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Development of Analytical Methods for Comprehensive Chemical and Physical Speciation of Arsenicals in Groundwater

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Project Summary

Title: Development of Analytical Methods for Comprehensive Chemical and Physical Speciation of Arsenicals in Groundwater

Project I.D.: DNR Project Number #154

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Period of Contract: September 2000 to September 2002

Background/Need: The basis for understanding the abundance and distribution of arsenic in groundwater is reliable chemical analysis. Improvements in sample collection, sample pre-treatment, and on-site and off-site laboratory determination methods are sought to insure that information about arsenic is of high quality.

Objectives: The objective of this project was to develop improved analytical methods to better characterize the specific forms of arsenic that are present in the groundwater formations of the Fox River Valley (FRV).

Methods: The primary arsenic compounds present in FRV groundwater are the trivalent and pentavalent oxyacids, arsenious and arsenic acids, respectively. These compounds are found predominantly as dissolved species (liquid or solution phase) but also can be adsorbed to small particles and colloidal matter (solid or particulate phase). There are three primary steps in chemical analysis: (a) sample collection, (b) sample pre-treatment, and (c) analyte determination. For sample collection, we optimized an existing method based on solid-phase extraction. We then developed an improved sample pre-treatment method using iron-chelating preservatives to improve the preservation of the

sample. For analyte determination, we expanded our existing potentiometric stripping method for on-site determination of both arsenious acid and arsenic acid, while we developed an improved method based on ion chromatography for off-site analyte determination. Additionally, we began studies of the particle-phase forms of the arsenic acids by field-flow fractionation.

Results and Discussion:

For **sample collection**, we optimized an existing method based on solid-phase extraction (SPE) (X.C. Le, S. Yalcin, M. Ma. *Environ. Sci. Technol.* **2000**, *34*, 2342). In this method, arsenite and arsenate are quantitatively sampled from groundwater samples by anion-exchange (displacement) chromatography. The SPE cartridge removes matrix interferences and pre-concentrates the analytes. We conducted three on-site sampling exercises to optimize the method conditions. Analyte determination was performed off-site by inductively-coupled plasma mass spectrometry (ICP-MS). This effort culminated in an optimized method with improved precision and a suitcase-sized "kit" for convenient field implementation.

For **sample pre-treatment**, we developed an improved method for the preservation of inorganic arsenicals in iron-rich water samples, which are commonly encountered in the FRV. The method chelates Fe(III) ions in solution because: (a) Fe(III) can rapidly oxidize As(III) to As(V), and (b) As(V) is quickly and tenaciously adsorbed by iron oxyhydroxides (colloidal FeOOH species) during a complex co-precipitation process. Precipitation of As(V) during sample transport and storage prior to off-site analysis introduces a "false negative" error because the precipitate is usually filtered away prior to analyte determination. Recently, several laboratories studying the analysis of arsenic in natural water samples have reported that ethylene diamine tetraacetic acid (EDTA) is the preferred reagent for the efficient chelation of iron. We studied EDTA as well as several other Fe-chelators — ones that have high formation constants for the complexation of iron ions and can be used optimally at neutral pH. The method we developed using either Oxine or Cupferron has a ~20% better recovery of arsenic than the reported EDTA-based methods.

For **on-site determination** of arsenite/arsenate, we improved upon our previous method using potentiometric stripping analysis (PSA). Arsenic can be speciated shortly after sample collection (i.e., to quantify the dissolved forms). Detection limits are <1 part

per billion (ppb) for each form of inorganic arsenic, and the measurement takes ~45 min to complete. The advantage of the on-site method is that it can minimize errors that are commonly introduced by sample handling, transport, and storage prior to conventional off-site laboratory determination.

For **off-site determination** of arsenite/arsenate, we developed a high-resolution laboratory method based on ion chromatography (IC) with optical detection. Although inorganic elemental analysis techniques such as ICP-MS with chromatographic "front-ends" are used widely in research laboratories for arsenic trace analysis, for *practical* implementation of the new EPA standard of 10 µg/L by municipal water treatment labs, IC with optical detection provides significant advantages because the capital and operating costs are drastically less than required for ICP-MS. Just as importantly, one can directly speciate inorganic arsenic at trace levels with high selectivity using the IC method. Method detection limits are comparable to the PSA method, but the IC method can better reject possible interferences because of the higher resolution chromatographic separation process.

For **physical characterization** of arsenic on particles and colloids, we assembled a flow field-flow fractionation (Flow FFF) system and began the development of a physical speciation method. Arsenicals in the small particle/colloidal phase (~50-500 nm) in groundwater samples are often ignored in conventional (solution phase) measurements. These species, however, may be found in drinking waters because they are small enough to pass through many filtration systems. We used fluorescence and light-scattering detectors to study the fractionated zones in FRV samples. We are continuing this work by directly interfacing the Flow FFF instrument to a mass spectrometer.

Conclusions/ Implications/ Recommendations: Our work has shown that sample collection methods based on solid-phase extraction (SPE) are effective for the chemical speciation of arsenite and arsenate. We improved an existing method by increasing its precision and practicality through the development of an "SPE field kit". Our studies of the preservation of total arsenic information led to the development of a method using either Oxine or Cupferron. The method is more effective than reported EDTA-based methods in preserving dissolved arsenic oxyanions. Both the sample collection and sample preservation methods require further field-

testing state-wide to assess their general applicability.

For the on-site quantitation of arsenite and arsenate, we developed a method based on potentiometric stripping analysis (PSA) with the requisite sensitivity and selectivity. However, the requirement to heat the sample to 95 °C (for arsenate reduction) is inconvenient for field use — we are presently developing an automated in-line heating module to address this problem.

The novel method that we developed based on ion chromatography with optical detection is well-suited for off-site quantitation of arsenite and arsenate. Validation of the IC method using NIST standards at the new drinking water standard (10 ppb) has the potential to facilitate nation-wide implementation of the new 10 ppb As standard.

Our studies using flow field-flow fractionation (Flow FFF) show promise for developing a better understanding of arsenic:particle/colloid distributions. The FFF method requires a significant effort to minimize the aggregation of particles and will no doubt benefit from the preservation chemistry that was also studied as part of the project.

Related Publications:

R.L. Johnson, J.H. Aldstadt. "Quantitative Trace-Level Speciation of Arsenite and Arsenate in Drinking Water by Ion Chromatography", *The Analyst (London)* **2002**, *127*, 1305-1311.

Key Words:

Arsenic, arsenite, arsenate, ion chromatography, potentiometric stripping, solid-phase extraction, field-flow fractionation

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A final report containing more detailed information on this project is available for loan at the Water Resources Institute Library, University of Wisconsin - Madison, 1975 Willow Drive, Madison, Wisconsin 53706 (608) 262-3069.

1.0 Background

Arsenic is ubiquitous in nature, and its chemical speciation encompasses five general classes of compounds: (a) inorganic arsenic minerals, common examples being orpiment and arsenopyrite; (b) inorganic arsenious and arsenic acids, the former trivalent and the latter pentavalent (hereafter referred to as As^{III} and As^{V}); (c) alkylated (usually methylated) derivatives of arsenic acid; (d) the arsines, reduced derivatives of arsenious acid that are the most toxic arsenic compounds; and (e) the arsenoribosides, a class of non-toxic organoarsenicals found in certain marine organisms.[1] In the groundwater and surface waters used for public drinking waters, the inorganic acids are the most common forms of arsenic that are present. Because of their chronic toxicity at low levels, there is particular concern over regulating their abundance and distribution in public drinking waters.[2]

While a 10 µg/L standard for total arsenic in drinking waters exists in the European Union, Japan, and more than a dozen other countries, the U.S. only recently approved lowering of the current standard of 50 µg/L (which was set in 1942 to this level).[3] This decision was primarily the result of a recent report from the National Academy of Sciences (NAS) based on their review of toxicological data.[4] They stated that the current 50 µg/L standard "...does not achieve the Environmental Protection Agency's (EPA) goal for public health protection and therefore requires downward revision as promptly as possible". In early 2001, the U.S. EPA proposed lowering the drinking water standard to 10 µg/L, for which executive approval was eventually received on October 31, 2001. Nation-wide implementation of the new arsenic standard is planned by 2006.[5]

The appearance of unusually high levels of inorganic arsenic in private wells in the Fox River Valley region is of concern to the Wisconsin Department of Natural Resources. Arsenic is released by oxidation of sulfide minerals such as pyrite and marcasite apparently as a result of the infiltration of atmospheric oxygen into wells that penetrate the aquifer.[6] While the mechanism and kinetics of arsenic release are the subject of other investigations,[7] improvements in the analytical chemistry of inorganic arsenic quantitation are the focus of our research.

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1 W.R. Cullen and K.J. Reimer, *Chem. Rev.* **1989**, *89*, 713, and references cited therein.

2 J. Nriagu, *Arsenic in the Environment: Cycling and Characterization*, Advances in Environmental Science and Technology, No. 26, John Wiley & Sons, New York, 1994.

3 *Drinking Water Standard for Arsenic*, U.S. Environmental Protection Agency, Technical Report EPA 815-F-00-015, U.S. Government Printing Office, Washington DC, 2001.

4 *Arsenic and Old Laws*, Natural Resources Defense Council, U.S Government Printing Office, Washington DC, 2000.

5 *Chemical & Engineering News* **2001**, 79, 45.

6 (a) Schreiber, M.E.; Simo, J.A.; Freiberg, P.G. *Hydrogeology Journal* **2000**, *8*, 161. (b) Riewe, T.; Weissbach, A.; Heinen, L.; Stoll, R. *Well Water Journal* **2000**, *6*, 24.

7 M. Gotkowitz, WGNHS, unpublished results, 2002.

Our approach to inorganic arsenic quantitation is based on the distribution of arsenic in two distinct phases: (a) arsenic as dissolved ionic species, and (b) arsenic associated with particulate or colloidal matter. Category (a) represents the "chemical speciation" of inorganic arsenic while Category (b) describes the "physical speciation" of inorganic arsenic. For Category (a), there are two forms: arsenite (the ionic form of monoprotic arsenious acid, O=As-OH) and arsenate (the ionic forms of triprotic arsenic acid $O=As(OH)_3$). Category (b) is more difficult to define, but an "operationally relevant" definition based on the material which is small enough to evade filtration is: "arsenicals (inorganic and organic) associated with particles and colloids that are <0.45 μm in average diameter."

A simplified depiction of our strategy for developing new methodologies and optimizing existing ones is shown in Figure 1. The questions that we endeavored to answer related to the measurement of arsenic in FRV groundwater were:

1. Can inorganic arsenicals at low ppb levels be measured on-site using field-portable instrumentation?
2. Are there improvements in precision and accuracy that can be realized for existing sample preservation and sample collection methods?
3. Can off-site analysis methods for inorganic arsenicals be developed such that adoption of the new As standard of 10 ppb is practical?
4. What can be learned about the abundance and distribution of arsenicals with colloidal and particulate matter?

In the following pages, we describe our work in attempting to answer these questions by discussing: (a) a new method for on-site determination of As(III) and As(V) using a field-portable instrument, (b) improved sample collection and preservation methods, (c) an improved method for off-site analysis, and (d) studies in the characterization of the physical speciation of inorganic arsenicals. This work has resulted in the writing of four manuscripts for contribution to the peer-reviewed scientific literature (one published and three in preparation).*

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* R.L. Johnson, J.H. Aldstadt. "Quantitative Trace-Level Speciation of Arsenite and Arsenate in Drinking Water by Ion Chromatography", *The Analyst (London)* **2002**, *127*, 1305-1311..

J. Scaffidi, J.H. Aldstadt. "A Study of the Optimal Conditions for the Reduction of Arsenate in the Potentiometric Stripping Determination of Inorganic Arsenicals in Natural Waters", in preparation.

A.R. Roerdink, J.H. Aldstadt. "A Method for the Determination of Arsenite, Arsenate and Feed Additive Arsenicals in Environmental Waters by Solid-Phase Extraction", in preparation.

C. Blaine, A.R. Roerdink, J.H. Aldstadt. "An Improved Method for the Accurate Quantitation of Arsenic in Natural Waters by Preservation Using Cupferron and Oxine", in preparation.

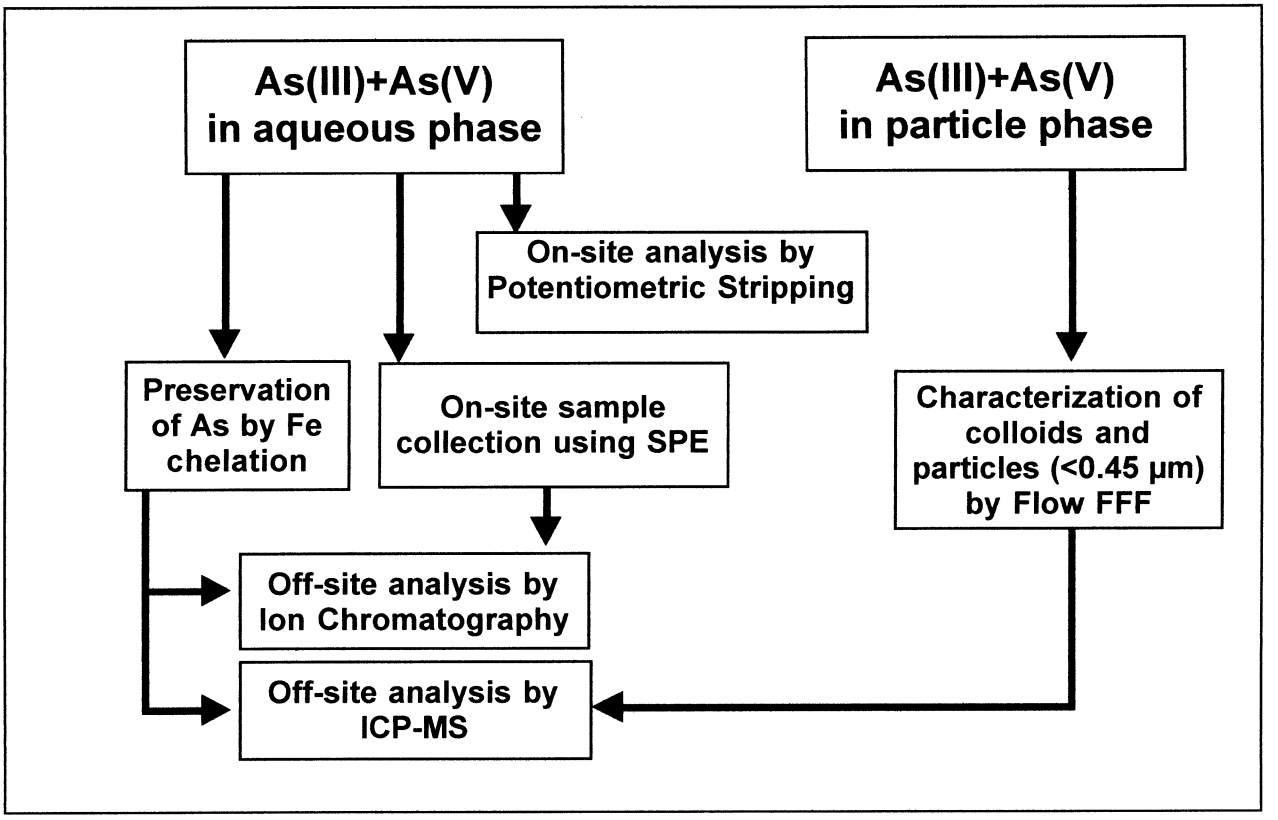

Figure 1. Analytical approach to studying inorganic arsenicals in groundwater.

2.0 Sample Collection & Pre-Treatment — Preservative Studies

2.1 Introduction

Sample collection is arguably the most important step in the overall analytical process. It is well known that most of the overall measurement variability — imprecision and inaccuracy — in environmental analysis can be attributed to errors made during the sampling step.[8] For groundwater samples, various chemical and physical changes (e.g., temperature, pressure, photochemistry, gas-phase chemistry) occur when a sample is brought to the surface. Furthermore, the introduction of microorganisms at the surface can significantly alter the sample integrity. For As(III) and As(V) in FRV groundwater, an additional complication of great consequence is the oxidation of As(III) to As(V). As(V) can readily adsorb onto iron oxyhydroxide (FeOOH) colloidal matter, which eventually precipitates.[9]

The objective of the preservative study described herein was to examine a variety of methods that would efficiently preserve inorganic arsenic species in groundwater samples from high iron-containing waters such as FRV groundwater. The common method for collecting samples of inorganic arsenicals consists of obtaining the raw sample from a properly purged well, placing it in a clean high-density polyethylene (HDPE) sample bottle, acidifying the sample (using a non-oxidizing acid such as HCl), and transporting it to the lab for storage. Preservation with acid is generally used in water analysis as a means to prevent the formation of the low solubility hydroxides of cationic species (M^{n+}). For arsenic and other oxyacids that form anions in solution, however, formation of hydroxides is irrelevant. Because of the growing awareness of the error in total arsenic concentration that can be introduced in high Fe-containing waters, new preservation methods have been recently reported in the literature.[10] These reported methods are all based on reacting the Fe with ethylenediamine tetraacetic acid (EDTA), the venerable complexing agent for dozens of metal cations (Figure 2). The reported work using EDTA sampled As at ambient pH in groundwater (approximately pH 6-8) and/or after acidification of the sample. However, the conditional formation constant for EDTA (K_f') is based on the chelator being in its fully ionized form (charge of –4), which is predominant at pH >10. Therefore, at pH=7 the K_f for the chelation of Fe(III) by EDTA (1.3×10^{25}) is reduced to 6.3×10^{21} because the fraction of the total forms of EDTA in the –4 charge state is only 5.0×10^{-4}.

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8 L. Keith. *Principles of Environmental Sampling.* American Chemical Society, Washington, D.C., 1988.

9 (a) Fuller, C.C.; Davis, J.A.; Waychunas, G.A. *Geochim. Cosmochim. Acta* **1993,** *57,* 2271; (b) Waychunas, G.A.; Rea, B.A.; Fuller, C.C.; Davis, J.A. *Geochim. Cosmochim. Acta* **1993,** *57,* 2251.

10 (a) Gallagher, P.A.; Schwegel, C.A.; Wei, X.; Creed, J.T. *J. Environ. Monit.* **2001**, *3*, 371. (b) Bednar, A.J.; Garbarino, J.R.; Ranville, J.F.; Wildeman, T.R. Environ. Sci. Technol. **2002**, *36*, 2213.

We therefore examined several other well known Fe-complexing agents that could be used — *without correction* — at neutral pH's. Our goal was to increase the aqueous solubility of Fe ions by their complexation to prevent formation of colloidal FeOOH species to which As(V) species readily adsorb. The ideal reagent would have a formation constant that is greater than EDTA for Fe(III) *and* have maximum binding efficiency in the pH range found that is typical of groundwater (pH=6 to 8). We found two reagents that met these criteria (Figure 2): **Cupferron** (N-Hydroxy-N-Nitrosobenzenamine) and **Oxine** (8-Hydroxyquinoline). We compared them to the disodium and tetrasodium salts of EDTA (Na_2EDTA and Na_4EDTA, respectively). The chelating agents were studied at concentrations of 250 and 500 ppm and at neutral pH and pH=4 to determine if the agents were more effective at chelating metals near their pK_a values (Table I).

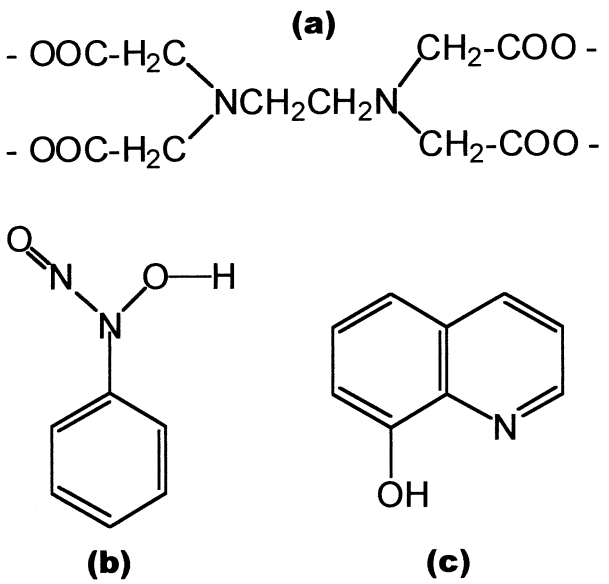

Figure 2. Structures of Fe(III) chelating agents studied. (a) tetravalent anionic form of EDTA, (b) Cupferron, and (c) Oxine are shown.

Chelating Agent	*Log_{10} K_f for Fe(III) (20°C, 0.1 μ)*	*pK_a*
EDTA	25.1	0.0, 1.5, 2.0, 2.7, 6.1, 10.2
Cupferron	~30 (estimate)	4.2
Oxine	35.0	4.9 (N-H), 9.8 (O-H)

Table I. Comparison of formation constants (log K_f) and acidity (pH) for the Fe(III) chelating agents studied.

2.2 Experimental

We conducted on-site sampling in August 2001, November 2001, and April 2002 at the Stilson residence in the Town of Algoma (northwest of Oshkosh, Figure 3). Sample solutions of HCl, Cupferron, Oxine, Na_2EDTA, and Na_4EDTA were prepared ahead of time in opaque high-density polyethylene (HDPE) bottles in 50.0 mL of reagent (18 MΩ-cm) water. At the sampling site, a 50.0 mL sample of unfiltered well water (after purging the well) was added to each of the respective sample bottles (n=4). The container of raw well water was shaken vigorously before transfer to the sample bottles. Quality control samples (blanks and 50 ppb standards) were also brought on-site. Samples were stored at 4 °C during shipment and agitated overnight (60 rpm rotator) before ICP-MS characterization. Samples were analyzed by ICP-MS the day after sample collection, and again 30 days later. Calibration models were constructed using seven standands, (0, 1, 10, 50, 100 250 and 500 ppb). The standard error of the estimate in the dependent variable (SEE_y) was < ±1 ppb. The standard correction for the presence of $Ar^{35}Cl^+$ species was used by monitoring m/z 77 for $Ar^{37}Cl^+$ while normalizing m/z 77 for the presence of minor isotopes of Se. Specifically:

Corrected Response = $^{75}As - (3.18 * {}^{77}ArCl) + (2.55 * {}^{82}Se)$

Total arsenic was determined using the instrumental settings listed in Table II.

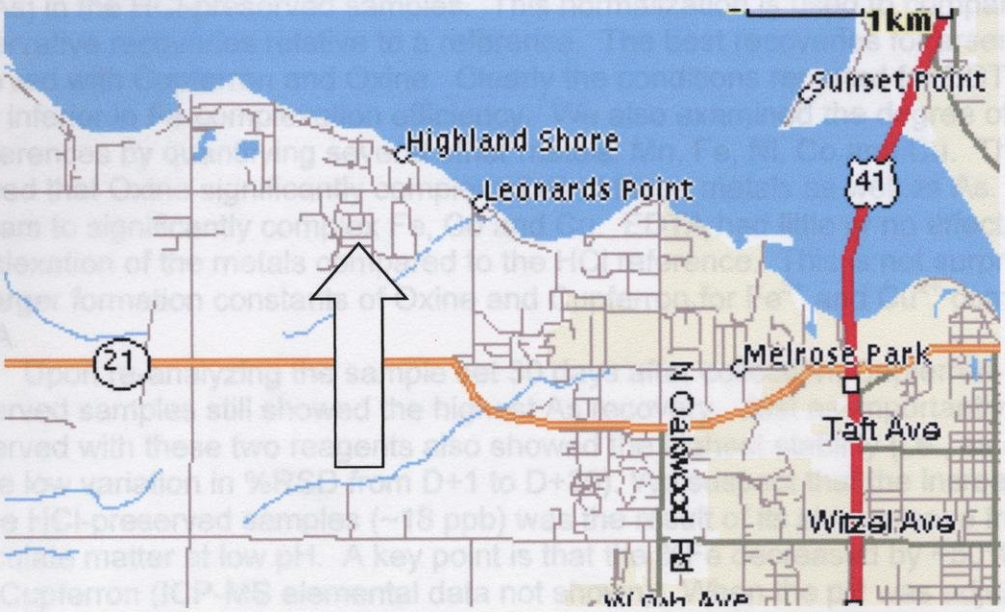

Figure 3. Location of Sampling Site, west of Oshkosh, approximately 1 km north of HWY 21 near Leonards Point Rd.

Factor	*Setting*
Forward Power (kW)	1.30
Coolant Ar (L/min)	13.5
Auxiliary Ar (L/min)	1.1
Nebulizer Ar (L/min)	0.8
Sample Uptake (mL/min)	1.1
Spray Chamber Temperature (°C)	4
Cone Voltage (V)	-70
Detector Voltage (V)	-500

Table II. Operational conditions for ICP-MS.

2.3 Results and Discussion

Figure 4 and Table III show the results of the preservative studies performed on the Stilson well water samples in November 2001. Table III lists the sample type, average As ppb (µg/L), number of samples run, standard deviation, and relative standard deviation of the data. The per cent recovery is the ratio of the average [As] to the [As] in the HCl-preserved samples. This normalization is used to compare the preservative recoveries relative to a reference. The best recoveries for arsenic were observed with Cupferron and Oxine. Clearly the conditions reported for EDTA show it to be inferior in Fe-complexation efficiency. We also examined the degree of matrix interferences by quantifying several other metals: Mn, Fe, Ni, Co and Cu. The data showed that Oxine significantly complexed all of these metals as well as As. Cupferron appears to significantly complex Fe, Co and Cu. EDTA had little or no effect on the complexation of the metals compared to the HCl reference. This is not surprising given the larger formation constants of Oxine and Cupferron for Fe^{3+} and Cu^{2+} compared to EDTA.

Upon re-analyzing the sample set 30 days after collection, Cupferron- and Oxine-preserved samples still showed the highest As recovery. Just as importantly, samples preserved with these two reagents also showed the highest stability (i.e., as evidenced by the low variation in %RSD from D+1 to D+30). We suspect that the increase in [As] for the HCl-preserved samples (~18 ppb) was the result of its slow release from particulate matter at low pH. A key point is that the %Fe decreased by ~50% with 250 ppm Cupferron (ICP-MS elemental data not shown). When the pH was adjusted to 4, the %Fe dropped to 8%, i.e., the lower pH helps to solubilize the Fe(III). The soluble Fe in the 250 ppm Oxine samples was less than 15%. Once again, the Oxine complexed with most of the metal ions and drastically decreased their solution concentrations. The EDTA salts appear to have had little effect on the metals in general. In fact, we found that both of EDTA salts (AR-grade) were contaminated with Cu and Fe (~1.5% w/w). Thus, Cupferron and Oxine appeared to be better preservatives than EDTA for inorganic arsenicals iron-rich groundwater samples.

A sampling trip six months later to the same well (April 2002) provided mixed results. We found that the arsenite and arsenate concentrations were quite different than we observed just six months earlier (using the SPE-ICP-MS method, *vide infra*). The average concentrations for unfiltered samples were (ppb):

Nov 01 arsenate / arsenite = 147 / 22
Apr 02 arsenate / arsenite = 7 / 62

The cause of these changes in the aquifer apparently affected the preservatives as well, especially in the case of the Oxine samples. The results we observed during the first two weeks of preservation were confusing. However, by D+14 the sample that were preserved with 500 ppm Cupferron (0.45 um filtered) showed high recovery and stability. We are continuing to study Cupferron to determine if more consistent results can be obtained for a variety of well locations at different times of the year.

			250 ppm	250 ppm	250 ppm
D+1	**Raw**	**0.1 M HCl**	**EDTA**	**Cupferron**	**Oxine**
ave [As], ppb	174.2	220.2	207.5	265.4	263.7
std dev	13.90	1.60	8.10	1.30	2.80
rel std dev	7.98%	0.73%	3.90%	0.49%	1.06%
% rec	79.1%	100.0%	94.2%	120.5%	119.8%
D+30					
ave [As], ppb	228.0	237.9	220.8	257.8	248.7
std dev	5.80	4.60	7.00	0.30	4.00
rel std dev	2.54%	1.93%	3.17%	0.12%	1.61%
% rec	103.5%	108.0%	100.3%	117.1%	112.9%
ave % rec	91.3%	104.0%	97.3%	118.8%	116.3%
Δ%	+24.43%	+8.04%	+6.04%	-3.5%	-6.8%

Table III. Comparison of preservatives the day after sampling (D+1) and 30 days after sampling (D+30) for Stilson well water sampled in November 2001.

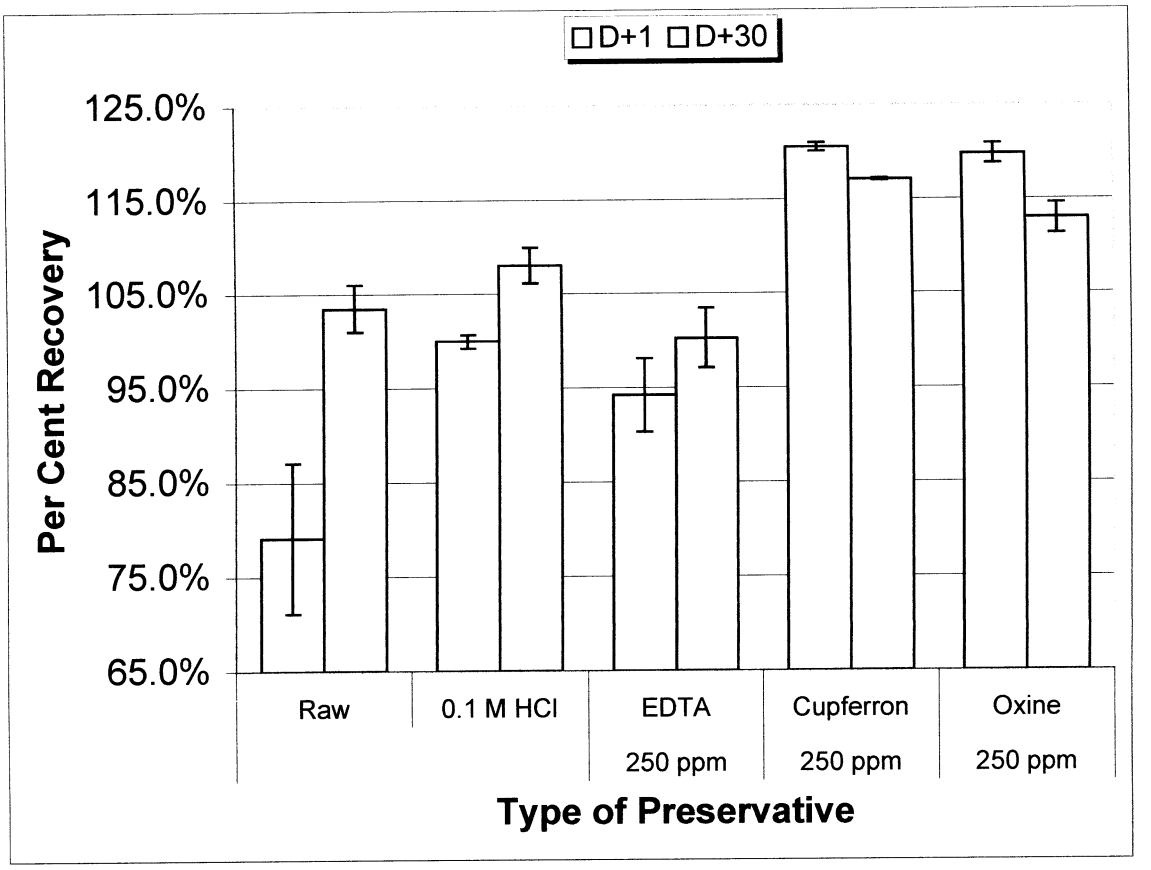

Figure 4. Comparison of preservative efficiency.

2.4 Solid-Phase Extraction

The preservation methods described above are useful when total inorganic arsenic information is sought. For collecting samples for which the chemical speciation of arsenic is desired, a simple method based on Solid-Phase Extraction (SPE) has been reported recently.[11] In this method, an aliquot of well water (~10 mL) is loaded onto the SPE cartridge (1.0 g bed volume) immediately after sampling the well. Because arsenite is protonated at pH < 9.2, neutral arsenious acid is unretained by the cartridge when samples of groundwater are collected (pH 6-8). Arsenate exists in anionic forms at neutral pH (primarily $HAsO_4^{2-}$ and $H_2AsO_4^-$) and is therefore retained by the cartridge. Once the original sample has been loaded and the 'wash' containing arsenious acid collected, a second container is used to collect the arsenate fraction which is released from the stationary phase by strong acid (i.e., thereby protonating the arsenate anions to become neutral arsenic acid). Thus this method is described as "displacment chromatograpy" rather than the more common "elution chromatography".

The strong anion-exchange (SAX) cartridges (manufactured by Supelco) are a proprietary quarternary ammonium-based stationary phase, covalently bound to the

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[11] X.C. Le, S. Yalcin, M. Ma. *Environ. Sci. Technol.* **2000**, *34*, 2342-2347.

inert support. The NR_4^+ functionality is a strong anion-exchanger. Typical results when using these cartridges are shown in Table IV. Arsenite (98.1% recovery) and arsenate (98.5% recovery) were easily separated. Whereas a negligible amount of arsenate (0.4%) is unretained during sample loading, a significant amount of arsenite is retained (4.9%), so a correction must be applied. In the published work, the arsenite retention was implied to be 100%. The precision of the method was also not reported. Because manual syringes were used for sample loading in the published method, we therefore designed and assembled a "field kit" (Figure 5). The kit uses a battery-operated balance to measure the mass of sample loaded onto the SPE cartridge by peristaltic pump. The ruggedized MIL-SPEC shipping case (22"x16") has customized foam inserts to protect the equipment during shipping. The procedure for using the kit is found in Appendix A.

As(III)	*Load*	*Elute*
Sample 1	37.1	1.8
Sample 2	37.6	1.9
Sample 3	32.1	1.5
Sample 4	32.1	1.7
Average	34.7	1.7
Std Dev	3.04	0.17
Target Conc	35.4	0.0
%Recv	98.1%	4.9%
95% (1 df)	27.3	1.5
As(V)	*Load*	*Elute*
Sample 1	0.0	49.2
Sample 2	0.1	49.9
Sample 3	0.4	52.4
Sample 4	0.3	53.0
Average	0.2	51.1
Std Dev	0.18	1.86
Target Conc	51.9	0.0
%Recv	0.4%	98.5%
95% (1 df)	1.6	16.7

Table IV. Typical results for inorganic arsenic speciation using the SPE method.

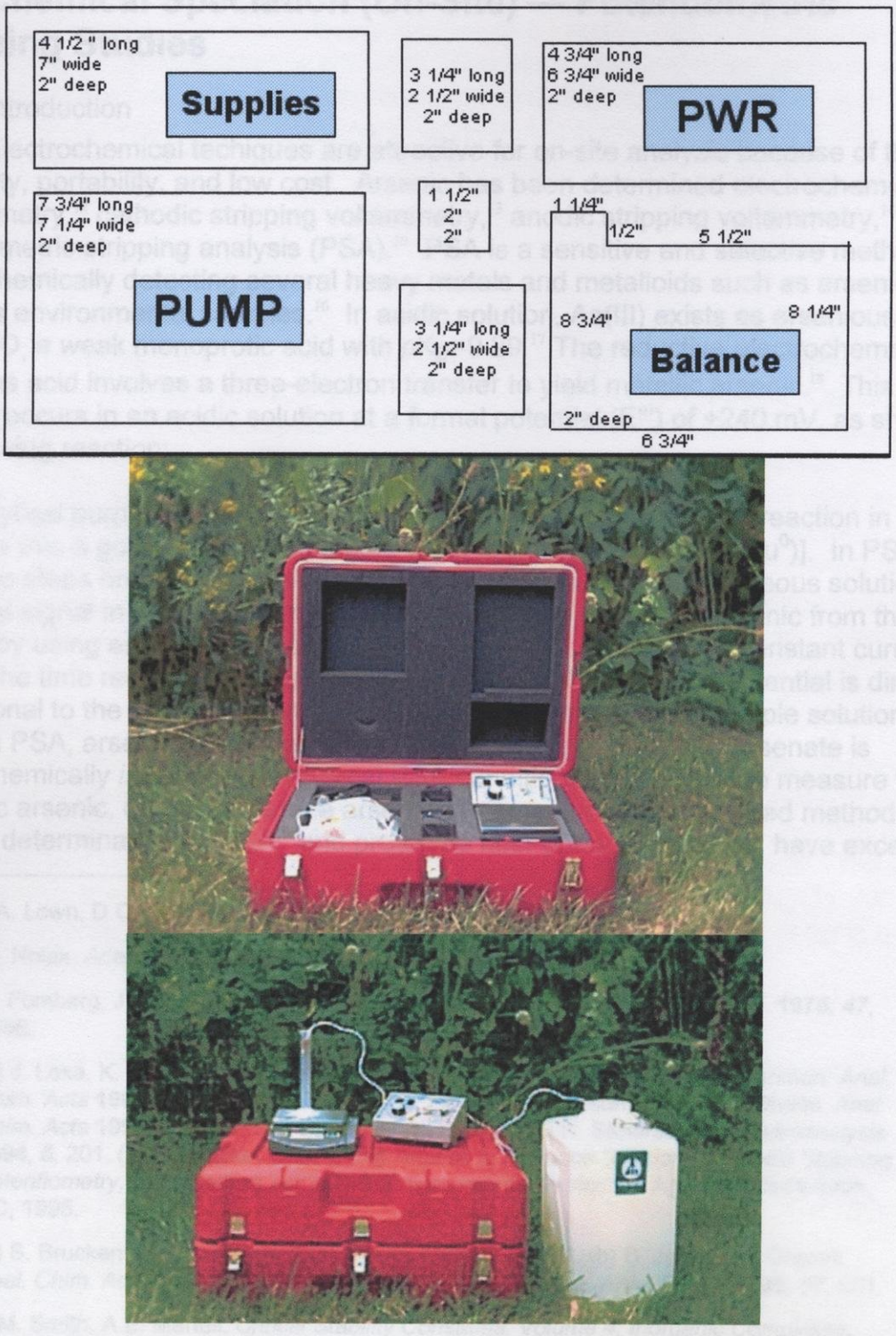

Figure 5. Schematic diagram of the SPE Kit (top) and photographs of the kit before (middle) and during (bottom) use.

3.0 Chemical Speciation (On-Site) — Potentiometric Stripping Studies

3.1 Introduction

Electrochemical techiques are attractive for on-site analysis because of their sensitivity, portability, and low cost. Arsenic has been determined electrochemically by amperometry,[12] cathodic stripping voltammetry,[13] anodic stripping voltammetry,[14] and potentiometric stripping analysis (PSA).[15] PSA is a sensitive and selective method for electrochemically detecting several heavy metals and metalloids such as arsenic in aqueous environmental samples.[16] In acidic solution, As(III) exists as arsenious acid, HO-As=O, a weak monoprotic acid with pK_a=9.29.[17] The reductive electrochemistry of arsenious acid involves a three-electron transfer to yield metallic arsenic.[18] This reaction occurs in an acidic solution at a formal potential (E°') of +240 mV, as shown in the following reaction:

$$HO\text{-}As{=}O + 3H^+ + 3e^- \rightleftharpoons As^0 + 2H_2O$$

For analytical purposes, metallic arsenic can then undergo a second reaction in which it dissolves into a gold electrode as a gold-arsenic amalgam [i.e., $As^0(Au^0)$]. In PSA, these two steps are used to pre-concentrate arsenious acid from aqueous solution. The analytical signal in PSA is then measured by oxidizing elemental arsenic from the gold surface by using either a chemical oxidant in solution or an applied constant current (or both). The time required to pass through the HO-As=O/As^0 redox potential is directly proportional to the concentration of arsenious acid in the original sample solution.

In PSA, arsenic in the trivalent state is measured directly — arsenate is electrochemically *inactive* under typical PSA conditions. Therefore, to measure total inorganic arsenic, one must reduce arsenate to arsenite. Our published methods for arsenite determination by PSA[19] and prototype field-portable system[20] have excellent

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12 J.A. Lown, D.C. Johnson. *Anal. Chim. Acta* **1980**, *116*, 41.

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14 G. Forsberg, J.W. O'Laughlin, R.G. Megargle, S.R. Koirtyohann. *Anal. Chem.* **1975**, *47*, 1586.

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18 A.P. Tomilov, N.E. Chomutov. *Encyclopedia of Electrochemistry of the Elements*, A.J. Bard (Ed.), Marcel Dekker, New York, 1974; vol. 2, pp. 43.

19 J.H. Aldstadt, H.D. Dewald. *Anal. Chem.* **1993**, *65*, 922. (b) J.H. Aldstadt, A.F. Martin. *Analyst (London)* **1996**, *121*, 1387.

figures of merit: linear calibration curves over two orders of magnitude, limits-of-detection < 0.5 µg/L, lack of interfering species (e.g., heavy metals), small sample size (< 500 µL), and high precision (< 5 % rsd). Therefore, the goal of the present work was to expand our method to inorganic arsenate by studying various oxidation approaches, including: electrolysis at very negative potentials, UV photochemical reduction, and chemical reduction by CuCl and $SnCl_2$, KI-ascorbic acid, and L-cysteine.

3.2 Experimental

Reagents

All chemicals were of analytical reagent grade quality and were prepared in high purity (18 MΩ-cm) water (NANOPure™ system, Barnstead-Thermolyne, Dubuque, Iowa). TraceMetal-grade acids were obtained from Fisher Scientific (Pittsburgh, Pennsylvania). Arsenic standards were prepared from As_2O_3 as described previously.[19] All As-containing solutions with concentrations less than 1 mg/L were prepared on the day of use by dilution with HCl. Gold plating solution (50 mg/L) was prepared by dilution of 1000 g/L $AuCl_3$ (Fisher) with 0.1 M HCl. All glassware and plasticware was washed and soaked for at least 48 hours in 5% (v/v) nitric acid (AR grade, Fisher) followed by copious rinsing with reagent water before use. Solutions were not deoxygenated.

Instrumentation

A Model PSU22 TraceLab Potentiometric Stripping Analyzer was obtained from Radiometer America, Inc. (West Lake, Ohio). A saturated Ag|AgCl reference electrode and Pt wire counter electrode (with salt bridge, 1 M HCl) were used along with a glassy carbon disk (3 mm-i.d.) working electrode (BAS; West Lafayette, Indiana). The working electrode was stored in 6 M HNO_3 and polished daily with 0.05 µm γ-Al_2O_3 All potentials reported herein are versus the Ag|AgCl reference electrode at 20°C unless otherwise indicated.

Procedures

The gold film electrode (GFE) was prepared by immersing the polished glassy carbon electrode in a solution of 50 mg/L $AuCl_3$ (0.1 M HCl) for 3 min at -1000 mV vs. Ag|AgCl with moderate stirring. The GFE was then conditioned by cycling through the method three times in 5 mg/L $AuCl_3$ (0.1 M HCl). A GFE prepared in this manner could be used reliably for 12 hr periods before a significant (>5%) change in the response to standards was observed.

The optimal PSA method conditions were determined during the course of this work. The PSA method was initiated by cycling the applied potential five times (5 s duration) between -700 mV and a potential 50 mV more cathodic than the potential at which the GFE began to oxidize (typically +600 mV for 0.1 M HCl, +150 mV for

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[20] J.H. Aldstadt. "A Flow Injection Trace Gas Analysis Method for On-Site Determination of Organoarsenicals", U.S. Patent and Trademark Office #5,641,686; issued June 24, 1997.

solutions containing 1% (w/v) KI, and +250 mV for solutions containing 0.01% (w/v) KI). Deposition was performed at a fixed potential of -700 mV for 30 s with rapid stirring. Stripping was performed in the constant-current mode (+10.0-µA applied current), and stripping data were treated by using a digital filter (7-point boxcar average, followed by a 9-point third-order Savitzky-Golay filter). Stripping was stopped at a potential ~50 mV cathodic of the GFE degradation potential. Background correction was performed immediately following analyte determination by plating for 1 s following by constant current stripping and data treatment as described above.

Quantitation

As was measured by calibration using a linear regression model or by the method of standard addition (MSA) for environmental samples. For calibration, As standards were prepared in carrier solution at 0, 0.5, 5, and 50 µg/L (n=4, in random order). Carryover between standards was less than 1% under these conditions. For MSA, the sample solution (in 1% (w/v) KI) was fortified with an As(V) standard and then adjusted to 0.01% (w/v) L-cysteine. Quantitative reduction of As(V) was observed after the solution was held at 95°C for 30 min with moderate stirring. Total As was then determined after addition of an As(III) standard.

3.3 Results and Discussion

We studied inorganic and organic reducing agents for transforming arsenate to arsenite. We initially examined CuCl[21] and $SnCl_2$[22] based on classic work reported in the literature, but we were unsurprised to find that these metal cations (and/or impurities) deposited on the GFE such that it was impossible to resolve the As(III) signal from the large reductant signal(s). Several organic reductants, including KI-ascorbate and L-cysteine have been reported.[23] We found that the mixture of 1% (w/v) KI and 1% (w/v) ascorbate will reduce As(V) to As(III), as shown in Figure 6. We observed a linear response for arsenite over the range of 1-100 µg/L, but we found that As(V) reduction was very slow (on the order of several hours) and relatively inefficient (<75% conversion).

L-cysteine has also been found to be an effective reducing agent.[24] We observed that As(V) reduction was fast and efficient, with a conversion efficiency approaching 100% after 30 min at 95°C (Figure 7). Beyond 30 min, we found increasing variability in the response. Therefore to insure quantitative and stable reduction, we chose a reaction time of 30 min. We observed that As oxidized from the GFE at approximately +50 mV in room temperature KI-containing solutions, and at approximately -100 mV in KI-containing solutions near 95°C. KI concentrations ranging between 0.01% and 1% (w/v) KI produced similar oxidation potentials (± 5 mV) from the GFE. As a result, these

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21 H.A. Laitenen. *Chemical Analysis*, McGraw-Hill, New York, 1960.

22 S. Tribalat. *Analytica Chimica Acta* **1947**, *1*, 149.

23 S. Nielsen, E.H. Hansen. *Analytica Chimica Acta* **1997**, *343*, 5.

24 (a) Y. Feng, H. Chen, L. Tain, H. Narasaki. *Analytica Chimica Acta* **1998**, *375*, 167. (b) S.B. Adeloju, T.M. Yound, D. Jagner, G.E. Batley. *Analytica Chimica Acta* **1999**, *381*, 207.

were chosen as the integration potentials under the above conditions. A typical calibration model for this method is shown in Figure 8.

In conclusion, we extended our arsenite method to arsenate by applying a reported method based on treatment with the L-cysteine at elevated temperature. Because the heating of the sample is impractical for on-site work, we are adapting the method to a continuous flow instrument that incorporates an in-line heating element. This modification will make use of the method in the field more convenient.

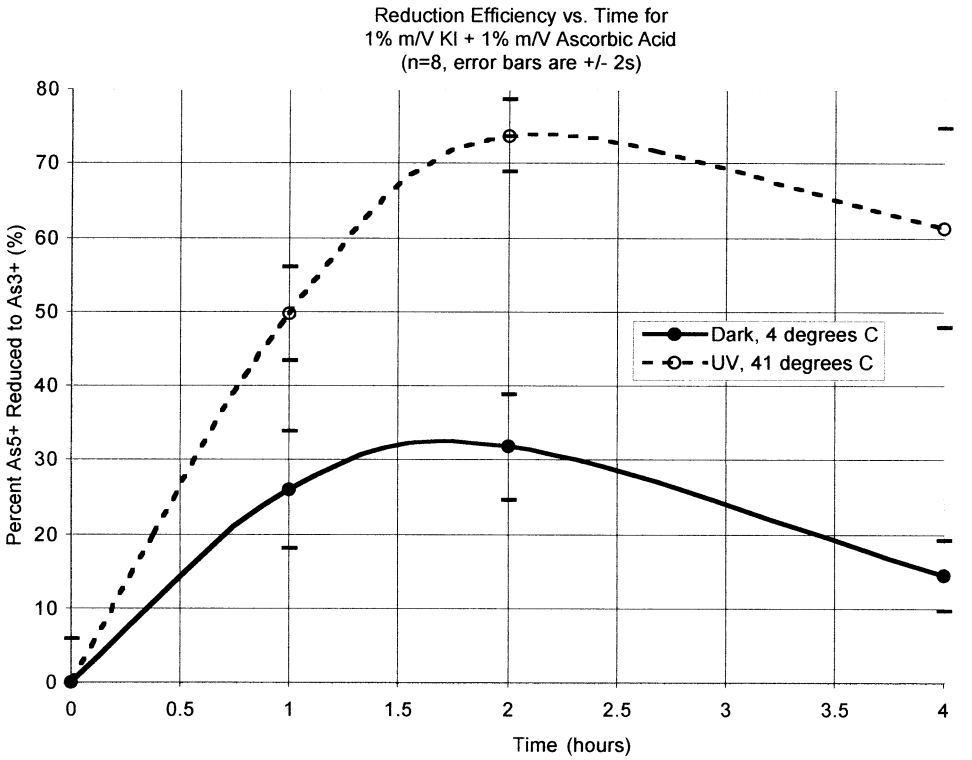

Figure 6. Comparison of the efficiency of reducing arsenate using the KI-ascorbate mixture (100 µg/L arsenate).

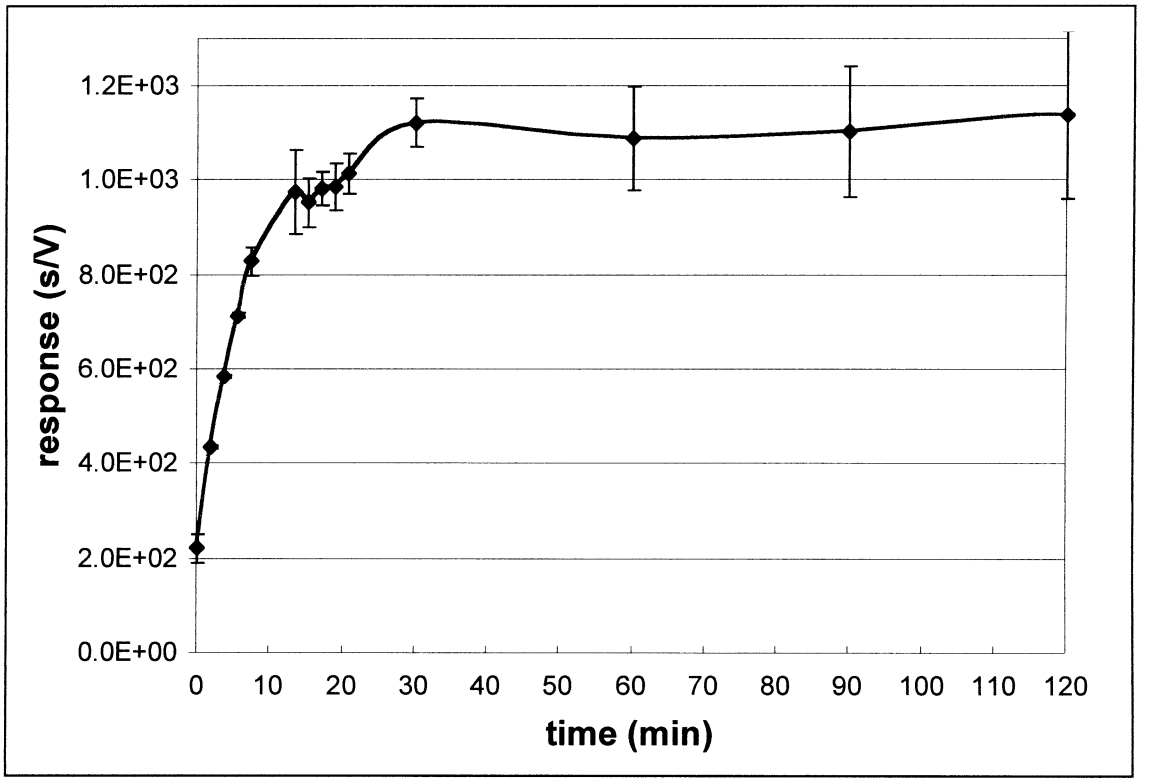

Figure 7. L-cysteine efficiency in reducing arsenate.

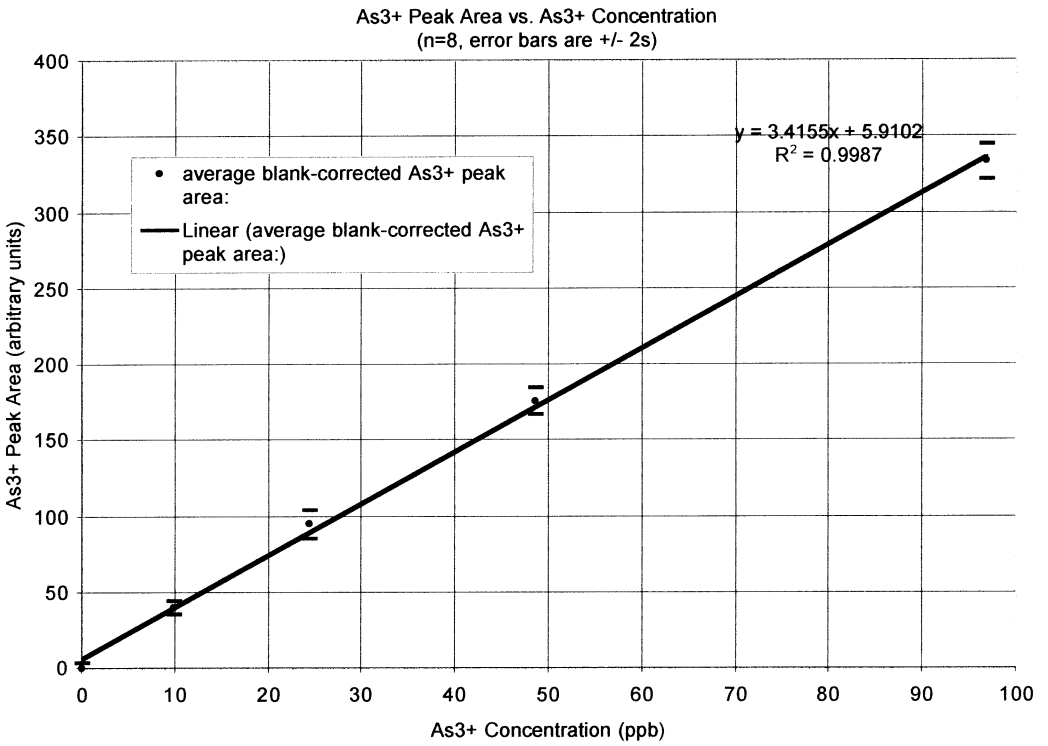

Figure 8. Example of an As(III+V) by PSA calibration model.

4.0 Chemical Speciation (Off-Site) — *Ion Chromatography Studies*

In this section, we describe our studies in developing an improved Ion Chromatography (IC) method for arsenic to permit precise, low parts-per-billion (µg/L) speciation of inorganic arsenicals in drinking waters. The method requires minor modifications to *existing* instrumentation — i.e., small laboratories will not have to resort to the large capital and operating costs of ICP-MS for trace As determinations. The method is based on comprehensive optimization of the anion-exchange ion chromatographic (IC) separation of arsenite and arsenate with post-column generation and detection of the arsenate-molybdate heteropoly acid (AMHPA) complex ion using a liquid-core waveguide.

4.1 Introduction

For water quality analysis laboratories to measure inorganic arsenicals at the new standard, there are several important considerations regarding choice of technique. The key analytical figures of merit are high sensitivity with low detection limits (on the order of 1 µg/L to provide a reliable way to monitor 10 µg/L) and high selectivity to reject interfering species. These requirements rule out several existing techniques for arsenicals, including electrochemical and colorimetric approaches (e.g., using molybdenum blue[25] and diethyldithiocarbamate[26]) which are incapable of quantifying arsenic at 10 µg/L in complex mixtures.[27] While methods based on Ion Chromatography (IC) with conductimetric, colorimetric, or ultraviolet absorption for detection may have the requisite selectivity, their high limits of detection (>100 µg/L) preclude their use as well.[28-30]

There are, of course, several robust methods for quantifying arsenicals in complex matrices at trace levels using atomic spectroscopic methods.[31-38] Mature

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25 J.E. Portman, J.P. Riley. *Anal. Chim. Acta* **1964**, ***31***, 509.

26 M.H. Arbab-Zavar, M. Hashemi. *Talanta* **2000**, **52**, 1007.

27 (a) W.R. Penrose. *CRC Crit. Rev. Environ. Control* **1974**, 465; (b) K.J. Irgolic, *Hazardous Metals in the Environment*, Ed. M. Stoeppler, Elsevier: Amsterdam, 1992, Chapter 11; (c) M. Burguera, J.L. Burguera. *Talanta* **1997**, *44*, 158.

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29 L.D. Hanson, B.E. Richter, D.K. Rollins, J.D. Lamb, D.J. Eatough. *Anal. Chem.* **1979**, *51*, 633.

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34 J. Gomez-Ariza, D. Sanchez-Rodas, I. Giraldez, E. Morales. *Talanta*, **2000**, *51*, 257.

35 M. Vilano, A. Padro, R. Rubio. *Anal. Chim. Acta* **2000**, *411*, 71.

methods exist based upon inductively-coupled plasma optical emission spectroscopy (ICP-OES), hydride generation atomic absorption spectroscopy (HG-AAS), and atomic fluorescence spectroscopy (AFS). And while the notorious isobaric $ArCl^+$ interference at m/z 75 was initially problematic for trace-level arsenic determination by ICP-mass spectrometry (ICP-MS), nowadays the reliable quantitation of arsenic by this technique is routine. Nevertheless, in addition to analytical performance, the candidate method for meeting the new standard should be economical for implementation at municipal water quality laboratories. This in general rules out many of the atomic methods because the capital and operating costs can be prohibitive. In fact, the American Water Works Association recently stated that the new arsenic standard was impractical from an analytical standpoint because only the largest cities could afford the requisite hardware, operational costs, and skilled staff.[39] This argument was predicated on the assumption that only an atomic spectroscopic method presently in practice could reliably measure inorganic arsenicals at <10 µg/L.

4.2 Experimental

Reagents

All chemical reagents used were analytical reagent grade or better. Reagent water (18 MΩ-cm) was prepared by passing deionized water through a NanoPure filtration system (Barnstead-Thermolyne, Dubuque, Iowa) equipped with an ultraviolet lamp (deuterium, 14 W). Stock standards were prepared as follows: arsenic acid from an Atomic Absorption Spectroscopic standard (Fisher Scientific, Pittsburgh, Pennsylvania), arsenious acid from sodium arsenite (Fisher), silicate from a saturated silicate solution (SiO_3) (Fisher), and phosphate from potassium phosphate (Aldrich, Milwaukee, Wisconsin). The pH of binary standards of pentavalent and trivalent inorganic arsenic (i.e., arsenic and arsenious acids) was adjusted to 10.2 with sodium hydroxide (Fisher) before use. Standards were stored in opaque high-density polyethylene (HDPE) bottles in the dark at 4 °C; standards less than 1 mg per L were made on the day of use. Arsenic standards and drinking water samples were passed through a 0.20 µm PTFE syringe filter (Fisher) prior to injection. The IC mobile phase was prepared with sodium carbonate and sodium hydrogen carbonate (Fisher); the solution was filtered under vacuum through a 0.45 µm Nylon 66 filter (Supelco, Bellefonte, Pennsylvania) and degassed by purging with 99.995% (v/v) nitrogen (Praxair, Milwaukee, Wisconsin) at approximately 5 mL/min for at least 10 min. A blanket of nitrogen was maintained over the solution during use. Reagents for the post-column derivatization studies — potassium persulfate, ammonium molybdate

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37 P. Terashade, M. Pantsar-Kallio, and P.K.G. Manninen. *J. Chromatogr.* **1996**, *750A*, 83-88.

38 J. Mattusch, R. Wennrich. *Anal. Chem.* **1998**, *70*, 3649.

39 *Analytical Chemistry of Arsenic in Drinking* Water, American Water Works Association Research Foundation, U.S. Government Printing Office, Washington DC, 1998.

tetrahydrate, ascorbic acid, glycerol, sulfuric acid, and nitric acid — were obtained from Fisher; the mineral acids were "TraceMetal" grade. Potassium antimonyl tartrate hydrate ($K(SbO)C_4H_4O_6$) was obtained from Aldrich. The derivatization reagent solution was prepared fresh on each day of use. All glassware and plasticware was washed and soaked for at least 36 hours in 5% (v/v) nitric acid (AR grade, Fisher) followed by copious rinsing with reagent water before use.

Four local drinking water samples were obtained for study. An aliquot of each sample was diluted 1:1 with mobile phase (2.5 mM sodium bicarbonate / 0.91 mM sodium carbonate) and filtered using a 0.45 µm PTFE syringe. A second aliquot of each sample was treated in the same manner but was fortified with 50 µg/L arsenious acid and 50 g per L arsenic acid. Drinking water samples were fortified with inorganic arsenic > 24 hours before determination.

Instrumentation

A schematic diagram of the system is shown in Figure 9. The ion chromatograph (Model DX-100, Dionex Corp., Sunnyvale, California) consisted of the following components: piston pump; IonPAC AG7 pre-column (50 mm x 4 mm i.d., ~5 µm particle diameter) and IonPAC AS7 analytical column (250 mm x 4 mm i.d., ~5 µm particle diameter) with column temperature controller (Model LC-22A, 500 mm, Fisher); and six-port two-position electrically-actuated injection valve with polyether ether ketone (PEEK) 20-100 µL sample loop. The post-column manifold (Global FIA, Gig Harbor, Washington) was composed of an ultraviolet lamp (254 nm, 8.0 W); a four-channel peristaltic pump with 0.89 mm i.d. Tygon tubing; reagent mixing coil (Teflon tubing, 5.0 m x 0.7 mm i.d.); oxidation coil (Teflon, 2.5 m x 0.7 mm i.d.); reaction coil (Teflon, 1.0 m x 0.7 mm i.d., knitted or "serpentine" design); back-pressure regulator (PEEK, 1.0 m x 0.25 mm i.d., conventional coil design); and stirrer/hot plate (Fisher). The optical system (Ocean Optics, Dunedin, Florida) consisted of a tungsten-halogen light source (Model LS-1, 6.5 W); optical fibers (400 µm o.d. x 2 m); flow cell (Model SMA-Z, Plexiglas, 1.0 cm path length, 1.5 mm i.d.) with sapphire windows or liquid-core waveguide flow cell (Model LCW-1, Teflon AF, 100 cm x 0.56 mm i.d.); and Model S2000 charge-coupled device spectrophotometer (2048-element silicon array with 12.5 µm x 200 µm per element; blazed at 750 nm with 120 lines per mm, 500-1100 nm spectral range).

System performance was monitored on each day of use by quality control charting. The IC system was monitored separately by injecting (25 µL) a 5.0 mg per L arsenic acid standard into the 5.5 mM Na_2CO_3/2.0 mM $NaHCO_3$ mobile phase (1.45 mL/min) and measuring the peak area by suppressed conductivity detection (Dionex Model ASRA-1 self-regenerating suppressor with thermally stabilized conductivity flow cell). The performance of the detector was monitored by injection (150 µL) of either 10 mM $CuSO_4$ (Fisher) into the short path (i.e., 1.0-cm) "Z" cell or by injection (150 µL) of 0.10 mM $CuCO_3$ into the long-path (100-cm) Teflon AF cell at a flow rate of 0.50 mL reagent water per min with

optical detection at 800 nm. After optimization of the method was completed, system performance was monitored by injecting a 100 µL binary sample of 50 µg/L arsenious acid and 50 µg/L arsenic acid through the entire system, with measurement of peak heights at 818 nm.

Data acquisition for the IC system was performed on a Pentium II personal computer using OOIBase 3.2 software (Ocean Optics); the instrument response was measured as maximum peak height. Sequential Simplex optimization was performed using MultiSimplex 2.04 (MultiSimplex AB, Karlskrona, Sweden).

4.3 Results and Discussion

The chromatographic separation, oxidation, and derivatization reactions were optimized and the method was applied to several fortified natural samples. A schematic diagram of the instrument is shown in Figure 9. The optimized method factors are listed in Table V.

Separation of Arsenite

The observed elution order for inorganic $As^{III/V}$ species on weak anion-exchange columns is: $HAsO_2 < H_2AsO_4^- < HAsO_4^{2-} < AsO_4^{3-}$ with the use of an eluent whose pH is above the first pK_a value of arsenic acid (pK_a=2.22). Thus elution of arsenious acid in the void volume can be problematic, dependent upon the matri. We therefore explored ways to increase the retention of arsenious acid by altering the stationary phase composition, eluent composition, and mobile phase flow rate. Through these efforts, the arsenite capacity factor (k') was improved from 0.081 to 0.13 by using a mobile phase (2.0 mL/min) composed of 2.5 mM Na_2CO_3 and 0.91 mM $NaHCO_3$ (pH 10.5). A typical chromatogram (pH=10.5) using the optimal conditions, with a retention time of 13 s for (neutral) arsenious acid and 472 s for arsenic acid (predominatly as its divalent anion), is shown in Figure 10.

Generation of Arsenate

The most powerful methods reported in the literature are oxidation-digestion procedures for decomposition of organoarsenic compounds to arsenic acid, carbon dioxide, and water.[40-43] In the two-step mechanism shown below, irradiation of persulfate ion at 254 nm results in the production of the highly-reactive hydroxyl radical (OH•):

$$S_2O_8^{2-} + h\nu \rightleftharpoons 2\ SO_4^- \qquad (1)$$

$$2\ SO_4^- + H_2O \rightleftharpoons HSO_4^- + OH\bullet \qquad (2)$$

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40 J. Golimowski, K. Golimowski. *Anal. Chim. Acta* **1996**, *325*, 111.

41 R. Rubio, A. Padro, J. Alberti, G. Rauret. *Anal. Chim. Acta* **1993**, *293*, 160.

42 W.R. Cullen, M. Dodd. *Appl. Organomet. Chem.* **1988**, *2*, 1.

43 R.H. Atallah, D.A. Kalman. *Talanta* **1991**, *38*, 167.

Arsenious acid will readily undergo oxidation to arsenic acid under these reported conditions. The post-column photo-oxidation reactor (2.5 m x 0.7 mm) was optimized (0.37 μM potassium persulfate at 0.50 mL/min) such that arsenite was converted to arsenate with 99.8% ± 4.2% efficiency.

Reaction Chemistry Optimization

The most sensitive and widely used IC method for arsenic is based upon post-column derivatization of arsenate by formation of its "molybdenum blue" complex. The reaction scheme for arsenate shown below has been well-known for many years for the determination of phosphate and silicate as well.[44] Application of the "molybdenum blue" colorimetric method has been reported for both batch [45] and flow injection (FI)[46,47] modes. In the first step (reaction 3 below), arsenate (i.e., arsenic acid) forms the arsenate-molybdate heteropoly acid (AMHPA).[48] The solution is heated (40-90 °C) and catalysts based on Sb,[45,47] Sn,[46] or Bi[49] have been reported. The second step (reaction 4 below) is the rate-determining step, a one-electron reduction that converts Mo from the +6 oxidation state to the +5 state to form the complex ion. Reducing agents such as ascorbic acid[45,47,50-52] or hydrazine sulfate[46] have been reported for this step. The reduction causes a color change from yellow to deep blue.

$$H_3AsO_4 + 12H_2MoO_4 \xrightleftharpoons{\Delta \text{ / catalyst}} H_3As(Mo_{12}O_{40}) + 12H_2O \qquad (3)$$

$$H_3As(Mo_{12}O_{40}) \xrightleftharpoons{\text{reducing agent}} H_3As(Mo_{12}O_{40})^{1-} \qquad (4)$$

Multi-variate optimization of the complexation reaction conditions yielded the following levels: 1.3 mM ammonium molybdate, 7.7 mM ascorbic acid, 0.48 M nitric acid, 0.17 mM potassium antimony tartrate, and 1.0 % (v/v) glycerol (Table V). A long-path length flow cell (Teflon AF, 100-cm) was used to measure the absorption of the AMHPA complex (818 ± 2 nm). Figures of merit for arsenite / arsenate include: limit of detection (1.6 / 0.40 μg/L); standard error in

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44 J. Murphy, J.P. Riley. *Anal. Chim. Acta* **1962**, *27*, 31.

45 W.K. Fowler, T.T. Hancock, J.J. Martin. *Anal. Letters* **1985**, *18*, 2381.

46 W. Frenzel, F. Titzenthaler, S. Elbel. *Talanta* **1994**, *41*, 1965.

47 P. Jones and R. Stanley, *Anal. Chim. Acta* **1991**, *249*, 539.

48 C.A. Housecroft, A.G. Sharp, *Inorganic Chemistry*, Pearson, Essex, UK, 2001, Chapter 22.

49 Z. Li, S. Mou, Z. Ni, J.M. Riviello. *Anal. Chim. Acta* **1995**, *307*, 79.

50 Y. Narusawa. *Anal. Chim. Acta* **1988**, *204*, 53.

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52 Z. Li, S. Mou, Z. Ni, J.M. Riviello. *Anal. Chim. Acta* **1995**, *307*, 79.

absorbance (5.1×10^{-3} / 3.5×10^{-3}); and sensitivity (2.9×10^{-3} / 2.2×10^{-3} absorbance units per ppb).

Method Application

The chemistry used in the IC system for generating the HPA complex is not specific to arsenic acid. Phosphate and silicate also form deep blue heteropolyacid (HPA) complexes and can therefore interfere with the determination of the inorganic arsenicals.[46,47,49,51] However, the interference of silicate (up to 10 mg per L) is negligible because it is unable to form a detectable HPA complex under the conditions used. Furthermore, we observed baseline separation of phosphate, arsenic acid, and arsenious acid in the chromatogram; R_s=5.48 ± 0.26 for arsenious acid and phosphate while R_s=3.47 ± 0.06 for phosphate and arsenate. Thus, as illustrated in Figure 10, arsenic acid and arsenious acid can be quantitatively determined in matrices that may contain species that would otherwise interference, in contrast to the suppressed ion conductivity method which only can be used to quantify much higher levels of pentavalent arsenic.

In Table VI, figures of merit and calibration models using the system equipped with either the 1-cm "Z"-cell or the 100-cm Teflon AF cell are shown. For the "Z"-cell, the linear regression model was constructed using arsenic and arsenious acid concentrations of 0.0, 0.10, 0.50, 1.0, and 2.5 mg per L (n=4); for the liquid-core waveguide cell, the concentrations were 0.0, 0.10, 2.5, 5.0, 25, 50, and 100 µg/L (n=4). The limits of detection (LOD, 3δ) and linear dynamic range (LDR) in Table VI clearly indicate that the method can be used to measure environmentally relevant levels.

The method was applied to quantification of inorganic arsenicals in drinking water samples. Drinking water samples were collected from four local sources: (a) the City of Oshkosh, with intake from Lake Winnebago; (b) the City of West Bend, with intake from a deep aquifer; and (c) the City of Milwaukee, with intake from Lake Michigan. A commercial bottled water, "Aqua Splash" from the Lafayette Springs was also tested. We fortified the samples to 50 ppb, which is the current standard until 2006 (work is in progress to validate the method using NIST standards at 10 ppb). Quantitative recovery of fortified samples for arsenic acid (97.9±1.4%) and arsenious acid (93.5±1.3%) was observed (Table VII); the latter required subtraction of a small, unknown, co-eluting peak (note that this was an interference because it was also observed *without* the UV photooxidation reactor in-line). The recoveries of the fortified analytes in the City of Milwaukee municipal water were, however, significantly lower: 60.6 % for arsenious acid and 85.4 % arsenic acid. Apparently, an unknown component of this matrix suppresses both arsenic signals, with the effect more pronounced for arsenious acid than for arsenic acid. Whether the observed suppression is affecting the separation, UV-photooxidation, and/or complexation chemistry steps is not known at this time. Additional experiments are underway to identify the interfering compound and its source.

In conclusion, we were able to extend the venerable "molybdenum blue" complexation chemistry to determine environmentally relevant levels of arsenate, a more than a ~100x improvement in sensitivity. A >30% improvement in the response and ~10-fold improvement in precision was realized by employing a multivariate optimization approach; a ~100-fold increase in path length provided by the Teflon AF flow cell was crucial as well. Additionally, by incorporation and optimization of an in-line photo-oxidation reactor, trace-level quantification of arsenite at relevant levels was also demonstrated. The improved IC method was successfully applied to several types of drinking water samples, and it proved to be an effective means to monitor arsenic in this type of matrix. Our current efforts are centered on extending the method to the measurement of organoarsenicals, e.g., feed additive organoarsenicals (e.g., Roxarsone) in agricultural run-off samples.

Anion-Exchange IC:	*Post-Column Derivatization:*
2.5 mM Na_2CO_3 / 0.91 mM $NaHCO_3$	1.3 mM ammonium molybdate
2.0 mL/min flow rate	7.7 mM ascorbic acid
100 μL injection volume	0.48 M nitric acid
	0.17 mM potassium antimony tartrate
UV Photo-Oxidation:	1.0 %(v/v) glycerol
0.37 μM potassium persulfate	5.0 m derivatization reagent coil
2.5 m oxidant coil	1.0 m reaction coil (serpentine geometry)
0.50 mL/min flow rate	90 °C water bath
	0.50 mL/min flow rate
	818 nm detection wavelength

Table V. Optimized conditions for the IC method.

Table VI. Comparison of figures of merit for the conventional flow cell (b=1 cm) and long-path cell (b=100 cm) for the optimized IC system.

SEEy = standard error of the estimate for absorbance; LOD = limit of detection (n = 10 for both configurations); LDR = linear dynamic range; and RSD = relative standard deviation (for b = 1 cm, n = 8 at 2.5 mg per L; for b = 100 cm, n = 8 at 2.5 µg per L).

	Arsenious Acid (b = 100 cm)	**Arsenic Acid (b = 100 cm)**	**Arsenious Acid (b = 1 cm)**	**Arsenic Acid (b = 1 cm)**
Model	$y = 2.9x10^{-3} x - 2.7x10^{-3}$	$y = 2.2x10^{-3} x + 1.0x10^{-3}$	$y = 4.1x10^{-1} x - 2.5x10^{-3}$	$y = 1.3x10^{-2} x - 3.0x10^{-3}$
SEEy	5.1×10^{-3}	3.5×10^{-3}	4.3×10^{-3}	1.3×10^{-2}
LDR	1.6-100 mg per L	0.40-100 mg/L	160-2500 mg/L	130-2500 mg/L
LOD (3s)	1.6 µg/L	0.40 µg/L	160 mg/L	130 mg/L
RSD	0.004	0.0052	0.028	0.041

Table VII. Results obtained for fortified inorganic arsenicals (each analyte at 50.0 µg/L) in several municipal drinking waters and a commercial bottle water.

Blank values, observed concentrations (based on linear regression calibration models), adjusted values (after blank subtraction), and per cent recovery (%Rec) relative to the fortified value (50.0 µg/L) are listed.

		[As(III)]	Error	%Rec	[As(V)]	Error	%Rec
Oshkosh	Blank	2.2	0.28		0.0	0.00	
	Observed	47.0	1.00		47.1	1.20	
	Adjusted	44.9	0.95	**89.8%**	47.1	1.20	**94.2%**
West Bend	Blank	6.5	0.82		0.0	0.00	
	Observed	52.8	1.00		48.2	1.60	
	Adjusted	46.3	1.30	**92.6%**	48.2	1.60	**96.4%**
Milwaukee	Blank	0.0	0.00		0.0	0.00	
	Observed	30.3	1.00		42.7	1.60	
	Adjusted	30.3	1.00	**60.6%**	42.7	1.60	**85.4%**
Aqua Splash	Blank	7.2	0.46		0.0	0.00	
	Observed	56.3	1.60		51.6	1.50	
	Adjusted	49.1	1.65	**98.2%**	51.6	1.50	**103.2%**
Overall				**85.3%**			**94.8%**

Figure 9. Schematic drawing of the IC system.

OxnC = oxidation coil; MC = mixing coil; RxnC = reaction coil; FO = fiber optic; CCD = charge-coupled device detector; BPR = back-pressure regulator. Inset: photograph of the liquid-core waveguide flow cell (Courtesy of Ocean Optics).

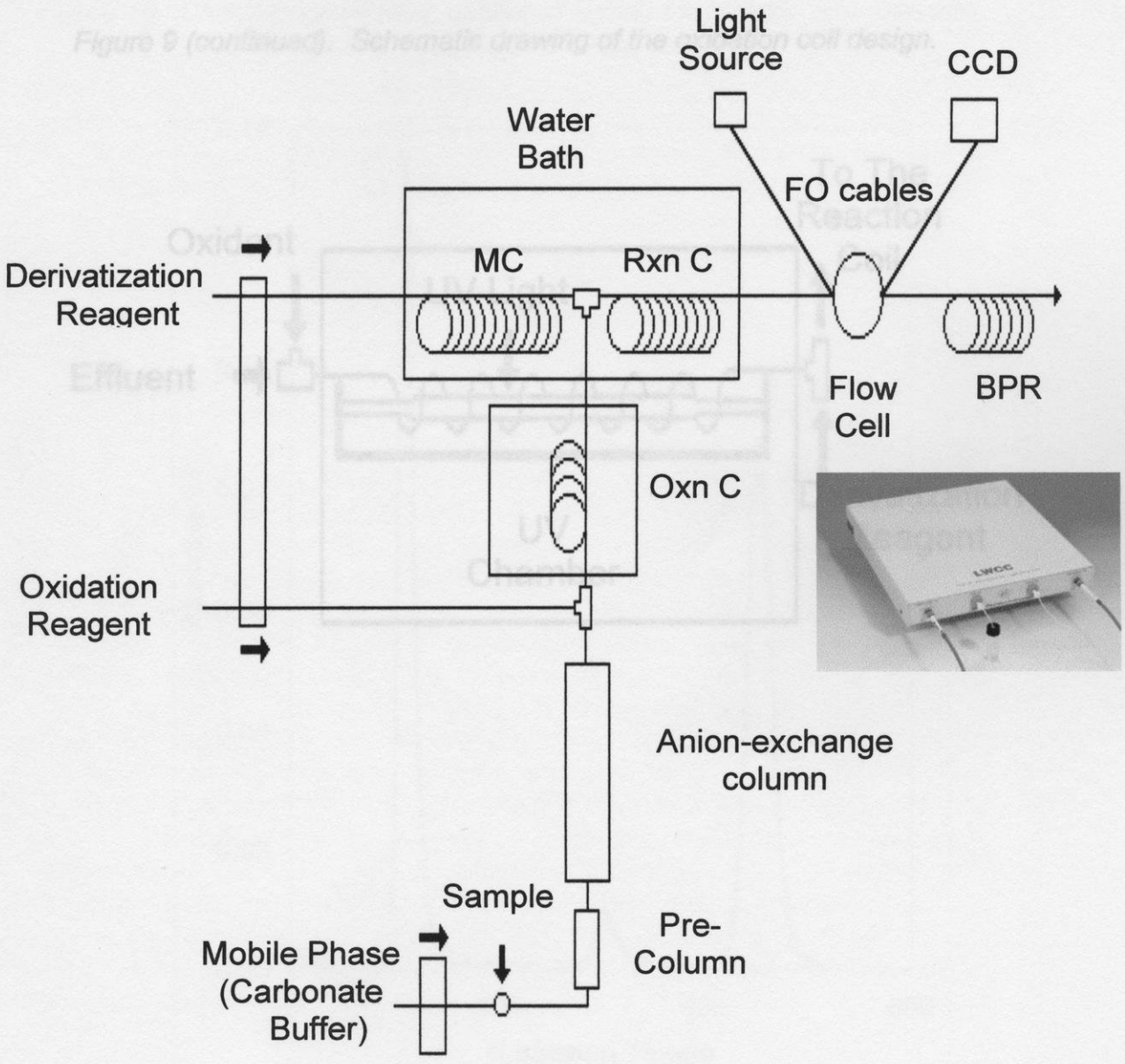


Figure 9 (continued). Schematic drawing of the oxidation coil design.

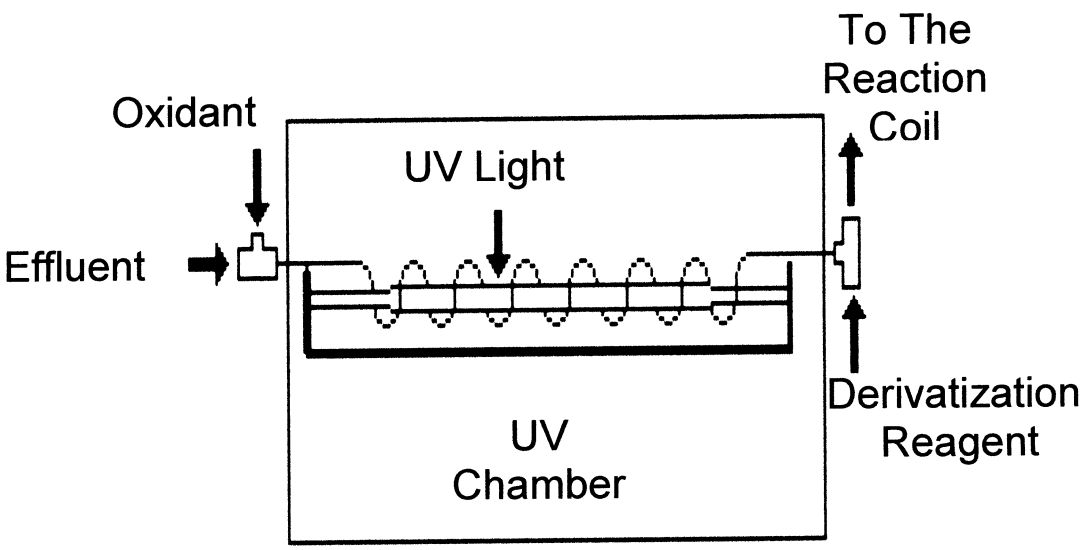
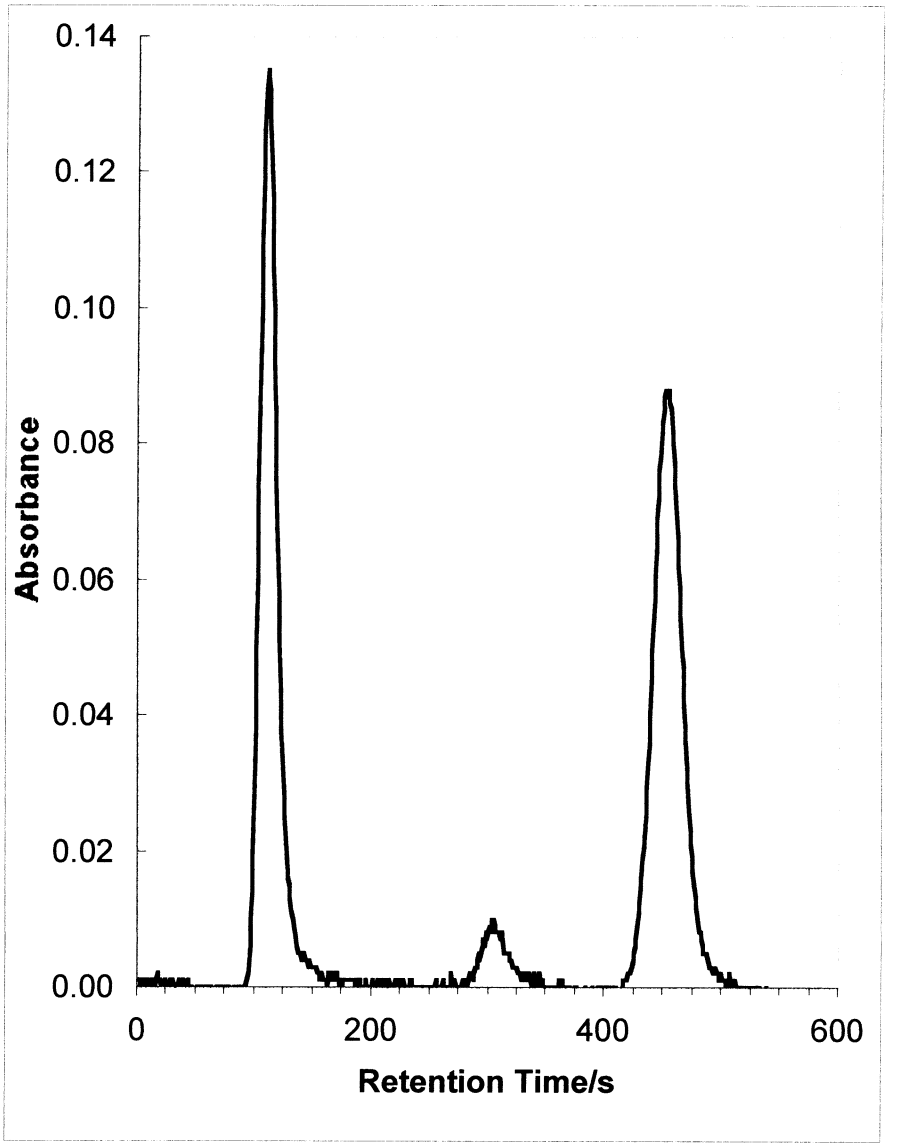


Figure 10. Chromatogram (raw, unfiltered signal) for arsenite and arsenate (each at 50 µg/L) in drinking water.



5.0 Physical Speciation — Field-Flow Fractionation Studies

5.1 Introduction

The on-line physical speciation of metals and metalloids in the sub-micron particle range is an area of research at the forefront of environmental chemistry. For example, the formation and stabilization of very small particles in the colloidal size range can greatly affect the transport of toxic metals in water. The distribution ratio of metals can be lowered by orders of magnitude and the risk factors associated with increased solubility are raised significantly. Current mathematical models fail to account for the sorption behavior of metals in systems with high solids-to-aqueous ratio.[53]

The techniques presently used to fractionate sub-micron particulate material (e.g., centrifugation, ultrafiltration) suffer from poor resolution and low sample throughput. Field-Flow Fractionation (FFF) is based on the use of a ribbon-like channel with permeable walls. In the "flow" mode of FFF, a field perpendicular to the flow through the channel is created by a cross-flow of liquid at a right angle to the channel flow. The cross-flow forces the sample particles against the accumulation wall (i.e., the membrane) where they form a "cloud" whose mean thickness depends on the diffusion coefficient of the particle and the linear velocity of the cross-flow stream. The normal laminar flow through the channel has a characteristic parabolic distribution of linear velocities across the thin dimension of the FFF channel. This means that the linear velocities close to the walls of the channel are lower than those in the center of the channel. The thicker sample clouds which are formed by particles of smaller diameter (larger diffusion coefficient) are caught up in the faster moving flow lines (close to the center of the channel) and are swept down the channel faster than those which form the more compact clouds (larger particles, smaller diffusion coefficients). This forms the basis of the separation of macromolecules, colloids, or particles with FFF.[54]

We used FFF to study the distribution of inorganic arsenicals as a function of particle size. We studied particulate and colloidal matter in the range of ~50 to 500 nm. Although FFF can be used to study larger particles using the “steric” mode, we chose to focus on the 50-500 nm range for two reasons: (a) the smallest particles and colloids are the most mobile in the environment (and have been mentioned in several geochemical studies of the FRV as potentially important)[55] and (b) samples of FRV groundwater that have been filtered (0.45 µm) were shown to contain small particles/colloids with adsorbed As. The presence of these species can affect the accuracy of the ICP-MS measurement because if the analyst agitates the sample beforehand, a significant increase in response can be observed.

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53 B. D. Honeyman, P. H. Santschi. *Environ. Sci. Technol.* **1988**, *22*, 862.

54 J.C. Giddings. *Science* **1993**, *260*, 1456-.

55 (a) Gotkowitz, M. “Report on the Preliminary Investigation of Arsenic in Groundwater Near Lake Geneva, WI”, Wisconsin Geological and Natural History Survey, 2000.

5.2 Experimental

The Flow FFF system (Model F1000; Postnova Analytics, Salt Lake City, UT) is shown schematically in Figure 11. Channel flow and cross flow solutions were buffered with 0.050 M *Tris*(hydroxy)-aminomethane hydrochloride (THAM) (pH=9.0). Particle standards were purchased from Duke Scientific (Palo Alto, CA) and Polysciences, Inc. (Warrington, PA). Standards of 50, 112, 214 and 402 nm were prepared at a concentration of 0.02% (w/v) in 0.01 %(w/v) Triton-X and 0.02 %(w/v) sodium azide, and the pH was buffered to 9.0 using THAM. Samples were injected (20 µL) into the channel flow (2.0 mL/min), and the cross flow was stepped at 2 min intervals from 2.0 mL/min to zero. A UV detector and right-angle laser light scattering (RALLS) detector were used in series. The UV detector monitored scattering at 254 nm while the primary line (488 nm) of an Argon ion laser (Uniphase) was used for RALLS.

5.3 Results and Discussion

Most of our effort for this aspect of the project was in developing the instrumental setup for the Flow FFF system. We assembled the system (Figure 11) and studied various standards and FRV groundwater samples (preserved and "raw") using three different detection methods. We examined humic acids using a fluorescence detector and more general particle/colloid detection by light scattering using an ultraviolet (UV) detector and a right-angle laser light scattering (RALLS) detector.

We initially used a flow-through fluorescence detector to characterize the humic acid (HA)-laden fraction (< 500 nm) of groundwater to "benchmark" the system. This configuration was found to be sensitive to ~1 mg/L HA using a commercial HA preparation (Aldrich). We then studied the UV and the RALLS detectors downstream of the fluorescence cell in the system to study species in the target size range. Particle standards (polystyrene spheres) were used to construct a model over the range of interest (Figure 12).

We are presently studying the separation of material in preserved samples of FRV groundwater. We are planning to examine the fractions by atomic and molecular MS to determine the distribution of arsenicals for suspended solid material in these samples.

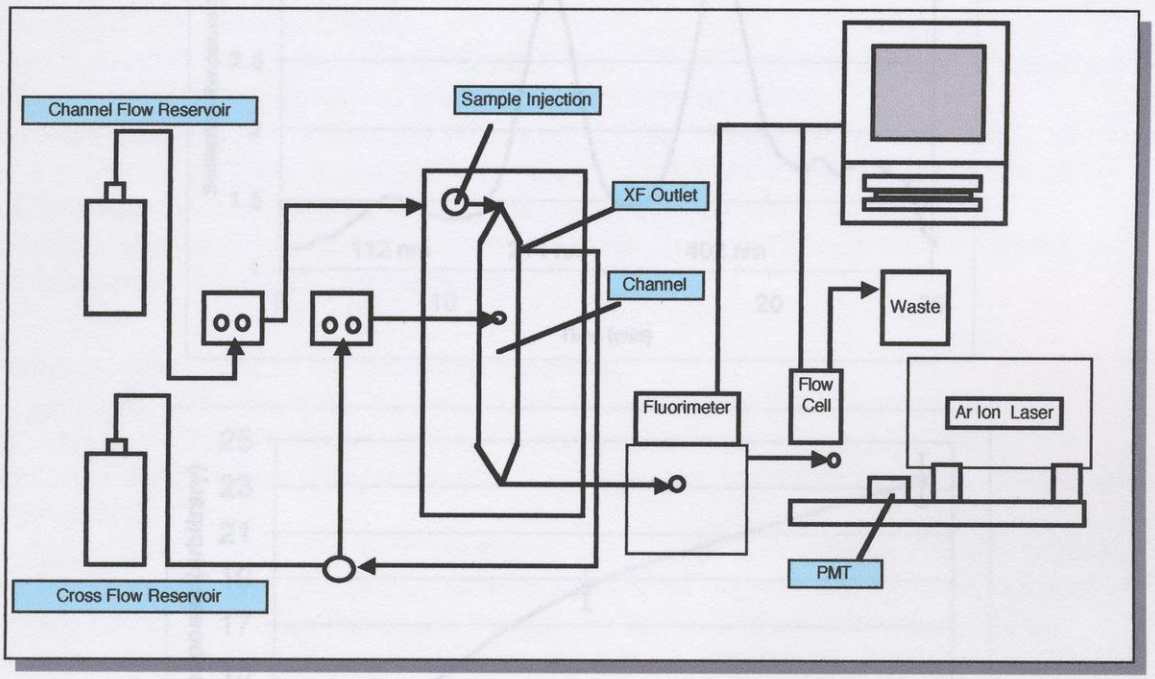

Figure 11. Schematic of the Flow FFF system.

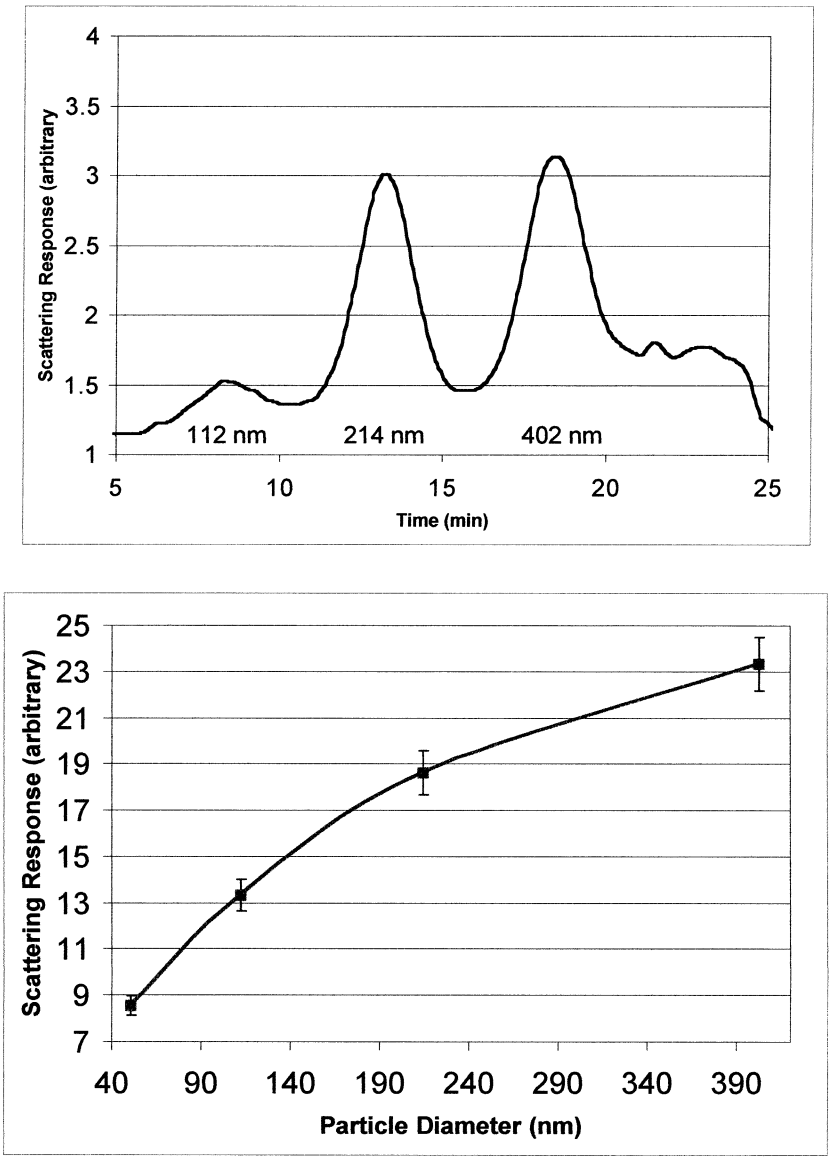

Figure 12. Calibration response (top) and model (bottom) prepared for the Flow FFF system using particle standards (50, 112, 214, 402 nm).

Acknowledgements

The research described in this report was performed by:

- **Rebecca Johnson** conducted the IC method development work as her Master of Science thesis, graduating in December 2001. Rebecca is now a chemist in the Analytical Development Group at Pharmacia (Kalamazoo, MI).
- **Jason Harb** performed the Flow FFF work. Jason is a student in the Ph.D. program in analytical chemistry at UWM.
- **Aaron Roerdink** performed the SPE work and ICP-MS measurements. Aaron is a student in the Ph.D. program in analytical chemistry at UWM.
- **Jonathan Scaffidi** performed the PSA work as his "Senior Thesis" at UWM. Jon is now a graduate student in analytical chemistry at the University of South Carolina.
- **Dr. Christine Blaine** conducted the preservative study while on a one-year sabbatical as a Visiting Professor from Carthage College (Kenosha, WI), where she is an Associate Professor of Chemistry.

This work was presented at the following meetings:

- R.L. Johnson, J.H. Aldstadt. Determination of Arsenic by Ion Chromatography: Optimization of the 'Molybdenum Blue' Complex Formation Reaction. 28th Federation of Analytical Chemistry and Spectroscopy Societies Meeting, Detroit, MI (October 2001).
- J.H. Aldstadt, R.L. Johnson, A.R. Roerdink, J.G. Harb, C.A. Blaine. Arsenic and Old Ligands: Development of Comprehensive Analytical Methods for Arsenic Speciation in Groundwater. 26th Meeting of the Wisconsin Section of the American Water Resources Association, Wisconsin Dells, WI (March 2002).
- R.L. Johnson, J.H. Aldstadt, Parts-Per-Billion Level Quantitation of Inorganic Arsenicals in Drinking Water by Ion Chromatography. 34th Great Lakes Regional Meeting of the American Chemical Society, Minneapolis, MN (June 2002).

Finally, we thank Dave Johnson and Tim Asplund of the Wisconsin Department of Natural Resources and Madeline Gotkowitz of the Wisconsin Geological and Natural History Survey for insightful discussions regarding environmental arsenic behaviour.

Appendix A. Sample Collection Procedure for Inorganic Arsenic Compounds in Well Water by Solid-Phase Extraction

Equipment:

- Strong Anion Exchange (SAX) Cartridges, 3 mL (Supelco)
- Mini-Pump Variable Flow Peristaltic Pump (Fisher Scientific)
- Tygon tubing with PE unions
- Mini-Balance (Fisher Scientific)
- Optima Grade Nitric Acid (Fisher Scientific)
- Parafilm
- 60 mL clean HDPE bottle
- Cooler with ice

Procedure:

The following steps are performed on-site:

1. Condition the SAX cartridge as specified by Supelco.
2. Set the Mini-Pump to PURGE and flush tubing with approximately 50 mL of well water sample.
3. Connect SAX cartridge to tubing. Place cartridge above a 60 mL clean HDPE bottle on the balance.
4. Set pump to FAST 2. At this setting the flow rate will be approximately 1.5-2.0 mL/min flow through the cartridge. Allow well water to pass through cartridge until 10.0 g is reached on the balance.
5. Cap and seal the sample bottle with Parafilm. This sample contains arsenite (As^{+3}).
6. Seal both ends of the SAX cartridge with Parafilm. The SAX cartridge contains arsenate (As^{+5}).
7. Place the sample bottle and cartridge on ice in the dark during shipment to the lab.

The following steps are performed in the lab:

8. Using the Mini-Pump set on PURGE, flush tubing with approximately 50 mL of 1.0 M HNO_3.
9. Place the cartridge above a 60 mL clean HDPE bottle on the balance.
10. Desorb the arsenate by connecting the SAX cartridge to the pump tubing and set pump to FAST 2. At this setting the flow rate will be approximately 1.5-2.0 mL/min flow through the cartridge. Allow the 1.0 M HNO_3 to pass through cartridge until the 10.0 g mark is reached on the balance.
11. Both arsenite and arsenate fractions are then analyzed by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS).