Development of a Gas-Phase Chemiluminescence System for the Measurement of Arsenic in Drinking Water

By

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1. INTRODUCTION

Inorganic arsenic is classified as a known human carcinogen by the International Agency for Research on Cancer (IARC) and the World Health Organization (WHO).[1-2] Arsenic poisoning is characterized by serious illnesses due to hyperkeratosis on palms and feet, fatigue symptoms of arsenicosis, cancer of the skin, bladder, lung, and other vital organs. Chronic exposure has also been linked to respiratory, reproductive, developmental, neurological and immunological defects.[3] Arsenic toxicity is related to its ability to inactivate several enzymes and it weakens the integrity of structural proteins that are involved in DNA repair and cellular metabolism.[4]

Prolonged consumption of water from arsenic contaminated aquifers lead to chronic arsenic poisoning in many parts of the world. The worst and most publicized situations exist in Bangladesh, India, Nepal, China, Argentina, New Mexico and Chile.[5] Toxic level concentration in groundwater has been documented in more than 26 countries around the globe.[6] In the 1970's about 10 million tubewells were drilled throughout Bangladesh in an effort to eliminate waterborne diseases originating from polluted surface water. It was later discovered that the water from these tubewells were contaminated with high levels of arsenic. It is estimated that 77 - 95 million people out of a total population of 140 million in Bangladesh are drinking groundwater containing more than the maximum contamination level (MCL) of 50 μg/L[7]. According to the United States Geological Survey, 32 million people in the US drink water containing 2.0 μg/L - 50 μg/L arsenic. In 2006, the U.S.EPA reduced the maximum permissible level

of arsenic in drinking water from 50 to 10 μg/L due to the increasing health hazard related to arsenic exposure[8]. The Canadian Federal Government has recently proposed a revised 5.0 μg/L arsenic limit for the Federal Drinking Water Guidelines. According to the guideline, the estimated lifetime cancer risk associated with the consumption of drinking water containing arsenic at 10.0 μg/L is greater than the range that is considered generally to be "essentially negligible".[9]

Clearly there is an urgent need to monitor and document drinking water sources around the globe. However, the lack of a rapid, reliable and field deployable method for the measurement of arsenic has been the challenge for decades. The primary motivation of this research is to develop an innovative technology based on chemiluminescence (CL) detection of arsine gas with ozone in a simple instrumental setup and computer controlled system that is amenable to remote laboratories in Bangladesh and elsewhere.

2. EXPERIMENTAL

2.1 Chemicals and Reagents

All chemicals used in this experiment are guaranteed reagents suitable for use in the chemistry laboratory which meet or exceed the American Chemical Society (ACS) requirements where required. Standard stock solutions of As (III) and As (V) were prepared at concentrations of 400 μg/L using sodium arsenite ($NaAsO_2$, Baker & Adams) and sodium arsenate heptahydrate ($Na_2HAsO_4 \cdot 7H_2O$, Fisher). Pure deionized water (18 MΩ-cm at 25^oC) was used for dilution of the stock solution. In some cases 2.0 M H_2SO_4 was used for dilution immediately before use. The reducing agent, 2%

NaBH$_4$ solution for hydride generation, was prepared from sodium borohydride (sodium tetrahydroborate, NaBH$_4$, 99% reagent plus, Sigma Aldrich) dissolved in 0.5 M NaOH prepared from solid sodium hydroxide pellets (NaOH, Amresco) and 1.0 mM disodium ethylenediamine tetraacetate (Na$_2$EDTA, Fisher). The NaOH was used to stabilize the borohydride and Na$_2$EDTA was added to form a highly stable coordination compound with the trace metals which are known to catalyze the reduction of borohydride. (Note: NaOH absorbs water from accessible air and its weight can be altered instantly if left in open air.) The 2.0 M H$_2$SO$_4$ solution prepared from concentrated H$_2$SO$_4$ (95 - 98% ACS Reagent, Sigma Aldrich) was used for total As (III) and As (V) determinations in acidic media.

Silver nitrate (AgNO$_3$, Fisher), ammonium hydroxide (NH$_4$OH, Sigma Aldrich), potassium hydroxide pellets (KOH, Fisher), dextrose sugar (C$_6$H$_{12}$O$_6$, J.T. Baker) and concentrated nitric acid (HNO$_3$, Sigma Aldrich) were used to prepare the silvering solution that was applied for the silver-coating of the reaction cell. Potassium iodide (KI, 99% Reagent plus, Sigma Aldrich) was used for detecting the presence of ozone in the flow system and to absorb excess ozone on the exit of the system. The clear potassium iodide solution turns to yellow color upon ozone absorption. Granular activated carbon was also used at the inlet of the air pump to capture biogenic organic contaminates and at the end of the system to destroy ozone. (HAZARD: activated carbon at higher temperature may cause fire upon high levels of ozone flow.)[10] Silica gel desiccant (SiO$_2$, Fisher) and Drierite (CaSO$_4$, Fisher) were used as drying agents. For sample analysis purposes, random unknown sample solutions were prepared from 1000 µg/L As stock solution. SRM (Standard Reference Material, Fisher) was used for validation

of results. Other tested samples included groundwater and surface water samples from different regions of Ethiopia and ground water sample from Siskiyon Mountain Oregon.

2.2 Materials and General Instrument Setup

For a batch measurement system it is necessary to deliver the reducing agent ($NaBH_4$) into the reaction cell as fast as possible so that the generation of arsine gas will be instantaneous. This was accomplished by an auto-pump (SP100VI-004 Variable flow pump, APT instruments), indicated as $NaBH_4$-Pump on the schematic diagram. The pump is connected to a control switch on the circuit board (CB) for computer automation through its serial port and powered by a 12 V DC power supply (PS). Experience shows that the strong alkaline borohydride solution is a very strong reducing agent and could destroy plastic materials and make them brittle. A 3 mm cylindrical teflon magnetic stirrer was used to swirl the sample continuously.

Samples are prepared from 2.0 mL arsenic containing water sample in 4.0 mL of 2.5 M H_2SO_4. The mixture is placed into a 40.0 mL glass vial with screw top Teflon line septa (Supelco). Two holes were made on the tightly closed Teflon septa. (Note: Teflon Septa has good leak resistance property, but it is important to check for leaks frequently.) The first hole is used for $NaBH_4$ inlet and it reaches midway into the vial. The second hole is used for AsH_3 gas outlet and it is set near the top of the vial. The two tubes are Fluorinated Ethylene Propylene (FEP, Upchurch Scientific) with 1/16 inch OD and 1/50 inch ID sizes. The length of the tube that connects the reaction vial to the cheiluminescence chamber (CC) is 45 cm and the tubing between the CC and the waste vial is 90 cm containing a 4 cm radius loop. Two flow controllers (Micro Metering Valves Assy P-446 and P-447, Upchurch Scientific) and two unidirectional check valves

(02-19CV0012N, Ark-Plas) were used for fine tuning the flow rate and prevent backflow on each side of the air pump and ozone lines. Fittings, nuts and unions (P-200, P-207, P-307, P-630, P-602 and U-665, Upchurch Scientific) were used for connecting tubes based on their sizes. An adjustable ozone generator with ozone output capability of 200 mg / hour (60 Hz Model OZX-300UB, Enaly Trade Co., Ltd. China) using a power source of 110 V - 120 V AC to generate the necessary ozone for the CL reaction from pure oxygen in ambient air. The ozone generator consumes a total power of 15 W to operate. The air is supplied to the ozone generator by a small air pump attached to the ozone generator which is packed in the same compartment sharing the same power supply. Two 250 mL cylindrical plastic containers (Enaly Trade Co. Ltd. China) containing drying agent and activated carbon (Drierite, $CaSO_4$) are attached to the inlet of the air pump to trap moisture and any organic matter.

A glass reaction cell, also referred as a chemiluminescence chamber (CC) designed in this laboratory with specific dimensions was used throughout the experiment. The glass cell has 1 mm thickness, 0.5 inch diameter and 1.75 inch vertical height. It has two 0.5 inch long by 0.0625 inch diameter inlets on the top portion of the cell for AsH_3 and O_3 flow and a third outlet of the same size at the bottom for reacted AsH_3 and O_3 exit flow. Figure1 shows the glass chemiluminescence reaction cell with its optical window mounted onto a photomultiplier tube. A glass waste vial made with Teflon septa screw top (40 mL, Supelco) filled halfway with activated carbon was used for waste drainage. The waste vial is bidirectionally linked using FEP tubing to the exit channel of the chemiluminescence chamber and to a 0.5 L plastic bottle containing

acidic potassium iodide (KI) solution, so that the ozone will be trapped before it gets
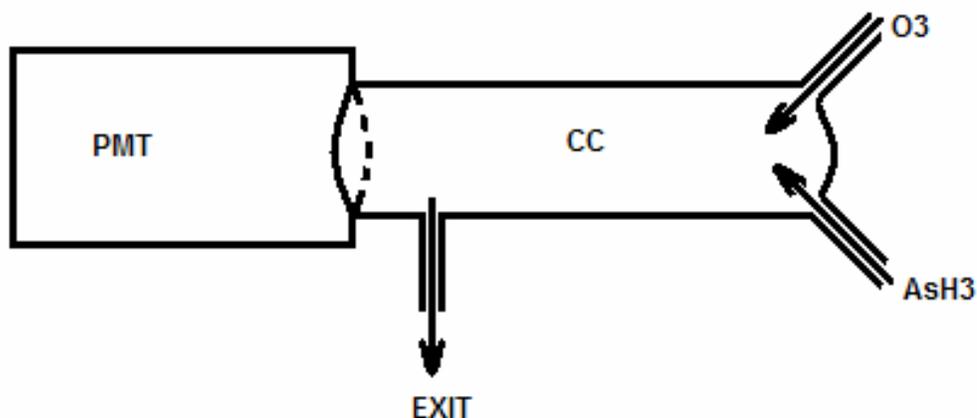
released to the environment.

Figure 1. Chemiluminescence Chamber (CC) with O_3 and AsH_3 inlets and exit

connection with its optical window mounted on the PMT.

A photomultiplier tube (PMT, Hamamatsu photodetector Model H5784, Japan)

was used to sense and amplify the emission of chemiluminescence (CL) resulted from

the AsH_3 and O_3 reaction. The PMT is connected to a circuit board (CB) sharing a 12

volt DC power supply (PS). A black opaque polyethylene box, 20 cm x 15 cm x 5 cm (l

x w x h) was used to keep the reaction cell and the PMT attached together in an opaque

surrounding, so that light can not get access and interfere with the CL detection. A

circuit board that contains op-amps (operational amplifiers) with variable gains up to

10000 was implemented in a network of feedback resistors. The circuit has a filter with

a 1 sec time constant to reduce the external noise such as the noise induced by air

pump fluctuation and the power supply. All data acquisition and control was maintained by a PMD USB-1408FS data card (Measurement Computing Inc. USA). The PMT gain was controlled by a 12 bit digital to analog converter (DAC) and all the data were acquired by a 14 bit analog to digital converter (ADC). A blue LED was attached inside the black box to test the performance of the PMT at the beginning of each experiment. The LED was controlled by the digital logic output from the data card. The software interface was written in-house using Delphi-6 (Borland Software Inc. USA), a Pascal programming language and it was loaded onto a Pentium 4, Windows XP class laptop PC.
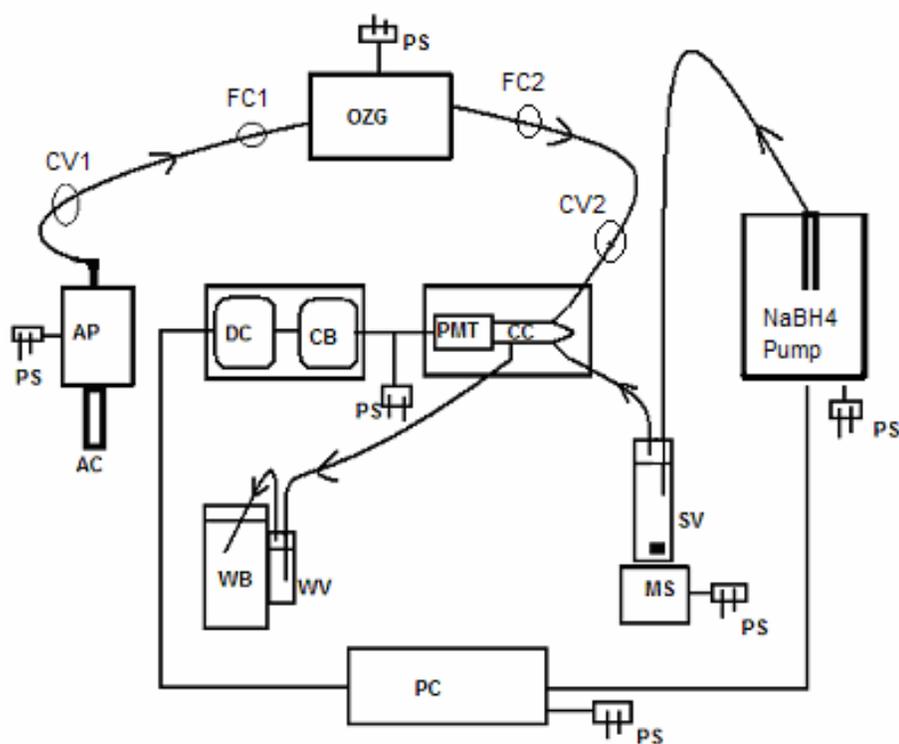

Figure 2. Schematic diagram of Gas-phase CL based arsenic analyzer. The abbreviations indicate: Power Supply (PS), Sample Vial (SV), Magnetic Stirrer (MS),

Flow Controller 1 & 2 (FC1, FC2), Check Valve 1 & 2 (CV1, CV2), Ozone Generator (OZG), Chemiluminscence Chamber (CC), Photo Multiplier Tube (PMT), Circuit Board (CB), Data Card (DC), Air Pump (AP), Activated Carbon (AC), Waste Bottle (WB), Waste Vial (WV),and Personal Computer (PC).

2.3 System Mechanization

It is very important that the exact amount of the required reagent is delivered into the reaction vial at a specific time, so that there will be enough time to collect enough data for the background signal as well as for the actual chemiluminescence signal. In this experiment the computer program was setup to allow a 20 seconds delay for collecting only the background signal then activate the auto-dispenser to add the $NaBH_4$ solution and continue collecting data for another 40 seconds until the signal dies down to reach near the background level. In general 30% of the total allocated time for running a single experiment is devoted to background signal collection and 70% of the total time is spent on collecting the chemiluminescence signal. The computer program subtracts the background signal from the total acquired signal and displays the integrated signal (mV-s) in the user window. The integrated signal (I Signal) generated from several standard solutions can be used to calculate the calibration factor (calibration factor = I Signal (mV-s) / Conc. (µg/L)). Once a calibration factor (slope of the linear calibration curve) is known, the concentration of unknown samples can be automatically calculated and displayed in the user interface window in units of µg/L. The program has different adjustable parameters that are applicable for testing and troubleshooting the instrument with a blue LED that is also controlled by the computer

interface. The full scale signal detection can be adjusted from 1 V to 20 V with a signal amplifier gain up to 10,000, and variable PMT gain from 0 mV to 900 mV. The number of data points that can be collected in a single experiment and the interval between these data points can also be adjusted in the user interface. The chemiluminescence data acquisition user window indicating the adjustable parameters is shown on Figure 2. The program has the option to digitally filter the data, enter a calibration factor for direct concentration display, reading data, printing data, copying and saving data files for post processing.
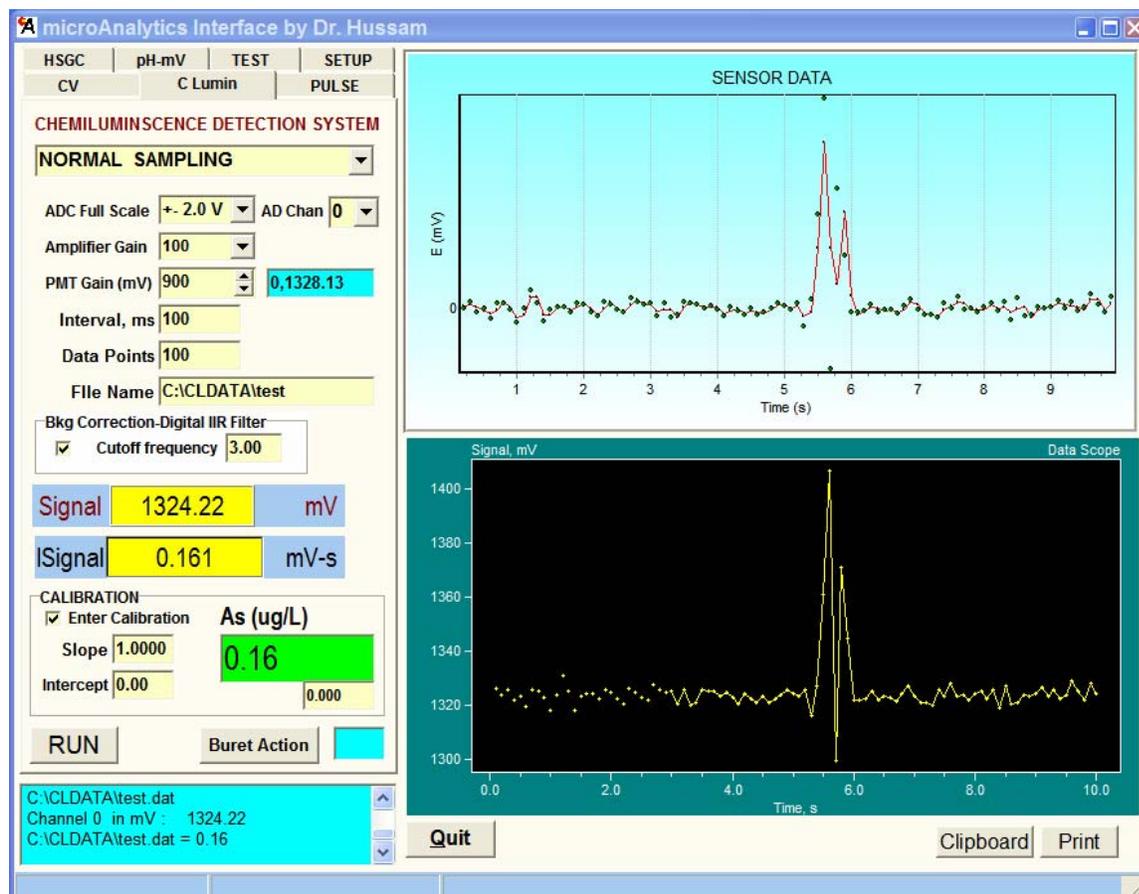
Figure 3. Data acquisition user window utilized for the gas-phase chemiluminescence based arsenic analyzer. Two real-time data plot windows are shown. The top window displays processed data and the bottom window displays the raw data.

2.4 Operational Order

The first step is the preparation of the sample solution and the required reagents. At the beginning of each test, the LED signal detection is performed to check the functionality of the PMT and the integrity of the data acquisition system. In a typical operational order, 1.00 mL of the reducing agent $NaBH_4$ is added into the acidified arsenic containing sample solution described above at a speed of 1.00 mL/s with the help of the auto-pump following a 20 sec background signal registration. Dispensing $NaBH_4$ into an acidic sample that contains arsenic reduces all of the As (V) to As (III) instantly and causes the formation of arsine gas (AsH_3). The arsine gas purges itself into the CC via the PTFE tube at the top of the reaction vial. Arsine reacts with ozone to give the expected CL that can be measured by the PMT. The total time for one complete run is approximately one minute for 600 data points in 100 ms data interval.

2.5 Light Detection and Processing

Chemiluminescence is a very weak intensity light that can be detected best by a sensitive PMT. The detection begins with the metal package PMT, a low-power consumption high-voltage supply and a low noise amplifier.[10] In general, a PMT contains a glass vacuum tube, a photocathode, a series of dynodes and an anode. The entry window of the PMT contains a thin deposit of a photocathode. Electrons emission

occurs as a result of the photoelectric effect upon the strike of photons on the photocathode deposit. The electrons are then accelerated towards the more positive potential dynodes and additional electrons are produced at each dynode. This cascading effect produces as many as 10^7 electrons for each photon striking the photocathode. Finally the accumulated charges reach the anode where it can be measured as current pulse.[12]

In this experiment a PMT for general application in the visible range was operated in the range of 300 – 650 nm, with $\pm$ 12 V external power supply to convert the output current into voltage. The specifications of the PMT are summarized in Table 1. The PMT gain was controlled by a DAC voltage control from the data card and was set to 800 – 900 mV for most CL work. The signal was further amplified by a two junction gate field-effect transistor (JFET-input) operational amplifiers (op-amps-OPA604AP). The first op-amp gain can be adjusted to 10X, 100X, 1000X and 10000X through four resistors in a current-to-voltage configuration. The second stage has a gain of 10 and a 1 µF – 10 µF capacitor to maintain a 1 second filter time constant. The analog signal from the PMT, which was further amplified by the op-amps, is then converted to a digital signal by an analog to digital converter (ADC) data card (USB-1408FS) and concurrently acquired by the data acquisition software.

Table 1. Specifications of the PMT (Hamamatsu, Module H5784, Japan)[11]

Parameters	Specifications
Input Voltage	$\pm$ 11.5 to $\pm$ 15.5 V
Max. Input Voltage	$\pm$ 18.0 V
Max. Input Current	+9/-1 mA
Max. Output Signal Voltage	+10.0 V (Load Resistance 10 kΩ)
Max. Control Voltage	+1.0 V (Input Impedance 100 kΩ)
Control Voltage Adjustment	+0.25 to 0.90 V
Current to Voltage Conversion Factor	1 V/μA
Effective Area	8 mm
Peak Sensitivity Wavelength	420 nm
Sensitivity Adjustment Range	$1:10^4$ x
Offset Voltage	$\pm$ 3 mV
Noise (peak to peak)	2 mV
Settling Time	2 s
Operating Temp.	+5.0 to 50.0 $^{\circ}$C
Weight	100 g

3. Results and Discussion

This section describes the basic CL formation from the AsH_3 – O_3 reaction in a reflective CC, the effect of silvering the CC, generation of hydride from $NaBH_4$, ozone and air flow, system response, determination of arsenic in standard solutions and unknown solutions, signal reproducibility, unique field water sample analysis and validation of results with other analytical techniques.

3.1 Chemiluminescence (CL) Formation

Chemiluminescence (CL) is the generation of electromagnetic radiation as light by the release of energy from chemical reactions. Electronically excited intermediates or products donate their energy to another nearby molecule and luminesce after the desired chemical reaction takes place. In principle, the light can be emitted in the visible, ultraviolet or infrared regions, but the visible light is the most common and detectable.[13] A typical CL signal generated from 50 ppb standard solution (SRM) solution is shown in figure 4.

It has long been known that the reactions of ozone with hydrides of arsenic (As), tin (Sn), selenium (Se) and antimony (Sb) produce simultaneous CL but, the arsine-ozone ($AsH_3 - O_3$) reaction generates a more intense CL than the others.[14] Kitao Fujiwara *et. al,* described the concept of the gas-phase chemiluminescence detection from arsine and ozone reaction for the first time in 1982.[15] The use of $AsH_3 - O_3$ reaction-based CL generation for the measurement of arsenic has not been fully pursued quite enough because of the complexity of the technique and its dependability on an expensive luminometer.[16] In this project, a gas-phase chemiluminescence reaction of $AsH_3 - O_3$ is studied under improved laboratory conditions and some of the problems are addressed.

The reaction of arsine and ozone is represented as follows. The extended CL emission is proved to be due to the excimer (excited molecule by a photon formation, $(AsO)_2$).[17]

$$AsH_3 + O_3 \rightarrow AsO^* + AsO^* \leftrightarrow (AsO)_2 \rightarrow 2AsO + h\upsilon$$

The CL formed due to this reaction is superimposed on a background, over and above the electronic noise. It is suspected that the background noise could be from isoprenes or light olefins resulting CL reaction with ozone. These impurities are sometimes occur naturally and may enter the system if not trapped by the granular activated carbon at the inlet of the air pump.
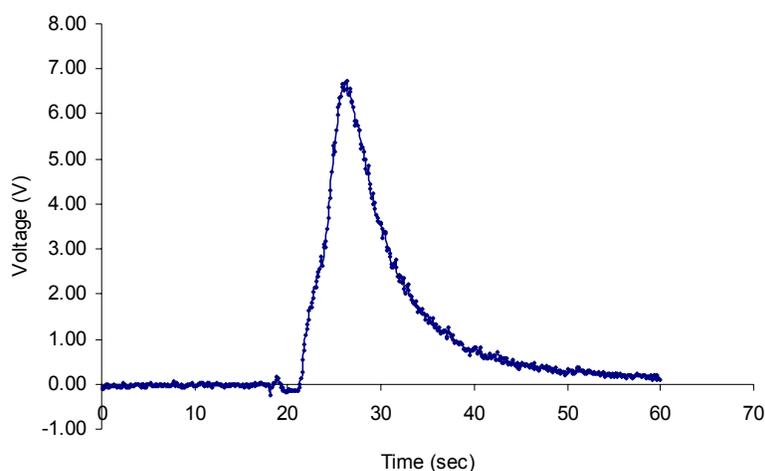

Figure 4. CL signal for 50 ppb As_{total} solution. The data was collected after the addition of sodium borohydride into the reaction vial.

3.2 Chemiluminescence Chamber

Optimizing the reflection of the intense CL in the CC is as crucial as the formation of the CL itself. To maximize the CL detection, the cell was silvered on the exterior with silver nitrate solution in accordance to silvering protocol and painted black on the top with black epoxy metal paint. At the same time, a similar reaction cell was wrapped on the exterior with a shinny silver paper (simply used for gift wrapping purposes) and secured on top using several rounds of white tape and black electrical tape. The two

reaction cells were compared for optimum CL generation. The sensitivity of the silver paper wrapped and black taped reaction cell was more superior to the silvered and black painted CC. The silvering was conducted on three similar CC and the resultant signals from all three were similarly lower than the silver-paper-wrapped CC. When a reaction cell is silvered from the outside, the inside becomes just like a mirror whereas wrapping a cell with silver paper does not have the same effect but regardless, it makes the interior of the cell very shiny. The experiment suggests that intense CL from AsH_3 - O_3 reaction is better reflected in shiny but not mirror-like surroundings. Figure 5 shows the comparison of the results in silvered and silver-paper wrapped reaction cells.
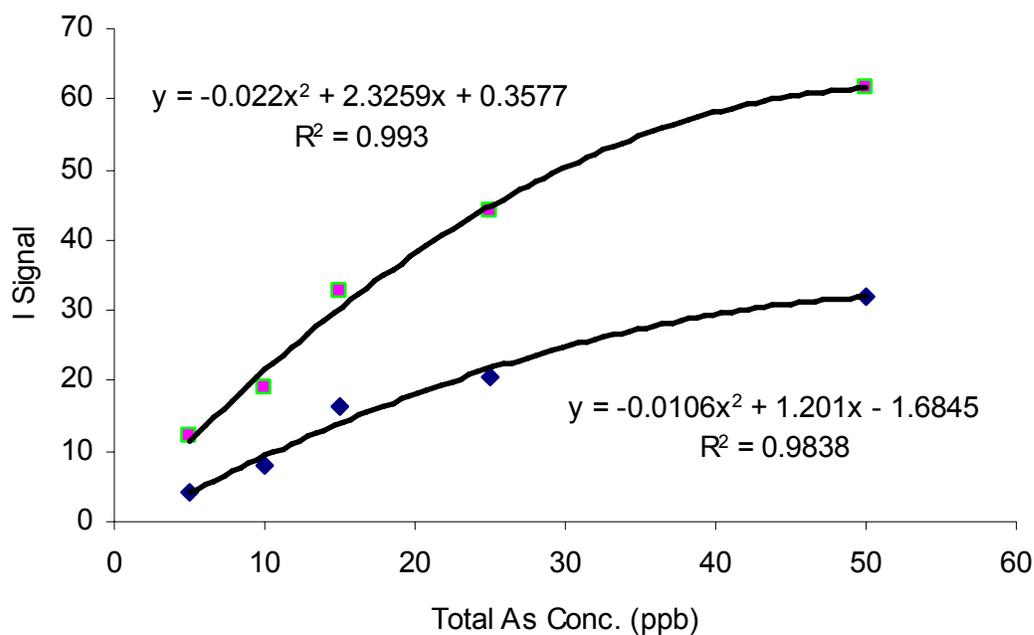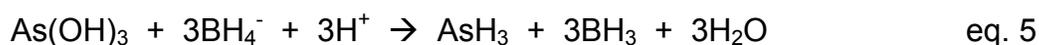

Figure 5. CL Signal response for 50.0 ppb, 25.0 ppb, 15.0 ppb, 10.0 ppb and 5.0 ppb total arsenic concentrations in silvered and silver-paper-wrapped CC. The top

polynomial regression represents the silver-paper-wrapped CC and the bottom one represents the silvered CC using $AgNO_3$ solution.

3.3 Sodium Borohydride ($NaBH_4$) Induced Hydride Generation

Arsenic forms a volatile hydride (arsine, AsH_3) when reduced with $NaBH_4$ under controlled pH.[18] $NaBH_4$ induced reduction of arsenic is unique among the other reducing agents such as Zn and HCl. It is exceptionally capable of differentiating arsenic oxidation states.[19] The overall hydride generation reaction including As (V) reduction to As (III) can be represented by the following chemical equations respectively.

$$As(O)(OH)_3 + H^+ + BH_4^- \rightarrow As(OH)_3 + BH_3 + H_2O \qquad \text{eq. 4}$$

$$As(OH)_3 + 3BH_4^- + 3H^+ \rightarrow AsH_3 + 3BH_3 + 3H_2O \qquad \text{eq. 5}$$

$$BH_3 + H_2O \rightarrow H_3BO_3 + H_2 \qquad \text{eq. 6}$$

One of the issues with using $NaBH_4$ as a reducing agent is the short-term stability. It is no longer a strong reducing agent passed 25 to 30 days after the first preparation. Addition of more concentrated NaOH could help extend the stability beyond one month but the downside is that it also increases the overall alkalinity. To reverse this effect more acid or buffer solution can be added to the sample, however increasing the amount of acid in the sample also undermines the hydride generation. Further, adding more solution in the sampling vial could take up more volume of the reaction vial and it may cause moisture transport into the CC via the PTFE tube. If moisture manages to get into the CC, it could absorb the intense CL and undermines the signal detection by the PMT. This problem had been encountered previously until

the specifics of the sample and reagents were determined based on numerous experiments. It has been established that $NaBH_4$ is a major contributor for the alkalinity.

The hydrolysis rate of $NaBH_4$ to BH_4^- and H^+ was previously reported as first order in other studies. It had been suggested that impurities may increase this first order hydrolysis rate. Metallic catalysts like nickel and cobalt accelerate the hydrolysis reaction for hydrogen generation that could also be used for H_2 fuel cells.[20] Beside the long term stability issue, the use of $NaBH_4$ for continuous onsite arsenic monitoring in developing countries could be very expensive.

3.4 System Response

The analyzer has been optimized to give the best possible signal based on a number of experiments. Figure 6 shows comparison of incremental CL signals resulted from the optimized system for a series of As_{total} concentrations. Different factors were identified as contributors for compromised CL signal. One of the problems was unwanted access of light into the black box where the CC and PMT are held together. Due to high sensitivity of the PMT, any stray of light that gets into the black box can be detected and may alter the result from the CL signal. This external light increases the background noise and decreases the signal noise (S/N) ratio. As a result, the detection limit cannot be determined or be dependable. All tubing connections of the CC were purposely made black in color to prevent external light contact. Transparent tubing used in previous experiments displayed noisy signals. In one instance the CL signal improved noticeably when an experiment was run in the dark with the room light turned off.

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The position of the CC is also a factor which affects the CL detection. Improved results were obtained when the exit connection of the CC was at 45° inclination. The inclined position provides a better drainage of the reacted AsH_3 - O_3 from the CC before a subsequent reaction takes place. The issue of moisture accumulation inside the CC has been resolved by this inclination. Accumulation of AsH_3 - O_3 on the surface of the inner CC forms a molecular or atomic film (adsorbate) that could adsorb the intense CL before it gets detected by the PMT.

Some of the connections could become vulnerable for leaks due to the relatively high pressured flow of the gas or liquid. To check leaks on various connections should be the first trouble shooting step when CL signal is diminished. Commercial soap water (Snoop ® Leak Detector) has been the preferred method for minuscule leak detections.

Addition of a magnetic stirrer greatly improved the CL signal detection by forcing more AsH_3 to be eluted uniformly from the sample vial. A sample with a magnetic stirrer spinning at ~400 rpm showed improved CL signal value and better reproducibility when compared with the one without a magnetic stirrer.

Signal disturbance due to vibration was avoided by securely packaging the circuit board and data card together in an aluminum box. Previously 1 μF and 45 μF capacitors were used for signal amplification, but when replaced with a 10 μF capacitor, the reproducibility of results greatly improved due to noise spike filtration. The 10 μF capacitor was chosen because the 45 μF capacitor was too big and it retains extra charge from previous signals and adds those charges to the successive signal. On the contrary, the 1 μF capacitor was too small to remove excess noise.

Extending the tube length between OZG and CC has also provided an improved CL signal. Longer tubing offers sufficient time for light olefins, such as ethane and isoprene, to react fully with O_3 before reaching the CC. Last but not least, the CL signal was remarkably optimized when a loop was included on the tube connecting the CC-exit to the waste vial. The loop exerts a back pressure in such a manner that the AsH_3-O_3 can not leave the CC without reacting and the luminous being detected. It has been proved that all the arsenic in a sample will not convert to arsine gas upon sodium-borohydride addition. Figure 7 shows consecutive spikes of the same 50 ppb total arsenic containing solution. It was found that it takes at least three replicate runs to extract all the arsenic in the sample solution as arsine gas in this setting.
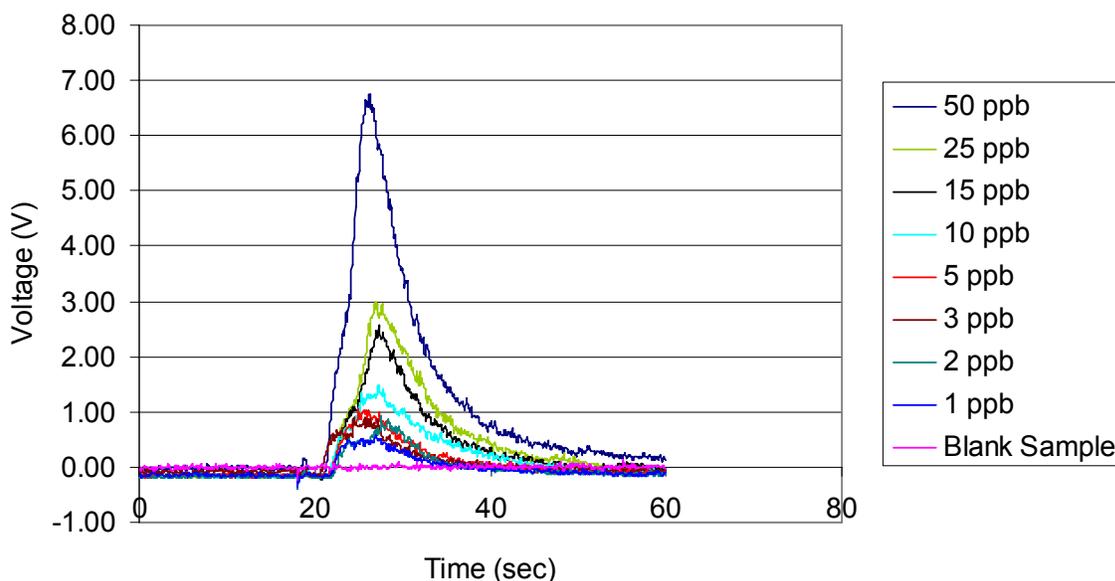

Figure 6. Comparison of incremental CL signals for a series As_{total} concentrations.

50 ppb As Solution First Run

50 ppb As Solution Second Run
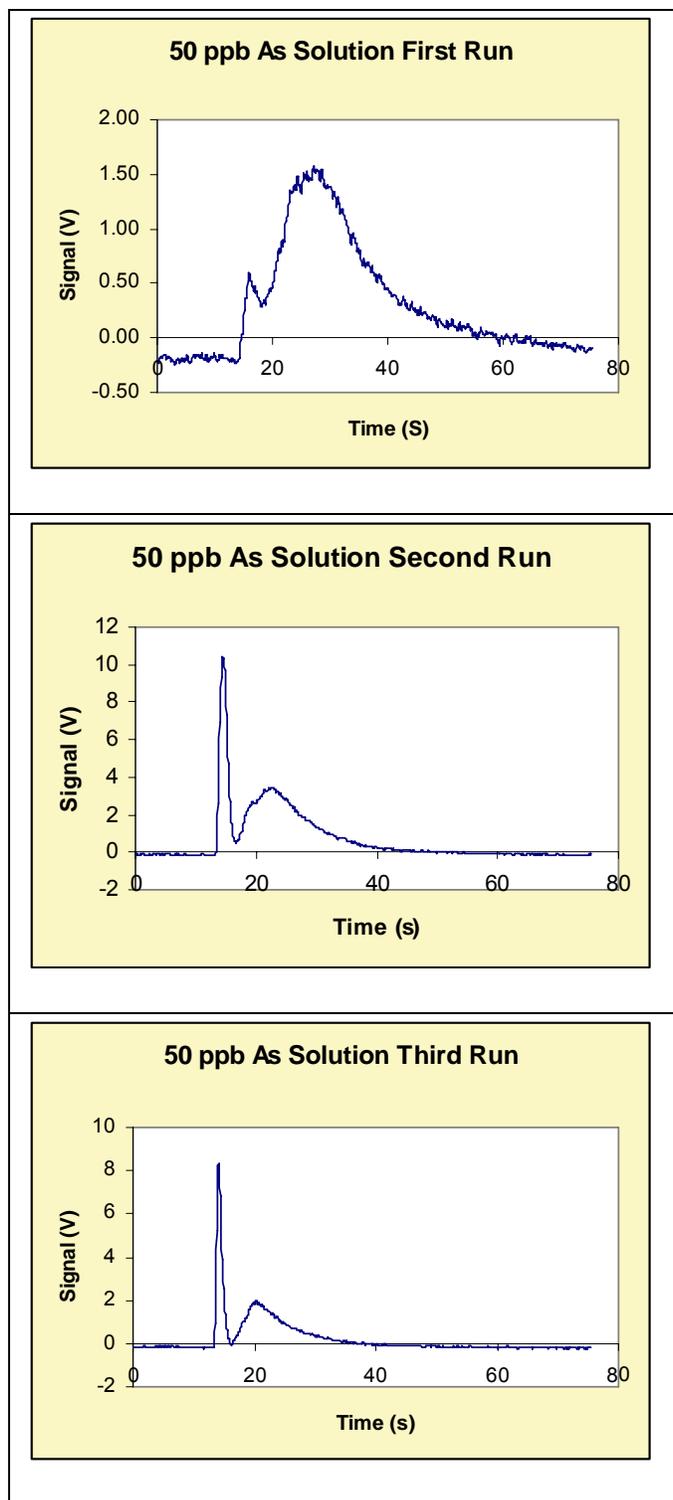
50 ppb As Solution Third Run

Figure 7. Three replicate runs of 50 ppb arsenic containing solution. The data was collected throughout the addition of sodium borohydride up to the end of hydride generation in a single addition.

The linear calibration equation of the second signal was used for the determination of arsenic concentration in unknown samples and standard solutions. The second signal was chosen among the first one and the third one due to the slight advantage of signal reproducibility and higher integrated peak area. The limit of detection (LOD) was determined to be 0.124 ppb (µg/L or 124 ppt) based on 3 runs of 50.0 ppb As_{total} standard solutions. The calibration equation for the second signal is shown as follows. Calculated concentrations of standard solutions based on the linear calibration curve are listed in table 4.

I Signal (mV-s) = (0.894 $\pm$ 0.02) As $_{(tot)}$ µg/L + (0.847 $\pm$ 0.02)
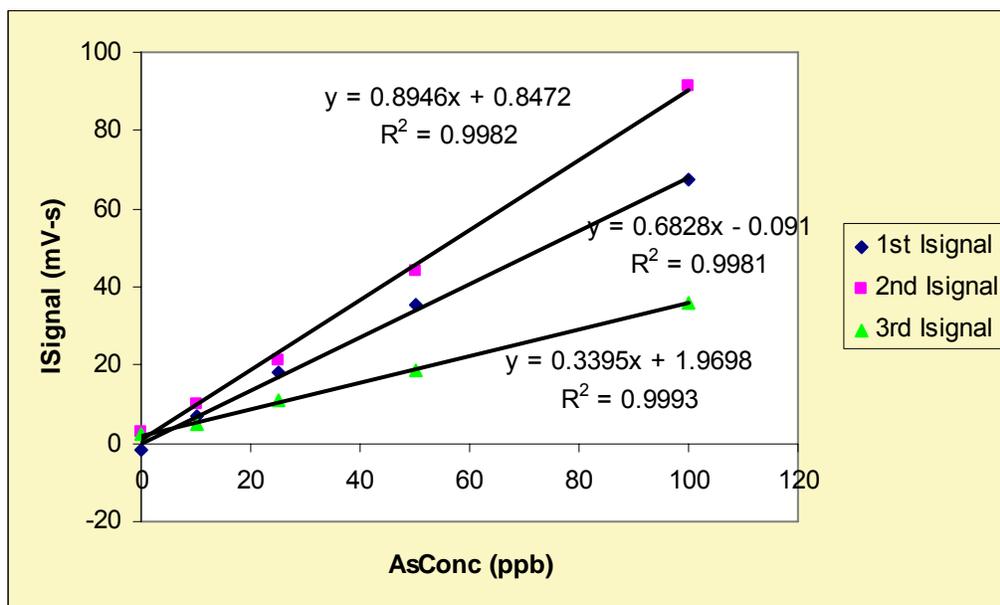
r^2 = 0.998

Figure 8. Calibration curves for first, second and third signals based on 0.0 ppb, 10.0 ppb, 25.0 ppb, 50.0 ppb and 100.0 ppb arsenic containing samples.

Table 4. Total arsenic determination for standard samples in ppb level based on the second standard addition linear calibration curve.

As$_{total}$ Standard (ppb)	I Signal (mV-s)	Calculated Conc. (ppb)
0	2.350 $\pm$ 0.536	1.681 $\pm$ 0.599
10.0	10.992 $\pm$ 1.787	11.348 $\pm$ 1.910
25.0	22.008 $\pm$ 1.434	23.670 $\pm$ 1.604
50.0	44.598 $\pm$ 3.123	48.938 $\pm$ 3.493
100.0	92.946 $\pm$ 3.320	103.019 $\pm$ 3.614

Table 5. Total arsenic determination in field water samples from Ethiopia

Sample ID	Calculated Conc. (ppb)
Legedadi	6.664 $\pm$ 0.370
Akaki	BDL
Awassa	14.589 $\pm$ 0.421
Lalibela	BDL
Nile	0.960 $\pm$ 0.337

Table 6. Total arsenic determination in field water sample from Syskiyon Mountain Oregon.

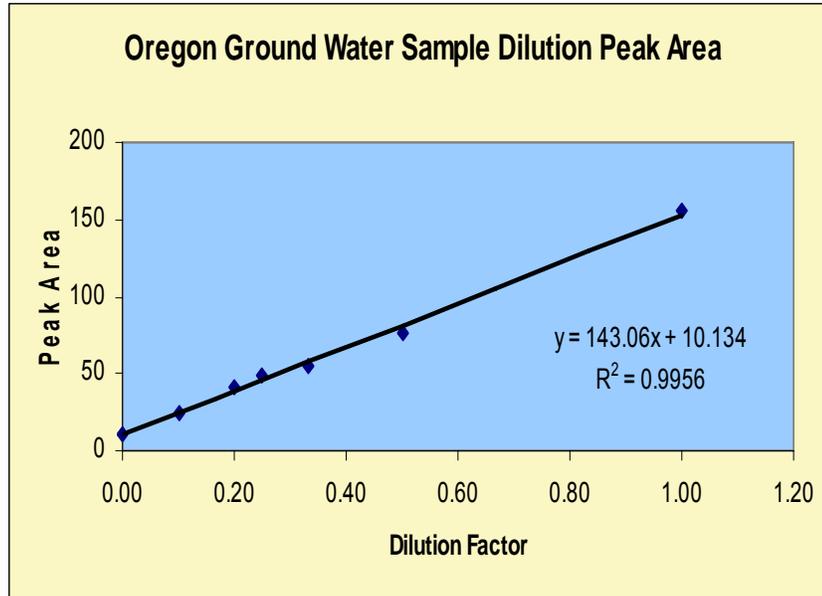| Sample ID	I Signal (mV-s)	Calculated Conc. (ppb)
Siskiyon (dilution factor = 5)	41.93 $\pm$ 2.840	207.75 $\pm$ 2.840

Figure 9. Dilution of Ground Water from Syskiyon Oregon. 207.75 ppb (µg/L) total arsenic was detected.

The results in Table 4 indicate that Awasa Lake water has the highest arsenic concentration followed by Legedadi Deep-Well water. Awasa Lake has a surface area of ~92 km^2 and maximum depth of ~23 m.[21] For a lake of this size, the measured 14.589 ppb As_{total} concentration in 1.0 mL sample is significantly high. (Note: The EPA maximum limit for arsenic concentration in drinking water is 10.0 ppb.) The distribution of arsenic in the entire lake may not be uniform for different reasons. More water samples need to be tested from diverse portions of the lake in order to determine the final arsenic concentration.

The arsenic contamination in Awasa Lake is possibly linked to industrial waste or the hot springs running parallel to the Awasa basin in the Rift Valley axis. (Note: Hot springs and volcanic activity can introduce potentially toxic high arsenic

concentrations.)[22]   The lake is fed by a number of streams which may also have contributed to the arsenic contamination. Legedadi Deep-Well water sample showed an average As_{total} concentration of 6.64 $\pm$ 0.370 ppb. Even though the concentration is below the EPA limit of 10.0 ppb, the water will not be safe to use it as a potable water source for extended periods of time. (Note: BDL implies Below Detection Limit). Table 5 indicates the water sample from Syskiyon Mountain, Oregon USA containing 51.768 $\pm$ 0.284 ppb As_{total} which is above the toxic limit and it should not be used as potable water source.

3.5 Validation with Other Techniques: Comparison Studies

Two popular laboratory instruments were used to validate our results from the gas-phase chemiluminescence based arsenic analyzer. The first one was Flow Injection Hydride Generation Atomic Absorption Spectrophotometer (FI-HG-AAS, Perkin-Elmer Model 5100, equipped with GEM software). The second instrument used for validation was fully automated Atomic Fluorescence Spectrometer (AFS, PS Analytical, Millennium System software). Table 7 and Table 8 show total arsenic measurements with FI-HG-AAS and AFS respectively. Upon comparing the gas-phase CL analyzer with those of FI-HG-AAS and AFS, the data for both agreed in the range of $\geq$ 90%.

Table 7. Total arsenic determination with FI-HG-AAS

Standard As$_{total}$ Conc. (ppb)	FI-HG-AAS Absorbance	Calculated As$_{total}$ Conc. (ppb)
5.0	0.006 $\pm$ 0.000	6.093 $\pm$ 0.218
10.0	0.010 $\pm$ 0.001	10.155 $\pm$ 0.156
20.0	0.019 $\pm$ 0.000	19.903 $\pm$ 0.213
30.0	0.028 $\pm$ 0.000	28.738 $\pm$ 0.696
40.0	0.040 $\pm$ 0.001	40.620 $\pm$ 0.537

Table 8. Total arsenic determination with AFS

Standard As$_{total}$ Conc. (ppb)	AFS Peak Height (au)	Calculated As$_{total}$ Conc. (ppb)
1.0	55.933 $\pm$ 2.879	1.029 $\pm$ 0.030
2.0	108.232 $\pm$ 2.392	2.177 $\pm$ 0.110
3.0	154.796 $\pm$ 2.697	3.300 $\pm$ 0.230
4.0	191.725 $\pm$ 1.676	4.157 $\pm$ 0.120
5.0	249.335 $\pm$ 3.377	5.493 $\pm$ 0.287
10.0	497.078 $\pm$ 2.825	11.239 $\pm$ 0.520

4. CONCLUSION

In this endeavor, we have developed a fully automated gas-phase chemiluminescence based arsenic analyzer. This field ready analyzer is very sensitive, robust, affordable and environmentally friendly with a detection limit of 0.124 ppb. It can readily be packaged into a size of 45 cm x 30 cm x 45 cm (l x w x h) wood or metal box for field use and can be powered by a car battery. This development has the potential to be very useful for arsenic contaminated water surveillance and arsenic filter monitoring in addition to its regular use in laboratory measurements. It is also a great addition to our state-of-the-art Anodic Stripping Voltammetry (ASV), electrochemical analyzer.

References

(1)   IARC *Monographs*, 23, 39-141, **1980**

(2)   WHO *Fact Sheet*, No 210, **2001**

(3)   IARC National Academy Press *Arsenic in Drinking Water* Washington, DC, **2001**.

(4)   Hartwig, A.; Blessing, H.; Schwerdtle, T.; Walter, I. *Toxicology* **2003**, *193*, 161-169.

(5)   Felsmann, J.; Lia, V.; Cullen, W.; Ma, M.; Lu, X.; Le, C. *Clinical Chem.* **1999**, *45*, 1988 - 1997.

(6)   Mandal, B.; Suzuki, K. *Talanta* **2002**, *58*, 201 - 235.

(7)   Hussam, A.; Munir, A. *J. Environ. Sci. and Health Part A* **2007**, *42*, 1869 - 1878.

(8)   In *Arsenic in Drinking Water. Date accessed 02/01/2009*
http://www.epa.gov/safewater/arsenic/basicinformation.html.

(9)   In *Guidelines for Canadian Drinking Water Quality, Technical Document   on Arsenic.* ; Federal-Provincial-Territorial Committee on Health and the Environment, Health Canada. Ottawa, Ontario: 2006.

(10)   Kawamura, S. *Integrated Design and Operation of Water Treatment Facilities*; John Wiley and Sons 2000.

(11)   *Hamamatsu Photomultiplier Tubes Basics and Application*; 3rd ed.; Hamamatsu Photonics KK, 2006.

(12)   Brain, M. In *PMT Design. Date accessed 02/01/2009*
http://elchem.kaist.ac.kr/vt/chem-ed/optics/detector/pmt.htm 1996

(13)   Garcia Campana, A.; Baeyens, W. *Chemiluminescence in Analytical Chemistry*; CRC Press, 2001.

(14)   Toby, S. *Amer. Chem. Soc. Chem. Review* **1984**, *84*, 277-285.

(15)   Fujiwara, K.; Watanabe, Y.; Fuwa, K.; Winefordner, J. *Anal. Chem.* **1982**, *54*, 125-128.

(16)   Idowu, A.; Dasgupta, P.; Genfa, Z.; Toda, K. *Anal. Chem.* **2006**, *78(20)*, 7088-7097.

(17)   Fraser, M.; Stedman, D. *J. Chem. Soc.* **1983**, *79*, 527-542.

(18)   Shraim, A.; Chiswell, B.; Olszowy, H. *Talanta* **1999**, *50*, 1109-1127.

(19)   Morita, M.; Edmonds, J. *Pure and Appl. Chem.* **1992**, *64*, 575-590.

(20)   Liu, B.; Li, Z.; Suda, S. *J. Alloys and Comp.* **2006**, *415*, 288-293.

(21)   Lamb, A.; Leng, M.; Lamb, H.; Telford, R.; Mohamed, U. *Quaternary Science Reviews* **2002**, *21*, 2199-2211.

(22)   In *GSA Annual Meeting*; America, G. S. O., Ed. Denver, 2007; Vol. 39, p 219.