Ion Chromatography Procedure

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A. Introduction

Ion chromatography (IC) is used to separate out various ions based on their charge. This technique is especially helpful for measuring the concentration of a particular ion in a water sample. A small volume of the sample (mobile phase) is pumped through a column of packed particles (stationary phase) and the time it takes for the ion to pass through is recorded—this is called the retention time. The retention time of the ion depends on how it interacts with the column both physically and chemically. Known standard concentrations are used to estimate sample concentrations.

In the Soil and Water lab, our IC machine is made to analyze the concentration of negative ions (anions). Some of the common analyzed ions are nitrate, sulfate, and phosphate.

B. Set Up

Lab Equipment/Supplies:

- IC machine
- Plunger for vial caps
- Cassettes (vial holders)
- Sharpie marker
- Jump-drive
- Various pipettors and tips
- 1000ppm stock solutions
- Glassware (beakers, 100mL flasks, etc.)
- Extra sterile containers for holding liquid
- Vials (0.5mL or 5 mL), with black caps
- Gloves
- Deionized, degassed water

Note: Additional lab equipment is necessary if 1000 ppm stock solutions have not been made.

C. Preparation Overview

- Thaw out frozen samples.
- Load vials with standards, blanks, and samples. These can be filled a few days in advance and stored in a bag in refrigerator.
- Run the IC machine in batch mode.
- Adjust calibration curves for breakthrough times.
- Export data from Chromeleon into Excel.
- Copy data onto a memory stick or jump-drive.

Note: Please reserve machine a few days in advance by writing your last name, number, and the type of samples you will be analyzing. This helps the lab keep track of how many total samples have been analyzed if a new machine part is needed.

D. Stock Solution Preparation

Remember that a 1000 ppm solution has 1 g of an element dissolved in 1000 mL of distilled, degassed water. For example, say you would like to make 1000 mL of 1000ppm Cl solution. The atomic weight of Cl is 35.45 and Na is 22.99. So NaCl salt has a total molecular weight of 58.44. Because we will make a Cl solution we take 58.44/35.45 to get 1.6485 g NaCl added to 1000 mL of distilled, degassed water to get 1000 ppm Cl solution. If you only want to make a final solution of 100 mL you divide 1.6485/10 to get 0.16485 g NaCl mixed with 100 mL of distilled, degassed water to also get 1000 ppm Cl solution.

Appropriate amounts of the desired salt must be calculated, weighed, and added to 100 mL flask. Deionized, degassed water can then be added to the 100 mL line on the flask. The mixture should be shaken vigorously until all the salt is dissolved.

If questions arise, contact Shree.

E. Calibration Standards Preparation

1. Throughout this procedure, be sure to wear gloves. Sweat on the hands can offset

2. Use deionized, degassed water for blanks and for each dilution. Each IC Run should begin with 4 blanks and end with 3 blanks. This will ensure that the column is thoroughly cleaned for the next user. Blanks are made from 100% deionized, degassed water.

3. For most runs, the following calibration standard concentrations should be adequate (S1 to S5). However, this can easily be modified depending on the experimenter's preferences. Shree suggests keeping the concentrations low. For example 1ppm, 2 ppm, 5 ppm, and 10 ppm. If your results are higher than 100 ppm you should dilute and rerun the sample. The two runs should be within 5% difference (difference over average * 100).

4. Calculate how much volume of each 1000 ppm stock solution you need to make 100 mL of the S5 calibration standards. To solve for the unknown volume, use the following relationship:

   volume1*concentration1=unknown volume2*concentration2

   Where volume 1 is the final volume of diluted solution you want (i.e. 100 mL), concentration is the final concentration of solution you want (i.e. 20 ppm), volume 2 is how much of the stock solution you need (this is unknown), and concentration 2 is the original concentration of the stock solution (i.e. 1000 ppm).

5. Prepare five 100mL glass flasks by washing them thoroughly with deionized water and letting them dry. Using a sharpie, label them with the concentration of each anion, the date, and your initials. If there is already writing on the flask, it can be cleaned off with some rubbing alcohol.

6. Clean the pipettor tips and beakers with deionized water before use.

   Note: for best precision, the scale can be used to measure the accuracy of the pipettors. Graduated cylinders are not accurate. It is often difficult to tell which pipettors in the lab are working well.

7. Make up the highest-value standard (S5) first. Add the calculated amounts of Cl, NO_3-N, and SO_4-S 1000 ppm stocks to the 100 mL glass flask using the appropriate pipettor. Pour from the stock bottle into a small glass beaker or extra container and then draw from here using the pipettor. This will ensure that the stock solution is not contaminated. After the three stocks are in the flask, top up to the 100 mL line with deionized, degassed water, cap, and mix well.

8. Pour about half of S5 into a clean spare beaker or container. Using the pipettor, draw 50 mL of S5 out and empty into the S4 flask. Fill the S4 flask up to the 100 mL line with deionized, degassed water. You now have S4 in the beaker.

9. Repeat this dilution technique for the remaining three standards (S3, S2, and S1).

Note: Steps 7, 8, and 9 represent one of several standard making procedures.

10. It is useful to have single standards for SO4-S, NO3-N, and other any other ions of interest, as well as the triples calibration standards. Concentrations of these single standards can be equal to the highest concentration of the ion found in the triple standards so the peak will be very clear. These single standards allow you to identify the peak order of sulfate and nitrate and also giving you an independent check of calibration. It is best to take these from a second stock solution source but this is not absolutely necessary; especially if it is not available.

F. Filling the Vials

1. All samples must be filtered through a 0.45 um filter before running through the IC. See section J for notes on preservation of samples including frozen samples. For best precision vials and caps should be rinsed thoroughly with deionized water. Be sure to wear gloves throughout the rinsing and filling process.

2. With a Sharpie marker, label the vials with sample numbers. For example S1 to S5 for the standards, BL for each of 7 blanks, S4r for a replicate of one standard. Individual standards can be labeled with the ion and the concentration, for example, S40 for 40 ppm Sulfate. If doing dilutions, mark more vials for them. For example, ‘03x5’ for a five-fold dilution of sample ‘03’ (see Part I.).

   Note: A five-fold dilution is one part sample and four parts deionized water.

3. Fill vials at least halfway and cap (~0.6 mL). The cap must fit entirely into the vial when pressed down so that is flesh with the top of the vial. Caps should be placed into the vial with the white filter end touching the fluid inside the vial. Please use the black capper to put on the vial caps as using a kim wipe can leave fibers in the filter that will eventually clog the machine.

4. Load vials into cassettes in this order: four blanks, five triple calibration standards, all samples, individual ion standards, and three blanks. If you know that you have samples with high concentrations you might want to put blanks between them two reduce the risk of carryover from one sample to the next. It is recommended to put a known check standard (mid-range concentration) after every 10 samples so you can calculate the instrument’s drift factor.

G. Running the IC Machine

1. Fill the eluent bottle with fresh deionized, degassed water and be sure to keep the tube submerged as you pour the fresh water into the bottle. If air gets into this tube the whole system has to be flushed out and refilled. Empty the waste bottle under the machine if it is more than half full.

2. Power up machine and computer. Set eluent level to full.

3. If machine screen is not on, go to 'Control Panel' on the file browser and click on 'ICS_2000_system_AS40.pan'.

4. Warm up the machine for a half hour (click on the 'Start up' button). If the pump pressure goes up and down there may be bubbles in the line. Turn off the pump, open the door to the IC, and turn waste pump valve (bottom left) just a little until it's open. Let the pump run until all the bubble are gone, turn the pump off, tighten the valve, and then try to turn the pump on again. The pump, eluent, and data acquisition should all be turned on. Watch the conductance display because it should be stabilized to below 0.5 before you can start the run. Warming up the machine also involves priming the pump to remove air bubbles from the tubing inside the machine. This can be done from the digital display on the IC machine.

5. On the computer (in the Chromeleon program), create a "sequence" by copying an earlier one. It is easiest to "save as" an earlier sequence under a different name. This can then be edited. All old results will be removed from this new saved file.

6. Place the cassettes into the auto-sampler with the first one to be tested in front and last one at the back. The dots on the cassettes go toward the center of auto-sampler.

7. When conductance is stable at 0.5 uS, turn off the acquisition, and start the new sequence. Pump should be at around 2000 psi if it's fluctuating frequently there may be bubbles in the tube. Please let Shree know if this happens.

   To start the new sequence:
   a. Close the machine lid.
   b. Press the hold/run button.
   c. Open Chromeleon and click the "Start/Stop Batch" icon. Click "Ready-check" and then click "Ok".
   d. Click "Start" to start the run.

8. It is okay to tweak some aspects of the sequence on the computer while it is running. For example, the "nitrate" program can have its standard concentrations adjusted. However, this really should be done before the sequence has started.

9. Wait overnight...

H. After Run Completion

1. Double click on one of the single standards and record the peak retention time for this ion. Repeat this for all other ions of interest.

2. If you need to add additional standards in the QNT editor, make sure you do this before saving any changes (step 5 below). You will not be able to add additional standards after you save the run.

3. Click on the nitrage.pgm program. This is the template that provides concentration standards, time window center, and time window width. (Is this the “QNT editor” button?)

4. Fill in the concentration standard values for S1 to S5 as well as the retention times for each ion. Be sure that the windows do not overlap otherwise peaks will not be distinguished.

5. Save. If you do not save then peaks will not be adjusted.

6. It is okay to drop one bad standard result from the calibration since there are five standards and the fit is linear. Note that this may truncate high or low values.

7. After the calibration looks good, check the computed concentrations for all the triple standards. Do the retention times observed in the single standards match these?

8. Browse results for all samples, looking at the time plots to ensure that peaks are properly labeled for each ion. One or more peaks can be missing, meaning; the concentration of that anion is low. Are there any other unlabelled peaks that interfere with the three? Also see Part I. below if sample concentration amounts are larger than the highest standard concentration.

9. Click on one of the samples and highlight the row with the ion of interest. Click on the “Summary” tab and copy the “Amt” column into Excel. Repeat this for all other ions.

10. Save the Excel file and export it to a memory stick/jump-drive.

11. Be sure to remove the spent vials from the machine before the next user.

I. Further Dilutions

A higher concentration of an anion comes out earlier (left on chart) than a lower concentration of the same anion. Sulfate and nitrate come out close together in time, which sometimes means that a high SO_4 will overlap with a low NO_3. The likelihood of the peaks merging increases as the column gets older. If there is a single peak left skewed and below the usual range of sulfate, this can indicate a very high sulfate value masking a low nitrate. Dilution will cut down the high sulfate placing its peak "later" and giving a separate (subdued) peak for nitrate. Use the first run results to decide about dilutions in the second and third replicates. However, if the column is old and you know you have high concentrations of both SO_4 and NO_3, it might be best to wait until the IC column has been changed. See below for sample preservation.

Dilutions are highly recommended if a sample’s predicted concentration is larger than the highest standard concentration. The IC will use the calibration curves to extrapolate the unknown concentration of the sample, which may or may not be accurate.

Note: A 10:1 (read as “ten to one”) dilution consists of one part sample and nine parts deionized, degassed water. Multiply the concentration results by 10.

J. Other Important Notes

Preserving Samples
Refrigerate samples immediately and analyze them within 48 hours for best results. Freezing samples can cause ions to precipitate out. This precipitate may be difficult to see but is detrimental to the IC. Shree recommends not to freeze samples but instead to add approximately 100 uL of concentrated H_2SO_4 to each full 50 mL centrifuge tube immediately after filtering your samples. If you have already frozen your samples, please re-filter the thawed sample before running them through the IC. Use a 0.45 um filter.

K. Credits: Sheila Saia, Tony Salvucci, and Wei Zhang.