Determination of total arsenic in biological samples by arsine generation and atomic absorption spectrometry

ARTICLE *in* ANALYTICAL CHEMISTRY · JANUARY 1982

Impact Factor: 5.64 · DOI: 10.1021/ac00237a017 · Source: PubMed

4 AUTHORS, INCLUDING:

Michael E. Collings
University of North Dakota
24 PUBLICATIONS **135** CITATIONS
SEE PROFILE

Forrest H Nielsen
United States Department of Agriculture
256 PUBLICATIONS **8,369** CITATIONS
SEE PROFILE

Published in Analytical Chemistry, December 1981, pp 2221–2224, by the American Chemical Society

Determination of Total Arsenic in Biological Samples by Arsine Generation and Atomic Absorption Spectrometry

Eric O. Uthus,*[1] Michael E. Collings,[1] W. E. Cornatzer,[1] and Forrest H. Nielsen

United States Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, P.O. Box 7166, University Station, Grand Forks, North Dakota 58202

Arsine generated from dry combusted biological samples was measured by using an atomic absorption spectrophotometer equipped with a graphite furnace. Innovations of the method included the introduction of arsine into the interior of the graphite tube via one internal purge gas port only, the use of three traps to remove water from the generated arsine, and the use of Erlenmeyer flasks in the generation of arsine. EDTA was added to the sample mixture to prevent interference from copper, iron, and nickel cations. With the described procedure, the arsenic found in various NBS standards was as follows (in μg/g dry material): bovine liver, 0.056; pine needles, 0.215; orchard leaves, 10.51; oyster tissue, 13.17. Sensitivity and absolute detection limits (IUPAC) of the method were 0.11 ng and 0.14 ng, respectively.

The capability to determine widely varying levels of arsenic in biological samples has become a necessity for individuals concerned with the role of arsenic in animal and human health. Arsenic in biological materials in mg/g quantities is toxic (*1*), while 30–50 ng/g quantities are essential for normal biological functions (*2*).

A number of methods employing the use of arsine generation and atomic absorption spectrophotometry have been described (*3–6*). However, in those methods the generated arsenic hydride was directed through a flame, or electrically heated, quartz tube atomizer. In 1973, Knudson and Christian (*7*) described a method in which electrothermal atomic absorption spectrophotometry was used. In that method, the generated arsine was collected in a liquid nitrogen cold trap and then volatilized and introduced with an inert gas into a Perkin-Elmer (Perkin-Elmer Corp., Norwalk, CT) Model 403 atomic absorption spectrophotometer with a HGA-2000 graphite furnace at a temperature of 1750 °C. This method was apparently adequate for measuring inorganic arsenic in standard solutions. However, in order to measure nanogram quantities of arsenic in samples with an unknown matrix (i.e., acid solution of sample ash), modifications of the method of Knudson and Christian (*7*) were necessary to reduce contamination and to improve sensitivity. These modifications are described.

Table I. Instrumental Operating Conditions

source	electrodeless discharge lamp
wavelength	193.7 nm
source power	8 W
slit width	0.7 nm
carrier gas	helium
gas flow rate	external: 9 cm^3/s
	internal: 14 cm^3/s
atomizing temp	2300 °C
recorder	10 mV

EXPERIMENTAL SECTION

Apparatus. A Perkin-Elmer Model 503 atomic absorption spectrophotometer equipped with a HGA-2100 controller, electrodeless discharge lamp (EDL), and a Hitachi Model 056-1005 recorder was used. The settings on the instruments are shown in Table I.

[1] Department of Biochemistry, University of North Dakota, Grand Forks, ND 58202.

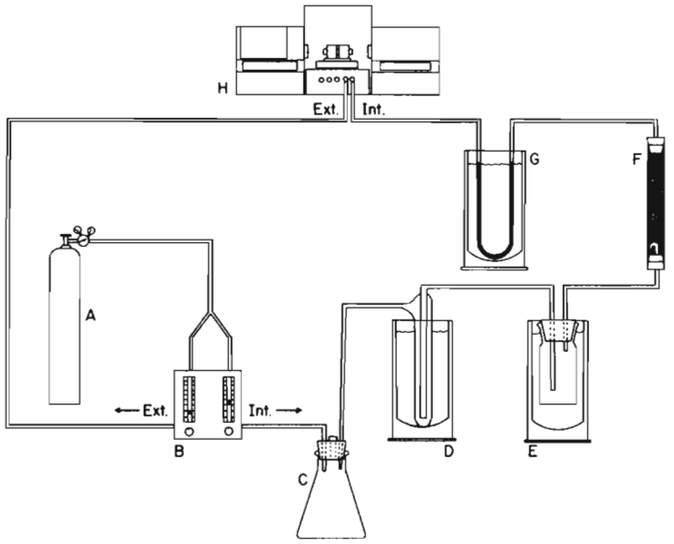

Figure 1. Apparatus used for the generation of arsine and its introduction into the graphite furnace: (A) helium; (B) flow meters for separate regulation of both internal and external gas flow; (C) generating flask; (D & E) cold traps to collect water; (F) $CaCl_2$ drying tube; (G) liquid nitrogen cold trap; (H) atomic absorption spectrophotometer.

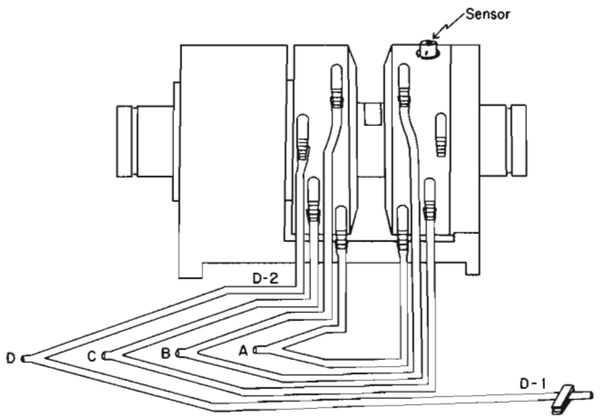

Figure 2. Rear view of the graphite furnace assembly showing water coolant and purge gas lines: (A) water in; (B) water out; (C) external purge gas; (D) internal purge gas. The internal purge gas line D-1 was disconnected and clamped off. Generated arsine entered the graphite tube through internal purge gas line D-2.

The basic apparatus used for the generation of arsine and its introduction into the graphite furnace is shown in Figures 1 and 2. Standard operating procedure and equipment for introducing gas into the graphite furnace were not used. As it came from the cylinder, the flow of helium was split, by using a glass Y joint, into two gas lines with separate flow meters. One line was connected directly to the inlet leading to the external flow around the graphite tube and adjusted to a flow rate of 9 cm^3/s. The other line was connected to the arsine generation flask and adjusted to a flow rate of 14 cm^3/s.

The arsine generation flask was a 125-mL Erlenmeyer flask (No. 4980, Corning, Medfield, MA) fitted with a No. 6 rubber stopper with three holes. In these holes were placed an inlet tube, outlet tube, and a rubber septum from a vacutainer tube (No. 4751, Becton, Dickinson, Rutherford, NJ). The outlet tube was connected by poly(vinyl chloride) tubing (Tygon, Norton Co., Akron, OH) to a series of three traps to collect water. The first trap was a cold finger condenser (LG–5235–112, Lab Glass, Inc., Vineland, NJ); the second was a 125-mL glass jar (No. 214205, Wheaton Scientific, Millville, NJ) fitted with a No. 8 rubber stopper equipped with inlet and outlet tubes. These two traps were placed in Dewar flasks containing an ice–ethanol slurry maintained at −20 °C. The third trap was a 16 × 65 mm column containing 8 mesh anhydrous calcium chloride (Mallinckrodt, St. Louis, MO). The traps were connected by poly(vinyl chloride) tubing to a cold trap. The cold trap was 7 mm o.d. Pyrex tubing with a "U" shape having enough length so that 160 mm of the "U"-shaped tubing, containing 1 mm glass beads, could be submerged in a Dewar flask containing liquid nitrogen. The cold trap was connected by 30 cm of poly(vinyl chloride) tubing to the internal gas flow inlet of the atomic absorption spectrophotometer. All poly(vinyl chloride) tubing was 6 mm i.d.

The arsine gas was introduced into the graphite tube via one port instead of the normal two. The internal purge gas line directly below the sensor of the graphite furnace was disconnected and the tubing clamped off.

Reagents. HCl (Instra-Analyzed, Baker Chemical Co., Phillipsburg, NJ) was used for both sample dissolution and arsine generation. The saturated solution of magnesium nitrate used for sample ashing was prepared from $Mg(NO_3)_2 \cdot 6H_2O$ (No. 2468, Baker Chemical Co., Phillipsburg, NJ) and deionized water (Super Q System, Millipore Corp., Bedford, MA). The 10% (w/v) aqueous $NaBH_4$ solution used for arsine generation was prepared by dissolving $NaBH_4$ (98% powder, Alfa Inorganics, Danvers, MA) in 0.5% (w/v) aqueous NaOH. The NaOH stabilized the $NaBH_4$. The $NaBH_4$ solution was filtered through No. 41 filter paper (Whatman, Clifton, NJ) before use. The complexing 10% (w/v) ethylenediaminetetraacetate (EDTA) solution was prepared from EDTA tetrasodium salt (Sigma Chemical Co., St. Louis, MO) and deionized water. The stock standard solution of arsenic (100 μg of As/mL) was prepared by dissolving 0.1320 g of As_2O_3 (Standard Reference Material 83C, National Bureau of Standards, U.S. Department of Commerce, Washington, DC) in 25 mL of 0.5 N NaOH and then diluting to 1000 mL with deionized water in a volumetric flask. The standard solution containing 10 ng of arsenic/mL was prepared by diluting the stock standard solution with deionized water.

The effect of varying the $NaBH_4$ concentration on arsine generation was assessed by using 1, 5, 10, and 15% $NaBH_4$ solutions. The effect of sample HCl concentration on arsine generation was assessed by using samples containing 10 ng of arsenic in HCl ranging from 0.25 to 1.5 N. To ascertain whether other elements interfere with the generation of arsine, we added 100 μg (unless noted otherwise) of the element in question to a standard sample containing 10 ng of arsenic. This possible interference was ascertained in both the absence or presence of EDTA. The elements used in the interference study were obtained by diluting atomic absorption standards (MCB, Cincinnati, OH).

Sample Preparation. The approximate weights of the biological samples (National Bureau of Standards, U.S. Department of Commerce, Washington, DC) used were 0.5 g of bovine liver (SRM 1577), 0.19 g of pine needles (SRM 1575), 0.1 g of orchard leaves (SRM 1571), and 0.075 g of oyster tissue (SRM 1566). Samples were weighed into 16 × 150 mm culture tubes (S/P dispo culture tubes, T1285-8, Scientific Products, Minneapolis, MN) and mixed with 0.5 g of α-cellulose (No. C8002, Sigma Chemical Co., St. Louis, MO). Two milliliters of saturated $Mg(NO_3)_2$ was added to the samples and mixed on a vortex mixer. Blanks containing just 2.0 mL of $Mg(NO_3)_2$ and 0.5 g of α-cellulose were also prepared. The culture tubes were heated to 200 °C, in an aluminum tube block (No. 2073, Lab-Line, Melrose Park, IL) placed on a hot plate. To ensure the samples were completely dry, we placed the culture tubes in a block heater for 30 min (Model No. 100C, GCA Corp., Chicago, IL) containing a tube block with 16-mm holes, maintained at 300 °C. The culture tubes were transferred to a stainless steel rack and placed in a cold muffle furnace (Model F-A1730, Thermolyne, Dubuque, IA) which was turned on and set at 600 °C. The tubes were removed 16 h later and allowed to cool to room temperature. The ashed sample was dissolved in 5 mL of 5 N HCl and quantitatively transferred with deionized water to 10 mL (blanks, bovine liver, and pine needles) or 100 mL (blanks, orchard leaves, and oyster) volumetric flasks and brought up to volume with deionized water. Sample volumes of 2.5 or 1.0 mL, respectively (containing 10 ng or less of arsenic), were used for analysis.

Arsine Generation. The sample was placed in a 125-mL Erlenmeyer flask with 1.0 mL of 10% EDTA, deionized water, and enough 5 N HCl to make 10 mL of 1 N solution. The stopper (see Apparatus) was fitted into the Erlenmeyer flask and the cold trap was allowed to cool for at least 15 s in the Dewar flask containing liquid nitrogen. With a 5-mL syringe fitted with a 21 g, $1^1/_2$ in. needle, 4.0 mL of 10% $NaBH_4$ was added to the sample through the rubber septum at a rate of about 0.2 mL/s. One minute later, the graphite furnace was turned on and allowed to stabilize (about 10 s) and then the recorder was turned on. The

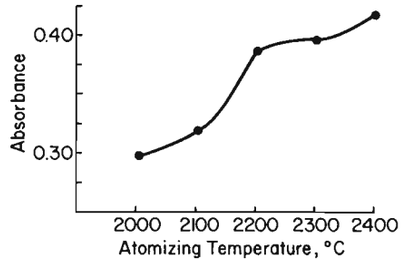

Figure 3. Effect of atomizing temperature on peak height response, converted to absorbance units, to 10 ng of arsenic.

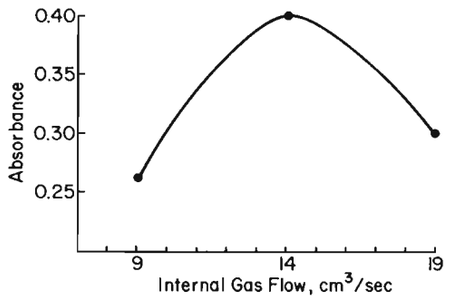

Figure 4. Effect of internal gas flow rate on peak height response, converted to absorbance units, to 10 ng of arsenic.

arsine introduced into the graphite furnace was volatilized by transferring the cold trap into a beaker of water maintained at about 40 °C.

RESULTS AND DISCUSSION

This method of analysis employs several new techniques. For example, the internal purge gas was not directed to two inlet ports. Instead, the arsine gas was directed into the interior of the graphite tube via one port. The other port was disconnected, the tubing clamped off, and the tubing connector on the graphite furnace left open. Thus, after the arsine was atomized and quantitated, the gas was expelled both through the hole in the graphite tube and internal purge gas line connecter on the graphite furnace. We found that, when the internal purge gas lines were affixed as described, the sensitivity of arsenic detection was elevated approximately 2.5 times. Furthermore, reproducibility was improved.

The use of Erlenmeyer flasks for generation of the hydride from arsenic present in samples was advantageous. The samples could be pipetted into flasks in advance of the time when the actual atomic absorption procedure was to be done. Also, the use of separate Erlenmeyer flasks eliminated the possibility of cross-contamination of samples which could occur in generators which must be washed, or flushed, after each sample run.

Three traps were used to collect water during the arsine generation procedure to assure that the gas was completely dry. Any water in the arsine significantly reduced the sensitivity of the method (*8*). Precaution should be taken to make certain the calcium chloride in the third trap does not become waterlogged.

Figure 3 shows the effect of atomizing temperature on the peak height response to a sample containing 10 ng of arsenic. The peak height increased with increasing atomizing temperature. Although higher temperatures gave slightly higher peak heights, we decided to use 2300 °C because higher temperatures shortened the life of the graphite tube and rings.

The effect of internal gas flow rate on peak height is shown in Figure 4. A flow rate of 14 cm^3/s gave a maximum peak height response to a sample containing 10 ng of arsenic. An external gas flow rate of 9 cm^3/s apparently was adequate to maintain an inert atmosphere around the exterior of the graphite tube and prevent its destruction.

Table II shows the effect on peak height of varying concentrations of HCl and $NaBH_4$ during arsine generation from

Table II. Effect of Varying HCl[a] and $NaBH_4$[b] Concentrations on the Peak Height (Absorbance) Response to 10 ng of Arsenic

% $NaBH_4$ (w/v)	absorbance		
	0.25 M HCl	1.0 M HCl	1.5 M HCl
1	0.049	0.070	0.070
5	0.100	0.271	0.232
10	0.176	0.397	0.335
15	0.143	0.385	0.327

[a] Values represent the HCl normality in the final 10 mL sample. [b] Arsine was generated by addition of 4.0 mL of $NaBH_4$ solution.

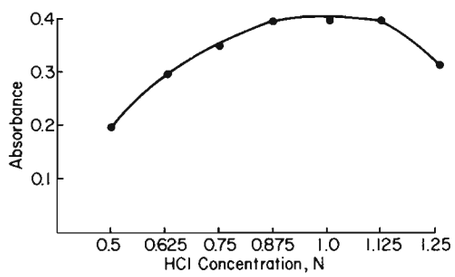

Figure 5. Effect of HCl concentration on peak height response, converted to absorbance units, to 10 ng of arsenic.

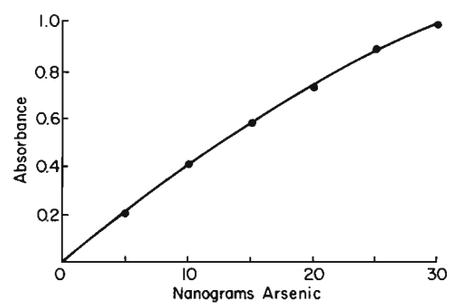

Figure 6. Standard curve. Arsine was generated from a 10-mL sample (1.0 N HCl) containing 0–30 ng of arsenic by using 4.0 mL of 10% $NaBH_4$.

a sample mixture containing 10 ng of arsenic. Of the concentrations tested, optimum peak height was found when the 10-ng arsenic sample was in 1.0 N HCl and 4.0 mL of 10% $NaBH_4$ was used to form the arsine. Further trials were done to ascertain the range of HCl normality in the sample which gave an optimal peak height response. As shown in Figure 5, maximal peak heights were found in the range of 0.875–1.25 N HCl. Thus, we chose a point in the middle of this range, 1.0 N, for use in our procedure.

The effect of various cations on arsine generation was studied. The findings showed that 100 μg of Mg^{2+}, Mn^{2+}, and Co^{2+} and 1 μg of Sn^{4+} did not affect the generation of arsine from a sample containing 10 ng of arsenic. However, Cu^{2+} at levels greater than 10 μg and Ni^{2+} at levels of 5 μg or greater depressed the generation of arsine. Also, the peak height response to 10 ng of arsenic was 10% higher with samples containing 100 μg of iron vs. no iron. The addition of 1.0 mL of 10% EDTA to the sample mixture prevented interference caused by 100 μg of copper, 100 μg of iron, and 25 μg of nickel. EDTA did not completely eliminate the interference caused by Ni^{2+} at tested levels greater than 25 μg. The copper, iron, and nickel findings explain why we routinely add 1.0 mL of 10% EDTA to the sample mixture containing digested biological material.

Levels of tin greater than 1 μg in the generating flask interfere with arsine generation. This interference was not eliminated by EDTA addition to sample mixture. Therefore, tin should not preclude the determination of arsenic by the described method unless the biological sample contains 200

Table III. Arsenic Content of NBS Reference Materials, μg/g (Dry Weight)

SRM	no. of samples	present study[a]	NBS certified value
1577, bovine liver	10	0.056 ± 0.003	0.055 ± 0.005
1575, pine needles	6	0.215 ± 0.006	0.21 ± 0.04
1571, orchard leaves	6	10.43 ± 0.22	10 ± 2
1566, oyster tissue	6	13.17 ± 0.34	13.4 ± 1.9

[a] Each value represents the mean ± standard deviation.

or more times as much tin as arsenic.

By use of the conditions described in the Experimental Section, sample blanks were determined to contain 2.5 ng of arsenic. Also, a calibration curve linear to 10 ng of arsenic (Figure 6) was obtained.

The digestion procedure used was chosen over wet digestion because residual acids (HNO_3, H_2SO_4, or $HClO_4$) from the latter procedure apparently interfere with arsenic determination by hydride generation methods (*9*). The cellulose was added during the digestion procedure to reduce sample foaming and, thus, to allow for faster drying of the sample. After dry combustion, arsenic will be present as As(V). Nonetheless, the samples were measured against an As(III) standard because, with experimental conditions used, hydride generation from an As(III) standard was identical with that from an As(V) standard. Recovery of 40 ng of arsenic as As_2O_3 in samples digested by the described procedure was determined to be 99.8 ± 1.1%.

Using our procedure, we found sensitivity and absolute detection limits (IUPAC) of the method were 0.11 ng and 0.14 ng, respectively. We also determined the arsenic content of various NBS standards. As shown in Table III, the values found agreed with the certified values.

LITERATURE CITED

(1) Fowler, B. A. In "Toxicology of Trace Elements"; Goyer, R. A., Mehlman, M. A., Eds.; Hemisphere Publishing Co.: Washington, DC, 1977; Chapter 3.
(2) Nielsen, F. H. In "Advances in Nutritional Research", Vol. 3; Draper, H. H., Ed.; Plenum Press: New York, 1980; Chapter 6.
(3) Evans, W. H.; Jackson, F. J.; Dellar, D. *Analyst (London)* **1979**, *104*, 16–34.
(4) Chapman, J. F.; Dale, L. S. *Anal. Chim. Acta* **1979**, *111*, 137–144.
(5) Brodie, K. G. *Am. Lab. (Fairfield, Conn.)* **1979**, *11*, 58–66.
(6) Peats, S. *At. Absopt. Newsl.* **1979**, *18*, 118–120.
(7) Knudson, E. J.; Christian, G. D. *Anal. Lett.* **1973**, *6*, 1039–1054.
(8) Braman, R. S.; Justen, L. L.; Foreback, C. C. *Anal. Chem.* **1972**, *44*, 2195–2199.
(9) Kang, H. K.; Valentine, J. L. *Anal. Chem.* **1977**, *49*, 1829–1832.

RECEIVED for review June 1, 1981. Accepted July 31, 1981. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.