Final Report Phase I SBIR

High Sensitivity, Low-Cost Fluorescence Detection System for Beryllium Particulates

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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. Beryllium metal (as metal or as a metal alloy) and beryllium oxide are the most important beryllium materials from an industrial perspective. The number of DOE workers sensitized to beryllium and who developed CBD are shown below in Fig 1 from a 2003 DOE report¹.

A summary for various US regulations related to beryllium is given in Table 1. The air borne levels are time weighted averages, typically over an eight hour shift.

Table 1: Comparison of US regulations on beryllium by various Agencies

Agency	Air contamination	Surface Contamination
Department Of Energy (DOE) Regulation 850CFR ²	0.2 $\mu\text{g}/\text{m}^3$	0.2 $\mu\text{g}/100\text{cm}^2$ for free release of equipment. 3 $\mu\text{g}/100\text{cm}^2$ for housekeeping and removal of equipment to another Be area
Occupational Safety and Health Administration (OSHA) ³	2 $\mu\text{g}/\text{m}^3$	
National institute of Occupational Safety and Health (NIOSH) ⁴	0.5 $\mu\text{g}/\text{m}^3$	
American Conference of Governmental Industrial Hygienists (ACGIH) ⁵	2 $\mu\text{g}/\text{m}^3$ (proposed) 0.02 $\mu\text{g}/\text{m}^3$	

Widely used laboratory methods to measure beryllium in workplace atmospheres (e.g., NIOSH 7102,⁶ OSHA ID-125G⁷ and ASTM D7035⁸) use atomic spectrometric instrumentation, typically Inductively Coupled Plasma- Atomic Emission Spectroscopy (ICP-AES). Preparation of samples for such analysis involves the use of highly toxic acids and high heat, and the necessary laboratory equipment is expensive. This instrumentation also requires highly trained personnel and is not field deployable.

Recently, 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)⁹, and includes a dissolution technique using dilute ammonium bifluoride solution^{10, 11}. 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 are commercially available. Since, fluorometers can be field deployable; personnel beryllium badges could be instantly analyzed to significantly increase safety of the workers.

This method of beryllium particulate detection was developed at Los Alamos National Laboratory (LANL)¹⁰ and licensed to Berylliant Inc in Tucson Arizona. The principals at AJJER along with others, formed a new company called Berylliant Inc and licensed the technology from LANL. Berylliant's sole current business is based on beryllium detection using fluorescence technology. While, Berylliant (also a small business) has undertaken commercialization, regulatory activities and standard test development methods, AJJER has supported most of its research and development activities. The system is commercialized under the name BeFinder by Berylliant Inc. The detection limits are similar to the industry accepted methods utilizing ICP-AES as listed above. Further, the BeFinder method lists several advantages as given below:

- Field or laboratory deployable
- Rapid turnaround (test results within one hour)
- High throughput (can process about 32 samples/90 minutes)
- Beryllium specific (Other metals do not interfere with Be results)
- Low capital cost (~ \$10,000)
- Quantification from 0.2 µg to 4 µg using Turner Fluorometer
- Detection limit down to 0.02 µg
- Uses only a fraction of material collected, allowing re-verification
- Chemicals are easy to transport and dispose
- Does not require highly trained lab personnel

The DOE detection limits are the lowest in the industry and are largely based on the detection limits imposed by the current instrumentation which is typically ICP-AES. For example, American Conference of Governmental Industrial Hygienists (ACGIH) has a notice of intended change⁵ to adopting short term exposure limit of 0.2µg/m³. At a sampling rate of 2liters/min will require an action level of 0.006µg. One of the major obstacles for this is seen as availability of suitable instrumentation to be able to detect at these levels. ICP-MS (Mass spectroscopy) can achieve this, but at a tremendous capital cost. A further complication has been that if the worker badges are modified so that they collect 10 times more air, then pumps become larger, expensive and cause inconvenience to workers. Alternatively, ability to measure lower amounts of beryllium allows less air to be sampled on a worker's detection badge permitting lighter weight badges to be available increasing worker comfort and flexibility. Fluorescence is a highly accepted method of detection for binding drugs and other molecules because it is

extremely sensitive and is routinely used for most sensitive biological measurements including evaluation of binding to DNA strands and proteins.

Technical objectives

The technical objective in Phase I was to enhance the detection limit of the fluorescence system by a minimum factor of 10. This was done by modifying both the chemistry and instrumentation. Both of these were done independently. In each case we were able to lower the detection limit as desired. The objectives in Phase II are to adapt these changes for commercial activity (chemicals and instrument changes including automation). For Phase II, we were also to conduct round robin testing at several laboratories so that standard ASTM or NIOSH methods may be amended to account for any changes in procedures resulting from the success of this program.

Phase I Results

Two main courses of action were pursued related to chemistry to get at least a ten fold improvement. The first being to increase the beryllium in the fluorescent measurement solution by adjusting the dilution ratio, and second being changes to the dye structure. Experiments with the dye structure were done in collaboration with Los Alamos National Lab in collaboration with Dr. Mark McCleskey and Dr. Anthony Burrell, and the inter-lab study with dilution ratios was performed in collaboration with Dr. Kevin Ashley at NIOSH. The results from the dilution ratios reported here were published recently¹² and have also been incorporated in the recently approved NIOSH procedures 7704¹³ and 9110¹⁴. The ASTM method 7202-05 has already been modified to include this and is now published as ASTM D7202-06¹⁵. Berylliant took immediate action to communicate these changes as this required only a slight modification of the procedure that can be easily implemented by the end users and derive the benefits thereof. The study with the different dyes showed that other effective dyes can be made, but the current HBQS dye has the strongest fluorescence.

Regarding instrumentation, the main objective was to decrease the thermal load so that the temperature variations in the sample during measurement can be minimized. This required changes to illumination, detection and several related components. Further, we also made changes where fluorescence and absorption were combined to increase the quality of the results.

Chemistry, adjustment of dilution ratio

The fluorometers used in this section were several types as follows: Ocean Optics S2000-FL (Dunedin, FL), 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).

Currently, the beryllium sample is dissolved in 5 ml of ammonium bifluoride, of which 0.1 ml is added to 1.9 ml of buffered HBQS solution^{10, 11}. In this process, only 2% of the available beryllium sample is used. The HBQS solution is prepared using a sodium hydroxide solution and is buffered using lysine. The buffer is used to maintain a high pH when an aliquot of the dissolution solution comprising beryllium (typical pH 4.5) is mixed with the HBQS solution. For the dyes to perform, i.e., bind with beryllium and fluoresce, a high pH of the mixed solution is necessary. When more of the dissolution solution is added to the dye solution to increase the amount of beryllium in an attempt to increase the detection limit, the pH falls as shown in Table 2. As seen in this table if one was able to use a ratio of 1:3 and measure all the beryllium in the solution, the detection can be improved by a factor of six (compared to 1:19 ratio which is used at present).

Table 2: Change in pH with change in dissolution solution:detection solution ratio

Dissolution solution (ml)	Detection solution (ml)	Ratio "R"	Dilution ratio	pH
0.1	1.9	1:19	20	12.46
0.4	1.6	1:4	5	12.16
0.5	1.5	1:3	4	11.39
1.0	1.0	1:1	2	8.55

Based on the pH changes it was decided to evaluate the dilution ratio of 5 and compare that to the standard dilution ratio of 20. Also, the high pH is responsible for precipitating out other metals such as iron and titanium which interfere with the results as they impart yellowness or cloudiness to the samples. Fluorescence spectra of extremely low concentrations of beryllium in the presence of HBQS, obtained using the Ocean Optics device, are presented in Fig. 2. Unreacted HBQS yields a fluorescence band with $\lambda_{\text{max}} \approx 590$ nm, while the fluorescence of the Be-HBQS complex is blue-shifted, with $\lambda_{\text{max}} \approx 475$ nm. Beryllium concentrations of less than 0.2 ng/mL were measured quantitatively using the HBQS fluorescence reagent with "Dilution ratio" of 5, offering a dramatic increase in sensitivity over beryllium fluorophores investigated previously.

These results gave us the confidence that a "Dilution ratio" of 5 would meet our sensitivity requirements. The method detection limit (MDL) 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 procedure⁸. This method of MDL estimation was 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/sample for the Turner instrument to ≈ 2 ng/filter for the Spex device, corresponding to solution beryllium concentrations of ≈ 0.03 ppb to ≈ 0.08 ppb. For purposes of comparison, it is noted that estimated MDLs for beryllium by ICP-MS of ≈ 0.03 ppb have been reported for environmental samples¹⁶.

Table 3. Estimated method detection limits for beryllium in MCE and Whatman 541 filters using different fluorometers.

Fluorometer	MDL, MCE filters	MDL, Whatman 541 filters
Turner Quantech	0.75 ng	0.78 ng
Ocean Optics S2000-FL	1.5 ng	1.6 ng
Spex Fluorolog2	1.9 ng	2.1 ng

Potential interferences from a number of other metals have been investigated in previous work and found to be negligible, with the exception of iron and titanium dioxide^{10, 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 TiO₂ or Fe at ten-fold and 100-fold excess, respectively. Negative interference from TiO₂ observed initially after extraction was alleviated via additional filtering through nylon microsyringe filters. 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.

Follow-up experiments were carried out to investigate the NH₄HF₂ extraction / HBQS fluorescence method from MCE filters spiked with low levels of BeO prepared from slurries. The spiked filters were prepared by a commercial Lab. 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 three different laboratories using three different fluorescence instruments, and it is reiterated that spike levels were unknown to each analyst. The results of Table 5 show mean reported values that are close to the spike levels, and precision estimates (as measured by relative standard deviations [RSDs]) range from $\approx 2\text{-}3\%$ for levels of 0.02-0.05 μg Be, to $\approx 13\text{-}19\%$ for MCE and Whatman 541 filters spiked at 0.002 μg . Results from blanks were all below the MDL for each fluorometer.

Table 4. Analysis results from MCE and Whatman 541 filters spiked with ultra-low levels of beryllium. Data are pooled from three different laboratories using three different fluorometers.

Spike level, μg Be (n=6)	Mean ($\pm$ SD), μg Be	RSD (%)
MCE Filters		
Blank	N.D.	—
0.002	0.0023 (± 0.00030)	13
0.005	0.0052 (± 0.00012)	2.3
0.020	0.0210 (± 0.00055)	2.6
0.050	0.0504 (± 0.0014)	2.8
Whatman 541 Filters		
Blank	N.D.	—
0.002	0.0025 (± 0.00048)	19
0.005	0.0056 (± 0.00035)	6.3
0.020	0.0209 (± 0.00049)	2.3
0.050	0.0507 (± 0.00013)	2.6

ND=Not detected, SD=Standard Deviation, RSD= Relative Standard Deviation

Chemistry, synthesis of new dye structures

HBQS

The development of the beryllium fluorescence detection technique based on the HBQS dye has taught us much about the way beryllium binds to mixed donor ligands.

One of the most obvious features of this dye system is the mixed ligand system where a typical hard donor, the OH group, is coupled with the soft donor of the pyridine nitrogen. It is believed that this combination is the key factor in the formation of strong beryllium complexes. In addition, the disruption of the strong hydrogen bond in the HBQS is the foundation of the fluorescence in the beryllium complex. There are several features of HBQS which could be improved. In particular the need

to change to a pH >11 before reading the fluorescence is more complex than if a simple pH 7 solution could be used.

Previous research has included binding of beryllium by bidentate ligands to make BeL and BeL_2 species with chelating ligands such as chromotropic acid and ligands designed to polynuclear Be_2L complexes. It has recently been suggested in literature that Be binding occurs through the displacement of strong hydrogen bonds. Strong hydrogen bonds occur when the distance between the two heteroatoms, typically O-H-O or O-H-N, is shorter than the sum of the Van der Waals radii and the energy barrier to hydrogen transfer between two atoms is on the order of the O-H vibrational zero-point-energy. The strong hydrogen bond provides two advantages. First, the O--O distance in a strong hydrogen bond is in the range 2.4 - 2.8 Å which brings two oxygen atoms into a predefined chelating binding site for beryllium that corresponds very well to the intraligand oxygen-oxygen atom distances of 2.26 to 2.86 Å observed in known beryllium structures in the Cambridge Structural Database. Second, the strong hydrogen bond provides a low barrier pathway to displace the proton without breaking a strong covalent O-H bond. The closer the oxygen atoms are in the hydrogen bond, the flatter the two-well potential energy surface becomes until the crossover energy becomes lower than the zero-point-energy of the O-H stretching mode. Near this point the proton can easily shift to the more acidic oxygen, allowing Be to interact with the basic oxygen without the energy cost of breaking a covalent O-H bond (-87.3(±1.5) kcal/mol). The dye indicator we have used in the past, HBQS has such a strong hydrogen bond. The displacement of the strong hydrogen bond leads to the fluorescence observed. As part of this SBIR we attempted to extend this strategy to a tridentate complex to improve the Be binding and lower the detection limit. Di phenol pyridine (DPP) was synthesized and found to have a strong hydrogen bond by NMR. In fact the NMR shows one broad resonance at 12 ppm for the phenol protons suggesting that both phenolic protons are equivalent and involved in a strong hydrogen bonding interaction. The potentially three coordinate binding site comprising the pyridine nitrogen and two phenols was shown to bind Be leading to fluorescence. Plots of the Be response to HBQS and DPP in HEPES buffer are shown below. The DPP molecule binds Be well and gives a strong fluorescence response. The fluorescence intensity is about a factor of 3 less than HBQS, so we chose to continue our efforts with HBQS.

Figure 3

Figure 4

Figure 5

Instrumentation

Fig 6 shows the change in fluorescent signal with change in temperature for the HBQS dye system. In practical situations this does not terribly interfere as the calibration standards could be used frequently for recalibration. The calibration takes less than a minute and in situations where temporal temperature changes are expected, calibration can be done repeatedly.

Figure 6

Fluctuations relate to the inconsistency between the samples during measurement, is due to the heating of the sample caused in the sample compartment of the fluorometer. The time for measurement is only a fraction of a second to a few seconds, but the operator inconsistencies can subject sample for different periods of time in the sample compartment. This results in differential heating by the absorption of the light from the source lamp within the sample (with the dye) and the inefficient removal of heat generated by electronics and source lamp. There are simple solutions to both of these by ensuring good airflow and heat transfer through the sample compartment and providing a shutter between the sample and the source lamps which only opens for the period of a fraction of a second or a few seconds during which the measurements are made and not while the sample is sitting within the measurement compartment. This also reduces the heating of the detector, as it does not have any fluorescent flux during the dead period. Further, the source may be pulsed rather than be of a continuous illumination type where a mechanical chopper may be used such that it is synchronized with the pulsation period. Highly efficient UV LEDs that emit within specific wavelengths to keep the thermal loads small may also be used. To compensate for small input signal, high sensitivity detectors and high transmission bandpass filters were required. More elaborate temperature controls can be implemented by using heating/cooling blocks as sample holders that could be controlled by thermoelectric (or Peltier) plates. The detector may also be cooled to reduce its dark current.

As a first step we implemented simple changes by designing a fluorometer from scratch to see which of these issues are important. We selected low power sources and high transmission filters and highly sensitive detectors (i.e. high efficiency components) to decrease the thermal load on the system. In this fluorometer we selected a UV LED lamp as an illumination source and a sensitive photomultiplier as diode for the detector coupled with a high transmission narrow band-pass filter. The LED light power output (source) was $45\mu\text{W}$. Thus the heat load on the sample-compartment and the samples is quite low. This lamp had a peak output at 380nm. This

was obtained as an integral unit with the power supply, fiber and LED from Ocean Optics. However, in future a more suitable LED may be picked with a peak output at 360-365nm so that it will coincide with the peak of the absorption in the measurement solution. The detector used had a high sensitivity of 6V/nW at 475nm. Further, the fluorescent filter had a peak transmission of greater than 90% at 475nm and fell sharply to almost 0% transmission at 460 and 488nm (bandwidth ± 10 nm). In addition, the sample compartment was isolated from the heat generated by the electronics. As a comparison, commercial Turner Barnstead instrument uses a tungsten lamp with an emission filter having a transmission of about 30% and an emission filter of 10% transmission. Since, high energy components are used along with filters that attenuate most of the energy, the typical temperature in the sample compartment (of the Barnstead instrument) due to the heat from the electronics and the lamp is about 5C higher as compared to the ambient. Thus the sample time inside the compartment can lead to inconsistencies, particularly when very low concentration needs to be detected. For the instrument set-up designed in Phase I, the sample compartment temperature was indistinguishable from the ambient.

To understand, how this change may influence sensitivity, a fluorometer was constructed on the lab bench using an optical breadboard as a base using intrinsically low energy and high efficiency components as described above. A photograph of this is shown in Figure 7. The following three series of standards were made to see the effectiveness of this solution as shown in Table 5.

Figure 7: Top view of Fluorometer assembly from Phase I

Table 5: Standards prepared for measurement, Dilution ratio 20X

Standards used in current procedure			Standards diluted by a factor of x10 compared to current procedure			Standards diluted by a factor of x100 compared to current procedure		
Concentration of Be (ppb) in standard	Concentration of Be (ppb) in Measurement solution	Corresponding Be (μg) on swipe or air filter	Concentration of Be (ppb) in standard	Concentration of Be (ppb) in Measurement solution*	Corresponding Be (μg) on swipe or air filter	Concentration of Be (ppb) in standard	Concentration of Be (ppb) in Measurement solution*	Corresponding Be (μg) on swipe or air filter
0	0	0	0	0	0	0	0	0
10	0.5	0.05	1	0.05	0.005	0.1	0.005	0.0005
40	2	0.2	4	0.2	0.02	0.4	0.02	0.002
200	10	1	20	1	0.1	2	0.1	0.01
800	40	4	80	4	0.4	8	0.4	0.04

The results in Table 6 show the measured values of these samples against the readings obtained on the fluorometer. For this, three series of standards were prepared, where the first standard was 10X more dilute than the first one and the third one that was 100X more dilute as compared to the first one. Also, just the method of sample preparation introduced successive errors with each dilution. The table shows that the measured values (red font) for these solutions are quite on the mark with all values in the first series and the second series. Thus it is easy to quantify at $0.005\mu\text{g}$ on the media. When one goes to the third dilution level, the calibration becomes difficult as the 0.0005 and $0.002\mu\text{g}$ values start merging with the background. Thus there were two ways of further improving the sensitivity without radical departure from the chemistry. One was to decrease the dilution ratio and the other is to reduce the background. Table 7 shows the results from using 5X dilution ratio which corresponds to the finest levels of μg on the media represented by the third series. The results show that we are able to quantify down to $0.0005\mu\text{g}$ on the media, which is 50X better improvement than the goals of Phase I and this is better or comparable to the most sensitive ICP-MS available today. However, as a part of Phase II as discussed later, we would like to reduce the background further so as to improve confidence and the quality of results at these low levels.

Although the tables below show quantification down to $0.0005\mu\text{g}$ it should be remembered that a true quantification limit is only established while considering interference from other elements, thus as discussed later, one of the issues will be to lower the background signal further so that quantification at these levels will be possible and confidence established by running samples at various laboratories and using matrices with other elements. Since, this test is beryllium specific, thus issues caused by interference of other elements are negligible.

Table 6: Standards prepared for measurement, Dilution ratio 20X

Standards used in current procedure			Standards diluted by a factor of x10 compared to current procedure			Standards diluted by a factor of x100 compared to current procedure		
Concentration of Be (ppb) in standard	Corresponding Be (μg) on swipe or air filter	Measured Be (μg) on swipe or filter	Concentration of Be (ppb) in standard	Corresponding Be (μg) on swipe or air filter	Measured Be (μg) on swipe or filter	Concentration of Be (ppb) in standard	Corresponding Be (μg) on swipe or air filter	Measured Be (μg) on swipe or filter
0	0	0.001	0	0	0.00006	0	0	BG*
10	0.05	0.049	1	0.005	0.0059	0.1	0.0005	BG*
40	0.2	0.195	4	0.02	0.0207	0.4	0.002	BG*
200	1	1.01	20	0.1	0.0981	2	0.01	0.0139
800	4	4.00	80	0.4	0.391	8	0.04	0.0398

*BG=Background

Table 7: Measurement of x100 dilution standards using a dilution ratio of 5.

Concentration of Be (ppb) in Measurement solution	Corresponding Be (μg) on swipe or air filter	Measured Be (μg) on swipe or filter
0	0	0.0001
0.02	0.0005	0.00045
0.08	0.002	0.00238
0.4	0.01	0.0099
1.6	0.04	0.0391

This method is specific to beryllium, however interferences from iron or titanium can cause the measurement solution to be yellow which reduces its efficacy. Some experiments related to this were conducted with samples which had about 100,000 fold excess iron (compared to beryllium). These samples when mixed with the dye solution were yellow in color. However, on standing these for two hours they cleared, and were measured after re-filtration. The absorption spectra of these solutions immediately after preparation and after standing for various time periods followed by filtration are shown in Figure 8. After two hours of standing, the spectra did not change, and secondly the yellowness that we see with eye is much more accentuated in the UV part of the spectrum. The amount of beryllium measured in the solutions is shown in Table 8. Thus an important source of error can be reduced if the fluorometer is able to simultaneously measure absorbance in wavelengths lower than 450nm and warn the user to wait before taking the measurements. This reduces the subjective evaluation by the user on the yellowness of the fluid. Since this absorption is quite pronounced in the UV, one may use the excitation lamp for fluorescence as the source and measure its absorbance. Further, since absorbance is measured for transmitted light and fluorescence at right angles to it, both measurements have the potential to be combined as a single unit.

Figure 8: Absorbance spectra of iron comprising samples

Table 8: Amount of beryllium measured in high iron sample.

Standing Time, hrs	Amount of beryllium (ppb)
0 (initial)	0.53
2	1.65
4	1.67
6	1.68
24	1.62

A fluorometer which combines all of the above attributes will result in enhanced sensitivity and accuracy due to the elimination of the interferences. This feature can be included at a small incremental cost. Further, it is not necessary to include heating/cooling blocks or thermo-electric devices which can increase cost, complexity, size and the analysis time to reach the thermal equilibrium. A preliminary costing of this instrument showed that for a commercial fluorometer, all the parts can be acquired in unit volumes below \$3,000 and the instrument can be made available to the end user for less than \$10,000. As discussed below, one of the priority tasks is to develop and make this instrument available to the end-users through Berylliant Inc within one year of the start of Phase II. Further, this will integrate several user conveniences that are not available in the fluorometers on the market today.

Table 9 shows how the background changes when different solutions and cuvettes are used. The background from the disposable cuvettes is quite high and most significant contributor. The disposable cuvettes are plastic and have a component that has inherent fluorescence. For the simplicity of the test, cost and user convenience, we would like to continue to employ plastic cuvettes. We may be able to use disposable polymeric cuvettes and eliminate most of the background emanating from them as discussed below. However, there are two other sources of background that may be substantially reduced by simple changes to the materials used and modifying the dye concentration. First, we need to isolate the source of fluorescence that is responsible for the difference between the results in water and the solution without dye. Once the cause is isolated we will have to check if background can be reduced by obtaining the problem material from a different source or introducing a purification step. Second, we may be able to use a lower dye concentration which will significantly help in the reduction of the background in the ultra-low range.

In all dye solutions, the dye concentration has been $63\mu\text{M}$ which compares to about $4\mu\text{M}$ of beryllium (at $4\mu\text{g}$ of beryllium on the media). As an alternative we could use a dye solution with a concentration of $30\mu\text{M}$ for all tests, or use $6\mu\text{M}$ for samples which had beryllium levels below $0.4\mu\text{g}$ on the media. In these experiments we will have to prove that lowering of dye concentration will have no effect on the results and interferences. In addition, we will have to choose a route which is easy to automate.

Table 9: Background Noise (relative arbitrary units)

Type of Cuvette	No solution, empty Cuvette	Water only	Detection solution w/o dye	Detection solution with $1/10^{\text{th}}$ dye concentration	Detection solution with normal dye concentration
Quartz	78	72	185	208	380
Disposable	250	755	990	1009	1030

Another change that can be implemented in terms of the optical layout is to minimize the fluorescence background from the cuvettes as mentioned earlier. This change is schematically shown in Figure 9.

Figure 9: Schematics of fluorometer configuration

The present fluorometer (in Figure 7) and commercial fluorometers are typically configured for excitation beam as shown by Source 1. In this case the cuvette walls are also

excited by the incoming beam. However, if the incoming beam is directed from the top into an open cuvette, then the fluorescence from the walls is avoided.

The Phase I work has resulted in two provisional patent applications that have been filed:

1. *Improved Methods to Analyze Beryllium by Fluorescence*
2. *Methods to reduce Background in Analysis of Beryllium by Fluorescence*

Since one of the chemical approaches using 5x dilution showed sufficient promise, we decided to accelerate its commercialization and Berylliant conducted round robin tests and successfully incorporated changes in the ASTM and the NIOSH protocols. Revised ASTM test has been published as D7202-2006¹³ and NIOSH procedures 7704¹⁴ and 9110¹⁵ for air and wipes respectively have also been approved. Further, Berylliant has been able to work with the beryllium community and added this test to the American Industrial Hygiene Association (AIHA)¹⁷ list so that the laboratories could be accredited for implementing this test. Thus part of the progress achieved in Phase I is being commercialized before the start of Phase II!

Commercialization and Phase II Work:

It is helpful to remember the relationship between AJJER and Berylliant Inc. Fluorescence method of beryllium particulate detection being used for this SBIR was initially developed at Los Alamos National Laboratory (LANL). The principles at AJJER along with others, formed a new company called Berylliant Inc and licensed the technology from LANL. Berylliant's sole current business is based on beryllium detection using fluorescence technology. Berylliant (also a small business) has undertaken commercialization, and standard test development methods. AJJER is an independent research and development company which has supported Berylliant's research programs. While this SBIR at AJJER will capitalize on the results of Phase I in development of instruments and prototypes as given below, Berylliant will simultaneously concentrate on commercial activities to bring these to the market immediately.

The two technical goals/tasks of Phase II can be summarized as:

1. **Ultra-sensitive detection:** Develop and demonstrate a system comprising of an inexpensive fluorometer and changes to chemicals in order to routinely quantify down to at least to 0.005 μ g of beryllium on the media, while keeping all the advantages of fieldability and portability.
2. **Automation:** While incorporating the above development to automate the sample preparation and measurement in order to allow customers to process at least 240samples/shift.

References:

1. Presented at Beryllium Health and safety Committee (BHSC) meeting in Nov 2003 held in Washington DC
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