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ABSTRACT: Hydride generation (HG) is an effective technique that eliminates interfering matrix species and enables hydride separation. Arsenic speciation analysis can be fulfilled by cryogenic trapping (CT) based on boiling points of resulting arsines using liquid nitrogen (LN2) as a coolant. In this work, LN2 was replaced by the thermoelectric effect using a cryogenic trap that consisted of a polytetrafluoroethylene (PTFE) body sandwiched by two Peltier modules. After the trap was precooled, the arsines flew along a zigzag channel in the body and reached a sorbent bed of 0.2 g of 15% OV-3 on Chromosorb W-AW-DMCS imbedded near the exit of the trap. CH3AsH2 and (CH3)2AsH were trapped, while AsH3, that passed the trap unaffected, was detected by atomic fluorescence spectrometry. Continuous operation led to enhanced throughput. For inorganic As, the limit of detection (LOD) was 1.1 ng/g and recovery was 101.0 ± 1.1%. Monomethylarsonic acid and dimethylarsinic acid did not interfere with 0.2 ± 1.2% and −0.3 ± 0.5% recoveries, respectively.

Hydride generation (HG), first demonstrated by Marsh in 1836,1 allows thorough separation of volatile arsines from interfering matrix components using a simple gas/liquid separator (G/L). HG is especially useful for separation of arsines of toxicologically relevant arsenic species (TRAS): inorganic As (iAs), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA). For elements that form volatile hydrides, HG dramatically enhances sensitivity leading to extensive implementation in atomic absorption spectrometry,2 atomic fluorescence spectrometry (AFS),3,4 inductively coupled plasma (ICP)-optical emission spectrometry,5 and ICP-mass spectrometry.6

Arsenic speciation analysis can be carried out either prior to or post HG. Schemes of pre-HG speciation analysis include high performance liquid chromatography (HPLC), solid phase extraction (SPE),7 and dispersive liquid–liquid microextraction.8 HG of TRAS could be performed under a set of conditions specifically tuned to favor iAs, leading to selective iAs quantitation without HPLC.9 Alternatively, HG could be carried out under 4 sets of conditions.10 For each set, a linear equation was set up to correlate AFS intensity to TRAS concentrations where coefficients were slopes of TRAS calibration curves. TRAS concentrations in unknown samples were then solved mathematically.

Cryogenic trapping (CT)11,12 and focusing (CF)13,14 are effective post-HG speciation schemes based on boiling points (BPs) of resulting arsine species: −55, 2, and 35.6 °C for AsH3, CH3AsH2, and (CH3)2AsH, respectively. The arsines of TRAS are first trapped in a U-tube immersed in liquid nitrogen (LN2). When condensation is complete, the U-tube is exposed to the ambient air or heated by a nichrome coil. Rising temperature causes trapped arsines to be released from the U-tube in the order of ascending BPs; the arsines are then swept by a carrier gas stream to an As specific detector. CT separates arsines of...
TRAS without using chemical reagents leading to low cost and green chemistry. Thus far, LN2 was used in all cases as a coolant. Though effective, LN2 is hazardous if safety procedures are not followed. In this work, LN2 was replaced by a pair of solid-state Peltier modules to promote personnel safety. Such a thermoelectric cryotrap is described in this manuscript for the first time including its design and operation for iAs quantification in rice.

Arsenic is a notoriously toxic environmental contaminant. Unlike workers in As-related mining and manufacturing industries, the general public is exposed to As mainly from drinking water and rice. Low-dose chronic intake adversely affects human health and may cause cancer in all organs. The International Agency for Research on Cancer identified As as a Group 1 human carcinogen. On the other hand, rice is the top energy source (20%) for humans and the dietary staple for half of world population. However, rice accumulates much more As in comparison to other terrestrial crops, in part due to anaerobic growing conditions. Among TRAS, iAs is more toxic than MMA and DMA and far more toxic than other organic arsenic species commonly found in seafood such as arsenobetaine and arselenocholine. The Food and Agriculture Organization/World Health Organization determined 3.0 μg/kg bw · d as iAs to be the lower limit on the benchmark dose for a 0.5% increased incidence of lung cancer (BMDL0.5). Currently, China has set a 200 ng/g iAs maximum level (ML) in rice; the Codex Committee on Contaminants in Foods proposed 200 and 300 ng/g draft iAs MLs in polished and raw rice, respectively. To meet regulatory requirements and protect consumers, sensitive iAs detection methods are much needed. Rice was selected as the model matrix due to its unique ability to accumulate As and its important role in human diet.

**EXPERIMENTAL SECTION**

Reagents and Solutions. AsIII standard solution (1000 μg/L in 1–5% HNO3) was purchased from Fluka (Milwaukee, WI, USA); AsV standard solution (1000 μg/L in ≤2.5% HNO3) was from PerkinElmer (Waltham, MA, USA). Solid MMA (≥99.5%) and DMA (≥99.0%) were purchased from Chem Service (West Chester, PA, USA) and Sigma-Aldrich (Milwaukee, WI, USA), respectively. MMA and DMA stock standard solutions were individually made by dissolving an accurate amount of the respective solid in 10 mL of deionized water (DIW); further dilution was done in DIW. NaBH4 (>99%), 30% silicon antifoam solution, ACS grade KI, HNO3, and l-ascorbic acid were from Sigma-Aldrich. ACS grade HCl and NaOH were from Mallinckrodt (Phillipsburg, NJ, USA). Rice flour 1568b standard reference material (SRM) was purchased from National Institute of Standard and Technologies (NIST, Gaithersburg, MD, USA). The sorbent used in the cryotrap, 15% OV-3 on Chromosorb W-AWDMCS 60/80, was purchased from Ohio Valley Specialty Company (Marietta, OH, USA).

A 0.28 N nitric acid digestion solution was prepared by diluting 4.45 mL of concentrated nitric acid in 100 mL of DIW in a 250 mL volumetric flask and filling to volume. A prereduction solution, also used as the reagent blank, was prepared by dissolving 300 mL of concentrated HCl, 40 g of KI, 4 g of l-ascorbic acid, and 1 mL of 30% silicone antifoam in 0.5 L of DIW and then diluting to 1 L. A 1% (w/v) NaBH4–0.1 M NaOH reduction solution was prepared daily by dissolving 10 g of NaBH4 and 4 g of NaOH in DIW, diluting to 1 L, and filtering through a 0.45 μm Supor-450 membrane filter (Pall Life Sciences, Port Washington, NY, USA) under vacuum and stored in a container with a loose cap. DIW prepared with a Barnstead E-pure system (Dubuque, IA, USA) was used to prepare all the solutions.

**Cryogenic Trap Design.** Shown in Figure 1 is the cryotrap assembly. At the core is a trap body made of polytetrafluoroethylene (PTFE) with 75 × 75 × 19 mm outer dimensions (Figure 2). The central portion of the body was 9 mm thick through which a 6.35 mm wide zigzag flow channel was cut. The channel had a cross section of 57 mm2 and a total length of 282 mm leading to a total volume of 16 mL. The body was sandwiched by two 50 × 50 mm Peltier modules (Model 19911-5M31-12CW-S, Custom Thermoelectric, Bishopville, MD, USA) with cold plates facing the trap body. The modules, each rated at 23.8 V and 12 A, were connected in parallel and powered by a variable power supply (Model HY3050EX, Acifca, San Jose, CA, USA). Installed near the exit of the channel was a bed of 0.2 g of Chromosorb sorbent with two sides held by the PTFE channel walls and two sides by the...
ceramic cold plates, and the remaining sides facing the flow and the exit, respectively, were confined by glass wool. Apiezon M vacuum grease (Apiezon Products, Manchester, UK) was applied on edges of the trap body to maintain airtightness.

Hot plates of the modules were in contact with two water-block heat exchangers (Model WBA-3-0-0.85-CU-01, Custom Thermoelectric). Arctic Silver 5 thermal compound (Arctic Silver, Visalia, CA, USA) was applied to hot plate—water block interfaces to promote heat conduction. The heat exchangers, of a copper shell design, were circulated with 15 °C water from a thermostatic bath (Model ESRB-7, Techne, Standon, UK).

**Microwave-Assisted Digestion.** Rice samples were purchased from local markets. A 10 g aliquot was ground using a small coffee mill (Model F203, Krups, Millville, NJ, USA); the resulting rice flour was kept in a desiccator before use. Aliquots of 250 ± 5 mg of rice flour were weighed into 100 mL PTFE vessels, to which 10 mL of 0.28 N nitric acid was added, followed by brief shaking. The vessels were then placed in a 14-position carousel of a Mars 5 microwave digestion system (CEM, Matthews, NC, USA). The temperature program consisted of a 2 min ramp to 95 °C and 30 min hold at this temperature. When samples cooled down to room temperature, the contents were transferred to 15 mL centrifuge tubes, followed by centrifugation at 3600 g for 5 min.

**Hydride Generation.** Supernatants (2 mL) were transferred to 10 mL volumetric flasks which were filled to the mark with the prereduction solution. The flasks were then capped; the contents were mixed by hand shaking and then allowed to stand for 1 h for complete reduction. As(III)–NaBH₄ reaction was carried out in flow injection mode. The resulting arsines were swept by argon carrier gas at 250 mL/min flow rate through a 48 in. long Nafion dryer (MD-110-48P, Perma-Pure, Farmingdale, NJ, USA) where most of the moisture permeated through a Nafion tubing wall into a counter-flowing nitrogen stream.

**Cryogenic Trapping.** As shown in Figure 3, the cryotrap was integrated into a Millennium Excalibur atomic fluorescence spectrometer (P S Analytical, Kent, UK) between the dryer and the detector. Prior to sample injection, the trap was precooled with the water bath set at 15 °C and the Peltier modules were powered at 10.0 V. Once trap temperature stabilized at 40 min, the trap was run in continuous cooling mode. At the end of the work shift, the cryotrap was powered off while dry argon continued to flow for 1 h to release trapped arsines from the cryotrap into an exhaust suction pipe.

**Atomic Fluorescence Spectrometry.** In the detection chamber of the Excalibur spectrometer, AsH₃ was atomized by a diffusion flame supported by hydrogen gas evolved from NaBH₄ acidification. The resulting atomic cloud was excited by a boosted discharge arsenic hollow cathode lamp (Model E033L001, Photon, Victoria, Australia); the resonance emission at 193.7 nm was collected at 90°, isolated by an interference emission filter, and detected by a solar blind photomultiplier tube. AFS operation was controlled by Millennium software (P S Analytical) under the same conditions as previously described.7

**Rice Analysis.** A standard curve was constructed daily using As(III) reagent standards. Rice samples were analyzed in triplicates; quantitation was based on peak height. NIST 1568b rice flour CRM was used for method validation.

## RESULTS AND DISCUSSION

**Peltier-Effect Cryotrap vs LN₂ Coolant.** CT as a physical approach fulfills separation of volatile hydrides based on BPs without using chemical reagents. The resulting method is thus safe, cost-effective, and environmentally friendly. Traditional cryotrap designs include a quartz U-trap (6 mm od × 200 mm l), a Pyrex U-tube (6 mm od × 400 mm l) half packed with 60–80 mesh glass beads, a PTFE tubing (3 mm id × 200 mm l), a glass U-tube (6 mm od × 150 mm l) packed with silanized glass wool, a glass U-tube (6 mm od × 160 mm l) packed with glass wool treated with dimethyl-dichlorosilane plus a PTFE chromatographic column (4000 mm l × 3.5 mm d) packed with Supelco Carbopack B HT 100 sorbent 40/60, and a glass tube (2.5 mm id × 305 mm l) filled with 0.8 g 15% OV-3 on Chromosorb W AW-DMCS 45/60 and wrapped with 15–20 Ω Ni80/Cr20 wire (0.51 mm diameter at 5.275 Ω/m). In the last two cases, sorbent was used to introduce gas chromatography (GC). Such an approach, known as CF, sharpens analyte peaks resulting in improved resolution and accuracy. In all cases, the cryotrap were immersed in a LN₂ bath. An icy water or alcohol bath may be used for precooling.

LN₂ is an excellent coolant with an extremely low BP (−195.8 °C). Such extreme temperature can cause cold burns to skin and eye upon brief contact. Furthermore, large liquid-to-gas expansion ratio (1:694 at 20 °C) of this coolant poses potential hazards due to asphyxiation, pressure buildup, or explosion. In this work, LN₂ was replaced by two Peltier modules; CT was carried out by thermoelectric effect, also known as Peltier effect. A pair of solid-state devices operated at low voltage was easier to handle and much safer than LN₂. With LN₂ eliminated, so was the need to frequently replenish coolant.

The limitation of a Peltier module is the maximum temperature difference between hot and cold plates, of ~62 °C in theory (without load) and far less than 62 °C in practice

Figure 3. Schematic diagram of the HG-CT-AFS system.
Table 1. Comparison of iAs-in-Rice Results by the SPE-HG-AFS vs HG-CT-AFS Method

<table>
<thead>
<tr>
<th>rice samples</th>
<th>country origin</th>
<th>SPE-HG-AFS (ng/g)</th>
<th>HG-CT-AFS (ng/g)</th>
<th>difference, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>med grain</td>
<td>USA</td>
<td>74 ± 2</td>
<td>72 ± 1</td>
<td>−2.7</td>
</tr>
<tr>
<td>jasmine</td>
<td>Thailand</td>
<td>77 ± 1</td>
<td>73 ± 1</td>
<td>−5.3</td>
</tr>
<tr>
<td>glutinous</td>
<td>USA</td>
<td>66 ± 2</td>
<td>67 ± 1</td>
<td>+1.5</td>
</tr>
<tr>
<td>enriched long-grain</td>
<td>USA</td>
<td>74 ± 1</td>
<td>72 ± 0</td>
<td>−2.7</td>
</tr>
<tr>
<td>brown med-grain</td>
<td>USA</td>
<td>94 ± 1</td>
<td>90 ± 2</td>
<td>−4.3</td>
</tr>
<tr>
<td>basmati</td>
<td>India</td>
<td>60 ± 3</td>
<td>51 ± 1</td>
<td>−16</td>
</tr>
<tr>
<td>matta</td>
<td>India</td>
<td>49 ± 2</td>
<td>52 ± 2</td>
<td>+5.9</td>
</tr>
<tr>
<td>jasmine</td>
<td>Vietnam</td>
<td>66 ± 2</td>
<td>76 ± 1</td>
<td>+14</td>
</tr>
<tr>
<td>brown basmati</td>
<td>India</td>
<td>235 ± 9</td>
<td>242 ± 5</td>
<td>+3.8</td>
</tr>
<tr>
<td>ponni</td>
<td>India</td>
<td>57 ± 2</td>
<td>64 ± 5</td>
<td>+15</td>
</tr>
<tr>
<td>organic haiga med-grain</td>
<td>USA</td>
<td>83 ± 5</td>
<td>80 ± 1</td>
<td>−3.7</td>
</tr>
<tr>
<td>organic basmati</td>
<td>India</td>
<td>46 ± 1</td>
<td>50 ± 2</td>
<td>+8.3</td>
</tr>
<tr>
<td>wild red</td>
<td>Thailand</td>
<td>86 ± 5</td>
<td>93 ± 3</td>
<td>+7.8</td>
</tr>
<tr>
<td>wild black</td>
<td>USA</td>
<td>45 ± 1</td>
<td>48 ± 0</td>
<td>+6.5</td>
</tr>
<tr>
<td>mixed rice flour</td>
<td>India</td>
<td>68 ± 3</td>
<td>70 ± 2</td>
<td>+5.8</td>
</tr>
<tr>
<td>NIST SRM 1568b@92 ± 10</td>
<td>USA</td>
<td>102 ± 5</td>
<td>92 ± 4</td>
<td>+0.0</td>
</tr>
</tbody>
</table>

(with load). As a result, it is impossible to condense AsH₃ (BP at −55 °C) using single-stage Peltier modules and room-temperature cooling water. However, a single-stage trap can completely retain CH₃AsH₂ (BP at 2 °C) using single-stage Peltier modules and room-temperature cooling water. However, a single-stage trap can completely retain CH₃AsH₂ (BP at 2 °C), (CH₃)₂AsH (BP at 35.6 °C), and (CH₃)₃As (BP at 56 °C). Due to higher toxicity, iAs is usually the only target of interest in most analysis, vide supra. Unlike LN₂ that is used in disruptive cooling—heating cycles, this cryotrap retained only methylated arsines allowing continuous detection of AsH₃. Consequently, sample throughput was enhanced.

**Cryogenic Trap Material.** CT or CF poses certain requirements on the material and design of the trap body: resistance to arsenic corrosion and ideally no irreversible adsorption. Metals excel in thermal conductivity; copper (Cu) is an example with $k_{Cu} = 401$ W/mK. However, Cu reacts with arsenic disqualifying it as trap material. Though chemical resistance can be improved by a layer of gold coating, adsorption of arsenic on the gold surface poses another technical obstacle. Graphite excels in thermal conductivity and machinability, but it also suffers from arsenic adsorption. On the other hand, hardness of many ceramic materials renders machining highly difficult.

In comparison, polymers are adequate in chemical inertness and yet less prone to irreversible adsorption. Among polymers, PTFE possesses superior chemical resistance and machinability, but the limited surface area of the PTFE gas channel plus the exposed surfaces of ceramic cold plates resulted in a small capacity to adsorb and condense methylated arsines. A sorbent bed was thus installed to increase surface area and introduce capacity to adsorb and condense methylated arsines. A sorbent bed was thus installed to increase surface area and introduce capacity to adsorb and condense methylated arsines. A sorbent bed was thus installed to increase surface area and introduce capacity to adsorb and condense methylated arsines. A sorbent bed was thus installed to increase surface area and introduce capacity to adsorb and condense methylated arsines. A sorbent bed was thus installed to increase surface area and introduce capacity to adsorb and condense methylated arsines. A sorbent bed was thus installed to increase surface area and introduce capacity to adsorb and condense methylated arsines. A sorbent bed was thus installed to increase surface area and introduce capacity to adsorb and condense methylated arsines. A sorbent bed was thus installed to increase surface area and introduce capacity to adsorb and condense methylated arsines. A sorbent bed was thus installed to increase surface area and introduce capacity to adsorb and condense methylated arsines. A sorbent bed was thus installed to increase surface area and introduce capacity to adsorb and condense methylated arsines. A sorbent bed was thus installed to increase surface area and introduce capacity to adsorb and condense methylated arsines. A sorbent bed was thus installed to increase surface area and introduce capacity to adsorb and condense methylated arsines.

**Use of Adsorbent.** CT in a continuous mode necessitates a trap capacity large enough to sustain adsorption of CH₃AsH₂ and (CH₃)₂AsH long enough without breakthrough. In this work, 0.2 g of 15% OV-3 on Chromosorb W-AW-DMCS 60/80 served this purpose. The sorbent also enabled GC leading to better resolved peaks of released methylated arsines. GC was not exploited in this work due to the fact that the only target, AsH₃, was slightly polar with a low (−55 °C) BP; hence, it did not condense or adsorb on the sorbent. The sorbent capacity was tested by repeated injection of 8.9 ng/g MMA 16 times plus 67.2 ng/g DMA 10 times which, based on a 200× dilution factor, was equivalent to 200 injections for a rice sample containing 140 ng/g MMA and 670 ng/g DMA, much higher than those in typical rice. No breakthrough was observed. For an 8-h work shift, this corresponded to 25 injections per hour. At the end of the shift, the cryotrap was flushed with argon for 1 h to release trapped arsines and to renew the trap. In practice, continuous operation mode not only enhances throughput but also maintains the integrity of the Peltier modules. It was observed that repeated cooling—heating cycles caused stress leading to microcracks on the cold ceramic plates which shortened module life and posed the hazard of toxic arsenic leakage.

**Cryotrap Performance.** The hydride yields were found to depend on the concentration of HCl in the reductant solution; the yield from DMA using 30% HCl was only about 40% of those from As³⁺ and As⁵⁺. Among the resulting arsine species, significant (19%) loss of (CH₃)₂AsH and total loss of (CH₃)₃As were previously reported through a 12 in. Naion dryer (MD-110-12FP, PermaPure) at a 90 mL/min flow rate. AsH₃ and CH₃AsH₂ on the contrary, experienced no loss. In this work, a 50% loss was estimated for (CH₃)₂AsH through a 48 in. dryer made of the same tubing at 250 mL/min Ar flow rate. A longer dryer was necessary in this work to better exclude moisture; otherwise, ice would gradually build up and finally clog the trap.

When the trap temperature stabilized at the end of the 40 min precleaning period, recovery study was carried out using a mixed rice sample from 30 common rice types. Low recoveries, 0.2 ± 1.3% for MMA at 186 ng/g and −0.3 ± 0.5% for DMA at 440 ng/g, implied that CH₃AsH₂ and the portion of (CH₃)₂AsH that survived the dryer were effectively retained by the sorbent bed inside the cryotrap. Quantitative (101.0 ± 1.1%) recovery for iAs at 100 ng/g, on the other hand, revealed AsH₃ was not trapped due to its low (−55 °C) BP. When HG was initiated, the temperature of the cryotrap interior was maintained at around −3 °C, measured using a HSTC-TT-J-245-36 thermocouple and a CN16PT-330 reader (Omega Engineering, Norwalk, CT, USA) on a similar PTFE cryotrap assembly under otherwise the same conditions. Under this temperature, total retention was expected for CH₃AsH₂, (CH₃)₂AsH, and (CH₃)₃As due to their higher BPs.
became incomplete when bath temperature was set at 20 °C. The Peltier modules were powered only by 10 V. A higher voltage would enhance cooling ability, but the heat dissipated to the heat exchangers would overwhelm the water bath thermostat leading to instability.

**Determination of iAs in Rice.** Adequate linearity (R > 0.999) was always observed in daily AsIII calibration curves. The limit of detection (LOD), 1.1 ng/g, was calculated (3σ) from 10 peak heights of reagent blanks. Calculation was performed with NIST 1568b rice flour SRM. Excellent agreement (Table 1) was found between the result (92 ± 4 ng/g) and certified iAs value (92 ± 10 ng/g). Table 1 also shows the results of 15 rice samples measured by SPE-HG-AFS7 vs HG-CT-AFS. Close agreement was usually the case except for three samples with >10% difference.

**CONCLUSIONS**

Design of a Peltier-effect cryotrap is presented here for the first time. Selective trapping of CH3AsH2 and (CH3)2AsH on a Chromosorb sorbent bed enabled rapid determination of untrapped AsH3 with high sensitivity and reproducibility. In contrast to pre-HG speciation, this unique physical method obviated chemical separation and eliminated chemical reagents, therefore gaining simplicity, cost, green chemistry, and safety advantages. Continuous operation also enhanced sample throughput.

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**Notes**

The authors declare no competing financial interest.

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