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Introduction to Module

This module provides an introduction to ***Ion Chromatography (IC)***. In this module the basic theory and applications of IC will be presented at a level that assumes a basic general chemistry background. It is designed to move through the module sequentially using the links above.

Ion chromatography is a subset of liquid chromatography methods: *ion exchange*, *ion exclusion*, *ion pair* chromatography.

IC is a useful tool for determining the presence and concentration of ions in samples and is utilized in numerous industrial and research settings including a [test for authentic tequila](#) and for environmental analyses such as the determination of anions (PO₄³⁻, Cl⁻, NO₃⁻, etc) in surface waters. Ion-exchange is the basic principle behind the removal of cations and anions from drinking water using most commercial, such as Brita®, water filters. Ion-exchange is also a natural process that occurs with clay substrates, resulting in the mobility of cations in soils.

This module presents the basic history, theory, and applications of IC. The module focuses on ion exchange chromatography, as it is the most common use of IC in environmental analysis, but includes the related ion exclusion and ion pair methods.

[Next](#)

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Learning Objectives:

Upon completion of this module you should be able to

1. Explain the basic principles, operation and application of IC.
2. Differentiate between IC and other chromatographic methods.
3. Explain the chemical basis for stationary phase effects and mobile phase effects.
4. Predict retention order given the relative dominance between stationary phase effects and mobile phase effects.
5. Differentiate between stationary phases used in anion exchange and cation exchange.
6. Explain the basis for the common IC detection methods.
7. Describe the general process of analyzing a sample by IC.
8. Explain the rationale for using a suppressor cartridge and how it works.
9. Predict the effects of overloading, eluting too quickly, eluting too slowly, baseline drift.

History

Ion Chromatography (IC) methods were first reported around 1850 when H.Thomson and J.T. Way used various clays as an ion exchange and extracted labile calcium, magnesium, and ammonium ions. In 1927, the first zeolite column was used to remove Mg_{2+} and Ca_{2+} from water. Cation exchange using a sulfonated polystyrene/divinylbenzene column was developed in the 1940s as part of the Manhattan project. Very large columns were used to concentrate and purify the radioactive nucleotides required for the atom bomb. In the late 1940s anion exchange was performed with the attachment of a quaternary ammonia on the polystyrene/divinylbenzene support. The industrialization of the technique occurred in the 1970s when Small et al ([Small, H.; Stevens, T.S.; Bauman, W.C. *Anal Chem*, 1975, 47, 1801](#)) developed a suppressor column that enabled conductivity detection.

For a more thorough description of the history see Small, H *J Chem Ed*, 2004, **81**(9), 1277-1284.

Basic Principles of Ion Chromatography

Basic process of IC

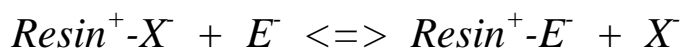
The basic process of chromatography using ion exchange can be represented in 5 steps: eluent loading, sample injection, separation of sample, elution of analyte A, and elution of analyte B, shown and explained below. Elution is the process where the compound of interest is moved through the column. This happens because the eluent, the solution used as the solvent in chromatography, is constantly pumped through the column. The chemical reactions below are for an anion exchange process. (Eluent ion = ▲, Ion A= ■, Ion B = ●)

Step 1: The eluent loaded onto the column displaces any anions bonded to the resin and saturates the resin surface with the eluent anion.



(key: Eluent ion = ▲, Ion A= ■, Ion B = ●)

This process of the eluent ion (E^-) displacing an anion (X^-) bonded to the resin can be expressed by the following chemical reaction:



Step 2: A sample containing anion A and anion B are injected onto the column. This sample could contain many different ions, but for simplicity this example uses just two different ions ready to be injected onto the column.



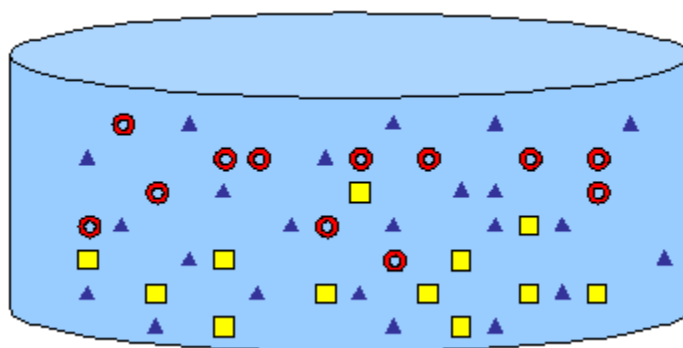
Step 3: After the sample has been injected, the continued addition of eluent causes a flow through the column. As the sample elutes (or moves through the column), anion A and anion B adhere to the column surface differently. The sample zones move through the column as eluent gradually displaces the analytes.

Question to consider: How would you write the chemical reaction for elution process with respect to anion A and anion B. How would you write the K_f expression for the two reactions? How would you sketch the elution process at this step using a figure similar to the figure in Step 1 if the K_f for anion A(□) is larger than the K_f for anion B(○)?

Step 3: The continued addition of the eluent causes a flow through the column. As sample elutes, anion A and anion B adhere to the column surface differently. The sample zones move through the column as eluent gradually displaces the analytes.



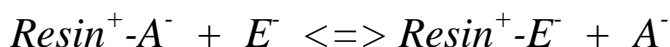
In reality not every eluent ion is removed from the surface of the column. It depends on the amount of analyte loaded. A better representation of the column can be seen by just looking at a slice of the column where the separation is occurring, as shown in the figure below.



Step 4: As the eluent continues to be added, the anion A moves through the column in a band and ultimately is eluted first.



This process can be represented by the chemical reaction showing the displacement of the bound anion (A^-) by the eluent anion (E^-).

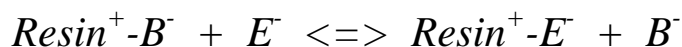


Question to consider: *If ion B had a very strong affinity for the resin, how would the elution time for ion B be affected? If it takes forever to come off, would this be useful in trying to determine the quantity of that ion present? When might this be useful? (Hint: go back to the introduction to the module and look at where ion-exchange is used...)*

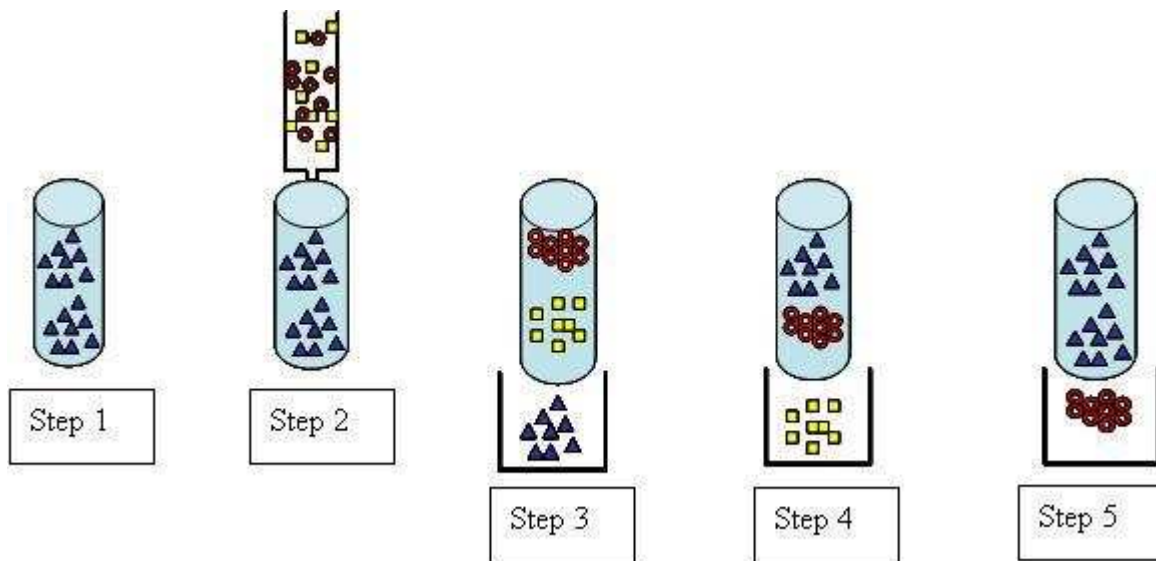
[Click here to check your answer](#)

(Answer: As the affinity ion B has for the resin increases, the elution time would increase. If the affinity becomes large enough, in essence anion B will stay on the column. This phenomena is utilized in water filtration where ion exchange is used to remove particular ions from the sample.)

Step 5: The eluent displaces anion B, and anion B is eluted off the column.



The overall 5 step process can be represented pictorally:



Stationary phase (or resin) composition

There are a number of different resins or stationary phases that have been developed for use in IC. The main classes of substances used are: *modified organic polymer resins, modified silica gels, inorganic salts, glasses, zeolites, metal oxides, and cellulose derivatives*. The most commonly used resins are the silica gels and polymer resins. As the sample is injected onto the column, the two different analytes briefly displace the eluent as the counter -ion to the charged resin. The analyte is briefly retained at the fixed charge on the resin surface. The analytes are subsequently displaced by the eluent ions as the eluent is added to the column. The different affinities (see the chemical reactions in the basic process section) are the basis for the separation. The K_f values of each reaction is also known as the selectivity coefficient. The greater the difference between the K_f values for the two analytes, the more the two analytes will be separated during the ion chromatography

process. In reality, the interaction between the solvent and the analyte can also have an impact on the order each analyte is eluted. For a more in-depth analysis of predicting the retention order [see the material by Dr. Thomas Wenzel](http://www.bates.edu/x65385.xml). (<http://www.bates.edu/x65385.xml>)

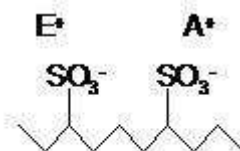


Figure 1: cation exchange surface

The common cation exchange resins are based on either polystyrene-divinylbenzene (PS-DVB) or methacrylate polymers. The surface of these polymers (Figure 1) is functionalized with a negatively charged sulfonated group ($-\text{SO}_3^-$). The cation in the eluent or the analyte of interest is the counter-ion in the vicinity of the charged functional group.

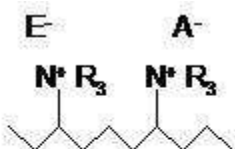


Figure 2: anion exchange surface. The R stands for some organic (C and H) chain.

The surface of the polymer is functionalize with a quaternary amine ($-\text{N}^+\text{R}_3$) for anion exchange (see Figure 2). The quaternary amine provides a positive charge to the surface, attracting negatively charged anions in the liquid phase. Just like the cation exchange resin, the anion of the eluent or the analyte of interest exists as the counter-ion in the vicinity of the positive charge residing on the amine.

Detection Methods

In order to have useful information, you need to be able to detect what comes out of the column. The analytes are ions that come out in separate sample bands, which means there is a small part of the solution coming out with a higher concentration of ions.

Based on your knowledge, how could you possibly detect ions or changes in ion concentrations coming off of the column?

There are many different possibilities of detecting ions. Due to its simplicity, most instruments use conductivity.

Conductivity- Conductivity is the measure of a material's ability to conduct electricity. Since conductivity is proportional to the number of ions in solution, it is the primary method of detection for ion chromatography. One problem with measuring conductivity is the high conductivity that may be present in the eluent. Conductivity became common with the use of a suppressor.

The suppressor is a cation or anion exchanger after the ion exchange column that replaces the eluent ions with either H^+ or OH^- . If you are performing cation analysis, the eluent is acid, and the exchanger replaces the eluent counterion with OH^- . This then converts much of the eluent to neutral H_2O . Thus the suppressor greatly reduces the conductivity contribution from the eluent, enabling the signal from the analyte of interest to be more readily detected.

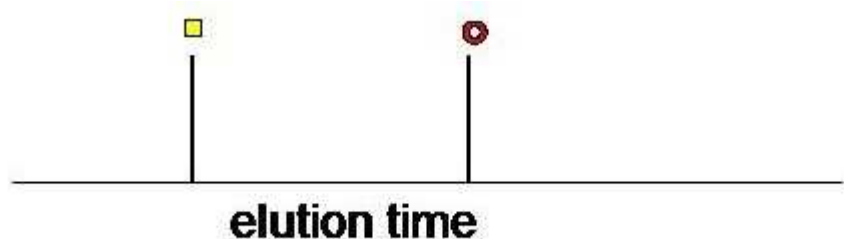
Other methods-

Other detection methods have been coupled with IC, including *mass spectrometry, atomic spectroscopy, fluorescence, luminescence, UV-Vis,* and *potentiometric*. Most require post-column reactions to generate the signal or are so selective they are not useful in detecting multiple

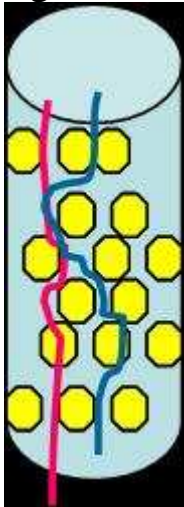
analytes simultaneously. Of these methods, the most likely to be broadly used is *mass spectrometry*-(i.e. the determination of [ionic compounds in toothpaste](#) Cavalli, S; Herrmann, H; Höfler, F; **LC GC Europe**, 2004, *17*(3), 160).

Chromatograms

The time to elute an analyte is a function of how long the analyte is retained on the column, therefore the output of IC is a graph of conductivity as a function of time, called a chromatogram. Based on the previous discussion of elution, you may expect a chromatogram to look something like:

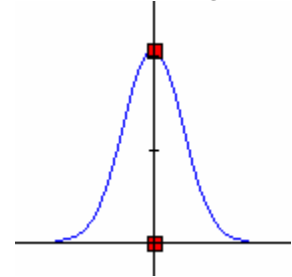


However, we need to consider another process that impacts shape of the elution peaks. As an analyte flows through the column, some of the analyte molecules may pass by through the length of the stationary phase faster or slower than would be expected due to diffusion processes or the formation of channels. An example of this can be seen in the figure with the path of each ion shown in the figure.



If the two ions are traveling at the same speed, set by the flow of the eluent, what can you say about when they will emerge? Will they emerge at the same time?

From the figure, you should be able to see that the path in red is much shorter than the path in blue. Since the path is shorter, and the ions are traveling at the same speed, the ion following the red path will emerge first. Thus a normal chromatogram peak will have a gaussian distribution, symmetric around the mean, as seen in the figure on the right. You can also peruse a more extensive mathematical modeling of the chromatogram peak [here](#) (link takes you to a different website



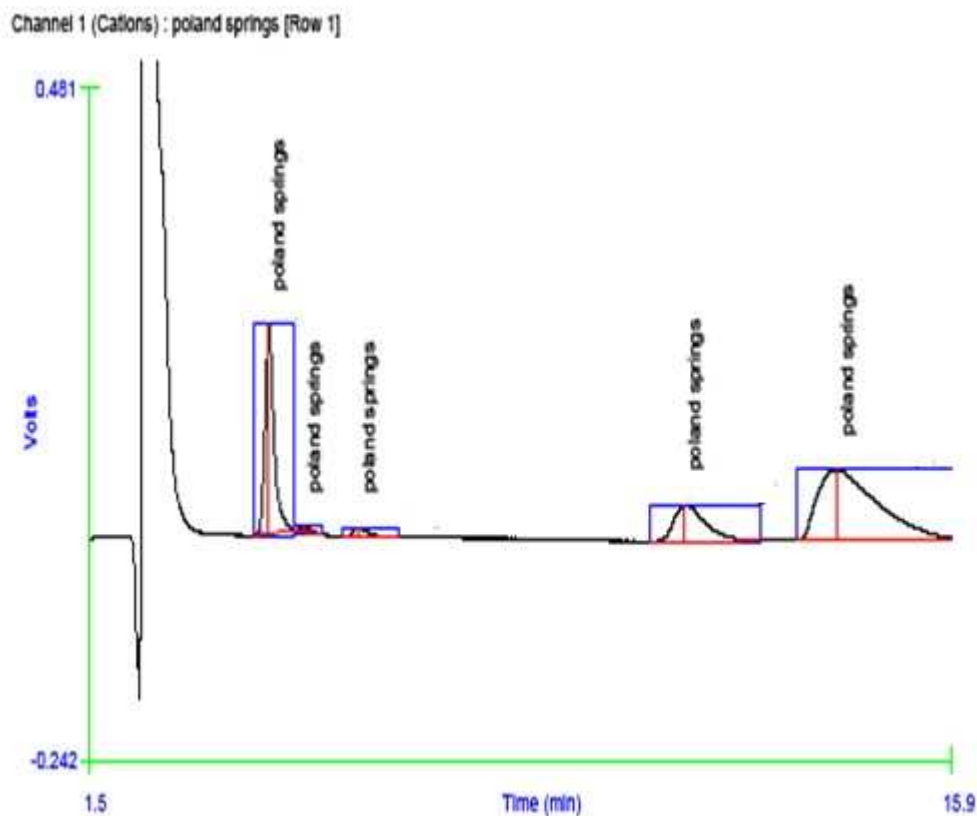
http://www.chem.uoa.gr/applets/AppletChrom/App1_Chrom2.html).

Since we are concerned with the concentration of ions present in the solution, how will the chromatogram change as you increase the amount of analyte loaded onto the column?

After considering the prior question: ***Since we are concerned with the concentration of ions present in the solution, how will the chromatogram change as you increase the amount of analyte loaded onto the column?***

The chromatogram peak will increase in height and concomitantly in area. Therefore when quantifying data, the peak height or area is used. In order to determine actual concentrations, a series of standards must be analyzed to calibrate the response between peak area and actual concentration for each ion.

An example chromatogram of Poland Springs bottled water. Each separate peak is due to a different cation.



The area under each peak is used to calculate the concentration of each ion. *What information would you need in order to determine the relationship between peak area and concentration?*

Just like any other quantitative method, you need to calibrate the response, in this case by analyzing a series of known standards and plotting a calibration curve.

If you notice, the last peak is not completely gaussian, there are other factors that do have an impact on peak shapes. For more in-depth information on peak shape, see the following material ([link is to an external website](http://www.bates.edu/Prebuilt/wenzel-chrom-text-revised-6-10.pdf) <http://www.bates.edu/Prebuilt/wenzel-chrom-text-revised-6-10.pdf>).

Basic Instrumentation

You now have an outline of the basic ion chromatography process. The loading of the sample onto the column varies with the instrument. The sample is eluted off of the column, through the detector. The signal from the detector is converted into the chromatograph. Lastly, considering you have the eluent flowing through a column with very fine particles. *How can you force water through a column containing very fine particles?*

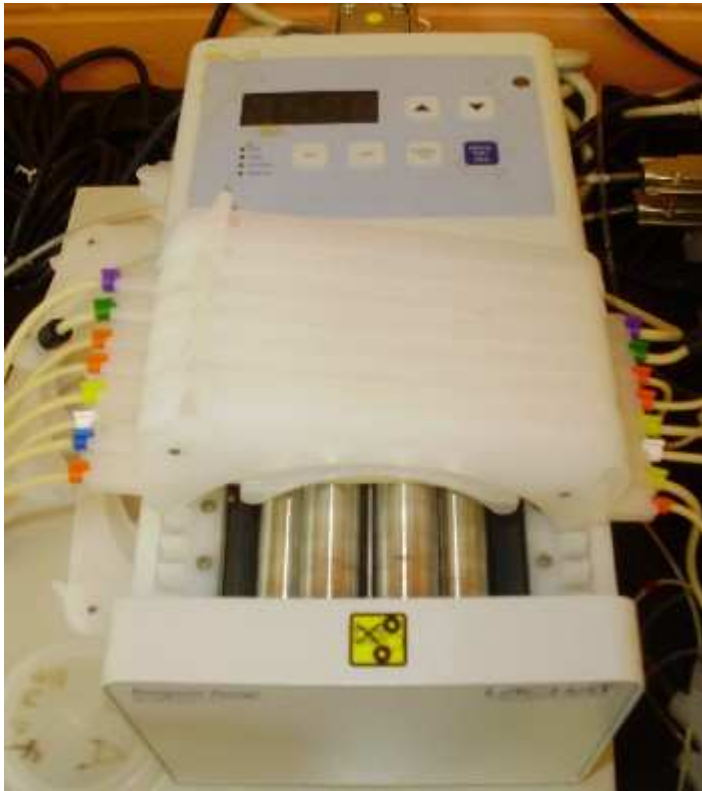
When a water filter is used in a pitcher, the force of gravity is used, and when a water filter is used on a tap, the water pressure in the pipes is used. In our situation, a much higher pressure is required, since the solid phase particles are much smaller than a water filter on a household tap.

The pressures required for most IC instruments is at least 600 psi. In order to achieve this pressure a double piston high pressure pump is used, one such example is shown in the picture.

The injection of the sample onto the column is performed using a multiport valve that is inline with the eluent tubing. Different instruments have slightly different styles, with the most common a direct port for injection.

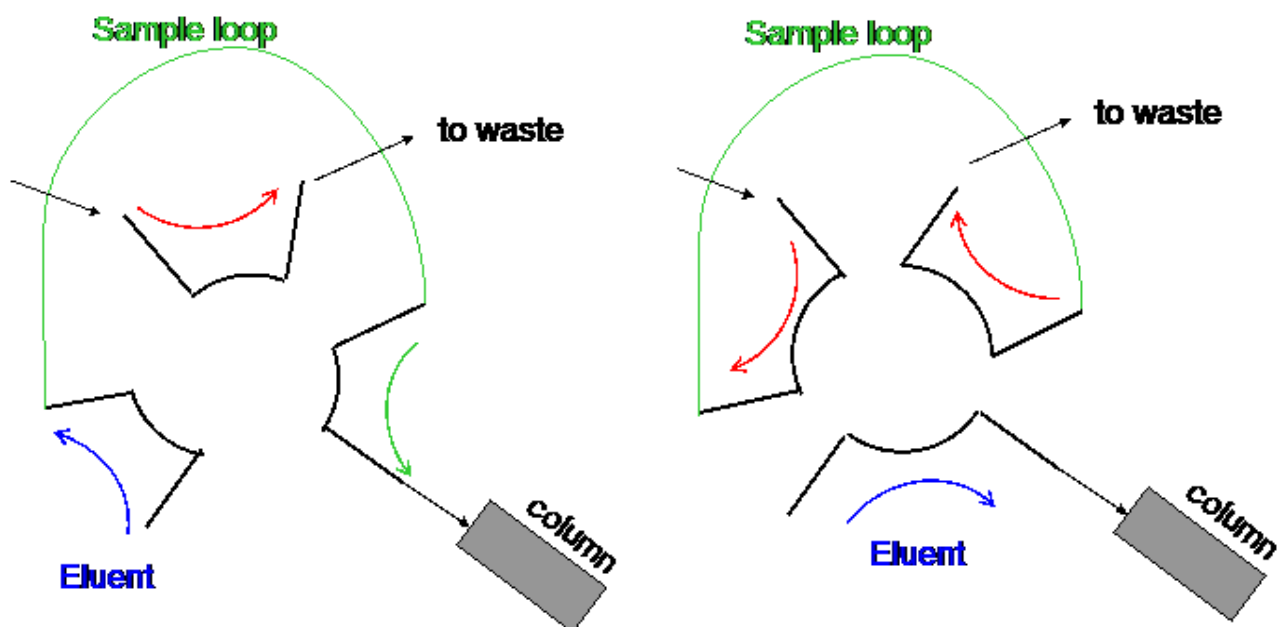


Another example is where an autosampler is used. In the photo to the left, an autosampler is shown, where a peristaltic pump (image below) pulls the sample up the autosampler tube and into the sample loop on a six-port valve. This is the setup used in the Lachat 8500 QuickChem series with an ion chromatography channel.

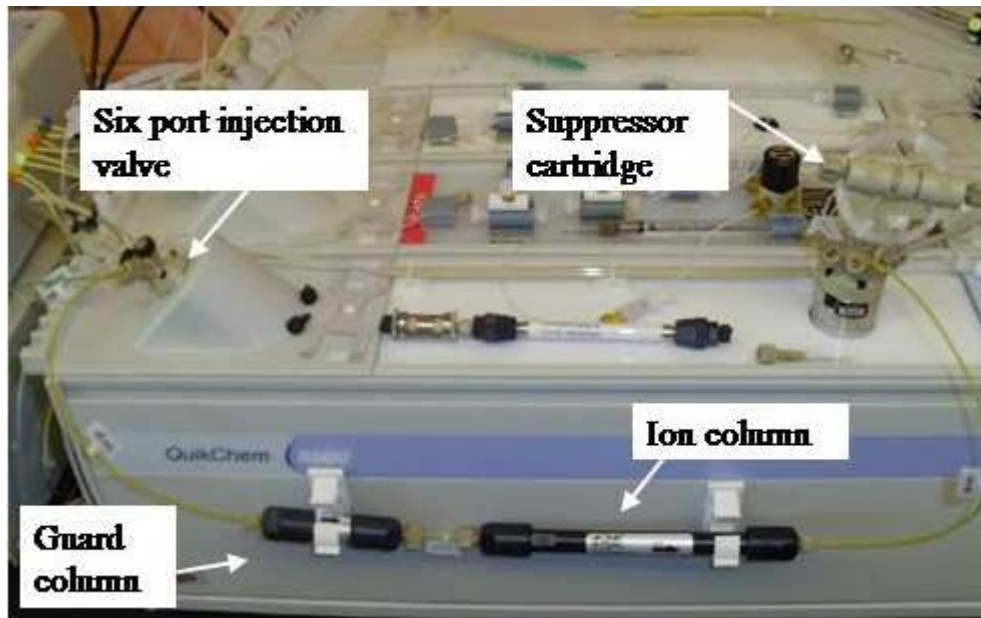


Software controls the six-port valve and at the appropriate time the valve switches to have the sample flow onto the column.

The six port valve works by first having the flow of the sample go through the sample loop (figure on the left) and then to the waste. This fills the sample loop with the sample. At a set period, the valve turns connecting different lines coming in. The eluent now forces the sample from the sample loop onto the column.



One instrument, a Lachat 8500 QuickChem with IC, is shown below illustrating a guard column, analytical column, and the suppressor cartridge. The guard column is used to protect the analytical ion column from contamination.



Six port injection valve

Suppressor cartridge

Ion column

Guard column

Experiments:

There are a number of different possible lab experiments that utilize IC analysis, from one week experiments to semester long projects.

1) An experiment to cover one four hour lab period is the analysis of the ion content of bottled water. If you wish to use this as a dry lab exercise, sample data can be provided to students with the [sample data set](#) (in Excel), and [chromatograms](#) (in pdf) collected on a suite of bottled water samples in 2009.

2) An experiment that would cover 2-3 four hour lab periods:
-evaluating whether a stream is contaminated by road salt applications. ([lab information](#))

3) An project that could cover multiple weeks with more sample preparation due to more complex samples. This could easily be adapted for project based learning experiences.

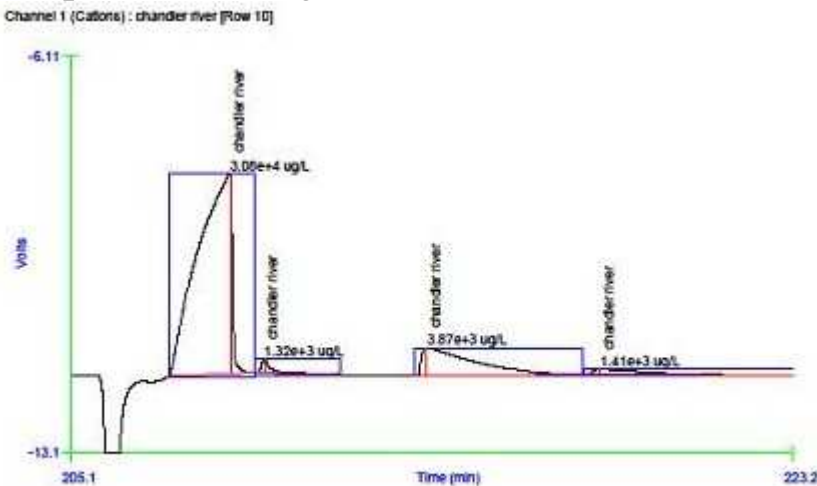
- anions in fruit juices see Whelan et al, J Chem Ed 81(9), Sep 2004, 1299-1302.

Troubleshooting

There are troubleshooting guides for HPLC, a related chromatographic technique. In many instances the troubleshooting is similar. The [HPLC troubleshooter](http://www.dq.fct.unl.pt/QOF/hplcts.html) (<http://www.dq.fct.unl.pt/QOF/hplcts.html>) online interactive guide can be useful for IC as well.

There are several common issues that show up in IC, see if you can think about these chromatograms and what might cause the problem.

Sample Chromatogram 1



If you look at the first big peak, the shape is quite a bit different than the other peaks. It is very broad and has fronting to the peak. This is due to overloading the column, thus there is not a great separation sodium peak, and it dwarfs any other peak that would have a shorter elution time. This sample was from a tidal river, thus the high sodium concentration.

Several other common issues that show up in IC are if the suppressor column starts to fail, the eluent solution changes either in pH or ion concentration, or the analytical column gets contaminated. These tend to cause baseline drifts or very poor peaks.