Methods and Materials Manual
Of Ion Chromatography Using DX-100 IC

Collecting Samples

Field Material:

LDPE bottle with cap (pre-rinsed with DI water)   thermometer
permanent marker   field notebook

Procedure:

1. Record site description in field notebook, along with corresponding site number.
2. Carefully lower bottle into stream in area that is flowing freely and fill with some water to rinse.
3. Repeat Rinse.
4. Lower bottle into stream again; fill to capacity, ensuring that no sediment from the bottom is included.
5. Measure temperature; record in field notebook.
6. Mark site number and date on the bottle.

Filtering your Samples

Samples must be filtered before they are run through the Ion Chromatograph. The filtering process removes large particulate matter, which would impede IC readings. Filtering is done by Gelman Acrodisc 13 mm syringe filters (0.45μ) membrane filters.

Creating Standards

Standards are used to calibrate the DX-100 machine and assure the highest quality of chromatograph readings. The standards created represent the expected range of ionic concentration in the samples to be tested. Often, this range can be estimated, for we anticipate certain ionic concentrations from certain types of samples. For instance, precipitation will have much lower concentrations than stream water, and therefore will require a smaller range of standards.

Each diluted standard has a specific ionic concentration that must be calculated using information given on the label of Dionex Seven Anion solutions.

Anion Analysis

Laboratory Material:

5-100mL/25ml volumetric flasks   200-1000 uL pipet   1000uL pipet tips
Deionised water   marking pen   parafilm
Dionex Seven Anion Standard solution (SAS), Part # 56933 (refrigerated when not in use); contains 20mg/L Fluoride, 30mg/L Chloride, 100mg/L Nitrite, 100 mg/L of Bromide, 100 mg/L of Nitrate, 150mg/L Phosphate, 150mg/L Sulfate

**Procedure:**
1. Rinse volumetric flasks few times (~3) each with DI water to remove possible contaminants.
2. Use marker to make labels for each flask:
   Standard 0 = 0mL SAS/ 100mL total (Blank);
   Standard 1 = 300 microlitre SAS/ 100mL total;
   Standard 2 = 600 uL SAS/ 100mL total;
   Standard 3 = 1.2mL SAS/ 100mL total;
   Standard 4 = 2.5ml SAS/100mL total;
   Standard 5 = 5.0mL SAS/100mL total.
3. Fill volumetric flasks about ¾ full with DI water; leave enough room to add SAS to each vial without exceeding 100mL line/25 ml line (depends of whichever flask you are using)
4. Fill the flask marked 0 completely with DI water.
5. Gently shake to homogenize diluted standards; invert flasks and agitate for few minutes.
6. Seal with parafilm to store; lasts approximately one week.

**Computation:**
The standard concentration range created can be calculated in the following manner:
Ex: Find the concentration of F⁻ in Standard 5.
Given 20mg/L = 20mg/1000mL = Xmg/5mL (use 5mL SAS in Standard 4); X = .10mg .10mg/8mL; add DI to make 100mL total → .10mg/100mL = 1.0mg/L
Concentration of F⁻ in Standard 5 = **1.0mg/L**

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**Prepare Sample Vials**

**Laboratory Material:**
- 5mL plastic vials with caps
- capping tool
- sample racks
- 100mL beaker of DI water
- 200-1000uL pipet
- pipet tips
- bottles of sample
- sample form
- anion
- anion/cation standard series

**Procedure:**

**A. Standards**
Always begin IC runs with a set of standards
1. Fill vial ¾ way with DI water to create “blank” vial.
2. Place cap in vial; push down with capping tool (side with hole in it) to straighten cap; invert capping tool (to flat side); push down until cap is level with mouth of vial.
3. Place vial in sample rack; record sample description, rack number and vial space on
sample form; check that these numbers correspond as they determine
the sampling order during an ion analysis run.
4. Repeat step 1 through step 3 for each standard; one entire rack should be filled.

B. Undiluted Samples

Undiluted samples must always be run through the Ion Chromatograph when
performing ion analysis. The resulting data will be more error-free than that of diluted
samples.
1. Fill vial ¾ way with sample.
2. Place cap in vial; push down with capping tool (side with hole in it) to straighten cap;
invert capping tool (to flat side); push down until cap is level with mouth of vial.
3. Place vial in sample rack; record rack number and vial space on sample form.
4. After every five samples, include either a blank (vial of DI water) or a check (vial of
anion/cation solution, depending upon the type of analysis to be performed).

C. Diluted Samples

If data is incomplete (i.e., peaks are too high to be read by the machine), after
running undiluted samples, create diluted samples. The actual dilution factor will vary,
depending upon how concentrated your samples are. Multiple runs of the same sample
are often required to complete a data set, so allow plenty of time (a few days to a week)
to finish experiment. The following is an example of how to prepare diluted samples
using a dilution factor of 20. That is, 0.25mL sample in 5mL DI water (1mL sample in
20mL DI).
1. Attach tip to pipet; set to 250uL (0.25mL).
2. Extract 250uL of sample; inject into vial.
3. Discard pipet tip; attach new tip.
4. Set pipet to 1000uL (1mL).
5. Extract 1000uL of DI water from beaker; inject into vial.
6. Repeat step 5 until there are 5000uL of DI in the vial.
7. Place cap in vial; push down with capping tool (side with hole in it) to straighten cap;
invert capping tool (to flat side); push down until cap is level with mouth of vial.
8. Place vial in tray; record tray number and vial space on sample form.
9. Discard pipet tip; attach new tip and repeat step 1 through step 8 until all samples have
been diluted. Be sure to change pipet tip for each sample extraction and for each
switch between sample and DI water. Also, after every five samples, create either a
blank or check vial.

Create a Schedule in PeakNet

Computer Lab Material:
computer        printer        PeakNet program        completed sample form

Procedure:
1. Turn on computer and printer.
2. Open PeakNet program by double clicking MenuDx icon.
3. Choose Schedule option on menu screen.
4. Fill in sample descriptions in the Sample column;
5. Choose “sample” for all the real samples, blank, standards in the Sample Type column.
6. Double click on the first box in the Method column; select analysis method from this list. The number refers to the number of injections of sample extracted for the run (which is usually 1).
7. Fill down method in spreadsheet;
8. Fill down for the Data File column. During the run, the computer automatically fills in this column with the path under which each data file is saved.
9. Volume, Weight and Int. Std. columns should all automatically fill in with the number “1.” If not, add and fill down.
10. Fill down the Dilution column with the dilution factor calculated. For undiluted samples, the factor is 1; for our example diluted run the dilution factor would be 20.

Prepare Eluent for Ion Chromatography: Anion Analysis

**A: Anion Stock Solution**

*Laboratory Material:*
- Sodium Carbonate (Na$_2$CO$_3$)
- Sodium Bicarbonate (NaHCO$_3$)
- rubber gloves
- 500 mL volumetric flask
- 1000mL graduated cylinder
- 2 Dionex plastic storage containers
- electronic balance
- DI water w/resistivity of 18.2 megaohm-cm or better

*Procedure:*
1. Tare balance; put on rubber gloves; measure out 26.49g of Na$_2$CO$_3$.
2. Fill graduated cylinder with 400mL DI water; pour into flask.
3. Add the Na$_2$CO$_3$ to the flask; agitate to dissolve.
4. Solution volume will increase; fill flask to the 500mL mark.
5. Stock solution is 0.5M sodium carbonate; pour into Dionex plastic container for storage.
6. Tare balance; measure out 21.00g of NaHCO$_3$.
7. Repeat steps 2 through 5 to create 0.5M sodium bicarbonate stock solution.

**Anion Eluent Solution**

*Laboratory Material:*
- 0.5M sodium carbonate stock solution
- 0.5M sodium bicarbonate stock solution
- 25 mL graduated cylinder
- 10mL graduated cylinder
- 2L volumetric flask
- DI water

*Procedure:*
1. Fill flask ⅔ full with DI water.
2. Measure 10.8mL of 0.5M sodium carbonate stock solution in 25mL graduated cylinder; carefully pour into flask.
3. Measure 1.2 mL of 0.5M sodium bicarbonate stock solution in 10mL graduated cylinder; carefully pour into flask.
4. Fill flask to 2L mark with DI water; the anion eluent solution is 2.7 mN Na$_2$CO$_3$ and 0.3 mM NaHCO$_3$. 


5. Aspirate solution for 5 minutes, or until most of the small bubbles are removed.

5. Cation eluent solution is 22mN H₂SO₄.

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**Start Up the Ion Chromatograph**

*Laboratory Material:*

- Dionex model DX-100 Ion Chromatograph
- Dionex model AS40 Automated Sampler
- N₂ gas tank
- 3 regenerant bottles + DI water
- waste bucket
- laboratory log book
- prepared eluent solution (either cation or anion, depending upon analysis type)

*Procedure:*

Refer to **Figure 4.**

1. Fill **eluent bottle** with prepared eluent if less than halfway full.
2. Fill **regenerant bottles** with DI water; make sure the DX-100 pump is off (usually the IC is not turned on at this point; however, if it is on and in Relay mode, use the shutdown method to turn the pump off).
3. Make sure the N₂ gas tank valves are closed.
4. Remove the caps of both regenerant bottles to release any built up pressure.
5. Fill the bottles with DI water using the extra regenerant bottle located to the left of the Automated Sampler.
6. Check the **waste bucket**; empty if more than half full.
7. Turn on the **Automater Sampler (AS40)** and **Ion Chromatograph (DX-100)**. The power switch for the AS40 is located on its back panel; the DX-100 has a switch on top right-hand side.
8. Open the **N₂ gas tank** by turning the right and left knobs in the directions indicated. DO NOT change middle knob.
9. Check the N₂ pressures (regenerant 10-15 psi, eluent 5-9 psi, N₂ regulator at 100 psi).
10. Check the **channel control settings**; should be set on eluent A for anion analysis, B for cation. THIS IS VERY IMPORTANT! INCORRECT SETTINGS WILL DAMAGE THE IC COLUMNS.
11. Set the **high pressure limit** at 2800 psi (hold down the button for 3 seconds).
12. Set the **range** at 30uS for anion analysis or 3uS for cation analysis.
13. Open the **transducer (bleed) valve** and turn the pump on. The pressure should be zero for at least one minute to flush out any air bubbles from the system. Close the bleed valve. (This step is not necessary if the DX-100 has been used recently. If it has been idle for more than a day, then it should be flushed.)
14. Make sure the flow rate (black knob, lower right corner inside IC) is set correctly at 1.5mL/min (150) for anions and 1.0mL/min (100) for cations. The valve sometimes shakes rapidly; watch out for this.
15. Allow the DX-100 to equilibrate with the pump on for 20 minutes. Check that the **background conductivity** has settled within the range 14-16 uS for anions or 0.6-0.8 for cations; record background conductivity in log book.
15. Record cell pressure and N₂ tank pressure in log book.

**Load and Run Samples**

*Laboratory Material:*
Dionex model DX-100 Ion Chromatograph/Dionex model AS40 Automated Sampler
Assembly
preparation sample racks prepared PeakNet schedule (saved on computer)
computer printer

*Procedure:*
1. Create new folder under C:/PeakNet/data. Recommended folder name includes analysis type and date.
2. Open MenuDx icon; select Run.
3. Go to File and Load Schedule; select the schedule created in Part V. A screen will come up, with an option to Browse data file path. Choose the folder you created in step 1.
4. Choose desired injection level on the AS40 Automated Sampler (usually already set at 1); switch AS40 from Hold to Run.
5. Switch IC from Local to Relay (press Control button). IF YOU DO NOT, NO READINGS WILL BE MADE BY THE IC!
6. MAKE SURE THE PRINTER IS ON AND THERE IS PLENTY OF PAPER!
   If there is no more paper, it is necessary to manually reprint each data sheet.
7. In the MenuDx Run window, select Run and then Start; the machine will automatically run.
8. Each sample takes about 15 minutes to run through the machine completely. Estimate when the analysis run will end. Return to shut down the apparatus (see Part X).

**Things to check periodically while running the IC:**
- Eluent and regenerant pressures: Increasing pressure may indicate a clog in the system.
- Eluent conductivity: If baseline conductivity increase quickly or falls outside the required range (14-16 uS for anions, 0.6-0.8 for cations), there may be a problem with the regenerant flow.
- Regenerant flow: The DI water should flow into the suppressor and bubbles should flow out.
- Eluent flow: Occasionally the flow rate knob shakes and changes the speed of eluent passing through the machine. If this happens, just dial the number back down (to either 100 for cations or 150 for anions) and lock it.
- Eluent level: The eluent bottle should NEVER run dry.

**Shut Down of the Ion Chromatograph**

*Procedure:*
1. The Shutdown method included in the schedule turns off the IC pump automatically.
2. If the IC will remain idle for more than a day, turn the channel setting to Rinse. Turn the pump back on for 2 minutes; switch back to eluent A/B and shut off pump.
3. Flip the power switches to turn off the DX-100 and AS40.
4. Close the N₂ valves TIGHTLY.
5. Close the PeakNet program.
6. Refill the eluent and regenerant bottles if the are low.
Computer Analysis: Excel Spreadsheets and Graphs

The Ion Chromatograph gives data in the form of computer printouts. When a specific ion is present, it will show up as a peak in conductivity on the graphs provided by the PeakNet program. Ions have various retention times (time it takes to separate out chemically), and so the computer program is able to label each peak. A separate table provides the exact area beneath the each peak, and the ion to which this number applies. We convert these peak areas to ionic concentrations through use of an excel spreadsheets.

Standards Spreadsheet

We can compare the previously calculated concentrations of prepared standards to the data received from the IC printouts (i.e., peak area conversions). The Excel Standard Spreadsheet provides a sensitivity (correcting) factor that is used later on to compute the ionic concentrations in the samples.

Procedure:
1. Open “IC Template” on computer desktop; the first page of this spreadsheet contains an outline for the Standards Spreadsheet, with all the necessary Excel equations already included.
2. Copy and paste this template onto a new spreadsheet; save as a new excel file preferably with the same name as you data file path (ex: anion.5.9.01).
3. Delete the old peak area numbers and input new data.
4. Delete the old mg/L and enter the series of concentrations computed for standards in Part III.
5. The computed slope for each ion is the previously mentioned sensitivity correction. The Standard Error (Std.Error) for each should be less than 5%.