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The photo-oxidation of solutions of arsenicals to arsenate: a convenient analytical procedure

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The separation of arylarsonic acids by HPLC on a reverse-phase C_{18} column is described. Solutions containing these arsenicals and others such as arsenobetaine are photo-oxidized to arsenate by ultraviolet (UV) radiation (1200 W, 1 h exposure). This allows the analysis of the solution for arsenic by hydride generation techniques. The method, UV HGAA, is developed and applied to the determination of arsenic in the methanol extracts of the Manila clam (*Verupis japonica*) and the Horse clam (*Schizothoerus nutalli*).

Keywords: Organoarsenic, analysis, hydride generation, photo-oxidation, marine clams, HPLC, GFAA

INTRODUCTION

Arsenic analysis by atomic absorption spectrometry (AA) has been the focus of much research. Although conventional flame AA is inherently insensitive for the determination of arsenic, the use of graphite furnace and hydride generation techniques has greatly reduced the detectable concentration levels. In particular, hydride generation techniques have achieved wide approval and use.[1–9]

Hydride generation usually involves the reduction of arsenate, arsenite, methylarsonate or dimethylarsinate by sodium borohydride to the appropriate arsine $Me_xH_{3-x}As$, $x=0$–2. The reduction is pH-dependent,[7] which allows some speciation of mixtures. Further speciation can be accomplished by trapping (cryofocusing)[8] the arsine *mixture* and making use of the relative volatility of the arsines in the warmed sample to effect separation. Alternatively, gas chromatography can be used to quantitate the mixture of arsines after cryofocusing.[9]

Most arsenicals, however, are not reduced to volatile arsines and this class includes many naturally occurring arsenic compounds such as arsenobetaine,[10–13] arsenocholine,[13] tetramethylarsonium salts[10] and arseno sugars.[14,15] All these arsenicals have to be converted to reducible derivatives if hydride generation techniques are to be employed in their analysis, and this is generally done by wet digestion of samples with various combinations of acid,[10,11,16,17] base[18] or by dry-ashing with magnesium nitrate.[19] However, these methods are difficult to apply directly if the sample is already in solution.

This work describes a photo-oxidation procedure which can be used to decompose organoarsenic compounds, in solution, to arsenate. A continuous hydride generation system can then be used to quantitate the arsenic present in the sample. The method is applied to arylarsenicals and to methanol extracts of marine clams. HPLC procedures for separating these classes of arsenicals are also described.

EXPERIMENTAL

Instrumentation

A Varian Techtron Model AA1275 atomic absorption spectrometer was used for arsenic determination. This was equipped with a hollow cathode lamp operating at 7 mA, a wavelength of 193.7 nm, and a slit width of 1 nm. The AA was also equipped with a deuterium background corrector and an HP 82905A printer. Graphite furnace atomization was achieved with a Varian Techtron GTA-95 accessory. The continuous hydride generation system used in this study consisted of a Gilson Miniplus 2 four-channel peristaltic pump which was used to withdraw the sample and mix it with hydrochloric acid. This solution was then reacted with sodium borohydride solution (4%) and introduced into the gas–liquid separator via a 20-turn mixing coil. The design of the apparatus is similar to that described previously.[20] The stripped gases are led into a conventional open-ended T-shaped quartz absorption cell [8.5 cm × 1 cm (o.d.)] mounted in the air/acetylene flame of a standard Varian burner.

A Waters HPLC instrument equipped with M45 and M510 pumps and automated gradient controller was used for HPLC separations on a C_{18} reverse-phase column [μBondpak™ C18, 3.9 mm (i.d.) × 30 cm steel, Waters]. Chromophoric groups were detected using a Waters M481 variable-wavelength detector (and associated Waters QA-1 Data System). Graphite furnace AA (GFAA) was used as an arsenic-specific detector; fractions were collected from the column and transferred to the automatic delivery system of the AA spectrometer.

Chemicals and reagents

All chemicals were of reagent grade or better. Arsenobetaine[21] and arsenocholine[22] were prepared by literature methods. Clams were collected from Thetis Island, BC, Canada, and frozen until needed.

Clam extracts

Frozen clams were first thawed, deshelled and weighed (wet). They were homogenized (10 g–1000 g in a blender with methanol (2.5 cm^3 g^{-1} tissue). The homogenized sample was then left on a mechanical shaker for a week. The sample was then filtered off and the filtrate analyzed. For the UV irradiation experiments, 50 cm^3 of the extract was used or, better, smaller volumes, usually 1 cm^3, were made up to 50 cm^3 with water.

Irradiation procedure

The sample (50 cm^3) in a quartz container (2.5 cm o.d.) was irradiated for a specified time using a 1200 W medium-pressure lamp (Hanovia). The sealed tubes (maximum 24) were arranged around the lamp in a fan-cooled carousel.[23] After irradiation the solutions were allowed to cool, reconstituted to volume, and examined by HPLC and/or hydride generation AA (HGAA). In many experiments, the GFAA signals and HGAA signals of the solution were measured before and after irradiation.

Method (HGAA)

Hydride generation from arsenate (1 ppm, μg cm^{-3}) was studied in order to establish optimum operating conditions which were used for all subsequent determinations. These conditions are given in Table 1.

Table 1 Operating conditions for hydride generation

Sample acidity	4 mol dm^{-3}
Sample flow	3.5 cm^3 mm^{-1}
$NaBH_4$ concentration	4% (in 0.05% NaOH)
$NaBH_4$ flow	3.5 cm^{-3} min^{-1}
Carrier gas flow (Ar)	0.6 dm^3 min^{-1}

The signal from hydride generated from arsenite was invariant over the concentration range 1–8 mol dm^{-3} for the hydrochloric acid. The signal from arsenate was lower over the same range of acid concentration and peaked in the range 3–5 mol dm^{-3}; hence 4 mol dm^{-3} acid was chosen for the standard conditions.

Graphite furnace (GFAA) analyses were carried out using the operating parameters listed in Table 2.

Nickel nitrate (20 μL, 100 μg cm^{-3}) as a matrix modifier was added to 20 μL of sample using the automatic sample delivery system. A lower concentration of modifier (50 μg cm^{-3}) can be used for non-biological samples.

Method (HPLC)

Separation of the arylarsenicals was carried out, Fig. 1, using 0.05 mol dm^{-3} acetic acid/methanol as the mobile phase and the gradient program listed in Table 3. Initial attempts to use a water/methanol mixture were unsuccessful. Arsenic-containing fractions were detected by GFAA.

Table 2 Parameters for GFAA analysis

Step	Temp. (°C)	Time (s)	Argon flow (dm^3 min^{-1})
1	75	5.0	3.0
2	90	30	3.0
3	120	10	3.0
4	1000	30	3.0
5	1000	2.0	3.0
6	2300	1.0	3.0
7	2300	2.0	3.0

Table 3 HPLC gradient program

Time (min)	Flow (cm^3 min^{-1})	CH_3COOH (0.05 mol dm^{-3})	MeOH (%)	Curve[a]
0.00	1	80	20	–
4.00	1	80	20	3
6.00	1	40	60	3
10.00	1	0	100	3

[a] Selected convex curve profile from options on automated gradient controller.

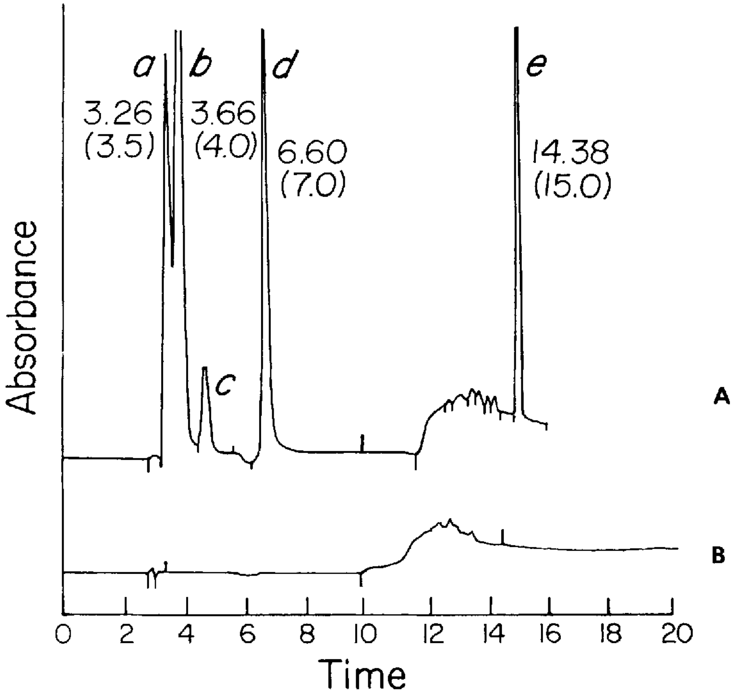

Figure 1 (A) HPLC trace of: *a*, mixture of a 2-OH-4-$NH_2C_6H_3AsO(OH)_2$; *b*, 4-$NH_2C_6H_4AsO(OH)_2$; *c*, 3-NO_2-4-OH-$C_6H_3AsO(OH)_2$; *d*, 4-$NO_2C_6H_4AsO(OH)_2$; *e*, $(C_6H_5)_3As$, each 10 μg cm^{-3} as As. C_{18} column, 10 μL injection, gradient program as listed in Table 3. The retention times (min) are given for detection at 254 nm. The figures in parentheses are retention times for detection by GFAA following fraction collection at 30 s intervals. The lower curve (B) is the chromatogram of the same mixture following UV irradiation (20 min).

RESULTS AND DISCUSSION

Armstrong *et al.*[23] reported that ultraviolet (UV) irradiation (1200 W lamp) of organic matter in sea-water results in rapid oxidation to carbon dioxide and that organic phosphorus compounds are photo-oxidized to inorganic phosphate. In their method a few drops of 30% hydrogen peroxide were added to the sample prior to exposure to UV light. Photo-oxidation was also used by Measures and Burton[24] as part of a procedure for determining selenium in sea-water; again a small amount of peroxide was added to the sample. These studies were models for the present investigation. During the preparation of this manuscript we became aware of two related papers. Tam[25] irradiated acidified (1 cm^3 of 1 mol dm^{-3} HNO_3) aqueous solutions of 2-$NH_2C_6H_4AsO(OH)_2$, $(CH_3)_2AsO(OH)$, and arsenazo [1-(2-arsonophenylazo)-2-hydroxy-3, 6-naphthalenedisulfonic acid sodium salt] with a 450 or 550 W mercury lamp (peroxide was added). The arsenate produced was subsequently reduced to arsenite which was extracted with diethylammonium diethyldithiocarbamate into carbon tetrachloride and determined using a carbon rod AA. Stringer and Attrep[26] found that arsenic present in waste-water solutions of $(C_6H_5)_3AsO$, $CH_3AsO(ONa)_2$, and $(CH_3)_2AsO(OH)$ could be determined by HGAA following photo-oxidation with a 450 W lamp for four hours (peroxide was added to the acidified sample).

Some arylarsenicals such as 4-$NH_2C_6H_4AsO(OH)$ (arsanilic acid) 3-NO_2-4-$OHC_6H_3AsO(OH)_2$ (Roxarsone), 4-$NO_2C_6H_4AsO(OH)_2$ (Nitarsone), and 4-$NH_2CONHC_6H_4AsO(OH)_2$ (Carbarsone) are approved as animal feed additives[27] and the parent compound phenylarsonic acid ($C_6H_5AsO(OH)_2$) has been found in oil shale,[28] in oil-shale process waters, and in shale oils.[29] Thus it is important to develop separation methods for this class of compounds. Previous workers have used HPLC on anion-exchange columns coupled with element-specific GFAA detection to separate $C_6H_5AsO(OH)_2$, $(C_6H_5)_2AsO(OH)$ and 4-$NH_2C_6H_4AsO(OH)_2$. We chose to study the use of a reversed-phase column and the chromatogram of the successful separation of a number of arylarsenicals is shown in Fig. 1(A). The use of acetic acid was found to be critical. We were unable to improve the separation of the first two compounds and the separation was insufficient to allow use of GFAA as detector following fraction collection.

As a test of the efficacy of the UV irradiation procedure the same mixture, whose chromatogram is shown in Fig. 1(A), was irradiated for 20 min and rechromatographed [Fig. 1(B)]. It is obvious that all aromatic groups have been destroyed. Separate ion-pair chromatography on the same reversed-phase column with tetrabutylammonium nitrate in water/methanol (95/5) as the eluant and GFAA as detector established that the product from the UV irradiation is arsenate.[30–32] The oxidation of arsenite to arsenate on UV irradiation has previously been described.[33]

The conditions established for the separation of the arylarsenicals (Table 1 and Fig. 1) were, however, not suitable for the separation of compounds such as $Me_2AsO(OH)_2$, $Me_3As^+CH_2COO^-$, arsenate, and arsenite.[32]

Because arsenate is the product of the photo-oxidation of solutions of arsenicals, hydride generation from these solutions should allow convenient determination of the concentration of arsenic. Some results relating to this proposition are given in Table 4.

One hour of irradiation was found to be sufficient to decompose all the samples. (HPLC with UV detection was also used to monitor the rate of photo-oxidation of the arylarsenicals). The GFAA results for the arylarsenicals show good agreement and indicate only minor matrix effects at worst. Hydride generation from the same class of compounds does occur but the low volatility of the compounds $ArAsH_2$ is probably responsible for the small signal. A similar result has been noted[34] where the response to the hydride generated from $4\text{-}NH_2C_6H_4AsO(OH)_2$ is about 200 times lower than that from inorganic sodium arsenate. These authors found that addition of persulfate resulted in decomposition of the arylarsenical to arsenate, and increased the relative response to become nearly equivalent to that of arsenate. Our own results show that a 10-fold increase in response is obtained by photo-oxidizing the arsenical to arsenate.

As expected, no volatile hydrides are produced by the borohydride reduction of the aliphatic arsenicals listed in Table 4. However, photo-oxidation to arsenate allows easy measurement of the hydride evolved. (These signals are slighly lower than the other entries in this column, presumably because of some slight change in experimental parameter with time, e.g. loss during ashing). The GFAA signals for the irradiated aliphatic compounds are in line, but there are problems with the values from the unirradiated solutions. The operating parameters listed in the Experimental section were not optimized for this type of compound and it seems that this would need to be done in order to get more reliable results. This does highlight the utility of the UV HGAA procedure.

The UV HGAA responses generated for

Table 4 Comparison of GFAA and UV HGAA techniques[a]

Compound	HGAA signal[b] Before UV irradiation	HGAA signal[b] After UV irradiation	GFAA signal[b] Before UV irradiation	GFAA signal[b] After UV irradiation
$4\text{-}NH_2C_6H_4AsO(OH)_2$	0.011	0.125	0.010	0.011
$3\text{-}OHC_6H_4AsO(OH)_2$	0.012	0.124	0.010	0.012
$3\text{-}NO_2\text{-}4\text{-}OHC_6H_3AsO(OH)_2$	0.011	0.123	0.011	0.010
$4\text{-}NO_2C_6H_4AsO(OH)_2$	0.009	0.122	0.012	0.013
$(CH_3)_3As^+CH_2COO^-$	0.000	0.133	0.004	0.012
$[(CH_3)_3AsCH_2CH_2OH]I$	0.000	0.110	0.009	0.012
$[(CH_3)_4As]I$	0.000	0.110	0.009	0.013

[a]The concentration of standard solutions was 10 ng cm^{-3}; 20 μL samples were injected for GFAA measurements. Solutions (50 cm^3) were irradiated for 1 h prior to the repeat measurements by GFAA or HGAA. [b]Average of four determinations.

arsanilic acid and arsenobetaine were studied in a number of acid media. Solutions containing 1 $\mu g\,cm^{-3}$ of As were prepared in various 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 $mol\,dm^{-3}$ acid solutions and irradiated for 1 h. Relative standard deviations from the means of the absorbance of each solution were 0.5% for HNO_3, 0.4% for HCl, 0.4% for H_2SO_4 and 0.4% for CH_3COOH. The signals generated from arsenobetaine solutions irradiated for 15 min and 30 min respectively were obtained in all acid media. The relative signal intensities indicate that higher acid concentrations require longer irradiation times to generate signals indicative of complete photo-oxidation. The longer time required can be attributed to the quenching effect of the other species introduced.[23]

The irradiations were also carried out in water/methanol, water/ethanol, and acetonitrile solutions of both arsanilic acid and arsenobetaine. The breakdown of the arsenic–carbon (As–C) bonds was essentially complete within 30 min of irradiation time over the concentration range 0–30 $\mu g\,cm^{-3}$ As and 0–100% of the organic solvent. Standard deviations of 0.5% were obtained. Interference from organic solvents seems to be much less than from acid solutions.

In order to study the concentration range over which the UV HGAA technique can be used, calibration graphs were obtained for the compounds in Table 4. A typical plot for arsenobetaine is shown in Fig. 2. Like most AA results the plot is linear at low arsenic concentrations (<100 $ng\,cm^{-3}$) but the curvature increases at higher levels.

Analysis of clam extracts

In order to establish that arsenobetaine, a common constituent in extracts of marine animals, would be photo-oxidized in 'real' samples and could be used as a standard, this arsenical was added to samples of clam extracts and the solutions were irradiated with the UV lamp. The results are shown in Table 5. The recoveries are in the satisfactory range 89.6–103.8% using HGAA as a measure of decomposition. A longer irradiation time is necessary to ensure complete decomposition.

Once efficient photo-oxidation had been confirmed the method of standard additions was used to analyze the total arsenic concentration in the two clam extracts. The data for the Manila

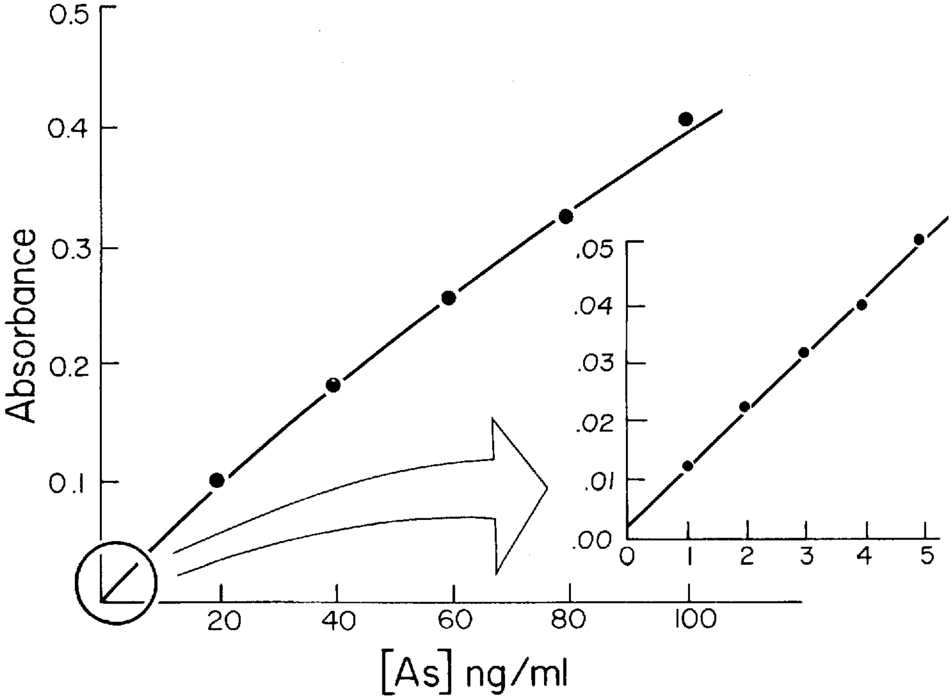

Figure 2 Calibration curve for arsenobetaine using UV irradiation followed by hydride generation AA, UV HGAA.

Table 5 Recovery of arsenic from samples of clam extracts

Arsenic added[a] (ng cm^{-3})	Arsenic found[b] (ng cm^{-3})	Recovered (%)
Manila clam extract		
0	10.6	—
10.0	20.5	99.1
20.0	30.8	101.8
30.0	40.4	98.1
40.0	49.5	89.6
Horse clam extract		
0	7.8	—
10	17.9	101.2
20	27.1	91.0
30	38.1	103.8
40	47.4	94.8
50	57.5	96.1

[a] As arsenobetaine (as [As]). [b] Arsenic determined by HGAA following UV irradiation for 2 h.

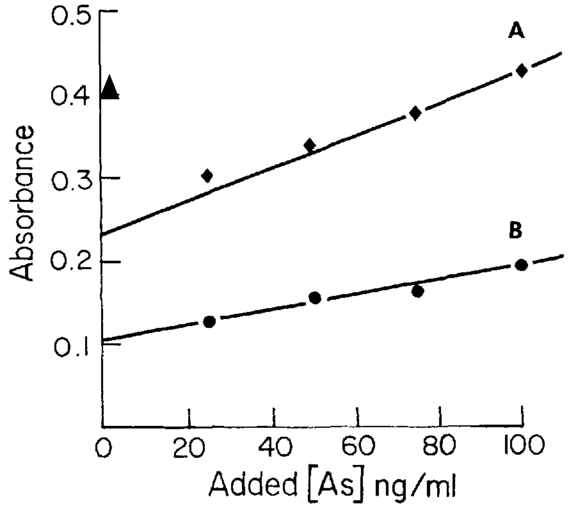

Figure 3 Standard addition curves from 1 cm^3 samples of extracts of Manila clams. (a) Each sample was made up to 50 cm^3 with water and irradiated for 2 h prior to HGAA. Standard amounts of arsenobetaine were added prior to the irradiation. (b) GFAA determination using standard arsenic solution for the addition.

clams extract are plotted in Fig. 3 as an example. Line B is that obtained by HGAA following standard addition of standard arsenite to an unirradiated sample. Line A is obtained by GFAA following addition of aliquots of arsenobetaine to samples of the extract. The extract and the amended extracts were photo-oxidized prior to the arsenic determination by HGAA.

The Horse clam extracts were treated similarly; arsenobetaine was used as the standard for the UV HGAA determination and arsenite as the standard for the GFAA determination. In the latter case the standards were mixed using the GTA 95 sample dispenser. The results obtained for the two analyses are shown in Table 6. The amount of arsenic found in both samples using the two techniques is comparable, 124.8 μg dm^{-3} and 123.9 μg dm^{-3} for the Manila clam extract and 28.9 μg dm^{-3} and 27.1 μg dm^{-3} for the Horse clam extract. The relative standard deviations are better using the UV HGAA method. The photo-oxidation followed by hydride generation minimizes the interferences from the organic matrix which affect the the GFAA determination.

The method eliminates the use of acids and bases for sample preparation and therefore inter-

Table 6 Analysis of clam extracts by standard additions[a]

	Manila clam[b]		Horse clam[c]	
	GFAA	UV HGAA	GFAA	UV HGAA
r	0.983	0.992	0.984	0.974
b (RSD)	0.233 (2.33)	0.104 (0.67)	0.0314 (2.44)	0.0102 (0)
$a \times 10^4$ (RSD)	18.8 (0.014)	8.36 (0.04)	10.8 (5.16)	3.76 (0)
[As] (ng cm^{-3})	123.9	142.8	27.1	28.9
[As] (μg g^{-1})[d]	1.7		2.4	

[a] r = coefficient of linear regression of $y = ax + b$. RSD = relative standard deviation (%). [b] Using premixed standards. [c] Standard mixed through GTA 95 sample dispenser. [d] Average value based on wet weight of clam sample.

ferences from these acids or bases. It is rapid as up to 24 samples can be irradiated at a time. Good reproducibility (0.9–1.4% RSD), low detection limit (around $0.3\,\mu g\,cm^{-3}$) and good recoveries (97.1 ± 4.4%) make the method suitable for determining arsenic in extracts from marine animals.

More recently, we have found that aliquots of standard arsenite solution can be used for the standard addition UV HGAA method. However, we prefer to add the aliquots of arsenite to the samples prior to irradiation rather than making standard additions to a single bulk photo-oxidized sample.

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