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Determination of inorganic selenium species by neutron activation analysis in aquatic species after preconcentration with ammonium pyrrolidinecarbodithionate

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Inorganic selenium species were determined in several parts of a freshwater fish of the species *Tilapia nilotica* found breeding in disused tin-mining pools. The inorganic selenium species in the monazite-rich ores can enter the human food chain through the consumption of the fish. The Se(IV) and Se(VI) species were preconcentrated by solvent extraction with APCDT-$CHCl_3$ before irradiation in a TRIGA Mk.II reactor. Total inorganic selenium species separation was done using Chelex-100 chelating resin. Quantitative interpretations of the distribution of inorganic selenium species in the fish are discussed with particular reference to the Se(IV)/Se(VI) ratio.

Introduction

Various methods have been commonly used in the determination of traces of selenium and they include gas chromatography,[1] atomic[2] and molecular[3] fluorescence spectrometry, inductively coupled plasma-mass spectrometry,[4] X-ray emission spectrometry,[5] cathodic stripping voltammetry,[6] neutron activation analysis[7] and hydride generation-atomic absorption spectrometry[8,9] (HGAAS). Most of the techniques used in the determination of inorganic selenium respond only to Se(IV) and as such other oxidation states namely the Se(VI) species must be converted to the Se(IV) species prior to their determination. Though selenium particularly the inorganic species, are well distributed in the environment, their concentrations are very low especially in natural water bodies where they are found as a result of seepage from seleniferous soils and also industrial wastes.[10] Some of these selenium undergo biogeochemical processes to form the organoselenium compounds, which are more toxic than the inorganic species. Though plants of the *Cruciferae* family have a special tendency to absorb selenium into their tissues, animals need to have selenium in trace amounts to maintain normal body metabolism.[11] Diseases of selenium deficiency, such as the myocardial disease known as Keshan disease in China have so far been recognized. Extensive studies were also carried out in the investigation of selenium as an essential constituent of the enzyme glutathione peroxidase which functions as cell protectors from highly oxidized products in lipids in higher vertebrates.

Because of its trace amounts present in most environmental samples and also it is both essential and toxic to animal and man within a relatively narrow concentration range, a reliable separation method followed by a relatively sensitive method of quantitative determination is required. Thus neutron activation analysis has been selected as the analytical method because of its high sensitivity but the presence of high matrix constituents in most biological samples impedes this method. Therefore, a suitable separation step has to be incorporated into the analysis to get rid of the interfering constituents. Several common separation techniques have been used including chelation and solvent extraction,[12] chelation and ion exchange,[13] electrolytic preconcentration[14] and co-precipitation[15] in an effort to preconcentrate the relevant species. The determination of Se(IV), Sb(III) and Sb(V) from seawater by APCDT extraction and subsequent adsorption on C_{18}-bonded silica gel was carried out by STURGEON et al.[16] since selenium exhibits properties similar to those of arsenic, a method previously used with good success on the preconcentration of As(III)[17] by APDTC in $CHCl_3$ is applied in this study.

In the present work, inorganic selenium species from aquatic samples taken from man-made water bodies were preconcentrated using a mixture of APDTC-$CHCl_3$. The $CHCl_3$ loaded with Se(IV) species from the extraction procedure were then irradiated to determine the Se(IV) concentrations by γ-spectrometry. The nature of inorganic selenium species uptake by certain aquatic life is also discussed.

Experimental

Since the concentration of selenium species in natural waters is governed by the rate of seepage from seleniferous environments, water bodies in disused tin mining pools could be a good source of selenium input as a result of the land-use activity related to dredging. The aquatic species found in these water bodies range from algae undergrowth found along the perimeter of the pools to a common, hardy freshwater fish, the *Tilapia nilotica*. This particular fish

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from the *tilapia* family is found in abundant in disused tin mining pools and because of its rapid breeding nature, they are also bred in man-made ponds and is a major contribution to the local aqua-culture industry.

All fish samples were collected from a fresh catch and selected to ensure only fish of similar sizes were used in the study. They were packed in ice and brought to the laboratory for further preparation. Samples were also taken from a man-made pond to be used as a control. The fish samples were cut and separated into three parts, namely the head, flesh and bones using pre-cleaned stainless steel blades. The wet weights were recorded before freeze-drying them using an Edward Mudalyo freeze-dryer and later ground to < 200 mesh size with a Herzog HSM-100 grinder. Samples were sealed in pre-treated polyethylene containers for further analysis. For quality assurance controls, a CRM *Lobster Hepatopancreas* marine reference material (TORT-1) supplied by the National Research Council of Canada (NRCC) and standard selenic acid (H_2SeO_3) were used.

All reagents used were of analytical grade (Analar), purified prior to use and prepaed by diluting Analar acids (15.8M HNO_3, 11.8M HCl and 18.1M H_2SO_4), base (7.1M NH_4OH) or salt (NH_4COOCH_3) with quartz-distilled water (Q-H_2O) to the appropriate concentrations. The chelating resin Chelex-100, in the sodium form used in the total inorganic selenium separation step, was obtained from Sigma Chemicals. Aqueous stock solutions of Se(IV) were prepared by dissolving H_2SeO_3 in Q-H_2O and made up to the mark. Ammonium pyrrolidinecarbodithionate (APCDT) used in the extraction step was obtained from Fluka. All containers were washed with Triton-X100 detergent solution before soaking overnight in 10% HNO_3. This was followed by rinsing with Q-H_2O and later stored in fume cupboards providing a Class-100 working environment.

Sample solutions for the extraction step with APCDT-$CHCl_3$ and preconcentration with Chelex-100 were prepared by dissolving 1 g of the dried material with 10 ml of HNO_3 (70%) in a Teflon pressure decomposition vessel of a CEM Model MDS-81D microwave digester. A safety valve was placed on the vessel, the cap tightened using a capping station and heated for 7 minutes at 48 °C at 30% power (190 kW). Since 12 vessels can be placed in the turntable, duplicates or several samples can be digested simultaneously. The resultant solutions were allowed to cool to ambient temperature before the addition of 1.5 ml of 30% H_2O_2 and finally made up to 250 ml for subsequent analyses. All CRMs and standards used in this study were similarly treated for consistency.

The extraction procedure for the Se(IV) species requires the preparation of a fresh batch of APCDT solution and this was done by dissolving 5 g of APCDT in 100 ml Q-H_2O. Bromine and other impurities were removed by shaking with $CHCl_3$ while insoluble materials were filtered off. Ethylenediaminetetraacetic acid (EDTA) solution was used as a masking agent and a 12% solution was prepared for this purpose. The details of the extraction procedure follows that described elsewhere.[17] Since inorganic selenium species exist in the Se(IV) and Se(VI) forms, the extraction was done on the Se(IV). The Se(VI) determination was achieved by first reducing the Se(VI) to Se(IV) with 25% sodium thiosulphate ($Na_2S_2O_3$) solution. Total selenium [hence, Se(VI) by difference] was determined on a separate batch of 1 g sample subjecting the same treatment done on the first batch. The difference in the two selenium concentrations constitutes the Se(VI) in the samples investigated.

The ion-exchange procedure using the chelating resin Chelex-100 was carried out to preconcentrate the elements prior to the total inorganic species determination. The procedure followed that of KINGSTON et al.[18] with some modifications.

The final solution obtained after the phase separation was used in the irradiation step to determine the inorganic Se content in the samples. Irradiations of 6 hours at 750 kW power were performed on 1 ml of the solution in polyethylene vials which were heat-sealed and packed into larger vials before irradiating them in a neutron flux of $4.0 \cdot 10^{12}$ n $\cdot$ cm^{-2} $\cdot$ s^{-1} from a TRIGA Mk.II reactor at the Malaysian Institute of Nuclear Technology and Research followed by about 2 weeks of cooling before counting commenced. A large volume coaxial HPGe detector with a resolution of 1.9 keV at 1332 keV ^{60}Co was used for counting the 264.7 keV and 136.0 keV γ-rays of ^{75}Se in the liquid sample over a period of 3600 seconds. Amplification and analysis of signals were done by an ADC ND592 (Nuclear Data) Analyzer connected to a ND6000 (Nuclear Data) multichannel analyzer.

Results and discussion

Figure 1 shows the map of the sampling locations where the species *Tilapia nilotica* were taken. The locations are clustered together because of localized tin mining activities. Fish of almost similar sizes were chosen for uniformity and to reduce the margin of error in the uptake of inorganic Se due to difference in age and length of exposure of the species in the water. Selective separation and extraction of the Se(IV) species from aqueous media by APDC and subsequent adsorption on C_{18}-bonded silica gel prior to determination by GFAAS[16] enabled concentrations of Se(IV) as low as 50 ng/l to be detected. Preconcentration of Se(IV) with the formation of a complex with 3-phenyl-5-mercapto-1,3,4-thiadiazole-2(3H)-thione potassium salt (Bismuthiol-II) have been successfully used by SAKAI et al.[19] where the complex was

Table 1. Concentrations of total inorganic selenium (ng/g) in different parts of the *Tilapia nilotica* species

Locations	Bone	Flesh	Head
1	297 ± 27	410 ± 31	93 ± 6
2	331 ± 28	410 ± 31	92 ± 7
3	367 ± 29	275 ± 23	82 ± 6
4	294 ± 26	289 ± 22	80 ± 7
5	381 ± 39	327 ± 26	88 ± 7
6	344 ± 24	316 ± 28	94 ± 8
7	362 ± 30	287 ± 21	76 ± 5
8	428 ± 36	371 ± 24	73 ± 5
9	349 ± 27	255 ± 17	75 ± 5
10	349 ± 24	248 ± 17	76 ± 6
11	417 ± 33	257 ± 19	77 ± 6
12	287 ± 22	267 ± 47	84 ± 7

Table 2. Concentrations of Se(IV) and Se(VI) in the bones of the *Tilapia nilotica* species

Locations	Se(IV), ng/g	Se(VI), ng/g	Se(IV)/Se(VI)
1	47 ± 3	233 ± 18	0.202
2	16 ± 1	280 ± 21	0.057
3	34 ± 3	293 ± 23	0.116
4	48 ± 4	221 ± 19	0.217
5	40 ± 3	302 ± 25	0.132
6	128 ± 10	195 ± 25	0.656
7	43 ± 4	279 ± 23	0.154
8	37 ± 4	318 ± 28	0.106
9	24 ± 3	287 ± 27	0.084
10	16 ± 2	290 ± 26	0.055
11	15 ± 1	358 ± 29	0.042
12	15 ± 1	241 ± 20	0.062

Table 3. Concentrations of Se(IV) and Se(VI) in the flesh of the *Tilapia nilotica* species

Locations	Se(IV), ng/g	Se(VI), ng/g	Se(IV)/Se(VI)
1	2 ± 0.15	395 ± 28	0.005
2	1 ± 0.05	390 ± 25	0.002
3	2 ± 0.05	260 ± 23	0.008
4	2 ± 0.05	214 ± 19	0.009
5	3 ± 0.08	312 ± 28	0.010
6	3 ± 0.06	301 ± 28	0.010
7	2 ± 0.05	272 ± 23	0.007
8	3 ± 0.05	355 ± 35	0.008
9	1 ± 0.04	240 ± 22	0.004
10	9 ± 0.15	213 ± 19	0.042
11	4 ± 0.08	242 ± 23	0.017
12	3 ± 0.05	212 ± 20	0.014

Table 4. Concentrations of Se(IV) and Se(VI) in the head of the *Tilapia nilotica* species

Locations	Se(IV), ng/g	Se(VI), ng/g	Se(IV)/Se(VI)
1	14 ± 0.5	68 ± 5	0.206
2	15 ± 0.8	72 ± 6	0.208
3	12 ± 0.9	56 ± 4	0.214
4	11 ± 0.9	58 ± 6	0.190
5	11 ± 0.8	56 ± 5	0.196
6	13 ± 0.7	68 ± 5	0.191
7	15 ± 0.7	51 ± 4	0.294
8	11 ± 0.6	49 ± 5	0.244
9	10 ± 0.6	53 ± 4	0.189
10	9 ± 0.3	57 ± 5	0.158
11	10 ± 0.6	50 ± 4	0.200
12	15 ± 0.9	55 ± 4	0.273

later adsorbed onto activated carbon and irradiated in a thermal neutron beam using the short-lived ^{77m}Se with a half-life of 17.5 seconds to calculate the amount of Se(IV) present in water samples. However, the success of the preconcentration and extraction step by APCDT-$CHCl_3$ followed by neutron activation analysis on aqueous samples for inorganic arsenic determination[17] prompted the application of this technique to the quantitative analysis of Se(IV) and Se(VI) in biological samples.

The *Lobster Hepatopancreas* TORT-1 SRM used in this study has a certified Se concentration of 6.88 ± ± 0.47 μg/g and the value obtained in this study using the Chelex-100 chelating resin separation is 6.22 ± ± 0.49 μg/g. This is equivalent to about 90.33% recovery and considered quite substantial, thus enabling this procedure to be used throughout the study. The microwave digestion with programmed heating was chosen insted of the normal heating to prevent massive loss of the volatile materials during the digestion step. The reproducibility of this technique in arsenic speciation was reported by YUSOF et al.[17] Table 1 shows

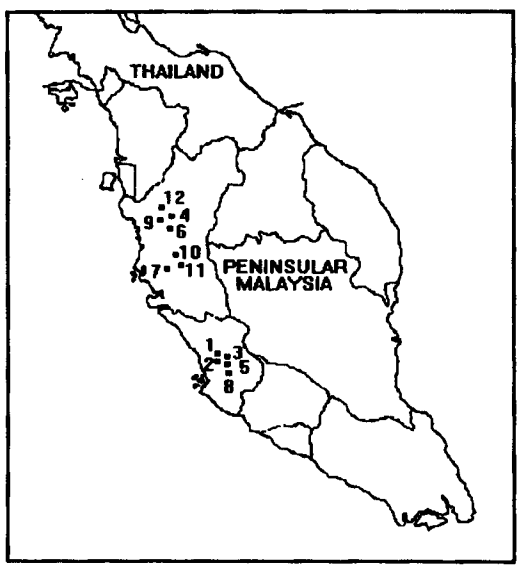

Fig. 1. Map of sampling locations for the species *Tilapia nilotica* samples

the concentrations of total inorganic Se in different parts of the *Tilapia nilotica* species using the Chelex-100 separation procedure. Comparatively, different parts of the fish seem to indicate special preference in the accumulation of the inorganic Se species. In general, the bones have relatively higher concentrations of inorganic Se than the flesh with the head showing the least affinity for this element. This trend is prevalent in all the samples taken from all the locations. Other parts of the fish might show different preference but since only the bone, head and flesh parts are normally used in the preparation of food, other parts are not relevant and therefore discarded.

The use of instrumental neutron activation analysis (INAA) on such trace elements as As, Se, Hg, Cr, Zn, Cd and Cu in biological samples could pose some serious problems due to the high ^{24}Na and ^{82}Br activities developed on irradiation. The broad Compton continuum of the ^{24}Na matrix activity will obscure the peak areas from the γ-rays from the ^{76}As and ^{75}Se. A post-irradiation chemical separation of the isotopes of interest, as reported by SHARIF et al.[20] is an alternative to overcome this problem. However, the extraction of Se(IV) species by APCDT at pH 1.5 has been successfully used[17] in eliminating the interfering alkali and alkaline earth metals and also the halogens. Interferences from other metallic ions in the determination of Se could be possible, especially by As and Bi. This is especially true in the hydride generation technique where not only As and Bi, but also Sn, Sb and Te have the tendency to interfere with the Se signals.[21] However, the use of INAA will overcome this problem because of the selective nature of the γ-rays analysis.

The Se(IV) and Se(VI) concentrations determined in the bone, flesh and head of the *Tilapia nilotica* species are presented in Tables 2, 3 and 4, respectively. The values given are expressed in μg/g dry weight. The normal weight used in the preparation of food is about five times more than the dry weight. Figures obtained for the Se(IV) concentrations in various parts of the *Tilapia nilotica* species show that the reduced form of the Se species is very much lower than the Se(VI) species. The bones of the *Tilapia nilotica* species have a greater tendency to concentrate the inorganic Se as compared to the head and flesh parts. This was also reflected in the figures obtained for the total inorganic Se contents using the Chelex-100 preconcentration step as shown in Table 1. The overall results indicate that most of the Se occur as the Se(VI) species and this is reflected in the low Se(IV)/Se(VI) ratio in the three parts of the *Tilapia nilotica* species investigated. The low Se(IV)/Se(VI) ratio also suggests that high oxidizing environment prevails in these water bodies thus favouring the formation of the Se(VI) than the Se(IV) counterpart. The inorganic Se uptake by the *Tilapia nilotica* species depends on the chemical forms of the element and its quantity, microbiological activities in the water and the age of the fish.

Very low levels of Se species are normally present in freshwater, usually in the range of 0.02–1.0 ng/ml[22] and this is one of the main difficulties in Se speciation. However, certain freshwater species have the tendency to accumulate the inorganic Se and this have been reported by SHARIF et al.[20] where the concentrations of inorganic Se from six different fish range from 2.96–6.27 μg/g. Concentrations of inorganic Se in the μg/g levels have also been found in fish flour[23] (1.8 μg/g) and in tuna fish,[24] as high as 5.1 and 6.2 μg/g based on dry weight. If the net weight of the fish during food preparation is about five times that of the dry weight, the average daily consumption is about 6 g per person and if the maximum total inorganic Se is about 0.833 μg/g (Table 1), then the estimated daily intake of inorganic Se would be in the region of less than 1 μg/g. If Se is as toxic as As, Pb and Cd, then the daily Se intake through fish is less than the maximum permissible level (MPL) of between 1–2 μg/g as recommended by the Ministry of Health[25] for fish and fish product. However, periodic monitoring of the Se levels in the freshwater species should be carried out to ensure that they do not exceed the tolerable limit which would then be detrimental to human health.

Conclusion

The simplicity of the preconcentration step using a complexing agent such as APCDT followed by INAA have eliminated the problem associated with interfering metallic ions and with sufficient cooling time after the irradiation (usually two weeks) most of the 264.7 and 136.0 keV photopeaks would be visible for peak area estimation. Most of the freshwater fish samples investigated showed inorganic Se contents well below the MPL of between 1–2 μg/g with most of the Se species in the higher oxidation state of Se(VI). This is also indicative of the highly oxidizing environment in the water bodies. Further studies would be needed to quantify the organoselenium constituents present in aquatic life from these disused mining pools for a complete speciation of Se species in these samples.

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