THE INTERNATIONAL JOURNAL OF SCIENCE & TECHNOLEDGE

Speciation of Se(IV) and Se(VI) in Human Blood Samples from Residents of Wassa West District, a Mining Impacted Area of Ghana

B. V. Samlafo
Department of Chemistry Education, University of Education, Winneba, Winneba, Ghana
L. H. Bobobee
Department of Chemistry Education, University of Education, Winneba, Winneba, Ghana
P. O. Yeboah
Department of Nuclear Sciences and Applications, Graduate School of Allied Sciences
University of Ghana, Atomic Campus, Accra, Ghana
Y. Serfor-Armah
Department of Nuclear Sciences and Applications, Graduate School of Allied Sciences
University of Ghana, Atomic Campus, Accra, Ghana

Abstract:
This paper examines the speciation of selenium(IV) (selenite) and selenium(VI) (selenate) levels in human whole blood samples from Tarkwa and its environs, a mining impacted area in Ghana, using hydride generation atomic absorption spectrophotometric technique.
Selenium(IV) in the experimental subjects ranged from 0.08±0.01 µg/mL to 0.50±0.02µg/mL with a mean of 0.29 µg/mL, while the controlsranged from 0.02±0.1 µg/mL to 0.21 ±0.05 µg/mL, with a mean of 0.07 µg/mL. Significant differences existed between the levels of selenium(IV)concentrations in experimental and controlsubjects($p=0.0001 <0.05$). The selenium(VI) in experimental subjects ranged from 2.97±0.52 µg/mL to 4.39±0.42 µg/mL,with amean of 3.63 µg/mL, while the control ranged from 0.60 µg/mL to 1.0 µg/mL, with a mean of 0.89 µg/mL.Significant differences exited between the levels of selenium(VI) in experimental and the control subjects ($p=5.98 \times 10^{-14}<0.05$). The measurement precision determined by relative standard deviation was within ±5%. The accuracy of the determination was evaluated by analysing certified standard human hair reference material GBW 09101. The observed valueswere within ±6% of the certified values.

***Keywords**: blood, selenium, speciation, health, toxicity, mining, nutrient, hospital*

1. Introduction

As the second of two paper series, part one (Samlafo et al, 2011) provided the general overview of the total selenium status in the blood samples of the inhabitants of Tarkwa and its environs, a mining impacted area in Ghana. This paper seeks to address the distributionof selenium species in whole blood samples of the residents in Tarkwa, since there is a relationship between chemical speciation, bioavailability, toxicity and metabolism of selenium species.

Although various elements provide different biological, toxicological or physiological functions in biological systems, such functions are more specific or characteristic, depending on their chemical forms even at the trace level. Presently,therefore, speciation analysis of various trace elements are receiving great attention in various scientific fields. (Hiroki Haraguchi, 2001).

Selenium is an essential microelement for animals, humans and in microorganisms. It has three levels of biological activity (Hamilton, 2004), trace concentrations are required for normal growth and development, moderate concentrations can be stored to maintain homeostatic functions and elevated concentrations can result in toxic effects. Although selenium is an essential trace nutrient, important to humans and most other animals as an antioxidant, it is toxic at high concentrations due to incorporation of selenium in place of sulphur in amino acids, with subsequent alteration of the three-dimensional structure of protein and impairment of enzymatic function (Amweg, 2003).

The environmental and biological factors that affect the biogeochemical cycling of Se in the environment have a profound influence on its subsequent availability and toxicity to organisms(Amweg, 2003). Selenium occurs in several different oxidation states in the aquatic environment that include oxidized forms of selenium(VI)(selenates, SeO_4^{2-}) and selenium(IV)(selenites SeO_3^{2-}).Elemental selenium(Se^0) and the reduced form of selenium, selenide(Se^{2-}), the primary form found in sediments, has little toxicological significance for most organisms. The selenide is more volatile and hence not bioavailable to plants.Selenium(VI), and selenium(IV)are both water soluble inorganic species, found typically in aerobic water sources. According to Lemly,2003, the more toxic an elemental species, the less bioavailable it is to living organisms,hence Selenium (IV), is approximately 5-10 times more toxic to organisms than selenium (VI) and less bioavailable to plants. In alkalioxidized soils, Selenium(VI) has been reported as the dominant soluble form of Selenium and adsorbed Selenium was mostly selenium(VI)(Fio and Fujii, 1990)

The main source of Selenium exposure to man is in food. Selenium enters the food chain through plants, which take it from soil(Reilly, 1996).The concentration of selenium in the plasma is about 80% of that in whole blood(Combs, 2001). Selenium concentration in smokers, chronically ill persons, the fragile elderly, children,pregnant and lactating women may be 25-30% lower than those in adult control subjects (Reyes, 2000).

The value for plasma selenium levels in defining Selenium deficiency remains uncertain. While studies on Selenium status and human immunodeficiency virus(HIV) disease have used a criterion deficiency of < 85.0µg/L, there is evidence that higher plasma levels are needed for optimal biological function(Levander, 1987). Low selenium status is also commonly observed in children suffering from Kwashiorkor. Significantly, lower selenium levels were also found in women who had either first-trimester or recurrent miscarriages. Low selenium status has alsobeen associated with significantly increased incidence of negative mood state such as depression and anxiety(Benton D, 1991).

The toxicity of selenium to animals varies with the amount and chemical form of selenium ingested, the duration and continuity of intake, and the type and nature of the diet, with regards to its protein and sulphate content (Shamberger, 1981).

Among selenium's clinical roles are its anticarcinogenic activity and its prevention of heavy metal toxic effects. Although Selenium is required for testosterone biosynthesis and the formation and normal development of spermatozoa,selenium supplementation significantly increases sperm motility,however, the nutritional required concentration range of selenium was found to be very narrow(Magos L, 1980). Overdose and toxicity of selenium is characterized by alkali disease and blind staggers(Levander, 1987). Moreover, the toxicity, availability and environmental mobility of selenium are strongly dependent upon its chemical forms(Shibata Y, 1992). Therefore, knowledge of the different species of selenium present in a particular system is required for an accurate assessment of the biological and environmental impact of this element.

There is a growing appreciation that, it is not just the total intake of dietary selenium that is important to health, but that the species of selenium ingested may also be important. Without adequate knowledge of selenium speciation, false conclusions may be drawn when assessing selenium requirements for optimal health.

2. Materials and methods

2.1. Study Area

The study area is Tarkwa and its environs in the Wassa West District of the Western region of Ghana. Tarkwa is the administrative capital of the Wassa West District,which lies between latitude 4ºN and 5º40''N and longitude 1º45'' W and 2º10''W. The District covers a total land area of 9235km^2. It is bordered to the north by the Wassa Amenfi District, to the south by the Mpohor- Wassa East and Ahanta West, to the east by the Mpohor-Wassa East and to the west by the Nzema East District as shown in figure 1.

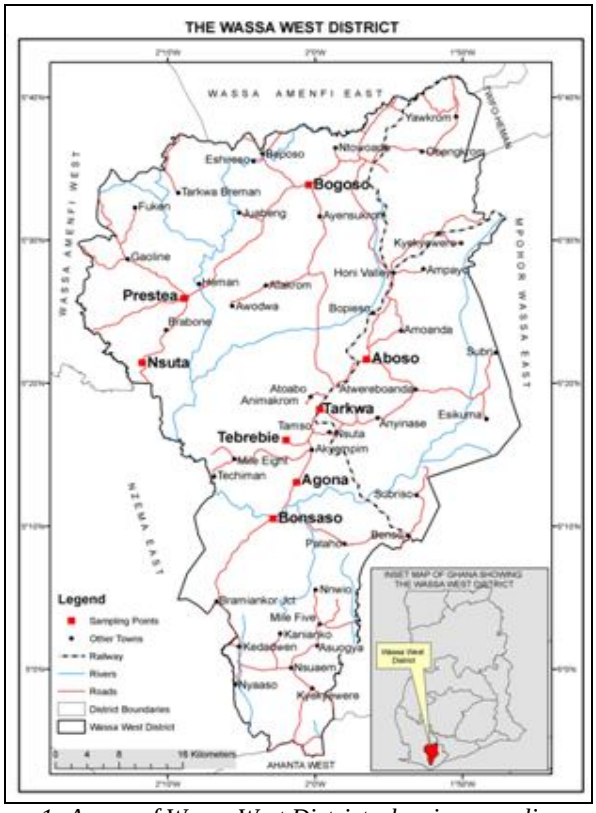

Figure 1: A map of Wassa West District, showing sampling points

2.2. Sample Collection

All the blood samples were collected from Tarkwa Government Hospital, ABA Gold Field Hospital(Tarkwa), Ghana Manganese Company Hospital (Nsuta), Ami Maternity Home (Tarkwa), GAG Hospital (Iduapriem),and Pentecost Clinic (Tarawa)with ethical approval from the Ghana Health Service. The control samples were taken from Akatsi and its environs, a distance of 480Km by road from Tarkwa, where there are no known mining activities

Blood samples were collected in tubes containing ethylenediaminetetraacetic acid EDTA, (BHD chemical,England) as an anticoagulant by a qualified Health Officer. The samples were stored in a fridge at 2-4^{O}C.

The subjects were made to respond to a detailed questionnaire regarding dietary habits, gender, and age profile. A total of115 blood samples were collected. However ten blood samples each withhigh total selenium levels were selected for the speciation studies in both experimental and control subjects.

2.2.1. Sample Preparation

One hundred milligrams (100mg) of whole blood samples from experimental subjects were weighed and each transferred into 100mL polytetraflouroethylene Teflon bombs. Twenty-five millilitres(25mL) of double distilled water was added to the sample. Ten millilitres(10mL) of concentrated nitric acid was added to the mixture and allowed to stand for 10 minutes. Five millilitres(5.0 mL) of 30% H_2O_2 was also added to the mixture to dissolve any organic matter that might be left in the mixture. The resulting mixture was put in a microwave, digested for 25minutes in a Milestone microwave oven (Ethos900) using the following operation parameters: 250W for 2minutes, 0W for 2minutes,250W for 6minutes, 400W for 5minutes, 650 W for 5minutes and 5minutes allowed for venting. After digestion, the Teflon bombs were cooled in a water bath for 5.0 minutes to reduce internal pressure and to allow volatilized material to re-solubilize to enhance analyte recovery and to ensure complete mixing. The digest was divided into two portions.

3. Reduction of selenium(VI) to selenium(IV) for total Se determination

Five millilitres of the first portion of the digested blood samples were taken into each of the ten test tubes and 5.0 mL of 5.0M HCl was added. The mixture was heated for 30 minutes to a temperature range of 90-100^{O}C in a water bath to reduce Selenium (VI) to Selenium (IV). Calibration standards were prepared and the digest was assayed for total selenium in five replicates using 6.0M HCl and 0.6%w/v$NaBH_4$ in 0.5% w/v NaOH as the reductant in Varian Fast Sequential Atomic Absorption Spectrometer, model AA240FS. Acetylene gas was used as the carrier gas. Inert argon was passed through the system to remove interfering gases between each reaction time. The instrument detection limit was 0.002µg/mL.

The second portion of the digest was determined directly for Selenium(IV) without taking it through the pre-reduction stage and the amount of Selenium(VI) was obtained by difference. The same procedure was repeated for the control samples from Akatsi and its environs.

3. Results and Discussion

An independent one-tailed t-test was used to compare the means of both the experimental and the control subjects in order to established statistical significant differencebetween the two groups. The p value for which significant differences occurred, was set at <0.05 at 95% confidence limit.

The precision and accuracy of the analytical technique were validated by analysing human hair standard reference material, GBW 09101. The values obtained compared favourably well with the recommended values as Spearman's correlation coefficient was +0.92. The values of the experimental samples were within ±4% of the recommended value. The measurement precision specified by the relative standard deviation was within ±5%. The error margins presented in the results were standard deviations

Table1: Mean concentrations of trace elements in µg/g of standard human hair reference material, GBW 09101 with standard deviations, (n=5).

Element	Recommended value	Experimental value
Se		
Hg	2.10±0.05	1.98±0.1
As	0.60±0.03	0.58±0.02
Ca	1091±81	1092±62
Na	264.60±22	266.10±18

Table 1

Selenium(IV), in the experimental blood samples (Table2) ranged from 0.08±0.01 µg/mL in B_8 to 0.50±0.02 µg/mL in B_{58}, with a mean of 0.29 µg/mL. Selenium(IV), in control samples ranged from 0.02±0.01 µg/mL in CB_{29} to0.21±0.05 µg/mL in $CB_{39}$with a mean of 0.07 µg/mL. There was significant difference between the experimental and control subjects ($p=0.0001<0.05$). This difference might be due to the mining activities in the area, especially from emission of selenium particles by coal-combustion engines (train systems), since these coal-combustion engines are the main source ofpossible selenium pollution in the area. Sulphide-gold containing ore is also a likely source of selenium pollution in the study area, since selenium has a high tendency to be incorporated into sulphide containing gold ores. These levels of selenium(IV) in the experimental subjects are of much concern, since this chemical species is the most toxic form of selenium.

	Sample Code									
Species	**B1**	**B8**	**B10**	**B31**	**B36**	**B39**	**B44**	**B45**	**B58**	**B71**
Se(IV)	0.18±0.02	0.08±0.01	0.41±0.10	0.18±0.01	0.36±0.08	0.42±0.12	0.20±0.05	0.17±0.04	0.50±0.02	0.42±0.05
Se(VI)	2.97±0.52	3.13±0.49	3.32±0.61	3.65±0.72	4.39±0.42	3.99±0.23	3.71±0.12	3.48±0.35	3.74±0.47	3.90±56
Total Se	3.15±0.80	3.21±0.08	3.73±0.90	3.83±0.90	4.75±0.80	4.41±0.96	3.91±0.50	3.65±0.60	4.24±0.62	4.32±1.10

Table 2 : Mean concentrations of Se(IV) and Se(VI) in experimental blood samples with standard deviations (µg/mL wet weight), (n=5)

	Sample Code									
Species	**CB25**	**CB26**	**CB28**	**CB29**	**CB30**	**CB31**	**CB36**	**CB37**	**CB38**	**CB39**
Se(IV)	0.05±0.01	0.09±0.02	0.05±0.01	0.02±0.01	0.04±0.01	0.08±0.02	0.03±0.01	0.06±0.02	0.06±0.02	0.21±0.05
Se(VI)	0.92±0.03	0.89±0.02	0.83±0.26	0.93±0.12	0.99±0.21	0.94±0.14	0.95±0.16	0.91±0.18	1.00±0.24	0.60±0.09
Total Se	0.97±0.02	0.98±0.01	0.87±0.08	0.95±0.06	1.03±0.02	1.02±0.04	0.98±0.10	0.97±0.02	1.06±0.01	0.81±0.04

Table 3: Mean concentrations of Se(IV) and Se(VI) in control blood samples with standard deviations (µg/mL wet weight), (n=5)

Selenium(VI),in experimental blood (Table2) ranged from 2.97±0.52 µg/mLin B_1 to 4.39±0.42µg/mL in B_{36}, with a mean of 3.63 µg/mL. The Selenium (VI), in control subjects (Table 2) ranged from 0.60±0.09 µg/mL to 1.0±0.24 µg/mL, with a mean of 0.89 µg/mL. There was significant difference between the experimental and the control subjects ($p= 5.98 \times 10^{-14} < 0.05$). This difference is due to the mining activities in the study area. Generally, the levels of Selenium(VI)in both experimental and control subjects were higher than Selenium(IV). This is probably because, althoughselenium (VI) is less stable than selenium (IV),Selenium (VI) is more soluble in water than Selenium(IV), hence more bioavailable to plants, a major source of selenium exposure(food crops and medicinal plants) to man.

According to Margaret P. (2008),selenium is excreted through urine and feaces, however, the inorganic selenium species are rapidly excreted in urine, in contrast to selenomethionine, which is retained. The total recovery of selenium in urine and feaces of selenium (VI) and selenium (IV) was 82-95% of the total dose. However, Methylated species, such as trimethylselenonium, contribute to only a minor fraction of selenium in urine, in variable amounts. Selenium is excreted into the urine in the form of monomethylated selenium (selenosugar) when rats are fed a diet with selenium sources at an adequate concentration. A small portion of selenium is excreted into the hair

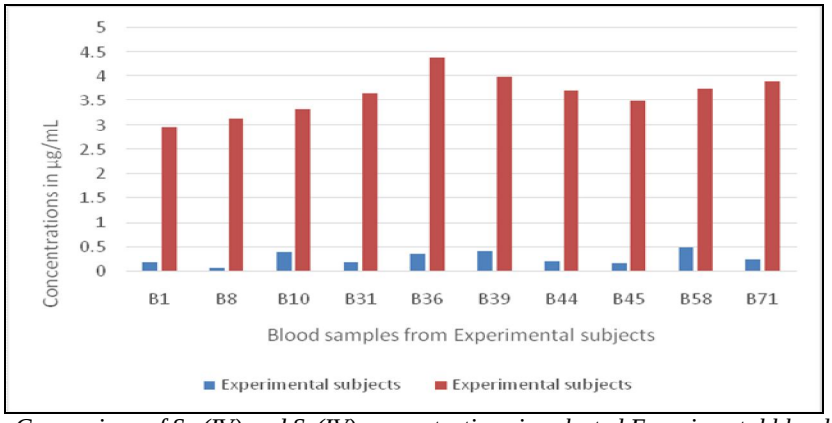

Figure 2: Comparison of Se (IV) and Se(IV) concentrations in selected Experimental blood samples

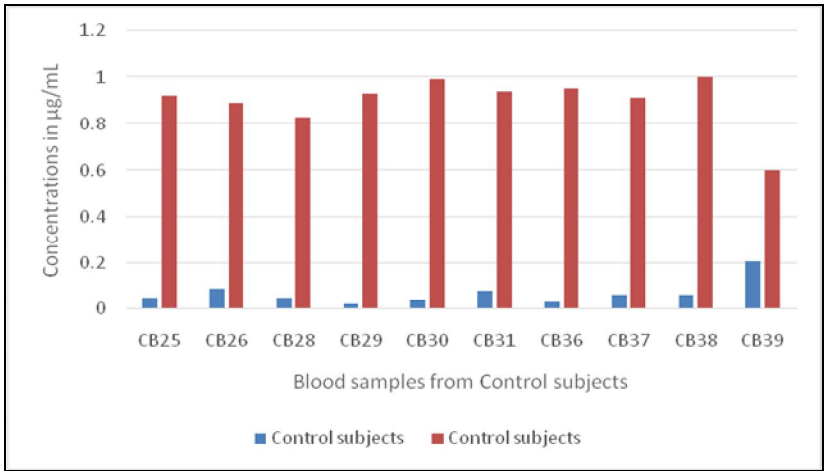

Figure 3: Comparison of Se (IV) and Se (IV) concentrations in selected control human blood samples

4. Conclusion

Selenium (VI) the less toxic species was found to be the dominant form of Se in whole blood in both the experimental and control subjects. Generally, the higher the total Se, the higher the toxic species of Selenium(IV),hence a higher level of total selenium predisposes the individual to selenium toxicity.

5. Conflict of Interests

The authors have no conflict of interests to declare.

6. References

1. Amweg, E. L. (2003). Comparactive bioavailability of selenium to aquatic organisms after biological treatment of agricultural drainage water. Aquatic Toxicology, 63, 13-25.
2. Benton, D. C. (1991). The impact of selenium supplementation on mood. Biol. Psychiatry, 29, 1092-1098.
3. Combs, G. F. (2001). Selenium in global food systems. Br. J. Nutr., 517-547.
4. Fio John L, a. F. (1990). Selenium speciation methods and application to soil saturation extracts from San Joaquin Valley, Califonia. Soil Science Society of America Journal, 54, 363-369.
5. Hamilton S J. (2004, June). Review of selenium toxicity in the aquatic food chain. Science of Total Environment, 29, 326-321.
6. HIroki Haraguchi, T. O. (2001). Speciation of drugs in blood serum by surfactant-mediated HPLC/ICP-MS with direct sample injection. Analytical Sciences, 17(37), 54-60.
7. Lemly A D, F. S. (1993). Sources and impacts of irrigation drainwater contaminants in arid wetlands. Environmental Toxicology and Chemistry, 12, 2265-2279.
8. Levander, O. A. (1987). Selenium in Food and Health. U S A: Springer.
9. Magos L, W. M. (September 2003). Toxicological profile for selenium. Atlanta Geogia: Agency for Toxic substances and Disease Registry .
10. Margaret, P. R. (2008). Food chain selenium and human health: sportlight on speciation. Guildford, University of Surrey, UK: Nutritional Sciences Division, Faculty of Health and Medical Sciences.
11. Reilly, C. (1996). Selenium in food and health. London: Blakie Academics and Professionals.
12. Reyes, H. B. (2000). Selenium, zinc and copper plasma levels in intrahepatic cholestasis of pregnancy, in normal pregnancies and in healthy individuals in Chile. J. Hepatol, 32, 542-549.
13. Samlafo, B. V.-A. (2011). Atomic Absorption Spectrophotometric Determination of Selenium Concentrations in Hair and Nails of Residents of Wassa West District of Ghana. Journal opf Applied Science and Technology, 16(1), 93-97.
14. Shamberger, R. J. (1981). Selenium in the environment. Science of the Total Evironment, 17, 59-74.
15. Shibata Y, M. M. (1992). Selenium and arsenic in biology: their chemical forms and biological functions . Advances in Biophysics, 28, 31-80