Automated Fluorescence Analysis of Beryllium Air Filters and Surface Swipes by Sequential-Injection Analysis

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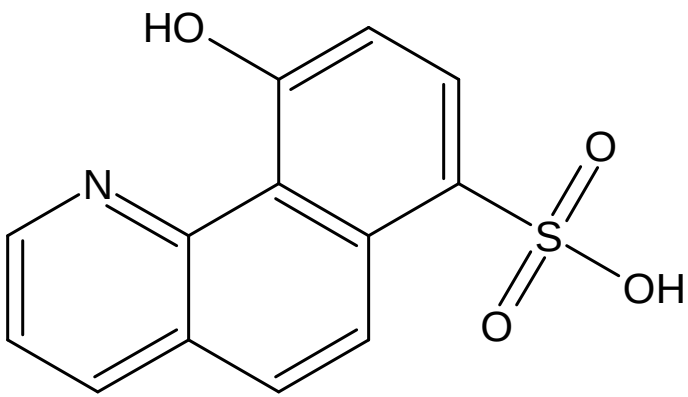
Fluorescence Method of Analyzing Be

- Method is based on the formation of a fluorescent complex between Be ions and the sodium salt of 10-hydroxybenzo[h]quinoline-7-sulfonic acid.



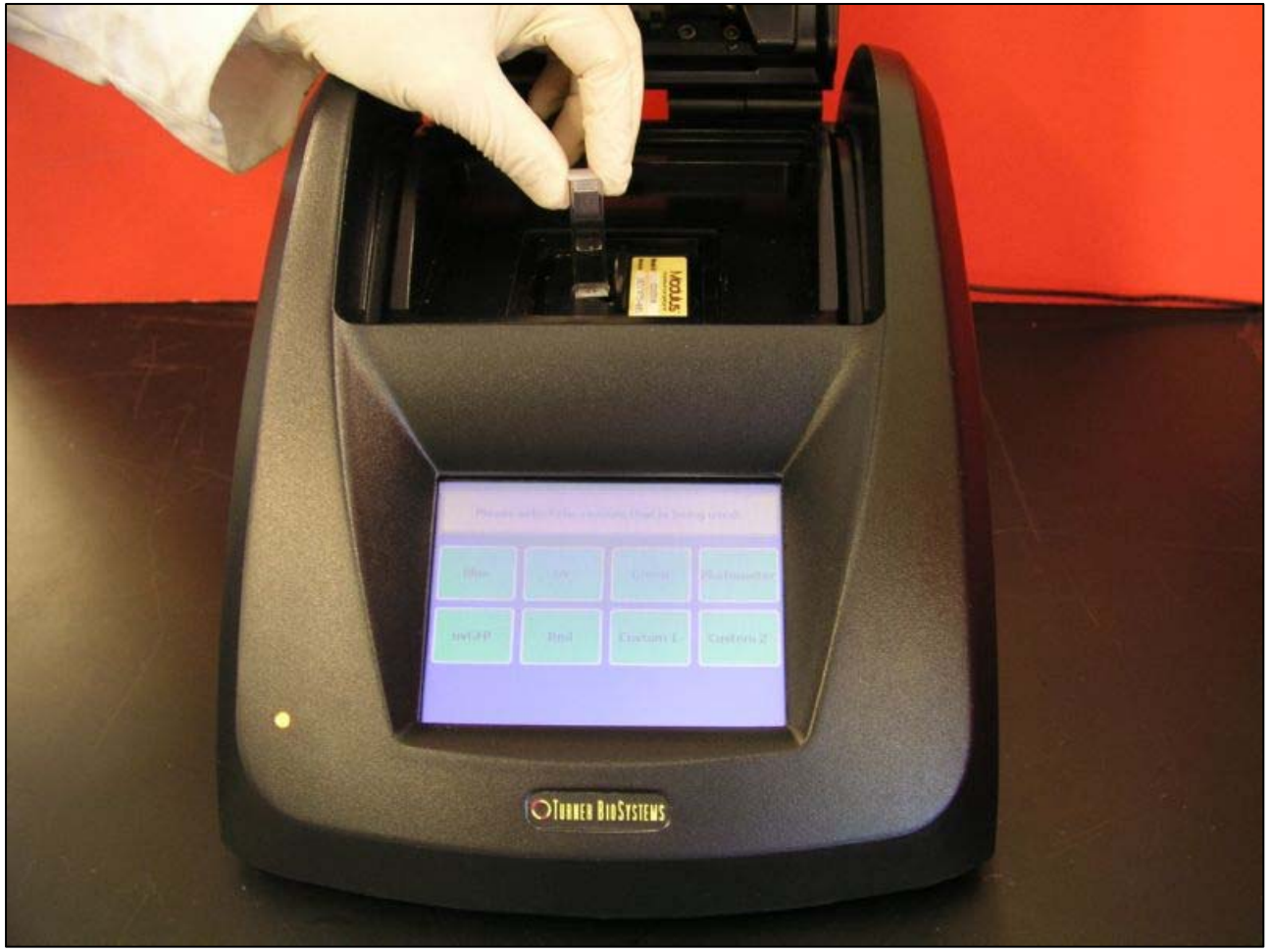
HBQS

- Approved methods:
  - ASTM D7202
  - NIOSH 7704
  - NIOSH 9110

Be Methods at Y-12

- Manual Method
  - Tedious
  - Samples are handled one at a time
  - Instrument time ~2 minutes per sample/standard
- Be Robotics Method
  - Automated
  - Good for large batches of samples (70)
  - Instrument time >3 minutes per sample (depends on batch size)
- Sequential-Injection Analysis
  - Automated
  - Instrument time ~3.5 minutes per sample/standard

Manual Fluorescence Method for Be



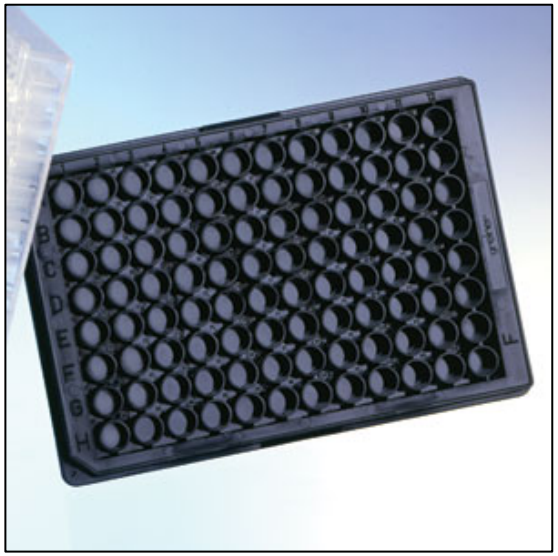
Manual Fluorescence Method for Be

- 0.1 ml sample plus 1.9 ml reagent solution in cuvette
- Field deployable
- Low sample throughput
- Detection down to ~0.004 µg Be
- Cost ~$3K
- Cuvettes are moved by hand

High-Throughput Fluorescence Method for Be

High-Throughput Fluorescence Method for Be

- High-throughput, automated instrument
- Robotic liquid-handling system automatically prepares a 96-well plate (70 samples +16 stds +10 QC stds)
- Gen5 Microplate reader measures fluorescence of each well
- Large analytical range (0.01µg - 20 µg)
- Cost $135K



Analytical Needs

- Need for an automated instrument to analyze small batches of rush samples
  - For example, need to dissolve and analyze a batch of 10 samples within ~2 hours

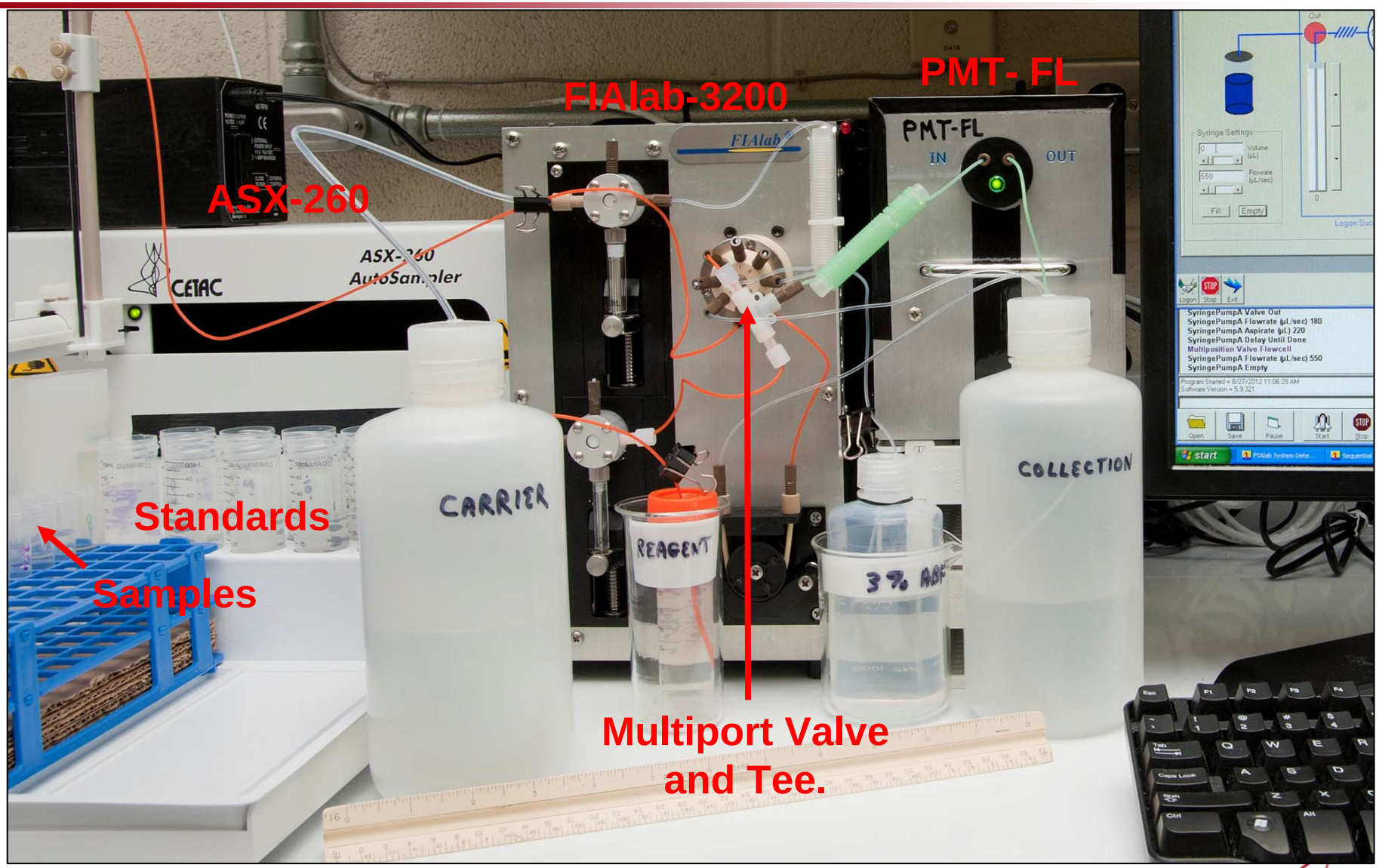
- Need to lower Be detection limit for air-filter samples
  - especially if small volumes of air are sampled

Proposed Instrument

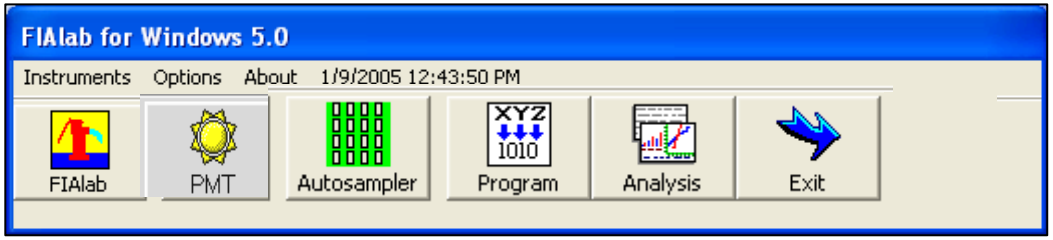
- FIAlab-3200 sequential-injection analyzer
  - PMT-FL detector with bandpass filters
  - Autosampler allows automatic operation
  - Computer
  - Sits on top of desk

- Total cost is $35K for hardware listed above

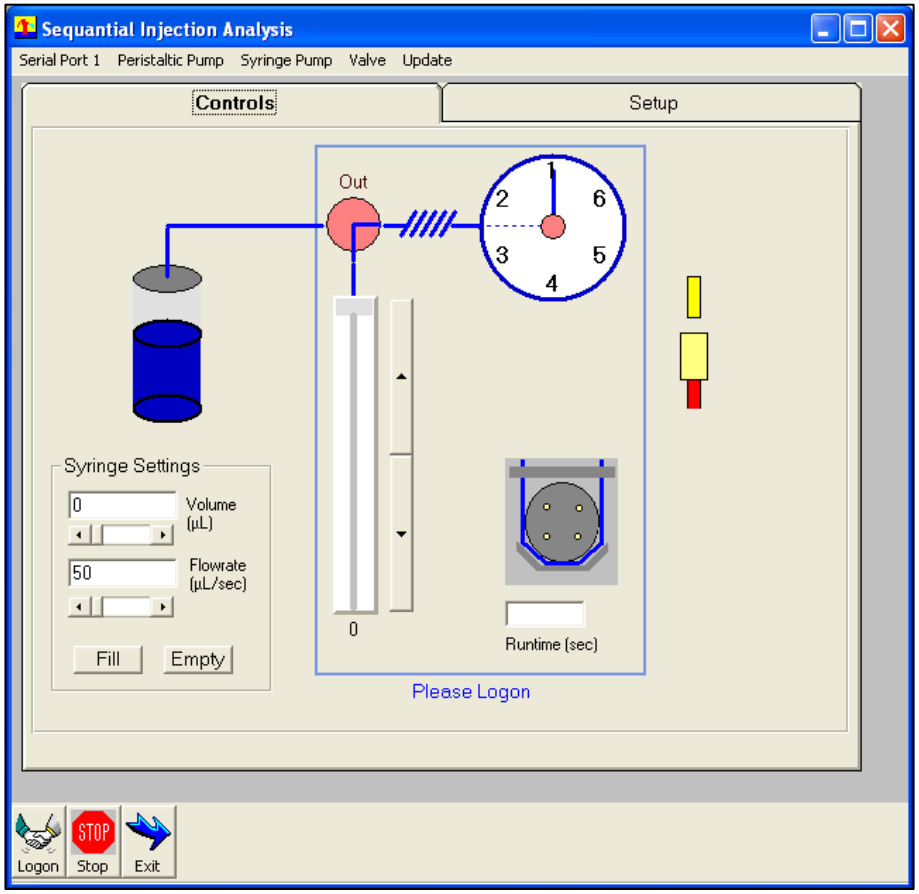
FIAlab-3200 with Detector and Autosampler



Sequential-Injection Analysis



Menu window



Hardware window is used to establish control of syringe pumps, 8-port valve, and peristaltic pump

Sample of Script Programming

Multiposition Valve Sample

' Aspirate Sample (delivered by peristaltic pump)

SyringePumpA Flowrate (µL/sec) 8
SyringePumpA Valve Out
SyringePumpA Aspirate (µL) 35
SyringePumpA Delay Until Done
Peristaltic Pump On

User develops software to operate the hardware.

Multiposition Valve Flowcell

' Dispense reagent and sample simultaneously

SyringePumpA Flowrate (µL/sec) 35
SyringePumpB Flowrate (µL/sec) 35
SyringePumpB Valve Out
SyringePumpA Dispense (µL) 35
SyringePumpB Dispense (µL) 35

35 µL of sample is mixed with 35 µL of reagent.

Interferences to SIA Method

We have identified 3 types of interferences to Be analyses by the SIA method:

1) **carryover** of Be between analyses,
2) **background fluorescence** in the sample, and
3) **dissolved iron** in the sample.

Carryover Interferences to SIA Method

Carryover

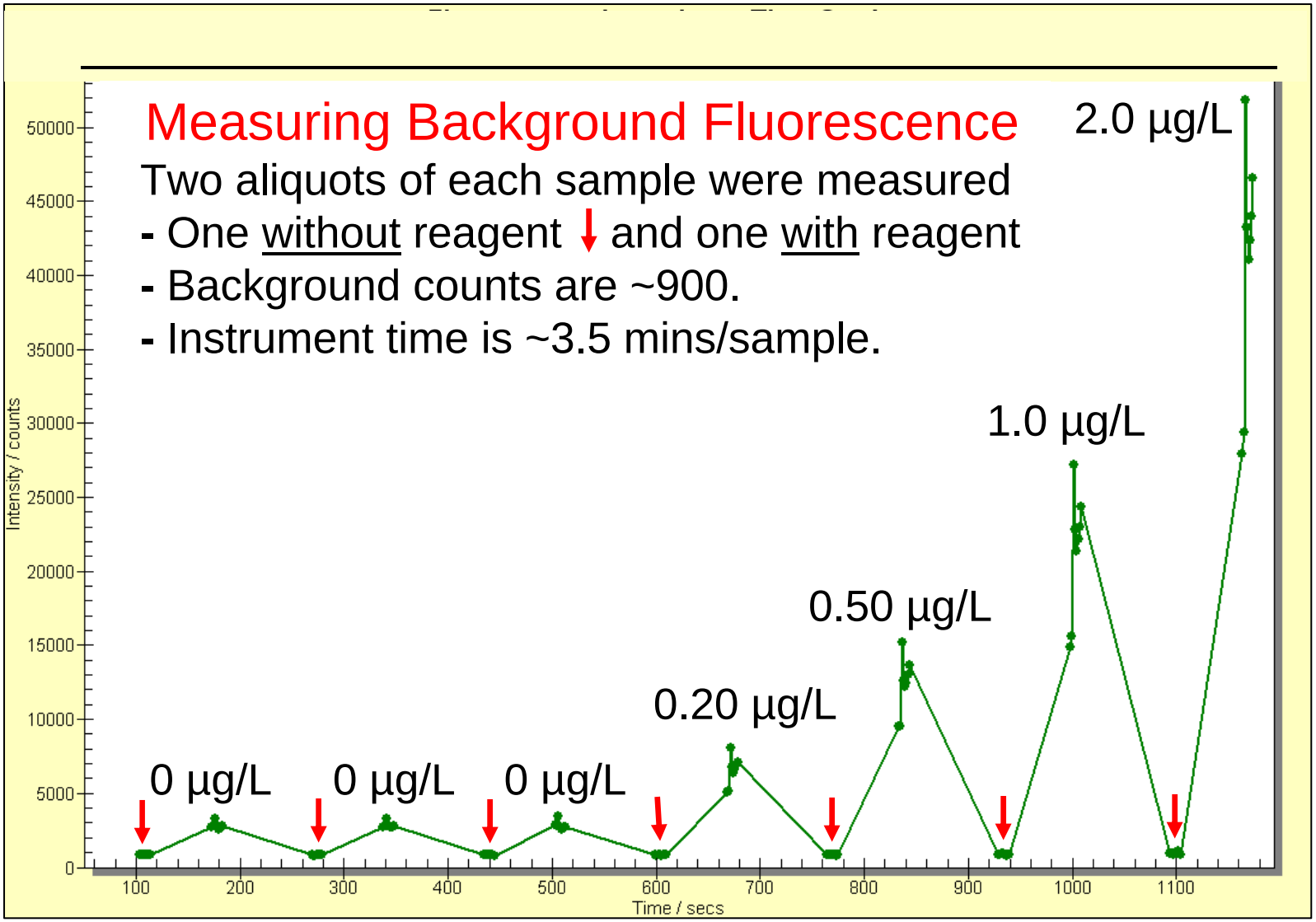
- Be in one sample can show up in the next sample
- Minimized by extensive rinsing after each analysis
- Eleven blanks, analyzed following analyses of Be check standards, gave calculated values of either 0.00 or 0.01 µg Be/L.

Background Interferences to SIA Method

Background Fluorescence

- Is more of a problem with surface-swipe samples
- Past work:
  - 1 mg of activated charcoal per ml of sample was added to adsorb unwanted, fluorescent species
- This work:
  - Aliquot <u>without reagent</u> is used to measure counts of background fluorescence
  - aliquot <u>with reagent</u> is used to measure counts of total fluorescence
  - Net counts = total counts - background counts

Background Interferences to SIA Method



Background Interferences to SIA Method

Whatman #41 filter-paper disc (swipe) were added to 4 check standards. (Normal background ~900 counts.)

Check Std	Background Fluorescence Counts due to Paper Disc (no reagent)	Calculated Concentration Without Background Subtraction	Calculated Concentration With Background Subtraction
Blank	~12,600	~ 0.5 µg Be/L	0.01 µg Be/L
0.10	~11,100	~ 0.6	0.12
0.20	~12,200	~ 0.7	0.24
0.50	~13,600	~ 1.0	0.51

Iron Interferences to SIA Method

Dissolved Iron

- Is a potential problem with surface-swipe samples only
- Dissolved iron is precipitated by HBQS solution
- Particles block light inside detector and reduce signal

- All samples and standards (in 3% ABF) were pre-treated with 8 vol% addition of 10-N NaOH solution to precipitate iron, if present:
  - i.e. 400 µL NaOH solution is added to 5 ml sample
  - Sample is centrifuged if solution is not clear.

Iron Interferences to SIA Method

Dissolved Iron

- Samples with 0.5 g Fe/L were prepared from Be standard solutions
- Iron precipitated as significant, red/brown mass after addition of 10-N NaOH solution
- Be recovery was >85% from resulting solution after sample was centrifuged

Results for Calibration Curve

Calibration curve (max height) = A + B conc + C conc^2 + D conc^3

From the calibration curve, the concentrations of standards (or unknowns) can be calculated from the measured counts:

Std	Conc	Calc	Net Counts
1	**0.00**	**0.00 µg Be/L**	2458
2	**0.20**	**0.19**	7216
3	**0.50**	**0.48**	14375
4	**1.00**	**0.97**	26448
5	**2.00**	**1.96**	51007
6	**5.00**	**4.96**	124391
7	**10.0**	**10.01**	246629
8	**20.0**	**20.02**	483301
9	**50.0**	**49.99**	1156720
10	**100.**	**100.00**	2219989

A = 2458.0
B = 24794.1
C = - 42.06
D = 0.159

R^2 = 0.9999994

Results for Check Standards

Check Std	Conc	Calc	Mass Be (µg) *
1	0.00	0.01 µg Be/L	0.0000
2	0.05	0.05	0.00025
3	0.10	0.09	0.0005
4	0.20	0.19	0.0010
5	0.50	0.48	0.0025
6	1.0	0.97	0.0050
7	2.0	1.96	0.010
8	5.0	4.96	0.025
9	10.	10.01	0.050
10	20.	20.02	0.100
11	50.	49.99	0.250
12	100.	100.00	0.500

***** Mass Be (µg) = Conc (µg Be/L) x 0.005 L (sample vol)

Summary

1) A fluorescence analysis for Be has been developed for a FIAlab-3200 sequential-injection analyzer with a PMT detector and autosampler. The software development was key to the methods development.
2) Problems of carryover, background fluorescence, and dissolved iron have been addressed favorably.
3) The instrument time is ~3.5 minutes per sample.
4) Only 35 μL of HBQS solution is required per sample.
5) This low-cost, automated, desktop instrument can measure Be concentrations down to ~0.05 μg Be/L.