturally occurring stable isotopes of most elements that constitute the samples are transformed into radioactive isotopes by neutron capture. The radioactive isotopes so formed decay according to their characteristic half-lives varying from seconds to years, emitting the γ -radiations with specific energies. The characteristic γ -rays emitted by radioactive isotopes are subsequently measured with semi-conductor γ -ray spectrometers to identify the source of these γ -radiations. Since each radionuclide emits γ -radiation of a specific wavelength or energy, the emitted γ -radiations are characteristic of the isotope formed and hence characteristic of the parent element (Bode and De Coeij, 1998).

In the last four decades, neutron activation analysis has been found to be extremely useful in the determination of trace and minor elements in many disciplines. These include environmental analysis applications, nutritional and health related studies, geological as well as material and forensic sciences (IAEA-TECDOC, 2001).

2.2 Basic Principles of NAA

The principle of neutron activation analysis is the nuclear reaction, specifically the neutron capture and subsequent gamma radiation emission through β -decay, called (n,γ) reaction. The radiative neutron capture has high probability for thermal (energy ~ 0.025 eV) neutrons and for elements having large cross-sections. The sequence of events occurring during the nuclear reaction of neutron capture or (n, γ) reaction used for neutron activation analysis is shown in Figure 2.1.

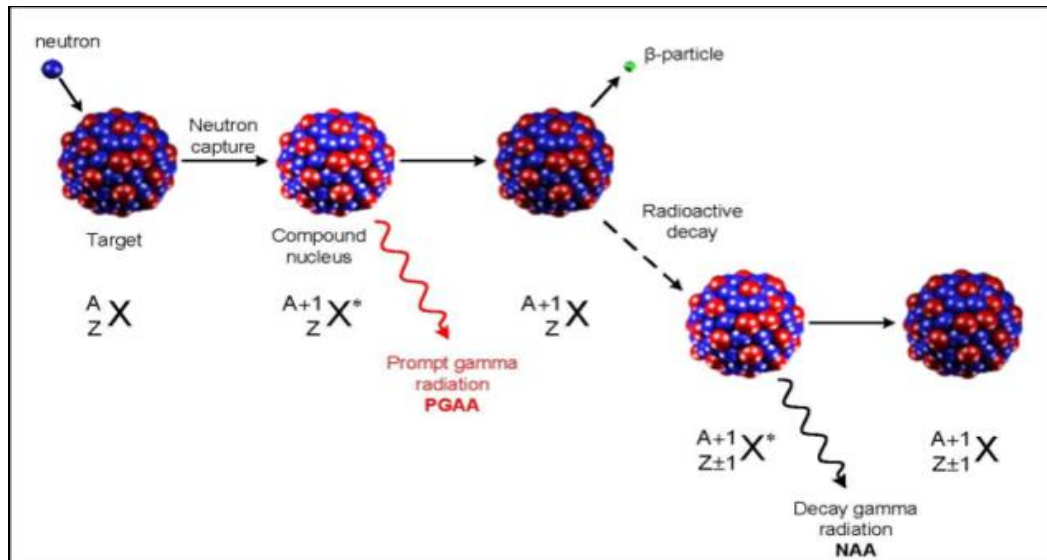


Figure 2.1: Diagram illustrating the process of neutron capture by a nucleus followed by the emission of gamma rays

In a neutron capture reaction when a neutron interacts with the target nucleus via an inelastic collision, a compound nucleus is formed which is in an excited state. The excitation energy of the compound nucleus is associated to the binding energy of the neutron with the nucleus. The compound nucleus de-excites nearly instantaneously into a more stable state by emitting one or more characteristic prompt gamma rays. In many cases, this new configuration yields a radioactive nucleus which decays typically by β -emission, the product nucleus being in an excited state may then de-excite by emission of one or more characteristic delayed γ -rays, but at a much slower rate according to the unique half-life of the radioactive nucleus. Depending upon the particular radioactive species, half-lives can range from fractions of a second to several years or more (Win, 2004).

2.3 Activation equation

As a result of the neutron capture reaction the product nuclide can be either stable or radioactive. In the former case, only gamma de-excitation occurs, resulting in an essentially prompt emission of discrete gamma radiation of usually high energy, up to 8-10 MeV. In the latter case, the radioactive reaction product undergoes one or more decays, and is eventually transformed into a stable nuclide. The radioactive decay is characterized by its half-life, which can be anywhere from a fraction of a second to many years. Although the continuous spectrum of beta electrons or positrons can also be utilized for activation analysis, it is most practical to observe the discrete gamma radiation of the reaction product. This is due to the higher selectivity and penetration of

the gamma decay radiation. The number of radioactive nuclides formed per unit time in a nuclear reaction is determined by the reaction rate:

$$R = \int_{E_{min}}^{E_{max}} N_0 \sigma(E) \phi(E) dE \quad (1)$$

where $\sigma(E)$ is the differential capture cross section (cm^2), $\phi(E)dE$ is the flux or fluence rate of particles with kinetic energy between E and $E+dE$ impinging on the sample (the differential flux $\phi(E)$ is measured in units of $\text{cm}^{-2} \text{s}^{-1} \text{eV}^{-1}$), N_0 is the number of target atoms of the nuclide that take part in the given reaction. N_0 is connected with the mass $m(\text{g})$ of the given element in the sample and is given by:

$$N_0 = m\theta N_A/M \quad (2)$$

Where M is the molar mass of the element (g mol^{-1}), θ is the isotopic abundance and N_A is Avogadro's constant. For thermal neutrons, the absorption cross section is usually inversely proportional to the neutron speed ("1/v" law). Using Equation (2), Equation (1) gives a reaction rate (s^{-1}) as below:

$$R = m(\theta N_A/M) \sigma \phi \quad (3)$$

The activation process and the resulting decays of excited nuclear states are controlled by the laws of nuclear physics and can be fully described by mathematical equations. In the activation reaction, a radionuclide is formed and subsequently decays. The rate of change of the number of radioactive atoms N is thus determined by the differential equation:

$$dN/dt = R - \lambda N \quad (4)$$

where λ is the decay constant (s^{-1}) giving probability of decay per unit time and Equation (4) accounts for simultaneous production and decay. Integration of equation (4) gives:

$$N(t) = R(1 - e^{-\lambda t})/\lambda \quad (5)$$

So activity A_t which gives number of disintegrations per unit time is given by:

$$A_t = A_0 e^{-\lambda t}$$

Where A_0 denotes the activity at time $t = 0$ (Zeisler et al., 211).

2.4 Quantitative analysis

Quantitative analyses are performed by measuring induced activity due to NAA from the radionuclide of interest. Assume that the sample has been activated and the measurements have to be done on this sample at $t = 0$. Since the induced activity is primarily the product of number of atoms ($\sim$ mass) of the element of interest, neutron capture cross-section and the neutron flux, this has to be corrected by multiplying with the fraction of the target isotope in the sample and saturation factor (determined by the half-life of the isotope formed and the time of irradiation). The induced activity of the sample will thus be given by:

$$A_t = (mN_A/M)\sigma\Phi\theta(1 - e^{-\lambda t_i})$$

In the above equation, A_t = Activity of the element of interest in the sample at time t , $N = (mN_A/M)$ is the number of atoms of the target element (where m is the weight of the element in grams, N_A = Avogadro number and M = atomic weight of the element), σ = neutron absorption cross section in cm^2 , Φ = neutron flux in $\text{neutron cm}^{-2} \text{ s}^{-1}$, θ = fraction of the target isotope in the element, $(1 - e^{-\lambda t_i})$ = saturation factor, $\lambda = 0.693/T_{1/2}$, $T_{1/2}$ = half-life for the radioisotope produced by the reaction and t_i is the time of irradiation. The

activity of the sample after a cooling or delay period (t_d) from the end of the irradiation that lasted for time t_i is given by:

$$A(t_d) = (mN_A/M)\sigma\Phi\theta (1 - e^{-\lambda t_i}) (e^{-\lambda t_d})$$

and the accounting for the number of radioactive nuclei that decay during counting interval t_c , the activity will be given as:

$$A = (mN_A/M)\sigma\Phi\theta (1 - e^{-\lambda t_i}) (e^{-\lambda t_d}) (e^{-\lambda t_c})$$

2.5 Detection limit of NAA

The detection limit represents the ability of a given NAA procedure to determine the minimum amounts of an element reliably. The detection limit for a given element by NAA may be different for each individual type of material, and analysis conditions. The detection limit depends on many factors, such as the amount of material to be irradiated, the duration of the irradiation time, the duration of the counting time, the decay conditions, the neutron flux, the detector size, the counting geometry, the background shielding, the interference situation including such things as the ambient background, the Compton continuum from higher energy-rays, as well as any γ -ray spectrum interferences (Naeem et al., 2013).

2.6 Types of NAA

The NAA process can be divided into two categories with respect to the time of measurement, i. e., prompt gamma neutron activation analysis (PGNAA) in which the measurements take place during irradiation and the delayed gamma neutron activation analysis (DGNAA), where the measurements follow radioactive decay after end of

irradiation. PGNAA is an instrumental procedure that makes use of the prompt gamma-ray emissions that occur immediately after neutron capture during the de-excitation of the newly formed compound nucleus. The capture can produce nuclear states with energies up to about 11 MeV above the ground state, which usually decay through a cascade of gamma rays. The complexity and broad energy range of these spectra distinguishes PGNAA from most other NAA procedures (Molnár and Lindstrom, 1998).

The main advantage of PGNAA is the ability to obtain gamma-ray spectra from the neutron capture of nuclides that do not produce radioactive isotopes through the (n,γ) reaction. PGNAA favorably complements NAA through efficient reactions with the light elements that generally do not produce radionuclides, and also a number of higher Z elements with high cross sections the normal decay products of which may be difficult to measure in the presence of high activities induced in certain matrices. The light elements most often investigated are H, B, C, N, Si, P, S, and Cl, and the heavier elements are Cd, Sm, and Gd (Vértes et al., 2010).

The DGNAA operational mode is more common. So when one mentions NAA, it is generally assumed that one refers to measurement of the delayed γ -rays or conventional NAA. About 70% of the elements have properties suitable for measurement by NAA. The qualitative characteristics of NAA are the energies of the emitted gamma-rays and the half-life of the nuclide whereas the quantitative characteristic is the intensity, which is the number of gamma quanta of energy measured per unit time (Njinga et al, 2013).

DGNAA is useful for the vast majority of elements that produce radioactive nuclides. The technique is flexible with respect to time such that the sensitivity for a long-lived radionuclide that suffers from interference by a shorter-lived radionuclide can be improved by waiting for the short-lived radionuclide to decay. Contrarily the sensitivity for short-lived isotopes can be improved by reducing the time of irradiation to minimize the interference of long-lived isotopes. This selectivity is a key advantage of DGNAA over other analytical methods (Verma, 2007).

The probability of a neutron interacting with a nucleus is a function of the neutron energy. This probability is referred to as the capture cross-section or neutron cross section (σ) which is a measure for the probability that a reaction will take place. Capture cross section varies strongly for different reaction types, elements and energy distributions of the bombarding neutrons. Each nuclide has its own neutron energy capture cross-section relationship. For many nuclides, the capture cross-section is greatest for low energy neutrons or thermal neutrons. Some nuclides have greater capture cross-sections for higher energy neutrons or epithermal neutrons. For routine neutron activation analysis, generally nuclides are activated by thermal neutrons (Vértes et al., 2010). Various reactions are possible during NAA with regard to emission of particles, i. e., alpha, proton, gamma, neutron or/and neutrons from the excited target. The most common reaction occurring in NAA is the (n,γ) reaction, but also reactions such as (n,p) , (n,α) , (n,n') and $(n,2n)$ are important (Gluscock and Neff, 2003).

2.7 Energy regions of neutrons

In NAA the energy of neutrons forming flux plays an important role in the activation process. The energy of neutrons produced through various sources varies depending upon the type of a bombarding particle, target and reaction taking place. Thus in a broader sense neutrons are categorized according to their energy such as thermal, epithermal, fast neutrons etc. In nuclear research reactors which are intense sources of neutrons, the neutron flux distribution consists of all these three categories. Figure 2.2 shows a typical reactor neutron energy spectrum with all three energy regions (Hamidatou et al., 2013).

Neutrons having energy of 0.025 eV are called thermal neutrons. They are also called slow neutrons because their most probable speed at room temperature is low, i. e., $v \sim 2,200$ m/s. Due to the $1/v$ -dependence of most capture cross section, thermal neutrons have the greatest probability of interacting with most target elements. For this reason, thermal NAA is the most simple and universal activation analysis technique and remains the workhorse in the activation analysis laboratory (Zeisler et al., 2011).

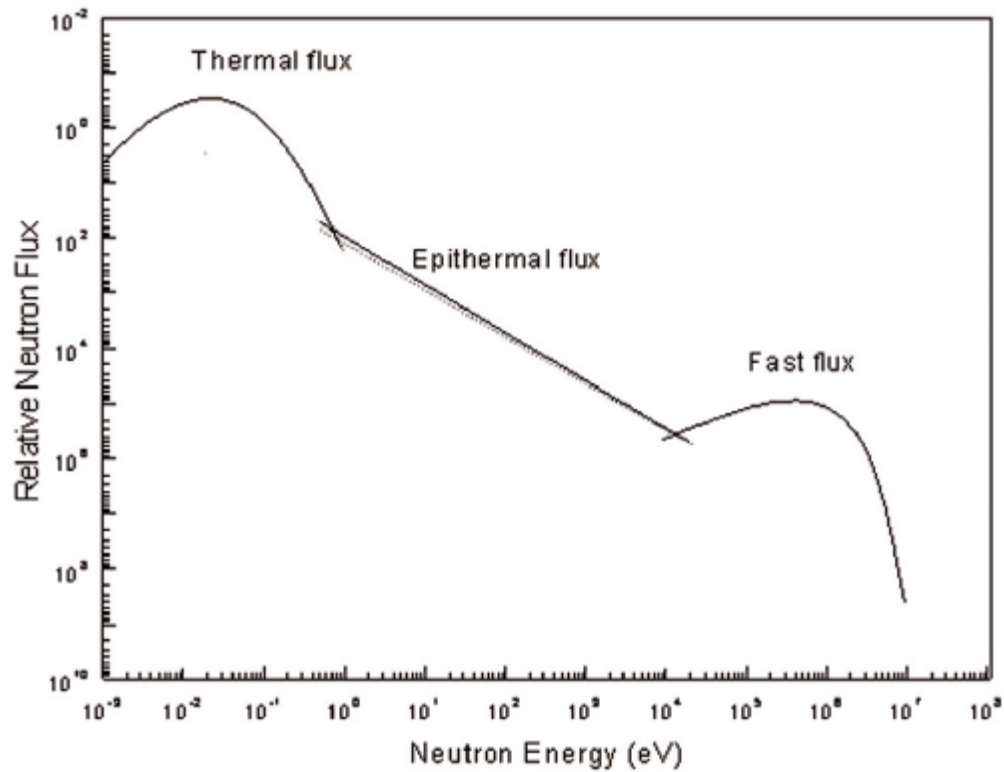


Figure 2.2: A typical reactor neutron energy spectrum with all three energy regions (Hamidatou et al., 2013)

Neutrons with energies (1 eV to 100 keV) slightly higher than the thermal neutrons are called epithermal neutrons. They serve as activation particles in the epithermal NAA (ENAA), which is based on the selective activation of certain nuclides the cross sections of which exhibit strong individual resonances in the lower part of this energy range. Special facilities to enhance ENAA reactions over thermal NAA are employed in the irradiation location. To suppress the thermal neutron activation of the matrix elements,

the sample is covered with a thermal neutron absorber, such as cadmium or boron. As a result, high sensitivities can be attained despite the relatively low activity induced by the epithermal neutrons (Alfassi, 2001).

Neutrons in the energy range from 1-20 MeV are called fast neutrons. The fission spectrum of a light-water-moderated reactor provides as many fast neutrons as thermal neutrons. Therefore, fast neutron activation of certain elements via (n, p) reactions is a very selective technique, complementary to thermal and epithermal NAA. A common and particularly useful form of FNAA is the instrumental analysis with 14 MeV neutrons that are produced by small accelerators known as neutron generators (Zeisler et al., 2011).

2.8 Neutron production sources

There are two basic essentials required to carry out an analysis of samples by NAA, first is the source of neutrons, and second is the gamma spectrometric system suitable for detecting gamma rays emitted from the compound nucleus. Neutrons can be produced through various sources, i. e., radioisotopic sources, particle accelerators and nuclear reactors. Radio-isotopic neutron emitters include $^{238}\text{Pu}(\text{Be})$, $^{226}\text{Ra}(\text{Be})$, $^{210}\text{Po}(\text{Be})$, $^{124}\text{Sb}(\text{Be})$, $^{241}\text{Am}(\text{Be})$, and ^{252}Cf (which is called a fission neutron source). The neutrons from isotopic sources have different energy distributions with a maximum energy typically in the order of ~ 10 MeV (Hamidatou et al., 2013).

Particle accelerators (or neutron generators) produce a high flux of neutrons through (p,n), (d,n), (α ,n) or other reactions. The particle accelerators are used to produce neutrons whereby a convenient target material is bombarded by accelerated charged

particles and the neutrons are produced in a nuclear reaction. The most common types are based on the acceleration of deuterium ions towards a target containing either deuterium or tritium, resulting in the reaction $2\text{H}(2\text{H},n)^3\text{He}$ and $3\text{H}(2\text{H},n)^4\text{He}$, respectively. These type of neutron generators are small accelerators whose output may be pulsed or continuous. The first reaction often denoted as (D,D), yields mono-energetic neutrons of 2.5 MeV and typical outputs in the order of 10^8 - 10^{10} s^{-1} , whereas the second reaction (D,T) results in mono-energetic neutrons of 14.7 MeV and outputs of 10^9 - 10^{11} s^{-1} . The (D,D) and (D,T) sources are also known as fusion neutron sources. In an effort to produce neutrons for the selective excitation of nuclear isomeric states by inelastic scattering, $^7\text{Li}(p, n)^7\text{Be}$ reaction has proved to be useful due to its relatively high yield and very narrow energy spread (Verma, 2007).

In Nuclear reactors, neutrons are produced through the fission reaction. The flux of neutrons produced by the reactors is of the order of 10^{12} – $10^{15} \text{ ncm}^{-2} \text{ s}^{-1}$. Since the thermal neutrons are required for NAA due to their maximum absorption cross-sections, the neutron beam has to be thermalized as the neutron beam from the reactor consists of thermal, epithermal, and fast neutrons. The nuclear reactors operating in the maximum thermal power region and with maximum neutron flux are most efficient neutron sources for high sensitivity activation analysis induced by epithermal and thermal neutrons because of the high cross section of neutron activation in the thermal region for a majority of elements (Arai & Crawford, 2009). Figure 2.3 shows the relation between neutron cross section and neutron energy for major actinides for (n, γ) reaction (Hamidatou et al., 2013).

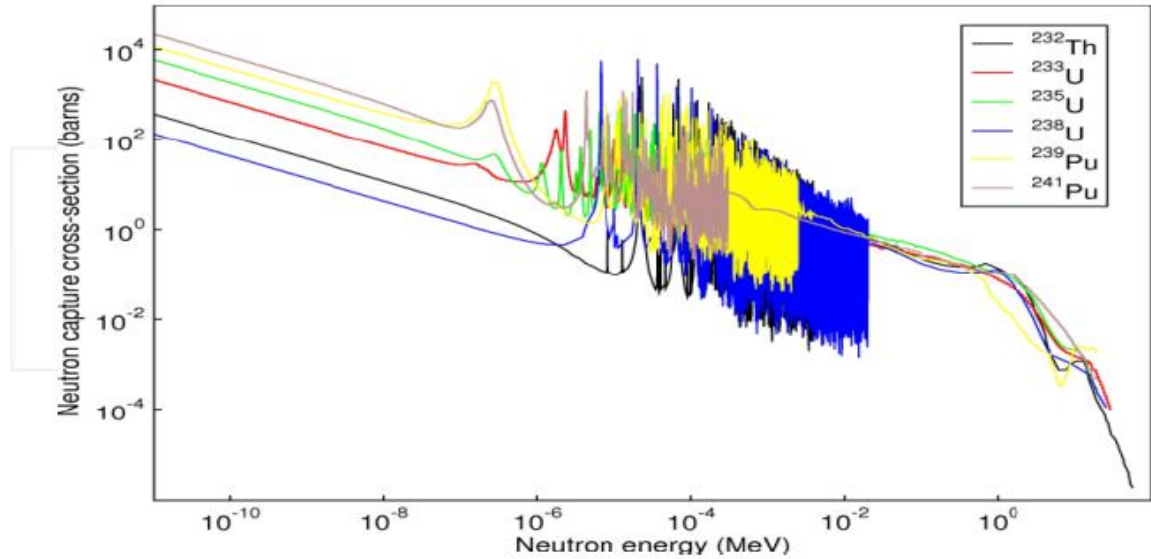


Figure 2.3: Relation between neutron cross section and neutron energy for major actinides (n,capture)

2.9 Gamma ray spectrometry

The instrumentation used to measure γ -rays from the radioactive samples due to neutron activation consists of a semiconductor detector, associated electronics, and a multichannel analyzer. Figure 2.4 shows a functional block diagram of NAA based gamma- ray detection system. In gamma-ray spectrometry, gamma rays are detected by HPGe/Ge(Li) semiconductor detectors and/or NaI(Tl) scintillation detectors. In the recent past a 4π configuration of NaI(Tl) with an array of nine detectors was made available for such experimentation in the McMaster Accelerator Lab (MAL) of McMaster University (Byun et al., 2004).

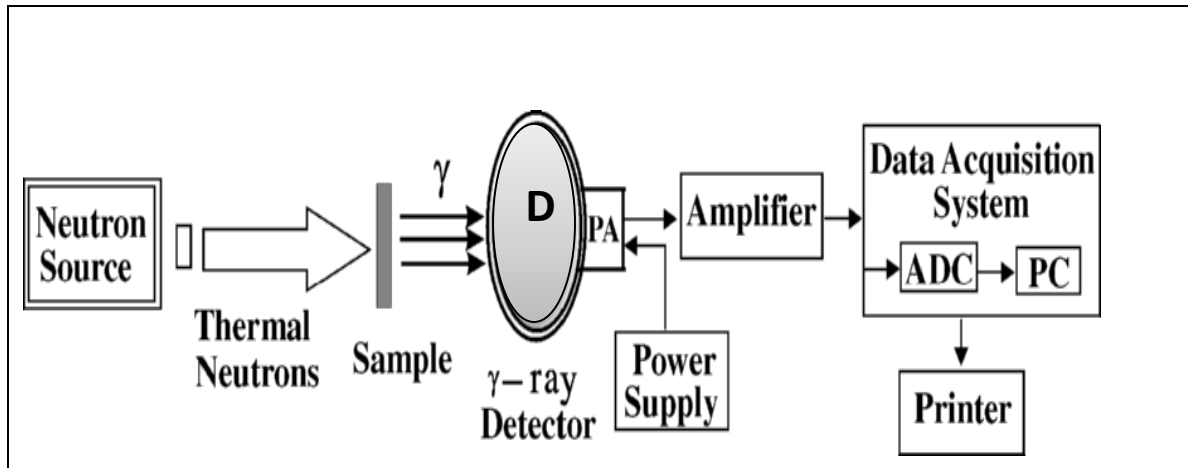


Figure 2.4: Functional block diagram of NAA based gamma ray spectrometer

2.10 HPGe and Ge(Li) detectors

The hyper-pure Ge (HPGe) and Ge (Li) are two types of germanium (semiconductor) detectors. The older version was a lithium drifted crystal of purified germanium used in Ge (Li) detector. It began to be phased out in the early 1980s when the production of high purity germanium became a more routine practice. During the interaction of γ -rays with the germanium detectors, photoelectrons are generated which are accelerated in the voltage field applied to the crystal, and this produce pulses at the detector's pre-amplifier which are proportional to the original gamma ray energy (Knoll, 2000).

Semiconductor detectors are basically P-I-N diodes, in which the intrinsic (I) region is created by depletion of charge carriers when a reverse bias is applied across the diode. When a photon interacts in the intrinsic region, tracks of electron-hole pairs are produced

(analogous to electron–positive ion pairs in a counting gas). In the presence of an electric field, these pairs separate and are swept rapidly to their respective collecting electrodes (detector contacts) by the electric field. The resultant charge is integrated by a charge sensitive preamplifier and converted to a voltage pulse with amplitude proportional to the original photon energy. These detectors are cooled to reduce the thermal leakage current. The detector is in thermal contact with the liquid nitrogen, which cools it to around 77°K (-196°C). Because of the greater sensitive region, the noise contribution from thermally generated electrons and holes is much reduced by cooling to liquid nitrogen temperatures.

The HpGe and Ge(Li) detectors typically have an energy resolution of ~1 keV (at 122 keV) and ~2 keV (at 1.332 MeV) respectively. HPGe detector is used for low energy γ -ray detection. Semiconductor detectors provide greatly improved energy resolution over other types of radiation detectors for many reasons. Fundamentally, the resolution advantage can be attributed to the small amount of energy required to produce a charge carrier and the consequent large “output signal” relative to other detector types for the same incident photon energy (Knoll, 2000).

2.11 NaI (TI) detector

The thallium doped sodium iodide crystal NaI(Tl) scintillation detector consists of thallium (Tl) activated NaI crystal (having a small amount of impurity of Tl) which is optically coupled to a photomultiplier tube. As the γ -rays interact with the scintillator material (through the process of photoelectric effect, Compton effect and pair production according to their energy range), optical photons are produced due to fluorescence/phosphorescence, i.e., the gamma ray transfers energy to the crystal through

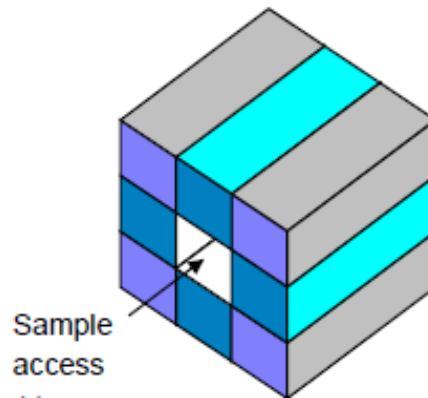
excitation of K-shell electrons via the photoelectric effect. These electrons migrate to the crystal's ground state giving off photons in the Ultraviolet to Visible energy range. These light photons are then converted to an electrical pulse by the photomultiplier tube or a photo diode. The high atomic number of iodine in NaI gives good efficiency for γ -ray detection.

The photo-multiplier (PM) tube consists of a photocathode, a focusing electrode and a series of dynodes that multiply the number of electrons striking them several times each. Sodium-iodide detectors are operated at room temperature, are very rugged, relatively cheap and easy to operate, and they are not significantly affected by temperature or humidity. However they have limited ability to distinguish between gamma ray peaks that are closer together. The commercially available NaI(Tl) detector includes a high resolution NaI(Tl) crystal and a photomultiplier tube. (Knoll, 2000).

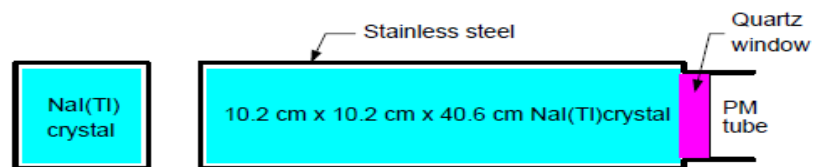
Compared to a sodium iodide detector, the HPGe detectors are relatively expensive, more difficult to operate, require constant liquid nitrogen conditions to operate, and are sensitive to environmental conditions of the counting room. However, the big advantage of the HPGe is that it has higher resolution i. e., it can resolve two gamma rays that are closely spaced.

2.12 NaI(Tl) detector in 4π configuration

In this study NAA of phantoms is planned to be performed using 4π NaI(Tl) detection system, which consists of detector array of nine square type detectors. The 4π geometry detector system is usually utilized for low level radioactivity measurements in γ -ray spectroscopy. The layout of the detector array is shown in Figure 2.5 (a-b). The



(a)



(b)

Figure 2.5: (a) Layout of 4π γ -ray detector array (b) Cross-sectional view of a detector unit

cross sectional size and length of eight NaI(Tl) crystal detectors are $10.2 \times 10.2 \times 40.6$ cm³ and ninth detector has dimensions of $10.2 \times 10.2 \times 10.2$ cm³. These detectors are arranged in an array with close to 4π geometry. It can be seen that the central square region is left void for positioning of the irradiated sample and calibration source. The solid angle of the detection geometry is 3.96π sr, so more than 95 % of the gamma rays emitted from the samples can reach the detector surface. In this way the 4π gamma-ray detection system provides highest improved geometry for sample detection. Figure 2.6 shows a block diagram of the original signal processing electronics (Byun et al., 2004) and Figure 2.7 shows the system upgraded to digital signal processing (Matysiak et al., 2013).

The gamma ray spectra in 4π array detection system can be acquired in singles, coincidence or anticoincidence modes. The single mode spectrum is accumulated without gating while the coincidence or anticoincidence spectrum is accumulated under the gating condition. The coincidence mode is activated when two or more detectors generate output pulses coincidently while the anticoincidence mode is confined to detection by one detector only at a time. The high voltage biases are applied from a single high voltage power supply with a sufficient current limit through a distribution box. The singles mode spectrum is accumulated by a simple configuration. The photomultiplier (PMT) tube output signals are all summed by a summing circuit before shaping and then the summed signal is fed into a spectroscopy amplifier and an analogue to digital converter (ADC). The shaping time of the amplifier and analogue to digital conversion gain of the ADC can be changed as per the experimental requirements.

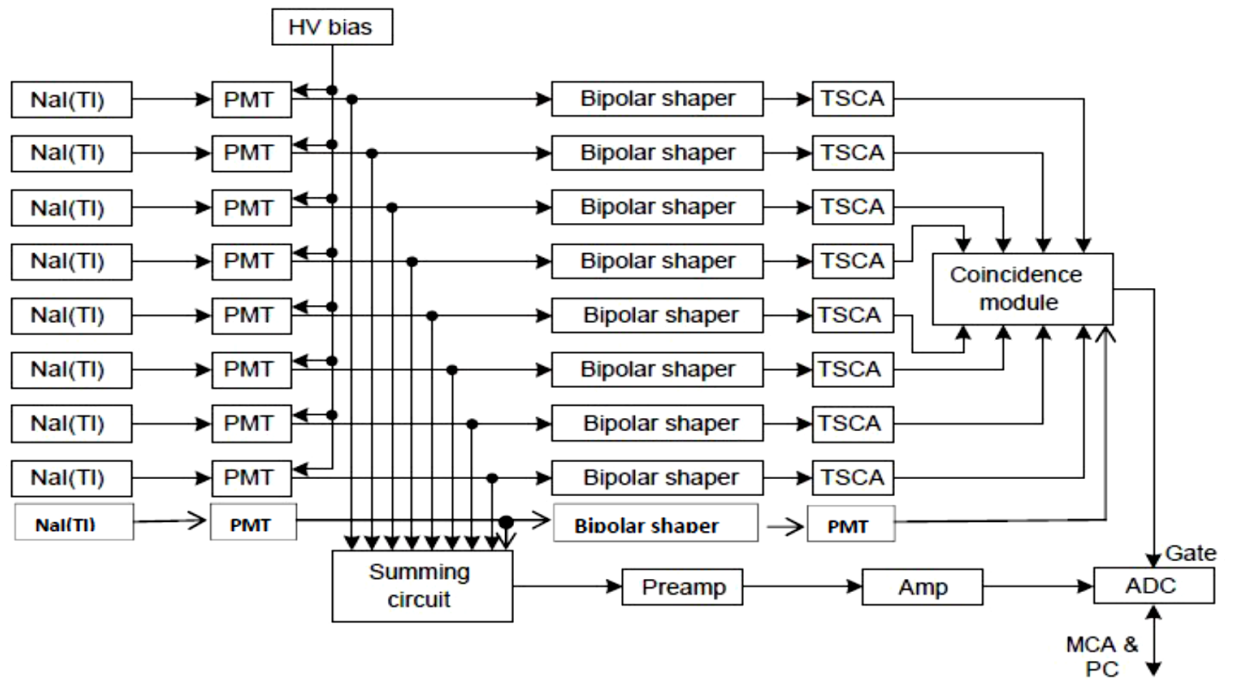


Figure 2.6: Block diagram of the signal processing electronics for 4π γ -ray detector array (Byun et al., 2004)

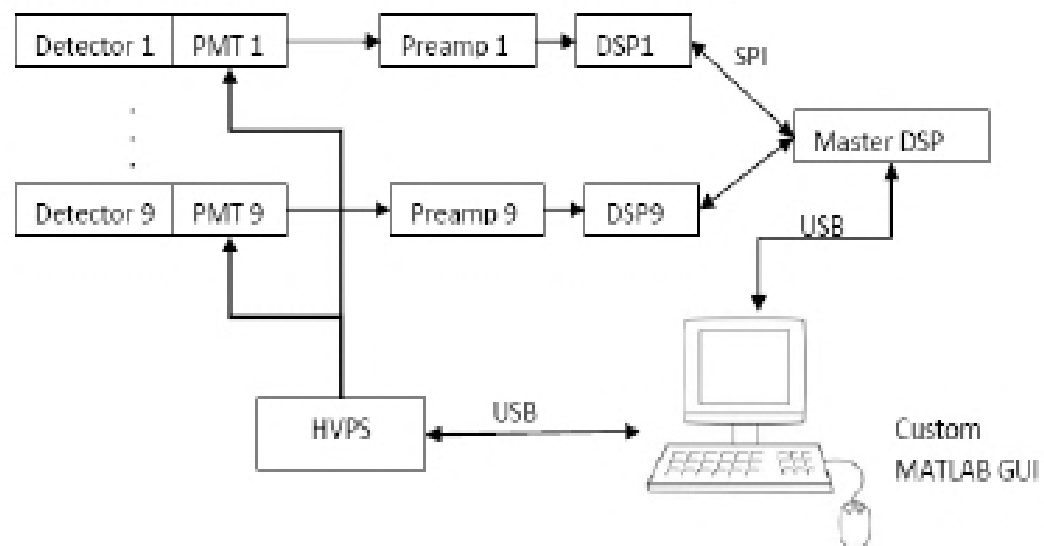


Figure 2.7: Functional components of the detection system (Matysiak et al., 2013)

Gain stability of individual detectors in a multi-detector array affects the energy resolution of the coincidence as well as anticoincidence spectra, and photo-multiplier tubes exhibit gain instabilities due to temperature variations and bias voltage drifts. Stabilizing a multi-detector system poses considerable difficulty. Moreover, the NaI(Tl) detectors in 4π array are equipped with different models of PMTs, each characterized by a different temperature sensitivity. It is therefore important to monitor the long-term gain stability of the system. (Matysiak et al., 2013).

Chapter 3

MATERIALS AND EXPERIMENTAL PROCEDURES

This chapter describes the materials and methods used to perform the experiments carried out in the present study. It includes preparation of the hand tissue equivalent phantoms, working of the irradiation cavity for neutron activation process, operation of the 4π NaI (TI) detection system, analysis of gamma spectra, initial feasibility test of selenium phantom; and irradiation of sets of phantoms with various concentrations of selenium element.

3.1 Phantom for initial feasibility test

Selenium can be identified and quantified in the gamma spectrum using the short lived ^{77m}Se ($T_{1/2} = 17.5$ s) induced isotope from NAA in $^{76}\text{Se}(n,\gamma)^{77m}\text{Se}$ reaction. ^{77m}Se emits gamma rays of energy 162 keV. To the best knowledge of the author, selenium concentrations in the hand tissue equivalent phantoms have not been measured using neutron activation analysis and the 4π detection system previously at the McMaster Accelerator Labs (MAL). Therefore at the first instance it was considered imperative to go for a feasibility test of a human hand tissue equivalent phantom.

The first hand tissue equivalent selenium phantom was prepared by adding 750 μg of Se (750 μl by volume from the stock solution) in 250 ml of de-ionized water (cylindrical water matrix) contained in a low density polyethylene (LDPE) Nalgene bottle. Selenium stock solution (Se standard for ICP) was obtained from Sigma Aldrich.

The other added elements in the phantom solution were Ca (14.9 g) from $\text{Ca}(\text{NO}_3)_4\text{H}_2\text{O}$, Cl (1.19 g) from NH_4Cl and Na (1.25 g) from NaNO_3 . The amounts of elements other than the selenium were added keeping in view the ICRP (1975) recommendations for a reference man of 70 kg mass.

The feasibility test of the first selenium phantom was conducted by irradiating the phantom in the irradiation cavity and by employing a 4π NaI (TI) array of detectors for gamma-ray counting. This facility is available at the McMaster Accelerator Lab (MAL). Before irradiation of the Se-phantom, all nine detectors of the detection system were aligned to the same channel number for detection of gamma rays of energy 162 keV from the irradiated phantom. The composition and the working operation of the 4π detection system has been described in chapter-2.

Generally at MAL, in various experiments while pursuing the detection of gamma signals using the 4π detector, γ -ray spectra in the energy regions usually above 511 keV are measured. For this purpose a Co-60 source (gamma peaks: 1170 keV and 1332 keV) is used for energy-channel calibration and centroid of 2nd peak from Co-60 (1332 keV) is aligned at channel number 525 ± 1 for all nine detectors. The alignment is done by controlling the gain voltage of the NaI(TI) detectors. This results in the contribution of gamma-ray signals of same energy from the radioisotope of interest from all detectors at one corresponding channel number in coincidence, anticoincidence and single modes of detection. The feasibility test of the Se-phantom was carried out by irradiating the phantom for 30 s at proton energy 2.3 MeV and proton current 400 μA in the irradiation cavity. Description of the irradiation cavity may be seen in next section 3.2.

3.2 Irradiation cavity

The layout of the irradiation cavity for IVNAA is shown in Figure 3.1 (Byun et al., 2007) and Figure 3.2 (Davis et al., 2008) depicts a view of the phantom access port of the irradiation cavity. The Tandetron accelerator installed at McMaster University is used to produce low energy fast neutrons of mean neutron energy less than 500 keV for neutron activation analysis (NAA). The accelerator can deliver protons up to 2.5 MeV. These neutrons are produced using thick lithium metal targets via the ${}^7\text{Li}(p,n){}^7\text{Be}$ reaction. These targets are mounted at the end of a beam duct and cooled with water to prevent excessive heating by the proton beam. The target holder used for experimentation has a copper backing between the water channel and the target.

This facility can be operated at proton beam currents in the range from 60 to 600 μA . The total neutron yield ($\text{n}/\mu\text{C}$) of a thick lithium target increases with increasing incident proton beam energy. This increase in total neutron yield of a thick lithium target with increase in beam energy is accompanied by an increase in the mean neutron energy. Regardless of the target material, target cooling is always challenging practically when high current operations are intended. The situation is a little worse in the case of lithium due to its relatively low melting temperature (180.5 $^{\circ}\text{C}$). At high proton currents the heat removal mechanism prevents the target melting.

Basically the irradiation cavity consists of three main components, i. e., moderator, reflector and shielding for the subject body. The detailed information on the cavity design

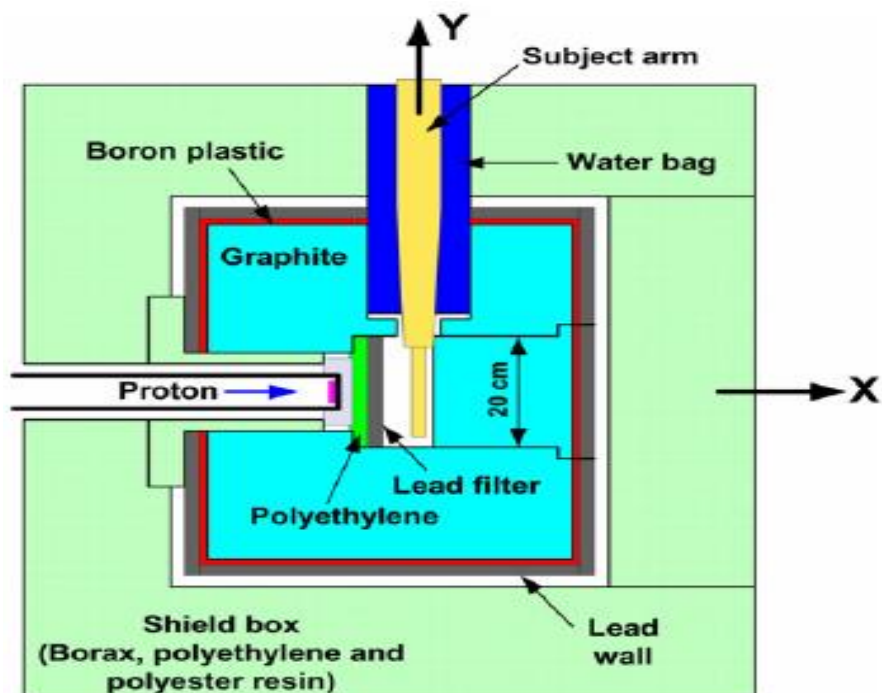


Figure 3.1: Irradiation cavity (Byun et al., 2007)



Figure 3.2: Outer view of phantom access port of the irradiation cavity (Davis et al., 2008)

has been reported by Pejovic'-Milic' et al. (2006). The moderator shifts the neutron spectrum down to the low energy region so that neutron captures occur efficiently in the sample under irradiation. For proton energy up to 2 MeV, polyethylene has been established to be the optimum moderator material. A lead filter is located next to the moderator to absorb the direct gamma-rays from Li target, ${}^7\text{Li}(p,p'\gamma)$ and the capture gamma-rays from the moderator, ${}^1\text{H}(n, \gamma)$, so that the gamma-ray dose contribution to the phantom/sample can be reduced.

The graphite reflector is used to utilize the neutron beam more efficiently and also to make the radiation shielding for a subject easier by moderating stray neutrons further. A boron plastic sheet and a lead wall absorb thermal neutrons and gamma-rays leaking out of the reflector. The outermost shield box of the cavity made of borax, polyethylene and polyester resin keep the radiation level outside the cavity within the licensed radiation level of the accelerator laboratory. For access to the irradiation position a hand access hole has been machined through the reflector which can be used to place phantoms or the hand of a subject inside the cavity at the irradiation position.

3.3 Spectrometry of Se phantom

The first irradiated phantom after the end of irradiation was transferred to the NaI (TI) detection system for detection of γ -ray signals. The phantom transfer time from the irradiation cavity to the 4π counting cavity was 12 s. The phantom was counted for 10 cycles each of which was for 10 s duration. The gamma spectra obtained in this way were analyzed. The gamma-ray peak from ${}^{77\text{m}}\text{Se}$ in the gamma spectrum was identified using

the anticoincidence mode data. The gamma-ray peak (162 keV) was found at channel # 35 on the gamma spectrum which can be seen in Figure 3.3.

As the selenium peak appeared close to the rising edge of the spectrum, therefore for more clarity of the peak position, the gain of the detection system was increased by increasing gain voltages of all nine detectors. At this time 2nd peak of Co-60 (1332 keV) was adjusted for all detectors at channel # 800 ± 1 instead of channel # 525 ± 1 . To check the new location of the Se-peak after increase of gain, a similar phantom containing same elemental concentrations as that of first Se-phantom was irradiated under the same irradiation parameters for proton energy, current and irradiation time as chosen for the first phantom. The measured gamma spectrum this time showed the location of the Se-peak at channel # 54 moved from channel # 35.

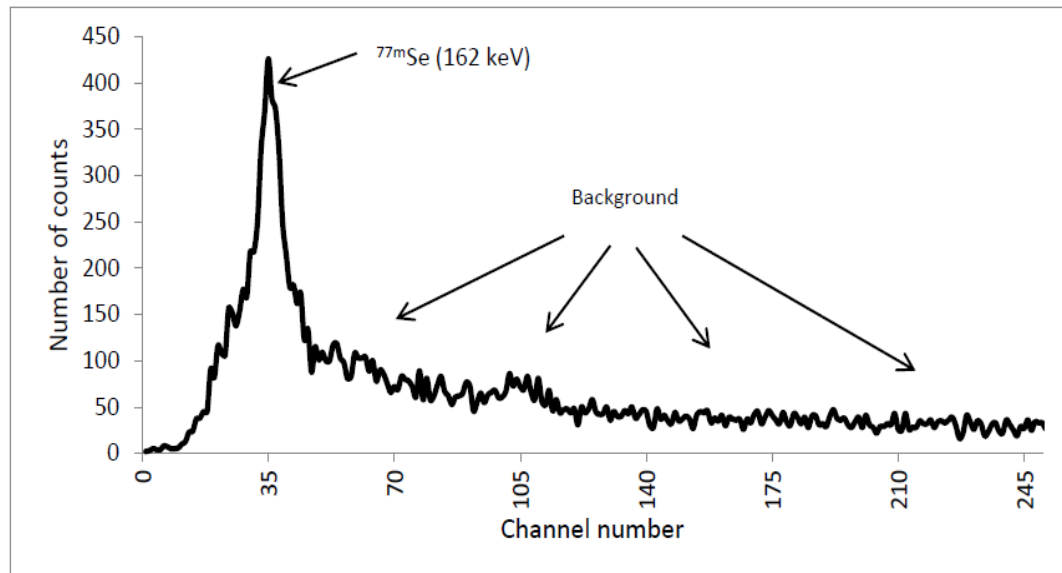


Figure 3.3: Location of Se-peak in the gamma spectrum of the hand tissue equivalent phantom

Location of Se-peak in the gamma spectrum for first cycle of 10 s counting interval after increasing the gain of detectors is shown in Figure 3.4. This initial experiment confirmed the detection of Se-peak in the gamma spectrum at the appropriate location using the 4π detection system. Energy-channel calibration line was also drawn using known sources of Cd-109 (88 keV) and Co-60 (1170 keV and 1332 keV). Gamma spectrum taken for 60 s duration of these sources and the energy-channel calibration line drawn thereof are shown in Figures 3.5-3.6 respectively.

3.4 Preparation of set of phantoms

For further investigations and to establish the minimum detection limit (MDL) of selenium in the hand tissue equivalent phantom, a set of phantoms consisting of eight selenium phantoms was prepared. For preparation of these phantoms selenium concentration in the range from zero to 750 μg was added in the LDPE Nalgene bottles filled with 250 ml deionized water in each of them. The contents of other elements of Ca, Cl and Na used in the feasibility test phantom were repeated in the same quantities and were kept constant in all eight phantoms. The quantities of the compounds added in the phantoms were recorded and shown in Table 3.1. All phantoms were properly labeled showing phantom #, amount of elemental concentrations added, date of preparation, date of irradiation, and name of the researcher.

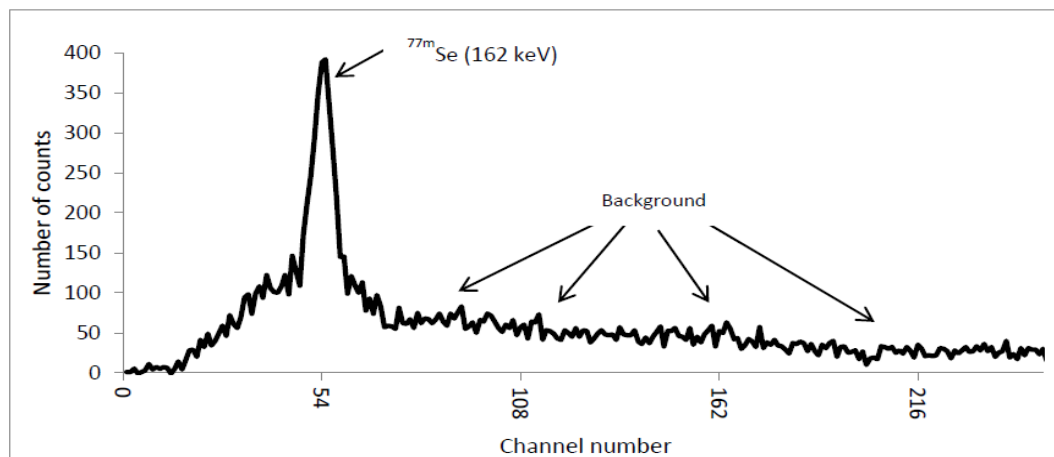


Figure 3.4: Location of Se-peak in the gamma spectrum of the hand tissue equivalent phantom after increase of gain

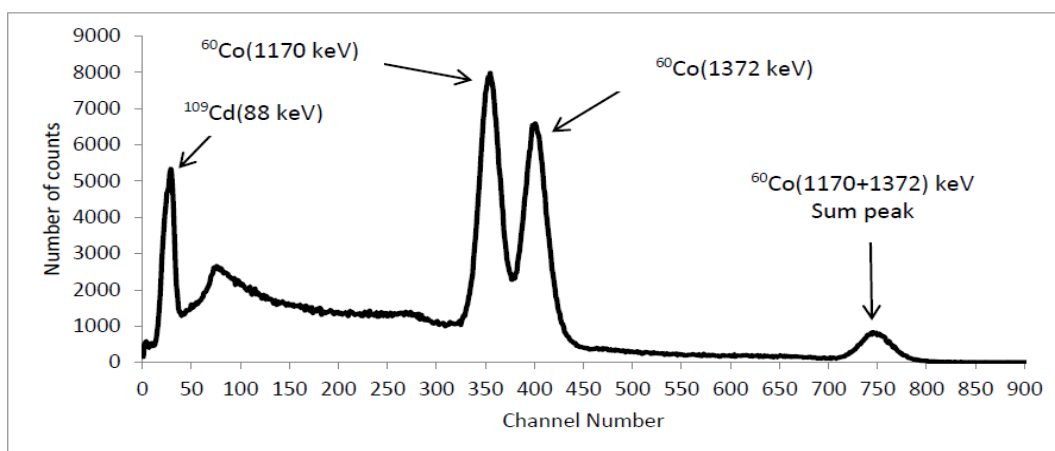


Figure 3.5: Gamma spectrum of calibration sources

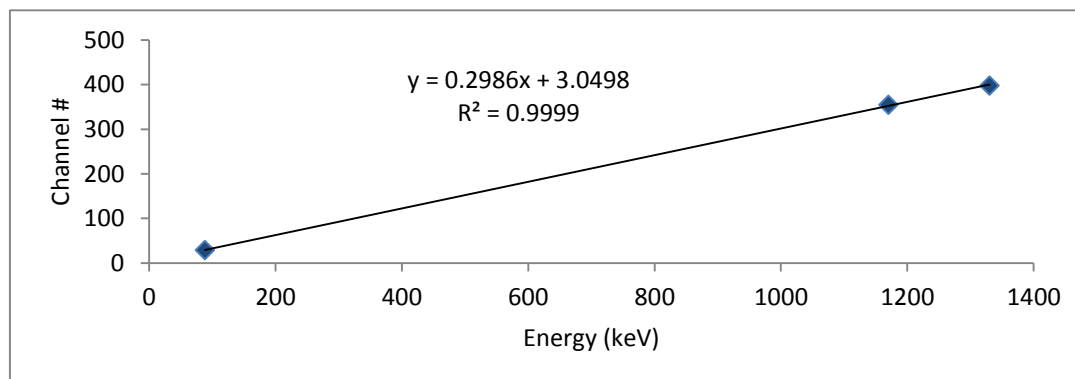


Figure 3.6: Energy-Channel calibration line

Table 3.1: Contents of Se, Ca, Cl and Na in various phantoms

Phantom #	Se (μg) <i>*(Se-standard for ICP)</i>	Ca (14.9 g) <i>(Ca(NO₃)₂4H₂O) (g)</i>	Cl (1.19 g) <i>(NH₄Cl) (g)</i>	Na (1.25 g) <i>(NaNO₃) (g)</i>
1	00	87.8	1.80	4.62
2	10	87.8	1.80	4.62
3	30	87.8	1.80	4.62
4	50	87.8	1.80	4.62
5	100	87.8	1.80	4.62
6	200	87.8	1.80	4.62
7	500	87.8	1.80	4.62
8	750	87.8	1.80	4.62

*Compound used are shown in italic parenthesis

3.5 Irradiation of phantoms and counting

All phantoms were irradiated one by one by placing them in the irradiation cavity. During irradiation proton current (I_p), energy (E_p) and irradiation time for each of phantoms were maintained at 400 μA , 2.3 MeV and 30 seconds respectively. However, the phantoms transfer time from the end of irradiation and the start of counting varied

slightly between phantoms, i. e., from 12-13 s. After each irradiation the respective phantom was counted for many cycles of 10 s duration in the 4π NaI (TI) detection system. Spectra were obtained in anticoincidence, coincidence and individual modes for all phantoms. Data collected were analyzed using a personal computer and Origin-(Data Analysis and Graphing) Software, version 8.6. In this way selenium and calcium peaks were identified and areas under the peaks were computed for further analysis.

3.6 Irradiation of second and third sets of phantoms

In order to pursue optimization conditions for irradiation parameters a second set of phantoms consisting of eight phantoms having same concentrations of selenium and other elements (i. e., Ca, Cl and Na) as used in the first set of phantoms (shown in Table 3.1) was arranged. These phantoms were irradiated at proton energy 2.3 MeV and current 550 μ A for 22 s duration. All eight irradiated phantoms were counted in the 4π detector for 10 cycles each of which was for 10 s duration. The phantoms transfer time from irradiation cavity to the 4π detector was in the range from 11-12 seconds. The counting data were obtained in anticoincidence, coincidence and singles modes of detection. The gamma spectra using the anticoincidence mode data were selected and analyzed to compute the areas under the Se and the Ca peaks. Similarly, a third set of phantoms identical (content and shape wise) to 1st and 2nd sets of phantom, was also arranged and irradiated for 21 s at proton energy 2.3 MeV and current 560 μ A; and then measured in 4π system.

3.7 Phantom preparation and irradiation for investigation of a feature at 197 keV

During the analysis of the first set of phantom data a feature was noticed at energy around 197 keV (channel # 65) which appeared just to the high energy side of selenium

peak (162 keV/channel # 54). As this unknown energy peak was causing spectral interference in the vicinity of selenium peak, it was considered appropriate to explore the origin of this peak. For this purpose two phantoms, one containing 2000 μg pure selenium in 250 ml deionized water and the other having 250 ml deionized water mixed with 40 μl of nitric acid were prepared in LDPE Nalgene bottles. Both the phantoms were irradiated for 30 s at proton energy and current of 2.3 MeV and 400 μA respectively. Phantoms were counted in the 4π detection system for many cycles of 10 s duration. The counting data obtained for both the phantoms were analyzed.

In order to get a higher number of counts two bigger water phantoms containing 1034 ml of deionized water mixed with 165 μl of nitric acid in the HDPE Nalgene bottle were also prepared. The first bigger phantom was irradiated for 30 s at proton energy 2.3 and proton current 400 μA . The second bigger phantom was irradiated for 22 s at proton energy 2.3 and proton current 550 μA in the irradiation cavity. Both the irradiated phantoms were counted in the same detection system and spectra saved were analyzed to investigate the characteristics of the feature peak.

Chapter 4

RESULTS AND DISCUSSION (Part-I)

This chapter presents the results of experiments performed to test the feasibility of measuring selenium in humans using the hand tissue equivalent phantoms by employing a non-invasive and non-destructive technique known as *in vivo* neutron activation analysis (IVNAA). In the recent past at the McMaster Accelerator Lab (MAL) a number of minor and trace elements in human hand have been identified and quantified using the IVNAA method coupled with the 4π detection system. However, the present study is the first of its kind at the MAL to investigate the gamma-ray signals from an irradiated hand equivalent Se-phantom and to examine the spectrum below an energy of 511 keV.

4.1 Initial feasibility test

The initial feasibility test for identification of the selenium peak in the gamma-ray spectrum was conducted by preparing a single hand tissue equivalent Se-phantom. Table 4.1 lists the element masses, compounds used, the respective reactions, half-life of activated radionuclides and capture cross-section of the target element. In the phantom Ca was added for normalization of the Se signal to the Ca signal, Cl was added to account for the spectral interference, and Na was added due to its significant contribution to the background continuum via $^{23}\text{Na}(n,\gamma)^{24}\text{Na}$ (Chamberlain et al., 2012). These elements were added in concentrations that were consistent with those cited in ICRP publication 23 (1975) for the hand of a Reference Man.

Table 4.1: Element masses used with their respective reaction, half-life and capture cross section in the feasibility test phantom

Element	Reaction	Mass	Compound used	Half life	Cross section
Selenium ^a	$^{76}\text{Se}(n,\gamma)^{77\text{m}}\text{Se}$	750 (μg)	$^{\text{c}}\text{Se}$	17.4 s	21 b
Calcium ^b	$^{48}\text{Ca}(n,\gamma)^{49}\text{Ca}$	14.9 (g)	$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	8.7 m	1.09 b
Chlorine ^b	$^{37}\text{Cl}(n,\gamma)^{38}\text{Cl}$	1.19 (g)	NH_4Cl	37.24 m	0.433 b
Sodium ^b	$^{23}\text{Na}(n,\gamma)^{24}\text{Na}$	1.25 (g)	NaNO_3	14.997 h	0.530 b

^aZhang and Chatt (2014), ^bBhatia et al (2014), ^cSe standard for ICP

The phantom was irradiated for 30 s at proton energy 2.3 MeV and proton current 400 μA . The irradiated phantom after delay of 12 s transfer time was counted in the 4π detection system for 10 s intervals. The gamma-ray spectrum obtained based on the counting data clearly showed the presence of the selenium peak. As the centroid of selenium peak was observed at channel # 35, the gain of the system was increased and the process of irradiation of phantom and its counting was repeated. This time selenium peak was visible at channel # 54. Figure 4.1 shows the presence of the selenium peak for cycles 1-3 of the phantom containing 750 μg selenium. This initial feasibility test confirmed that the selenium peak can be identified in the phantom at the above indicated irradiation parameters by using 4π detection system for counting of γ -ray spectrum.

4.2 Analysis of set of selenium phantoms

The successful identification of the Se-peak in the gamma-ray spectrum encouraged the preparation of a set of selenium phantoms in various concentrations. Therefore eight

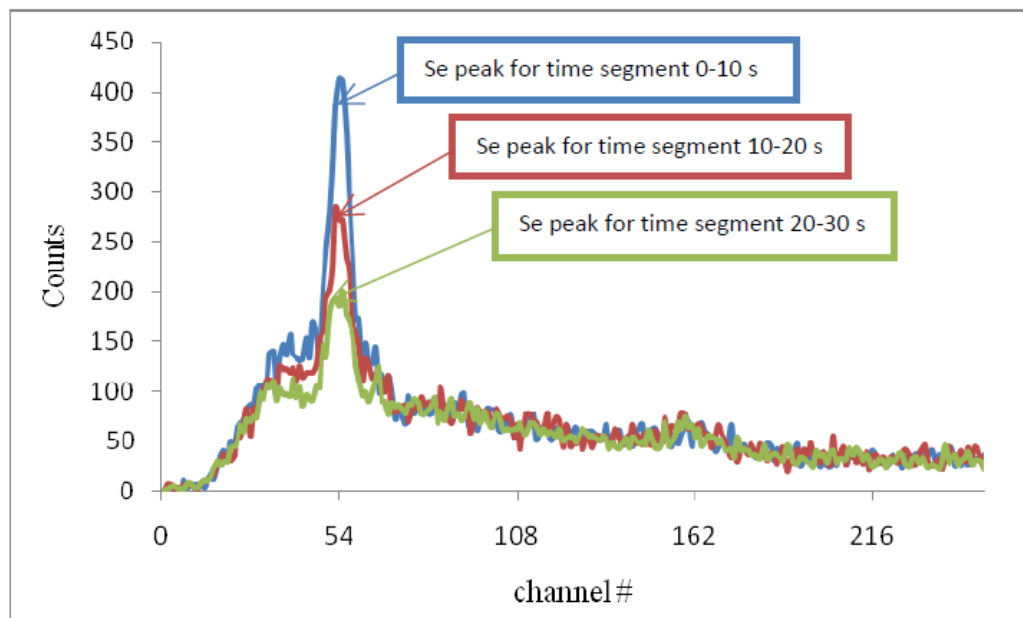


Figure 4.1: Selenium peaks in the feasibility test phantom for 1st three cycles

selenium phantoms were prepared in which selenium concentration varies from 0-750 μg , whereas concentrations of Ca, Cl and Na was kept constant in all phantoms as mentioned above in Table 4.1. The shape of the phantoms also remained the same, i.e., cylindrical (bottles).

The distribution of all the elements including selenium and calcium were made as uniform as possible throughout the entire volume of the phantom by vigorous shaking. The phantoms are not an exact match for the human hand in terms of shape, density and dimension which may introduce small differences in activation between the actual human subject and water phantoms. Similarly target quality, proton beam current and incident neutron flux variations, thickness of overlying soft tissues in case of real hand subject, and counting geometry also results in some inconsistency in the measured data.

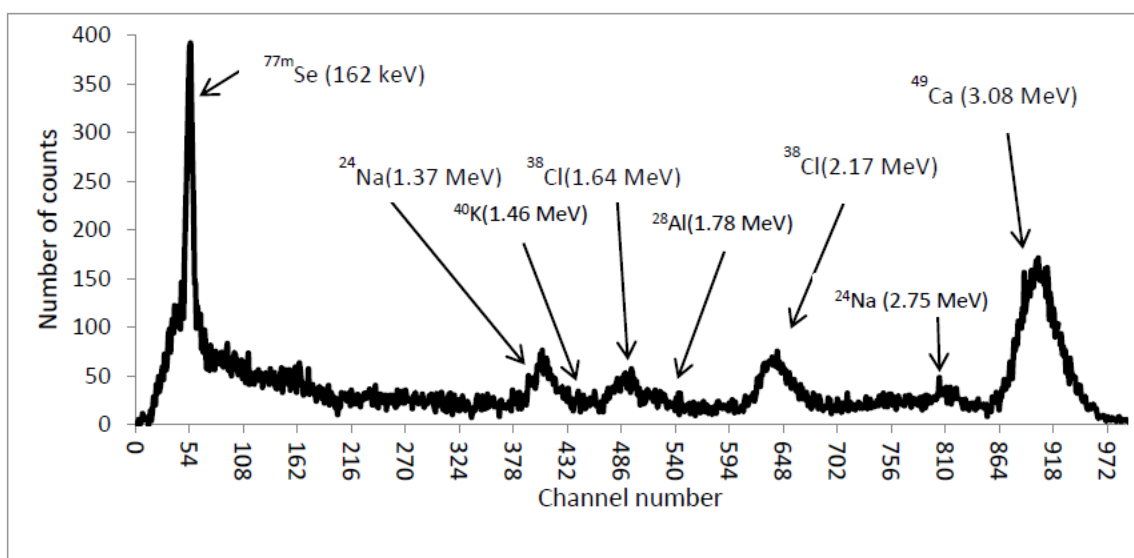
Therefore to overcome this problem the measured signals (count rate) from the elements of interest (Se in the present case) are normalized to Ca signals to make the accuracy of measurement independent of above mentioned factors (Bhatia et al., 2014) under the assumption that this normalization adequately compensates for these differences. This process of normalization is a common practice and is a useful index for comparing results from different studies (Aslam et al., 2008, Davis et al., 2008, Chamberlain et al., 2012).

Irradiations of the set of phantoms was performed at proton energy of 2.3 MeV and proton current of 400 μ A for 30 s and the detected spectra were consisted of 10 s intervals. For each phantom integrated proton current (IC), fission counts (FC) and transfer time from the end of irradiation to start of counting were noted as shown in Table 4.2. The irradiated phantoms were counted in the 4π NaI (TI) γ -ray spectrometer. The spectra were obtained in anticoincidence, coincidence and singles modes of detection. The anticoincidence mode data were used to plot gamma-ray spectra from the activated radioisotopes. One such spectrum using counting data for time segment 0-10 s from the phantom containing 750 μ g selenium is shown in Figure 4.2.

The presence of gamma-ray peaks from ^{77m}Se (162 keV), ^{24}Na (1.37 and 2.75 MeV), ^{40}K (1.46 MeV), ^{38}Cl (1.64 and 2.17 MeV), ^{28}Al (1.78 MeV) and ^{49}Ca (3.08 MeV) is evident in the spectrum. Except for peaks ^{40}K (1.46 MeV) which is from the background material and ^{28}Al (1.78 MeV) which is associated with an aluminum

Table 4.2: Integrated proton current, fission counts and transfer time for phantoms

Selenium (μg)	Integrated proton Current (μC)	Fission Counts	Transfer time (sec)
750	12370	321159	13
500	12394	316794	13
200	12307	313854	12
100	12314	312353	13
500	12370	334413	13
30	12014	325224	12
10	12230	333987	12
00	12570	344406	13

Figure 4.2: Spectrum showing gamma-ray peaks from ^{77m}Se , ^{24}Na , ^{40}K , ^{38}Cl , ^{28}Al and ^{49}Ca in 750 μg selenium phantom

impurity within the plastic of the bottle used in phantom preparation (Mostafaei et al., 2013), all other gamma-ray peaks originate from the activated isotopes of the added elements of selenium, calcium, chlorine and sodium, in the Se-phantoms.

4.3 Computation of Se and Ca counts

Selenium and calcium counts were computed by analyzing the peaks in the gamma spectrum. For the analysis, the computer software Origin version 8.6 was used to determine the peak area of the photo-peaks of interest. This user friendly program uses the Levenberg-Marquardt fitting algorithm to fit each peak in the spectrum (Chamberlain et al., 2012). Selenium and Calcium peaks were fitted using a Gaussian curve of the active data plot on a constant background according to the following equation (Fice, 2008):

$$y = y_0 + A/(w \cdot \sqrt{\pi}/2) \cdot \exp[-2(x-x_0)^2/w^2]$$

Where, y_0 = Baseline offset, x_0 = Center (mean) of the peak, A = Total area under the curve from the baseline, w = Width of the peak (two sigma ≈ 0.849 the width of the peak at half height, while half of the width, i.e., $w/2$ gives the standard deviation). While applying the Gaussian fitting function, centroid and width parameters were fixed using the data obtained in anticoincidence mode of detection.

Analysis of peaks with fixed parameters of centroid and width reduces the uncertainty in the computed areas under the peaks. For choosing the appropriate position of centroids and width to be fixed for peaks of interests for spectra from all phantoms, initially the center points and widths were determined from the higher concentrations at

which well-defined peaks were observed. This was achieved by fitting the data saved in anticoincidence and summing of singles modes. Fitting of peaks was done for fixed and variable positions of center points and widths of peaks with the best possible chi-squared values. For higher concentration phantoms, the fits were very well defined, however for lower concentration phantoms this was not the case because they had fewer counts. The quality of fitting model was assessed by observing that the net areas of the analyzed peaks followed the exponential decay law for successive time intervals.

From the fitting data obtained for the whole set of phantoms, three separate estimates of selenium and calcium contents were obtained after making corrections for transfer and decay time from first three time segments, i. e., 0-10, 10-20, and 20-30 s. For a set of eight selenium phantoms computed values of selenium and calcium counts and their respective ratio (showing normalization of Se peak area to Ca peak area) are reported in Table 4.3.

Since $^{77\text{m}}\text{Se}$ has a short half of 17.4 s, it means that more counts are detected in the first ten seconds than in the second and third ten seconds. The detection process of radiation events inside the detector obeys Poisson distribution function in which the uncertainty on detected signals N is expressed as $\sqrt{N}$. As the signal N decreases with time the relative uncertainty on the signal increases as $\sqrt{N}/N$ (or $1/\sqrt{N}$). Therefore as compared to time segment 0-10 s, the relative uncertainty in the subsequent counting intervals (i. e., 10-20, and 20-30 s) of events measurement becomes worse.

Table 4.3: Se and Ca counts and their respective ratio computed for all phantoms

Se (μg)	Ca (g)	Se/ Ca counts	(0-10 s)	(11-20 s)	(21-30 s)
00	14.9	Se counts	-81 $\pm$ 50	-202 $\pm$ 75	-302 $\pm$ 104
		Ca counts	5171 $\pm$ 130	5244 $\pm$ 118	5366 $\pm$ 128
		Se/Ca counts ratio	-0.0156642 $\pm$ 0.0096772	-0.0385202 $\pm$ 0.0143282	-0.0562802 $\pm$ 0.0194277
		Se/Ca ($\mu\text{g/g}$) ratio	0	0	0
10	14.9	Se counts	-92 $\pm$ 56	132 $\pm$ 82	64 $\pm$ 129
		Ca counts	5544 $\pm$ 117	5618 $\pm$ 118	5507 $\pm$ 135
		Se/Ca counts ratio	-0.0165945 $\pm$ 0.0061558	0.0234959 $\pm$ 0.0146042	0.0116215 $\pm$ 0.0116215
		Se/Ca ($\mu\text{g/g}$) ratio	0.6711409	0.6711409	0.6711409
30	14.9	Se counts	121 $\pm$ 58	62 $\pm$ 96	79 $\pm$ 157
		Ca counts	5380 $\pm$ 112	5597 $\pm$ 120	5252 $\pm$ 131
		Se/Ca counts ratio	0.0224907 $\pm$ 0.0107908	0.0110773 $\pm$ 0.0171535	0.0150418 $\pm$ 0.0298955
		Se/Ca ($\mu\text{g/g}$) ratio	2.0134228	2.0134228	2.0134228
50	14.9	Se counts	190 $\pm$ 64	95 $\pm$ 80	156 $\pm$ 123
		Ca counts	5539 $\pm$ 130	5708 $\pm$ 138	5622 $\pm$ 110
		Se/Ca counts ratio	0.0343022 $\pm$ 0.0115824	0.0166433 $\pm$ 0.0140211	0.0277481 $\pm$ 0.0218850
		Se/Ca ($\mu\text{g/g}$) ratio	3.3557047	3.3557047	3.3557047
100	14.9	Se counts	421 $\pm$ 74	377 $\pm$ 95	309 $\pm$ 129
		Ca counts	5863 $\pm$ 130	5783 $\pm$ 121	5687 $\pm$ 123
		Se/Ca counts ratio	0.0718062 $\pm$ 0.0127215	0.0651910 $\pm$ 0.0164839	0.0543344 $\pm$ 0.0227137
		Se/Ca ($\mu\text{g/g}$) ratio	6.7114094	6.7114094	6.7114094

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200	14.9	Se counts	819±81	682±106	626±157
		Ca counts	6066±132	6174±130	6326±139
		Se/Ca counts ratio	0.1350148±0.0136725	0.1104632±0.0173256	0.0989566±0.0249132
		Se/Ca (µg/g) ratio	13.4228190	13.4228190	13.4228190
500	14.9	Se counts	2400±153	2374±185	2133±220
		Ca counts	6743±132	6849±133	6844±142
		Se/Ca counts ratio	0.3559246±0.0237358	0.3466199±0.0278372	0.3116598±0.0327888
		Se/Ca (µg/g) ratio	33.5570470	33.5570470	33.5570470
750	14.9	Se counts	3818±242	3364±210	3238±194
		Ca counts	6611±130	6743±133	6769±125
		Se/Ca counts ratio	0.5775223±0.0383268	0.4988877±0.0326609	0.4783572±0.0299905
		Se/Ca (µg/g) ratio	50.335570	50.335570	50.335570

4.4 Calibration lines and minimum detection limit

After applying the required corrections for the transfer and counting time, the count rate of selenium was normalized to calcium count rate and linear calibration lines were drawn by plotting the Se/Ca concentration ratio against Se/Ca peak area (counts) ratio for all eight phantoms. Se/Ca counts ratio was taken to normalize the selenium signals to the calcium signals. During peak fittings the chi-squared, which looks at the goodness of the fits to the phantoms data, for time segment 0-10 s was found in the range from 0.98- 4.6 with an average value of 1.7 ± 1.2 . The highest value of chi-squared, i.e., 4.6 was

observed for the phantom containing highest concentration of 750 μg of selenium. At this concentration, # of selenium counts were also highest among the whole set of phantoms. The higher chi-squared values at higher concentration of selenium indicates that the fit becomes worse with higher count rates. However, with reducing concentration of selenium in phantoms, # of counts was decreasing and chi-squared was improving approaching to 1. The fit deviations in phantoms of various concentration of selenium may be attributed to some random variation in the chi-squared value per channel. The second possibility for large fit deviations lies in the assumption of a Gaussian fit or the lack of data points available for the fit. As the peak area increases, the observed shape deviates inevitably from a true Gaussian, which may explain the observed increasing trend in the chi-squared values with increasing concentration of selenium in phantoms.

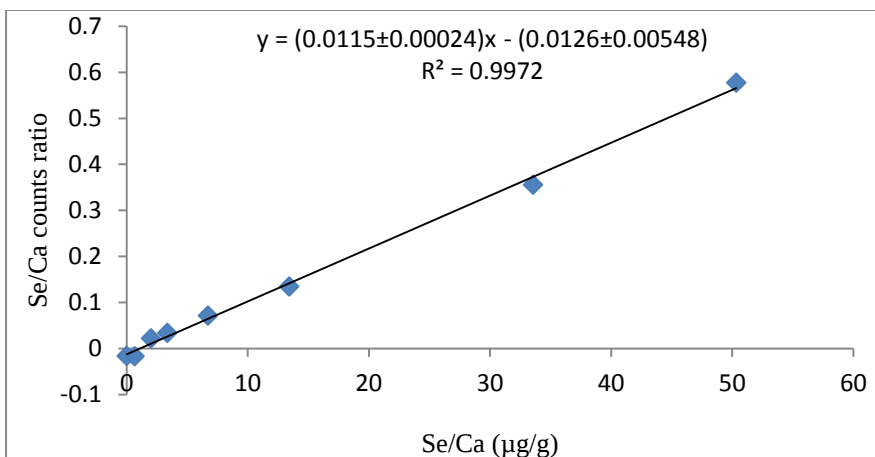
Another more likely possibility is that the fit of a Gaussian on a linear background is not accounting for all the features in the spectrum. It is quite possible that there are Compton edges resulting from concentrations of other elements of calcium, chlorine and sodium (which were added in all phantoms in uniform quantity) that are affecting the fit. It is also suspected that the # of channels selected to collect the spectrum was not appropriate to obtain good statistics.

The exceptionally poor fit could also be due to some other additional features in the spectrum that are not being accounted for in the fit. One such feature peak was found in the vicinity of the selenium peak. The selenium peak was found at channel # 54 (162 keV), whereas this additional feature peak was found at channel # 65 (197 keV). The rising edge of the feature peak probably causing spectral interference with the ending tail

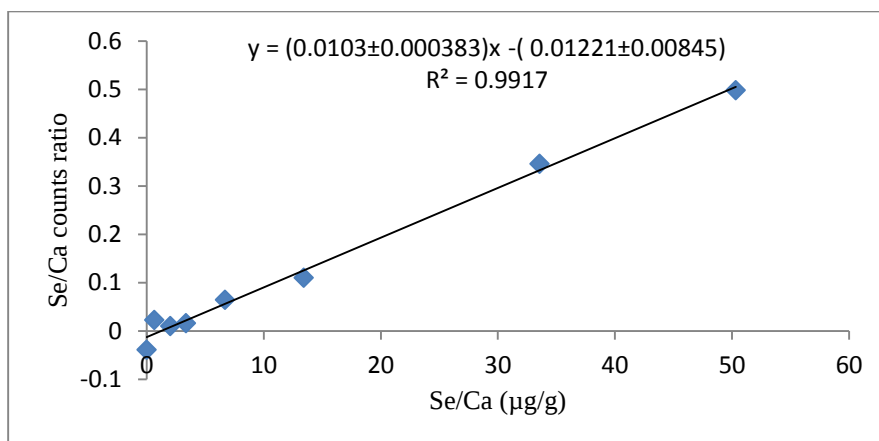
of selenium peak. This issue has been thoroughly investigated and is described in chapter five. Anyhow, as the average value of chi-squared came out to be 1.7, it was assumed that the fitting model used reasonably fits the data.

The reason for acceptance of fit results was that the calibration lines obtained for various time intervals resulted in a good linear fit and any further improvement in fitting of data should only slightly improve the detection limit. Finally the calibration lines were drawn between Se/Ca concentration and Se/Ca counts per channel. Figure 4.3 (a-c) depicts the calibration lines derived from three counting intervals, 0-10, 10-20 and 20-30 s. The counting intervals are comprised of points from all eight phantoms counted. From each phantom measurement the corresponding data points for a specific concentration of selenium and calcium lie within 2σ or 95 % confidence level. It is evident from the calibration curves that the measured activities (in terms of # of counts) of each of two elements (i. e., selenium and calcium) are linearly related to the concentrations of the respective element present in the phantoms.

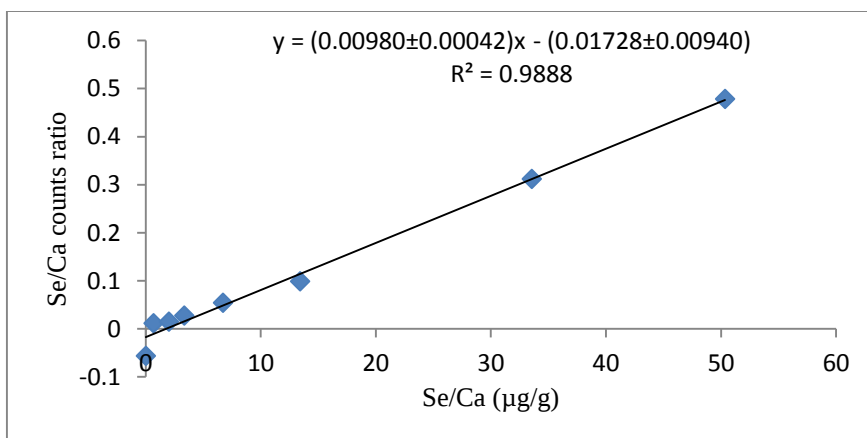
The slopes of regression and the intercepts were found to be 0.0115 ± 0.00024 ($r^2 = 0.997$) and -0.0126 ± 0.0054 for the 1st counting interval 0-10 s, 0.0103 ± 0.00038 ($r^2 = 0.991$) and -0.0122 ± 0.00845 for the 2nd counting interval 10-20 s; and 0.0098 ± 0.00042 ($r^2 = 0.988$) and -0.0172 ± 0.0094 for the 3rd counting interval 20-30 s.



(a)



(b)



(c)

Figure 4.3: Calibration lines for time segments (a) 0-10 s, (b) 10-20 s and (c) 20-30s

The slope of the calibration lines also describes the net Se/Ca count rate ratio and can be used to determine the Se/Ca mass ratio in phantoms. The slopes also predict Se/Ca sensitivity in terms of counts ratio per unit mass ratio of element, which in our case are 0.0115 ± 0.00024 , 0.0103 ± 0.00038 and 0.0098 ± 0.00042 Se-counts/Ca-counts per $\mu\text{g-Se/g-Ca}$ for time intervals, 0-10, 10-20 and 20-30 s respectively. Among three time intervals, the counts ratio sensitivity for time interval, 0-10 s was found highest, whereas the same was found lowest for the time interval 20-30 s. This means that sensitivity of counts ratio of two elements (Se/Ca) decreases for the subsequent intervals. From the calibration lines, the MDL (minimum detection limit) can be obtained. The MDL refers to the minimum amount of selenium in the human hand equivalent phantom which can be distinguishable from zero selenium in the phantom with 95% confidence level.

The MDL was estimated as twice as the uncertainty (i. e. 2σ) of zero selenium phantom over the slope of the calibration line using the below given relationship (Atiya, 2012):

$$MDL = 2\sigma_{x_{exp}}$$

Where

$$\sigma_{x_{exp}}^2 = \left(\frac{\sigma_{y_{exp}}}{m}\right)^2 + \left(\frac{\sigma_b}{m}\right)^2 + \frac{\sigma_m^2(b - y_{exp})^2}{m^4} + 2\left(\frac{y_{exp} - b}{m^3}\right)\left(-\frac{\sigma_y^2 \sum_{i=1}^N x_i}{D}\right)$$

The above equation not only accounts for the standard uncertainty in the area of the zero concentration calibration standard (or phantom) but also non zero uncertainty in the intercept “b” of the calibration line. In this equation, m = slope, σ_m = error in slope, b = y-intercept, σ_b = error in y-intercept, y_{exp} = normalized area of zero concentration phantom &

σ_{yexp} is its error, $\sigma^2_{b,m}$ = covariance of b and m, N= number of data points on fitting,

$$\sigma_y^2 = \frac{\sum_{i=1}^N [y_i - (mx_i + b)]^2}{N-2} \quad \text{and} \quad D = N \sum_{i=1}^N x_i^2 - (\sum_{i=1}^N x_i)^2.$$

The computed values of minimum detection limits (MDL) for three time segments are shown in Table 4.4. As discussed earlier that the inherent relative uncertainty gets worse in the late intervals after the first counting interval, i. e., intervals 10-20 s and 20-30 s in the instant case, therefore in order to take this effect into account in a final combined estimate of Se/Ca content, the overall mean MDL was calculated as inverse variance weighted mean (IVWM) MDL from three MDLs rather than just taking simple mean value of the results to determine the final detection limit using the following Equation (Chamberlain et al., 2012):

$$\text{MDL (IVWM)} = \{1/[1/(\text{MDL}_{0-10 \text{ s}})^2] + [1/(\text{MDL}_{10-20 \text{ s}})^2] + [1/(\text{MDL}_{20-30 \text{ s}})^2]\}^{1/2}$$

Table 4.4 shows the inverse variance weighted mean MDL calculated from three time segments, 0-10 s, 10-20 s and 20-30 s. Data reported in Table 4.4 illustrate that the majority of selenium signal was obtained in the first time segment of detection. However, as time lapses uncertainty increases and signal to noise ratio becomes poorer in the latter two detection time segments and relatively adding more noise and less signal. The IVWM value of MDL from three time segments of detection was calculated to be 1.58 $\mu\text{g Se/g Ca}$, which is lower than the MDLs obtained from each of three separate time intervals. The thumb rule in MDL measurement is that as the MDL decreases, the detection system improves. Therefore for three time segments procedure, the IVWM method provides the better final detection limit.

Table 4.4: MDLs for three time segments and inverse variance weighted (IVW) mean MDL

<i>Time segment (s)</i>	<i>MDL ($\mu\text{g Se/g Ca}$)</i>
0-10	1.96
10-20	3.28
20-30	4.48
<i>IVW mean MDL</i>	<i>1.575$\approx$1.58</i>

The MDL of selenium concentration has been reported in the present study after normalizing the selenium to the calcium concentration in the hand tissue equivalent phantoms. The MDL value thus obtained is difficult to compare directly with the reported data in literature whereby selenium contents measured in different biological organs are traditionally expressed in terms of $\mu\text{g (Se)}$ per g of organ mass collected as sample specimen for analysis. Also no other study could be found where by Se concentration was measured in hand phantoms or a real subject in which case the results were expressed in terms of Se/Ca concentration ratio measured using INAA. However if the results are expressed in terms of Se concentration (μg) per total mass of phantom (g), i. e., $\mu\text{g/g}$, then the results of the present study can be conveniently compared with the reported data. Applying this analogy the IVWM value of MDL shown in Table 4.4 is found equivalent to 94.2 (ng/g). A comparison of the present study results with the reported data can be seen in section 4.9.

4.5 Dosimetry

In measuring the elemental concentrations in real subjects using the in vivo neutron activation analysis technique, the radiation dose to be given to the subject has always been a limiting factor. This limiting factor ensures use of radiation dose within the recommended protocol to avoid health risks associated with over exposures. Therefore it is important to estimate the radiation dose received by the phantom within the irradiation cavity during irradiation for 30 s interval from neutron at proton energy of 2.3 MeV and current of 400 μA .

Using a tissue equivalent proportional counter (TEPC), Darvish Molla (2015) has recently measured the dose equivalent of 0.9363 mSv/min- μA from neutrons and 0.0230 mSv/min- μA from gammas at the center of the irradiation cavity at proton energy of 2.3 MeV. Assuming that phantoms in the present study were positioned at the center of cavity during irradiation, the total dose equivalent is calculated to be 191.9 mSv. To bring down the equivalent dose further we need to optimize the irradiation conditions.

4.6 Optimization of detection limit

For the purpose of optimization of detection limits as reported in Table 4.4, a set of eight phantoms was irradiated for different values of proton current and irradiation time while keeping proton energy same as done in case of irradiation of first set of eight phantoms. This time irradiation parameters were set at proton energy: 2.3 MeV, proton current: 550 μA and irradiation time: 22 s. Detail of selenium phantoms, integrated current, fission counts and transfer time are shown in Table 4.5. After peaks fitting and making corrections for transfer and counting time for all eight phantoms, selenium to

calcium count ratio and selenium to calcium concentration ratio were determined and presented in Table 4.6.

Table 4.5: Integrated charge, fission counts and transfer time for 2nd set of phantoms at E_p : 2.3 MeV, I_p : 550 μ A and T_i : 22 s

Selenium (μ g)	Integrated proton current (μ C)	Fission counts	Transfer time (sec)
750	12933	176631	12
500	12817	173823	12
200	12531	166423	11
100	12373	166012	11
500	12436	166595	11
30	12620	171397	12
10	12889	171905	12
00	12720	169155	12

Table 4.6: Se and Ca counts and their respective ratio for 2nd set of phantoms

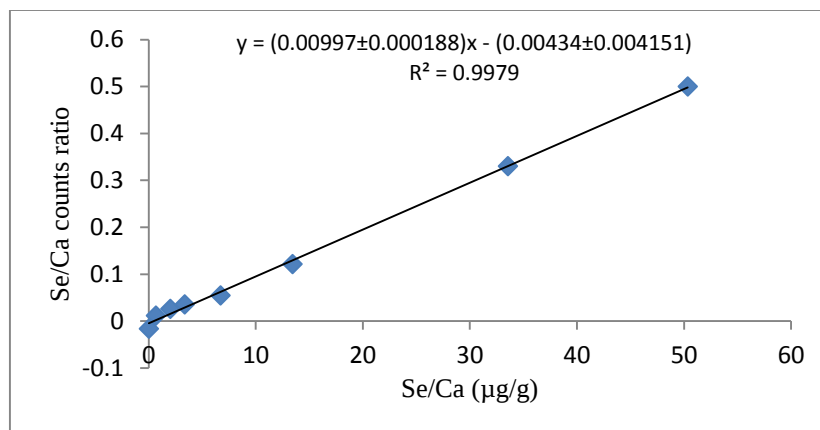
<i>Se</i> (μg)	<i>Ca</i> (<i>g</i>)	Counts	(0-10 s)	(11-20 s)	(21-30 s)
00	14.9	Se counts	-58 $\pm$ 42	-17 $\pm$ 86	18 $\pm$ 104
		Ca counts	3503 $\pm$ 166	3470 $\pm$ 152	3428 $\pm$ 109
		Se/Ca counts ratio	-0.0165572 $\pm$ 0.0120153	-0.0048991 $\pm$ 0.0247847	0.0052508 $\pm$ 0.0303388
		Se/Ca ($\mu\text{g/g}$) ratio	0	0	0
10	14.9	Se counts	40 $\pm$ 44	-74 $\pm$ 62	-68 $\pm$ 82
		Ca counts	3444 $\pm$ 150	3625 $\pm$ 156	3406 $\pm$ 147
		Se/Ca counts ratio	0.0116144 $\pm$ 0.0127858	-0.0204137 $\pm$ 0.0171259	-0.0199647 $\pm$ 0.0240905
		Se/Ca ($\mu\text{g/g}$) ratio	0.6711409	0.6711409	0.6711409
30	14.9	Se counts	90 $\pm$ 50	51 $\pm$ 69	-110 $\pm$ 93
		Ca counts	3464 $\pm$ 150	3524 $\pm$ 151	3505 $\pm$ 140
		Se/Ca counts ratio	0.0259815 $\pm$ 0.0144779	0.0144721 $\pm$ 0.0195898	-0.0313837 $\pm$ 0.0265631
		Se/Ca ($\mu\text{g/g}$) ratio	2.0134228	2.0134228	2.0134228
50	14.9	Se counts	122 $\pm$ 64	5 $\pm$ 65	258 $\pm$ 93
		Ca counts	3445 $\pm$ 141	3436 $\pm$ 163	3381 $\pm$ 127
		Se/Ca counts ratio	0.0354136 $\pm$ 0.0186341	0.0014551 $\pm$ 0.0189174	0.0763087 $\pm$ 0.0276555
		Se/Ca ($\mu\text{g/g}$) ratio	3.3557047	3.3557047	3.3557047
100	14.9	Se counts	184 $\pm$ 53	85 $\pm$ 78	234 $\pm$ 100
		Ca counts	3357 $\pm$ 148	3731 $\pm$ 152	3584 $\pm$ 155
		Se/Ca counts ratio	0.0548108 $\pm$ 0.0159717	0.0227820 $\pm$ 0.0209265	0.0652901 $\pm$ 0.0280442
		Se/Ca ($\mu\text{g/g}$) ratio	6.7114094	6.7114094	6.7114094

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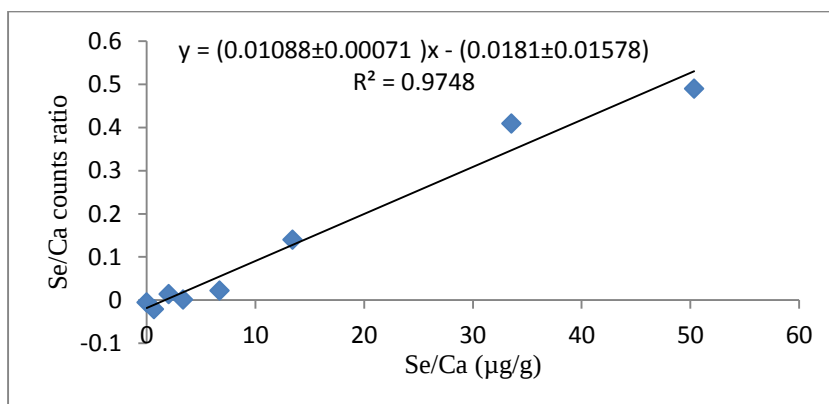
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200	14.9	Se counts	431±76	502±110	537±122
		Ca counts	3538±146	3577±154	3460±140
		Se/Ca counts ratio	0.1218202±0.0220614	0.1403410±0.0313399	0.1552023±0.0358149
		Se/Ca (µg/g) ratio	13.4228190	13.4228190	13.4228190
500	14.9	Se counts	1193±110	1518±132	1002±165
		Ca counts	3614±150	3706±150	3666±159
		Se/Ca counts ratio	0.3301051±0.0333787	0.4096060±0.0392872	0.2733224±0.0465431
		Se/Ca (µg/g) ratio	33.5570470	33.5570470	33.5570470
750	14.9	Se counts	2026±187	1981±156	1745±168
		Ca counts	4050±149	4041±186	4146±154
		Se/Ca counts ratio	0.5002469±0.0497055	0.4902251±0.0447150	0.4208876±0.0434322
		Se/Ca (µg/g) ratio	50.335570	50.335570	50.335570

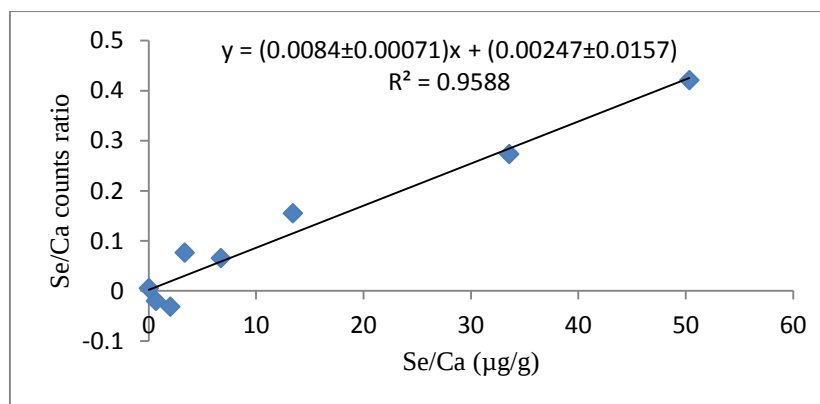
The calibration lines derived from three counting intervals, i. e., 0-10, 10-20 and 20-30 s are shown in Figure 4.4. The minimum detection limit (MDL) calculated for each of three time segments and inverse variance weighted mean MDL from three MDLs comes out to be 2.22 µg Se/g Ca, which is equivalent to 0.13 µg (Se)/g of phantom mass (Table 4.7). It is seen from Table 4.7 that MDL calculated for each of individual time segment and inverse variance weighted mean value of MDL from three time segments is higher than what has been reported in Table 4.4 in case of 30 s irradiation at proton current of 400 µA. This means that with changed irradiation parameters the detection



(a)



(b)



(c)

Figure 4.4: Calibration lines for time segment (a) 0-10 s, (b) 10-20 s and (c) 20-30 s from 2nd set of phantoms

Table 4.7: Inverse variance weighted mean MDL from 2nd set of phantoms

<i>Time segment (s)</i>	<i>MDL ($\mu\text{g Se/g Ca}$)</i>
0-10	2.55
10-20	5.52
20-30	7.90
<i>Inv. Variance Weighted Mean</i>	<i>2.221$\approx$2.22</i>

limit could not be improved. The reason for this was investigated by reviewing the irradiation parameters for two sets of phantom and number of fission counts reported in Tables 4.2 and 4.5. It was found that the # of fission counts counted by the neutron counter (installed inside the irradiation cavity) for the 2nd set of phantom irradiated at $E_p=2.3$ MeV and $I_p= 550$ μA for 22 s, was smaller than the # of counts recorded during the irradiation of 1st set of phantoms at $E_p=2.3$ MeV and $I_p= 400$ μA for 30 s. A comparison of two sets of irradiation parameters regarding # of fission counts (FC) and integrated proton current (IC) for all phantoms is shown in Table 4.8.

The average values of IC and FC are calculated to be 12321 ± 158 μC and 325274 ± 11412 counts for 1st set of phantoms, whereas for 2nd set of phantoms IC and FC values are in the order of 12665 ± 209 μC and 170243 ± 3867 counts respectively. The difference between average values of IC from two sets of phantoms is not significant. Moreover the inter-phantom variation of IC for the 1st set is 1.3 % against 1.7 % for the 2nd set of phantoms, which is also not uncommon. However a significant reduction in # of

Table 4.8: IC & FC values for 1st and 2nd set of phantoms

Se (μg)	(2.3 MeV, 400 μA , 30 sec)		(2.3 MeV, 550 μA , 22 sec)	
	Integrated proton current (μC)	Fission counts (FC)	Integrated proton current (μC)	Fission counts (FC)
750	12370	321159	12933	176631
500	12394	316794	12817	173823
200	12307	313854	12531	166423
100	12314	312353	12373	166012
50	12370	334413	12436	166595
30	12014	325224	12620	171397
10	12230	333987	12889	171905
00	12570	344406	12720	169155
<i>Av$\pm$SD</i>	<i>12321$\pm$158 μC</i>	<i>325273.8$\pm$11412.3</i>	<i>12664.9$\pm$209 μC</i>	<i>170242.6$\pm$3867.4</i>
	<i>Average fission counts/μC= 27.1</i>		<i>Average fission counts/μC= 14.1</i>	
	<i>Average fission counts/μA= 26.4</i>		<i>Average fission counts/μA= 13.4</i>	

fission counts is observed for 2nd set of phantoms which has affected the average fission counts per unit charge and per unit current. Calculated values of fission counts per unit charge and fission counts per unit current come out to be 27.1 FC/ μC and 26.4 FC/ μA for the 1st set of phantom and; 14.1 FC/ μC and 13.4 FC/ μA for the 2nd set of phantoms respectively. This implies that there was a significant reduction in average number of fission counts per unit charge and fission counts per unit current for the 2nd set of

phantoms during irradiation. This could be attributed to the malfunctioning of the irradiation system inside the irradiation cavity at the time of irradiation of 2nd set of phantoms.

4.7 Irradiation of 3rd set of phantoms

For further pursuing the optimization of irradiation parameters, a 3rd set of phantoms identical to 1st and 2nd sets of phantom (in terms of elemental contents, shape and material of bottles) was irradiated at proton energy 2.3 MeV and proton current 560 μA for 21 s. This time average value of FC counts per μA current for all eight phantoms was observed to be 19.1 FC counts/ μA , against values of 13.4 and 26.4 FC counts/ μA observed during the irradiation of 2nd and 1st sets of phantom respectively. The irradiated phantoms were counted one by one after each successive irradiation in the counting cavity of the 4π detector for 10 s intervals. After peaks fitting and making corrections for transfer and counting time for all phantoms, selenium to calcium count ratio and selenium to calcium concentration ratio were computed and are presented in Table 4.9.

The calibration lines derived from three counting intervals, i. e., 0-10, 10-20 and 20-30 s are shown in Figure 4.5. The minimum detection limit (MDL) calculated for each of three time segments and inverse variance weighted mean (IVWM) MDL from three MDLs are shown in Table 4.10. The obtained IVWM value of MDL of 1.36 $\mu\text{g Se/g Ca}$ is equivalent to 81 ng (Se)/g (phantom mass). It is evident from Table 4.10 that MDL calculated for each of individual time segment and inverse variance weighted mean value

Table 4.9: Se and Ca counts and their respective ratio for 3rd set of phantoms

Se (μg)	Ca (g)	Counts	(0-10 s)	(10-20 s)	(20-30 s)
00	14.9	Se counts	-58 $\pm$ 55	-195 $\pm$ 80	-307 $\pm$ 109
		Ca counts	6761 $\pm$ 139	6604 $\pm$ 131	6778 $\pm$ 142
		Se/Ca counts ratio	0.0085786 $\pm$ 0.0081368	0.0295275 $\pm$ 0.0121280	0.0452935 $\pm$ 0.0161094
		Se/Ca (μ g/g) ratio	0	0	0
10	14.9	Se counts	55 $\pm$ 58	24 $\pm$ 78	-59 $\pm$ 126
		Ca counts	6241 $\pm$ 126	6294 $\pm$ 113	6029 $\pm$ 131
		Se/Ca counts ratio	0.0088126 $\pm$ 0.0092950	0.0038131 $\pm$ 0.0112392	0.0097860 $\pm$ 0.0209000
		Se/Ca (μ g/g) ratio	0.6711409	0.6711409	0.6711409
30	14.9	Se counts	70 $\pm$ 76	108 $\pm$ 72	38 $\pm$ 141
		Ca counts	6808 $\pm$ 155	6790 $\pm$ 141	6872 $\pm$ 154
		Se/Ca counts ratio	0.0102820 $\pm$ 0.0112706	0.0159057 $\pm$ 0.0106089	0.0055296 $\pm$ 0.0205183
		Se/Ca (μ g/g) ratio	2.0134228	2.0134228	2.0134228
50	14.9	Se counts	308 $\pm$ 71	184 $\pm$ 85	238 $\pm$ 112
		Ca counts	6711 $\pm$ 147	6746 $\pm$ 168	6876 $\pm$ 143
		Se/Ca counts ratio	0.0458947 $\pm$ 0.0106273	0.0272754 $\pm$ 0.0126183	0.0346131 $\pm$ 0.0163044
		Se/Ca (μ g/g) ratio	3.3557047	3.3557047	3.3557047
100	14.9	Se counts	448 $\pm$ 64	413 $\pm$ 85	364 $\pm$ 144
		Ca counts	6359 $\pm$ 150	6391 $\pm$ 147	6606 $\pm$ 126
		Se/Ca counts ratio	0.0704513 $\pm$ 0.0102015	0.0642212 $\pm$ 0.0133827	0.0551014 $\pm$ 0.0218236
		Se/Ca (μ g/g) ratio	6.7114094	6.7114094	6.7114094

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200	14.9	Se counts	834±68	778±90	526±155
		Ca counts	6373±149	6420±140	6288±144
		Se/Ca counts ratio	0.1308645±0.011000	0.1211838±0.0142655	0.0836513±0.0247244
		Se/Ca (µg/g) ratio	13.4228190	13.4228190	13.4228190
500	14.9	Se counts	2279±132	2322±168	2282±200
		Ca counts	6486±159	6689±153	6533±171
		Se/Ca counts ratio	0.3513721±0.0220993	0.3471370±0.0319499	0.3493035±0.0319499
		Se/Ca (µg/g) ratio	33.5570470	33.5570470	33.5570470
750	14.9	Se counts	3842±127	3605±246	3539±282
		Ca counts	6650±147	6652±149	6519±166
		Se/Ca counts ratio	0.5777443±0.0229744	0.5419422±0.0389227	0.5428746±0.0454132
		Se/Ca (µg/g) ratio	50.335570	50.335570	50.335570

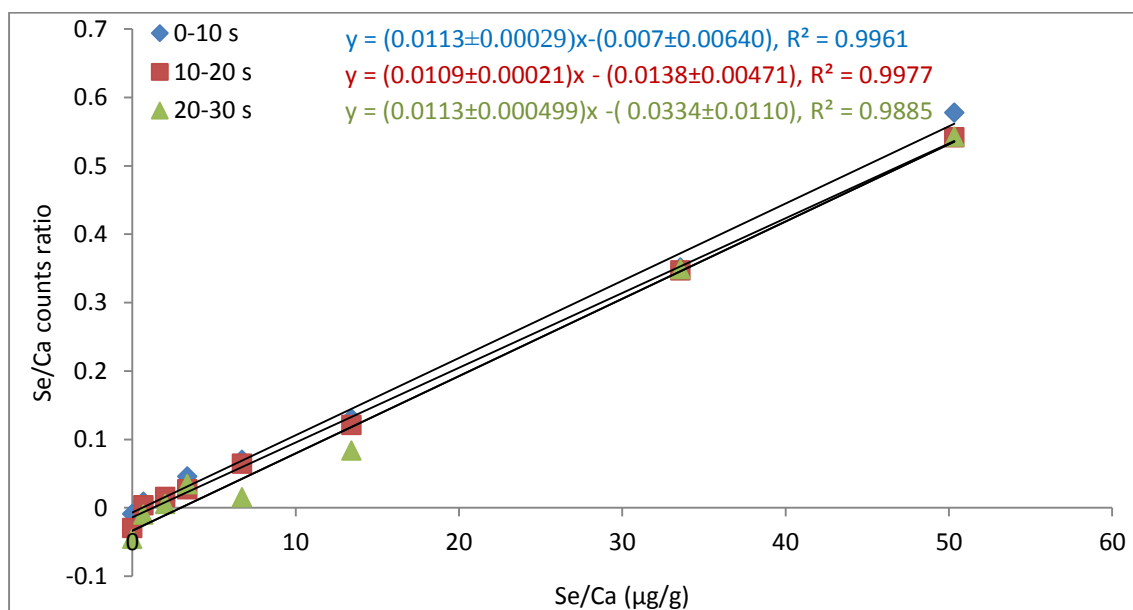


Figure 4.5: Calibration lines for time segments: 0-10, 10-20 and 20-30 s from 3rd set of phantoms

Table 4.10: Inverse variance weighted mean MDL from 3rd set of phantom

<i>Time segment (s)</i>	<i>MDL ($\mu\text{g Se/g Ca}$)</i>
0-10	1.85
10-20	2.40
20-30	3.60
<i>Inv. Variance Weighted Mean</i>	<i>1.357$\approx$1.36</i>

of MDL from three time segments is lower than the values reported in Table 4.4 (for 30 s irradiation at proton current of 400 μA) and Table 4.7 (for 22 s irradiation at proton current of 550 μA). The IVWM MDL of 1.36 $\mu\text{g Se/g Ca}$ shown in Table 4.10 is less by 13.9 % (from 1.58 $\mu\text{g Se/g Ca}$) and 38.7 % (from 2.22 $\mu\text{g Se/g Ca}$) from IVWM MDL values obtained for 1st and 2nd sets of phantoms respectively. Therefore among all three sets of irradiation parameters, the optimized irradiation parameters are obtained at proton energy 2.3 MeV, proton current 560 μA and irradiation time of 21 s.

4.8 Projected estimation of MDL for late counting cycles

In our experiments all three sets of phantom were irradiated at fixed proton energy of 2.3 MeV, whereas proton current and irradiation time varied, i. e., 400 μA for 30 s irradiation, 550 μA for 22 s irradiation and 560 μA for 21 s irradiation. However the counting duration for all phantoms in the irradiation cavity remained same, i. e., 10 cycles each of which was for 10 s duration. At fixed proton energy, radiation dose from neutrons to the target phantom (or subject) depends on proton current. Therefore one would expect that dose received by the phantom would be proportional to proton current.

This also implies that dose is proportional to counts indicated by fission chamber (FC) which is installed at the end of the irradiation cavity. In other words, more # of FC counts is an indication of higher dose received by the phantom and vice versa. As inside the irradiation cavity a phantom is exposed to beam of neutrons, the total activation of one particular element or all active-able elements is also expected to be proportional to the FC counts. This phenomenon can be confirmed by taking into account the fitted areas of Ca-peaks computed for three sets of phantom irradiated under different irradiation parameters. For instance considering the phantom with highest concentration of selenium (i.e., 750 μg) in all three sets of phantom measured for equal counting intervals, i. e., 0-10, 10-20 and 20-30 s, it is observed that average Ca counts were in the order of 6708 ± 85 , 4078 ± 58 and 6607 ± 76 for 1st, 2nd and 3rd set of phantom.

A comparison of FC counts and an integrated proton current (μA) for all three sets of phantom observed during the course of experiments is shown in Table 4.11. Now looking at the ratio of average # of FC counts and the integrated current, i. e., FC/ μA values (Table 4.11), it is evident that average value of FC/ μA increased as FC counts increased. This pattern looked true even for Ca-counts, whose average value also increased as the ratio of FC/ μA value went up. This suggests that FC counts are proportional to neutron flux and are a reliable indicator of dose from neutrons streaming out from the lithium target on which protons were incident. From this analogy it is inferred that # of decays (of activated isotopes) are proportional to # of counts (measured

Table 4.11: Integrated proton current (IC) and fission chamber (FC) counts values for three sets of phantom

Phantom Se (μg)	Irradiation parameters: E _p = 2.3 MeV, I _p =400 μA, T _i =30 s		Irradiation parameters: E _p = 2.3 MeV, I _p =550 μA, T _i =22 s		Irradiation parameters: E _p = 2.3 MeV, I _p =560 μA, T _i =21 s	
	IC (μC)	FC counts	IC (μC)	FC counts	IC (μC)	FC counts
750	12370	321159	12933	176631	12431	236913
500	12394	316794	12817	173823	11987	228640
200	12307	313854	12531	166423	11864	224337
100	12314	312353	12373	166012	11892	227411
50	12370	334413	12436	166595	11976	227639
30	12014	325224	12620	171397	11935	228491
10	12230	333987	12889	171905	12075	235119
00	12570	344406	12720	169155	12076	233899
Av± SD	12321±158	325273.8±11412.3	12664.9±209	170242.6±3867.4	12029.5±179	230306.1±4423
FC/IC=26.4 FC/μA		FC/IC=13.4 FC/μA		FC/IC=19.1 FC/μA		

from fitted data), which in turn are proportional to dose received on phantom at time of irradiation. The above analysis concludes that two factors are mainly responsible for determining detection limit of an activated element of interest, i. e., irradiation time and dose to the phantom. Therefore for a given dose the detection limit would improve with shorter time of irradiation. Since the radioactive decay is a random phenomenon which is

represented by the Poisson distribution, so keeping in view the analysis given in the preceding paragraphs, it appears that the detection limit (DL) varies as the square root of number of counts detected from the activated sample.

Now considering the results (Se and Ca counts detected) from all three sets of phantoms in which irradiation parameters (proton current and irradiation time) were different, the detection limit of selenium in phantom can be predicted approximately. For instance if we fit the counting data obtained from first set of phantoms for time segments, i. e., 0-10, 10-20 and 20-30 s, we see that the detection time and detection limit are correlated. Thus we reach to the result that the detection limit varies as the square root of $e^{t_c/T_{1/2}}$. Where t_c and $T_{1/2}$ represents counting time (s) and half-life of element of interest. The generalization of this relationship between the counting time and the detection limit assumes that all the detected counts (signals) from activated selenium for various time segments obey the above relation for estimation of detection limit. Applying the above said relationship for detection limit, the inverse variance weighted mean value of MDL for 0-10, 10-20 and 20-30 s time segments is calculated to be 1.49 $\mu\text{g}(\text{Se})/\text{g}(\text{Ca})$, which is close (94.3 %) to the experimentally computed MDL of 1.58 $\mu\text{g}(\text{Se})/\text{g}(\text{Ca})$ reported in Table 4.4. Likewise, for the remaining seven time segments of detection, i. e., from 30-40, up to 90-100 s, the IVWM value of MDL can be projected as 4.47 $\mu\text{g}(\text{Se})/\text{g}(\text{Ca})$.

A further comparison of ratio of FC counts and integrated current (IC) (Table 4.11) with Ca counts, i.e., (FC counts/Ca counts) and (IC value/Ca counts) obtained in all three sets of irradiation, it appears from 3rd set (i.e., 560 μA , 21 s) that the fission chamber was under reading and the integrated current was the better indicator of

activation (and hence dose), which emphasises the value of taking the ratio of Se to Ca concentration in presenting the results.

4.9 Comparison of MDL with previous studies

The optimal MDL obtained in the present study (Table 4.10), along with a comparison with previously reported concentration of selenium measured in various biological samples (human and animal organs) using different analytical techniques, are presented in Table 4.12. As shown in this table, the optimal observed MDL value of 81 ng/g for selenium in hand tissue equivalent phantoms is found much below the previous measurements made in different organs of humans and animals. As shown in Table 4.12, Nicolaou et al. (1982) and Tsakovski et al. (2010) used NAA and reported Se concentration below 1 μg (Se)/g of organ mass.

A survey of the published literature reveals that data on selenium concentration particularly in human bone tissue are limited. Only a few studies report the selenium contents measured in bone tissue. Lindh et al. (1980) investigated selenium in bone tissue from the autopsy specimens of femur (bone) of industrially exposed workers and a control group of individuals from unpolluted area. They used neutron activation analysis method for measurement of selenium in bone tissue and found selenium in the range from < 0.02 - 0.58 and < 0.06 - 0.12 $\mu\text{g/g}$ in the industrially exposed workers and the control group respectively. Zachara et al. (2001) while employing the Fluorometric method (FM) determined the selenium level in bone tissue of cadavers of 46 healthy subjects. They assumed total bone tissue mass in body as 11000 g of a 70 kg reference man and found 0.830 μg of selenium per g of bone tissue.

Table 4.12: Se-concentration measured in various organs of humans and animals

Literature Reference	*Technique used	Organ (tissue)	Se ($\mu\text{g/g}$)
Dickson & Tomlinson (1967)	RAA	Human liver (02 adults) Human kidney (02 adults) Human heart (02 adults)	0.39 0.63 0.22
Blotcky et al. (1973)	NAA	Rat liver	1.11
Mckown & Morris (1978)	INAA	Bovine liver (43 adults)	1.138 $\pm$ 0.076
Subramaniam & Meranger (1982)	AAS	Human liver (44 adults)	0.35-0.51
Nicolaou et al. (1982)	CNAA	Pig's liver	0.65 $\pm$ 0.05
Milman et al. (1983)	XRF	Human liver (28 adults)	0.20
Aadland et al. (1987)	AAS	Human liver	2.2
Oster et al.(1988)	AAS	Human kidney (15 adults) Human liver (18 adults) Human lungs (14 adults) Human skeleton (17 adults)	0.77$\pm$0.169 0.291$\pm$0.078 0.132$\pm$0.033 0.111$\pm$0.017
Molokhia & Molokhia (1990)	NAA	Human liver	1.58 $\pm$ 0.32
Czauderna (1996)	NAA	Mice liver Mice lung	4.14$\pm$0.12 1.62$\pm$0.11
Zachara et al. (2001)	FM	Human skeletal muscle	1.431
Hac et al. (2003)	AAS	Human liver (64 adults)	0.289 $\pm$ 0.084
Xing el al. (2006)	NAA	Human liver	1.18-2.14
Tsakovski et al. (2010)	INAA	Toenail (20 adults)	0.764 $\pm$ 0.03
Roy et al. (2010)	XRF	0.7 mm solitary nail	1.522 $\pm$ 0.038
This work (2015)	IVNAA	Hand tissue equil. phantom	0.081

*RAA= Radio-activation analysis, FM= Fluorometric Method NAA= Neutron activation analysis, INAA= Instrumental NAA, AAS= Atomic absorption spectrometry, CNAA= Cyclic NAA, XRF= X-ray fluorescence, IVNAA= In vivo NAA

Chapter 5

RESULTS AND DISCUSSION (Part-II)

This chapter presents the contents of a draft article submitted to the Journal of Applied Radiation and Isotopes for publication. The work presented in this article was performed by the author of this thesis under the supervision of Dr. David R. Chettle, who also corrected the draft article.

This article investigates the origin of a feature peak noticed at energy 0.197 MeV during the analysis of gamma-ray spectra obtained from the irradiation and the counting of water based hand tissue equivalent selenium phantoms. While pursuing the identification and quantification of the selenium peak at energy 0.162 MeV in various selenium phantoms, a peak appeared just to the high energy side of the selenium peak at energy 0.197 MeV, which was causing spectral interference in the vicinity of selenium peak. Investigations made thereof to explore the characteristics of this peak and the conclusion drawn thereof has been demonstrated in this article, which is reproduced as under:

5.1 Identification of oxygen-19 during *in-vivo* neutron activation analysis of water phantoms

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HIGH LIGHTS

- During (n, γ) reaction, ^{18}O in water phantom is activated and becomes radioactive ^{19}O
 - ^{19}O emits γ -ray peaks of 0.197 and 1.357 MeV, which can be measured by a 4π NaI(Tl) detection system
-

ABSTRACT

Hand bone equivalent phantoms (250 ml) carrying concentrations of selenium in various amounts were irradiated and counted for IVNAA by employing a 4π NaI(Tl) based detection system. During the analysis of counting data, a feature at a higher energy than the gamma-ray peak from $^{77\text{m}}\text{Se}$ (0.162 MeV) was observed at 0.197 MeV. Further investigations were made by preparing pure water phantoms containing only de-ionized water in 250 ml and 1034 ml quantities. Neutrons were produced by the $^7\text{Li}(p,n)^7\text{Be}$ reaction using the high beam current Tandetron accelerator. Phantoms were irradiated at fixed proton energy of 2.3 MeV and proton currents of 400 μA and 550 μA for 30 s & 22 s respectively. The counting data saved using the 4π NaI(Tl) detection system for 10 s intervals in anticoincidence, coincidence and singles modes of detection were analyzed. Areas under gamma peaks at energies 0.197 MeV and 1.357 MeV were computed and half-lives from the number of counts for the two peaks were established. It was concluded that during neutron activation of water phantoms, Oxygen-18 is activated, producing short lived radioactive ^{19}O having $T_{1/2}=26.9$ s. Induced activity from ^{19}O may contribute spectral interference in the gamma-ray spectrum. This effect may need to be taken into account by researchers while carrying out IVNAA of biological subjects.

Key words: ^{19}O , IVNAA, water phantom, Tandetron accelerator, 4π NaI(Tl) detection system

Water based phantoms are commonly employed at the experimental stages to investigate the radiation effects in lieu of *in-vivo* irradiation of real biological subjects.

Water is used as soft tissue equivalent material because its density is considered as equal to that of soft tissue. Water is also considered as the universal solvent because it dissolves more substances than any other liquid owing to its chemical composition and physical attributes (Pidwirny, 2006). For the purpose of measuring concentrations of different elements in various organs of the human body, organ equivalent phantoms based on water are designed. These water phantoms have elemental concentrations in various amounts added depending upon the biological characteristics and nature of the organ of interest to simulate the respective organ (Caldas et al., 2010).

The authors were carrying out experiments to test the feasibility of measuring selenium in human hand bone using *in-vivo* neutron activation analysis (IVNAA). The IVNAA technique has been in use for many decades and it provides a non-invasive and non-destructive procedure for detection of many major, minor, and trace elements in the human body for medical research, clinical diagnosis, and occupational health purposes (Chettle and Fremlin, 1984, Davis et al., 2008). In our experiment the elemental composition of hand equivalent phantoms was based on ICRP publication 23 (1975) recommended values. Each phantom was of cylindrical shape in 250 ml Nalgene bottles containing deionized water and concentrations of elements. Selenium was used in various concentrations in the phantoms. Phantoms were irradiated in the irradiation cavity which has been created in McMaster Accelerator Lab (MAL) for IVNAA of elements in a human hand.

The physical components and the operation of irradiation cavity have been reported by Byun et al. (2007) elsewhere. Phantoms were irradiated for 10 s intervals at

an incident proton energy of 2.3 MeV and current 400 μA . The irradiated phantoms were transferred to the gamma-ray detection system for counting at 10 s intervals. The gamma-ray detection system contained nine NaI(Tl) detectors, configured in a 4π geometry. The counting data can be saved in anticoincidence (1024 # of channels), coincidence (1024 # of channels), and singles (2048 # of channels), modes of detection. Chamberlain et al. (2012) have thoroughly explained the working of the detection system. The performance of the system was tested experimentally by using calibrated sources and measuring background before irradiation of phantoms. After acquiring the spectral data, gamma-ray peaks at 0.162 MeV were fitted using anticoincidence mode data for all spectra using a Gaussian function on a linear background. For data fitting, Origin 8.6 software was used. Peak fittings were done at fixed centroid and peak width parameters as this method offers reduced uncertainty in the computed areas under peaks.

During these analyses a peak was noticed at energy around 0.197 MeV which appeared just to the high energy side of the selenium peak (0.162 MeV) in the gamma spectrum. As this unknown energy peak was causing spectral interference in the vicinity of the selenium peak, it was considered imperative by the authors to explore the origin of this peak. For this purpose data were fitted at energy of 0.197 MeV. The features of this peak were found to be comparatively more prominent in phantoms of lower selenium concentrations as compared to phantoms having higher concentration of selenium. Figure 1 shows the presence of the peak at 0.197 MeV, where gamma energy peak at 0.162 MeV from 50 μg selenium phantom can also be seen.

To explore the characteristics of this peak further and comparing it with the selenium peak, a comparison set of phantoms were prepared which consisted of two 250 ml phantoms, one contained 2000 μg selenium in deionized water and the other contained deionized water added with 40 μg nitric acid (i. e., 2% of 2000 μg). Nitric acid was added in the second phantom which contained only de-ionized water because selenium stock solution itself contained 2% nitric acid.

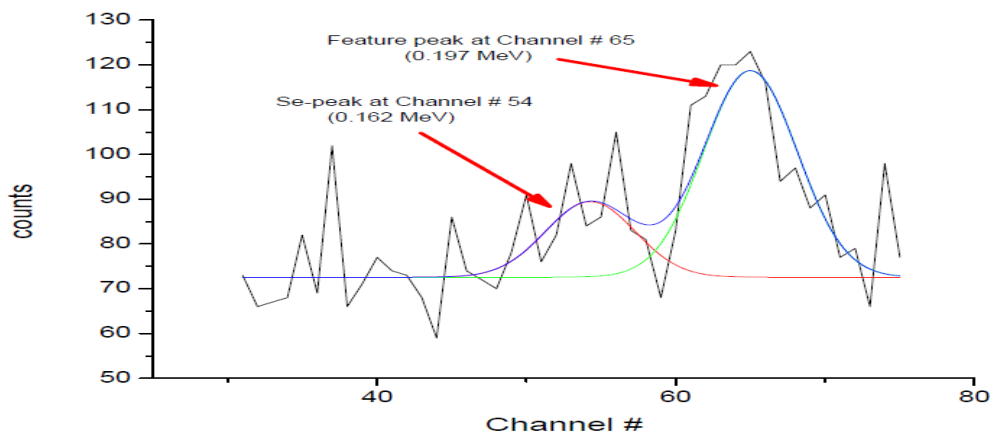


Figure 1: Selenium peak at 0.162 MeV and feature peak at 0.197 MeV using the anticoincidence counting data of 50 μg selenium phantom

Both phantoms were irradiated at same irradiation parameters (i.e., 2.3 MeV proton energy, 400 μA proton current) for 30 s. Both phantoms were counted for many cycles of 10 s duration and the data acquired in anticoincidence mode were analyzed. In the spectrum of pure selenium, the selenium peak at 0.162 MeV was dominant (Figure 2), whereas fitting data acquired for different time segments from the pure water phantom provided good evidence of presence of a peak at 0.197 MeV as is evident from Table 1

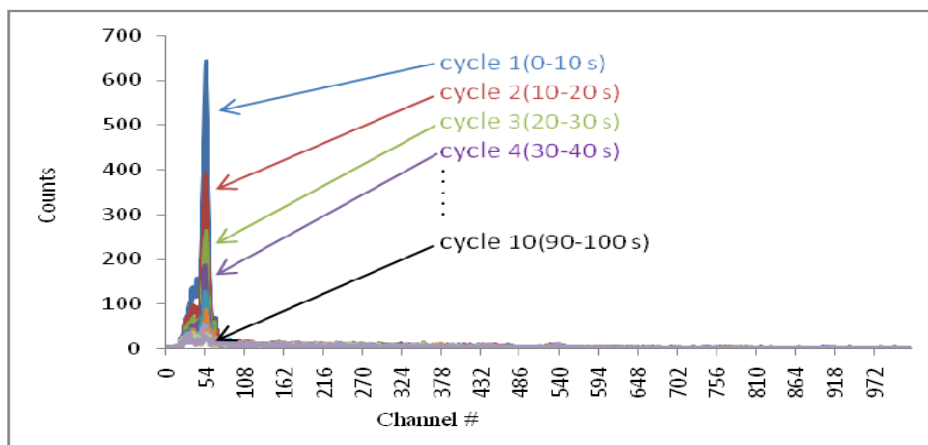


Figure 2: Se-peak in pure selenium phantom spectrum

Table 1: Areas under peaks computed using anti-coin data for various time segments at 0.197 MeV

Time (s)	# Counts
0.235	321±35
11.454	250±36
22.672	198±30
33.891	136±26
45.11	104±16
56.297	65±19
67.532	57±16
78.766	60±14
89.985	42±16
101.219	26±19

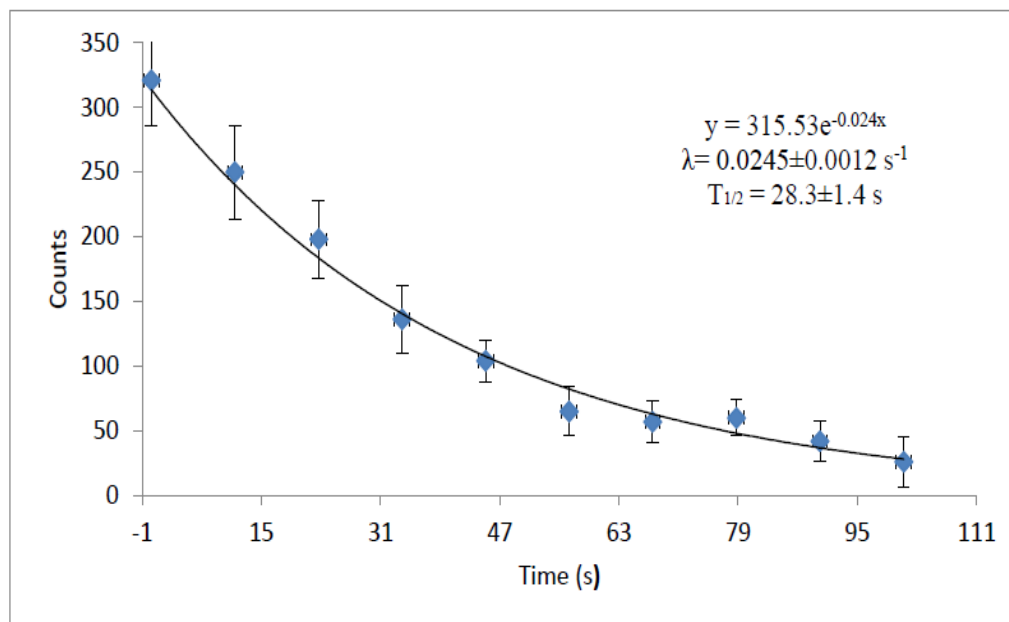


Figure 3: Exponential decay of counts with time

and the graph in Figure 3 which shows exponential decay of number of counts with time (s). Figure 3 indicates that the unknown peak appears in the gamma spectrum of pure water phantom is due to the presence of an isotope in water phantom whose half-life has been calculated to be 28.3 ± 1.4 s. On comparing the half-life measured from the graph shown in Figure 3 with the half-lives of short lived isotopes with the library data, it is found that the feature peak is consistent with that emitted from Oxygen-19, which is produced during $^{18}\text{O}(n,\gamma)^{19}\text{O}$ reaction (Kamemoto, 1964), the half-life of which has been reported as 26.9 s (Stepišnik et al., 2009). However, according to the table of isotopes (1996), during the decay of ^{19}O , two gamma-ray energy peaks are emitted, i. e., 0.197 MeV and 1.357 MeV. Our data in anticoincidence mode analyzed in the energy range up to 1.357 MeV did not show a clear peak at 1.357 MeV. ^{18}O which is activated during (n,

γ) reaction has natural abundance of 0.2 % (Kendall and McDonnell, 1998). This means that water phantom of 250 ml water contains 0.44 g of ^{18}O . It is mentioned here that for the same irradiation parameters the ratio of expected number of $^{77\text{m}}\text{Se}$ atoms activated in 50 μg selenium phantom to number of ^{19}O atoms activated in pure water phantom of 250 g (250 ml) is calculated to be ~ 1.4 , taking into account the fractional abundance, neutron capture cross section, element mass and atomic mass of the respective isotopes.

To assess the possibility of identification of 1.357 MeV peak simultaneously with 0.197 MeV peak in the gamma spectrum as the result of decay of Oxygen-19, a bigger water phantom whose mass was four times (approx.) higher than 250 ml water phantom was irradiated for 30 s at proton energy 2.3 MeV and proton current 400 μA . At the end of irradiation the phantom was transferred from irradiation cavity to the detection system in 19 s and counted for twenty cycles each of which was of 10 s duration. As per the decay scheme of ^{19}O shown in Figure 4 (Cooper and Crasemann, 1970), the 1.357 MeV peak is nearly all in coincidence with the 0.197 MeV peak, whereas a significant amount of the 0.197 MeV peak is not in coincidence with the 1.357 MeV peak. So the anticoincidence mode of detection is expected to suppress the 1.357 MeV peak more than the 0.197 MeV peak. Taking into account this phenomenon, the counting data obtained in singles mode of detection were summed up for all nine detectors and fitted for both peaks at 0.197 MeV and 1.357 MeV.

coincidence data. The coincidence mode of detection data of the bigger pure water phantom this time was fitted using a triple Gaussian function which covered three energy peaks of 1.357 MeV from ^{19}O , 1.554 MeV from the sum peak and 1.78 MeV from ^{28}Al (Table 3).

Table 2: Areas under peaks computed using summing of singles data

Time (s)	Peak area (counts) (0.197 MeV)	Peak area (counts) (1.357 MeV)	Peak area (counts) (1.78 MeV)
0.203	1209±90	424±20	304±20
11.438	863±60	263±18	289±18
22.641	662±52	208±17	270±23
33.828	523±43	202±17	232±17
45.078	406±40	159±16	270±16
56.281	278±47	125±15	207±15
67.50	232±31	119±16	199±16
78.719	159±29	86±15	201±15
89.938	103±23	78±14	190±14
101.156	110±27	71±15	191±15
112.406	81±20	66±14	192±14
123.609	38±21	78±16	161±14
134.875	17±20	54±13	141±13
146.140	---	47±13	166±14
157.360	---	---	133±12

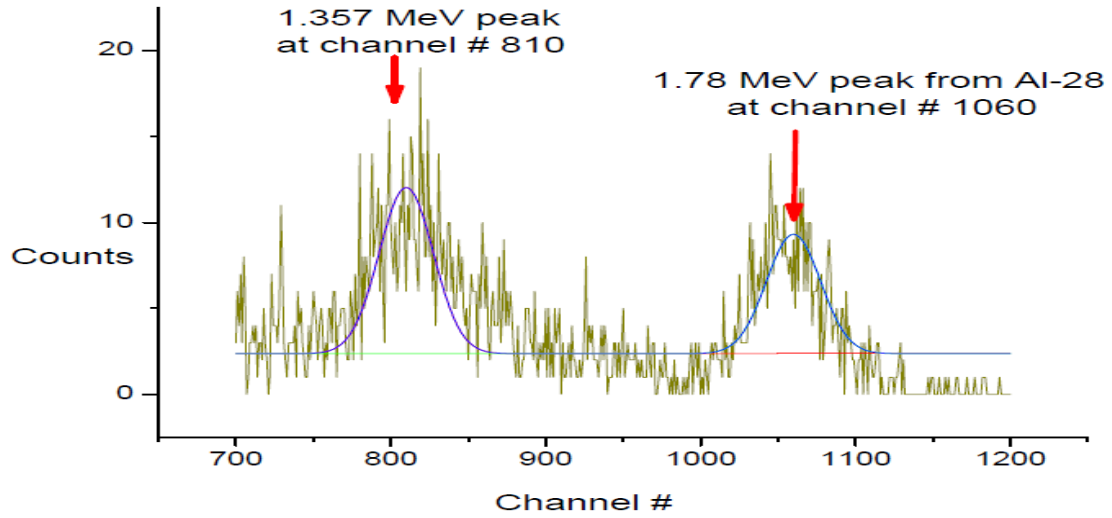


Figure 5: Fitting of peaks 1.357 MeV from ^{19}O and 1.78 MeV from ^{28}Al using summing of singles mode detection data

Figure 6 shows fitting of the sum energy peak at 1.554 MeV along with the 1.357 MeV and 1.78 MeV peaks using the coincidence mode data for time duration 0-10 s. Area under peaks (counts) computed for various time segments can be seen in Table 3 and a graph showing the decay pattern of the sum peak (1.554 MeV) with time is presented in Figure 7.

According to the Table of Isotopes (1996), the intensity ratio of two gamma peaks (0.197 MeV and 1.357 MeV) emitted from oxygen-19 isotope is 1.9. This ratio is based on the fractional abundance of gamma peaks and response of the detector at lower and higher energies. However, Byun et al. (2004) measured the peak efficiency and the total efficiency Of the 4π NaI (TI) gamma-ray detector in the energy range from 0.07-5.0 MeV using the experimental and the Monte Carlo simulation methods. Considering these values the expected ratio of peak efficiency at 0.197 MeV to 1.357 MeV for 4π gamma-ray detector has been computed to be 1.448 (≈ 1.5).

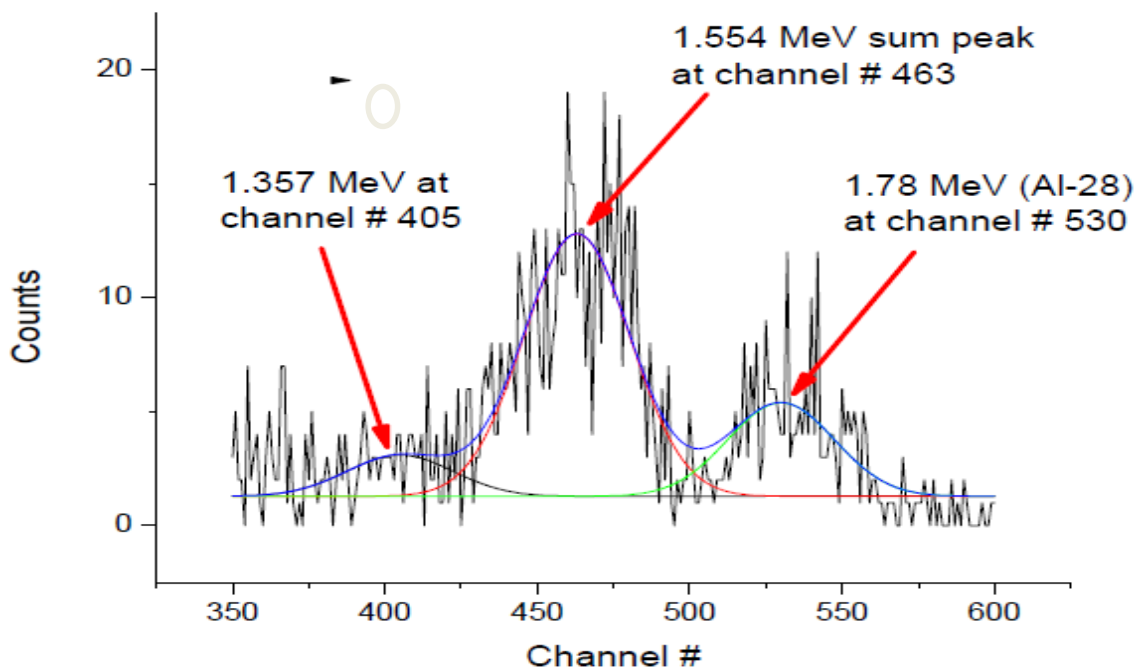


Figure 6: Fitting of sum energy peak at 1.554 MeV along with 1.357 MeV from ^{19}O and 1.78 MeV from ^{28}Al using coincidence mode data for time duration 0-10 s

Table 3: Peak areas using coincidence data

Time (t)	1.357 MeV	1.554 MeV	1.78 MeV
0.203	80±24	505±24	180±25
11.438	94±18	355±18	152±18
22.641	63±18	258±18	157±19
33.828	78±16	202±16	127±16
45.078	66±17	156±16	150±17
56.281	59±16	142±15	150±16
67.50	78±16	106±16	136±16
78.719	55±14	66±14	119±14
89.938	43±13	--	129±13

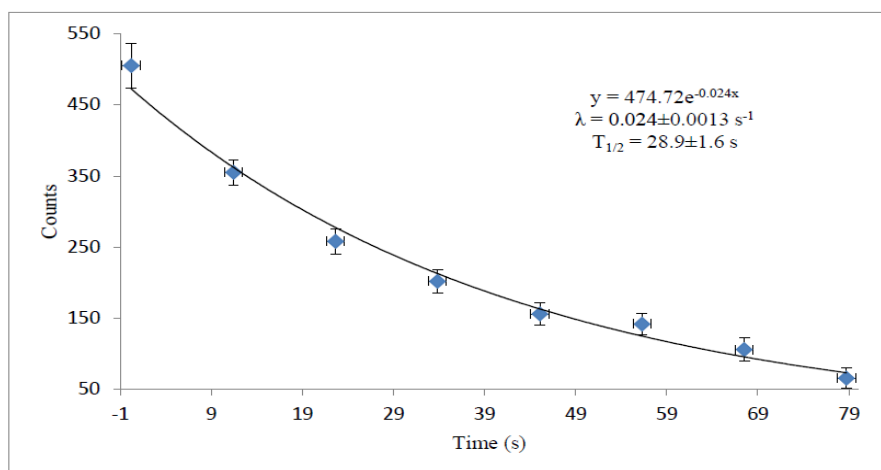


Figure 7: Decay of sum peak (1.554 MeV) counts with time

Keeping in view the calculated value of intensity ratio between two energy peaks from Oxygen-19 and response of 4π NaI (TI) based spectrometric system at low and high energies, we can expect the number of counts detected at 1.357 MeV to be reduced by a factor of 2.75 as compared to the number of counts at 0.197 MeV. Stepišnik et al., (2003) measured induced Oxygen-19 activity owing to the (n,γ) reaction at 0.197 MeV and 1.357 MeV in the primary cooling water of a TRIGA MARK II research reactor. It will be pertinent to mention here that Nicolaou et al. also showed the presence of ^{19}O peak at 0.197 MeV in the γ -ray cumulative spectrum obtained by the cyclic activation of selenium in liver phantom (peak 5 in Figure 5 of Nicolaou et al., 1982).

It is evident from Table 2 that the number of counts computed for energy peak at 0.197 MeV are three times (approx.) higher than the counts detected at 1.357 MeV energy for initial counting cycles. However for late time segments (cycles) this ratio was found inconsistent with the initial counting cycles. The inverse weighted mean of the

number of counts ratio of two energies for first five cycles is 2.882 ± 0.139 , whereas the figure for the second five counting intervals is 1.763 ± 0.190 . There may also be some background or longer lived counts contributing to the 1.357 MeV peak. The relative uncertainty in the subsequent counting intervals of events measurement also becomes worse due to fact that as the signal N decreases with time the relative uncertainty on the signal increases according to relation $\sqrt{N}/N$ (or $1/\sqrt{N}$).

From the preceding analysis we conclude that gamma-ray peaks detected at energies of 0.197 and 1.357 MeV during counting of irradiated water phantoms are associated to the decay of Oxygen-19, which is produced as result of the $^{18}\text{O}(n,\gamma)^{19}\text{O}$ reaction. In these experiments we generated neutron flux from $^7\text{Li}(p,n)^7\text{Be}$ reaction in which proton energy was kept at 2.3 MeV. At this energy and in the irradiation cavity used (Byun et al., 2007), thermal neutrons are a major component of the neutron fluence and it is these which are mainly responsible for the activation process in the target atoms of the phantom contents. This suggests that natural oxygen in water contained by phantoms can be activated by the interaction of thermal neutrons. Therefore those who carry out the irradiation of water based phantoms using thermal neutrons should take into account the possible production of Oxygen-19.

Employing this technique the possibility of measuring oxygen in human body may also be explored by further improving the measurement methodology and the detection system. Some studies have been conducted in past whereby oxygen was determined in water and biological samples by various methods. Volborth and Banta (1963) determined oxygen in water and other materials using $^{16}\text{O}(n,p)^{16}\text{N}$ reaction in

which a 14 MeV energy neutron flux was used. Kamemoto (1964) suggested a rapid non-destructive analysis of oxygen by neutron irradiation of natural oxygen to form oxygen-19 in $^{18}\text{O}(n,\gamma)^{19}\text{O}$ reaction. Anders and Briden (1964) reported the measurement of oxygen in water and other analytical samples by inelastic scattering reaction (n,n') using fast neutrons. Wood et al. (1975) precisely measured ^{18}O in water contained in biological samples via $^{18}\text{O}(p,n)^{18}\text{F}$ reaction and their results were comparable in accuracy to the mass spectrometry technique. Kehayias and Zhuang (1993) also demonstrated the feasibility of detecting *in-vivo* oxygen in humans through inelastic scattering reaction of fast neutrons while using the fast neutrons from a D-T source.

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Chapter 6

CONCLUSIONS AND FUTURE SUGGESTIONS

6.1 Conclusions

This study has led to the following conclusions:

- (i) The non-invasive and non-destructive neutron activation analysis technique, using the McMaster University Tandetron Accelerator coupled with the 4π NaI(Tl) detection system would be a feasible means of performing *in vivo* measurements of selenium in human hand tissue equivalent phantoms. This technique may be extended as a quantitative and analytical approach for quantifying selenium in human hands and other organs.
- (ii) Based on the preliminary measurements the inverse variance weighted mean value of minimum detectable limit has been calculated to be $1.58 \mu\text{g Se/g Ca}$ for first three separate time intervals with each for 10 s duration. Whereas, an equivalent dose of 191.9 mSv is estimated for these measurements from neutron and gamma components of the incident beam at proton energy 2.3 MeV and proton current 400 μA .
- (iii) During the neutron activation of water phantoms, Oxygen-18 is activated, producing short lived radioactive ^{19}O having $T_{1/2}=26.9$ s. Induced activity from ^{19}O may contribute spectral interference in the gamma-ray spectrum. This effect may need to be taken into account by researchers while carrying

out IVNAA of biological subjects. A comparison of peak areas computed for ^{19}O using anticoincidence and summing of singles modes of detection shows that signals (counts) are partially suppressed in anticoincidence as compared to sum of singles.

- (iv) The sources of uncertainties in these measurements included counting statistics, phantom transfer time from the irradiation to the counting systems, shifting of gain in the detector during measurements and the data extraction methodology.
- (v) The sensitivity of measurements to the proton current and neutron flux variations, positioning of phantoms upon irradiation and detection geometry were taken into account by normalizing the measured amount of selenium to the calcium.
- (vi) Currently the data on *in vivo* measurement of selenium in humans are limited. This study would greatly contribute to the current data.

6.2 Future suggestions

Before transition to actual human subjects trials, the method developed in the present study to measure selenium definitely needs improvements for which a significant amount of work is required to be done. This will help a lot in developing a comprehensive system to monitor selenium levels in human body by employing *in vivo* neutron activation technique. In this respect the following suggestions are made for future research:

- (i) The preliminary testing of selenium measurement has been done only at one proton energy of 2.3 MeV with different proton current values and irradiation intervals. It is therefore worth seeing to try proton energy at lower levels below 2.3 MeV in combination with varying current and exposure intervals. This may help in further improving the MDL and reduction in radiation dose received locally by the phantom. Therefore beam parameters for irradiation may be optimally selected.
- (ii) ^{77m}Se is a short lived isotope with half life of 17.4 s. The irradiated sample transfer from the irradiation cavity to the detection system normally take 11-13 s, during which a significant decay takes place resulting lower number of detected counts especially from lower selenium concentrations phantoms. Therefore for obtaining more counts in the initial cycles of counting, detection system may be installed closer to the irradiation cavity to reduce the sample transfer time. This modification will consequently result detection of more counts and an improvement in the MDL. To avoid higher background, the shielding level of the detection system may be improved.
- (iii) During fitting of detection spectra, a simple model of Gaussian peak on a linear background was used for analysis of selenium and calcium peaks. The chi-squared values observed from fits give an impression that the fitting model used did not provide a perfect fit. The goodness of fit which provides the basis of chi-squared value may be limited by additional features such as the Compton edges in the spectra which could have been taken into

consideration. This aspect may be considered and a more detailed analysis of the fit may be done in order to identify the additional features in the areas of the fit. This may provide more accurate fit and lead to a reduction in the MDL to some extent if not significantly.

- (iv) During analysis of fission chamber (FC) counts and integrated current (IC) obtained for three different irradiation parameters, it was observed that FC counts and IC value affect the activation of Se and Ca. Therefore a careful evaluation of these parameters is warranted in future measurements.
- (v) For comparing the cross validity of experimental results, the Monte Carlo simulations may be performed in order to produce a better assessment of the feasibility study demonstrated in this thesis.
- (vi) Irradiation and detection systems may be improved and the feasibility of measuring selenium in other sites such as nails, liver, heart, kidneys etc., which are storage places for selenium in the body, may be assessed using IVNAA.

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