

Investigation of Poor Analytical Recovery Using Borosilicate Glass Containers in Selenium Determination by Atomic Absorption Spectrometry with Hydride Generation

Suminda Hapuarachchi and Thomas G. Chasteen*

Department of Chemistry

1003 Bowers Boulevard, CFS317e

Sam Houston State University

Huntsville, TX 77340, USA

E-mail: chasteen@shsu.edu

Abstract

The chemical interferences of Hg^{2+} , Sn^{4+} , Pb^{2+} , Fe^{3+} , Bi^{3+} , and Te^{4+} in the hydride generation atomic absorption spectrometry (HG-AAS) determination of selenium have been studied but interference from the glassware commonly used in these analyses has not been systematically determined. When a glass test tube, which was employed to prepare selenium samples in the required Se(IV) oxidation state for HG-AAS, was used without proper pretreatment it caused a large negative interference in selenium analysis (10-90%). In this study, we have quantitatively determined the magnitude of interferences caused by borosilicate glassware in HG-AAS selenium analysis. A series of glass pretreatments were also evaluated to remove or minimize those interferences. We found that treating glass test tubes with concentrated nitric acid prior to the sample preparation, resulted in the highest recovery of selenium compared to separate pretreatments of either distilled water, a commercial laboratory detergent, aqua regia, chromic acid, or concentrated hydrochloric or sulfuric acids. Surprisingly, HCl pretreatment resulted in the lowest average recovery rate of all the methods of pretreatment examined.

Keywords: HG-AAS, HGAAS, interference, glass surface, recovery, acid pretreatment

INTRODUCTION

Atomic absorption spectrometry with hydride generation (HG-AAS) is a commonly used method to analyze selenium due to the method's high sensitivity and selectivity [1-4]. There are numerous studies that discuss different types of interference in the determination of selenium by this technique. Workers have determined the permissible concentrations of foreign ions for the analysis of selenium(IV) [5,6], and Akl *et al.* have proposed means of precipitating and separating interfering ions using a chelation reagent [7] to increase Se recoveries. More recently masking agents—such as thiourea and EDTA—to reduce interferences have also been used [8]. Dedina and Tselav's book-length review of HG-AAS has also categorized matrix interferences in common Se analytical methods [9]; however, almost no previous work directly addresses the role that glass surfaces in sample containers play in the analysis of selenium by HG-AAS. In 1980 Raptis and coworkers describe the advantages of using quartz over glass [10]; however, in our work with biological samples harvested from a bioreactor whose bacterial culture has been amended with various metalloids, we require the analysis of approximately 25 individual screw capped test tubes per bioreactor run. The availability and cost of quartz containers—or even more specialized PTFE lined containers—has led us to use the more commonly available borosilicate glass test tubes for routine HG-AAS analyses. While the literature often suggests soaking vessels in acidic solutions before trace metal analysis [11-13], a useful evaluation or quantification of the benefit of this process has not been discussed in the literature for Se analysis via HG-AAS or what we considered to be reasonable accounting of alternate glass pretreatment methods. We have focussed here only on quantifying a type of interference/sample loss in analysis of selenium(IV) which is due to glassware used in this process, and we also report investigation of pretreatment methods aimed at increasing Se recovery.

To analyze selenium using HG-AAS, all the sample's selenium ions are converted to selenium (IV) since this is the oxidation state in HG-AAS required to form the volatile hydride. Most commonly

50% HCl is used as the reducing agent [8,14,15], though SnCl_2 can also be used [16]. In our work with biological samples containing metalloids for analysis, we use either inductively coupled plasma spectrometry [17] or HG-AAS [18]. For our HG-AAS work the process of Se reduction is carried out in a borosilicate glass test tube. In our experiments, we observed that this container has caused poor recoveries of Se in our samples analyzed by HG-AAS. In the past, we have also discussed this problem with a commercial laboratory which carries out Se determinations with this method and which was also having difficulty with Se recoveries in biological samples [19]. Moreover low recovery rates in Se AAS analyses have also been reported by others [20-22]. Further studies in our lab have shown that when a (borosilicate) glass tube was used without any pretreatment, it could cause (10-90%) negative interference in selenium analysis. The aim of this study was to determine the magnitude of the interference of glassware and also to provide a chemical pretreatment to maximize the recovery rates in determination of selenium in HG-AAS.

MATERIALS AND METHODS

Reagents

All the chemicals used in this research were analytical grade chemicals and were used as received.

Selenium dioxide (SeO_2), selenium standard solution for HG-AAS and concentrated hydrochloric acid (HCl) were purchased from Fisher Scientific (Houston, TX, USA). Nitric acid (HNO_3) was ordered from Aldrich Chemicals (Milwaukee, WI, USA). Sulfuric acid was purchased from J. T. Baker Inc. (Phillipsburg, NJ, USA) and Extran[®], commercial detergent, was ordered from EM Science (Darmstadt, Germany). Selenium atomic absorption calibration standards were prepared from 1000 ppm standard solutions in 50% v/v HCl, which was also used as the sample matrix.

Tube preparation for sample analysis

Borosilicate glass test tubes (Corning Inc., Corning, NY, USA) used for Se reduction (screw cap; 16 mm x 125 mm) were purchased from VWR Scientific (Sugarland, TX, USA). The test tube glass composition was SiO_2 , B_2O_3 , Na_2O , Al_2O_3 and K_2O 80.6%, 13.0%, 4.0%, 2.3%, and 0.1% respectively as determined by the manufacturer (type I, class A borosilicate glass; DD-G-54; ASTM E-438) (Corning.com site). In these experiments, new glass tubes were used for each analysis. Tubes were treated with different chemical acids or commercially available detergents followed by washing with DI water before being used in the Se sample preparation. The following solutions were used to treat test tubes:

1. untreated—directly from shipping container
2. deionized water
3. concentrated HCl [23]
4. concentrated H_2SO_4
5. 0.2% Extran[®] solution in deionized water [24,25]
6. 2% Extran soaked for 12+ hrs.

7. aqua regia (3 HCl:1 HNO₃ v/v) [26,27]
8. chromic acid (2.5g of Na₂Cr₂O₇ dissolved in 250 Con. H₂SO₄) [28]
9. concentrated HNO₃

First, test tubes were rinsed with the above solutions followed by rinsing with distilled water thoroughly and then air-dried before use; the untreated tubes were used as received from the manufacturer without any washing whatsoever. All rinsing except the overnight Extran soaking involved soaking tubes for 15 minutes in the specified reagent, followed by rinsing in deionized water.

Sample preparation in tubes

Standard selenium-containing solutions prepared for instrument calibration (in 50% HCl) were used as samples in this analysis. Those samples were heated in a water bath (30 min) in capped, treated or untreated test tubes then analyzed by HG-AAS using the procedure below.

HG-AAS analysis

Samples prepared in test tubes as described above were analyzed by HG-AAS [1,29,30]. A Varian FS 220 AAS with a hydride generation module was used, and instrumental parameters were set according to the user manual guidelines: 196.0 nm Se line, 0.2 nm slit width, air/acetylene flame to heat the quartz tube atomizer, and N₂ as carrier gas [31].

Ten molar HCl and 0.6% NaBH₄ (prepared in 0.5% NaOH) were used as hydride generation reagents. Sample, acid and borohydride solution flow rates were maintained respectively at 8 mL, 1.2 mL and 1.2 mL min⁻¹. Recalibration (reslope) was carried after every fifth sample to minimize instrumental drift. Sample blanks were 50% HCl. The working range was up to 20 ppb Se and R² values for calibrations were routinely > 0.99.

RESULT AND DISCUSSION

When glass test tubes were used in our selenium analysis without any kind of pretreatment, that is, straight out of the manufacturer's shipping container, a large experimental error occurred. Samples were reduced in test tubes without washing to determine the magnitude of this interference (see Table I below). All tabulated values are an average of 3 replicate samples with standard deviation in parenthesis, and although the three Se standard samples analyzed in untreated tube showed a relatively well-grouped recovery (30.3–37.0 %) the overall recovery was clearly unacceptable without pretreatment. Distilled water rinsing also produced poor recoveries.

Next we tried to treat our test tubes with a commercially available detergent, Extran, which is a powder, forms a mildly basic solution in water, and has been used for cleaning glassware in metalloid analysis [20,24,25,32]. Different concentrations of Extran solutions were evaluated: tube were rinsed with 0.2% Extran or 2.0% Extran and tubes were soaked in 2.0% Extran for 24 hr and then rinsed with deionized water. The Table's data show that this reagent was not capable of substantially reducing the negative interference in selenium analysis and indeed the recovery rate for the 15 ppb Se samples for both Extran concentrations were less than 8%.

Aqua regia (3 HCl:1 HNO₃ v/v) is a plausible choice to treat glass tubes due to its highly oxidative and metal dissolving ability [6,33-35] and these results display some kind of improvement in results with the percent recovery approaching 100% except in the 15 ppb samples (Table I). For low concentration Se samples this might be an alternative or there is a possibility that much longer pretreatment times might solve the problem; however, we did not extend the 15 min time to experiment with this.

Similar to aqua regia, chromic acid solution is also a strong oxidant and therefore possibly a useful means of cleaning glass surfaces for these sorts of analysis [28]. H₂CrO₄ was evaluated to see whether it could remove the problematic glass effects, but data from those experiments also show that it

was not capable of removing glass interference; results showed both positive *and* negative errors.

Although this is the only pretreatment method we evaluated that introduced metal atoms in the pretreated reagent, there has been no report of significant interference of Cr in Se HG-AAS analysis [9].

Hydrochloric acid is widely used for glass/container pretreatment in metalloid/metal analysis. Literature methods suggest that HCl alone [36,37] or HCl mixed with other acids [13,16,35] can be used to treat sample containers or to stabilize Se sample for AAS. Among all the acid pretreatments utilized in this study, samples prepared in HCl treated tubes showed the lowest % Se recovery (average recovery 11.7%, see Table 1). Average sulfuric acid recoveries were much better but the spread between the H₂SO₄ sample recoveries was larger.

According to data in Table 1, nitric acid is capable of removing glass interference in selenium determination in HG-AAS with reasonable effectiveness. The percent recovery range for nitric acid-treated tubes was the highest of the nine treatments studied (103–121%), with an average recovery rate of 111%. Similar recovery rates for Se in AAS and atomic fluorescence spectrometry have been reported by others for samples in the same concentration range, that is, close to the detection limit [20,38,39]. Our 5 ppb Se samples showed the highest recovery (121% recovery for three replicates) for the HNO₃ treatment. This can be rationalized by the larger standard deviation at this, the lowest concentration studied which was only at about 3 times the detection limit (2 ppb at 3S/N), and, again reasonable, the standard deviations for the replicate nitric acid pretreatment samples decreased as sample concentration increased (Table 1). This suggests that recoveries even closer to 100% might be expected for methods with better sensitivity.

Even though the composition of the glass in the test tubes used in these experiments is known (see above), it is not apparent what elements in the glass makeup would interfere with selenium analysis given the interfering ions reported in the literature [9,40]. The chemical interferences of Hg²⁺, Sn⁴⁺, Pb²⁺, Fe³⁺, Bi³⁺, and Te⁴⁺ (among others) in HG-AAS determination of selenium [9] and Cu, Ag, and Au in hydride generation atomic fluorescence spectrometry [6] have been studied; however, none of these

interferants is a major component in the borosilicate glass used in these studies. And possibly most important, the concentrations of interfering ions that are known to cause Se in HG-AAS as reported in the literature are very probably orders of magnitude lower in our tubes' solutions than would be present from their dissolution out/off of the glass surfaces were they present as surface contaminants [9]. Instead the poor recovery rates (almost always low) suggest **adsorption of Se to the glass** instead of dissolution and contribution of interfering ions to sample solution during the analysis.

According to the glass treatment data reported here, rinsing borosilicate test tubes with concentrated nitric acid for 15 min, followed by rinsing with deionized water, is most successful at reducing the poor recovery rates for Se determination using HG-AAS. From average recovery rates of 34% for untreated tubes, surfaces treated with concentrated HNO_3 averaged 111%. It is still not clear why other oxidants like chromic acid or aqua regia did not work effectively for this, but this suggests that an oxidizing acid wash of the surface is, in a simple way, not the mechanism that eliminates interference. Furthermore simply filling empty chemical sites in the glass surface with anions also does not appear a reasonable answer because high H^+ or Cl^- or HSO_4^- (or SO_4^{2-}) or $\text{Cr}_2\text{O}_7^{2-}$ (or HCr_2O_7^-) in other pretreatments we tried doesn't solve the low recovery problem either. Indeed earlier workers have reported poor recovery rates for selenite in the presence of chloride [36]; however, all our samples had a final matrix of 50% HCl going into the hydride generation step.

Shendrikar and West [41] and others [35] have reported that storing selenium samples in nitric acid prevented sample loss; however, they did not systematically address pretreatment issues and since HNO_3 is itself an interferant/unwanted oxidant in the hydride generation process that we and many others use, this is not an alternative final matrix for HG-AAS analysis; although apparently a useful *pretreatment* reagent, nitric acid must be washed away with deionized water or decomposed before the HCl reduction step common to almost all Se hydride generation analyses. Gómez-Ariza *et al.* [12] do suggest rinsing glassware with 0.08 M nitric acid; however, our use of 30% nitric acid solution (aqua regia) while successful in the 5 and 10 ppb samples did not reduce recoveries successfully for the higher

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15 ppb range. Only the concentrated HNO_3 pretreatment results in the highest overall yield. (We did not try other concentrations of HNO_3 besides concentrated and aqua regia.) Gómez Ariza *et al.* in other work [42] have determined storage of inorganic Se species in borosilicate to be more stable than storage in quartz. While we have not worked with quartz test tubes this makes us even less inclined to do so.

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Table 1. Percent recoveries for different borosilicate glass test tube treatments

| Pretreatment Reagent | Expected Se ppb | Determined Se ppb (SD, n = 3) | % Recovery |
|------------------------------------|-----------------|----------------------------------|--------------|
| Untreated | 5 | 1.85(±1.73) | 37.0 |
| | 10 | 3.47(±4.77) | 34.7 |
| | 15 | 4.54(±6.03) | 30.3 |
| | | Average | 34% |
| Distilled water | 5 | 2.76(±2.48) | 55.2 |
| | 10 | 3.17(±4.62) | 31.0 |
| | 15 | 5.35(±6.02) | 35.7 |
| | | Average | 40.6% |
| 0.2 % Extran | 5 | 4.18(±2.04) | 83.6 |
| | 10 | 1.94(±1.34) | 18.4 |
| | 15 | 1.10(±0.45) | 7.3 |
| | | Average | 36.4% |
| 2 % Extran (24 hours) | 5 | 5.16(±0.32) | 103.2 |
| | 10 | 6.65(±4.64) | 66.5 |
| | 15 | 1.06(±0.66) | 7.1 |
| | | Average | 58.9% |
| Aqua regia | 5 | 5.52(±1.32) | 110.5 |
| | 10 | 10.17(±0.48) | 101.7 |
| | 15 | 10.82(±6.32) | 72.1 |
| | | Average | 94.8% |
| Chromic acid | 5 | 6.84(±0.91) | 136.8 |
| | 10 | 6.20(±3.18) | 62.0 |
| | 15 | 9.52(±3.3) | 63.5 |
| | | Average | 87.4% |
| HCl | 5 | 0.51(±0.09) | 10.1 |
| | 10 | 0.83(±1.36) | 8.3 |
| | 15 | 2.53(±1.88) | 16.7 |
| | | Average | 11.7% |
| H₂SO₄ | 5 | 3.01(±2.68) | 60.2 |
| | 10 | 9.93(±0.18) | 99.3 |
| | 15 | 3.64(±3.85) | 24.2 |
| | | Average | 61.2% |
| Nitric acid | 5 | 6.06(±0.90) | 121 |
| | 10 | 10.99(±0.46) | 109 |
| | 15 | 15.49(±0.36) | 103 |
| | | Average | 111% |