**Dionex Protocol\_ revised 3/7/14**

Dual ICS-2000 Ion Chromatography Systems

System 1: AERS 500 4mm– self regenerating suppressor & AS-18 column, KOH eluent generator cartridge

System 2: CERS 500 4mm self regenerating suppressor & CS-12a column, MSA eluent generator cartridge

Anion standards range: Cl-, SO4-, Br-: 0.01-30 mg L-1; NO3--N, PO43--P: 0.001-3 mg L-1

Cation standards range: Na+, Ca2+, Mg2+: 0.3-100 mg L-1; K+: 0.03-10 mg L-1

Initial Steps \*\*NEW\*\*

1. Turn on instrument(s) (on back, upper right)
2. Refill eluent bottle (s) with fresh nanopure water (18.2 M-Ohms), using dedicated flask
3. Login to computer locally (to machine: NS-K216-01, not WIN network) as NS-K216-01\LocoBio, password = b1ology
4. Connect to Chromeleon Server Monitor (right-click the chameleon- should have a red x through it if not connected- in the lower right hand menu), click ‘Start server’
5. Check system setup in Server Configuration
   * 1. Open server configuration (should be listed in windows start menu; otherwise, programs\Chromeleon\Server Configuration)
     2. Click on plus box next to timebase you wish to run to expand.
        1. Anions= TERRA
        2. Cations = Cations
        3. Both= Simultaneous
     3. Check that device you wish to run has a serial number listed after the system # and that it doesn’t say ‘virtual’

e.g., Dionex ICS-2000 IC System [04080140] (Single Cartridge)

* + - 1. If it does say ‘virtual’, need to make the instrument active by making the same instrument ‘virtual’ in another timebase.
         1. In the Server Configuration, expand details for all timebases by clicking on plus boxes next to them
         2. Identify systems that have serial numbers after them; these are the live systems.

04100760 is the anion machine (Systems 1 and 3)

04080140 is the cation machine (Systems 2 and 4)

* + - * 1. Right click on the live system you wish to make virtual/simulated and select ‘**Properties**’ \*note\* you cannot be connected to the timebase of any machine in a Chromeleon panel to do this!
        2. On the ‘**General**’ tab in the properties box that pops up, click on the radio button next to ‘simulated’ under Mode.
        3. Click ‘**ok**’ to close properties box.
        4. Right click on the virtual system you wish to make live and select ‘**Properties**’
        5. On the ‘**General**’ tab in the properties box that pops up, click on the radio button next to ‘live’ under Mode. In the Module Serial Number dropdown box, select the appropriate serial number.
        6. Click ‘**ok**’ to close properties box.
        7. Save the server configuration

1. Check that the correct columns are installed in the anion instrument:
   1. TERRA for soil porewater or high concentration samples
   2. RIOS for streamwater samples

Chromeleon Software

1. Double-click the chameleon icon on the Desktop to start the Chromeleon Software
   1. The previously open sequence will appear in the Browser window.
2. Open the panel window (“AnionCationSimultaneous”) to interface with the instrument
   1. In the Browser window, left panel, click on the plus box next to ‘NS-K216\_01\_local’
   2. In the right panel, double- click on ‘**AnionCationSimultaneous.pts**.’ This will open the control panel window.
   3. Expand the window you want for analysis
      1. Terra = anions only
      2. Cations= cations only
      3. Simultaneous= anions & cations from single vial (MUST use 5 ml vials!)
3. Click on ‘connected’ check box on appropriate timebase to connect instrument to software control
   1. ‘Terra’ is anion system timebase
   2. ‘Cations’ is cation system timebase
   3. ‘Simultaneous’ is dual injection timebase
      1. In simultaneous window, ‘System’ = anions (on left side of screen); ‘System 2” = cations (on right side of screen)
   4. Check that the ‘connected’ boxes in the other timebases are unchecked.

Setup

Priming the system

If the system is not running, it needs to be primed

1. Eluent lines:
   1. Click on box “**Pump Settings**”
      1. Then click Eluent Valve “**OPEN”**
   2. Insert a 10ml syringe into the right hand knob (Priming Valve).
   3. Turn the knob counterclockwise 2 quarter turns, then slowly pull back on the plunger. This should draw out air bubbles and eluent.
   4. Withdraw at least 20mL of eluent to remove old eluent from the lines and discard down drain.
   5. Click ‘**close’** eluent priming valve
2. Pump:
   1. Still in Pump Settings, click on “**Prime**”
   2. A warning will pop up to tell you to loosen the Waste Valve (left hand knob, opposite the Priming Valve) before priming.
   3. Turn the knob ½ to 1 turn and click “**OK”**.
      1. Note: don’t open the waste valve too far, or it will let air into system
      2. If pressure jumps up past 150 psi, you didn’t open the waste valve enough; adjust as needed- otherwise pump will shut down.
   4. Prime the system for ~ 5 minutes, or until there are no more air bubbles\* exiting the waste line.
   5. When finished priming, click “**off** ‘ to turn off the pump. Close the waste valve
   6. Proceed with System startup, in next section.
3. *[If you need to prime via the instrument touch-screen panel instead of computer:]*
   1. In lower right corner, touch arrow next to ‘home’. This brings up a menu
   2. Select ‘**Pump**”
   3. In the eluent valve box (lower left), touch ‘**open’**
      1. follow 1b-1d above
      2. when finished removing eluent, touch ‘**closed’**
   4. click the house icon in lower right, next to the menu arrow. This takes you to the home screen.
   5. On left side of screen, halfway down, touch ‘**prime’**
      1. follow 2b-2d above
      2. when finished priming, touch ‘**off’**

Starting the System

1. Adjust the eluent level to reflect the new eluent volume. When the 2 L bottle is full, the eluent level in the control panel should be set to 1.7 L. (This is to prevent running out of eluent, which introduces air to the system)
   1. Click on ‘**pump settings’**, highlight value in eluent bottle box, entered desired value, hit ‘**enter’**. Close box.
2. Click ‘**Startup’** to start the pump, suppressor, CR-TC and conductivity detector simultaneously.
3. Check settings
   1. Suppressor
      1. Anions: ON, 85 mA as default; change to 120 mA for ~10 min, then change back to 85 mA
      2. Cations: ON, 59 mA as default
      3. To change, click on box ‘**Detector Settings**’, highlight value next to ‘Supp. Current’, type in desired value and hit ‘**enter**’. Close box.
   2. Eluent Concentration, CR-TC
      1. Anions: ON, 32 mM as default, CR-TC on; change to 40 mM for ~10 min, then change back to 32 mM
      2. Cations: ON, 20 mM as default, CR-TC on
      3. To change, click on box ‘**EG Settings**’, highlight value next to ‘Target Conc’, type in desired value and hit ‘**enter**’. Close box.
   3. Pump flow rate: default is 1 mL/min
4. Listen for clicking in the pump. \*\*If there’s an irregular noise, click on ‘**shutdown**’ to stop system and prime again for a few minutes. If there’s still clicking, may need to take apart pump and replace seals and o-rings.
5. Once system is running, allow system to stabilize. Total Conductivity should be under 1 before acquiring data to establish baseline.
6. Acquire baseline; click blue button that reads ‘**Acquire Data’** when you hover over it. ECD\_1 channel should be checked in the box that pops up; click ‘**ok**’.
7. Allow system to stabilize before running samples. When baseline is stable, click on blue circle ‘**Acquire Data’** button to stop recording baseline. DO NOT click on red square button; this will stop flow, but leave on suppressor-bad!!!
8. While the baseline is stabilizing, you should set up your sequence and prep samples and standards.
9. Check autosampler connection (green relay on back of autosampler to green relay on back of ICS2000)
   1. If running anions only or simultaneous systems, relay to autosampler should be connected to anion system
   2. If running cations only, relay to autosampler should be connected to cation system
10. Before starting a run, record the pump pressure, background conductivity, settings, and ion count on appropriate tab of the log sheet workbook on computer desktop.

Starting a Run

1. Set up Sequence: in Chromeleon, go to Window → Browser
   1. 2 ways to set up sequence: copy an old sequence, or use sequence wizard. To ensure accuracy of settings, use sequence wizard!
      1. **Using sequence wizard**
         1. to **File** → **New**
         2. Choose ‘Sequence (using Wizard)’ from list → ok
         3. Specify timebase (‘TERRA”, “Cations” or “Simultaneous” from the window)
         4. Choose a sequence name (usually date and project name)
         5. Enter sample information; make sure to leave room at the beginning for 2 or 3 blanks, standards,QC’s (i.e., start position ≠ 1)
            1. Note: can also insert blanks and QC’s once finished with sequence wizard steps
            2. Injection volume = 25 or 50 ul (check sample loop).
            3. Click ‘next’
         6. Enter Standard information
            1. Injection volume = 25 or 50 ul (check sample loop)
            2. ‘next’
         7. Specify program file and quantification file
            1. Program file takes the form xxx.pgm
            2. Quantification file takes the form xxx.qnt
         8. Specify sequence name and save location
            1. Datasource should be NS-K216-01\_local
         9. Press ‘done’
      2. **Using old run**: Note: must use sequence run since 11/20/12, or computer will reference incorrect timebase and database
         1. old run that has similar settings (program, method) to what you want to run. \*\*
            1. Check injection volume (sample loop size)
            2. Check eluent conc and suppressor current
         2. go to **File** → **Save As** and save as your file name (include date and project name)
         3. In the sample list, add or delete samples and standards and rename accordingly. Make sure you have at least 2 or 3 blanks in the beginning of the sample run before you run your standards. (otherwise, you will not have a good standard curve)
         4. **File** → **Save** when you are finished editing your sample list.
2. If not already on, turn on power switch on Autosampler (lower right side of rear of AS40)
   1. If running simultaneous injection, make sure that the sample splitter is installed; this is the mixing tee with orange tubing
      1. Disconnect the blue sample line at the union near the autosampler
      2. Connect the empty tee port to the free autosampler fitting
      3. Connect the free ends of the orange tubing to each system’s sample port on the injection valve (‘S’) note; will have to remove blue sample line from whichever ‘S’ port it is in.
   2. If running only anions or only cations, make sure blue sample line is correctly installed
      1. If necessary, disconnect orange tubing from sample ports on injection valves and disconnect the mixing tee from the autosampler fitting.
      2. Connect blue sample line from autosampler union to the sample port on the injection valve of instrument you’re running.
3. Load cassettes of samples: pull back sliding lever and load with the black dot towards the middle of the autosampler (notched track should be facing front of autosampler)
4. Press the Hold/Run button to switch to ‘Run’. This should advance the first cassette into position and be in ‘ready’ mode
5. In Chromeleon, go to **Batch** → **Edit** to open batch list
6. Click on ‘**Add’** and select your sample file and click on ‘**open’**. This will add your sample file to the batch list.
7. Once your file is added to the batch, you can click on ‘**Ready Check’** which will tell you how much eluent and disk size your file requires. Then you can click on ‘**Start’** to start the run. If the autosampler is on ‘Run’ you should hear the autosampler arm start moving to the first sample.
8. When run ends, you need to shut down machine. On control panel, click on ‘**Shutdown’** in the System box near top. Note: if running simultaneous panel, must shutdown each system individually.
   1. Alternatively, you can add a shutdown sequence to your batch list
   2. Make sure you open the appropriate sequence for your timebase [ ‘Shutdown Terra’, ‘Simultaneous\_shutdown’ or ‘cation\_shutdown’) in the browser and change the status column from ‘finished’ to ‘single’ and hit ‘**save’**

During a Run

1. Make sure Eluent bottle stays above 0.3 L. If lower volume than this, could introduce air to system via end line filter.
   1. When you refill the eluent bottle mid-run, make sure to adjust the level in the control panel. A box will pop up saying you are intercepting a run, hit “**ok”**.
2. If the autosampler doesn’t advance to the next cassette or puts itself on hold, try restarting run. If this doesn’t work, try shutting down the IC, turning off the autosampler, and restarting the computer and software. Usually it’s a communication problem between the computer, IC and autosampler, and rebooting everything resolves this issue.

Maintenance

1. The IC should be run for at least 30 mins every week. This prevents salt build-up in the lines and in the suppressor, and also saves the trouble of removing and capping the suppressor and column for longterm storage. Following this procedure will extend the life of the suppressor, and will allow the system to generate more stable baselines.
2. If you notice any maintenance needs, please bring them to the lab manager’s attention
3. Record any maintenance in the maintenance log.

Notes

* Bubbles: The only place there should be bubbles in the flowpath is in the Regen Out line, exiting the suppressor. If there are bubbles in the line from the Eluent Bottle, the end filter on the intake line may need to be replaced.

If using the RIOS columns, you need to change the method ECD\_1.AcqOff time from 11 minutes to 13 minutes. Otherwise you’ll have phosphate peaks coming through on the following sample’s chromatogram.

Example Program:

RIOS\_100mA\_35mM Program

Pressure.LowerLimit = 200

Pressure.UpperLimit = 3000

%A.Equate = "%ADI"

CR\_TC = On

LoadPosition

Data\_Collection\_Rate = 5.0

CellTemperature = 30.0

ColumnTemperature = 30.0

Suppressor\_Type = ASRS\_4mm

; Pump\_ECD.Carbonate = 0.0

; Pump\_ECD.Bicarbonate = 0.0

; Pump\_ECD.Hydroxide = 35.0

; Pump\_ECD.Tetraborate = 0.0

; Pump\_ECD.Other eluent = 0.0

; Pump\_ECD.Recommended Current = 87

Suppressor\_Current = 100

Concentration = 35.00

Curve = 5

Flow = 1.00

-1.100 Pump\_ECD\_Relay\_1.Closed Duration=138.00

0.000 Autozero

ECD\_1.AcqOn

Pump\_InjectValve.InjectPosition Duration=30.00

11.000 ECD\_1.AcqOff

End