

Analysis of Beryllium in Samples by Optical Fluorescence: Method Adaptation for Field and High Throughput (HT) Analysis - 11467

Anoop Agrawal, Lori L. Adams, Juan Carlos L. Tonazzi and John P. Cronin

Berylliant Inc, Tucson, AZ 85712

ABSTRACT

Traditionally, beryllium analysis of wipe, air and bulk samples has been conducted by laboratories using highly trained personnel and expensive capital equipment. In addition, such methods are labor intensive, particularly the steps involving sample preparation. Recently a new method to determine beryllium using optical fluorescence, which can be used in the field, has been introduced. This new method uses low-cost instrumentation and set up, is simple to use, and is non-destructible, as only a small fraction of the dissolved sample is analyzed. The new method has been used for wipes, air samples, and bulk samples such as soils, rocks, sediments and fly ash.

Test methods based on this technology have been approved by National Institute of Occupational Safety and Health (NIOSH) and American Society of Testing Materials (ASTM). Laboratories can also be accredited by American Industrial Hygienists' Association (AIHA). The new method meets the analytical requirements to satisfy the Department of Energy requirements and also the proposed regulations by the American Conference of Government Industrial Hygienists (ACGIH). This method is particularly beneficial for field use where the results can be obtained within two hours, allowing a more efficient use of equipment and manpower. More recently, an automated system, which uses a 96 micro-well plate to analyze the wipe and air samples, has been introduced. The samples for analysis are prepared automatically and the samples are analyzed in a plate reader. This method results in a tremendous increase in productivity, and the plate with 96 samples is read in less than three minutes, where the conventional methods may take six to ten hours to analyze similar numbers of samples. The new method is particularly suitable for facilities that annually analyze in excess of 20,000 samples. The high throughput method meets the goals of increased productivity, while also meeting the standard analytical method guidelines and the detection sensitivity requirements to meet the Department of Energy and other regulations.

INTRODUCTION

Analysis of beryllium by optical fluorescence has been recently established [1-4] and is approved as a valid test method for determining beryllium particles on wipe samples, air filters and soil samples. These methods are available as ASTM methods D7202 [5] and D7458 [6], and NIOSH analytical methods as 7704 and 9102 [7]. Other methods used to analyze beryllium are graphite furnace atomic absorption spectrometry (GFAAS), inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively couple plasma mass spectrometry (ICP-MS) [8-9]. The optical fluorescence method using the dye hydroxybenzoquinoline sulfonate (HBQS) is not

sensitive to interference by other metals that may be present [10] and has beryllium detection limits comparable to the most sensitive method using mass spectrometry. More recently, an automated system that prepares the samples in a 96 micro-well plate for analysis in a plate reader has been introduced. This method results in a tremendous increase in productivity as compared to the other methods; the plate with 96 samples is read in less than three minutes, whereas the conventional methods may take six to ten hours to analyze a similar number of samples.

For analysis of beryllium by fluorescence the samples (filters or wipes) are first subjected to a dissolution process by placing them in 5ml of 1% ammonium bifluoride and heating to 80 or 90°C for 30 to 60 minutes. For manual analysis, fluorescence measurement solutions are then prepared in cuvettes by adding 0.1 ml of the dissolution solution to 1.9 ml of a detection solution (20x dilution) containing 1.1mM HBQS, 1mM ethylenediamine tetraacetic acid and 100mM L-Lysine monochloride with the solution pH adjusted to 12.85. The solutions are then measured for beryllium by using an excitation beam with peak maximum in a range of 360 to 380 nm and for emission with peak transmission in a range of 460 to 485 nm. The fluorometer is calibrated using beryllium standard solutions. Typical standards for routine analysis to meet DOE's 10CFR850 [10] use beryllium concentrations of 0, 10, 40, 200 and 800µg/L. When these standards are mixed with HBQS (20X dilution), the beryllium concentrations are 0, 0.5, 2, 10 and 40 ppb in the measurement solution.

The phenolic group in the HBQS dye binds specifically to beryllium where the six-member ring has the ideal distance between O-O or N-O for chelating Be [11]. The chemical structure of HBQS is shown in Figure 1.

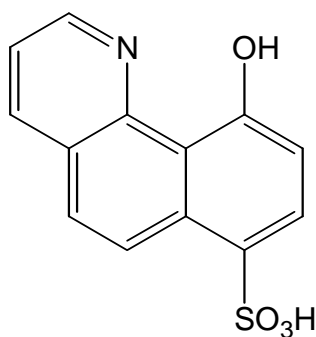


Fig. 1. Structure of hydroxybenzoquinoline sulfonate (HBQS) fluorophore

Figure 2 shows the excitation spectra for the measurement solutions with different amounts of beryllium. Figure 3 shows the emission spectra for all of these solutions at an excitation wavelength of 365 nm. The maximum fluorescence emission is seen at 475 nm for the dye solution with beryllium. A tightly bound hydrogen bonded proton (without the dye) leads to a weak triplet emission at 580 nm for the dye solution. When the proton is displaced by beryllium, fluorescence emission is observed at 475 nm [11].

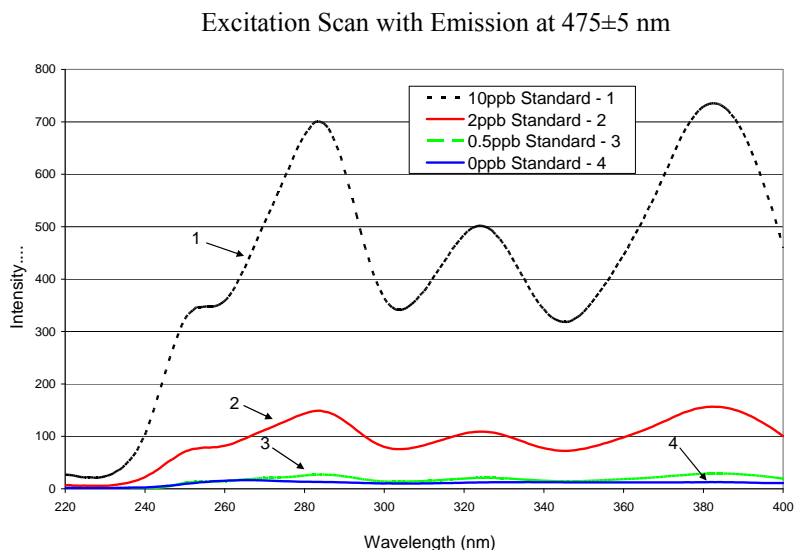


Fig. 2. Excitation scan of hydroxybenzoquinoline sulfonate (HBQS) dye with different concentrations of beryllium

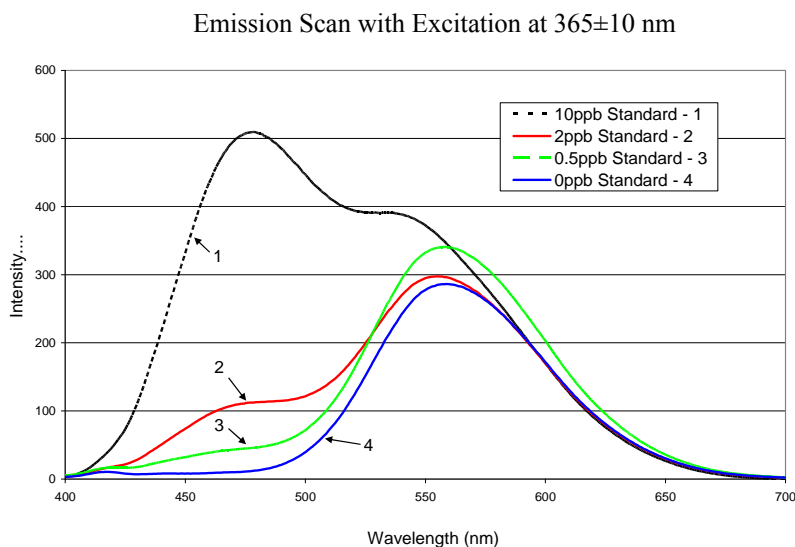


Fig. 3. Emission spectra of hydroxybenzoquinoline sulfonate (HBQS) dye with different concentrations of beryllium

DISCUSSION

A manual system comprised of portable units was developed; its simplicity made it acceptable for field use as part of mobile or field based laboratory [3]. The sequence of operations is shown in Figure 4. All the manipulations were conducted using ergonomic pipettes and dissolution using a hot block [12]. The final samples were made as solutions in optical cuvettes that were placed in a fluorometer and could be optically read in a few seconds. Because there were no moving parts and flow channels in the fluorometer, this is considered “maintenance free,” which

provides an advantage in the field. The fluorometer was a small lightweight (3kg) unit that could be powered via AC or a DC input. In addition to its low capital cost, the system also was capable of analyzing wipe and air samples in less than two hours. This allowed field workers to rapidly analyze the samples at their work-sites without long waiting periods and expensive arrangements of transporting samples to distant laboratories and waiting to hear from them before they could contemplate their next steps.

Since the sample analysis or reading used optical fluorescence, a technique routinely used to analyze biological samples in a high throughput mode, we decided to build a similar system for analyzing environmental samples. The samples are contained on a plate (about 8.9x12.7cm), where each plate has multiple wells to contain liquid samples. Typically most common plates have 24, 96, 384 or 1536 wells (see Figure 6 which shows a 96 well plate). Multiple samples can be located on one plate including samples for calibration with known amount of analyte. Further, robotic instruments are used to prepare the samples in these plates. Using such an instrument would allow to reduce labor, and raise productivity for laboratories, which could allow them to process hundreds to thousands of samples/day. Using traditional analytical methods, an operator and dedicated equipment are required for processing a set of 50 to 60 samples/shift; labor requirements scale linearly with the number of samples to be analyzed.

A robotic system that would automate both the sample preparation and its analysis was designed. Further, a standard 96 well plate was used to hold up to 96 samples and the calibration standards. All 96 wells on a plate could be read in less than 3 minutes. Sample preparation steps are shown in the block diagram in Figure 4.

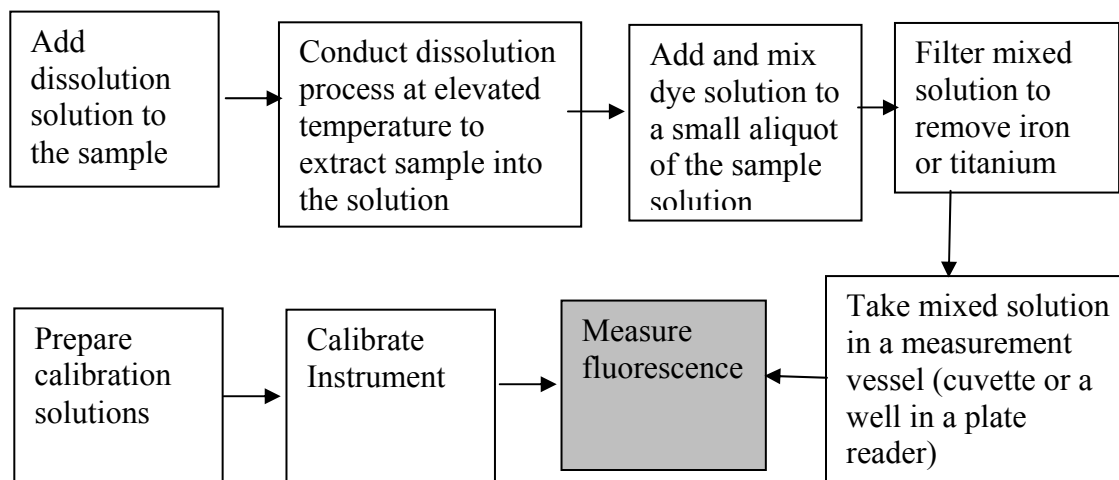


Fig. 4: Flow chart of process steps to prepare and analyze samples for beryllium by optical fluorescence

In the high-throughput (HT) system all of the above steps were automated by using a Janus robotic liquid-handling system from Perkin Elmer (Waltham, MA) and customizing the hardware and the software for the purpose. A tray of sample tubes is placed on the equipment deck, where dissolution solution is dispensed. The tray is removed for the dissolution process where the samples are treated at an elevated temperature for a period of time. The sample tray is then

placed on the equipment deck, and all of the above steps including calibration samples are prepared automatically, wherein the final sample measurement vessel comprises of a disposable plate with 96 wells (12x8 array format), each with a capacity of about 0.3 ml. This plate can also hold additional blank samples or known spike samples to verify the process. Typically, 70-80 unknown samples are included for analysis, depending on the desired configuration. The sample tray is picked robotically and inserted into a plate reader (Synergy 2 from BioTek, located in Winooski, VT) where the entire tray is read and analyzed in under three minutes. Figure 5 shows a robotic liquid handling system with four pipette tips along with a plate reader. Figure 6 shows a microwell plate with 96 compartments or wells. The throughput for the system was to be between 240 samples to 320 samples/eight hour shift, which mainly depended on the plate layout. The throughput of the system can be increased by increasing the number of pipette tips to eight. In addition, because the system is automated, the technician does not have to spend all of his time preparing samples and running the analysis, but only needs to address periodic changing of the sample trays, removing and placing the sample trays after the dissolution process, and placing new plates and filtration modules after each plate is processed. This allows a technician to conduct other jobs or even to manage multiple machines, which results in high productivity. This method is typically suitable for laboratories analyzing 20,000 or more samples/year and can be highly economical when the sample load increases to more than 50,000 samples per year.

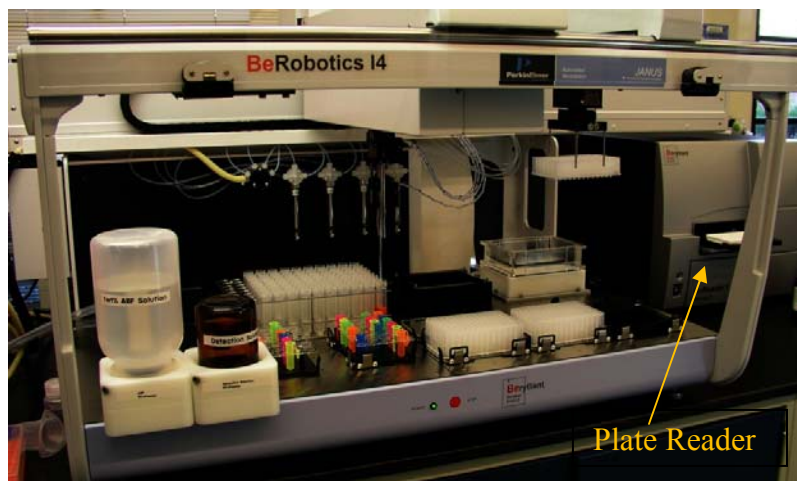


Fig. 5: Liquid handling system and a plate reader

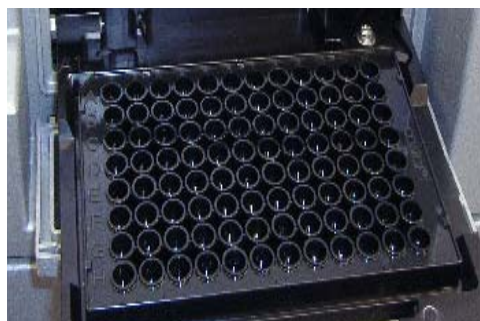


Fig. 6: 96 microwell plate, size 3.5x5 inch

A HT system [13] was implemented at a beryllium processing facility for which the plate layout is shown in Figure 7 [14]. As shown, this plate comprised of

- Standards
- Initial calibration verification (ICV) using a second source standard
- Blank level checking: calibration (CCB) and method blanks
- Reporting limit verification
- Duplicate lab controls (aqueous BeO)
- Method precision from lab control system (LCS) duplicates
- End of plate verification (CCV)

Including the various checks within the plate ensured that the data quality was good and provided a check on the instrument performance. The machine detection limits were found to be 0.0008 µg/wipe and the analytical range was 0.01µg - 20 µg. This sensitivity and the range were sufficient to meet the DOE requirements as laid out in 10CFR850 [10] and the American Conference of Government Industrial Hygienists (ACGIH) [15] recommendations. Table 1 [14] shows a quick comparison of the time taken in various steps as compared to a standard competing analytical technique using inductively coupled plasma- atomic emission spectroscopy (ICP-AES). It should be noted that the dissolution period (minimum of 45 minutes including a cooling period of fifteen minutes or any user-defined period, which in this case is 140 minutes) can be used by the HT machine to prepare another set of samples or prepare a new series of calibration samples, in order to optimize the total productivity of the operator and the equipment.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	ICV									
B	Std #1	Std #5	ICB									
C	Std #2	Std #6	RLV									
D	Std #3	Std #7	Well left empty									
E	Blank	Blank	Method blank#1									Method blank#2
F	Std #4	Std #8	LCS-1									LCS-2
G	Std #5	Std #9										CCB
H	Blank	Blank										CCV

Fig. 7: Plate layout for typical HT implementation

The system results in several advantages, which are summarized:

- Reduced cost due to
 - Reduced labor.
 - Reduced capital- fewer analytical instruments and less laboratory space is needed.
 - Reduced turn-around time.
- Increased safety
 - Reduced worker injury from repetitive motions.
 - Less interaction between humans and chemicals.
- Improved consistency

Table 1: Comparison of time taken by the two analytical methods

	ICP-AES	HT Fluorescence
Batch size	45 samples	70 samples
Sample digestion time	63 minutes	140 minutes
Sample analysis time (for all)	180 minutes	2 minutes
Total sample digestion and analysis time	243 minutes	147 minutes
Time/sample	5.4 minutes	2.1 minutes

CONCLUSIONS

An automated system was implemented to analyze beryllium contamination in wipe and air samples by optical fluorescence. This system utilized a robotic liquid handling system for automating sample preparation and a 96 micro-well plate reader to analyze the samples. This method is suitable for laboratories where large numbers of samples are analyzed so that an increase in productivity and cost-reduction can be realized.

ACKNOWLEDGEMENTS

Financial support to undertake the initial work on the high throughput system was supported by a grant from the US Department of Energy under contract DE-FG02-06ER84587. We are grateful for this support.

REFERENCES

1. CRONIN, J.P., A. AGRAWAL, L. ADAMS, J.C.L. TONAZZI, M.J. BRISSON, K.T. WHITE, D. MARLOW, AND K. ASHLEY: Interlaboratory evaluation of an extraction and fluorescence method for the determination of trace beryllium in soils. *J. Environ. Monit.*, (2008).
2. ASHLEY, K., A. AGRAWAL, J. CRONIN, J. TONAZZI, T.M. MCCLESKEY, A.K. BURRELL AND D.S. EHLER: Ultra-trace determination of beryllium in occupational hygiene samples by ammonium bifluoride extraction and fluorescence detection using hydroxybenzoquinoline sulfonate. *Analytica Chimica Acta* 584: 281-286 (2007).
3. AGRAWAL, A., 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. SCHLECT, P. SONTALIA AND K. ASHLEY: Validation of a Portable Fluorescence Method for the Measurement of Trace Beryllium in the Workplace Air and Wipe Samples. *J. of Environmental Monitoring*, 8: 619-624 (2006).
4. ASHLEY, K., T. M. MCCLESKEY, M. BRISSON, G. GOODYEAR, J. CRONIN AND A. AGRAWAL: Interlaboratory Evaluation of a Portable Fluorescence Method for the Measurement of Trace Beryllium in the Workplace. *J. of ASTM International*, Vol. 2 (9), paper ID JAI13156 (2005).
 5. ASTM D7202, Standard Test Method for Determination of Beryllium in the Workplace Using Field-Based Extraction and Fluorescence Detection. ASTM International, West Conshohocken, PA (USA), (2005).
 6. ASTM D7458, - 08, 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, PA (USA), (2008).
 7. US National Institute for Occupational Safety and Health (NIOSH), NIOSH Manual of Analytical Methods, 5th ed., Method Nos. 7704: BERYLLIUM in Air by Field-Portable Fluorometry., and Method Nos. 9110: BERYLLIUM in Surface Wipes by Field-Portable Fluorometry. NIOSH, Cincinnati, OH (2007).
 8. US National Institute for Occupational Safety and Health (NIOSH), NIOSH Manual of Analytical Methods, 4th ed., Method Nos. 7102 and 7300, NIOSH, Cincinnati, OH (1994).
 9. US Environmental Protection Agency (EPA), Trace Elements in Water & Wastes – ICP-MS, Method No. 200.8, EPA, Washington, DC, (1994).
 10. Code of Federal Regulations, 10 CFR Part 850, *Chronic Beryllium Disease Prevention Program*. U.S. Department of Energy: Washington, DC (1999).
 11. MINOGUE, E.M., A.K. BURRELL, T.M. MCCLESKEY, T.P. Taylor: *Development of a new fluorescence method for the detection of beryllium on surfaces*, *J. ASTM Int.*2 (10): (2006).
 12. “Portable System” Berylliant Inc, Tucson, AZ, <http://www.berylliant.net/products.html> (2010)
 13. “High Throughput System” Berylliant Inc, Tucson, AZ, <http://www.berylliant.net/products.html> (2010)
 14. OATTS, T., Presented at the Beryllium Health and Safety Committee (BHSC) Spring Meeting in Washington DC, 2010.
 15. American Conference of Governmental Industrial Hygienists Adopts TLV for beryllium of 0.05 µg/m³ http://www.hss.energy.gov/HealthSafety/WSHP/be/ACGIH_05_ug_TLV.pdf (2009).