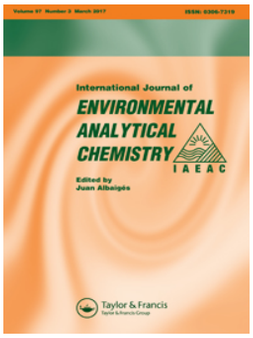

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ARTICLE

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Optical molecular fluorescence determination of ultra-trace beryllium in occupational and environmental samples using highly alkaline conditions

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ABSTRACT

Exposures to beryllium (Be), even at extremely low levels, can cause severe health effects in a percentage of those exposed; consequently, occupational exposure limits (OELs) promulgated for this element are the lowest established for any element. This work describes the advantages of using highly alkaline dye solutions for determination of Be in occupational hygiene and environmental samples by means of an optical molecular fluorescence technique after sample extraction in 1–3% ($w{\cdot}w^{-1}$) aqueous ammonium bifluoride (NH_4HF_2). Improved attributes include the ability to further enhance the detection limits of Be in extraction solutions of high acidity with minimal dilution, which is particularly beneficial when NH_4HF_2 solutions of higher concentration are used for extraction of Be from soil samples. Significant improvements in Be method detection limits (MDLs) are obtained at levels manyfold below those reported previously for this methodology. Notably, MDLs for Be of <0.01 ng L^{-1} /0.1 ng per sample have been attained, which are superior to MDLs routinely reported for this element by means of the most widely used ultra-trace elemental measurement technique, inductively coupled plasma mass spectrometry (ICP-MS). Very low MDLs for Be are essential in consideration of reductions in OELs for this element in workplace air by health organisations and regulatory agencies in the USA and internationally. Applications of enhanced Be measurements to air filter samples, surface wipe samples, soils and newly designed occupational air sampler inserts are illustrated.

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KEYWORDS
Beryllium; extraction; fluorescence; occupational hygiene; soils; surface dust; ultra-trace analysis; workplace air

1. Introduction

Beryllium (Be) is an extremely useful but highly toxic element that is a key constituent in numerous materials such as high-performance ceramics, composites and specialty alloys. Be-containing components have myriad industrial, energy and defence uses including applications in microelectronic circuitry, medical devices, nuclear reactors, aerospace and even sports equipment. In the USA, it is estimated that about 35,000 workers are exposed to Be during their work activities [1]. Exposure by inhalation or surface contact can cause immune sensitization in a percentage of those exposed. Once sensitised,

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succeeding exposure by inhalation can lead to chronic beryllium disease, a debilitating, incurable and potentially fatal lung disease. Exposure to airborne Be may also cause lung cancer [1]. Consequently, there are efforts to prevent occupational exposures to Be through safety and health recommendations and regulations, which in turn raise challenges concerning measurement performance requirements for sampling and determination of trace amounts of Be in workplaces.

Accurate measurement of Be concentrations at ultra-trace levels in work air samples is necessary in consideration of the very low occupational exposure limits (OELs) that have been promulgated for this element [2,3]. It has been shown in previous work that a molecular fluorescence detection technique using hydroxybenzoquinoline sulfonate (HBQS) as fluorophore [4], after sample dissolution in dilute ammonium bifluoride (NH_4HF_2) [5], offers method detection limits (MDLs) which are low enough to allow for accurate Be measurements at levels well below current OELs in the USA and Europe [6,7]. Adequately low Be MDLs for current OELs in the USA are also provided by graphite furnace atomic absorption spectrometric [8] and inductively coupled plasma mass spectrometric (ICP-MS) [9] methods after digestion of samples in appropriate dissolution reagents [10].

All of the previous work on the use of HBQS fluorescent dye was based on the report from Matsumiya et al. [4] Those workers demonstrated that it is important that the pH of the dye solution and the sample solution be about 12.2 in order to obtain a high fluorescence signal. If the pH increased slightly, there was a rapid decrease of fluorescent intensity. In addition, Matsumiya et al. [4] also showed that the fluorescent intensity decreases below pH 12.2, and this decrease continues to a pH of about 4.5, below which there is no signal observed above the background. Thus, the previous work using dilute ammonium bifluoride simplified the method by adding lysine to the dye solution (a buffer) so that when the dye solution was mixed with acidic solution (dilute ammonium bifluoride) containing the extracted beryllium, there was minimal pH shift, thus there was no need for extra titration steps to bring the pH back into the desired narrow range near 12.2. From this, we postulated that there was no need of buffer if we could maintain a highly alkaline pH by adding more base. As is discussed in this paper, adding more base had many benefits: first, one could dissolve the samples in more acidic solutions and the higher alkalinity of the dye solutions still preserved a high enough pH of the mixture to provide good fluorescence signal for beryllium quantification; second, one could mix higher amounts of the sample solution relative to the dye solution to obtain superior detection limits and third, since lysine comes from natural sources and sometimes it can have naturally fluorescing organic contaminants, its elimination simplified the process of making the dye solution and lowered the background signal. The aim of this paper was to experimentally demonstrate that removing lysine and increasing alkalinity of the dye solution significantly benefits the method.

Since even further improvements in MDLs for airborne Be are desired in anticipation of decreasing OELs for this element, including significantly reduced short-term exposure limits (STELs), the above improvements, if borne out, can be of high importance. For instance, the US Occupational Safety and Health Administration (OSHA) has established a tenfold reduction in the permissible exposure limit (PEL), to 0.2 μg m^{-3} as an 8-h time-weighted average (TWA) [11], which is in the neighbourhood of the OEL for Be promulgated previously by the US Department of Energy (DOE) [12]. OSHA has also established

a STEL (15 min) that is on the order of its previous PEL. The American Conference of Governmental Industrial Hygienists Threshold Limit Value (TLV®) for Be is 0.05 μg m^{-3} as an 8-h TWA (inhalable particles) [2]. Outside of the USA, the lowest Be OELs have been promulgated in Germany [3]: 0.14 μg m^{-3} (inhalable particles) /0.06 μg m^{-3} (respirable particles); these figures apply to 2-h TWA sampling as well as a 15-min STEL. In consideration of the above OELs for Be and air sample collection flow rates of 2–10 l min^{-1} over time durations as little as 15 min, MDLs in the sub-nanogram per sample range are required to allow for quantitative measurements of Be in air at these very low levels.

Apart from air samples, there is a need for accurate measurements of trace levels of Be in other occupational and environmental matrices such as surfaces, dusts, soils, etc. [13]. Regulatory limits for Be on surfaces (of equipment, workrooms, etc.) have been established by DOE, with 0.2 μg Be per 100 cm^2 sampling area as the lowest limit for demonstration of a contamination-free work environment and for purposes of equipment release [12]. Consequently, methods for sampling Be in surface dust have been developed, evaluated, validated and standardised [14–16]. Measurement of trace Be in soils is also important as this enables assessment of potential anthropogenic sources of suspected Be-contaminated environments [17–19]. Molecular fluorescence detection after dilute NH_4HF_2 extraction with heating has been shown to yield Be trace measurement results that are comparable to data obtained from atomic spectrometric determination following strong acid digestion [17,20].

This work describes further improvements to the dilute NH_4HF_2 extraction/HBQS fluorometric detection method, with demonstrated successful applications to ultra-trace Be measurements in air filters, surface wipe samples, soils and newly available occupational air sampler inserts. It is shown that the use of highly alkaline HBQS dye solution for Be determination by fluorescence results in significant improvement in MDLs for occupational hygiene and environmental samples.

2. Experimental

Ethylenediaminetetraacetic acid disodium salt dehydrate (EDTA, ≥99%), ferric chloride hexahydrate (reagent grade) and L-lysine monohydrochloride (lysine, ≥98%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ammonium bifluoride (NH_4HF_2, ≥98%) was purchased from Fisher Scientific (Hampton, NH, USA). HBQS was prepared and purified after the procedure of Matsumiya et al. [4] from HBQ precursor obtained from Sigma-Aldrich. Aqueous beryllium standard solution (1000 μg mL^{-1}) came from Spex Certiprep (Metuchen, NJ, USA). Sodium hydroxide solution (2.5 M), plastic centrifuge tubes (15 ml and 50 ml) and plastic syringes with Luer-lock apparatus (5 ml and 10 ml) were obtained from Fisher Scientific. GH Polypro™ (GHP) hydrophilic polypropylene Acrodisc® syringe filters (25-mm dia., 0.2-μm pore size) and nylon bottletop filters (500-ml capacity, 0.2-μm pore size) were purchased from Pall Corporation (Port Washington, NY, USA). Mixed-cellulose ester (MCE) filters (37-mm dia., 0.8-μm pore size) were obtained from SKC (Eighty-Four, PA, USA) and cellulosic cassette inserts (Solu-Serts®) were provided by Zefon International (Ocala, FL, USA). Whatman 541 filters (47-mm diameter) were obtained from Sigma-Aldrich. Typically, MCE filters are used for air sampling and Whatman filters have been used for surface sampling. Certified MCE filters

[21] spiked with known quantities of aqueous beryllium standards (diluted from 1000 µg mL^{-1} standard solution) and National Institute of Standards and Technology Standard Reference Material (NIST SRM®) 1877 beryllium oxide (NIST, Gaithersburg, MD, USA) were provided by High-Purity Standards (North Charleston, SC, USA). Certified reference material (CRM) soils with established beryllium contents were obtained from NIST and from the Canadian Certified Reference Materials Project (CCRMP, Ottawa, ON, Canada). Mechanical pipets (with plastic pipet tips to fit) of various sizes, used in carrying out most experiments, were purchased from Eppendorf (Hamburg, Germany).

Measurements of pH were conducted using an Orion 290A+ meter (Thermo Electron, Beverly, MA, USA) calibrated using buffer standards of pH 4.0, 7.0, 10.0 and 13.0. Standard solutions of pH 4, 7 and 10 were obtained from ACROS (Geel, Belgium) and that for pH 13 was obtained from Ricca Chemical (Arlington, TX, USA). Deionised (18 MΩ-cm resistivity) water (DI), used in all experiments, was prepared using a Barnstead® purification system (Thermo Fisher Scientific, Waltham, MA, USA). Where necessary for sample agitation, a Labquake® rotator (Thermo Fisher) was used for this purpose.

HBQS fluorometric dye solution containing lysine was made following the procedure outlined in ASTM D7202 [22]: Lysine, 19.508 g (intended for high-pH buffering) [23], 2.208 g of EDTA and 0.0382 g of HBQS were added to 1800 ml of DI water. This mixture was stirred at room temperature until all constituents had dissolved; the pH of the resultant solution was 4.65. For adjustment of the pH to alkaline conditions, to this solution was added 150 ml of 2.5 M NaOH; the measured pH of the resulting mixture was 12.77. An additional 17 ml of 2.5 M NaOH was added stepwise to bring the pH to 12.86; finally to this 33 ml of DI water was added and the final pH remained at 12.86. The solution was then filtered through a 2-µm GHP polypropylene filter. A highly basic dye solution without lysine was prepared as follows: 1.104 g of EDTA, 0.019 g of HBQS and 900 ml of DI water were stirred at room temperature until a clear yellow solution was obtained. To this, 114.5 ml of 2.5 M NaOH was added. After mixing, a clear yellow solution was obtained; this had a measured pH of 13.17. The resultant solution was then filtered through a 2-µm filter as above. The concentration of the HBQS dye in solutions with and without lysine was the same, at 61 µM. These dye solutions were found to be stable (by pH and fluorescence measurements) for at least 12 months.

For fluorescence measurements, 2–4 ml of analyte solution was placed into 10-mm path length plastic cuvettes with transmittance >330 nm (Sarstedt, Nümbrecht, Germany). Fluorescence measurements with excitation at λ of 365 nm or 384 nm were carried out using a Modulus fluorometer (Turner Biosystems, Sunnyvale, CA, USA). Some experiments using excitation at λ = 384 nm were conducted on a RF 5301PC Spectrofluorometer (Shimadzu Scientific, Columbia, MD, USA). Fluorescence emission was monitored at the maximum for the HBQS-Be adduct of 480 (±5) nm [4].

3. Results and discussion

To investigate optimised analytical conditions, a suite of experiments was carried out in order to characterise the performance of the ultra-trace Be fluorescence measurement methodology in lysine-free and lysine-containing dye solutions [22,24,25]. Influence on background fluorescence signal was studied and a battery of analytical tests on representative samples of interest in occupational and environmental hygiene was carried

out. Of particular interest were comparisons of the analysis results obtained between HBQS dye with and without lysine described above, as well as performance studies on a new workplace air sampling apparatus (i.e. a cellulosic sampler insert) [26].

Solutions were made up in DI water with varying NH_4HF_2 contents ranging from 1% to 20% ($w \cdot w^{-1}$). In an initial experiment, these NH_4HF_2 solutions were added to the two dye solutions prepared above in a volumetric ratio of 1:19 (20× dilution) and the resulting pH was measured (Figure 1). It can be seen that the pH of the solution mixtures (measurement solutions) made using dye solution with lysine fell below 12 when these were mixed with 6% NH_4HF_2 solution, whereas the pH for lysine-free dye solution remained above 12 for solutions containing up to 17% NH_4HF_2. The lysine-free dye solution was able to tolerate more added acid (i.e. NH_4HF_2) as compared with the solution containing lysine, which is a triprotic amino acid ($pK_1 \approx 2.2$, $pK_2 \approx 9.0$, $pK_3 \approx 10.5$) [27]. The results of Figure 1 show that lysine does not provide effective high-pH buffering properties, as has been proposed [23]; on the contrary, better acid tolerance is observed and high pH is maintained when lysine is absent. Both the NH_4HF_2 and lysine neutralise the hydroxide used in the preparation of the dye solutions, such that more NH_4HF_2 can be added to lysine-free solutions before there are any significant changes in pH. These results are important analytically as it is desired to maintain very high pH for optimal fluorescence of the HBQS-Be adduct [4]. It is also shown that the addition of lysine is not only unnecessary but can also be disadvantageous when the dye solution is challenged with acidic sample extract.

The above solutions with varying percentages of NH_4HF_2, but containing 4 μg L^{-1} of beryllium in the final mixture (for analysis), were prepared. These solutions were subjected to fluorescence measurements, with results (uncorrected for background) shown in Figure 2. It can be seen that the fluorescence signal remains high for lysine-free solutions at much higher NH_4HF_2 content vs. HBQS dye solutions containing lysine. Figure 3 shows the dependence of fluorescence intensity on the pH of dye solutions with and without beryllium. The results from both lysine-containing and lysine-free dye solutions do not show a rapid decrease in fluorescence intensity above a pH of about

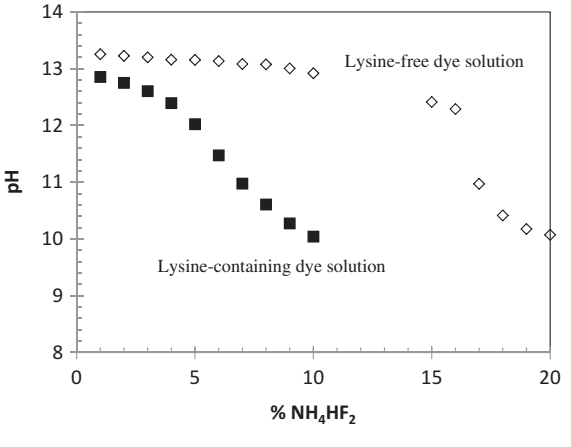

Figure 1. Effect of NH_4HF_2 solution concentration on pH when mixed with HBQS dye solution at a ratio of 1:19. Open diamonds: lysine-free dye solution; closed squares: dye solution contains lysine.

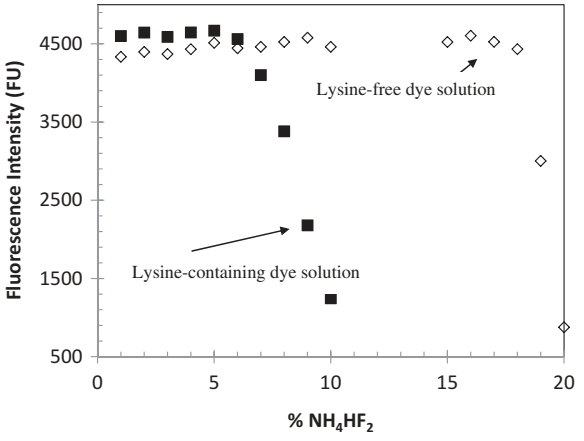

Figure 2. Effect of NH_4HF_2 solution concentration on HBQS-Be fluorescence intensity when mixed with the dye solution at a ratio of 1:19 (4 µg L^{-1} beryllium). Open diamonds: lysine-free dye solution; closed squares: dye solution contains lysine.

12.6. A rapid drop in fluorescence intensity is observed at a pH value of about 11 and below (Figure 3). For comparison, the change in fluorescence with pH data from Matsumiya et al. [4] is shown in Figure 3. (Since this is relative fluorescent intensity, the data of Matsumiya et al. [4] were normalised so that peak intensity of their results coincide with the maximum of our data shown in this figure.) When we carried out more detailed analysis, we observed a very different trend between data from this study and the results of Matsumiya et al. First there was no sharp drop-off the fluorescent intensity at higher alkalinity (i.e. higher than 12.2), and second, that there was no discernible fluorescent signal below pH 10, and thus the method is not practically usable below pH

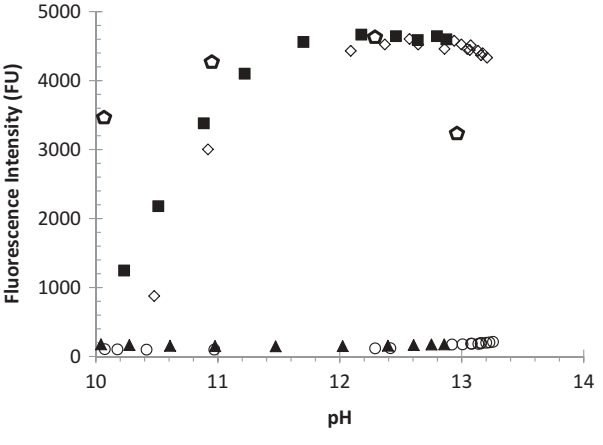

Figure 3. Effect of pH on fluorescence intensity when mixed with the dye solution at a ratio of 1:19 (with and without 4 µg L^{-1} Be). Open circles and diamonds: lysine-free dye solution (blank solution and Be-containing, respectively); closed triangles and squares: dye solution contains lysine (blank and containing Be, respectively). Open pentagons represent data from Reference 4 (Matsumiya, et al.).

11; preferably the pH should be at or greater than 11.5. As a practical guideline, the pH of the mixture of the acidic extraction solution and the dye solution should be about 12 or higher for effective determination of beryllium by this method. It is noted (Figure 2) that the pH drop in lysine-free solution occurs around 19% NH_4HF_2, whereas the same happens at about 7% NH_4HF_2 for solution containing lysine. This demonstrates that lysine-free HBQS dye solution can be used with highly acidic starting solutions, a decided advantage for certain samples requiring stronger NH_4HF_2 concentrations for effective extraction of Be prior to fluorescence analysis. In all cases, the fluorescence intensity resulting from the HBQS-Be adduct when using excitation at 384 nm was about double that obtained with excitation at 365 nm.

Dye solutions described above (i.e. with and without lysine) were mixed with 1% and 3% NH_4HF_2 solutions at 20× and 5× dilution and the resultant pH values were measured (Table 1). The reason for the choice of these dilutions and concentrations of NH_4HF_2 solutions is that such conditions have been used to analyse surface wipe and air filter samples for Be content, which are typically extracted in 1% NH_4HF_2 [5,6], as well as soil samples, where 3% NH_4HF_2 is used for Be extraction [17,20]. Usage of highly alkaline solutions allows for the use of 5× dilution with 3% NH_4HF_2 and hence enables Be to be detected with higher sensitivity when these solutions are used for extracting Be into an aqueous phase. One may even increase the alkalinity of the dye solutions further for applications with yet higher concentration of NH_4HF_2 and/or lower dilutions. When the fluorescence at 480 nm of the above solutions was monitored as a function of [Be] over the range 0–20 μg L^{-1}, it was found that the sensitivity of the method was greater when using lysine-free solution vs. solution containing lysine only because in the former a higher pH could be maintained. This is a benefit of the use of highly alkaline conditions *sans* lysine throughout the measurement process.

Method detection limits (MDLs) for Be were estimated by analysing 10 blank media and reporting the MDL as three times the standard deviation of the mean blank signal [22]. Calibration solutions were made with known amounts of Be in 1% NH_4HF_2 solutions and were mixed with either dye solution using 20×, 5× or 3× dilution. Batches of filters were prepared and analysed in the presence of soluble Be [5] at low concentrations (0.05–2 μg L^{-1}). Also, filters spiked with high-fired beryllium oxide (BeO) were subjected to sample preparation and fluorescence analysis. The certified values for Be on BeO-spiked filters were within ±15% for all spiking levels. The filters were placed into 15-ml plastic centrifuge tubes with 5 ml of 1% NH_4HF_2 solution, capped and heated for 1 h at 90°C to extract Be into the dilute aqueous NH_4HF_2 solution. Solutions were analysed by mixing with highly alkaline lysine-free dye solution and also with dye solution which

Table 1. Measurements of pH at 20×, 5× and 3× dilutions with 1% and 3% NH_4HF_2 (aqueous) when mixed with HBQS dye solution with and without lysine.

NH_4HF_2 (%, w·w^{-1})	Dilution ratio	pH of lysine-containing HBQS solution	pH of lysine-free HBQS solution
1	20×	12.8	13.2
1	5×	12.1	13.1
1	3×	**9.4**	12.5
3	20×	12.6	13.1
3	5×	**9.4**	12.2
3	3×	**5.6**	**8.7**

pH values for which the method will not be sensitive for beryllium determination are bolded.

contained lysine. Analyses of Be-spiked media were done at least in triplicate using dilutions of 5× and 20× (and in some cases 3×) in accordance with procedures delineated in ASTM D7202 [22]. Analysis results for Whatman 541 filters are shown in Table 2 and data for MCE filters are presented in Table 3.

In another set of experiments, 37-mm diameter cellulosic sampler inserts consisting of MCE filters melded to cellulose acetate housings (for insertion into 37-mm air-sampling cassettes: Solu-Serts) [28] were tested. The Solu-Serts were spiked using predetermined quantities of NH_4HF_2 solutions containing soluble beryllium and dried. Blank media as well as spikes were evaluated in a similar manner as that described earlier for surface sampling media. These cassette inserts were folded and inserted into 15-ml centrifuge tubes (in a similar fashion as was done for Whatman and MCE filters) and extracted in 5 ml of 1% NH_4HF_2 solution at 90°C for 60 min before being allowed to cool to room temperature. Fluorescence at 480 nm was measured after mixing these extract solutions with the lysine-free HBQS dye by a 3× dilution factor. The fluorescence values were evaluated against two calibration curves for low-level calibration standards ('Low Cal-1' which used 0, 0.05, 0.1, 0.2 and 0.8 μg L^{-1} of Be as calibration standards after mixing the dye and the standard solution and 'Low Cal-2' which used 0, 0.2, 0.5, 0.8 and 4 μg L^{-1} of Be); the results are shown in Table 4. The fluorescence values were corrected for any background contributed by the blank sampling media. Optimal results were found using the 'Low Cal-2' calibration curve (Table 4) and show that the method may be used to quantify to as low as 0.5 ng Be per sample in cellulosic sampler inserts.

Table 5 shows results on MCE filters spiked with high-fired BeO. The spiked filters were obtained commercially. Beryllium was extracted in 5 ml of 1% NH_4HF_2 solutions as described earlier. The table shows the nominal values of Be (values supplied by the commercial supplier) and the expected nominal concentration of beryllium in solution (assuming 100% recovery) after mixing a portion of the extracted NH_4HF_2 solution with the dye solution in a dilution ratio of 3×. For calibration, solutions were made with soluble beryllium in 1% NH_4HF_2 solutions after mixing them with the dye solutions in a ratio of 1:2 (3× dilution). Beryllium in the calibration solutions was 0, 0.15, 0.3, 0.6 and 2.4 μg L^{-1}. The results on the spiked samples in Table 5 show excellent agreement with the expected values.

Experiments were carried out to investigate if there would be any effect of interference from other metals (besides Be) when using highly alkaline solutions. Iron (Fe) was chosen for these trials as it has been shown in the past that this element (and also

Table 2. Analytical figures of merit for fluorescence analysis of Whatman 541 filters (media blanks and in 0.05–2 μg L^{-1} [Be]) after extraction in 1% (aqueous) NH_4HF_2.

	HBQS dye solution with lysine		HBQS dye solution without lysine	
Dilution factor	20×	5×	20×	5×
Average media blank fluorescence reading ± Std. dev. (n = 10)	193 ± 4.2	264 ± 9.1	225 ± 3.5	233 ± 13
Calibration standards, μg L^{-1} Be	0, 0.05, 0.2, 0.5, 2	0, 0.05, 0.1, 0.2, 0.8	0, 0.05, 0.2, 0.5, 2	0, 0.05, 0.1, 0.2, 0.8
Calibration fit, slope	1102	1141	1021	1076
Calibration fit, intercept	174	147	207	169
Calibration fit, R^2	0.9999	0.9998	1.0000	0.9997
MDL, μg L^{-1} Be	0.0096	0.0337	0.0123	0.0255
MDL, μg Be	0.00096	0.00084	0.0012	0.00064

Table 3. Analytical figures of merit for fluorescence analysis of MCE filters (media blanks and in 0.05–2 µg L^{-1} [Be]) after extraction in 1% (aqueous) NH_4HF_2.

	HBQS dye solution with lysine		HBQS dye solution without lysine		
Dilution factor:	20×	5×	20×	5×	3×
Avg. media blank fluorescence reading ± Std. dev. (n = 10)	211 ± 2.5	179 ± 7.5	179 ± 2.1	161 ± 5.7	150 ± 2.5
Calibration stds. µg L^{-1} Be	0, 0.05, 0.2, 0.5, 2	0, 0.05, 0.1, 0.2, 0.8	0, 0.05, 0.2, 0.5, 2	0, 0.05, 0.1, 0.2, 0.8	0, 0.05, 0.1, 0.2, 0.8
Calibration fit, slope	1021	1076	1102	1141	1225
Calibration fit, intercept	207.0	169.2	173.8	146.5	118.8
Calibration fit, R^2	1.0000	0.9997	0.9999	0.9998	0.9999
MDL, µg L^{-1} Be	0.0072	0.021	0.0056	0.015	0.0062
MDL, µg Be	0.00072	0.00054	0.00056	0.00037	0.000090

Table 4. Fluorescence analysis of soluble beryllium-spiked cellulosic filter cassette inserts (Solu-Serts) using 3× dilution.

[Be], µg L^{-1}, after mixing extract with dye solution; nominal µg Be per filter	[Be] measured (µg L^{-1}) using 'Low Cal-1' ± Std. dev., n = 3	µg Be measured using 'Low Cal-1'	% Recovery	[Be] measured (µg L^{-1}) using 'Low Cal-2' ± Std. dev., n = 3	µg Be measured using 'Low Cal-2'	% Recovery
–0-; –0- (media blank)	–0.014 ± 0.0025	–0.0002	–	–0.004 ± 0.0025	–0.0001	–
0.033; 0.0005	0.024 ± 0.0025	0.00036	72%	0.033 ± 0.0024	0.0005	99%
0.067; 0.001	0.053 ± 0.0017	0.0008	79%	0.061 ± 0.0017	0.0009	92%
0.133; 0.002	0.127 ± 0.0072	0.0019	95%	0.133 ± 0.0071	0.0020	100%
0.333; 0.005	0.298 ± 0.0043	0.0045	90%	0.301 ± 0.0042	0.0045	90%
3.334; 0.050	3.098 ± 0.069	0.0464	93%	3.043 ± 0.0675	0.0456	91%
32.00; 0.480	32.01 ± 0.27	0.457	95%	29.87 ± 0.26	0.448	93%

to some extent titanium) can cause interference to the fluorescence measurement of the HBQS-Be adduct by imparting a yellow colour to the measurement solution [5]. This interferes with fluorescence measurement and biases the results towards lower amounts of measured beryllium [29]. The yellowness in the solutions may be removed by immediate filtering through GHP hydrophilic polypropylene filters of pore size 0.2 µm or finer; alternatively, it is an option to wait for at least 2 h for these metal impurities to settle out before filtering. Solutions were made up with beryllium and ferric chloride hexahydrate (as a source of Fe) in dilute NH_4HF_2 (1% and 3% NH_4HF_2 in water) and then mixed with the appropriate dye solution (lysine free or with lysine) using 5× and 20× dilution. In one set the solutions were immediately filtered through Acrodisc GHP 25-mm syringe filters with hydrophilic polypropylene 0.2 µm membrane. Another set of solutions was left standing for 2 h and then filtered using similar filters. Control samples with the same concentration of beryllium but without Fe were also measured. In each series (i.e. for a specific dye solution used, NH_4HF_2 concentration and dilution factor) the fluorescence intensity was normalised to the sample without Fe within the same series. In all of the solutions (mixtures of the dye and the sample solutions) the Be concentration was 0.1 µg L^{-1}. The Fe concentration in solutions with 20× dilution was 1.1 mg L^{-1} and for 5× it was 4.4 mg L^{-1}. In each case, the Fe concentration was more than 10,000 times the Be concentration. The results of these experiments are shown in Table 6 and demonstrate that Fe interference to Be fluorescence measurement can be effectively eliminated in lysine-free (as well as lysine-containing [5]) HBQS dye solutions.

Experiments were conducted on representative CRM soils to investigate the performance of the two dye solutions (i.e. with lysine and lysine free) as made up above and in

Table 5. Analysis of high-fired beryllium oxide spiked MCE filters using 3× dilution ratio.

Nominal mass of beryllium on filter (µg) and [Be] (µg L^{-1}) after mixing extract with dye solution	[Be], µg L^{-1} measured (±Std dev., n = 3)	µg Be measured	% Recovery
0.0000, 0.000	0.000 ± 0.0008	0.0000	–
0.0005, 0.033	0.029 ± 0.0012	0.0004	89%
0.001, 0.067	0.059 ± 0.0016	0.0009	88%
0.002, 0.133	0.121 ± 0.0027	0.0018	91%
0.005, 0.333	0.289 ± 0.0009	0.0043	87%
0.050, 3.33	2.98 ± 0.037	0.0446	89%
0.480, 32.01	27.9 ± 0.23	0.418	87%

Table 6. Treatment of iron interference on beryllium measurements; [Fe] (as ferric chloride) >10,000× [Be].

	HBQS dye solution containing lysine				HBQS dye solution without lysine			
% NH_4HF_2 (aqueous, w·w^{-1})	1%		3%		1%		3%	
Dilution factor	20×	5×	20×	5×	20×	5×	20×	5×
Relative fluorescence intensity, no Fe	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Relative fluorescence intensity (Fe present), filtered immediately	100.1	93.1	98.6	96.2	99.9	100.1	97.1	100.9
Relative fluorescence intensity (Fe present), filtered after 2 h	100.8	99.3	102.3	97.5	99.9	102.2	100.7	99.8

accordance with ASTM D7458 [30]. NIST SRM 2710, Montana soil ([Be] = 2.5 µg g^{-1}) and CCRMP Till-1 soil ([Be] = 2.4 µg g^{-1}) were analysed for beryllium content in the following manner. The extraction solutions (using 3% aqueous NH_4HF_2) used in this analysis were the same as used in previously published studies [17,20]. Each sample (0.5 g) was extracted in 50 ml 3% NH_4HF_2 at 90°C for 40 h. Three aliquots of each extracted solutions were mixed with the respective dye solutions at a 5× dilution ratio. The pH of the dye solution with lysine mixed with the sample solution was 9.4, while that for highly alkaline, lysine-free solutions was 12.4. Measured [Be] values (n = 3) using the high-pH mixture were 2.49 ± 0.014 µg g^{-1} and 2.04 ± 0.046 µg g^{-1} (99.5% and 84.9% recovery, respectively), while the low-pH (lysine-containing) dye solution yielded Be measurement results for both soils that were below the MDL. These trials demonstrate that the use of highly alkaline conditions can enable the measurement of low levels of beryllium in bulk environmental samples such as soils.

4. Conclusions

The experiments described herein show that there are several advantages in using highly alkaline, lysine-free HBQS dye solutions for trace and ultra-trace measurement of Be in occupational and environmental samples. Some of the important advantages include: (a) facile preparation of HBQS dye solutions *sans* lysine, which is not only superfluous but can also be deleterious to high-pH ultra-trace Be fluorometric analysis; (b) solutions of higher acidity can be used to extract Be from refractory and silicate materials, without affecting analytical sensitivity; (c) standard acidic solutions used for analysis of surface wipes and air samples containing 1% NH_4HF_2 can be mixed with the dye solutions in 3× dilution to enhance the MDLs of Be to below 0.1 ng (and quantification from <0.5 ng); (d) these dye solutions may be used to enhance the MDLs of Be in

bulk sample analysis such as soils by using 5× dilution where 3% NH_4HF_2 solutions are used for extraction and (e) interference from Fe is effectively eliminated. The results are important in consideration of very low OELs for Be that have been established globally and in newly promulgated regulations in the USA. The extremely low MDLs for Be will enable short-term airborne workplace exposures to be reliably monitored by means of a field-portable technique. The method offers promise for applications to a wider range of acidic extraction solutions, which could potentially be used to extract Be from challenging metallic samples such as aluminium and specialty alloys as well as ceramic materials.

5. Disclaimers

Mention of any company or product names does not constitute endorsement by the National Institute for Occupational Safety and Health (NIOSH). In addition, citations to websites external to NIOSH do not constitute NIOSH endorsement of the sponsoring organisations or their programmes or products; furthermore, NIOSH is not responsible for the content of these websites. All web addresses referenced in this document were accessible as of the submission date of the original manuscript. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of NIOSH. This article was prepared in part by the US Government employees as an element of their official duties and legally may not be copyrighted in the USA.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

[1] US Occupational Safety and Health Administration (OSHA), *Safety and Health Topics: Beryllium*, (OSHA, Salt Lake City, 2015). <www.osha.gov/SLTC/beryllium/index.html>. (14 February 2017).
[2] American Conference of Governmental Industrial Hygienists (ACGIH), *Threshold Limit Values and Biological Exposure Indices* (ACGIH, Cincinnati, 2016).
[3] Institut für Arbeitsschutz der Deutschen Gesetzlichen Unfallversicherung (IFA), *GESTIS International Limit Values for Chemical Agents: Occupational Exposure Limits*, (IFA, Sankt Augustin, 2017). <www.dguv.de/ifa/en/gestis/limit_values>. (21 February 2017).
[4] H. Matsumiya, H. Hoshino and T. Yotsuyanagi, Analyst **126**, 2082 (2001). doi:10.1039/b102300b.
[5] A. Agrawal, J. Cronin, J. Tonazzi, T.M. McCleskey, D.S. Ehler, E.M. Minogue, G. Whitney, C. Brink, A.K. Burrell, B. Warner, M.J. Goldcamp, P.C. Schlecht, P. Sonthalia and K. Ashley, J. Environ. Monit. **8**, 619 (2006). doi:10.1039/b601524g.
[6] K. Ashley, A. Agrawal, J. Cronin, J. Tonazzi, T.M. McCleskey, A.K. Burrell and D.S. Ehler, Anal. Chim. Acta. **584**, 281 (2007). doi:10.1016/j.aca.2006.11.066.
[7] K. Ashley, J. Chem. Health Saf. **18**, 26 (2011). doi:10.1016/j.jchas.2011.01.015.
[8] US National Institute for Occupational Safety and Health (NIOSH), in *NIOSH Manual of Analytical Methods*, 4th ed. edited by P.C. Schlecht and P.F. O'Connor (NIOSH, Cincinnati, 2003).
[9] K. Ashley, M.J. Brisson, A.M. Howe and D.L. Bartley, J. Occup. Environ. Hyg. **6**, 745 (2009). doi:10.1080/15459620903022605.
[10] K. Ashley and T.J. Oatts, in *Beryllium: Environmental Analysis and Monitoring*, edited by M.J. Brisson and A.A. Ekechukwu (Royal Society of Chemistry, Cambridge, 2009).

[11] OSHA, *Occupational Exposure to Beryllium; Final Rule*, Fed. Regist. **82**, 2470 (2017).
[12] US Department of Energy (DOE), *Chronic Beryllium Disease Prevention Program; 10 CFR Part 850* (DOE, Washington, 1999).
[13] K. Ashley, editor, *Beryllium: Sampling and Analysis; ASTM STP 1473* (ASTM International, West Conshohocken, 2006).
[14] G. Rondeau, in *Beryllium: Environmental Analysis and Monitoring*, edited by M.J. Brisson and A. A. Ekechukwu (Royal Society of Chemistry, Cambridge, 2009).
[15] K. Ashley, G. Braybrooke, S.D. Jahn, M.J. Brisson and K.T. White, J. Occup. Environ. Hyg. **6**, D97–D100 (2009). doi:10.1080/15459620903022597.
[16] K.L. Creek, G. Whitney and K. Ashley, J. Environ. Monit. **8**, 612 (2006). doi:10.1039/b601572g.
[17] A. Agrawal, J.P. Cronin, A. Agrawal, J.C.L. Tonazzi, L. Adams, K. Ashley, M.J. Brisson, B. Duran, G. Whitney, A.K. Burrell, T.M. McCleskey, J. Robbins and K.T. White, Environ. Sci. Technol. **42**, 2066 (2008). doi:10.1021/es702481h.
[18] A. Kabata-Pendias, *Trace Elements in Soils and Plants*, 4th ed. (Chap. 7). (CRC Press, Boca Raton, 2010).
[19] T.M. McCleskey, in *Beryllium: Environmental Analysis and Monitoring*, edited by M.J. Brisson and A.A. Ekechukwu (Royal Society of Chemistry, Cambridge, 2009).
[20] J.P. Cronin, A. Agrawal, L. Adams, J.C.L. Tonazzi, M.J. Brisson, K.T. White, D. Marlow and K. Ashley, J. Environ. Monit. **10**, 955 (2008). doi:10.1039/b804313b.
[21] T.J. Oatts, C.E. Hicks, A.R. Adams, M.J. Brisson, L.D. Youmans-McDonald, M.D. Hoover and K. Ashley, J. Environ. Monit. **14**, 391 (2012). doi:10.1039/C1EM10688K.
[22] ASTM D7202, *Standard Test Method for Determination of Beryllium in the Workplace by Extraction and Optical Fluorescence Detection* (ASTM International, West Conshohocken, 2014).
[23] E.M. Minogue, D.S. Ehler, A.K. Burrell, T.M. McCleskey and T.P. Taylor, J. ASTM Int. **2** (9), 9 (2005). doi:10.1520/JAI13168.
[24] NIOSH, Method No. 7704 in *NIOSH Manual of Analytical Methods*, 5th ed. edited by K. Ashley and P.F. O'Connor (NIOSH, Cincinnati, 2016).
[25] NIOSH, Method No. 9110 in *NIOSH Manual of Analytical Methods*, 5th ed. edited by K. Ashley and P.F. O'Connor (NIOSH, Cincinnati, 2016).
[26] M. Harper and K. Ashley, J. Occup. Environ. Hyg. **10**, 297 (2013). doi:10.1080/15459624.2013.777310.
[27] D.L. Nelson and M.M. Cox, *Lehninger Principles of Biochemistry*, 5th ed. (W. H. Freeman & Co., New York, 2008).
[28] R.N. Andrews, H.A. Feng and K. Ashley, J. Occup. Environ. Hyg. **13**, 40 (2016). doi:10.1080/15459624.2015.1072635.
[29] A. Agrawal, L. Adams, A. Agrawal, J.P. Cronin, J.C.L. Tonazzi and B. Duran, J. ASTM Int. **8** (8), 10 (2011). doi:10.1520/JAI103411.
[30] ASTM D7458, *Standard Test Method for Determination of Beryllium in Soil, Rock, Sediment, and Fly Ash Using Ammonium Bifluoride Extraction and Fluorescence Detection* (ASTM International, West Conshohocken, 2014).