USGS New York Water Science Center
Soil and Low Ionic Strength Water Quality Laboratory

Flow Injection Analysis

Standard Operating Procedure

1. Scope and Application

1.1 Analytes

Total Monomeric Aluminum and Organic Monomeric Aluminum

1.2 Reporting Limit

1.5 μmol Al/L

1.3 Applicable Matrices

This method is used to determine total monomeric aluminum and organic monomeric aluminum concentrations in precipitation, dilute surface waters and soil waters.

1.4 Dynamic Range

The analytical range for this determination is 0.1 to 37.06 μmol Al/L. Samples concentrations that exceed this range must be diluted and reanalyzed.

2. Summary of Procedure

This analysis for total monomeric aluminum and organic monomeric aluminum is an automated colorimetric reaction on a dual channel flow injection analyzer (FIA). Both channels are identical except for the addition of a cation-exchange column in the organic monomeric aluminum manifold. Each sample is systematically introduced into the FIA reaction manifold. In the organic monomeric aluminum manifold, the sample is initially passed through the resin column, which removes inorganic aluminum. In both manifolds, the sample is then mixed with a 1,10-phenanthroline/hydroxlyamine hydrochloride reagent, which eliminates the interference of iron. The sample subsequently reacts with a pyrocathecol violet (PCV) solution that turns blue-gray in the presence of monomeric aluminum. Aluminum polymers and strongly complexed Al monomers will not react with PCV. A hexamethylenetetramine buffer is added to adjust the pH to about 6.2. The absorbance of the color complex is measured at a wavelength of 580 nm. Inorganic aluminum is subsequently calculated by subtracting the organic monomeric aluminum concentration from the total monomeric aluminum concentration for each sample.

3. Safety Issues

3.1 Chemical Hazards

A. All strong acids and bases should be mixed in a fume hood.

B. Gloves, safety glasses, and lab coats should be worn when preparing and performing this analysis.
C. For proper handling techniques for specific chemicals, consult the appropriate Material Safety Data Sheets (MSDS).

4. Sample Preservation, Containers, Processing and Analysis Times

4.1 Sample Preservation

Samples are stored at 4°C without being filtered or acidified.

4.2 Containers

Samples are stored in 250-mL acid-washed polyethylene bottles.

4.3 Processing and Analysis Times

Sample processing: one week
Lab analysis: two weeks
LIMS entry: one week

5. Reagents and Standards

5.1 General Information

All reagents are commercially purchased and should be stored in the original container. Date the reagent bottles when received and when opened. Note expiration date, if any. No verification of the reagents is necessary.

5.2 Reagents

A. Degassed Deionized (DI) water
   1. Degas Milli-Q water by bubbling with commercial-grade helium for about 2 minutes.
   2. Degassed Milli-Q water is used for carrier water and for the preparation of all reagents.
   3. Prepare daily.
B. 1,10-Phenanthroline/Hydroxlyamine Hydrochloride
   1. Use 99+% purity hydroxylamine hydrochloride ($H_2NOH{\cdot}HCL$) and 99+% purity anhydrous 1,10-phenanthroline ($C_{12}H_8N_2$).
   2. Add 7.6 g hydroxlyamine hydrochloride to a 1,000-mL volumetric flask that contains about 500 mL Milli-Q water.
   3. Add 0.56 g anhydrous 1,10-phenanthroline to the same flask and stir until dissolved (about 20 minutes).
   4. Fill close to final volume with Milli-Q water and mix thoroughly with stir bar.
   5. Remove stir bar, fill to final volume and mix.
   6. Store in a polyethylene bottle at 4°C; label and date.
   7. Prepare every other day.
C. Pyrocatechol Violet (PCV)
   1. Use PCV that is about 90% pure.
   2. Add 0.386 g PCV to a 1,000-mL volumetric flask that contains about 500 mL Milli-Q water.
   3. Swirl until the PCV has dissolved.

4. Fill close to final volume with Milli-Q water, mix, then fill to final volume and mix again.
5. Store in a polyethylene bottle at 4°C; label and date.
6. Prepare every other day.

D. Hexamethylenetetramine Buffer
1. Use 99+% hexamethylenetetramine [$(CH_2)_6N_4$].
2. Add 168.0 g hexamethylenetetramine to a 2,000-mL volumetric flask that contains about 1,000 mL Milli-Q water.
3. Mix until all solid is dissolved.
4. Fill close to final volume with Milli-Q water, mix, then fill to final volume and mix again.
5. Store in a polyethylene bottle at 4°C; label and date.
6. Prepare every other day.
7. Due to the aromatic nature of this chemical, this reagent should be prepared in a chemical safety hood.

E. 0.1 M Hydrochloric Acid
1. Use commercially purchased 0.1 M hydrochloric acid (HCl).
2. Replace solution when the expiration date has passed.

F. 1 mM Sodium Chloride
1. Add 0.058 g sodium chloride (NaCl) to a 1,000-mL volumetric flask that contains about 500 mL Milli-Q water.
2. Swirl to dissolve solid.
3. Fill close to final volume with Milli-Q water, mix, then fill to final volume and mix again.
4. Store in a polyethylene bottle; label and date.
5. Prepare every 6 months.

G. Cation-Exchange Resin (pH about 5.0)
1. Add 100 mL Milli-Q water to a 250-mL beaker containing 100 g Amberlite IR (plus) ion-exchange resin-sodium form and 1.00 g Amberlite IR (plus) ion-exchange resin-acidic form.
2. Mix thoroughly and decant the liquid.
3. Wash again with Milli-Q water and then twice with 100 mL portions of 1 mM NaCl.
4. Add 100 mL of 1 mM NaCl to the beaker.
5. Store in a wide mouth container at 4°C; label and date.
6. Prepare every 6 months.

H. Cation-Exchange Column
1. Remove the fittings and foam plugs at both ends of the glass column and rinse the column with Milli-Q water.
2. Replace the foam plug at one end using forceps.
3. Draw about 10 cc resin and supernatant into a 20-cc syringe.
4. With the plugged side down, inject the resin into the top of the column in one motion without allowing air into the column. Once the column

is full, cover the bottom end with a gloved finger and plug the top end with foam. Replace the fitting on the top end, then turn the column over and replace the fitting on the other end.

5. Keep the column closed until the system is ready for the column addition. If the liquid drains out or air is introduced in to the column, the column must be rinsed and repacked.
6. The column is repacked daily and the resin is not recycled.

I. Cleaning Solution
1. Add 65 g sodium hydroxide (NaOH) to a 1,000-mL volumetric flask that contains about 800 mL Milli-Q water.
2. Swirl the flask until the pellets have dissolved.
3. Add 6 g disodium ethylenediamine tetraacetic acid dihydrate ($Na_2EDTA \cdot 2H_2O$) to the flask.
4. Allow to cool.
5. Fill close to final volume with Milli-Q water, mix, then fill to final volume and mix again.
6. Store in a polyethylene bottle; label and date.
7. Prepare every 6 months.

5.3 Standards

E. Aluminum Standard Stock Solution, 1,000 mg Al/L
1. Use a commercially purchased 1,000-mg/L aluminum standard.
2. Replace with a new stock when the expiration date has passed.
3. To avoid contamination, aliquots of stock solution must not be withdrawn directly from the bottle.

F. Aluminum Standard Substock Solution, 50 mg Al/L
1. Pipet 25.0 mL aluminum standard into a 500-mL volumetric flask.
2. Fill close to final volume with Milli-Q water, mix, then fill to final volume and mix again.
3. Store in a polyethylene bottle; label and date.
4. Prepare every 6 months.

G. Aluminum Working Standards
1. Pipet desired amount of standard substock into a designated 100-mL polyethylene volumetric flask.
2. Add 1.0 mL 0.1 M HCl.
3. Fill close to final volume with Milli-Q water, mix, then fill to final volume and mix again.
4. Label and date.
5. Prepare weekly.

Working Standard	Aluminum Concentration	Standard Substock Added (mL)
A	37.06 μmol/L (1.00 mg/L)	2.0
B	27.80 μmol/L (0.75 mg/L)	1.5
C	18.53 μmol/L (0.50 mg/L)	1.0
D	11.12 μmol/L (0.30 mg/L)	0.6
E	7.41 μmol/L (0.20 mg/L)	0.4
F	3.71 μmol/L (0.10 mg/L)	0.2
G	1.85 μmol/L (0.05 mg/L)	0.1
H	0.00 μmol/L (0.00 mg/L)	0.0

D. Aluminum Quality Control (QC) Stock Solution, 1,000 mg Al/L
   1. Use a commercially purchased 1,000-mg/L aluminum standard. This stock must be from a manufacturer or lot different from the standard stock.
   2. Replace with a new stock when the expiration date has passed.
   3. To avoid contamination, aliquots of stock solution must not be withdrawn directly from the bottle.

E. Aluminum QC Substock Solution, 50 mg Al/L
   1. Pipet 25.0 mL aluminum standard into a 500-mL volumetric flask.
   2. Fill close to final volume with Milli-Q water, mix, then fill to final volume and mix again.
   3. Store in a polyethylene bottle; label and date.
   4. Prepare every 6 months

F. Aluminum QC Samples
   1. Pipet desired amount of QC substock into a designated 250-mL polyethylene volumetric flask.
   2. Add 2.5 mL 0.1 M HCl.
   3. Fill close to final volume with Milli-Q water, mix, then fill to final volume and mix again.
   4. Label and date.
   5. Prepare weekly.

QC Sample	Aluminum Concentration	QC Substock Added (mL)
High	18.53 μmol/L (0.50 mg/L)	2.5
Low	7.41 μmol/L (0.20 mg/L)	1.0

6. QC Procedure

A. The standard curve is a third order curve obtained by plotting standard concentration vs. peak area. The best-fit line is drawn and the curve is accepted if the correlation coefficient is 0.998 or greater.

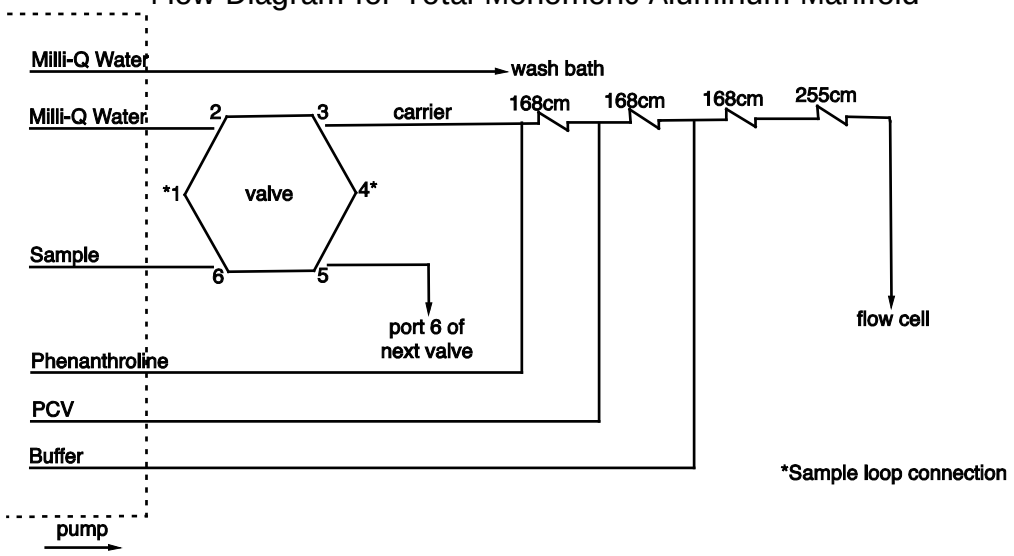
B. Quality-control samples are analyzed at the start of the run, after every 10 samples during the run, and at the end of the run.
C. A quality-control sample is acceptable if the analyzed value is within 10 percent of the QC-high range known value and 15 percent of the QC-low range known value.
D. If one of the QC samples fails the acceptance criteria, the run is stopped and the QC sample is re-run. If the QC sample fails again the run is stopped, the QC sample is remade from the substock, and/or the instrument is re-calibrated. Samples associated with the failed QC samples are re-analyzed.
E. QC samples are not currently available for the organic monomeric aluminum analysis. The QC sample is a check of column efficiency. The resin column should remove all aluminum from the QC sample. If the QC produces a peak, the column should be repacked.

7. Chemical Analysis Procedure

7.1 Instrumentation

Lachat QuickChem 8000 dual channel flow injection analyzer
Omnion software v 3.0.219H

Flow Diagram for Total Monomeric Aluminum Manifold



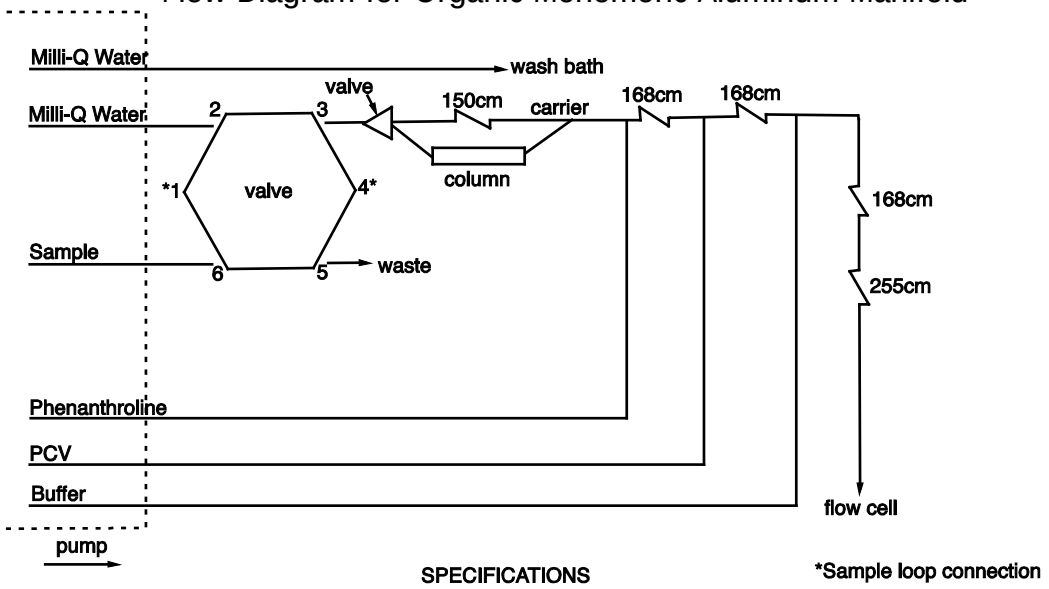
SPECIFICATIONS

Manifold tubing: 0.8 mm i.d.
Sample loop: 150 cm (780µL)
Flow cell: 10 mm
Interference filter: 580 nm
Pump setting: 35

Pump tubes:
Milli-Q Water/wash bath: 2.00 mL/min (green)
Milli-Q/carrier: 1.60 mL/min (blue)
Sample: 2.00 mL/min (green)

Phenanthroline: 0.32 mL/min (black)
PCV: 0.32 mL/min (black)
Buffer: 0.80 mL/min (red)

Flow Diagram for Organic Monomeric Aluminum Manifold



Manifold tubing: 0.8 mm i.d.
Sample loop: 150 cm (780μL)
Flow cell: 10 mm
Interference filter: 580 nm
Pump setting: 35

Pump tubes:
Milli-Q Water/wash bath: 2.00 mL/min (green)
Milli-Q/carrier: 1.60 mL/min (blue)
Sample: 2.00 mL/min (green)

Phenanthroline: 0.32 mL/min (black)
PCV: 0.32 mL/min (black)
Buffer: 0.80 mL/min (red)

7.2 Timing

Method Cycle Period - 55 seconds
Auto-sampler Timing Sample Period - 22 seconds
Minimum Probe in Wash Period - 24 seconds

Setting	Total Monomeric Al Channel (seconds)	Organic Monomeric Al Channel(seconds)
Load Period	17	18
Inject Period	38	37
Time to Valve	17	19
Expected Inject to Peak Start	20	30
Expected Peak Base Width	40	40

7.3 Start-Up

A. Turn the surge protector power on.
B. On the computer desktop, double click the **Omnion 3.0** icon.
C. Check the waste container daily and empty if nearing capacity.
D. When the Omnion data system screen appears, click on **Configuration** and choose **Autosamplers**. Click on the **Initialize Autosampler** button; then close the pop-up window.
E. Fill the carrier bottle with Milli-Q water.

F. Place pump tubes in appropriate reagent containers- see diagram. Cover reagent bottle openings with parafilm.
G. Tighten pump tubes and turn pump on. Pump should be set at remote and speed setting should be 35.
H. Allow reagents and Milli-Q water to pass through system for at least 10 minutes. Observe manifold for any leaks, clogs and/or bubbles in the lines.
I. Turn manifold valve to allow flow through resin column. Put resin column in-line. Allow the carrier to flow through the column for a few minutes, and then turn the valve to bypass the column.

7.4 Calibration

A. Click on the **OPEN** folder; double click the **aluminum calibration only.omn** file.
B. Fill appropriate tubes on calibration rack with standards and QC samples.
C. Click the green **START** arrow.
D. If the calibration is within acceptance criteria, the instrument will analyze the QC samples. If the calibration fails, a message will pop up, with options to proceed. In most cases choose **Recalibrate**.
E. If the calibration and QC samples pass, set up a sample tray.

7.5 Analysis

A. Take aluminum samples out of the refrigerator and let warm to room temperature.
B. Turn manifold valve to allow flow through the resin column.
C. Click on the **OPEN** folder; double click the **speciated default.omn** file.
D. Recalibrate if necessary, or delete the calibration standard rows from the run worksheet.
E. Enter the sample serial numbers (SSNs) into the run worksheet. Use the Auto Sample ID increment feature if the SSNs are consecutive.
F. From menu bar select **RUN**. Click **Export Worksheet Data**. Note date on run worksheet printout.
G. Fill the autosampler tubes with appropriate samples.
H. Click on the green **START** arrow.
I. Fill QC containers and carrier bottle as needed.
J. QC samples will be run automatically every 10 samples. If all QC samples pass, the run will proceed until the tray is finished.
K. If a QC sample fails, a message will pop up and analysis will cease. Click the **Stop Now** option. Remove SSNs that have been analyzed from the run worksheet.
L. Click green **START** arrow.
M. If QC samples pass, the run will continue. If QC samples fail, remake QC samples from substock and/or recalibrate.
N. Review the sample peaks and analysis data as the run progresses. Note any air bubbles, bad peaks, and/or samples requiring dilutions or re-runs.

7.6 Shut Down

A. Turn the manifold valve to bypass the resin column.
B. Change the pump setting to override standby.
C. Remove pump tubes from reagent bottles and rinse lines and weights with Milli-Q water. Place lines in a beaker of Milli-Q water.
D. After several minutes, place the PCV pump tube lines in the cleaning solution. Allow to pump about 15 seconds.
E. Rinse PCV pump tube reagent lines with Milli-Q water and place in beaker of Milli-Q water again. Allow to pump several more minutes.
F. Remove lines from beaker and pump air until no more water is moving through manifold.
G. Release tension of pump cartridges.
H. Exit Omnion.
I. Turn red button on surge protector off.

7.7 Maintenance

A. All pump tubes should be replaced as they become worn or stretched; the frequency depends upon the number of samples analyzed. The sample and wash bath tubes should be changed as they become discolored or clogged. Note dates in instrument notebook.
B. Interference filters should be cleaned with lens paper twice a year or whenever they become dusty or soiled.
C. Manifold tubing should be replaced as it becomes discolored or clogged; note in instrument notebook.
D. Sample tubes are rinsed, soaked in DI water overnight, and oven dried at 60°C between uses.
E. Buildup and clogging in the waste lines may require periodic replacement of the lines; note in instrument notebook.

7.8 Data Processing and LIMS Entry

A. In Microsoft Excel, open all files for the run date. They are stored in C:\Program Files\Lachat\Omnion\Data. All files are in a comma delimited text format.
B. Edit files as needed and copy/paste them all into the same file.
C. Save as a comma delimited file (.csv file extension), using the format MMDDYYYY.csv.
D. Print a copy, note the date on the copy, and close the file.
E. Double click **Watershed LIMS** icon.
F. Click **Custom**, then **Import Data**.
G. Under the Import drop down, choose **Monomeric Aluminum data**.
H. Choose and open the desired file.
I. Choose **Client**, **Units**, type in **Test Date**, and choose **Analyst**.
J. Exclude and/or edit any data necessary.
K. Click **Client ID to Sample No**.
L. Click **Set Data**.

M. Investigate problems for data that did not transfer or are duplicated.

8. Archiving

8.1 Data

Data files are backed-up daily by an automatic back-up program. Hard copies of the runs are filed and kept indefinitely. The laboratory LIMS system is backed up daily by automatic back-up program.

8.2 Samples

Samples are stored at room temperature until data can be verified. Sample bottles are cleaned and reused for new samples.

9. References

Lachat Instruments, 1997, Methods manual for the QuikChem automated ion analyzer – Method no. 10-113-33-1-A: Milwaukee, Wisc., Lachat Instruments (variously paged).

Lachat Instruments, 1997, Methods manual for the QuikChem automated ion analyzer Method no. 10-113-34-1-B: Milwaukee, Wisc., Lachat Instruments (variously paged).

Peden, M.E. and others, 1989, Evaluation of aluminum speciation using synthetic and natural samples – final report:Champaign, Ill., Illinois State Water Survey Contract Report 463.

U.S. Environmental Protection Agency, 1987, Handbook of methods for acid deposition studies – laboratory analysis for surface water chemistry, Washington, D.C., U.S. Environmental Protection Agency, EPA 600-4-87/026 (variously paged).

10. Key Words

Flow injection analysis, total monomeric aluminum, organic monomeric aluminum, water, soil water.