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THE SPECIATION OF ARSENIC IN SEAWATER AND MARINE SPECIES

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Dissolved inorganic arsenic species were determined in natural waters and selected marine species. Prior to irradiation by NAA samples were preconcentrated and the As(III) and As(V) species separated by APCDT-chloroform extraction. Marine samples were digested by microwave heating. Interpretation of data was achieved by comparing the ratio of As(III)/As(V) in the samples and correlating it with the prevailing environmental conditions.

Arsenic occurs extensively in the environment in different chemical species especially in natural water systems where it generally occurs in the oxidation states As(III) and As(V) as well as in food chain in various other forms. Since arsenic is both toxic and possibly carcinogenic, it has become one of the most widely measured trace metals in many environmental programs compared to other multiple oxidation-state elements such as iodine, chromium, antimony and selenium. The difficulty of isolating arsenic fractions in natural water systems explains the scarcity of data related to arsenic species distribution though much work has been done in the characterization of arsenic in fauna and flora primarily because of toxicological interest in food chain species. However the determination of As(III) and As(V) concentrations in natural waters has been extensively studied by FICKLIN[1], SUBRAMANIAM and MERANGER[2], AGGETT and KHADWANI[3] and MOK et al.[4] The presence of As(III), As(V), monomethylarsonic and dimethylarsinic acids have been measured in fresh estuarine and open seawaters by BRAMAN[5] and ANDREAE.[6] Biologically, As(III) is more toxic than As(V) and the concentrations of total arsenic in most natural water systems are in the range of 10^{-9} - 10^{-8} g/L.[4]

Though there was no evidence of the presence of trimethylarsenic species in seawater, a number of the heavier organo-arsenicals such as arsenobetaine, arsenocholine, acetylarsenocholine, arsenolipids, arsenosugars and the trimethylarsenic species have been detected in samples from biotic environments. Some of these species of lipid- and water-soluble arsenicals have also been isolated from the fauna and flora of natural water habitats and this suggests that the most widely observed biochemical pathway of arsenic in the environment is methylation.[7] The bioavailability and physiological or toxicological effects of arsenic on human beings depend on its chemical form and the order of toxicity for arsenic species decreases as follows : arsenite > arsenate > monomethylarsonic > dimethylarsinic acid > arsenobetaine.[8]

In view of the sub-nanogram levels of arsenic species in natural water systems, analytical techniques of high sensitivity should be employed in the determination. Various forms of chromatography, such as ion-chromatography, high-performance liquid chromatography, gas chromatography or selective volatilization of arsenic derivatives and also by graphite furnace atomic absorption spectroscopy have been employed. In any case the analytical procedure should be sensitive, simple and selective especially in the measurement of sub-nanogram levels of As(III) and As(V) for studying the chemistry of arsenic species in aquatic environment.

This paper describes a method that utilizes an extraction procedure for the separation and preconcentration of As(III) and As(V) from natural waters and selected marine species prior to neutron activation analysis (NAA). This method enables low levels of arsenic species to be determined with good extraction recovery. Extraction and preconcentration of arsenic species prior to irradiation were deemed necessary because firstly, the oxidation states of arsenic in the original samples irradiated

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cannot be distinguished after radiochemical separation and secondly the use of large volume of samples for irradiation without preconcentration is unsuitable due to the small concentration factor involved. The separation step was thought to be ideal to create an interference-free environment in the matrix during the activation period. Marine samples were subjected to digestion by microwave heating choosing a suitable program to prevent the change in oxidation states of arsenic species during the heating.

Materials and Methods

Natural surface water samples were collected from the coastal areas of Johore, Malaysia using a 1.5 L polyethylene bottle and later filtered through a 0.45 μm Millipore membrane filter in a Class-100 laminar flow hood. Marine samples were obtained from a fresh catch at the same locations where the water samples were collected. Preparation of the samples was done in less than 72 h prior to preconcentration and separation. A synthetic seawater used as a reference sample was prepared according to a prescribed procedure.[9] An SRM Lobster Hepatopancreas marine reference material (TORT-1) supplied by the National Research Council of Canada (NRCC) was used as reference material in the determination of arsenic species in marine samples by the comparison method. Marine species used in this study are swimming crabs (*Portunus pelagicus*) and blood clams (*Anadara granosa*).

All reagents were analytical grade and purified prior to use. Concentrated hydrochloric acid (10M) and nitric acid (16M) were prepared by sub-boiling distillation in a quartz still. Aqueous stock solutions of As(III) and As(V) were prepared by dissolving arsenic trioxide (As_2O_3) in 1 M HCl and sodium arsenate (Na_2AsO_3) in concentrated sulphuric acid (H_2SO_4) and diluted to the mark with deionized distilled water (DDW) respectively. Ammonium pyrrolidinecarbodithioate (APCDT) was purchased from Sigma Chemicals and the chloroform used in the extraction was obtained from Fluka. All containers were washed with Triton-X100 detergent solution and then soaked for at least 24 h in 10% HNO_3. After the soaking period the containers were rinsed with DDW and stored in a Class-100 clean hood equipped with a laminar flow filter.

A fresh batch of extraction solution was always prepared and this was done by dissolving 5 g of APCDT in 100 mL of DDW. Insoluble materials were filtered off and the removal of bromine and other impurities was achieved through shaking with chloroform. A 12% freshly prepared ethylenediamminetetraacetic acid (EDTA) solution was used as a masking agent in the extraction procedure. At least 200 mL of water sample was required in the extraction procedure. Prior to the As(III) extraction 100 mL of water sample was adjusted to pH 1.5 with 1 M HNO_3 followed by 2 mL of extraction solution, 4 mL of EDTA solution and 100 mL of chloroform. With the aid of a mechanical wrist action shaker, the samples were shaken for 10 min. and allowed to stand for 10 min. for phase separation. The organic phase was washed a few times with 5 mL batch of DDW. Then 8 mL of the organic phase was transferred to a flask and 2 mL of 50% HNO_3 was added to back extract arsenic. The same separation procedure was carried out and finally after phase separation 1 mL of the solution was transferred into a polyethylene vial and sealed for neutron irradiation. A second aliquot of the water sample adjusted to pH 1.0 with 1 M HNO_3 was required in the determination of As(V). Reduction of As(V) to As(III) was achieved using 1 mL of 25% sodium thiosulfate ($Na_2S_2O_3$) solution followed by 1 min. of shaking. Then 2 mL of the extraction solution, 4 mL of EDTA solution and 10 mL of chloroform was added to the flask and the total As(III) was extracted using the same procedure. The difference in the two arsenic concentrations represents the total As(V) in the sample.

Marine samples were freeze-dried and later ground to < 200 mesh size. Samples were sealed in pre-treated containers for further analysis. To digest marine samples, 1 g of the dry materials was placed in a closed Teflon PFA vessel and 5 mL of 70% HNO_3 added prior to heating in a microwave digester model MDS-81D. A safety valve was placed on the PFA vessel and the cap tightened using a capping station. The vessel was then placed in the turntable and a venting tube attached before the heating program commenced. Since 12 PFA vessels can be placed in the turntable, duplicates or several samples can be digested simultaneously. A heating program that requires 5 min. of heating at 48°C running at 30% power was suitable to digest the samples without apparent loss of arsenic. This

was achieved by running samples spiked with As(III) and As(V) as well as Lobster Hepatopancreas TORT-1 as reference material. The resultant solutions were allowed to cool for about 30 min. before preparing the stock solutions for analysis. Preconcentration and separation for As(III) and As(V) were carried out similarly to natural water samples.

Since liquid samples were used in the irradiation step special care was taken when samples were sealed in the polyethylene vials. Standards used were supplied by Merck. All samples and standards were irradiated for 6 h in a 1-MW TRIGA Mark-II reactor at the Nuclear Energy Unit in a neutron flux of 4×10^{12} n/cm^2/s followed by 3 days of cooling before counting commenced. Signals from the 559-keV γ-rays from ^{76}As were used to determine the arsenic concentration in the samples. Counting was done over a period of 3600 s using a detection system supplied by by EG&G ORTEC incorporating a large volume coaxial hyperpure germanium detector with a resolution of 1.9 keV at 1332 keV ^{60}Co. The signals were then amplified and analyzed by ADC ND592 (Nuclear Data) Analyzer connected to a ND6000 (Nuclear Data) multichannel analyzer. Spectra from the MCA were analyzed using PEAK program supplied by Nuclear Data with a ND6680 computer.

Results and Discussion

The approximate location of the sampling stations is depicted in Fig. 1. Sampling sites were chosen in coincidence with areas where fishing villages are located enabling marine species to be collected. In an earlier work,[10] sediments were collected at the prescribed stations and an elemental analysis carried out. Blood clams (*Anadara granosa*) and swimming crabs (*Portunus pelagicus*) are possibly the two species commonly found feeding at the sea-bed as other non-localized species would not give a true representation of the environment chosen.

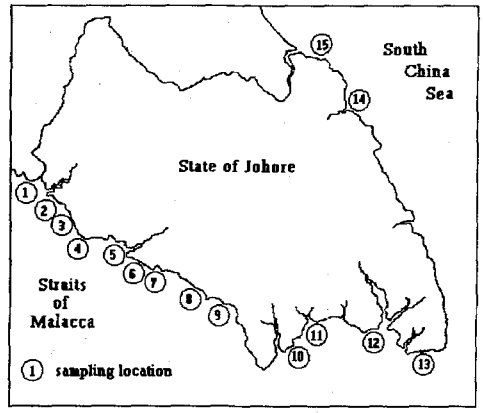

Fig.1
Map of sampling locations for seawater and marine samples

Inorganic As(III) species are readily extracted by APCDT at pH 1.5, thus eliminating the interfering matrix species including the alkali and alkaline earth metals as well as the halogens especially ^{23}Na and ^{81}Br which will interfere in the γ-ray analysis of ^{76}As at 559 keV. With the elimination of bromine possible overlapping of the 556 keV γ-rays emmited by ^{82}Br is overcome. The percentage efficiency of As(III) and As(V) extraction is given in Table 1. Lobster Hepatopancreas TORT-1 was used as a reference material besides samples spiked with As(III) and As(V). It was found

Table 1
Recovery of spiked As(III) and As(V) in samples by extraction with APCDT-chloroform

Samples	As(III) added (ng)	As(V) added (ng)	% recovery	
			As(III)	As(V)
seawater(100 mL)	100	100	87.9 ± 2.0	85.0 ± 2.8
seawater[a]	100	100	100.4 ± 4.2	96.0 ± 5.7
seawater[b]	100	100	43.2 ± 2.8	55.3 ± 3.4
		As(total) μg/g	% recovery of As (total)	
TORT-1[a]		24.6 ± 2.2	101.6 ± 3.2	
TORT-1[b]		24.6 ± 2.2	74.1 ± 3.5	

a digestion at 48°C at 30% power
b digestion at 86°C at 30% power

that substantial amounts of As(III) and As(V) were recovered from the spiked natural water samples, 87.9 ± 2.0% and 85.0 ± 2.8% respectively, thus enabling this method to be used throughout this study. The slight drop in the percentage recovered for As(V) could be due to partial extraction by APCDT when the samples were not shaken immediately after the addition of thiosulfate solution leading to incomplete reduction of the As(V) species. Seawater samples spiked with As(III) and As(V) were also subjected to microwave heating at different heating programs to simulate digestion of aqueous marine samples. Better recoveries of As(III) and As(V) were obtained with a lower heating digestion. However digestion at 86°C resulted in massive loss of arsenic species. The back-extraction was necessary to get the organic-based As(III) in chloroform back into the aqueous phase before irradiating the samples. A high concentration factor could be obtained using this procedure and the use of concentrated HNO_3 does not interfere with the NAA method in arsenic determination. Complete digestion of marine samples was achieved by programmed heating and cooling with good recovery for the As(III) and As(V) species spiked in the standard solution and the results are shown in Table 1. This procedure was found suitable in marine sample analysis prior to the extraction step and was used in the analysis of NRCC Lobster Hepatopancreas TORT-1 to determine the recovery of the certified arsenic content of 24.6 ± 2.2 μg/g. No information was given regarding the arsenic species in the reference material, therefore it was assumed that the arsenic extracted was total arsenic. The amount recovered using NAA and hydride generation AAS was 23.7 ± 2.6 μg/g and this agrees well with the certified value. The results of selective determination of As(III) and As(V) species in seawater samples taken from various sites using the proposed extraction method followed by NAA are presented in Table 2.

The concentration of total dissolved arsenic in seawater is normally between 1.0 and 2.0 ng/L mainly in the form of arsenate [As(V)], arsenite [As(III)] and the methylarsenic. The total dissolved arsenic obtained in this work is between 0.65 - 1.78 ng/L with an average value of 0.95 ± 0.35 ng/L and is within the range for most natural waters. Since As(III) is a thermodynamically unstable oxidation state, the predicted ratio of As(III)/As(V) under oxidative conditions is 10^{-26}.[11] However, in exceptional cases, mostly in coastal waters as carried out in this study, As(V) becomes a relatively minor fraction probably due to continuous discharge of arsenic compounds through anthropogenic activities thus increasing the arsenic content of many estuarine waters. This trend is clearly seen from Table 2 where the As(III)/As(V) ratio is rather high in most cases. Inorganic As(III) constitutes more than 19% of the total dissolved arsenic species although the total dissolved arsenic content in most of the samples falls within the average value of 1.0 - 2.0 ng/L. The rather high As(III) ratio in the shallow

sub-surface region of the water depth profile could be attributed to heterotrophic (bacterioplankton) activity especially in the zone of mixing of surface and intermediate waters. Arsenic (III) has been reported to be the dominant inorganic species in surface waters of tropical gyres and the high internal cycling of phosphorous results in the rapid uptake of As(V) by phytoplanktons in the warm waters and rapidly excreting it in the reduced As(III) form.[12] The settlement of industrial zones along the coastal areas particularly in the southern region of the study area attributes to the increased level of arsenic

Table 2
Concentration of As(III) and As(V) determined in water samples

sample location	As(III) ng/mL	As(V) ng/mL	As(total) ng/mL	As(III)/As(V)
1	0.26 ± 0.02	0.36 ± 0.02	0.63 ± 0.02	0.75
2	0.23 ± 0.01	0.54 ± 0.02	0.77 ± 0.02	0.43
3	0.36 ± 0.02	0.42 ± 0.02	0.78 ± 0.03	0.86
4	0.16 ± 0.02	0.23 ± 0.02	0.38 ± 0.02	0.70
5	0.32 ± 0.03	0.48 ± 0.02	0.80 ± 0.03	0.67
6	0.48 ± 0.02	0.44 ± 0.07	0.91 ± 0.09	0.92
7	0.38 ± 0.02	0.50 ± 0.04	0.89 ± 0.03	0.76
8	0.26 ± 0.04	0.39 ± 0.04	0.65 ± 0.05	0.67
9	0.20 ± 0.03	0.85 ± 0.04	1.05 ± 0.04	0.24
10	0.40 ± 0.02	0.48 ± 0.03	0.88 ± 0.03	0.83
11	0.30 ± 0.03	1.25 ± 0.02	1.55 ± 0.03	0.24
12	0.36 ± 0.02	1.41 ± 0.03	1.78 ± 0.04	0.25
13	0.33 ± 0.03	0.83 ± 0.02	1.17 ± 0.03	0.39
14	0.32 ± 0.02	0.84 ± 0.02	1.17 ± 0.03	0.38
15	0.32 ± 0.01	0.56 ± 0.03	0.89 ± 0.04	0.57

discharged together with some phosphorous-based compounds. Streams and rivers are usually used to discharge treated industrial effluents, nevertheless on reaching the estuaries gross changes in arsenic speciation occur where reduced and some methylated species can exceed inorganic As(V) concentration.

In general marine organisms accumulate more arsenic than fresh-water organisms. The high arsenic levels found in certain marine organisms reflect the prevailing high environmental arsenic levels either in the water or sediment. Although the dissolved arsenic concentrations are normally low certain localized marine species feeding at the seabed can accumulate arsenate.[13] The two marine species studied, the swimming crabs and blood clams, commonly served as a popular local delicacy were chosen and they were collected from the specified sites. The concentrations of dissolved As(III) and As(V) determined in these species together with the total arsenic found in the corresponding sediment samples are presented in Table 3. The uptake of dissolved arsenic is more pronounced in the blood clams compared to the swimming crabs. An average value of 11.22 ± 4.29 ng/g and 3.93 ± 1.99 ng/g was determined in blood clam and swimming crab samples, respectively. There are no clear patterns governing the uptake of dissolved arsenic even when related to the total arsenic contents in the sediments taken from the same location. However, in general the As(V) concentrations were relatively higher than that of As(III) in all the samples. Total organo-arsenicals isolated from the meat of red crab (*Chinoecete opilio*)[14] was reported to be about 2.1 μg/g while those of several types of clams[15]

ranges from 1.2 - 2.2 μg/g. The total arsenic determined in the muscle of some molluscs collected from the South Australian marine environment[16] exhibited values ranging from 3.9 - 47 μg/g. These figures are relatively high and showed no correlation patterns in the same species. Accumulated evidence shows that the major arsenicals found in marine species occur as the non-toxic organo-arsenic with only small quantities present as the more toxic inorganic species.[15] This evidence explains the low level of dissolved arsenic reported in this work with the average values ranging from 1.7 - 7.5 ng/g in the swimming crabs and from 6.9 - 15.5 ng/g in the blood clams.

Table 3
Selective determination of As(III) and As(V) concentrations in marine species

sample	As(III) ng/mL	As(V) ng/mL	As(total) ng/mL	As(total) in sediments (μg/g)
SC(5)	1.59 ± 0.16	3.09 ± 0.23	4.68 ± 0.09	8.00 ± 0.50
SC(8)	1.54 ± 0.06	1.60 ± 0.14	3.22 ± 0.09	10.70 ± 0.80
SC(9)	1.73 ± 0.24	3.32 ± 0.16	5.06 ± 0.09	12.30 ± 1.05
SC(12)	0.91 ± 0.12	0.86 ± 0.12	1.77 ± 0.12	36.40 ± 2.60
SC(13)	0.21 ± 0.05	1.92 ± 0.06	2.13 ± 0.08	7.30 ± 0.60
SC(14)	1.02 ± 0.07	6.52 ± 0.12	7.54 ± 0.17	16.80 ± 1.20
SC(15)	0.70 ± 0.06	2.40 ± 0.05	3.12 ± 0.04	6.90 ± 0.50
BC(6)	1.86 ± 0.11	9.40 ± 0.14	11.26± 0.17	8.20 ± 0.60
BC(9)	1.82 ± 0.10	5.09 ± 0.10	6.91 ± 0.10	12.30 ± 1.05
BC(13)	2.60 ± 0.16	12.80± 0.21	15.49± 0.26	7.30 ± 0.60

SC - swimming crab (*Portunus pelagicus*) (5) - location
BC - blood clam (*Anadara granosa*)

Threshold values in arsenic uptake vary depending on the organism.[17] Dissolved arsenic concentrations are normally low (1 - 2 μg/L) and marine organisms have the ability to concentrate arsenic. Arsenic uptake is either proportional to the arsenate concentration until the threshold value is reached, or independent of arsenate concentration. However there are cases where these criteria are not met probably species like mussels decrease their filtering rate in response to increased external arsenic concentration[18] Studies conducted on a large number of fish, crustaceans and molluscs[19] showed that the concentrations of inorganic arsenic present in these species were insignificant when compared to the total arsenic concentrations. Among the total organo-arsenic compounds found in marine species, arsenobetaine ($(CH_3)_3As^+CH_2COO^-$) appears to be widely distributed in marine animals at different trophic levels and is probably the end-product of arsenic metabolism in the marine ecosystem. The varying amount of arsenic uptake by the species could be related directly to their food source which in turn depends on the prevailing environmental conditions such as surface temperature, salinity and also exposure that govern the phytoplankton population and which varies from one sampling area to another.

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