BACKUP DATA - Method Nos. 7704 and 9110 / Beryllium
Issue 1

Authors: T. Mark McCleskey (Los Alamos National Laboratory, Los Alamos, NM, USA),
Anoop Agrawal (Berylliant, Inc., Tucson, AZ, USA), and Kevin Ashley (CDC/NIOSH, Cincinnati, OH, USA)

Substance: Beryllium (Be)

Exposure Limits:

Airborne Exposures:

Occupational Safety and Health Administration (OSHA): $2 \mu g/m^3$; Ceiling $5 \mu g/m^3$

American conference of Governmental Industrial Hygienists (ACGIH): $2 \mu g/m^3$ (suspect Carcinogen)

National Institute for Occupational Safety and Health (NIOSH): Not to exceed $0.5 \mu g/m^3$ (suspect Carcinogen)

Department of Energy (DOE): 10CFR850 : Airborne beryllium action level (10CFR 850.23) $0.2 \mu g/m^3$ for 8 hour time weighted average

OTHER: International Standards: The UK, Sweden, Austria, France and Spain have 8-hour Be limit values (LV) of $0.002 mg/m^3$, while in Denmark the full-shift LV is $0.001 mg/m^3$.

Surface wipes:

DOE : $3 \mu g/100cm^2$ for surface contamination limit for housekeeping (10 CFR 850.30), and for release of equipment (10 CFR 850.31) as $0.2 \mu g/100cm^2$, or the concentration of beryllium in the soil at the release point (whichever is higher).

OTHER: N/A

1.0 INTRODUCTION

The unique properties of beryllium (Be) have led to many applications in aerospace, the nuclear industry, manufacturing, electronics, and even sports equipment. Beryllium metal is light in weight and has high strength, and alloying beryllium with copper and aluminum results in materials with high corrosion resistance, stiffness and low stress relaxation. Beryllium alloys are used in high-end electrical connectors, springs, bearings and other components. The high thermal conductivity of beryllium oxide, while also being electrically insulative, is a key component to the dissipation of heat in integrated circuits. Unfortunately, beryllium is a Class A EPA carcinogen and its ingestion can cause the incurable and potentially fatal lung disease, chronic beryllium disease (CBD) [1,2]. Further, it has also been shown that skin exposure may result in

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1 Sanderson, W.T., Ward, E.M., Steenland, K., Petersen, M.R., *Lung Cancer Case-Control Study of Beryllium Workers*, American Journal of Industrial Medicine, 40(3) p. 284 [2001].

sensitization towards CBD[3]. Beryllium contamination has also been found in coal slag and bauxite, an aluminum ore. Thus, monitoring of beryllium in occupational environments is of vital importance. Beryllium metal (as metal and as a metal alloy) and beryllium oxide are the most important beryllium materials from an industrial perspective. Thus, one has to monitor and limit exposure of workers in industrial workplaces to beryllium particulate matter which may be inhaled or may come in contact with the skin. Some fraction of inhaled particles via breathing are deposited in the upper airways and cleared via the mucociliary escalator to the gastrointestinal tract and some fraction is retained in the lung. This latter lung-retained fraction may interact with the immune system to cause adverse health effect.

Current methods to detect beryllium (e.g. NIOSH 7102 and 7300) use atomic spectrometric instrumentation. Preparation of samples for such analysis involves the use of highly toxic acids, and the laboratory instrumentation is expensive. This instrumentation also requires highly trained personnel and is not field deployable.

To overcome these issues, a rapid, quantitative and a sensitive test for the detection of beryllium has been developed using fluorescence. The method is based on the fluorescence of beryllium bound to sulfonated hydroxybenzoquinoline (HBQS), and includes a novel dissolution technique using a dilute ammonium bifluoride solution. The intensity of fluorescence is linear with respect to beryllium concentration. A detection limit of lower than 0.02 µg Be/ 100 cm^2 has been achieved, which meets DOE regulations.[4] Interference studies have been carried out with a variety of metals, with minimal or no interferences found for the detection of Beryllium at 100 nM in the presence 0.4 mM of the other metals. The specificity for beryllium has been achieved using a number of mechanisms, such as (a) the use of ethylenediamine tetraacetic acid (EDTA), which binds larger metals, (b) the use of high pH in the detection solution, which causes unbound metals to precipitate, and (c) the use of HBQS, which is only able to accommodate beryllium in its complexation structure. The method has been proven successful under various operating conditions, including the detection of beryllium both in laboratory settings and in field trials. It fulfills the requirements for a fast, inexpensive, field deployable method of detection of beryllium. Details on the chemistry and development of the test method are in an attached reference from Los Alamos National Laboratory (LANL) in Appendix 4.

2 Schuler CR, Kent MS, Deubner DC, Berakis MT, McCawley M, Henneberger PK, Rossman MD, Kriess K. *Process Related Risk of Beryllium Sensitization and Disease in a Copper-Beryllium Alloy Facility*, American Journal of Industrial Medicine, 47(3) p-195 [2005]..

3 Tinkle SS, Antonini JM, Rich BA, Roberts JR, Salmen R, DePree K, Adkins EJ, *Skin as a Route of Exposure and Sensitization in Chronic Beryllium Disease*, Environmental Health Perspectives, 111(9) p-1202 [2003].

4 CFR (Code of Federal Regulations), Title 10, Energy, Part 850, Department of Energy, parts 500 to end [2001].

There are several advantages of the system which are summarized below:

- •Rapid (test results within one hour)
- •High throughput
- •Beryllium-specific
- •Low capital cost (less than $10,000)
- •Field or laboratory deployable
- •Detection limit for wipes lower than 0.02 µg/100 cm^2 or 0.02 µg on air filters.
- •Uses less hazardous solutions than methods using strong acid digestion
- •Uses only a fraction of material collected, an advantage for re-verification
- •Does not require highly trained lab personnel

These reports comprises data suitable for air monitoring and/or wipe sampling for occupational exposures to beryllium. Air samples would be taken using appropriate filter media in an applicable sampler. Surface wipe samples would be taken also using cellulosic wipe materials. The analytical method uses fluorescence detection of beryllium using the indicator 10-hydroxybenzo[h]quinoline-7-sulfonate (10-HBQS). More analytical details are also included in an attached reference in Appendix 7.

2.0 Data Sets
The data below are from different experiments having different objectives.

2.1 Data Set 1: Analytical issues and field data (Appendix 1)*
The tables A1-1 to A1-12 in Appendix 1 are from Los Alamos National Laboratory (LANL), covering a variety of analytical issues and evaluation of field data.

In many of these tables, concentration of beryllium in the measurement solution in units of parts per billion (ppb) is related to the quantity of beryllium on the media (e.g., filter paper) in units of µg. For sample preparation where 20X dilution is used, one starts out with 5ml of dissolution solution (1% ammonium bifluoride) to dissolute the beryllium on the media. Of this, 0.1ml of the dissolution solution is added to 1.9ml of the dye solution (20X dilution) for measurement. The "Standard Solutions" used for calibration have beryllium concentration measured in ppb. Table 1, below, shows typical standards used and how the beryllium concentration in the solution in ppb correlates to the amount of beryllium in µg on the media.

Table 1

Preparation of Standard Solutions	Concentration of beryllium (ppb) in cuvets comprising calibration standards and detector solution	Comments
0.1 ml of **0 ppb standard** + 1.9 ml of detection solution	0.0	Corresponds to 0.00 µg beryllium on media
0.1 ml of **10 ppb standard** + 1.9 ml of detection solution	0.5	Corresponds to 0.05 µg beryllium on media
0.1 ml of **40 ppb standard** + 1.9 ml of detection solution	2.0	Corresponds to 0.2 µg beryllium on media
0.1 ml of **200 ppb standard** + 1.9 ml of detection solution	10.0	Corresponds to 1 µg beryllium on media
0.1 ml of **800 ppb standard** + 1.9 ml of detection solution	40.0	Corresponds to 4 µg beryllium on media

Most of the data in this report were taken at 20x dilution (unless reported otherwise for increased sensitivity), which means that the volumetric ratio of the sample solution (in dissolution solution) to the detection solution was 1:19. Mechanical agitation or heating was used for obtaining most of these results, which was carried out by rotating the sample tube for 30 minutes or by heating at 80 °C. Most of the data were generated using an Ocean Optics Fluorometer and Turner Quantech Fluorometer unless reported otherwise in these tables. For more details on instrument settings and other instruments, please see section 2.4 below (and within these tables).

Tables A1-3 and A1-4 show supportive data on the effectiveness of using 1% ammonium bifluoride as dissolution solution. The results show that this solution and the method used is highly effective. Table A1-5 shows results from different lots of Whatman 541 filters. Different filter lots may have differing amounts of residual acid. The results show that all lots gave identical results.

2.2 Data Set 2: Evaluation of ammonium bifluoride extraction of various compounds of beryllium and various sampling media (See Appendix 2)

These experiments were conducted at NIOSH. The fluorometer used in this experiment was from Ocean Optics. The purpose of this experiment was to evaluate if 1% ammonium bifluoride was suitable in extracting non-water-soluble beryllium compounds such as beryllium oxide (BeO) and water-soluble compounds such as beryllium sulfate obtained from Sigma-Aldrich Co. According to a specialist from Sigma Aldrich, beryllium oxide powders supplied by them are processed at 2000 °C ("High Fired"). The ammonium bifluoride was found suitable for dissolution of beryllium compounds. The dissolution solution was agitated mechanically and ultrasonically and both methods were found suitable.
The wipe results indicate that Ghost Wipes were not suitable. Furthermore, some batches of Ghost Wipes have been found to be contaminated with beryllium.

2.3 Data Set 3: Comparison of dissolution methods using a difficult to solubilize commercial beryllium oxide material (See Appendix 3)

These experiments were conducted at LANL using UOX125 BeO (obtained from Brush Wellman and considered "High Fired"). The purpose of the experiment was to investigate the applicability of the method on a commercial BeO powder, and also to evaluate different methods of dissolution using 1% ammonium bifluoride. Slurries of these powders were prepared and Whatman 541 filters were spiked with BeO. These samples were then extracted using 1% ammonium bifluoride using mechanical agitation (which is done at room temperature), and also by heating the solutions to 75, 80 and 90 °C without mechanical agitation. Aliquots from these samples were then analyzed using a Turner-Quantech fluorometer and by an inductively coupled plasma-atomic emissions spectrometer (ICP-AES). These results are shown in Appendix 3. This data shows that when heating is used, 1% ammonium bifluoride dissolution is as effective as acid dissolution protocols used in ICP analysis. The results also show that if the amount of beryllium oxide present on the sampling medium is up to the regulation limit of 0. 2 μg, then either mechanical agitation or heating may be used.

If beryllium oxide is present in higher quantities, then heating at or in excess of 75 °C is recommended. However, it was concluded that mechanical agitation using 1% ammonium bifluoride is sufficient to raise a "red flag" in case the difficult to solubilize beryllium oxide is present anywhere near the regulation limit.

2.4 Data Set 4: Interlaboratory agreement (See Appendix 5)

The purpose of this experiment was to check for interlaboratory consistency on filters (Whatman® 541 and mixed cellulose ester (MCE) filters) spiked with pre-determined amounts of beryllium. The amount of beryllium on the filters was not revealed to the labs that conducted the analyses. All labs used 1% ammonium bifluoride for extracting beryllium and a dye detection solution supplied by a single source, but used different fluorescence instruments to analyze the samples. Appendix 5 lists the experimental details, the results, the instruments used, as well as important parameters.

The Fluorometers which have been found suitable for this method are: (a) Turner Quantech FM109515 from Barnstead Thermolyne (Dubuque, Iowa); (b) RF1501 from Shimadzu (Columbia, Maryland); (c) USB2000-FLG from Ocean Optics (Dunedin, Fl); and (d) SPEX Fluorolog 2 (Horiba, Irvine, CA).

Parameters used for Turner Quantech: Excitation band-pass filter with peak transmission at 360 nm and bandpass of 40 nm (NB360 part number from Barnstead LE1095X30). Emission band-pass filter with a peak transmission at 460 nm with a bandpass of 10 nm (NB460 part number from Barnstead LE1095X12). Small volume position of sample holder was used. Autogain parameter was used.

Parameters for Shimadzu RF1501: Excitation at 360 nm, emission at 475 nm (bandpass was ±10 nm for both).

For the Ocean Optics instrument a light emitting diode (LED) with peak emission at 380 nm was used (LED380), and the emission was integrated at 475 nm; a 1 mm diameter fiber optic cable (part number P1000-2-UV-Vis) was used to connect the sample holder and the spectrometer.

A SPEX Fluorolog 2 instrument, made by Jobin-Yvon SPEX (now Horiba), was also used. The excitation wavelength was 380 nm and the emission wavelength was 475 nm. In both cases the bandpass was 5 nm and integration time was 1 second. The light source was a 450 W Xenon arc lamp.

Data from this study was statistically analyzed for bias and repeatability and reproducibility. These results are discussed and explained in **Appendix 5** and the results tabulated in **Appendix 6**.

3.0 Source of Reagents and Solutions

1. Spiked filters for performance evaluation material samples (PEMs) by inter-laboratory testing were prepared by spiking with beryllium nitrate in deionized water. Both type of filters, i.e., Whatman 541 and mixed cellulose ester (MCE) were spiked. The Whatman and MCE filters are, respectively, used for surface sampling and for collecting particulate matter by air sampling.

2. Sources of beryllium oxide were Acros Chemicals (99%) and Sigma-Aldrich Chemical Company (99.98%). The high-fired BeO, UOX125 was obtained from Brush Wellman. See Appendices 3, 4 and 5 for details on which kind of beryllium oxide was used in specific tests. Sources of beryllium sulfate were Acros and Sigma-Aldrich.

4.0 Analytical Aspects

In all data sets the dissolution solution was 1% ammonium bifluoride in deionized (DI) water (w/w). The detection reagent was prepared by the addition of 12.5 mL of 10.7 mM ethylenediaminetetraacetic acid (EDTA) and 25 mL of 107 mM L-lysine monohydrochloride to 3 mL of 1.1 mM 10-HBQS (10-hydroxybenzo[h]quinoline-7-sulfonate). The pH was adjusted to 12.85 with the careful addition of 10 M sodium hydroxide (NaOH), and water was added to achieve a total volume of 50 mL.

The reagents used in the Inter-laboratory testing in Data Set 4 were purchased from Fisher Scientific (Pittsburgh, PA), with the exception of 10-HBQS, which was synthesized in the laboratory.

This method is not restricted to a particular type of fluorometer. As long as good calibration is obtained (correlation coefficient of equal or greater than 0.99) using the standards within and close to the regulation range, any fluorometer may be used. The list below includes fluorometers used successfully in the various data sets generated.

As stated above, the fluorometers which have been found suitable for this method are Turner Quantech FM109515 from Barnstead Thermolyne (Dubuque, Iowa), RF1501 from Shimadzu (Columbia, Maryland) and USB2000-FLG from Ocean Optics (Dunedin, Fl). The instrument parameters found suitable are discussed above in section 2.4.

4.1 Sampling Aspects
This test is on the analytical aspects of the media with beryllium particulates, and not on the sampling issues. Specific sampling studies were not undertaken here for the following reasons: (a) Air sampling aspects for aerosols (for subsequent metals determination) have been covered in previous work in support of NIOSH methods 7102 and 7300; (b) The efficacy of surface sampling using wipes has been covered in previous studies involving lead, and these results are extendable to other metals such as beryllium[5,6].

4.2 Dissolution Efficiency
Recovery of sample during dissolution was not an issue in the materials examined so far, nor in the concentrations used which span the regulation requirements. This can be seen in Tables A1-3, A1-4, A1-5, A1-6, A1-10, A1-11 of Appendix 1, Table A2-1in Appendix 2 (with the exception of Ghost Wipes) and UOX125 BeO in Table A3-1 and Table A3-2 in Appendix 3. Heating during dissolution is particularly preferred when high fired BeO (e.g., UOX 125) is present in excess of $0.2\mu g$ in the media. From these data on UOX125 it is preferable to dissolute high fired BeO by heating ammonium bifluoride in the range of 75 and 90°C.

This method was developed for use in the analysis of beryllium by sampling of air or surfaces. The volumes for time-weighted average (TWA) or short-term exposure limit (STEL) air samples are expected to be the same or better as were used in the development of NIOSH 7102 and NIOSH 7300 since this method has low detection limits. Due to the extreme health risk of beryllium exposure, dynamically generated test atmospheres were not created; however, air sampling capacities and breakthrough volumes are not expected to be similar to NIOSH Method 7102 or NIOSH Method 7300. Breakthrough studies and sampling in high or low humidity were not tested with this method; these aspects have been dealt with extensively in previous studies of aerosol sampling for metals using membrane filters[7].

5. Chavalitnitikul C, Levin L: A laboratory evaluation of wipe testing based on lead-oxide surface contamination. *Am. Ind. Hyg. Assoc. J.* **45**: 311-317 [1984].
6. Millson M, Eller PM, Ashley K: Evaluation of wipe sampling materials for lead in surface dust. *Am. Ind. Hyg. Assoc. J.* **55**: 339-343 [1994].

7. Lee KW, Ramamurthi M. Chapter 10, Filter Collection. In: Willeke K, Baron PA, eds. Aerosol Measurement. Van Nostrand Reinhold: New York [1993].

For the development of the sampling methods, different procedures were used to generate the performance evaluation samples. These included spiking of Whatman 541 and MCE filters by beryllium-soluble compounds in water, slurries of oxides, and wipe samples collected from the field. Please see details in various data sets (appendices) for more information.

Please see Table A1-4 (Appendix 1) for the data used in determining the analytical precision and bias for beryllium. A calibration curve was done using 6 levels of beryllium with 6 replicates at each level over the range of 0.02 to 3.00 µg/sample. The calibration curve gave a calculated NIOSH method accuracy of 14.4% at the upper 95% confidence limit, a precision of 4% relative standard deviation (RSD), and an estimated bias of -0.0088. These calculations were done using the formulas in Chapter O of the National Institute for Occupational Safety and Health Manual of Analytical Methods (NMAM)[8]. The NIOSH Guidelines for Air Sampling and Analytical Method Development and Evaluation calls for the method accuracy to be within ± 25% of the true concentration.

4.3 Determination of sampling and analytical Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The data for calculating limit of detection for the method and the instrument is shown in Appendix 1 as Tables A1-1 and A1-2. Since a detection limit of 0.02µg is required (for detecting an exposure limit of 0.2µg), we assumed this as the limit and designed a test with five samples at 0.02µg, one at 1µg (5X) and five at 0.2µg (10X) according to NIOSH procedures explained in Appendix 4 (see section on detection limit). When this data was fitted, we obtained a detection limit of 0.0136µg (or 13.6ng/filter paper).

To evaluate the Ocean Optics Instrument detection limit, we assumed 0.006µg as the detection limit which following a similar experimentation and analysis as above, resulted in the instrument detection limit of 4.15ng/filter.

All of the above limits were established using a dilution of 20X where 0.1ml of the dissolution solution with beryllium is mixed with 1.9 ml of the detection solution for fluorescence evaluation (see section 2.1 and Table 1).

More recently, samples with 5X dilution were evaluated where 0.4ml of the dissolution solution with beryllium was mixed with 1.6ml of the detection solution to look at lower amounts of beryllium. It was found that using the same instruments as before the limit of detection was 0.002µg of beryllium on the media and the samples could be easily quantified better than 0.02µg of beryllium on the media. This is given in more detail in Appendix 8. Thus it would be possible to sample air for short periods of time as shown in Table 2.

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8. Kennedy ER, Fishbach TJ, Song R, Eller PM, Schulman SA. *Guidelines for Air Sampling and Analytical Method Development and Evaluation.* DHHS, PHS, CDC, NIOSH, May 1995. US Government publication number: DHHS (NIOSH) 95-117.

Table 2

Sampling Rate (l/min)	1	1	1	1	4	4	4	1
Sampling Time (min)	60	480	120	480	60	480	60	120
Be concentration in air ($\mu g/m^3$)	2	0.2	0.2	0.02	2	0.02	0.2	2
Amount of Be on filter (μg)	0.12	0.096	0.024	0.0096	0.48	0.0384	0.048	0.24

4.4 Storage Stability

Since beryllium and beryllium oxide are inorganic materials, the storage conditions used in testing storage stability are not stringent.   and they The test materials are not expected to degrade. Room temperature storage is fine suitable for the storage of these materials. Analysis of Variance (ANOVA) indicated no difference in the data from day 1 compared to the data analyzed on day 30 (See Data in Table A1-8). Table A1-9 shows that the dye solution (detector solution) is stable for at least 12 weeks when stored on a laboratory bench.

Storage stability was studied in Agrawal et al.[9] and samples were stable for at least 30 days.

4.5 Interference with other elements

Table A1-7 shows that this method is not sensitive to interference by other elements. This is due to several reasons. First, EDTA in the detection solution binds many of the other elements; second, the high pH of the measured solution, pH 12+, causes most other metals to precipitate; and third, the attachment center in the dye is only able to fit a specific sized moiety, which is beryllium ion (see LANL paper in Appendix 4 for a more thorough discussion). Note that if there is a very high concentration of another element which results in coloration or haziness of the measurement solution due to suspended particles, one needs to either wait until the precipitate settles to the bottom of the cuvet or re-filter (syringe filter) the solution so that the measurement solution is always colorless and clear. **Any color or haziness in the measurement solution will interfere with fluorescence measurement**. Color and haziness may be produced if the interferents are in very high concentration, as was the case in Table A1-7 for iron (which formed a colored precipitate) or for titanium, where there were still some suspended titanium oxide particles in the measurement solution.

9. Agrawal A, Cronin J, Tonazzi J, McCleskey TM, Ehler DS, Minogue EM, Whitney G, Brink C, Burrell, Warner B, Goldcamp MJ, Schlecht PC, Sonthalia P, Ashley K. Validation of a standardized portable fluorescence method for determining trace beryllium in workplace air and wipe samples. *J. Environ. Monit.* **8**: 619-624 [2006].

APPENDIX 1

In many of these tables, the procedures that were followed are given in NIOSH Technical Report, Guidelines for air sampling, analytical method development and evaluation.[8] *Page numbers from these reports are listed in individual tables.*

Table A1-1: *Method Detection Limit Data for 20x Dilution*

- Media used: 541 filter paper
- 20x dilution (Volumetric ratio of detection to dissolution solution 19:1)

sample	μg	irradiance
1	0.02	0.01
2	0.02	0.009
3	0.02	0.01
4	0.02	0.009
5	0.02	0.009
6	0.1	0.03
7	0.2	0.054
8	0.2	0.057
9	0.2	0.057
10	0.2	0.054
11	0.2	0.054

Detection limit was calculated to be 13.6ng/filter paper as given in Appendix 3, p-65, NIOSH Technical Report. For this method we assumed a detection limit of 0.02μg and analyzed 5 samples at this level, one at 5X level and five at 10X level. Calculation method is also given in Appendix 4 - See section on "detection limits"

Table A1-2: *Ocean Optics Instrument Detection Limits Data: HBQS test*

- 20x dilution (Volumetric ratio of detection to dissolution solution 19:1)

sample	μg	irradiance measurement
1	0.006	0.026
2	0.006	0.027
3	0.006	0.025
4	0.006	0.027
5	0.006	0.026
6	0.030	0.044
7	0.060	0.071
8	0.060	0.070
9	0.060	0.071
10	0.060	0.070
11	0.060	0.068

Detection limit was calculated to be 4.15ng/filter paper as given in Appendix 3, p-65, NIOSH Technical Report.[8] For this method we assumed a detection limit of 0.06μg (6ng) and analyzed 5 samples at this level, one at 5X level and five at 10X level. Calculation method is also given in Appendix 4- See section on "detection limits".

Table A1-3: *Recovery of Beryllium from Spiked Whatman 541 Filters.*

Sample: Concentrations chosen around DOE Action limit of 0.2 µg/filter
Date: 11/12/03
Instrument: Ocean Optics, 2-sec integration, 3 avg., measured at 475.42nm
Dilution: 20x dilution (Volumetric ratio of detection to dissolution solution 19:1)

Standard, ppb	0.0	0.2	1.0	2.0	4.0	10.0	Slope	Intercept
Intensity	0.012	0.026	0.081	0.155	0.283	0.710	0.069649	0.01151

Calibration curve fit			
Standard, ppb	Intensity	Calculated Concentration, ppb	%Error
0	0.012	0.007	
0.2	0.026	0.208	-4.04
1	0.081	0.998	0.22
2	0.155	2.060	-3.01
4	0.283	3.898	2.55
10	0.710	10.029	-0.29

Results on Samples

Sample ID		Intensity	Be, µg/swipe
	1	0.080	0.098
	2	0.081	0.100
0.1µg	3	0.081	0.100
Be	4	0.081	0.100
	5	0.081	0.100
	6	0.081	0.100
	1	0.133	0.174
0.2µg	2	0.154	0.205
Be	3	0.152	0.202
	4	0.146	0.193
	5	0.152	0.202
	6	0.139	0.183
	1	0.298	0.411
0.4µg	2	0.278	0.383
Be	3	0.310	0.429
	4	0.307	0.424
	5	0.291	0.401
	6	0.291	0.401

Table A1-4: *Analysis of spiked Whatman 541 filters by fluorescence and ICP and recovery confirmation of beryllium using 1% ammonium bifluoride solution*

Recovery results for two action levels of 0.2 and 3 ug/swipe. Six sets of samples at 0.1x, 0.5x 1.0x and 2x the action limit per the NIOSH Technical manual (Section A page 10). Numbers for 0.4 ug/swipe loading are in Table A1-3. After dissolution, the filters were analyzed by ICP and no appreciable beryllium was found.

The spiked Whatman 541 filter (wipe) was treated with 1% ammonium bifluoride by mechanical agitation and the filtrate analyzed by fluorescence and ICP. To evaluate residual beryllium, on the wipe it was further treated with standard ICP protocols with acids and analyzed.

DOE ACTION LEVEL 1: 0.2µg/wipe	[Be] µg		DOE ACTION LEVEL 2: 3µg/wipe	[Be] µg	
Spike Level Be, µg	Fluorescence	ICP Filtrate	Spike Level, Be, µg	Fluorescence	ICP Filtrate
0	0.000	0.002	0.3	0.297	0.309
0	0.000	0.002	0.3	0.297	0.311
0	0.000	0.002	0.3	0.310	0.312
0	0.000	0.002	0.3	0.310	0.306
0	0.000	0.001	0.3	0.310	0.316
0	0.000	0.001	0.3	0.300	0.312
0.02	0.019	0.022	1.5	1.364	1.400
0.02	0.021	0.023	1.5	1.578	1.578
0.02	0.019	0.023	1.5	1.690	1.674
0.02	0.021	0.023	1.5	1.696	1.623
0.02	0.019	0.023	1.5	1.616	1.582
0.02	0.019	0.024	1.5	1.578	1.538
0.1	0.093	0.106	3	3.031	2.965
0.1	0.089	0.107	3	3.034	2.948
0.1	0.099	0.106	3	3.050	3.014
0.1	0.090	0.107	3	3.222	3.071
0.1	0.098	0.105	3	3.066	2.930
0.1	0.096	0.107	3	2.734	2.623
0.2	0.189	0.207	6	0.555	5.921
0.2	0.190	0.213	6	0.569	6.070
0.2	0.184	0.219	6	0.586	6.251
0.2	0.193	0.217	6	0.584	6.229
0.2	0.196	0.219	6	0.586	6.251
0.2	0.198	0.221	6	0.590	6.293

Table A1-5: *Analysis of Spiked #541 Filters by Fluorescence*

Different lots of Whatman 541 filters have different amounts of residual acid. To ensure that this issue does not interfere with the results, five different lots of filters were spiked and analyzed. The results show that different filter lots still give similar results.

Date: 10/29/2003
20x dilution (Volumetric ratio of detection to dissolution solution 19:1)
Fluorescence Intensity: 2-second integration at 475.42nm, average of three readings

Concentration (ppb)	STANDARDS ppb					Slope	Intercept
	0.0	0.2	1.0	2.0	4.0		
Intensity	0.012	0.027	0.082	0.153	0.274	0.065618	0.015109

Calibration curve fit			
ppb	Intensity	Calculated Concentration, ppb	%Error
0	0.012	-0.047	
0.2	0.027	0.181	9.40
1	0.082	1.019	-1.94
2	0.153	2.101	-5.07
4	0.274	3.945	1.37

Analysis of spiked filters. Each set of four filters was a different #541 lot.

Sample ID	Intensity	µg/filter
B1108930 Be 0	0.014	-0.002
B1108930 Be-1	0.150	0.206
B1108930 Be-2	0.148	0.203
B1108930 Be-3	0.155	0.213
814411 Be 0	0.013	-0.003
814411 Be-1	0.147	0.201
814411 Be-2	0.148	0.203
814411 Be-3	0.150	0.206
D1291860 Be 0	0.012	-0.005
D1291860 Be1	0.152	0.209
D1291860 Be2	0.151	0.207
D1291860 Be3	0.148	0.203
B1078249 Be 0	0.012	-0.005
B1078249 Be-1	0.157	0.216
B1078249 Be-2	0.149	0.204
B1078249 Be-3	0.148	0.203
D1330798 Be 0	0.012	-0.005
D1330798 Be1	0.147	0.201
D1330798 Be2	0.153	0.210
D1330798 Be3	0.151	0.207

Table A1-6: *Analysis of Spiked MCE Filters by Fluorescence*

Sample: Be (as solution) recovery from MCE air filters, in triplicate + sample with no filter
Date: 2/9/2004
20x dilution, 2-second integration, 3 avg.

Calibration data at 475.42nm from Ocean Optics Instrument

	STANDARDS ppb							
Concentration ppb	0.0	0.2	1.0	2.0	10.0	20.0	Slope	Intercept
Intensity	0.011	0.02	0.063	0.11	0.51	1.024	0.050571	0.01

Calibration data fit

	Intensity	Calculated Concentration, ppb	%Error
Blanks	0.011	0.023	
0.2	0.020	0.201	-0.43
1	0.063	1.051	-5.12
2	0.110	1.981	0.97
10	0.510	9.890	1.10
20	1.024	20.054	-0.27

Sample ID	Intensity	Be, µg/wipe
0.02µg Be no filter	0.025	0.03
0.02µg Be-A	0.021	0.02
0.02µg Be-B	0.022	0.02
0.02µg Be-C	0.025	0.03
0.2µg Be no filter	0.112	0.20
0.2µg Be-A	0.115	0.21
0.2µg Be-B	0.106	0.19
0.2µg Be-C	0.118	0.21
2µg Be no filter	1.035	2.03
2µg Be-A	1.032	2.02
2µg Be-B	1.039	2.04
2µg Be-C	1.027	2.01

When the results in this table are compared to the others (e.g., A1-6), it shows that the results are as expected and that use of either Whatman 541 or MCE does not change the conclusions. This is also seen in the inter-laboratory study shown in Appendices 5 and 6.

Table A1-7: *Interference Study with Other Metals*

Intensities taken from the Ocean Optics Flourometer
20x dilution (Volumetric ratio of detection to dissolution solution 19:1)

	0 Be	100nM Be	% Difference	1uM Be	% Difference
Be	0.005	0.112	-	1.078	-
0.4mM Al	0.004	0.112	0.00	1.054	0.00
0.4mM U	0.004	0.11	1.79	1.06	-0.57
2mM Ca	0.004	0.112	0.00	1.057	-0.28
0.04mM Li	0.004	0.112	0.00	1.06	-0.57
0.4mMPb	0.004	0.111	0.89	1.105	-4.84
0.4mM Zn	0.003	0.112	0.00	1.103	-4.65
0.4mM Fe	0.003	0.101	9.82	0.925	12.24
0.4mM V	0.003	0.114	-1.79	1.083	-2.75
0.4mM Sn	0.003	0.113	-0.89	1.105	-4.84
0.4mM W	0.003	0.116	-3.57	1.103	-4.65
0.4mM Cu	0.003	0.114	-1.79	1.062	-0.76
0.4mM Ni	0.004	0.114	-1.79	1.074	-1.90
0.4mM Co	0.005	0.111	0.89	1.03	2.28

Fe has the highest interference and some precipitate was noticed at the bottom of the cuvet.
When these samples were looked at again after standing (four hours), the solution had cleared
and no interference was seen. (The numbers below do not match the numbers above as these are
raw intensities and done on different days. Further in the table below the 100nM and 1µM
experiments were done at different times thus they do not have a linear relationship with the
concentration).

	0 Be	100nM Be	% Difference	1uM Be	% Difference
Be	0.009	0.215		1.403	-
0.4mM Fe	0.01	0.215	0.00	1.403	0.00

Interference data on Titanium*
Fluorometer used: Turner Quantech, 20x dilution

Amount of Be on filter, µg	Amount of TiO$_2$ on filter, mg	Beryllium (µg) by fluorescence	
			after additional filtration step
0.20	0.00	0.20	0.20
2.00	0.00	2.02	2.03
0.20	10.00	0.17	0.20
2.00	10.00	1.64	2.02
0.20	20.00	0.17	0.21
2.00	20.00	1.65	2.04

Titanium was analyzed separately on the request of Beryllium Health and Safety Committee.
This was done as titania powders are often used in paint formulations, and thus it is frequently
present when swiping painted surfaces or in atmospheric sampling.

Table A1-8: *Long Term Experiment to Determine Aging of Samples on Wipes*

Sample on spiked 541 filter aged for various periods (storing conditions –Lab bench)

Whatman 541
0.1 μg Be/filter
20x dilution (Volumetric ratio of detection to dissolution solution 19:1)

Sample#	Intensity		Sample#	Intensity		Sample#	Intensity
1-day			7-days			14-days	
#1	0.095		#13	0.099		#22	0.095
#2	0.096		#14	0.093		#23	0.098
#3	0.099		#15	0.096		#24	0.099
#4	0.095		#16	0.098		21-days	
#5	0.097		#17	0.097		#25	0.095
#6	0.096		#18	0.096		#26	0.094
#7	0.098		10-days			#27	0.096
#8	0.094		#19	0.096		30-days	
#9	0.096		#20	0.097		#28	0.098
#10	0.096		#21	0.099		#29	0.099
#11	0.097					#30	0.094
#12	0.096						

Table A1-9: *Long Term Experiment to Determine Stability of Detection solution*

Instrument Used: Ocean Optics
Storage Condition: Stored in a dark colored bottle on a lab bench.

Berylllium concentration ppb	Aged for 12 weeks	new prep
0	0.012	0.012
0.2	0.024	0.023
1	0.065	0.07
2	0.123	0.123
4	0.239	0.239
10	0.558	0.58
30	1.72	1.72

Table A1-10: *Field data from a LANL Machine Shop (sample numbers* 200301922*) or a Firing Point (sample numbers 200301845)-Comparison with ICP*

Side by side samples were collected at a firing point or a machine shop where beryllium contamination was expected by using wipes (Whatman 541) on a surface area of 100 square cm.
Wipe 1 was analyzed by ICP-AES (modified OSHA 125G*).
Wipe 2 was analyzed with the fluorometric method. An aliquot of the same dissolution solution was also analyzed by ICP-AES.
Both type of samples are form using beryllium metal, but an oxide skin is usually formed on the beryllium particles, which is expected to be more for the samples from the firing range.
Although wipe 1 and wipe 2 (taken from adjacent areas) are expected to result in generally similar low levels of beryllium contamination, there is a finite probability that there may be a large particle in one of these adjacent areas resulting in large variation of beryllium concentration between the two.
The samples from the firing range are highly variable in particle sizes and some particles may be as big as small chips. The samples from the machine shop were airborne and then settled on the surfaces.

Sample #		Wipe 1	Wipe 2			RPD	RPD
		ICP Result $\mu g/100\ cm^2$	Fluorometric Result $\mu g/100\ cm^2$	ICP of Fluorometric Dissolution Solution $\mu g/100\ cm^2$		Wipe 1 vs. Wipe 2	Wipe 2 ICP vs. Fluorometric
200301845	A	0.04	0.03	0.03		28.6%	0.0%
200301845	B	< 0.03	0.02	< 0.02			
200301845	C	0.29	0.11	0.13		87.1%	13.1%
200301845	D	27.8	8.86	11.0		103.3%	21.6%
200301845	E	0.06	0.07	0.06		9.5%	9.5%
200301845	D	< 0.03	0.02	< 0.02			
200301845	G	< 0.03	0.01	< 0.02			
200301845	H	< 0.03	0.01	< 0.02			
200301845	I	< 0.03	0.01	< 0.02			
200301845	J	< 0.03	0.01	< 0.02			
200301845	K	< 0.03	0.00	< 0.02			
200301845	L	< 0.03	0.02	< 0.02			
200301845	M	< 0.03	0.02	< 0.02			
200301922	A	0.74	1.02	1.03		31.9%	0.9%
200301922	B	0.38	0.34	0.33		10.4%	5.2%
200301922	C	0.32	0.30	0.27		4.9%	11.0%
200301922	E	6.83	8.50	8.79		21.8%	3.3%
200301922	D	7.94	7.75	7.71		2.4%	0.5%
200301922	G	5.69	7.35	7.86		25.4%	6.7%
200301922	H	12.7	15.1	15.3		17.8%	1.0%
200301922	I	14.4	14.3	14.7		0.6%	2.7%
200301922	J	12.8	13.6	12.7		6.7%	7.2%
200301922	K	10.8	11.0	10.8		1.6%	1.6%
200301922	L	21.5	19.0	17.5		12.6%	8.0%
200301922	N	6.98	9.08	9.00		26.1%	0.9%
200301922	O	18.8	25.3	25.2		29.4%	0.4%
200301922	P	6.79	11.7	10.9		52.7%	7.0%
200301922	Q	15.6	8.82	8.79		55.4%	0.4%
200301922	R	10.3	8.70	10.0		17.1%	13.9%
200301922	S	15.0	13.3	14.0		11.9%	5.1%
200301922	T	14.0	11.0	11.2		24.4%	1.9%
				Average of RPDs =		26.4%	5.5%
Average of Samples =		9.5	8.9	9.0		7.2%	1.0%

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*Both modified protocol and 125G utilize 1:1 sulfuric acid, concentrated nitric acid, concentrated hydrochloric acid and hydrogen peroxide. 125G optionally utilizes perchloric acid.

Modified protocol uses 1mL of sulfuric acid and 2mL of concentrated nitric and heated on a hot plate while adding hydrogen peroxide dropwise until the solution clears. The solution is then cooled, 1mL of hydrochloric acid is added, reheated, cooled again and diluted to 10mL and the resultant solution is taken to be 10% hydrochloric acid (HCl) and 4% sulfuric acid (H2SO4).

125G uses 2-4mL 1:1 sulfuric acid, 2mL concentrated nitric followed by a 1hour wait. Then some hydrogen peroxide (H2O2) is added and the solution is heated with more H2O2 added to clear the solution as it browns. They then cool the solution and add 2 to 4mL of concentrated HCl and reheat to near boiling and finally cool and dilute to 50mL. The resultant acid concentration is taken to be 8% HCl and 4% H2SO4.

Thus the 125G H2SO4/HNO3 ratio is higher than ours, and there is a waiting period built into 125G that is not used in the modified process, but the two methods are otherwise very much the same.

Table A1-11: *Field data from a LANL Firing Range (samples RW) and the machine shop (samples GW)-Comparison with ICP and Dissolution Recovery using Ammonium Bifluoride Solution. This data is same to the one in Table A1-10 where the sampling and analysis are reported without correcting for the area swiped)*

20x dilution (Volumetric ratio of detection to dissolution solution 19:1)
Date: 10/3/2003

Samples using Whatman 541 filters as wipes were analyzed with the fluorometric method using 1%ammonium bifluoride dissolution. An aliquot of the same dissolution solution (filtrate) was also analyzed by ICP-AES. Further, the residue on the dissoluted filter was analyzed by following a standard ICP acid digestion to evaluate how much of beryllium remained after the filters were dissoluted using ammonium bifluoride solution. The results showed that the dissolution procedure using 1% ammonium bifluoride was effective.

SAMPLE	fluorometric test	ICP Filtrate	ICP Dissoluted filter (residue)	SAMPLE	fluorometric test	ICP Filtrate	ICP Dissoluted filter (residue)
	µg Be/filter	µg Be/filter			µg Be/filter	µg Be/filter	
GW 2003-01923				RW 2003-01845			
A	0.347	0.350	bdl	A	0.030	0.030	bdl
B	0.137	0.130	bdl	B	0.017	<0.02	bdl
C	0.134	0.120	bdl	C	0.114	0.130	bdl
D	0.002	0.020	bdl	D	8.858	11.000	
E	5.950	6.150	0.048	E	0.066	0.060	bdl
F	5.425	5.400	0.052	F	0.023	<0.02	bdl
G	5.143	5.500	0.035	G	0.011	<0.02	bdl
H	3.179	3.210	0.047	H	0.009	<0.02	bdl
I	6.423	6.600	0.192	I	0.010	<0.02	bdl
J	2.182	2.030	0.034	J	0.012	<0.02	bdl
K	4.236	4.170	0.099	K	0.002	<0.02	bdl
L	1.137	1.050	bdl	L	0.017	<0.02	bdl
M	0.007	0.020	bdl	M	0.016	<0.02	bdl
N	3.813	3.780	0.128				
O	5.313	5.290	0.116				
P	1.631	1.520	0.026				
Q	6.173	6.150	0.223				
R	10.440	12.000					
S	6.987	7.350	0.289				
T	7.027	7.160	0.142				

bdl: below detection limit
ICP analysis performed by Chris Brinks

Appendix 2

Table A2-1: *Experiments to evaluate effectiveness of Ammonium Bifluoride as recovery solvent*

Using the LANL fluorescence method, good recoveries were obtained from both soluble and insoluble Be compounds; see the table below:

Table: Summary of results from Be extraction in 5 or 10 mL of 1% ammonium bifluoride & analysis by fluorometry; mechanical agitation or sonication for 1 hour. (Masses treated were 10-20 mg for $BeSO_4 4H_2O$ and 5-10 mg for BeO.)

Sample	Agitation method	Recovery(%) ± std. dev. (n = 3)
Be sulfate	mechanical	99.8 ± 4.4
Be sulfate	sonication	106.6 ± 12.0
BeO	sonication	83 ± 3
Be sulfate / MCE filters	mechanical	98.6 ± 1.6
Be sulfate / MCE filters	sonication	110.4 ± 4.7
BeO / MCE filters	mechanical	94 ± 6
BeO / MCE filters	sonication	93 ± 6
Be sulfate / Whatman 541 filters	mechanica	98.1 ± 3.3
Be sulfate / Whatman 541 filters	sonication	101.3 ± 2.5
BeO / Whatman 541 filters	mechanical	86 ± 8
BeO / Whatman 541 filters	sonication	96 ± 5
Be sulfate / Ghost wipes	sonication	79.3 ± 2.4
BeO / Ghost wipes	sonication	40 ± 4
Be sulfate / Palintest wipes	sonication	87.6 ± 2.5
BeO / Palintest wipes	sonication	84 ± 2

The results from the work summarized in the table show promise for the potential use of this extraction method for on-site analysis. These experiments also indicate that Ghost wipes, used widely for surface sampling by industrial hygienists, may not be appropriate for field-based monitoring of beryllium. Recovery results above 75% are acceptable[10].

Beryllium oxide and Beryllium sulfate samples were obtained from Sigma Aldrich. Please see Fig 2 in Appendix 8 for particle morphology and size characteristics.

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10 Kennedy, E.R., Fischbach, T.J., Song, R., Eller, P.M., Shulman, S.A., [1995]: *Guidelines for Air Sampling and Analytical Method Development and Evaluation.* CDC/NIOSH: Cincinnati, OH; DHHS (NIOSH) Publication No. 95-117 [1995].

Appendix 3

It should be noted that the recovery of beryllium from a collected sample into dissolution solution under standard conditions may not be complete for larger particle sizes (in excess of 100 microns aerodynamic diameter). High fired beryllium oxide (UOX 125) has been well characterized [11]. In addition a current initiative within the Beryllium Health and Safety Committee and Department of Energy (DOE), National Institute of Standards and Technology (NIST), and National Institute for Occupational Safety and Health (NIOSH) has been to make UOX125 a standard reference material [12].

Table A3-1: *Analysis of spiked Filters using suspension of Beryllium oxide (UOX125 from Brush Wellman)*

- Comparison of mechanical agitation and heating (without agitation) on dissolution process using 1% ammonium bifluoride Whatman 541 filters spiked from a suspension
- Instrument used: Turner Quantech
- Expected numbers show a range as it was difficult to control the uniformity of the suspension.

Calibration data

ppb pre-dilute	ppb pre-dilute	std. Final ppb	Readback, final, ppb
0	0.36	0	0.02
10	10.16	0.5	0.51
40	39.56	2	1.98
200	196.35	10	9.82
800	791.68	40	39.58

Summary of results in µg Be/filter

BeO, µl	expected	rotated	75 °C	90 °C
3	0.2	0.19	0.23	0.21
15	1 to 1.2	0.65	1.11	1.20
60	4 to 5	2.68	4.54	4.20

Data Details (see summary above)

	Rotated for 30 minutes	
Sample	Expected, µg/filter	µg/filter
Blank	0	0.00
3 A	0.2	0.21
3 B	0.2	0.21
3 C	0.2	0.15
15 A	1 to 1.2	0.85
15 B	1 to 1.2	0.60
15 C	1 to 1.2	0.49
60 B	4 to 5	2.87
60 C	4 to 5	2.48

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11 Stefaniak, A.B., Hoover, M.D., Day, G.A., Ekechukwu, A.A., Whitney, G., Brink, C.A., Scripsick, R.C.: Characteristics of beryllium oxide and beryllium metal powders for use as reference materials. *J. ASTM International.* 2(10); DOI 10.1520/JAI13174 [2005]

12 Watters, R.L., Hoover, M.D., Day, G.A., Stefaniak, A.B.: Opportunities for development of reference materials for beryllium. *J. ASTM International.* 3(1); DOI 10.1520/JAI13171 [2006]

75C for 30 minutes			90C for 30 minutes		
sample	Expected, μg/filter	μg/filter	sample	Expected, μg/filter	μg/filter
Blank	0	0.00	Blank	0	0.00
3 A	0.2	0.28	3 A	0.2	0.18
3 B	0.2	0.19	3 B	0.2	0.23
3 C	0.2	0.22	3 C	0.2	0.22
			15 A	1 to 1.2	1.42
15 B	1 to 1.2	1.16	15 B	1 to 1.2	1.15
15 C	1 to 1.2	1.06	15 C	1 to 1.2	1.02
60 A	4 to 5	4.39	60 A	4 to 5	4.42
60 B	4 to 5	4.99	60 B	4 to 5	3.79
60 C	4 to 5	4.25	60 C	4 to 5	4.38

Table A3-2: *Analysis of Spiked Filters using Freshly Prepared Suspension of Beryllium Oxide (UOX125 from Brush Wellman)*

Instrument used: Turner Quantech,
Dissolution: Sample heated to 80°C (using a heating block) without stirring in 1% ammonium bifluoride
After dissolution, the solution was analyzed using fluorescence and ICP. (NA=Not analyzed). Some of the samples were analyzed using accepted acid digestion and ICP protocol to ensure that the filters had the anticipated amount of beryllium oxide.

Spike Level		μg/filter (Whatman 541)		
Be μg	sample	fluor	ICP of filtrate	ICP digestion
0	A	0.00	0.00	
0	B	0.00	NA	
0.1	A	0.08	0.08	
0.1	B	0.06	0.06	
0.1	C	0.08	0.08	
0.1	D	0.11	NA	
0.1	E	0.09	NA	
0.1	F			0.13
	Average	0.08	0.07	
	Std Dev	0.018	0.012	
0.2	A	0.18	0.18	
0.2	B	0.19	0.19	
0.2	C	0.20	0.20	
0.2	D	0.15	NA	
0.2	E			0.20
0.2	F	NA	NA	0.26
	Average	0.18	0.19	
	Std Dev	0.022	0.010	
0.3	A	0.28	0.28	
0.3	B	0.26	0.26	
0.3	C	0.29	0.28	
0.3	D	0.27	NA	
0.3	E	0.23	NA	
0.3	F			0.29
	Average	0.27	0.27	
	Std Dev	0.023	0.012	

Spike Level		μg/filter (Whatman 541)		
Be μg	sample	fluor	ICP of filtrate	ICP digestion
0.0	C	0.00	0.00	
0.0	D	0.00	NA	
0.0	E	0.00	NA	
0.0	F	0.00	NA	
0.0	G	0.00	NA	
0.02	A	0.01	0.01	
0.02	B	0.02	0.02	
0.02	C	0.02	0.02	
0.02	D	0.01	NA	
0.02	E	0.01	NA	
	Average	0.014	0.02	
	Std Dev	0.0055	0.006	
1.5	A	1.17	1.20	
1.5	B	1.11	1.10	
1.5	C	1.35	1.33	
1.5	D	1.78	NA	
1.5	E	1.52	NA	
1.5	F			1.47
	Average	1.39	1.21	
	Std Dev	0.2726	0.115	
3.0	A	3.40	3.42	
3.0	B	3.82	4.06	
3.0	C	3.06	3.02	
3.0	D	3.09	NA	
3.0	E	3.13	NA	
3.0	F			2.70
	Average	3.30	3.50	
	Std Dev	0.3205	0.525	

Appendix 4

Development of a New Standard Method for the Detection of Beryllium on Surfaces

Edel M. Minogue, Deborah S. Ehler, Anthony K. Burrell**, T. Mark McCleskey**, Tammy P. Taylor

Chemistry Division (C-SIC), Los Alamos National Laboratory, MS J514, Los Alamos, New Mexico 87545, USA.

ABSTRACT

A rapid, quantitative, sensitive test for the detection of beryllium on surfaces has been developed. The method is based on the fluorescence of beryllium bound to sulfonated hydroxybenzoquinoline at pH 12.8, which emits at 475 nm when excited at 380 nm, and includes a novel dissolution technique. The intensity of fluorescence is linear with respect to beryllium concentration. A detection limit of 0.02 μg Be/ 100 cm^2 has been achieved, which is ten times lower than the DOE recommended working limit for non-beryllium work areas[13]. Interference studies have been carried out with a variety of metals including Al, Fe, Pb, U, Cd, Cr, Hg, Ca, W, Ni, Co and Cu with minimal or no interferences found for detection of Be at 100 nM in the presence 0.4 mM of the other metal. The method has been proven successful under various operating conditions including the detection of beryllium on a variety of surfaces both in laboratory settings and in field trials. It fulfills the requirements for a fast, inexpensive, field deployable method of detection of beryllium on surfaces. KEYWORDS: Beryllium, fluorimetric detection, HBQS, environmental monitoring

INTRODUCTION

The unique properties of beryllium (Be) have lead to many applications ranging from the aerospace and nuclear industry to manufacturing and electronics. Unfortunately, beryllium is a Class A EPA carcinogen and when inhaled into the lungs can cause the incurable and potentially fatal lung disease, chronic beryllium disease (CBD). Therefore, the monitoring of beryllium in occupational environments is of vital importance. Congress has recently passed limits of Be exposure of 2 μg / m^3 [14], and DOE facilities have adopted even more stringent levels that include 0.2 μg / m^3 for

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**Correspondence may be addressed to either author. E-mail (A.K.B.): burrell@lanl.gov, (T.M.M.) tmark@lanl.gov

13 CFR (Code of Federal Regulations), Title 10, Energy, Part 850, Department of Energy, **2001** parts 500 to end.

14 A Comparison and Critique of Historical and Current Exposure Assessment Methods for Beryllium: Implications for Evaluating Risk of Chronic Beryllium Disease; Kolanz, M.E. *Appl. Occupational and Environ. Hygiene* **2001a**, 16 5 559-567.

airborne levels and $0.2 \, \mu g / 100 \, cm^2$ as a surface level for the release of items from beryllium areas[13]. For release to another DOE facility working with beryllium, contamination levels are not to exceed $3.0 \, \mu g / 100 \, cm^{2}$ [13].

To date, the standard method for the detection of beryllium on surfaces is a surface swipe technique described by OSHA (ID-125G) [15]. The method involves swiping a 10 cm x 10 cm area with a cellulose ester membrane and subsequently digesting the membrane with hydrogen peroxide and sulfuric acid. Inductively coupled plasma atomic emission spectroscopy (ICP-AES) is used to quantify beryllium in the samples. Although straightforward, the procedure can be costly, turnaround time is slow, and it is unsuitable for field use. In addition, the current OSHA method requires consumption of the entire sample in order to meet detection levels. Consequently, verification of results can be difficult if concerns arise post-analysis. There have been attempts to develop a swipe analysis technique based on absorbance changes [16,17] but they have been unable to obtain the necessary quantitative detection limits of $0.02 \, \mu g / 100 \, cm^2$ for NIOSH approval.

Fluorescence is an ideal method of detection because it is extremely sensitive, non-destructive and can be performed quickly. Fluorescent detection of Be has been reported since the 1950s with literature reports on a variety of fluorescent indicators including morin [18,19,20], chromotropic acid [21], and Schiff bases [22]. Despite the many reports of fluorescent indicators for Be, a complete system for the fluorescence detection of Be has yet to be approved by NIOSH, and there is no commercial fluorescent Be detector kit. A complete, robust fluorescent detection method requires three key features: a dissolution method that is able to dissolve Be and BeO and remains compatible with the fluorescence indicator; tolerance to a wide variety of interferences; a minimal number of simple steps from dissolution to detection. Typical dissolution methods for the dissolution of BeO from a swipe involve concentrated inorganic acid and heating; in addition some methods use hydrogen peroxide. Such conditions are not compatible with any known fluorescent indicator, so the solution must be evaporated to dryness and further treated before it can be added to the fluorescent indicator. The work presented herein is a description of the development of a rapid fluorescence

15 Metal and Metalloid Particulates in Workplace Atmospheres (ICP Analysis), Method No. ID-125G, Control No. T-ID-125G-FV-03-0209-M,
http://www.osha.gov/dts/sltc/methods/inorganic/id125g/id125g.html#table1

16 Beryllium Colorimetric Detection for High Speed Monitoring of Laboratory Environments; Taylor, T.P.; Sauer, N.N. *J. Hazard. Mater.* B93 **2002** 271-283.

17 Beryllium in the Environment: A Review; Taylor, T.P.; Ding, M.; Ehler, D.S.; Foreman, T.M.; Kaszuba, J.P.; Sauer, N.N. *J. Environ. Sci. Health* A38 **2003** *2* 439-469.

18 Fluorometric Determination of Traces of Beryllium; Laitinen, H.A.; Kivalo P.; *Anal. Chem.* **1952** 24 9 *1* 467-1471.

19 Determination of Beryllium in Water by Ion Exchange Spectrofluorimetry; Capitan, F.; Manzano, E.; Navalon, A.; Vilchez, J.L.; Capitan-Vallvey, L.F. *Analyst* **1989** 114 *8* 969-973.

20 Optical Sensor for Beryllium Based on Immobilized Morin Fluorescence; Saari, L.A.; Seitz, W.R. *Analyst* **1984** 109 *5* 655-657.

21 Chromotropic Acid as a Fluorogenic Reagent. 1. Fluorometric-Determination of Beryllium; Pal, B.K.; Baksi, K. *Microchim Acta* **1992** *108* 275-283.

22 Metal-Complexes of Aromatic Schiff-Base Compound. 2. Fluorescence of Beryllium and Scandium Complexes and Their Use in Fluorimetry; Morisige, K. *Anal. Chim. Acta* **1974** 73 *2* 245-254.

method for the quantitative detection of beryllium on surfaces using the indicator 10-hydroxybenzo[h]quinoline-7-sulfonate (10-HBQS). The method is beryllium specific, inexpensive, applicable to different swipe materials, and field deployable. Detection limits of 0.02 µg beryllium / 100 cm^2 swiped surface (one tenth of the DOE required action level of 0.2 µg / 100 cm^2) have been achieved. We are currently working with the National Institute for Occupational Safety and Health (NIOSH) for approval of this method for beryllium detection.

In order to eliminate the time-consuming and non-fieldable digestion steps of current standard methods, the use of a fluoride-based medium to dissolve Be was investigated. It was found that Be metal was dissolved within seconds in 1% ammonium bifluoride (NH$_4$)HF$_2$.. However, high-fired BeO is the most difficult form of Be to dissolve. We tested the dissolution of 10 mg quantities of BeO with 50 mL of 1% (NH$_4$)HF$_2$ to demonstrate that 80% of the oxide form could be dissolved in just 15 minutes with minimal agitation. Fluoride, usually in the form of HF, is well noted for its ability to penetrate and dissolve metal oxides [23]. Most fluorescent indicators reported do not tolerate the presence of fluoride. The few reports of indicators that tolerate fluoride have complicated procedures involving heating with acid for dissolution and a titration process to obtain the final pH. The duration and complexity of those procedures do not lend themselves easily to field analysis.

Having screened several potential ligands, 10-HBQS, a water-soluble fluorescent dye, was selected for the development of the fluorescence method. In an previous study, The selection of 10-HBQS stemmed from work done in a previous study by Matsumiya et al [24] where they studied beryllium in urban air and showed that 10-HBQS, hydroxybenzoquinoline (HBQ) chelated the Be(II) ion. In another work, they used the precursor HBQ as a pre-column chelating reagent for the determination of beryllium in water by reversed-phased high-performance liquid chromatography [25]. HBQ fluorescent detection involves the formation of a six-membered chelate ring with Be. A tightly bound hydrogen bonded proton leads to weak triplet emission at 580 nm. When the proton is displaced by a metal such as beryllium, fluorescence emission is observed at 475 nm. However, because HBQ is sparingly soluble in water, we selected the sulfonated derivative 10-HBQS for our studies. Although HBQ was previously commercially available, neither HBQ nor 10-HBQS are currently commercially available nor are there useful synthetic procedures published. Therefore, we developed synthetic pathways for both of these compounds [26].

EXPERIMENTAL

23 Cotton, F.A.; Wilkinson, G.; Advanced Inorganic Chemistry 5thed. Wiley-Intersciemce: New York, 1988.

24 A Novel Fluorescence Reagent 10-hydroxybenzo[h]quinoine-7-sulfonate, for Selective Determination of Beryllium(II) Ion at pg cm^{-3} Levels; Matsumiya, H.: Hoshino, H.; Yotsuyanagi, T. *Analyst* **2001**, 126, 2082-2086.

25 Selective Determination of Beryllium (II) Ion at Picomole per Decimeter Cubed Levels by Kinetic Differentiation Mode Reversed-Phase High-Performance Liquid Chromatography with Fluorometric Detection Using 2-(2'-Hydroxyphenyl)-10-hydroxybenxo[h]quinoline as Precolumn Chelating Reagent; Matsumiya, H.; Hoshino, H.; *Anal. Chem.* **2003** 75 413-419.

26 The Generation and Trapping of 7,8-quinolyne: A New Synthesis of 10-hydroxybenzo[*h*]quinoline and Access to 7-substituted Derivatives; Collis, G.E., Burrell, A.K.; *Tetrahedron Lett*; submitted.

Apparatus: A miniature fluorescence spectrometer from Ocean Optics (S2000-FL) was customized to incorporate a UV LED with an excitation wavelength of 380 nm (continuous mode). Instrument calibration was carried out using a LS-1-CAL white light source. Detection was carried out using the USB2000 Miniature Fiber Optic Spectrometer connected to the serial port of a laptop computer. Spectra were obtained in the relative irradiance mode using Ocean Optics OOIBase32 Software. The wavelength of emission is 475 nm. The detection limit of the set-up was 0.06 ppb Be. Results were verified by ICP-AES, Jobin Yvon Inc., Edison, New Jersey. This particular instrument has a detection limit of approximately 100 ppt Be, allowing good comparison with the low levels of detection obtainable with our fluorimetric method. Verification of side-by-side swipes were carried out by the standard method for detection of beryllium on surfaces (i.e. digestion of swipe and then ICP-AES). The pH was measured using an Orion pH / ISE Model 710 meter which was calibrated using pH 4, 7 and 10 buffer solutions (Fisher Scientific Inc.).

Reagents and Solutions: Solid forms of beryllium used included beryllium oxide (BeO 99%, Acros) and beryllium sulfate (BeSO$_4$, Acros). All solid forms of beryllium were handled in a HEPA-filtered glove box by a beryllium-trained worker. The following stock solutions were prepared: (NH$_4$)HF$_2$ (Aldrich), 1 % wt / vol in water, 1.1 mM HBQS pH adjusted to pH 12 with 10 M NaOH (Fisher), 100 mM L-Lysine monohydrochloride (Aldrich) at pH 11-12, and 1 mM EDTA disodium dihydrate (J.T. Baker, Inc.). ICP standard solutions (1000 µg / mL metal; SPEX Centriprep) of the following metals were used in interference studies: Al, U, Ca, Li, Pb, Zn, Fe, V, Sn, W, Cu, Ni, Co, Cd, Cr and Hg. Deionized water (MilliQ®) was used throughout.

Whatman® 541 filters (47 mm diameter) are used as the standard swipe in our experiment. These cellulosic filters are currently utilized by Los Alamos National Laboratory industrial hygienists for the NIOSH approved method of Be testing and from this point will be referred to as *swipes*. The term *filter* will be used when a surface has not been swiped (e.g. for experiments where filters are spiked with known concentrations of Be).

METHOD

General Procedure: The detection reagent was prepared by the addition of 12.5 mL of 10.7 mM EDTA and 25 mL of 107 mM L-lysine monohydrochloride to 3 mL of 1.1 mM 10-HBQS. The pH was adjusted to 12.85 with the careful addition of 10 M NaOH and water added to a total of 50 mL. Beryllium standards were generated using Be spectrometric standard solutions diluted into 1% (NH$_4$)HF$_2$ for the desired concentrations. For calibration curves a 0.1-mL aliquot of each standard solution was added to 1.9 mL of the detection reagent, and spectra were taken at a set integration time. A linear increase in intensity at 475 nm with respect to increasing beryllium concentration was observed (Fig. 1). This enabled the conversion of intensities to concentrations. The amount of Be (µg / 100 cm^2) in the area swiped (A) was then obtained by equation 1, whereby C_s (µg / L) is the concentration for a given sample with a volume of V_s (L), and C_b (µg / L) is the concentration of the blank with a volume of V_b (L). F_d is the dilution factor in this method:

$$[Be](\mu g/100cm^2) = \frac{F_d \times [C_s V_s - C_b V_b]}{A} \qquad (1)$$

A result of 2 ppb in our method corresponds to 0.2 μg Be on the swipe. Results must be normalized if an area greater than 100 cm^2 is swiped. If the concentration of beryllium is out of range (too high), then the instrument is recalibrated using higher standards and a shorter integration time. In this way, the range of analysis can be extended. For quality control purposes, a calibration standard and a reagent blank are analyzed at least once every 20 samples.

Dissolution study: The dissolution study was comprised of two areas of interest: the suitability of the Be-dissolving agent and the time-minimization of this step. Preliminary studies of dissolution show 1% $(NH_4)HF_2$ dissolves Be and BeO at levels within the required detection range (i.e. 0.02 μg – 3.0 μg Be / swipe). Moreover, $(NH_4)HF_2$ does not interfere with 10-HBQS, the ligand of choice. Time analyses were carried out in order to minimize the dissolution time while ensuring that beryllium was dissolved. A 0.15 μg BeO / mL suspension was made by adding 7.5 μg of BeO to 50 mL H_2O. A filter was spiked with a 5-μL aliquot of the suspension. The spiked filter was placed in a tube, and 5 mL of 1% $(NH_4)HF_2$ was added, the tube capped and then rotated. A 0.5-mL aliquot was taken at set intervals and added to 1.5 mL of the dye reagent mix in a cuvette. Spectra were taken for each interval, and the intensity at 475 nm observed.

Interference Study: The following metal solutions were made by dissolving the standard ICP metal solution with 1% $(NH_4)HF_2$ such that the end concentration of the 0.1 mL aliquot in the 1.9 mL dye mix was between 0.04 mM and 2.0 mM: 0.4 mM Al, 0.4 mM U, 2.0 mM Ca, 0.4 mM Li, 0.4 mM Pb, 0.4 mM Zn, 0.4 mM Fe, 0.4 mM V, 0.4 mM Sn, 0.4 mM W, 0.4 mM Cu, 0.4 mM Ni, 0.4 mM Co, 0.04 mM Cd, 0.04 mM Cr, 0.04 mM Hg. Each sample was prepared in triplicate with (100 nM and 1 μM Be) and without Be. The interference metals were in $\geq$50,000 fold molar excess to the Be present. Spectra were taken for each sample, and the intensity at 475 nm observed.

Stability Study: Both the stability of the detection reagent solution and the Be-$(NH_4)HF_2$ detection reagent solution were studied over time. A 100-mL solution of the detection reagent containing 10-HBQS, EDTA and buffer was made as previously described. 1.9-ml aliquots? were removed at set time intervals, 0.1 ml of Be standards in $(NH_4)HF_2$ were added and analyzed fluorimetrically. The stability of the final samples was tested by keeping the first set of standards, sealed in cuvettes, which were subsequently fluorimetrically analyzed on a weekly basis.

Detection Limit: The current required NIOSH detection limit is 0.2 μg Be/ 100cm^2. In order to quantify the method detection limit, the following standards were prepared: five low-level standards (0.02 μg - ten times lower than the required detection limit), five standards at the detection limit of 0.2 μg , one standard of 0.1 μg and a reagent blank. Filters were spiked with the standards and dried for 20 minutes after which time 5 mL of $(NH_4)HF_2$ was added, followed by fluorimetric analysis.

Procedure for the Swipe Test: A 100-cm^2 surface was swiped with a Whatman® 541 filter moistened with deionized water, in accordance with the procedure described in OSHA ID-125G[15]

and in ASTM D6966 (27). The swipe was then placed into a 15-mL polypropylene tube, and 5 mL of the 1% -(NH$_4$)HF$_2$ solution was added. The tube was capped and then rotated (Barnstead / Labquake tube rotator) for 30 min during which time the Be was dissolved. The solution was filtered through a luer-locked PTFE (Millipore) or nylon 0.45 μm syringe filter. In a disposable, clear-sided cuvette, 0.1 mL of the filtrate was added to 1.9 mL of the dye solution mix (20x dilution). The cuvette was capped and briefly shaken, and a fluorescence spectrum taken ($\lambda_{excitation}$ = 380 nm; $\lambda_{emission}$ = 475 nm). A set of Be standards using the same dye mix was also prepared and the fluorescence spectra taken for each set of samples. A calibration curve of the intensities of Be at 475 nm versus beryllium concentration was plotted. From this the beryllium concentration in the sample was obtained. The remaining Be filtrate was analyzed using ICP-AES, providing corroborative results.

Field Trials: The implementation of our fluorimetric method on swipes from different environments was investigated. Potentially Be-contaminated surfaces were swiped according to OSHA and NIOSH procedures[15] by an industrial hygienist at Los Alamos National Laboratory in the laboratory, in the beryllium workshop areas, and also in the field. A 100-cm^2 area was swiped and the swipe placed in a tube. A 5-mL aliquot of (NH$_4$)HF$_2$ was added to the tube, which was subsequently rotated for 30 min. The Be-(NH$_4$)HF$_2$ solution was decanted into a luer-locked syringe filter and filtered. A 0.1-mL aliquot of the filtrate was added to 1.9 mL of the detection reagent and the sample was fluorimetrically tested for Be. The remaining filtrate was sent for ICP-AES for confirmational results.

In addition to this, 100 μL of potential interferents such as ethylene glycol, oil, and cleaning agents were added to Be-spiked filters. The filters were then subject to fluorimetric analysis. This was carried out in duplicate.

Side-by-side swipes from both a Be contaminated shop and firing points including surfaces such as steel, aluminum and paint were also collected, with one swipe analyzed by the fluorimetric method and the other by the digestion / ICP-AES method. The remainder of the Be-(NH$_4$)HF$_2$ filtrate was also analyzed by ICP-AES.

RESULTS AND DISCUSSION

Fluoride interference with indicator: Based on preliminary experiments involving the dissolution of BeO with (NH$_4$)HF$_2$, we needed a fluorescent indicator that could tolerate large concentrations of fluoride. HBQS had previously been reported to tolerate up to 20,000,000 equivalents of fluoride[24]. Most other Be fluorescent indicators are readily susceptible to fluoride interference at only 300 equivalents. We tested the response of HBQS in the presence of 0.25% fluoride and found that it responded well. The increase of intensity at 475 nm with respect to beryllium concentration as exhibited in Fig. 1 is not only a indication of the effectiveness of the ligand 10-HBQS but also a proof of the effectiveness of the ligand in the matrix containing (NH$_4$)HF$_2$.

Dissolution study: The dissolution of Be from the swipe into the (NH$_4$)HF$_2$ solution is the time-limiting step for this otherwise instantaneous method. We minimized this by investigating the time

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27 ASTM D6966 (2003) Standard Practice for Collection of Surface Wipe Samples for Subsequent Determination of Metals. American Society for Testing and Materials (ASTM); West Conshohocken, PA.

dependence for the dissolution of high fired BeO, one of the most inert forms of Be, spiked onto a Whatman® 541 filter. The intensity of the sample at 475 nm increased with increasing dissolution time up until 25 min. A direct overlap of the intensities at 25 min and 30 min was observed. No further increase of the fluorescence was observed. Therefore 30 min was chosen as the dissolution time for our experiments, providing a quick response time and near-complete dissolution.

Interference Study: Interference studies with a range of other metals have shown that even in 50,000-fold molar excess over Be, metals such as Pb, U, Hg or Cr show little (<1%) or no interference (Table 1). The exception was that high concentrations of Fe (i.e. > 20 µM Fe) have a negative effect on Be intensity of approximately 10% because suspended Fe precipitate absorbs light at 380 nm. If, however, the Fe precipitate is allowed to settle for 4 hours or is filtered using a PTFE or nylon filter, and is then reanalyzed, there is no interference. Having the Fe precipitate is an advantage of working at a high pH. Therefore, it is recommended that, with fluorimetric analysis of beryllium, if high iron content is suspected (e.g. due to swiping a rusty surface) or is evident from the gold-orange color that appears when the HBQS mix is added, filter the solution or allow the solution to settle until clear and colorless, and then carry out the fluorimetric analysis.

Stability Study: For the development of a field deployable method, it is essential that the reagents are stable over a given period of time. Therefore, the stability of the dye mix solution (stored in brown Nalgene HDPE bottles) was studied over time by running Be calibration curves made with the aging dye. After 120 days, no decrease in response was observed. Beryllium standard solutions, which contained the dye mix solution, were also studied over time. They remained stable over 28 days, thus enabling rapid on-site detection of beryllium with pre-prepared reagents and standards. It should be noted that if the beryllium standards including the dye mix are to be stored for longer than a week, the solutions should be stored in a screw-topped, sealable container.

Detection Limit: The method limit of detection (LOD) and the instrument detection limit were determined according to NIOSH procedures [28]. The low-level calibration standards were analyzed and the average result obtained for replicate aliquots. The results obtained were graphed against the mass of Be, and the linear regression equation $Y = mX + c$ enabled the evaluation of responses, Y^*_i, for Be mass. The standard error of regression was calculated using equation 2 where N is the number of data points:

$$S_y = \left[\frac{\Sigma \left(Y^*_i - Y_i \right)^2}{(N-2)} \right]^{\frac{1}{2}} \qquad (2)$$

A limit of detection of 13.6 ng / swipe (0.136 ppb) was achieved from equation 3 below:

28 Guidelines for Air Sampling and Analytical Method Development and Evaluation; Kennedy, E.R., Fischbach, T.J., Song, R., Eller, P. M., Shulman, S. A.: **1995** DHHS (NIOSH) Publication No. 95-117.

$$LOD = \left(\frac{3s_y}{m} \right) \qquad (3)$$

Field trial of swipe test: The Be-(NH$_4$)HF$_2$ solutions from field swipes were analyzed by both the fluorimetric method and ICP-AES. The recovery rate was 99.5 %, reinforcing the suitability of the method to realistic environments (Table 3). Beryllium levels ranged from below the fluorimetric detection limit <0.02 µg to 10 µg per area swiped which far exceeds the threshold limit of 0.2 µg Be / 100 cm^2. All were detectable using the method developed and were in concurrence with the results obtained from ICP-AES. No interference was detected when possible contaminants were added to Be-spiked filters. In fact, a 100% Be recovery rate was observed from filters contaminated with lubricating oil, cutting fluid and certain cleaners, the exception being Fantastic® spray cleaner for which a 96% Be recovery rate was observed. A comparison of results from side-by-side swipe analysis highlights the accuracy of this method when compared with the ICP-AES method (Fig. 2). It is difficult to compare side-by-side swipes, as they are not actually swiping the exact same area but these results indicate that the fluorimetric method can stand up to even the toughest test. Neither method showed consistently higher or lower biased values.

CONCLUSIONS

The method developed has been submitted to NIOSH for inclusion into the NIOSH Manual of Analytical Methods as the standard method for the detection of beryllium on surfaces. It is a rapid technique in which beryllium can be extracted from a swipe, bound to a fluorescent dye and analyzed by fluorescence. We have developed the first complete system for Be detection that dissolves both Be and BeO, detects Be down to 0.02 µg / swipe, tolerates a wide range of interferences, and is simple to use. Our method involves placing a swipe in a dissolution solution, mixing for 30 minutes, transferring a small aliquot to a detection solution and measuring the fluorescence at 475 nm. The potential portability of the fluorimetric device coupled with simplicity and specificity of the chemistry lends itself well to field analysis. Work is currently being carried out on the integration of this method into a portable sensor platform.

ACKNOWLEDGMENTS

Figure 1. Characteristic spectra for HBQS bound (475 nm) and unbound (580 nm) to Be.

Table 1. Interference Study.

Added Interferent	Relative Intensity at 475 nm		
	0 Be	100 nM Be	1 μM Be
No Interferent	0.005	0.112	1.078
0.4 mM Al	0.004	0.112	1.054
0.4 mM U	0.004	0.110	1.060
2.0 mM Ca	0.004	0.112	1.057
0.04 mM Li	0.004	0.112	1.060
0.4 mM Pb	0.004	0.111	1.105
0.4 mM Zn	0.003	0.112	1.103
0.4 mM Fe	0.003	0.101	0.925
0.4 mM V	0.003	0.114	1.083
0.4 mM Sn	0.003	0.113	1.105
0.4 mM W	0.003	0.116	1.103
0.4 mM Cu	0.003	0.114	1.062
0.4 mM Ni	0.004	0.114	1.074
0.4 mM Co	0.005	0.111	1.030

Table 2. Beryllium recovery analysis from samples taken in field trials.

Sample No.	Be (μg /100 cm^2)			
	Filtrate*	Filtrate#	Residual on Swipe#	% Recovery
2003-01923				
A	0.347	0.350	ND	100
B	0.137	0.130	ND	100
C	0.134	0.120	ND	100
D	0.002	0.020	ND	100
E	5.950	6.150	0.048	99.20
F	5.425	5.400	0.052	99.05
G	5.143	5.500	0.035	99.32
H	3.179	3.210	0.047	98.54
I	6.423	6.600	0.192	97.10
J	2.182	2.030	0.034	98.46
K	4.236	4.170	0.099	97.72
L	1.137	1.050	ND	ND
M	0.007	0.020	ND	ND

* Measured by fluorimetric method

Measured using ICP-AES, ND: Not detected

Fig. 2. Comparison of results obtained from side-by-side swipes including the comparison of the results obtained by the fluorimetric method and the ICP analysis of the fluorimetric solution.

APPENDIX 5

Kevin Ashley,[1] T. Mark McCleskey,[2] Michael J. Brisson,[3] Gordon Goodyear,[4] John Cronin,[4] and Anoop Agrawal[4]

Interlaboratory Evaluation of a Portable Fluorescence Method for the Measurement of Trace Beryllium in the Workplace*

ABSTRACT: Researchers at Los Alamos National Laboratory (LANL) developed a field-portable fluorescence method for the measurement of trace beryllium in workplace samples such as surface dust and air filters. The technology has been privately licensed and is commercially available. In cooperation with the Analytical Subcommittee of the Beryllium Health and Safety Committee, we have carried out a collaborative interlaboratory evaluation of the LANL field-portable fluorescence method. The interlaboratory study was conducted for the purpose of providing performance data that can be used to support standard methods. Mixed cellulose ester (MCE) membrane filters and Whatman 541 filters were spiked with beryllium standard solutions so that the filters spanned the range $\approx 0.05 - \approx 0.5$ µg Be per sample. Sets of these filters were then coded (to ensure blind analysis) and sent to participating laboratories, where they were analyzed. Analysis consisted of the following steps: 1. Removal of the filters from transport cassettes and placement of them into 15-mL centrifuge tubes; 2. mechanically-assisted extraction of the filters in 5 mL of 1 % ammonium bifluoride solution (aqueous) for 30 min; 3.-4. filtration and transfer of sample extract aliquots (100 µL) into fluorescence cuvettes; 5. introduction of 1.9 mL of detection solution (to effect reaction of the fluorescence reagent with beryllium in the extracted sample); and 6. measurement of fluorescence at ≈ 475 nm using a portable fluorometer. This work presents performance data in support of a procedure that is targeted for publication as a National Institute for Occupational Safety and Health (NIOSH) method and as an ASTM International standard.

KEYWORDS: beryllium, field-portable, fluorescence, interlaboratory evaluation, on-site monitoring, trace analysis, workplace

Introduction

Occupational exposure to beryllium can cause insidious and sometimes fatal disease, and new exposure limits for beryllium in air and on surfaces have been established in efforts to reduce exposure risks to potentially affected workers [1]. Advances in sampling and analytical methods for beryllium are needed in order to meet the challenges relating to exposure assessment and risk reduction. Accurate knowledge of the level of beryllium metal present in the workplace environment is crucial for the determination of the health risks posed to workers.

Field-portable techniques for the accurate, expeditious, and cost-effective monitoring of beryllium are desired to enable rapid assessment of potential worker exposures to this toxic metal

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[1] U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, 4676 Columbia Parkway, Mail Stop R-7, Cincinnati, OH 45226-1998 (USA); tel. +1(513)841-4402; fax +1(513)841-4500; e-mail: KAshley@cdc.gov.

[2] Los Alamos National Laboratory, P.O. Box 1663, MS J-582, Los Alamos, NM 87545, USA.

[3] Westinghouse Savannah River Company, Savannah River Site 707-F, Aiken, SC 29808, USA.

[4] Berylliant, Inc., 4541 E. Fort Lowell Road, Tucson, AZ 85712, USA.

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in the occupational environment. These considerations have resulted in efforts to develop field-portable analytical methods for measuring trace concentrations of beryllium on-site in the workplace. Candidate techniques for beryllium field monitoring have included fluorescence [2] and electroanalysis [3].

In the last few years, a field-portable fluorometric method was developed by researchers at Los Alamos National Laboratory (LANL) [4]; this method has recently been licensed and marketed commercially [5]. Owing primarily to the use of a novel fluorophore for Be^{2+} ion [6], hydroxybenzoquinoline sulfonate, the LANL field method offers significantly better limits of detection (LODs) for beryllium than were attainable by using fluorometric reagents investigated earlier. The previous methods [7,8] relied on fluorescence reagents that demonstrate insufficient sensitivity for trace measurements of beryllium, which are now required in workplace settings. More recent investigations have proposed new fluorometric techniques using reagents that enable ultratrace beryllium measurement in the laboratory [4,9].

The objective of the present study was to carry out an interlaboratory evaluation of the on-site fluorometric method for beryllium as it is currently marketed. An aim of this work was to establish estimates of method performance based on a collaborative interlaboratory analysis. These method performance parameters can then be used to support governmental methods such as those published by the National Institute for Occupational Safety and Health (NIOSH) [10]. Also, it is intended that method performance data obtained through this interlaboratory trial will be used as a basis for voluntary consensus standards, such as those published by ASTM International [11].

Performance Evaluation Samples

Performance evaluation material samples (PEMs) consisted of beryllium (in solution and diluted from standard beryllium nitrate solutions using deionized water) pipetted onto mixed-cellulose ester (MCE) membrane filters (Millipore, Billerica, MA) and Whatman® 541 cellulose fiber filters (SKC, Inc., Eighty-Four, PA). The filters were fortified at known levels between ≈0.05 and ≈0.5 μg Be per sample; the volume of the spiking aliquot was 0.1 mL. Also included were blanks of each sample medium ("spiked" with pure deionized water). After spiking by using micropipettes, the spiked filters were then allowed to dry in air at ambient temperature.

To ensure consistency with an ASTM International standard practice pertaining to interlaboratory testing [12], PEMs consisting of blanks plus sampling media spiked at four loading levels (0.050, 0.10, 0.20, and 0.40 μg Be per sample) were prepared. These PEMs were prepared with beryllium loadings targeted to bracket new action levels of 0.2 μg per 100-cm² sampling area for surface wipe samples [1] and 0.2 μg m⁻³ for 8-h time-weighted average (TWA) air filter samples [13]. The PEMs were prepared at a contract laboratory (Environmental Resource Associates, Arvada, CO; Lot no. 0809-04-04) under the oversight of LANL. PEMs were subsequently repackaged by the CDC/NIOSH Quality Assurance Coordinator to ensure blind analyses by the participating laboratories.

Interlaboratory Evaluation

Participating laboratories consisted of a subset of prospective participants that were identified by members of the Analytical Subcommittee of the Beryllium Health and Safety Committee [14]. PEMs were mailed to each volunteering laboratory by the coordinating laboratory

(CDC/NIOSH, Cincinnati, OH).   Each participating laboratory, along with associated PEM samples, was assigned a numerical code in order to ensure anonymity.

It was requested that the participating laboratories prepare and analyze the PEMs in accordance with the marketed procedure and kit [5]. Briefly, the analysis procedure consisted of the following steps (schematized in Fig. 1):

1. Removal of the filter samples from transport cassettes and placement of them into 15-mL plastic centrifuge tubes
2. Mechanically-assisted extraction of the filters in 5 mL of 1 % ammonium bifluoride solution (aqueous) for 30 min (in 15-mL centrifuge tubes mounted in a mechanical shaker)
3. Filtration of the extracted solutions through plastic syringe microfilters
4. Transfer of sample extract aliquots (100 μL) into fluorescence cuvettes using mechanical pipettes
5. Introduction of 1.9 mL of fluorescent dye detection solution to effect reaction of the fluorescence reagent with beryllium in the extracted sample
6. Measurement of fluorescence at ~475 nm using a portable fluorometer

The participating laboratories were asked to report analysis results in units of mass of beryllium (in μg) per PEM sample. (This required comparison of results for unknowns with calibration standards, along with consideration of appropriate dilution and correction factors, to convert fluorescence intensity to mass [5].)

FIG. 1—*Scheme for field-based preparation and fluorescence analysis of workplace samples for determination of beryllium content.*

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Precision, Bias, and Statistical Analysis

ASTM International voluntary consensus standard test methods require estimates of measurement uncertainty, and this can be in the form of precision and bias data [15]. Precision estimates are preferably obtained through data from interlaboratory evaluations. Bias of a test procedure must be estimated by evaluating the performance of the test method in question against a reference method, or from determinations of recoveries from the analysis of reference material samples, or both.

In this investigation, interlaboratory precision of analytical results from the volunteer laboratories was examined using statistics from overall interlaboratory analysis results. For purposes of satisfying ASTM International standard criteria, the analysis was done using statistics described in ASTM Standard E 691 [12], which requires a minimum of six participating laboratories. This standard practice also recommends a minimum of four samples for each type of matrix, with duplicate analyses of each of the four samples. Thus, each laboratory received a total of ten PEM samples (five for each filter matrix) for analysis by the field-portable fluorescence method for beryllium. An analogous interlaboratory validation study has been carried out previously in order to evaluate field-portable electroanalytical procedures for on-site determination of lead in environmental samples [16].

Repeatability and reproducibility were calculated for each of the four beryllium levels in the PEMs analyzed by the participating laboratories. Repeatability is an estimate of within-laboratory variability, while reproducibility is an estimate of the variability of both within- and between-laboratory results. Repeatability was calculated by averaging the squares of the standard deviations of within-laboratory results for each beryllium level; hence the average within-laboratory variance is given by the repeatability variance, $(S_r)^2$. Reproducibility variance is expressed by:

$$(S_R)^2 = (S_r)^2 + (S_L)^2$$

where S_L is the sample standard deviation of the mean value estimated from the average of reported interlaboratory test results for a given PEM. Relative standard deviations (RSDs) for repeatability and reproducibility (RSD_r and RSD_R, respectively) are then computed by dividing the standard deviations S_r and S_R by the mean interlaboratory test result for a particular PEM. The RSDs calculated can then be compared with the minimum precision that is desired (e.g., RSD=0.20 [17]) for the test method under evaluation.

Estimates of analytical bias, B, were computed by simply dividing the difference between the measurand and the reference value by the reference value:

$$B = (\mu_i - R_i) / R_i$$

Here, μ_i and R_i are the mean and reference beryllium contents, respectively, for the ith beryllium loading level in each PEM sample.

Results of the Interlaboratory Evaluation

Of the candidate volunteer laboratories that were identified by members of the Analytical Subcommittee of the Beryllium Health and Safety Committee, PEM filter samples were sent to eleven prospective laboratories. Analysis results were subsequently reported from eight laboratories, thereby exceeding the minimum number (for evaluation of an ASTM International test method) of six participants. Hence, for purposes of this round-robin evaluation, recruitment of a sufficient number of volunteers was achieved.

Analytical results reported by the eight individual laboratories that participated in the interlaboratory exercise are summarized in Table 1 for MCE filters and in Table 2 for Whatman 541 filters. For six of the eight laboratories, duplicate analyses were reported for PEM samples at each beryllium loading level for different sample aliquots run using (a) different portable fluorescence spectrometers, or (b) different fluorescence intensity integration times, or (c) both. Overall means were computed based on the pooled means for the average of the two results reported by each laboratory for each sample (excepting the two laboratories that reported a single result). Data from blank measurements were all near to or below the reported LOD of the analytical method (≈ 0.01 µg Be per sample) [5].

Results for repeatability and reproducibility for the two PEM filter matrices, determined in accordance with ASTM E 691 [12], are summarized in Table 3. Bias estimates for each PEM sample containing beryllium are presented in Table 4; overall mean values μ_i used in estimations of bias were taken from Tables 1 and 2 (for MCE and Whatman filter PEM samples, respectively).

TABLE 1—*Results from measurement of beryllium content in MCE filters, as reported by laboratories participating in the interlaboratory evaluation. Reference values for beryllium loadings on the PEM filter samples are given in parentheses in the column headings.*

Laboratory Number (n = 8)	Low (0.05 µg Be)	Medium Low (0.10 µg Be)	Medium High (0.20 µg Be)	High (0.40 µg Be)
001	0.0512; 0.0504	0.104; 0.105	0.203; 0.207	0.468; 0.484
003	0.060; 0.050	0.11; 0.10	0.21; 0.20	0.43; 0.40
005	0.052; 0.063	0.103; 0.125	0.222; 0.273	0.459; 0.503
006	0.050	0.10	0.21	0.41
007	0.0505; 0.0490	0.103; 0.103	0.210; 0.198	0.406; 0.396
009	0.051; 0.041	0.103; 0.092	0.208; 0.199	0.421; 0.421
010	0.053; 0.053	0.104; 0.107	0.197; 0.194	0.412; 0.415
011	0.053	0.105	0.203	0.404
Overall mean ± sample standard deviation	0.052 ± 0.0038	0.10 ± 0.0048	0.21 ± 0.016	0.43 ± 0.032
Relative Standard deviation	0.073	0.048	0.076	0.074

TABLE 2—*Results from measurement of beryllium content in Whatman 541 filters, as reported by laboratories participating in the interlaboratory evaluation. Reference values for beryllium loadings on the PEM filter samples are given in parentheses in the column headings.*

Laboratory Number (n = 8)	Low (0.05 µg Be)	Medium Low (0.10 µg Be)	Medium High (0.20 µg Be)	High (0.40 µg Be)
001	0.0528; 0.0519	0.103; 0.104	0.198; 0.203	0.399; 0.406
003	0.060; 0.050	0.11; 0.10	0.22; 0.20	0.42; 0.40
005	0.055; 0.063	0.114; 0.145	0.198; 0.251	0.437; 0.492
006	0.050	0.11	0.21	0.40
007	0.0530; 0.0500	0.103; 0.099	0.203; 0.198	0.401; 0.393
009	0.056; 0.052	0.101; 0.093	0.207; 0.198	0.409; 0.410
010	0.053; 0.057	0.106; 0.104	0.205; 0.209	0.403; 0.412
011	0.056	0.104	0.207	0.409
Overall mean ± sample standard deviation	0.054 ± 0.0028	0.11 ± 0.0099	0.21 ± 0.0078	0.41 ± 0.022
Relative Standard Deviation	0.052	0.090	0.037	0.054

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TABLE 3—*Repeatability and reproducibility for beryllium measurements from performance evaluation MCE and Whatman 541 filters, as computed using values reported by laboratories (n = 8) participating in the interlaboratory evaluation.*

Beryllium Level	Average (μg Be)	S_r	S_R	RSD_r	RSD_R
MCE Filters					
Low	0.052	0.0034	0.0051	0.065	0.098
Medium Low	0.10	0.0052	0.0071	0.052	0.071
Medium High	0.21	0.012	0.020	0.057	0.095
High	0.43	0.0080	0.033	0.019	0.077
Whatman 541 Filters					
Low	0.054	0.0027	0.0039	0.050	0.072
Medium Low	0.11	0.0068	0.012	0.062	0.11
Medium High	0.21	0.012	0.014	0.057	0.067
High	0.41	0.012	0.025	0.029	0.061

TABLE 4—*Bias estimates for beryllium measurements from performance evaluation MCE and Whatman 541 filters, computed using mean values from Tables 1–3. Reference values for beryllium loadings on the filters are given in parentheses.*

PEM Matrix	Low ($0.05\ \mu$g Be)	Medium Low ($0.10\ \mu$g Be)	Medium High ($0.20\ \mu$g Be)	High ($0.40\ \mu$g Be)
MCE filters	0.040	0.0	0.050	0.075
Whatman 541 filters	0.080	0.10	0.050	0.025

Discussion

Results shown in Tables 1 and 2 give estimates of interlaboratory precision (as measured by the relative standard deviation, RSD) that are similar for both MCE and Whatman 541 filters. For each loading level there are no statistically significant differences between the mean beryllium contents measured in the two different sampling media (*t*-tests for independent means; n = 8). Also, for both media (Tables 1–3), there is no apparent trend of precision changing as a function of beryllium loading. It is noted that no outlier tests were conducted on the data which were reported by the participating laboratories; all results were included and treated statistically, despite the possibility of statistical outliers. The highest intralaboratory RSD encountered is 0.065, and all interlaboratory RSDs are 0.11 or less (Tables 1–3). Ordinarily, interlaboratory precision estimates of 0.15 and below are regarded as acceptable for PEMs such as these, that is, consisting of liquid spikes on sampling media [16].

The results summarized in Table 3 show that figures for within-laboratory precision RSD_r spanned the range $\approx$0.02 – $\approx$0.07, while data for between-laboratory precision RSD_R were slightly greater, ranging from $\approx$0.06 – $\approx$0.11. These precision estimates compare very favorably with precision estimates from interlaboratory results for PEMs consisting of MCE filters spiked with beryllium in liquid form at similar levels (Beryllium Proficiency Analytical Testing [BePAT] program, American Industrial Hygiene Association [AIHA], 2003) [18]. For AIHA BePAT PEM samples, interlaboratory RSDs of $\approx$0.06 – $\approx$0.15 (n = 25) were computed from five different loading levels ranging from $\approx$0.15 – $\approx$0.6 μg Be per filter. The AIHA BePAT samples were prepared and analyzed by laboratories using reference analytical methods involving concentrated acid digestion and atomic spectrometric analysis, for example NIOSH Method 7102 [19]. Thus, it is shown that, for filter samples, the interlaboratory precision of the field-portable fluorescence method is at least as good as that of fixed-site laboratory methods.

Bias estimates were negligible or positive for all beryllium loadings for both PEMs (Table 4), and ranged from 0.0–0.10. In terms of recovery, mean values determined for beryllium loadings for all of the PEM samples (e.g., see Tables 1 and 2) were within ±10 % of the reference values. Typically, recoveries of 100 % ±15 % are regarded as acceptable for meeting the requirements of quantitative analytical methods [20].

A limitation of this study is that this collaborative interlaboratory evaluation did not utilize real aerosol samples generated from beryllium-containing materials. Generally, it is desirable to evaluate methods using performance evaluation samples that are as realistic as possible. But because of the serious health hazards and high costs associated with the generation of beryllium aerosols, it was not deemed feasible to prepare PEMs from beryllium-containing aerosols for this study. It would also be of interest to evaluate the portable fluorescence method on-site in the field, but such an effort is outside the scope of this investigation.

In summary, the results of the interlaboratory evaluation of the field-portable extraction and fluorescence method for beryllium indicate that the method is effective for the quantitative measurement of soluble forms of trace beryllium in MCE and Whatman 541 filter samples. Estimates of within-laboratory and between-laboratory precision compared favorably with interlaboratory precision estimates from a beryllium proficiency testing program, and bias estimates were 10 % or below for each performance evaluation sample tested. Performance data obtained here represent the minimum that is required for NIOSH methods and ASTM International standards. It is intended that future studies will address real-world sample matrices and on-site evaluations of the portable fluorescence method.

Disclaimer

Mention of company names or products does not constitute endorsement by the Centers for Disease Control and Prevention.

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APPENDIX 6

Precision and Bias Estimates: From Study in Appendix 5

Two matrices: MCE & Whatman 541
Four loading levels: ~0.05 „Ÿ ~0.5 µg Be/filter   (prepared by LANL contract lab)
Media blanks included
Laboratory anonymity maintained by coding

$$(S_R)^2 = (S_r)^2 + (S_L)^2$$

S_R = reproducibility std dev
Sr = repeatability std dev
SL = sample std dev

$$B = (\mu_i - R_i) / R_i$$

μ_i = mean values
R_i = reference values

Results from Be-spiked MCE filters (n=8)

	Low (0.05 µg)	Med low (0.10 µg)	Med hi (0.20 µg)	High (0.40 µg)
μ_i (S_L)	0.052 (0.0038)	0.10 (0.0048)	0.21 (0.016)	0.43 (0.032)
RSD(%	7.3	4.8	7.6	7.4

Results from Be-spiked Whatmans (n=8)

	Low (0.05 µg)	Med low (0.10 µg)	Med hi (0.20 µg)	High (0.40 µg)
μ_i (S_L)	0.054 (0.0028)	0.11 (0.0099)	0.21 (0.0078)	0.41 (0.022)
RSD(%	5.2	9.0	3.7	5.4

APPENDIX 7

Validation of a standardized portable fluorescence method for determining trace beryllium in workplace air and wipe samples†‡§

Anoop Agrawal,[a] John Cronin,[a] Juan Tonazzi,[a] T. Mark McCleskey,[b] Deborah S. Ehler,[b] Edel M. Minogue,[b] Gary Whitney,[b] Christopher Brink,[b] Anthony K. Burrell,[b] Benjamin Warner,[b] Michael J. Goldcamp,[c] Paul C. Schlecht,[d] Prerna Sonthalia[d] and Kevin Ashley*[d]

Beryllium is widely used in industry for its unique properties; however, occupational exposure to beryllium particles can cause potentially fatal disease. Consequently, exposure limits for beryllium particles in air and action levels on surfaces have been established to reduce exposure risks for workers. Field-portable monitoring methods for beryllium are desired in order to facilitate on-site measurement of beryllium in the workplace, so that immediate action can be taken to protect human health. In this work, a standardized, portable fluorescence method for the determination of trace beryllium in workplace samples, i.e., air filters and dust wipes, was validated through intra- and inter-laboratory testing. The procedure entails extraction of beryllium in 1% ammonium bifluoride (NH_4HF_2, aqueous), followed by fluorescence measurement of the complex formed between beryllium ion and hydroxybenzoquinoline sulfonate (HBQS). The method detection limit was estimated to be less than 0.02 µg Be per air filter or wipe sample, with a dynamic range up to greater than 10 µg. The overall method accuracy was shown to satisfy the accuracy criterion ($A \leq \pm 25\%$) for analytical methods promulgated by the US National Institute for Occupational Safety and Health (NIOSH). Interferences from numerous metals tested (in >400-fold excess concentration compared to that of beryllium) were negligible or minimal. The procedure was shown to be effective for the dissolution and quantitative detection of beryllium extracted from refractory beryllium oxide particles. An American Society for Testing and Materials (ASTM) International voluntary consensus standard based on the methodology has recently been published.

Introduction

The unique properties of beryllium (Be) have led to many applications in the aerospace industry, the nuclear industry, manufacturing, electronics, and even sports equipment.[1] Beryllium metal is lightweight and has high strength, and alloying beryllium with copper and aluminum results in materials with high corrosion resistance, stiffness and low stress relaxation.

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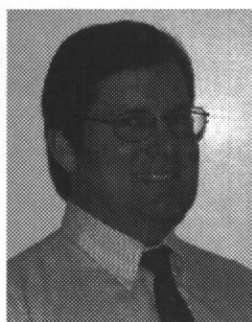[b] Los Alamos National Laboratory, P.O. Box 1663, Los Alamos, NM 87545, USA
[c] Wilmington College, Department of Chemistry, Wilmington, OH 45177, USA
[d] US Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, 4676 Columbia Parkway, Mail Stop R-7, Cincinnati, OH 45226-1998, USA. E-mail: KAshley@cdc.gov; Fax: +1 513 458 7189; Tel: +1 513 841 4402
† Presented at the 2nd Symposium on Beryllium Particulates and Their Detection, 8th–9th November 2005, University Park Marriot, Salt Lake City, UT, USA.
‡ Disclaimer: Mention of company names or products does not constitute endorsement by the Centers for Disease Control and Prevention or the US Department of Energy. The findings and conclusions in this paper are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.
§ This article was prepared by US Government employees as part of their official duties and legally may not be copyrighted in the United States of America.

Kevin Ashley was born in Hammond, Indiana (USA), in 1958. He received his PhD in physical-analytical chemistry from the University of Utah in 1987. In 1988, Ashley joined San Jose State University as an assistant professor of chemistry. Since 1991 he has been a research chemist in the Division of Applied Research and Technology of the National Institute for Occupational Safety and Health in Cincinnati, Ohio (USA). Dr. Ashley's research at NIOSH has focused on the development and evaluation of methods for metals monitoring in workplaces, including field-portable techniques. He is chair of ASTM International Subcommittee D22.04 on Sampling and Analysis of Workplace Atmospheres.

Trace beryllium is also often found in coal slag and aluminum ore. The high thermal conductivity of beryllium oxide, while also being electrically insulating, is a key component to the dissipation of heat in integrated circuits. Beryllium alloys are also used in high-end electrical connectors, springs, bearings and other components of a wide range of products.

Unfortunately, beryllium is a Class A EPA carcinogen, and its inhalation can cause an incurable and potentially fatal lung ailment, chronic beryllium disease (CBD).[2,3] Hence, monitoring of airborne beryllium in occupational environments is of vital importance. Further, it has also been reported that skin exposure may result in sensitization towards Be.[4] Thus, it is desirable to monitor and limit exposure of workers in industrial workplaces to particulate matter containing beryllium which can be inhaled or might come into contact with the skin. Beryllium metal (as metal or as a metal alloy) and beryllium oxide are the most important beryllium materials from an industrial perspective.

Widely used laboratory methods to measure beryllium in workplace atmospheres (e.g., NIOSH 7102,[5] OSHA ID-125G[6] and ASTM D7035[7]) use atomic spectrometric instrumentation. Preparation of samples for such analysis involves the use of strong acids and high heat, and the necessary laboratory equipment can be expensive. This instrumentation also requires highly trained personnel and is not easily field deployable. To overcome these issues, a rapid, quantitative and sensitive test for the detection of beryllium has been developed using fluorescence. The method is based on the fluorescence of beryllium bound to hydroxybenzoquinoline sulfonate (HBQS),[8] and includes a dissolution technique using dilute ammonium bifluoride solution.[9] Dilute ammonium bifluoride has been proposed as a field extraction medium which is much less hazardous than the strong acids used ordinarily in digestion of beryllium samples in the laboratory. Also, portable fluorometers that can be hand-carried to the field and powered by battery are commercially available. Given these and other considerations, it is of interest to thoroughly evaluate the on-site extraction and fluorescence method for beryllium in workplace samples.

A suite of experiments was carried out in order to validate the field method, which has been published as a new voluntary consensus standard by the American Society for Testing and Materials (ASTM) International. In this study, it was found that the intensity of fluorescence is linear with respect to beryllium concentration over several orders of magnitude. A detection limit of less than 0.02 μg Be per sample has been achieved, which allows for detection of Be at an order of magnitude lower than the lowest applicable DOE regulatory levels. Interference studies have been carried out with a variety of commonly co-occurring metals, with minimal or no interferences found for the detection of trace beryllium in the presence of many-fold excess of the other metals. Specificity for beryllium has been achieved using a number of strategies, such as the use of (a) ethylenediaminetetraacetic acid (EDTA), which binds larger metals, (b) high pH in the detection solution, which causes unbound metals to precipitate, and (c) HBQS, which binds selectively with beryllium. Experiments conducted in support of the method validation are described in detail in the following sections.

Experimental

Reagents and solutions

Sources of beryllium in solid form included beryllium sulfate (99.99% $BeSO_4 \cdot 4H_2O$, Aldrich, Milwaukee, WI, USA), beryllium oxide (99.98% BeO, Aldrich; and 99.5 + % UOX-125 BeO, Brush-Wellman, Elmore, OH, USA), and beryllium metal powder (99 + % Be, Matheson, Norwood, OH, USA). 1% (w/v) aqueous ammonium bifluoride (NH_4HF_2) extraction solution was provided by Berylliant (Tucson, AZ, USA). The detection solution, also from Berylliant, contained 1.1 mM HBQS, 1 mM EDTA and 100 mM L-lysine monohydrochloride; the pH was adjusted to 12.8 (± 0.1) with 10 M NaOH (Fisher Scientific, Hampton, NH, USA). Standard solutions consisting of ≈ 1000 μg mL^{-1} metal (Al, Be, Ca, Co, Cu, Fe, Li, Ni, Pb, Sn, U, V, W, Zn) concentration were obtained from Inorganic Ventures (Lakewood, NJ, USA). Titanium dioxide (TiO_2) came from Aldrich. Deionized water (18 MΩ cm) used for all experiments was prepared using a MilliQ® purification system (Millipore, Billerica, MA, USA).

Materials and equipment

Mixed-cellulose ester (MCE) filters, 37 mm dia., 0.8 μm pore size, and Whatman 541 filters, 47 mm dia., were obtained from SKC (Eighty-Four, PA, USA). Palintest and Ghost Wipe® dust sampling wipes were purchased from Palintest USA (Erlanger, KY, USA) and Environmental Express (Mt. Pleasant, SC, USA), respectively. Performance evaluation materials consisting of MCE and Whatman 541 filters spiked with beryllium sulfate to give levels between 0.05 and 0.5 μg Be per filter were prepared at a contract laboratory (Environmental Resource Associates, Arvada, CO, USA). Beryllium oxide-spiked filters (MCE and Whatman 541) were prepared from aqueous BeO suspensions;[10] the spiking level was 0.18 ± 0.01 μg Be per filter. Plastic centrifuge tubes (15 and 50 mL), 0.45 μm pore size, 25 mm dia., nylon plastic 5 mL syringe microfilters, and disposable fluorescence cuvettes (10 mm dia.) were obtained from Fisher. An analytical balance (Mettler Toledo AT261, Columbus, OH, USA) was used for high-precision weighing. Agitation was effected by using a rotator (Labquake®, Barnstead, Dubuque, IA, USA) or sonicator (Fisher FS110H). Sample heating was done using a VWR Digital Heatblock (VWR, West Chester, PA, USA). Where necessary, pH was measured using an Orion model 710 pH meter (Thermo, Beverly, MA, USA) that was calibrated with pH 4.0, 7.0 and 10.0 buffers (Fisher).

Fluorescence instrumentation

Portable fluorometers used were Turner Quantech® (Barnstead) and Ocean Optics S2000-FL (Dunedin, FL, USA) devices, respectively. The Turner Quantech instrument utilizes bandpass filters in the paths of incident and fluorescent beams, with excitation radiation of 360–390 nm and a detection spectral window of at least 440–490 nm. The Ocean Optics device employs a 380 nm light-emitting diode (LED) for excitation and a diode array detector for spectral measurements over a wavelength range of 300 to 800 nm; optical fibers

are used to transmit the excitation beam and the radiation detected at 90° to the incident 380 nm radiation.

Experimental methods

The objective of this work was to validate the field-portable fluorescence method in accordance with standard guidelines. Within-laboratory and field studies were carried out following strategies outlined by NIOSH for applications in workplace air sampling and analysis.[11] Interlaboratory collaborative testing was conducted as described in a pertinent ASTM International voluntary consensus standard.[12]

The overall analysis method[13] entailed the following steps: (1) Placement of filter or wipe samples into plastic centrifuge tubes of appropriate size. (2) Extraction of the samples in 1% (w/v) ammonium bifluoride solution for at least 30 minutes using agitation or heating. (3) Filtration of extract solutions through nylon plastic syringe microfilters. (4) Transfer of aliquots of sample extracts into disposable fluorescence cuvettes using mechanical micropipettes. (5) Reaction of the sample aliquots with detection solution containing the fluorescent dye (HBQS). (6) Measurement of fluorescence at ≈475 nm using a portable fluorometer.

Experiments were conducted to evaluate the analytical recovery, dynamic range, method detection limit, long-term sample stability; interferences, performance on field samples, and interlaboratory precision. For the purposes of this work, analysis results were ordinarily reported in units of mass of beryllium (in µg) per sample. This required comparison of results for unknowns with calibration standards, along with consideration of appropriate dilution and correction factors, to convert fluorescence response to mass. Confirmatory analysis was carried out using acid digestion and atomic spectrometry by NIOSH method 7102,[5] OSHA method 125G,[6] or equivalent.

Results

Representative solution fluorescence spectra for trace levels of beryllium in the presence of HBQS are shown in Fig. 1. Unreacted HBQS reagent fluoresces at $\lambda_{max} \approx 590$ nm, while the intense fluorescence of the Be–HBQS adduct is blue shifted to $\lambda_{max} \approx 475$ nm. With this fluorescence reagent, sub-ppb

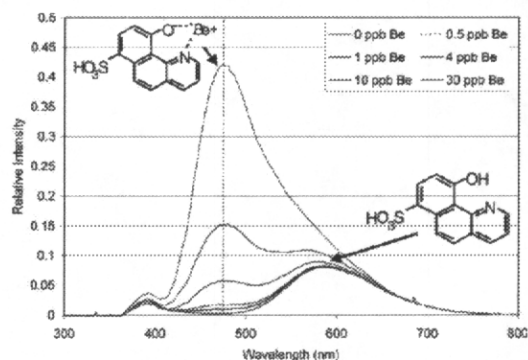concentrations of beryllium can be measured quantitatively. The HBQS fluorophore offers much higher sensitivity than do fluorescence reagents previously employed for beryllium detection.[14-16] Concentrations of less than 0.1 ppb Be can be determined using the HBQS reagent.

The method detection limit (MDL) was estimated according to the generalized NIOSH procedure.[11] An Ocean Optics S2000-FL portable fluorescence device (5 s signal integration time) was used for these trials. Low-level media spikes (MCE and Whatman 541 filters) corresponding to between 0.006 and 0.060 µg Be per sample were analyzed (along with low-level calibration standards), and the mean results were obtained for replicate samples at each spiking level. From this treatment the MDL was estimated to be 4.2 ng Be per sample for each sampling medium. An alternative method for estimating MDL involves the measurement of multiple blanks.[7] MDLs estimated in this fashion were found to be comparable to those obtained using the NIOSH protocol.

The analytical range of the method was evaluated using an Ocean Optics fluorometer. With this device it was possible to quantitatively measure from the method quantitation limit, ≈14 ng Be, up to at least 6 µg Be per sample without further dilution (fluorescence intensity $y = 0.0696[\text{Be}] + 0.0115$; $r^2 = 0.9998$; 2 s integration). Still greater masses, up to at least 10 µg Be per sample, can be measured directly using the Turner Quantech device using the low sensitivity setting, which applies to higher beryllium concentrations.

Results for analytical recoveries of beryllium from beryllium sulfate or beryllium oxide, and sampling media spiked with these beryllium compounds at levels of ≈0.02–2.0 µg Be per sample, are summarized in Table 1. Mechanical agitation or heating at 80 °C was used during sample extraction in 1% NH_4HF_2. Reference values for samples containing beryllium oxide were established by using a combination of NIOSH 7102 (sample preparation by hotplate digestion in nitric/sulfuric acids) and NIOSH 7300 (Be measurement by inductively coupled plasma atomic emission spectrometry, ICP-AES).[5] Recoveries from BeO were found to be higher when heating was used during extraction (Table 1). Beryllium recoveries from samples with larger masses of material (5–20 mg), weighed on a high-precision analytical balance prior to

Fig. 1 HBQS ($\lambda_{max} \approx 590$ nm) and Be–HBQS ($\lambda_{max} \approx 475$ nm) fluorescence spectra.

Table 1 Beryllium recoveries from extraction in 1% ammonium bifluoride (>30 min) and analysis by portable fluorometry; sampling media were spiked with beryllium sulfate (from solution) or beryllium oxide (Brush-Wellman UOX125 BeO in suspension) at masses of 0.02–2 µg Be per sample

Sample/media	Extraction technique	Mean recovery (%)	Relative standard deviation (%)
$BeSO_4$ ($n = 3$)	Mechanical	102	4.4
$BeSO_4$/MCE ($n = 9$)	Mechanical	105	6.1
$BeSO_4$/Whatman 541 ($n = 12$)	Mechanical	99.4	4.7
BeO ($n = 6$)	Mechanical	85.6	6.8
BeO ($n = 3$)	Heat	95.0	9.8
BeO/MCE ($n = 12$)	Mechanical	89.5	5.1
BeO/MCE ($n = 3$)	Heat	97.4	9.5
BeO/Whatman 541 ($n = 12$)	Mechanical	84.2	4.6
BeO/Whatman 541 ($n = 3$)	Heat	90.1	8.3

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Table 2 Summary of beryllium recoveries ($\pm$ standard deviations) from extraction in 1% ammonium bifluoride and analysis by fluorometry, using mechanical rotation (> 30 min) or sonication (1 h). Masses treated were 10–20 mg for $BeSO_4 \cdot 4H_2O$ and 5–10 mg for Be metal powder and BeO (Aldrich)

Sample/media ($n = 3$)	Mechanical rotation	Sonication
$BeSO_4 \cdot 4H_2O$ (no medium)	99.8 ± 4.4	106.6 ± 12.0
Be metal (no medium)	96 ± 3	----
BeO (no medium)	90 ± 5	83 ± 3
$BeSO_4 \cdot 4H_2O$/MCE	98.6 ± 1.6	110.4 ± 4.7
Be metal/MCE	93 ± 7	----
BeO/MCE	94 ± 6	93 ± 6
$BeSO_4 \cdot 4H_2O$/Whatman 541	98.1 ± 3.3	101.3 ± 2.5
Be metal/Whatman 541	95 ± 4	----
BeO/Whatman 541	86 ± 8	96 ± 5
$BeSO_4 \cdot 4H_2O$/Palintest	----	87.6 ± 2.5
BeO/Palintest	----	84 ± 2
$BeSO_4 \cdot 4H_2O$/Ghost Wipe	----	79.3 ± 2.4
BeO/Ghost Wipe	----	40 ± 4

Table 4 Interference study of beryllium fluorescence measurement (a.u.) in presence of titanium dioxide

Mass of Be on filter/μg	Mass of TiO_2 on filter/mg	Beryllium/μg, determined by fluorescence	
		Initial intensity measurement	Measurement after additional filtration step
0.20	0.00	0.20	0.20
2.00	0.00	2.02	2.03
0.20	10.00	0.17	0.20
2.00	10.00	1.64	2.02
0.20	20.00	0.17	0.21
2.00	20.00	1.65	2.04

spiking, are shown in Table 2. Agitation by rotation or sonication was used in these trials. Analytical recoveries from these samples were computed after extraction and fluorescence measurement based on the mass of originally weighed material.

For 13 metals, interference studies were carried out by diluting the appropriate metal standard solution with 1% NH_4HF_2, such that the final concentration of the metal in the detection solution was 0.04, 0.4 or 2 mM. The potentially interfering metals were in ≥ 400-fold molar excess to the beryllium that was present. The results from these trials, which were carried out using an Ocean Optics device (2 s integration), are shown in Table 3. Significant positive interference from iron which was measured initially (Table 3) disappeared several hours later, after allowing for precipitation to occur prior to reanalysis. Interference from titanium in the form of TiO_2 was investigated in a separate experiment using a Turner Quantech fluorometer, where beryllium and TiO_2 were spiked onto Whatman 541 filters at the levels shown in Table 4. Negative interference from titanium dioxide observed initially

Table 3 Fluorescence measurements (a.u.) from interference study of 13 metals present in solution in many-fold excess compared to 0–1 μM concentrations of beryllium

Metal, concentration	Fluorescence intensity (0 μM Be)	Fluorescence intensity (0.1 μM Be)	% difference	Fluorescence intensity (1 μM Be)	% difference
(None)	0.005	0.112	----	1.078	----
0.4 mM Al	0.004	0.112	0.00	1.054	−2.22
0.4 mM U	0.004	0.110	−1.79	1.060	−1.67
2 mM Ca	0.004	0.112	0.00	1.057	−1.95
0.04 mM Li	0.004	0.112	0.00	1.060	−1.67
0.4 mM Pb	0.004	0.111	−0.89	1.105	2.50
0.4 mM Zn	0.003	0.112	0.00	1.103	1.02
0.4 mM Fe	0.003	0.101	−9.82	0.925	−14.2
0.4 mM V	0.003	0.114	1.79	1.083	0.46
0.4 mM Sn	0.003	0.113	0.89	1.105	2.50
0.4 mM W	0.003	0.116	3.57	1.103	2.32
0.4 mM Cu	0.003	0.114	1.79	1.062	−1.49
0.4 mM Ni	0.004	0.114	1.79	1.074	−0.37
0.4 mM Co	0.005	0.111	−0.89	1.030	−4.45

in fluorometric measurement was alleviated after a second filtration step was performed using nylon filters (Table 4).

Long-term sample stability studies of beryllium on MCE filters have been conducted in previous work to support NIOSH methods 7102 and 7300.[5] To supplement those data, an investigation of long-term stability was carried out whereby Whatman 541 filters were spiked with beryllium in solution form at 0.1 μg Be per sample, and analyses were carried out for up to 30 days, as per NIOSH guidelines.[11] Samples were stored at room temperature, and HBQS fluorescence measurements were conducted using an Ocean Optics instrument (2 s integration time) after mechanical extraction in 1% NH_4HF_2. Results from these experiments showed no significant change in fluorescence intensity over the 30 day period. In a related study, long-term stability of the HBQS detection solution was investigated. Standard curves of beryllium concentration *versus* fluorescence intensity were obtained for freshly prepared detection solution and for the solution stored in a dark bottle at room temperature for a 12 week period. An Ocean Optics fluorometer (2 s integration time) was employed for these experiments. Fluorescence results from trace beryllium (0–30 ppb) with freshly prepared HBQS detection solution *vs.* 12 week old solution were essentially identical.

Field wipe samples (using Whatman 541 filters wetted with deionized water) were obtained from a machine shop and from a firing range at Los Alamos National Laboratory. These samples, which were taken from a variety of surfaces, were first processed and analyzed using the 1% NH_4HF_2 extraction/ portable fluorescence method.[13] The remaining extract solution was subsequently analyzed using ICP-AES.[5,6] Only 100 μL of the 5 mL sample extract is required for the fluorescence measurement, so 98% of the remaining solution is available for subsequent confirmatory analysis. Results from this comparison, which consisted of the paired analysis of results from 50 wipe samples taken in the field, are shown in Fig. 2. The plot is very close to linear (slope = 1.007) and the correlation is extremely close to unity ($r^2 = 0.9958$). For paired results above the respective method detection limits (*i.e.*, ≈ 0.02 μg Be sample^{-1}), the average relative percent difference between data obtained using the portable extraction/fluorescence method *vs.* ICP-AES analysis was 4.7% ($n = 39$). For a subset of the field samples treated by extraction in 1% NH_4HF_2 (*i.e.*, $n = 19$), the remaining, undissolved wipe material was digested and analyzed by OSHA method 125-G.[6] No beryllium was detected in these samples: all results were below the MDL.
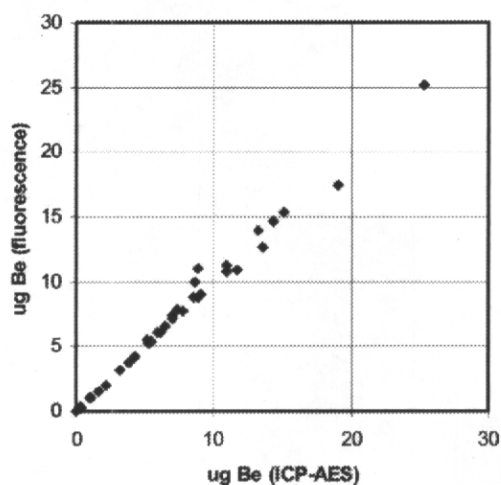
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Fig. 2 Field data from wipe samples: Comparison of extraction and portable fluorescence measurement *vs.* ICP-AES measurement of extract solutions.

Interlaboratory round-robin analysis results from nine participating laboratories that returned results are summarized in Table 5 (data are updated from ref. 17). The volunteer laboratories were requested to carry out the field extraction/fluorescence method for beryllium, and to report the results in terms of µg Be per sample. Results were reported for MCE and Whatman 541 filters spiked with beryllium (from beryllium sulfate solution) at levels between 0.05 and 0.5 µg Be per sample. Overall mean values were computed based on the pooled mean values (if applicable) reported by each laboratory for each sample. Results from blank measurements were all below the reported method detection limit (<0.02 µg Be per sample). Interlaboratory relative standard deviations (RSDs) were found to range from ≈5% to ≈10%. Bias estimates of overall means were calculated *vs.* the reference values listed in the first column of Table 5.

Discussion

It is pertinent to consider applicable action levels for beryllium in workplace air and on surfaces in view of the MDL estimated for the portable fluorescence method. Generally, in order to ensure that quantitative measurements can be achieved at the limit value of concern, it is desired that the MDL be at least an order of magnitude less than the applicable action level.[11] Several action levels for beryllium that have been established by government agencies in the US and in Europe are summarized in Table 6. The lowest applicable action levels for beryllium have been promulgated by the US Department of Energy (DOE).[18] For full-shift air samples and for surface dust samples of 100 cm^2 area, it can be seen that an estimated MDL of ≈0.004 µg Be per sample is more than 10 times less than all of the action levels listed in Table 6. Therefore, the portable fluorescence method can be used to quantitatively measure beryllium at the trace levels required (see, *e.g.*, Fig. 1).

Table 5 Interlaboratory round-robin analysis results ($n = 9$) from MCE and Whatman 541 filters spiked with beryllium sulfate

Beryllium level/µg Be sample^{-1}	Reported average ± std. dev./µg Be sample^{-1}	Interlaboratory RSD^a (%)	Estimated bias
MCE filters			
0.050	0.052 ± 0.0034	6.5	0.040
0.10	0.10 ± 0.0048	4.8	0.00
0.20	0.21 ± 0.018	8.6	0.050
0.40	0.42 ± 0.040	9.5	0.050
Whatman 541 filters			
0.050	0.053 ± 0.0054	10.2	0.060
0.10	0.11 ± 0.011	10.0	0.10
0.20	0.21 ± 0.0094	4.5	0.050
0.40	0.41 ± 0.025	6.1	0.025

^{a} (Relative standard deviation).

Beryllium recoveries from a representative soluble compound (*i.e.*, beryllium sulfate) are quantitative, as indicated by recovery values close to 100% (Tables 1 and 2). Beryllium oxide, which is highly refractory, is more difficult to extract, as evidenced by somewhat lower recoveries from BeO obtained by mechanical extraction (Tables 1 and 2). BeO recoveries are improved to 90% and better if heating is applied during extraction (Table 1). Recoveries of beryllium from spiked MCE and Whatman 541 filters are quantitative for both sampling media (Tables 1 and 2); this is true for beryllium sulfate, beryllium metal powder and beryllium oxide (especially if heating is used in extraction of BeO). However, somewhat lower recoveries are obtained from Palintest wipes spiked with beryllium sulfate or beryllium oxide, and beryllium recoveries from Ghost Wipes are unacceptably low, presumably due to matrix interferences (Table 2). The results shown in Tables 1 and 2 indicate that 1% ammonium bifluoride is an effective medium for the quantitative extraction of beryllium, even from challenging matrices such as high-fired beryllium oxide. The data of Table 2 indicate that large masses of beryllium can be quantitatively extracted and measured, thereby demonstrating the robustness of the method for

Table 6 European and US action levels for beryllium in workplace air and in surface dust

Country (organization)	8 h TWA^a action level (air)
Austria, France, Germany, Spain, Sweden, UK, US (OSHA)	2.0 µg m^{-3}
Denmark	1.0 µg m^{-3}
US (NIOSH)	0.5 µg m^{-3}
	Ceiling action level (air)
Austria	8.0 µg m^{-3}
US (OSHA)	5.0 µg m^{-3}
Denmark	2.0 µg m^{-3}
US (DOE)	0.2 µg m^{-3}
	Surface action level
US (DOE)	3.0 µg/100 cm^2 (housekeeping)
US (DOE)	0.2 µg/100 cm^2 (equipment release)

^{a} Time-weighted average.

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samples that may contain beryllium in extremely high excess with respect to the action levels summarized in Table 6.

The data presented in Tables 3 and 4 show that, with the exception of iron and titanium dioxide, the portable fluorescence method does not suffer from interferences from other metals that are present in significant excess. Moreover, interferences from Fe and TiO_2, which can be detected visually (by yellowish color or by turbidity, respectively), are effectively eliminated through further sample processing. Sample stability and the stability of the detection solution are excellent, as indicated by experimental results (not shown).

Results from field samples (Fig. 2) and from interlaboratory round-robin analysis (Table 5) offer further evidence of the robustness of the fluorescence method. The agreement observed for extracts of wipe samples between the portable fluorescence method and ICP-AES is outstanding (Fig. 2). Also, interlaboratory agreement when using the extraction/portable fluorescence method is excellent (Table 5). For air filter samples, pooled estimates of precision and bias (for the range 0.05–0.40 µg Be sample^{-1}) were 0.074 (±0.021) and 0.035 (±0.024), respectively. Based on the interlaboratory data, the overall method performance for air filter samples yielded an analytical accuracy estimate of 16% (±5%), which meets NIOSH criteria for method accuracy.[11] For wipe samples, pooled estimates of precision and bias were 0.077 (±0.028) and 0.059 (±0.031), respectively. Based on the interlaboratory results, the overall method performance for wipe samples gave an analytical accuracy estimate of 19% (±6%), which also meets NIOSH accuracy criteria.

In conclusion, the fluorescence method described has been demonstrated to operate successfully under various operating conditions, including the detection of beryllium both in laboratory settings and in field trials. The procedure fulfills the requirements for a fast, inexpensive, field-deployable method for the determination of beryllium in workplace samples. There are several advantages of the system: it is rapid (test results within one hour); high throughput; beryllium-specific; low capital cost ($\approx$ \$10,000); field or laboratory deployable; detection limit less than 0.02 mg Be per sample; uses less hazardous solutions; uses only a fraction of material collected, an advantage for re-verification; does not require highly trained laboratory personnel.

The method described has been published as an ASTM International voluntary consensus standard,[13] and has also been submitted for publication as a NIOSH analytical method.

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Ultra-trace determination of beryllium in occupational hygiene samples by ammonium bifluoride extraction and fluorescence detection using hydroxybenzoquinoline sulfonate[☆],[☆☆]

Kevin Ashley [a],*, Anoop Agrawal [b], John Cronin [b], Juan Tonazzi [b], T. Mark McCleskey [c], Anthony K. Burrell [c], Deborah S. Ehler [c]

[a] *U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, 4676 Columbia Parkway, M.S. R-7, Cincinnati, OH 45226-1998, USA*
[b] *Berylliant, Inc., 4541 E. Fort Lowell Road, Tucson, AZ 85712, USA*
[c] *Los Alamos National Laboratory, P.O. Box 1663, Los Alamos, NM 87545, USA*

Abstract

A highly sensitive molecular fluorescence method for measuring ultra-trace levels of beryllium has been previously described. The method entails extraction of beryllium workplace samples by 1% ammonium bifluoride (NH_4HF_2, aqueous), followed by fluorescence detection using hydroxybenzoquinoline sulfonate (HBQS). In this work, modification of the existing procedure resulted in a significant improvement in detection power, thereby enabling ultra-trace determination of beryllium in air filter and surface wipe samples. Such low detection limits may be necessary in view of expected decreases in applicable occupational exposure limits (OELs) for beryllium. Attributes of the modified NH_4HF_2 extraction/HBQS fluorescence method include method detection limits (MDLs) of <0.8 ng to ≈2 ng Be per sample (depending on the fluorometer used), quantitative recoveries from beryllium oxide, a dynamic range of several orders of magnitude, and freedom from interferences. Other key advantages of the technique are field portability, relatively low cost, and high sample throughput. The method performance compares favorably with that of inductively coupled plasma-mass spectrometry (ICP-MS).
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Keywords: Air monitoring; Beryllium; Extraction; Fluorescence; Trace analysis; Workplace

1. Introduction

Beryllium is a lightweight but durable metal that has numerous industrial and defense materials applications, including in aerospace, nuclear energy and weapons, electronics and communications, and automobile manufacturing [1]. When beryllium is alloyed with copper, aluminum or other metals, high-performance materials with a variety of attributes can be fabricated. Beryllium oxide has high thermal conductivity and is electrically insulating, properties that enable heat to be effectively dissipated in microscale integrated circuits. However, human exposures to beryllium can cause an immune system response known as beryllium sensitization [2], and can lead to the development of chronic beryllium disease (CBD), an incurable and potentially fatal progressive lung ailment [3]. The prevention of occupational exposures to beryllium particles by inhalation and/or skin contact requires the ability to measure this element at extremely low levels in workplace air and on surfaces.

Analytical methods for determining trace levels of beryllium in occupational hygiene samples have been recently reviewed [4]. While a variety of analytical techniques have been applied to trace beryllium measurement in the workplace, atomic spectrometric methods such as graphite furnace atomic absorption

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* Corresponding author. Tel.: +1 513 841 4402; fax: +1 513 458 7189.
  E-mail address: kashley@cdc.gov (K. Ashley).

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spectrometry (GFAAS) and inductively coupled-plasma atomic emission spectrometry (ICP-AES) are the most widely used methodologies for industrial hygiene monitoring purposes [5]. In pursuit of greater analytical sensitivity, inductively couple plasma-mass spectrometry (ICP-MS) for environmental analysis [6] has recently become more widely used for beryllium measurements in the occupational hygiene field. Compared to GFAAS and ICP-AES, ICP-MS offers lower detection limits for beryllium (and other elements), enabling ultra-trace analysis that may be required for short-term measurements and/or reduced occupational exposure limits (OELs).

Because of the complexity and expense of atomic spectrometric instrumentation, alternative methodologies have been investigated for trace beryllium measurements, including portable techniques such as colorimetry [7], electroanalysis [8,9], and fluorescence [10]. Recently a portable extraction/fluorescence procedure with an estimated method detection limit (MDL) that is competitive with GFAAS and ICP-AES was standardized and validated [11]. The method entails extraction of beryllium using a dilute aqueous solution of ammonium bifluoride, followed by fluorescence detection of beryllium using hydrobenzoquinoline sulfonate [12]. In this work we report on the modification of the fluorescence technique, which has enabled achievement of an estimated MDL that is comparable to that of ICP-MS. The modified fluorescence method has been applied to the determination of ultra-trace quantities of beryllium in occupational hygiene samples.

2. Experimental

2.1. Reagents, materials, and equipment

High-purity beryllium oxide powder (99.98% BeO) was obtained from Aldrich (Milwaukee, WI, USA). Beryllium metal (>99% Be) powder came from Matheson (Norwood, OH, USA). Ammonium bifluoride extraction solution (1% NH_4HF_2, aqueous), as well as fluorescence detection solution (aqueous) containing hydroxybenzoquinoline sulfonate (HBQS, 1.1 mM), ethylenediamine tetraacetic acid (EDTA, 1 mM), and L-lysine monohydrochloride (100 mM), were provided by Berylliant, Inc. (Tucson, AZ, USA). Low-concentration beryllium calibration standards (0–0.2 μg mL^{-1}) were prepared daily by dilution of aliquots from standard stock solutions (Berylliant). The pH of the detection solution was adjusted to 12.8 ± 0.1 with 10 M NaOH (Fisher Scientific, Hampton, NH, USA) with the aid of mechanical micropipets (Eppendorf, Hamburg, Germany) and an Orion model 710 pH meter (Thermo, Beverly, MA, USA) that was calibrated using pH 4.0, 7.0, and 10.0 buffers (Fisher Scientific). Deionized water (18 MΩ cm resistivity), prepared using a MilliQ® (Millipore, Billerica, MA, USA) water purification system, was used for all experiments.

Mixed-cellulose ester (MCE) filters (37 mm diameter, 0.8 μm pore size) and Whatman 541 cellulosic filters (47 mm diameter) were purchased from SKC, Inc. (Eighty-Four, PA, USA). Performance evaluation materials consisting of MCE and Whatman 541 filters spiked with extremely low levels of beryllium (0.002–0.05 μg Be per filter) were prepared by a contract laboratory (Environmental Resource Associates, Arvada, CO, USA) from an aqueous 1000 μg mL^{-1} beryllium standard solution (Inorganic Ventures, Lakewood, NJ, USA). MCE filters spiked with BeO (Aldrich) at levels of 0.2 and 0.5 μg per filter (±1%), prepared from aqueous suspensions [13], were provided by High-Purity Standards (Charleston, SC, USA). Where applicable, materials were weighed to the nearest 0.0001 g using a Mettler Toledo (Columbus, OH, USA) model AT 261 high-precision analytical balance.

Plastic 15 mL centrifuge tubes, 25 mm diameter nylon microfilters (0.45 μm pore size), plastic 5 mL syringes, and disposable fluorescence cuvettes (10 mm diameter) were obtained from Fisher Scientific. A Labquake® rotator (Barnstead, Dubuque, IA, USA) was used for mechanical extraction of some samples at room temperature (23 ± 1 °C), and a VWR Digital Heatblock (West Chester, PA, USA) was employed for sample heating at 85 ± 2 °C, where necessary.

Fluorometers used were Ocean Optics S2000-FL (Dunedin, FL, USA), Turner Quantech® (Barnstead), and Spex Fluorolog2 (Horiba Ltd., Tokyo, Japan) instruments. The Ocean Optics instrument utilizes a 380 nm light-emitting diode (LED) as an excitation source, and a diode array detector records the fluorescence signal over the range 300–800 nm; optical fibers transmit the source beam and the detected radiation at 90° to the incident source radiation. The Turner Quantech fluorometer employs bandpass filters to attenuate the excitation and detection beams, with source radiation of 360–390 nm and a detection spectral window of 440–490 nm. A quartz halogen lamp provides source radiation and a photomultiplier tube is used for detection. The Spex Fluorolog2 device utilizes a xenon arc lamp source (360–460 nm), monochromators for source and emission radiation (3 nm slit widths), and a photomultiplier tube detector (240–850 nm).

2.2. Electron microscopy

Scanning electron microscopy (SEM) [14] was performed using a Hitachi S-3000N instrument (Hitachi America, Brisbane, CA, USA). SEM images were obtained at 15 kV accelerating voltage and 300× magnification using a secondary electron detector.

2.3. Extraction and fluorescence measurement

To attain extremely low method detection limits for beryllium, and also to obtain quantitative recoveries from refractory beryllium compounds that are extremely difficult to dissolve, the procedure required significant modification of the published test method [15]. Specific differences entailed heating (versus mechanical agitation) to dissolve refractory BeO, and 1:5 dilution (versus 1:20) of extract solutions with the detection solution. Experiments on performance evaluation samples were carried out blind by analysts at four different laboratories, using one or more of the three fluorometric instruments.

First, the MCE and Whatman 541 filter samples containing trace beryllium were placed into 15 mL plastic centrifuge tubes. Then 5 mL of 1% NH_4HF_2 (aqueous) was added to each

centrifuge tube containing the filter sample, and each tube was capped securely. If necessary, a clean stirring rod was used to push the filter to the bottom of the centrifuge tube to ensure that the filter was completely immersed in the extraction solution. Extraction was carried out for 30 min either by mechanical agitation at room temperature using the rotator, or by heating at 85 °C using the hot block. (Samples containing soluble beryllium or beryllium metal were treated using mechanical extraction, while filters spiked with BeO were subjected to heating to facilitate dissolution.) After extraction (and cooling of sample extracts to room temperature, if necessary), sample extracts were filtered using the nylon microfilters attached to 5 mL syringes. Then 0.4 mL of extract solution was pipetted into a fluorescence cuvette, to which 1.6 mL of detection solution containing the fluorescence reagent (HBQS) was added. (The reaction between dissolved beryllium and HBQS to form the fluorophore adduct is instantaneous.) The fluorescence of the Be-HBQS complex was subsequently measured ($\lambda_{max} \approx 475$ nm) using one of the three fluorometers.

3. Results

Fluorescence spectra of extremely low concentrations of beryllium in the presence of HBQS, obtained using the Ocean Optics device, are presented in Fig. 1. Unreacted HBQS yields a fluorescence band with $\lambda_{max} \approx 590$ nm, while the fluorescence of the Be-HBQS complex is blue-shifted, with $\lambda_{max} \approx 475$ nm. Beryllium concentrations of less than 0.2 ng mL^{-1} were measured quantitatively using the HBQS fluorescence reagent with 1:5 dilution, offering a dramatic increase in sensitivity over beryllium fluorophores investigated previously [16–21]. This technique also offers a significant improvement in detection power compared to the method employing 1:20 dilution of the sample with detection solution [22]. For calibration standards in the range of a few parts-per-billion and below (i.e., 0.0, 0.2, 0.5, 0.8, 2.0, and 4.0 µg mL^{-1}), the measured coefficient of estimation $r^2 = 1.00$.

Recovery data from samples of beryllium metal and BeO in the absence and presence of sampling media (i.e., MCE
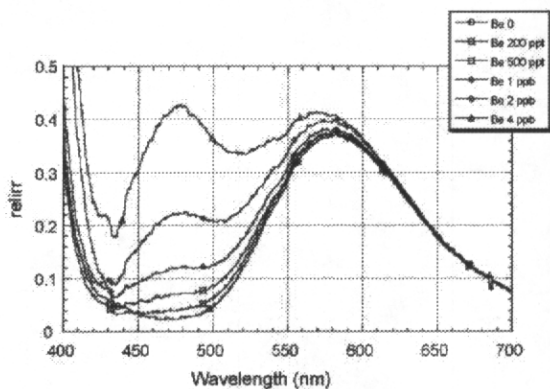
Table 1
Beryllium recoveries from beryllium metal and BeO in the absence and presence of sampling media

Sample/media ($n = 3$)	Extraction	Mean recovery (%)	R.S.D.[a] (%)
Be (no media)	Rotator	96	3.1
Be/MCE	Rotator	93	7.3
Be/Whatman 541	Rotator	95	4.2
BeO (no media)	Rotator	86	6.8
BeO (no media)	Heat block	97	6.6
BeO/MCE	Rotator	86	5.9
BeO/MCE	Heat block	99	7.7
BeO/Whatman 541	Rotator	86	7.8
BeO/Whatman 541	Heat block	96	6.2

Masses treated were 5–10 mg per sample.
[a] Relative standard deviation.

and Whatman 541 filters) are shown in Table 1. Recoveries were computed after extraction of 5–10 mg samples in 5 mL of 1% NH$_4$HF$_2$ and HBQS fluorescence measurement at 475 nm. These results show that mechanical extraction at room temperature (using the laboratory rotator) is effective for obtaining near quantitative (>90%) recoveries of beryllium metal. However, heating (at $\approx$85 °C by means of the heating block) is required in order to yield quantitative recoveries of BeO. The presence of active fluoride ions (from HF by dissociation of NH$_4$HF$_2$ in acidic medium) enables dissolution of refractory materials such as BeO.

Follow-up experiments were carried out to investigate the NH$_4$HF$_2$ extraction/HBQS fluorescence method from MCE filters spiked with low levels of BeO prepared from slurries. An SEM image from a representative filter fortified with BeO in this manner is shown in Fig. 2, and performance data from the BeO-spiked filters are presented in Table 2. The BeO particle in the SEM image consists of an agglomerate of a few micrometers in average diameter, comprised of primary particles of about 200 nm diameter. The results of Table 2 indicate that mechanical agitation of BeO-spiked MCE filter samples at room temperature yields beryllium recoveries of about 70%, while heating block extraction at $\approx$85 °C gives recoveries near 100%.

Fig. 1. Fluorescence spectra of trace concentrations of beryllium in the presence of hydroxybenzoquinoline sulfonate (HBQS).
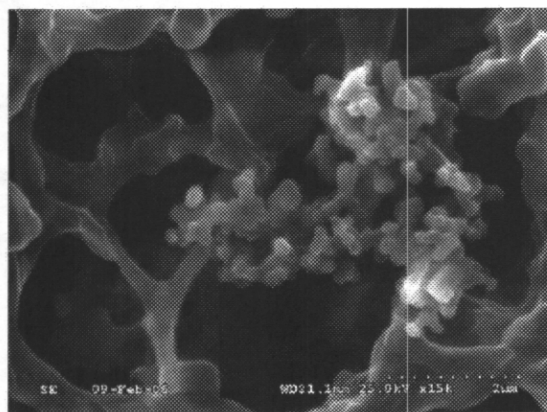

Fig. 2. Scanning electron micrograph of BeO particle on MCE filter (BeO-spiked filters courtesy of High-Purity Standards).

Table 2

Beryllium masses measured from MCE filters spiked with traces of BeO (prepared from slurries [13])

Beryllium loading level	Mass extracted by rotation at $\approx$23 °C ($n=4$)	Mass extracted by heating at $\approx$85 °C ($n=6$)
Media blank	N.D.[a]	N.D.
0.20 µg	0.135 µg ($\pm$0.0034)[b]	0.201 µg ($\pm$0.0074)
0.50 µg	0.365 µg ($\pm$0.040)	0.506 µg ($\pm$0.0068)

[a] None detected (<MDL).

[b] $\pm$Values are standard deviations.

The method detection limit was estimated by measuring a minimum of ten clean (unspiked) filters, and reporting the MDL as three times the standard deviation of repeat media blank measurements, in accordance with a consensus standard procedure [23]. This method of MDL estimation is intended to take into account the potential nonuniformity in blank sampling media. Results from these trials are presented in Table 3 for the three fluorometric instruments investigated in this work. Estimated MDLs for beryllium ranged from <0.8 ng per filter for the Turner instrument to $\approx$2 ng per filter for the Spex device, corresponding to solution beryllium concentrations of $\approx$0.03 to $\approx$0.08 µg L^{-1}. For purposes of comparison, it is noted that estimated MDLs for beryllium by ICP-MS of $\approx$0.03 µg L^{-1} been reported for environmental samples [6,24].

Potential interferences from a number of other metals have been investigated in previous work and have been found to be negligible, with the exception of iron and titanium dioxide [11]. In subsequent work, interference studies with iron and titanium dioxide were carried out at beryllium levels of 2–5 ng per filter, and with TiO_2 or Fe at 10-fold and 100-fold excess, respectively. Negative interference from TiO_2 observed initially after extraction was alleviated via additional filtering through nylon microsyringe filters: initial recovery of 83% ($\pm$4%) improved to 100% ($\pm$3%) after filtering. Negative interference from Fe seen at first was diminished after allowing the extract solution to sit for several hours to allow for precipitation of suspended hydrates: initial recovery of 75% ($\pm$6%) improved to 98% ($\pm$2%) after settling.

Analytical results from MCE and Whatman 541 filters spiked with beryllium at levels of 0–0.05 µg per sample are summarized in Table 4. Data were reported from four different laboratories using three different fluorescence instruments, and it is reiterated that spike levels were unknown to each analyst. The results of Table 4 show mean reported values that are close to the spike levels, and precision estimates (as measured by relative standard deviations [R.S.D.s]) range from $\approx$2–3% for levels of 0.02–0.05 µg Be, to $\approx$13–19% for MCE and Whatman 541 fil-

Table 3

Estimated method detection limits for beryllium in MCE and Whatman 541 filters from three different fluorometers

Fluorometer	MDL, MCE filters	MDL, Whatman 541 filters
Turner Quantech	0.00075 µg	0.00078 µg
Ocean Optics S2000-FL	0.0015 µg	0.0016 µg
Spex Fluorolog2	0.0019 µg	0.0021 µg

Table 4

Analysis results from MCE and Whatman 541 filters spiked with ultra-low levels of beryllium

Spike level (µg Be) ($n=6$)	Mean ($\pm$S.D.)[a] (µg Be)	R.S.D.[b] (%)
MCE filters		
Blank	N.D.[c]	–
0.002	0.0023 ($\pm$0.00030)	13
0.005	0.0052 ($\pm$0.00012)	2.3
0.020	0.0210 ($\pm$0.00055)	2.6
0.050	0.0504 ($\pm$0.0014)	2.8
Whatman 541 filters		
Blank	N.D.	–
0.002	0.0025 ($\pm$0.00048)	19
0.005	0.0056 ($\pm$0.00035)	6.3
0.020	0.0209 ($\pm$0.00049)	2.3
0.050	0.0507 ($\pm$0.00013)	2.6

Data are pooled from three different laboratories using three different fluorometers.

[a] Standard deviation.

[b] Relative standard deviation.

[c] None detected (<MDL).

ters spiked at 0.002 µg. Results from blanks were all below the MDL for each fluorometer. In consideration of the data shown in Tables 1–4, the fluorescence method demonstrates a dynamic range of over four orders of magnitude.

4. Discussion

GFAAS and ICP-AES techniques offer estimated MDLs of 0.005 and 0.009 µg Be per sample, respectively [25]. These MDLs are adequately low for beyllium measurements with reference to OELs that have been established by regulatory bodies such as the US Occupational Health and Safety Administration (OSHA) [26]. The OSHA beryllium permissible exposure limit (PELs) for 8 h time-weighted average (TWA) sampling is 2.0 µg m^{-3}, which translates to approximately 2 µg of beryllium per sample (assuming an 8 h sampling time and a sampling flow rate of about 2 L min^{-1}). This is a mass that is well greater than 10$\times$ the MDLs for GFAAS and ICP-AES, and can therefore be measured quantitatively by these techniques. Also, beryllium measurements pertaining to the OSHA permissible short-term exposure limit (STEL) of 5.0 µg m^{-3} do not require lower MDLs than those offered by GFAAS or ICP-AES. Assuming a 15 min minimum sampling time at 2 L min^{-1} typical sampling rate, the OSHA PEL (STEL) is about 0.15 µg of beryllium per sample, a mass that can still be quantified by GFAAS and/or ICP-AES.

Recently there has been a trend toward reduced airborne beryllium OELs, resulting in a need for even lower analytical method detection limits for this element. The US Department of Energy (DOE) promulgated an OEL for beryllium (8 h TWA) of 0.2 µg m^{-3}, and also established a surface beryllium contamination limit of 0.2 µg per 100 cm^2 of sampled surface area [27]. These limit values are nonetheless accessible by GFAAS and ICP-AES. Yet based on research suggesting beryllium sensitization and disease from extremely low exposures to this element [28], OSHA is considering a significant reduction in the beryllium permissible exposure limit (PEL) [29]. In 2005

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the American Conference of Governmental Industrial Hygienists (ACGIH) issued a Notice of Intended Change (NIC) to the beryllium Threshold Limit Value (TLV®), which entailed a 100-fold reduction in the TLV, from 2 to 0.02 $\mu g\,m^{-3}$ of sampled air [30]. Such a reduction in the limit value would necessitate a lower MDL than that offered by GFAAS or ICP-AES. A revised NIC was published by ACGIH a year later, which proposed TLVs for 8 h TWA and STEL sampling of 0.05 and 0.2 $\mu g\,m^{-3}$, respectively [31]. The use of ICP-MS or another highly sensitive technique such as the HBQS fluorescence method would be needed to achieve MDLs necessary for determination of the ultra-trace levels of beryllium that could be required in view of reduced OELs for this element.

It is of interest to investigate the ability of the HBQS fluorescence method to determine beryllium at the lowest potentially applicable OEL. Consider a hypothetical situation in which a personal air sample is obtained to measure beryllium in the workplace with respect to the proposed ACGIH STEL. If a 15 min sample is collected using a typical sampling flow rate of 2 L min^{-1}, the 0.2 $\mu g\,m^{-3}$ STEL would correspond to approximately 0.006 μg of beryllium. The results of Table 4 show R.S.D.s of about 2% for beryllium levels near this value, thereby demonstrating that beryllium can be measured quantitatively at this low level. In view of short-term exposure measurement, it is especially noteworthy that the NH_4HF_2 extraction/HBQS fluorometry method is field-portable, thereby allowing for on-site monitoring of beryllium with respect to the proposed STEL. The portable extraction/fluorescence method is also applicable to field-based measurement of trace beryllium on surfaces at levels well below the DOE action level of 0.2 μg Be per 100 cm^2 sample area. Although such low levels of beryllium can be measured by ICP-MS, this spectrometric technique is not field-portable.

Apart from issues of analytical sensitivity, it is also noteworthy that the use of 1% NH_4HF_2 and heating affords the ability to quantitatively extract beryllium from BeO (Tables 1 and 2), which is refractory. This obviates the need for the use of strong acid/high heat digestions (using, e.g. HF and H_2SO_4), which are required for BeO dissolution when using reference spectrometric methods. We also note that the BeO-spiked filters are useful as representative performance evaluation materials (PEMs), in that they present a more realistic and challenging matrix to laboratories. The morphology of the BeO particle shown in the SEM image (Fig. 2) is consistent with that of airborne particles of BeO fired at temperatures of >2000 °C [32]. It is planned to prepare BeO-spiked PEMs for proficiency testing programs, to which the NH_4HF_2 extraction/HBQS fluorescence method could be used as a recognized analytical test method by laboratory accreditation organizations such as the American Industrial Hygiene Association (AIHA) [33].

In conclusion, ultra-trace determination of beryllium can be carried out by means a field-portable extraction procedure, using fluorescence for detection. The method performance is comparable to that of ICP-MS, but fluorescence analyses can be carried out at a fraction of the cost of atomic spectrometry. Analytical attributes of the NH_4HF_2 extraction/HBQS fluorescence method are: (1) the estimated MDLs (<0.8 to ≈2 ng per sample) are comparable to those of ICP-MS; (2) high specificity

for beryllium, as evidenced by lack of interferences from other metals; (3) recoveries from refractory BeO are quantitative; and (4) the dynamic range extends over four orders of magnitude. Other key advantages of the technique include field portability, low cost (compared to, e.g. ICP-MS), and high throughput (≈30 samples per 90 min). If necessary in the future, even lower detection limits for beryllium in workplace samples should be attainable through the use of preconcentration techniques such as solid-phase extraction [34].

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