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Refractory arsenic species in estuarine waters

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Total digestion of estuarine water samples by dry ashing shows that a significant fraction of dissolved arsenic does not form hydrides with sodium tetrahydroborane ($NaBH_4$) and is therefore not detected by the normal hydride generation–atomic absorption analytical technique. It is also refractory to alkaline digestion with sodium hydroxide. Sequential ultrafiltration suggests a molecular weight below 210 for this new arsenic fraction. The magnitude and molecular weight of this fraction may open new perspectives on the biogeochemical cycling of arsenic. Ecological reasons can explain the discrepancy between these findings and previous research.

Keywords: organoarsenic, hydride generation, digestion, transmethylation, refractory, biogeochemical cycling, arsenobetaine

INTRODUCTION

Arsenic speciation in the marine environment is an active field of research as new arsenic forms are detected and identified in different compartments.[1-11] The limitations of the analytical methods available for the determination of arsenic species[12-14] make it likely, however, that a number of stable compounds remain undetected in estuarine waters. Whilst highly sensitive and selective, the methods used to date for the determination of arsenic species in natural waters require the derivatization of dissolved arsenic species to volatile hydrides, and thus will not detect species which do not form hydrides under the reaction conditions employed.

In the course of our investigations of the origin of trimethylated forms of arsenic in the waters of the Tagus we have detected a new arsenical fraction which is refractory to the hydride method.[15,16] This fraction could be broken down to various methylarsenicals (monomethylarsenic, MMA; dimethylarsenic, DMA; trimethylarsenic, TMA) by alkaline digestion ($0.5\ \mathrm{mol\ dm^{-3}}$ NaOH/80 °C/24 h). DMA usually predominates, in this digest.[15,17] Further processing of a separate water sample through sequential ultrafiltration combined with alkaline digestion suggests a molecular weight of less than 210 for this component.[15] In these experiments, the eventuality that the methylarsenicals found could be produced during alkaline digestion through transmethylation of reduced forms of arsenic (e.g. methyl(hydroxy)arsines,[18,19] although not compatible with the models that have been studied,[17,20,21] could not be entirely ruled out.

In order to resolve these issues we collected a water sample in the Tagus estuary in August 1987 and analysed it for total arsenic after total digestion by dry-ashing with $MgO/Mg(NO_3)_2$. Total digestion of some of the ultafiltration fractions that were obtained previously was also performed. The results of this study are presented in this paper.

MATERIALS AND METHODS

Water samples were collected and preserved according to standard procedures.[15] As discussed previously,[15] the sample for ultrafiltration was a composite sample, collected in winter in the middle and lower estuary of the Tagus (30 January 1987). A simplified scheme of the ultrafiltration sequence is depicted in Fig. 1. Ultrafilters used were Schleicher & Schuell (50 000 Da), Millipore PCAC (1000 Da) and Amicon Diaflo YCO5 (210 Da). For total digestion the terminal fractions of the ultrafiltration sequence (C_1 and D_1) were chosen; they are separated by the cut-off (NMWL) of the last ultrafilter, at about 210 Da.[22,23] Sample G was collected in summer (26 August 1987) at Praia do Alfeite.

Alkaline digestions were performed as indicated in the Introduction. For total digestion we adapted the classical dry-ashing procedure to the digestion of water samples.[24] An adequate volume ($2\ \mathrm{cm^3}$ per $50\ \mathrm{cm^3}$ of sample) of ashing-aid (a

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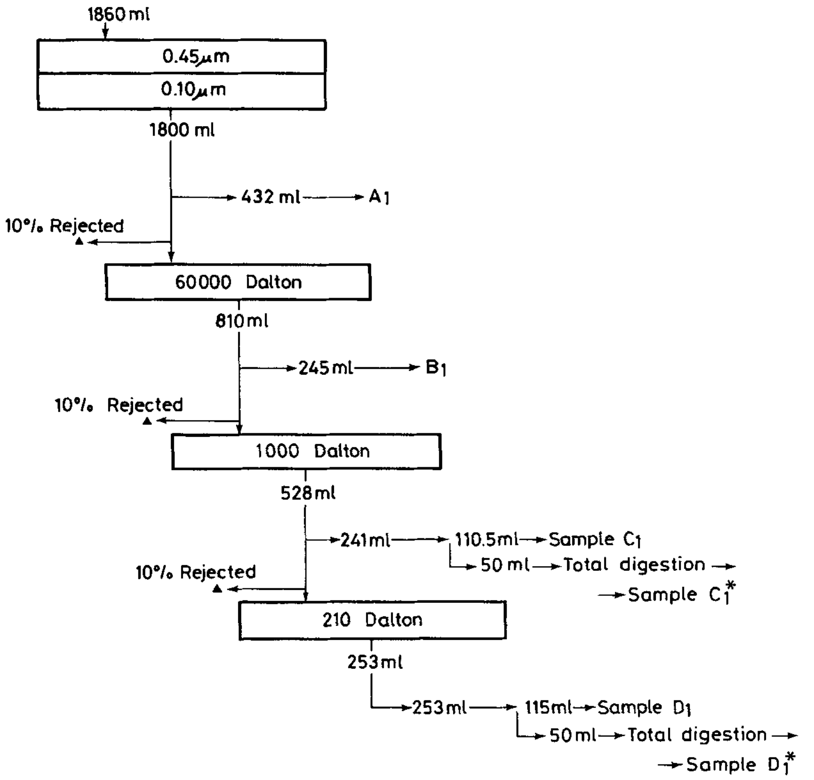

Figure 1 Ultrafiltration sequence. C_1, D_1, undigested fractions; C_1^*, D_1^*, subsamples of fractions C_1 and D_1 that were totally digested (dry-ashing).

slurry of 7 g $MgO + 10.5$ g $Mg(NO_3)_2.6H_2O$, in 100 cm^3 of Milli-Q water) is added to the sample, the mixture dried at 80 °C overnight and heated (1 h/200 °C + 1 h/300 °C + 12 h/460 °C) in a muffle furnace. The dry residue is taken up with 2M-HCl and made up to 50 cm^3 with double-distilled, deionized Milli-Q water, prior to analysis. Blanks of Milli-Q water were run in the same way and standards of synthetic arsenocholine ($(CH_3)_3\overset{+}{A}sCH_2CH_2OH$) (1 000 ng cm^{-3}) added to subsamples in order to determine the yield of the digestions.

All arsenic forms were detected and quantified by a sensitive and species-selective version of the hydride method, which has been described in detail previously.[25, 26]

RESULTS

In Table 1 we present the results of total (dry-ashing) digestions performed in two subsamples of sample G (G_1 and G_2). G_1 was taken as an undigested subsample, while G2 is a subsample of G that has been previously digested with sodium hydroxide. We also present in this table the concentrations of total inorganic ($As^{5+} + As^{3+}$) and methylated hydride-forming arsenic species (MMA + DMA + TMA) present in these subsamples prior to total (dry-ashing) digestion (2nd and 3rd columns). The yields of the total digestions performed are given in column 5. The corrected values for total (dry-ashing) arsenic (TA) are presented in column 6.

Total refractory arsenic (TRA) (7th column, 3rd row) is given by the difference between the corrected total arsenic (6th column, 2nd row) and total hydride-forming arsenic (total methylated + total inorganic) for subsample G_1 (4th column, 1st row). Refractory undigestible arsenic (RUA) (7th column, 7th row), on the other hand, is obtained by the difference between the corrected total arsenic (6th column, 6th row) and total hydride-forming arsenic of subsample G_2 digested beforehand with sodium hydroxide. A refractory 'digestible' fraction (RDA) can be computed from these two refractory fractions

Table 1 Total digestion (Sample G)

Fraction	As (μg As dm^{-3} ± SD) Inorganic	 Methylated hydrides	Total As (μg As dm^{-3} ± SD)	Yield (%)	Total As, corrected (μg As dm^{-3} ± SD)	As, refractory (μg As dm^{-3} ± SD)
G_1	19.2 ± 0.1	0.51 ± 0.07	19.7 ± 0.1			
G_1^*	24.0 ± 1.1	N.D.	24.0 ± 1.1		24.3 ± 1.1	
$G_1^* - G_1$				94.2		4.6 ± 1.1
Blank 1	N.D.	N.D.	N.D.			
G_2	18.7 ± 0.1	1.45 ± 0.23	20.2 ± 0.3			
G_2	23.3 ± 0.6	N.D.	23.3 ± 0.6		24.2 ± 0.8	
$G_2^* - G_2$				79.0		4.0 ± 0.8
Blank 2	N.D.	N.D.	N.D.			

Abbreviations: SD, standard deviation; N.D., below detection limit; * denotes total (dry-ashing) digestion.

(RDA = total refractory − refractory undigestible).

Figure 2 depicts the partitioning of sample G between its total inorganic (TIA), methylated hydride-forming (MHA), refractory 'digestible' (RDA) and refractory 'undigestible' (RUA), arsenic fractions.

Table 2 presents a complete fractionation of sample G. Total inorganic (TIA) and total (dry-ashing) arsenic (TA) are averaged between subsamples G_1 and G_2; the table further shows the resulting concentrations of total hydride-forming (THA), refractory 'digestible' (RDA), refractory 'undigestible' (RUA), total refractory (TRA = RDA + RUA) and total 'organic' arsenic (methylated hydride-forming + total refractory) of the same sample. The methylated hydride-forming arsenic (MHA) is the same as in Table 1 (G_1).

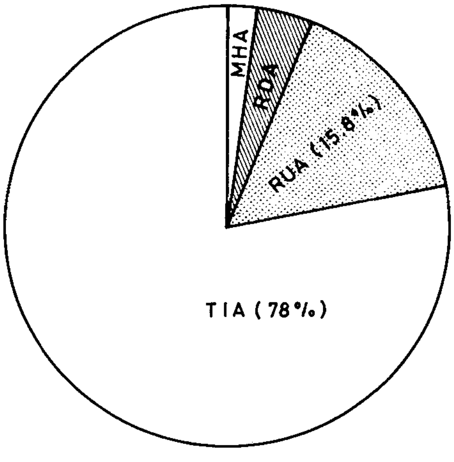

Figure 2 Partitioning of arsenic in sample G. TIA, total inorganic arsenic; MHA, methylated hydride-forming arsenic (MMA + DMA + TMA); RDA, refractory 'digestible' arsenic; RUA, refractory 'undigestible' arsenic.

In Table 3 total arsenic and the fractionation within total inorganic (TIA), methylated hydride-forming (MHA), refractory 'digestible' (RDA) and refractory 'undigestible' arsenic (RUA) for the low-molecular-weight fractions (C_1 and D_1) of the January sample are presented. The methylated hydride-forming (MHA) and the refractory 'digestible' arsenic concentrations were determined previously. TIA was averaged over the undigested subsamples of the ultrafiltration sequence.[15]

Total refractory arsenic corresponds to about 20 and 19% of the total content of arsenic in summer and winter (ultrafiltration) samples, respectively. On the other hand, the content of refractory 'undigestible' arsenic reaches 16 and 18%, being clearly the dominant portion of the refractory fraction in both cases (80 and 97%, respectively). A simplified version of the

Table 2 Fractionation of sample G

As fraction	Concentration (μg As dm^{-3} ± SD)	Percentage
Inorganic (TIA)	18.9 ± 0.1	78.2
Methylated hydrides (MHA)	0.51 ± 0.1	2.1
Total hydrides (THA)	19.5 ± 0.1	80.3
Refractory 'digestible' (RDA)	0.95 ± 0.2	3.9
Refractory 'undigestible' (RUA)	3.8 ± 0.3	15.8
Total refractory (TRA)	4.8 ± 0.2	19.7
'Organic' total (TOA)	5.3 ± 0.2	21.8
Total (TA)	24.2 ± 0.2	100.0

Table 3 Total digestion and fractionation of ultrafiltration fractions

		As content (μg As dm^{-3} ± SD)				
Fraction	Cut-off	TIA	MHA	RDA	TA[a]	RUA
C_1	$210 \leq$ NMWL ≤ 1000	5.96 ± 0.09	0.092 ± 0.044	0.054 ± 0.006	7.22 ± 0.29	1.25 ± 0.30
D_1	NWML ≤ 210	5.96 ± 0.09	0.087 ± 0.007	0.038 ± 0.005	7.37 ± 0.36	1.41 ± 0.36

[a] Total (dry-ashing) digestion.

Abbreviations: SD, standard deviation; NMWL, nominal molecular weight limit.

Student's *t* test[27] shows all these differences (total refractory and refractory 'undigestible' arsenic of sample G) to be significant at the 95% level. This test further establishes refractory 'undigestible' arsenic (and also total arsenic) as not being significantly different between consecutive stages of the ultrafiltration sequence (C_1, D_1, Table 3), at the same level of confidence. So, apparently, the molecular weight of the refractory species must be below 210.

The results provide clear evidence of the presence in the estuarine waters of the Tagus of a component refractory to the hydride method and also undetectable through sodium hydroxide digestion, at least under our operating conditions. This component seems to be present in the Tagus both in summer and in winter time.

DISCUSSION

These results are in contrast with observations from most previous studies. Andreae[28, 29] subjected water samples collected in the Gulf of Mexico to total (dry-ashing) digestion. Although the same method was used as was applied here for the Tagus samples, the samples from the Gulf of Mexico showed no significant difference between digested (total) and undigested aliquots. Peterson and Carpenter[30] did find a discrepancy between total arsenic (determined by co-precipitation with ferric hydroxide followed by neutron activation analysis) and total hydride-forming arsenic concentrations in the aerobic layer of an intermittently anoxic fjord. However they did not consider the difference to be significant. Other authors arrive at similar conclusions although they do not make clear the method employed.[31, 32]

The results presented here are apparently consistent with the evidence obtained later by Howard and Comber for Southampton Water.[33] These authors found, in water samples, a fraction of arsenic that is undetectable by the direct application of the hydride method but can be observed following UV irradiation. A 79% increase of the DMA content was found under these conditions. On average, this fraction represents about 25% of all the arsenic present in the water, which is quite consistent with our own findings (19–20%). However the ultraviolet (UV)-liberated arsenic detected in Southampton water showed a seasonality that is similar to that observed for methylated arsenic in the same region,[12, 34] whereas no evidence of seasonal variation can be deduced from our own results for the Tagus. Also it is by no means proved that the methods used in these investigations [total (dry-ashing) digestion in the Tagus; UV irradiation in Southampton Water] are equivalent. So, although an obvious convergence is evident, it is not clear at all that these two fractions of refractory arsenic are the same.

Our results make it clear, however, that the chemistry of arsenic in the aquatic environment can no more be considered as the chemistry of its inorganic ions and simple methylated forms, as has been done formerly.[29, 35] In fact the content of refractory arsenic found (1.3–4.6 μg As dm^{-3}) clearly exceeds the concentrations of 'total' arsenic believed to be typical for seawater (1.7–2.2 μg As dm^{-3}).[17, 36] The refractory 'undigestible' arsenic also represents 16 to 18% of the true total As content in Tagus waters, which is about the same proportion as the average arsenite (As^{3+}) fraction in coastal seawater[37] and by far exceeds both the simple methylated forms and the refractory 'digestible' arsenic previously found, which represents between 1 and 2%.[15, 17]

The magnitude of this fraction further suggests a preferential pathway for arsenic cycling through the water phase. This hypothesis can be of some significance in controversial issues such as the question of the origin of arsenobetaine in the tissues of marine animals. In fact the ubiquitous presence of this compound in these tissues[31] has been up to now, explained through its concentration along food-chain pathways[38–40] due to the lack of empirical evidence that could support the concurrent hypothesis of a preferential transfer through the liquid phase.[21, 28, 29, 31, 35, 41]

This is not without difficulties, however. Repeated experiments with a variety of arsenic substrates including arsenosugars fed to different target organisms known to accumulate arsenobetaine in nature (fish, crustacea, bivalve molluscs and copepoda) proved to be unsuccessful in the production of this compound in tissues.[43–46] On the other hand what is known about the physicochemical properties of arsenobetaine and some of its suggested precursors [dimethyloxyarsylethanol, does not favour the selectivity presumed to be required for accumulation along a food chain.[42] The biosynthesis of arsenobetaine and particularly the metabolic quaternization of the arsenic atom is also far from being clearly understood.[17, 31, 42] Moreover, detailed studies on a wide base of evidence show that there is no obvious relationship between the arsenobetaine content in tissues and the trophic position of an organism.[47] Consequently, despite some recent claims of success with mice,[48] the food-chain pathway hypothesis remains controversial as a basic explanation for the origin of arsenobetaine in marine animals.[31, 42]

In this connection the detection of a significant fraction of the estuarine arsenic pool in the water compartment seems to open new perspectives. The new refractory arsenic found in water samples should therefore be a focal point of further research on the biogeochemical processes of arsenic cycling in the estuarine marine environment.

Our findings also suggest that the new refractory 'undigestible' arsenic fraction should have a molecular weight not exceeding 210, which is consistent with the upper limit previously found for the 'digestible' refractory component, RDA, in the same sample.[15]

A number of compounds known to occur in the estuarine environment, like arsenocholine, are refractory to hydride production, undigestible with sodium hydroxide and have a molecular weight lower than 210.[7, 16, 49] These compounds are likely candidates for the identity of the refractory arsenic species we observed.[17]

However, instead of speculating on their chemical nature, which is certainly premature, it appears more important to resolve the obvious divergence between the results presented here and those of previous research.[28, 30–32] Ecological and ecophysical differences are a possible line of explanation. In fact, considerable differences prevail in this domain between estuarine and ocean phytoplankton species, particularly concerning their ability to biotransform and recycle arsenic species.[50] Estuarine phytoplankton communities, dominated by rapidly growing opportunistic species adapted to a changing environment relatively rich in nutrients,[51] are expected to process greater quantities of arsenic than ocean species adapted to a more stable low-nutrient environment.[50] Consequently higher levels of organoarsenic metabolites are likely to be present in estuarine waters than in coastal and offshore waters. Furthermore, it is possible that organisms dependent on estuarine and near-shore habitats and not present in the open ocean (e.g. benthic macrophytes) are the dominant producers of the refractory arsenical fraction(s) now detected.

The stable nature of the compounds in question, a reasonable assumption based on their refractory and undigestible character, will facilitate their accumulation to concentrations above the detection limits.

We are aware of the fact that the present findings raise more questions than they settle. Much more information will have to be collected, in field and experimental situations, to clarify these issues. It seems likely that the positive identification of the newly observed component(s) will be an important step towards the development of a comprehensive picture of arsenic speciation and biogeochemistry in the estuarine environment.

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