

Original Research Paper

Evaluation of sample collection containers for selenium quantification

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Abstract: Naturally occurring concentrations of selenium (Se) are very low: 0.02 $\mu\text{g L}^{-1}$ in freshwater and 0.01-2 mg kg^{-1} in soil. Mining operations must discharge water in accordance with the United States Environmental Protection Agency's established maximum contaminant level and may be required to discharge at even lower limits set by the state. For accurate analysis, sample collection containers and labware must be carefully chosen to prevent changes in composition through interactions between sample and container. Our objective was to assess four materials (glass, silanized glass, polytetrafluoroethylene, and polypropylene) for use in Se quantification. Varying Se stock solutions (0, 10, 25, and 50 $\mu\text{g L}^{-1}$) were prepared in 0.1 M sodium chloride, delivered to each container, and equilibrated for 6, 12, 24, or 48 hours. Selenium was lost to all containers with the least loss occurring in the silanized-treated glass (1-12% across all concentrations). Silanized glassware is recommended for Se quantification as it improved sample integrity.

Keywords: silanization; polypropylene (PP); polytetrafluoroethylene (PTFE); quality control

Introduction

The selenium (Se) maximum contaminant level (MCL) and maximum contaminant level goal (MCLG) is 50 $\mu\text{g L}^{-1}$ in drinking water as established by the United States Environmental Protection Agency (US EPA 2018). The MCLG is the concentration in drinking water at which there is no known or expected health risk. MCLGs are not enforceable but are intended goals to ensure public health. The MCL, which is enforceable, is the greatest concentration at which a contaminant can be present in drinking water. Overconsumption of Se resulting in toxicity or selenosis generally causes hair and nail loss (Fordyce, 2013), but nervous system disorders, skin disorders, and decreased fertility have also been reported

(Fordyce, 2013; Mistry et al., 2011). Aquatic life criteria, which are not enforceable, are defined as greatest concentration aquatic life may be exposed to over the long-term without exhibiting increased mortality and decreased reproduction (USEPA 2014). Fish exposed to concentrations exceeding the aquatic life criterion exhibit decreased fecundity, larval and juvenile deformity, and mortality. Mining operations must discharge water to receiving waterbodies at $\leq 50 \mu\text{g L}^{-1}$ to meet the US EPA's drinking water standards and as low as $\leq 5 \mu\text{g L}^{-1}$ assuming the US EPA's aquatic life criteria is adopted by the state. Therefore, sampling methodology and containers must minimize possible losses so standards can be enforced correctly.

Methods have been developed to quantify low concentrations of Se. However, loss of Se to glassware and sampling containers has not been assessed. Exploratory work found that up to 15% of the initial Se^{IV} concentration could be lost to glassware (Waltemeyer 2015). Losses to glassware could explain differences between the sum of Se species and total Se that have been reported (EPRI 2005; EPRI 2006). Loss of target compounds from solution to container surfaces has been recognized as an issue in trace compound quantification, especially in speciation analyses (Zief and Mitchell 1976; Kosta 1982). Differences have been interpreted as an artifact of sample storage time/method, precipitates formed during storage, interactions with other species, and/or measurement methodology. Losses to sample containers and labware are often considered to be minimal, but at low regulatory limits any loss can impact concentration reported and misrepresent actual concentrations.

Limited work has quantified potential losses of Se to labware or sample containers, but this is necessary for the establishment of methodology which can be used to enforce accurately the US EPA discharge limits. US EPA methods (1994; 2016) state that samples can be collected in plastic or glass, while plastic is preferred. The purpose of this study was to test and compare the loss of Se on common labware and sampling containers. Borosilicate glass, silanized glass, polypropylene (PP) plastic, and polytetrafluoroethylene (PTFE) plastic were selected for evaluation due to use of these materials in laboratories. Aqueous Se concentrations were selected with a maximum concentration of $50 \mu\text{g L}^{-1}$ which is the established Se MCL.

Materials and Methods

Apparatus

Samples were analyzed with a PSA 20.400 autosampler and PSA 10.055 Millennium Excalibur HG-AFS (Orpington, Kent, United Kingdom) operating with a Se boosted-discharge hollow cathode lamp with primary and secondary discharges of 20.0 and 25.5 mA respectively. Homologous PTFE tubes (Environmental Express, Charleston, SC) were used to hold all samples on the autosampler. These tubes are recommended for use by the vendor. Losses to these

containers were not of analytical concern. Preliminary method development found that there was no analytical loss to the homologous PTFE tubes during the time in which samples would be held on the autosampler.

Glassware and PTFE tubes (Oak Ridge Style 3114; Spectrum Chemical, NJ) were cleaned according to laboratory standards before cleaning in a two-stage acid bath: 7.0 M nitric acid for a minimum of 24 hours followed by 1.0 M hydrochloric acid (HCl) for a minimum of 24 hours. Containers were rinsed with distilled deionized water and air-dried prior to use. Glassware was silanized once dry by filling each vial fully with Sigmacote[®] (Millipore-Sigma, St. Louis, MO) for 10 seconds and then emptying it. Silanized vials air-dried for 24 hours before use. PP tubes (Thermo Fisher Scientific, Hampton, NH) were used only once.

Experimental Procedure

Varying Se stock solutions (0, 10, 25, and $50 \mu\text{g L}^{-1}$) were prepared in 0.1 M NaCl in triplicate. Samples (40 mL volume) were placed on a rotary shaker (Glas-Col, Terre Haute, IN) at 30 revolutions per minute for 6, 12, 24, or 48 hours to increase interaction with the container. The times selected were to evaluate the ability of the selected materials to suitably hold samples for later work, but these times are also reflective of sample hold times for regulatory analysis. Samples were prepared for each individual time interval. After equilibration, samples were transferred to homologous PP tubes for immediate determination of total Se by HG-AFS. Trace metal grade HCl (37%) was added at 20% v v⁻¹ as required for Se determination, which results in dilution. Target concentrations were 8, 20, and $40 \mu\text{g L}^{-1}$. Samples were prepared in triplicate in a completely randomized design (n=180).

Statistical Analysis

All statistical analyses were conducted with parametric procedures as only 7% of the data was not normally distributed (SAS 9.4; SAS Institute Inc., Cary, NC). Analysis of covariance (ANCOVA) was performed using proc glm with a response variable of normalized concentration and an interval variable of time. Normalized concentration was used because the HCl used for acidification contains trace levels of Se ($\leq 1 \mu\text{g L}^{-1}$ per manufacturer specification). Time was

the covariate and reflects conditions under which the experiment was carried out, in this case time intervals at which samples were allowed to equilibrate. The time for which the samples equilibrated was assumed not to be impacted by the factor levels of the experiment. Figures were generated using JMP Pro 14 (SAS Institute Inc., Cary, NC).

Results

Time was not independently statistically significant for any concentration ($p > 0.05$; Table 1). The interaction of treatment (container material) and time was statistically significant for both the 25 and 50 $\mu\text{g L}^{-1}$ model, whereas treatment was statistically significant for the 25 $\mu\text{g L}^{-1}$ model. Therefore, the concentration of Se measured varied with both equilibration time and container type. Slope of the regression (Table 2) for these models is slightly positive for silanized glass and PP. The slight positive slopes indicate a slight increase in concentration over time. However, these slopes are minimal (Figure 1). Slopes for PTFE were positive at 25 $\mu\text{g L}^{-1}$ and negative for the 50 $\mu\text{g L}^{-1}$ samples. Slope of the regression for glass was negative across all concentrations indicating loss over time.

Table 1. Overall model fit and fixed effects from ANCOVA analyses for labware evaluation by nominal concentration.

Nominal Concentration $\mu\text{g L}^{-1}$	Overall Model	Time	Treatment	Treatment \times Time
10	0.17	0.34	0.07	0.45
25	<0.0001*	0.06	0.005*	<0.0001*
50	0.02*	0.51	0.20	0.001*

Table 2. ANCOVA regression parameters by labware type and sample nominal concentration.

Nominal Concentration $\mu\text{g L}^{-1}$	Intercept			
	Glass	Silanized Glass	PP	PTFE
10	6.57	6.98	6.41	6.41
25	20.67	19.42	17.56	17.23
50	37.32	35.63	35.52	34.78
Nominal Concentration $\mu\text{g L}^{-1}$	Slope			
	Glass	Silanized Glass	PP	PTFE
10	-0.02	0.002	0.01	-0.02
25	-0.20	0.02	0.03	0.03
50	-0.18	0.05	0.08	-0.03

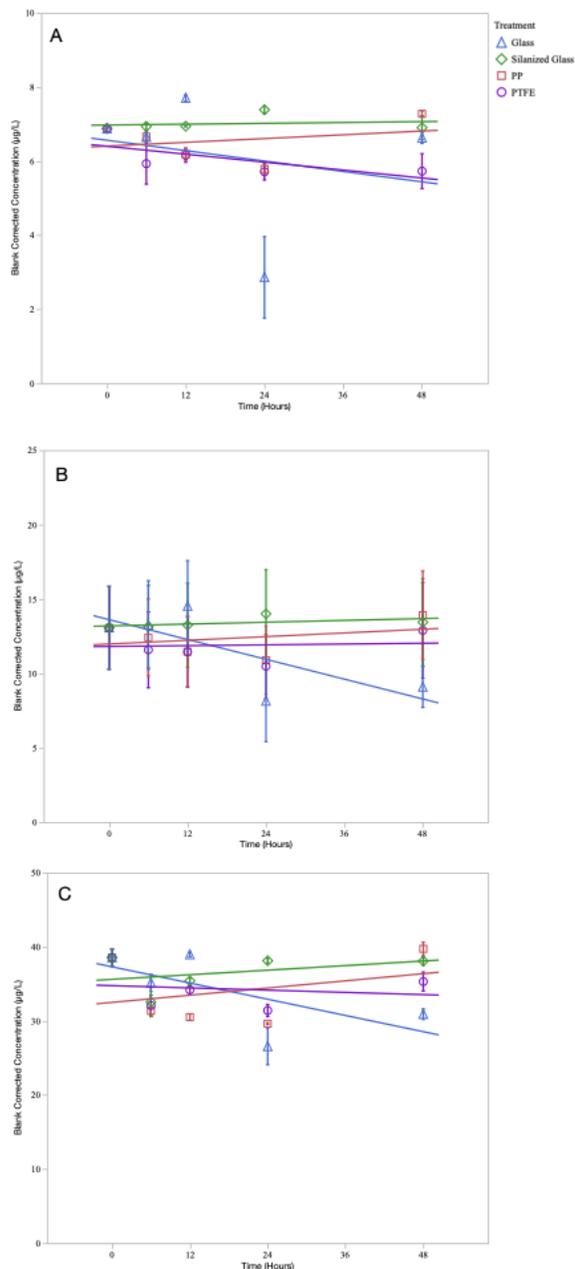


Figure 1. Change in Se concentration by labware over 48 hours for samples with an initial concentration of 10 $\mu\text{g L}^{-1}$ (A), 25 $\mu\text{g L}^{-1}$ (B), and 50 $\mu\text{g L}^{-1}$ (C). Error bars represent one standard error of triplicate samples.

The y-intercepts for the models can be used to evaluate container suitability. Ideally the intercept would be the target concentration of 8, 20, and 35 $\mu\text{g L}^{-1}$ when accounting for sample acidification. Silanized glass had y-intercepts of 6.98, 19.42, and 35.63 $\mu\text{g L}^{-1}$ (Table 2). Coupled with the minimal positive slope of the regression models, these intercepts suggest silanized glass is suitable for Se

work as it maintains concentration across time. While glass also exhibited y-intercepts close to the target concentration, the negative slope of the regression indicates that Se concentration can only be maintained for a short period of time without loss to the container. The y-intercepts for PP and PTFE were the lowest of all the materials analyzed, so there may be an initial loss to the container that once satisfied does not result in further loss.

Average percent loss (Table 3) was calculated across all time points analyzed for each container type by prepared concentration. All container types exhibited the greatest loss at the low concentration prepared. Silanized glass corresponded with the least loss 1.2%-12.3%. Glass exhibited the greatest loss 14.5-23.2%. PP and PTFE corresponded with moderate loss 9.2-18.0% and 11.4-23.9%. Loss across all material, including silanized glass, could be loss to caps. While PP and PTFE containers had caps of the same material, glass vials had polypropylene caps with PTFE septum. Silanization was not performed on the caps. There may have been losses to the PTFE septum which was in contact with the sample in both glass containers used. Losses in the silanized treatment could be attributable to improper sealing of all ion-exchange sites. If silanization resulted in a patchy seal, sorption of Se to unsealed exchange sites could occur.

Table 3. Average percent loss across time points \pm one standard error by container type.

Nominal Concentration	Glass	Silanized Glass	PP	PTFE
$\mu\text{g L}^{-1}$	-----%			
10	23.2 \pm 6.1	12.3 \pm 1.0	18.0 \pm 1.8	23.9 \pm 1.9
25	14.5 \pm 5.9	1.2 \pm 0.9	9.2 \pm 2.3	11.4 \pm 2.5
50	14.9 \pm 3.4	8.6 \pm 1.8	15.1 \pm 2.9	15.1 \pm 2.9

Discussion

While the US EPA (1994; 2016) recommends plastic, both PP and PTFE exhibited moderate analyte loss likely because of the surface properties. PP is widely used because it is resistant to most chemicals, has a high operational temperature range, and is a hydrophobic non-polar material (Gaillard and Strauss 1998). Unless specified, PP is a non-homologous thermoplastic with three possible polymer arrangements which can create areas of small charge imbalances that may promote differing

degrees of analyte attraction (Ochiai et al. 1971). The polymerization process results in a porous surface which allow for sorption of metal cations and organics to the PP surface (Muldrew et al. 1982; Teuten et al. 2007; Rochman et al. 2014). Further, PP plastic was found unsuitable for sampling and storage of natural water samples for metal analyses (Batley and Gardner 1977).

PTFE performed similarly to PP in this experiment but has a different surface chemistry which reduces analyte loss. Unlike PP, PTFE is a homologous thermoplastic in which only one possible arrangement of polymers. Its non-polar surface area that minimizes sorption of polar ions (Knapp and Schramel, 2003), especially at low temperatures (Batley and Gardner, 1977; Pinheiro and Bosker, 2004.) However, PTFE has a porous structure which can allow for losses of analyte at the trace level (Versieck, 1983).

Glass is commonly selected for sampling and lab analyses because of its properties: low coefficient of thermal expansion, inertness, and resistance to most chemicals. Samples in glass can be stored in several environmental conditions without additional transfers to other containers, which minimizes loss. However, silicate and silanol groups in glass can act as ion-exchange centers (Pepe and Byrne 1980), thereby altering the concentration of trace ions in solution (Seed 2000), and adsorptive losses to glass can be significant. Metal cations sorb to glass near pH of 6 (Pinheiro and Bosker 2004), a common pH of natural waters. Sorption of anions occurs primarily at low pH and in the presence of protonated surfaces (John et al., 2018). Therefore, sample pH and surface charge of glass may result in losses and underreporting of Se concentration.

Silanization is a common technique employed in microbiology which seals exchange sites with polysiloxane chains (Seed 2000). This proven to be especially useful when working with small volumes (Farrell 2014). In this experiment, samples contained in silanized glass had the greatest measured concentration and the lowest percent loss at all prepared concentrations, which was attributed to the sealing of ion-exchange. Glass quality, rather than instrumentation, can be more important at low analyte concentrations (Schaaf et al. 2018).

Therefore, silanization could reduce the need to purchase high quality glass to minimize analyte loss and improve data accuracy.

Samples were not acidified as is standard in (US EPA 1994). Anions, including Se oxyanions, sorb at low pH. If samples are acidified for storage, losses to sample containers and labware could be larger than those reported in this study. Therefore, the use of silanized glass would be ideal. Sample collection and storage in silanized glass would result in minimal loss to the sample vessel itself and increase accuracy in reporting Se concentrations in mining discharge waters.

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