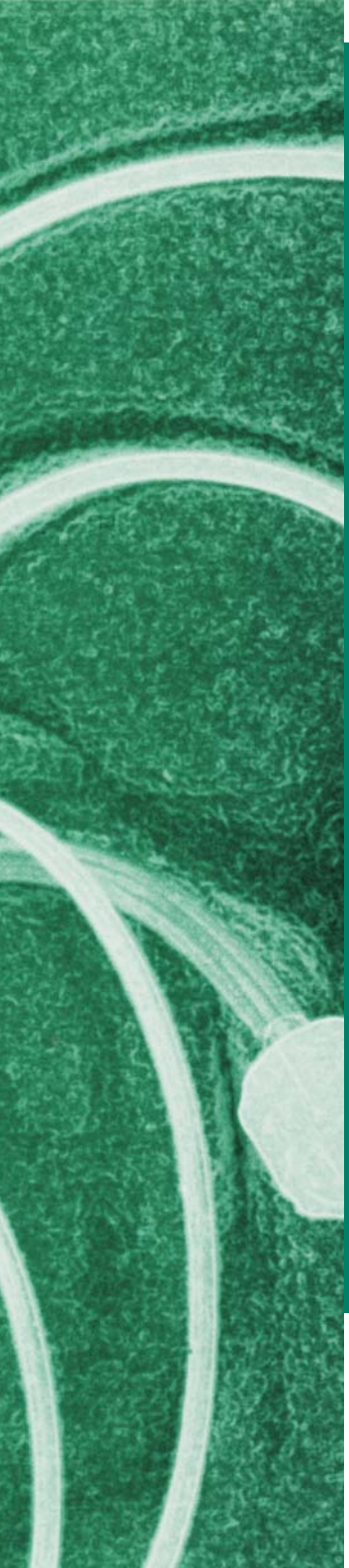

A Practical Guide to Ion Chromatography

AN INTRODUCTION AND TROUBLESHOOTING MANUAL

SeQuant

Innovators in Chemical Analysis™

A PRACTICAL GUIDE TO ION CHROMATOGRAPHY

An introduction and troubleshooting manual

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A Practical Guide to Ion Chromatography

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A PRACTICAL GUIDE TO ION CHROMATOGRAPHY

An introduction and troubleshooting manual

Foreword

This guide will show you some of the most common practical issues that occur during analysis of *anions* by ion chromatography. Many users find the technique difficult and hard to understand. It is our hope that we will be able to explain a few of the most fundamental prerequisites for analysis of water samples. We also want to give you guidance on troubleshooting of the instruments. If your ion chromatographic problems cannot be solved with this guide, SeQuant will be happy to assist you.

First of all, we recommend that you visit our web pages (http://www.sequant.com), where you will find more information on applications and our products.

The Ion Chromatographic System

Figure 1 shows an ion chromatographic system with suppressor. Eluent is pumped by an eluent pump through an injection valve, where exactly reproducible sample volumes are injected.

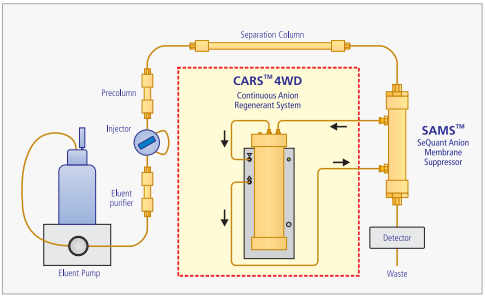

Figure 1: Anion chromatography system comprising a SAMS™ suppressor with CARS™ 4WD for continuous regeneration.

The sample ions are carried by an eluent flow to a separation column, where interaction with fixed ions of opposite charge (*i.e.*, positive) take place. The ions are thereby slowed down to an extent characteristic of each ion and arrive separated at the *"suppressor"*, where the eluent and analyte ions are transformed before they reach the detector.

The detector measures the electrolytic conductivity of the eluate and the function of the suppressor is both to reduce the background of the eluent and to increase the sensitivity for the sample ions. The detector signals can be evaluated manually from recorder charts or automatically with an integrator or a computer-based data-acquisition system with suitable chromatography software.

As with all chromatographic techniques it is very important to recognize the chromatographic terms *efficiency*, *capacity* and *selectivity*. If these factors are kept in mind, it is easier to achieve good separations in a time-saving and economic way:

Efficiency

An efficient chromatographic system will generally produce high and narrow chromatographic peaks, and thus provide good sensitivity. The efficiency is controlled by the column choice and careful selection of other components of the flow system.

Capacity

The capacity term has to do with the ability of the column to attract ions and the eluent strength required to elute these through the column. To put it simply, the capacity relates to the retention time of the ions and the ability to accommodate samples with a large span in ion concentrations.

Selectivity

Selectivity refers to the capability of the separation system in achieving separation between different analytes. The selectivity depends on the chemical and physical qualities of the column, that result in interactions with the ions to be separated, and also on the choice of eluent.

The Eluent

The eluent transports the sample through the system and contributes to the selectivity of the separation. The eluent is a solution of a salt in water, alternatively a solution of several salts, that also act as a buffer, providing a stable pH. The table below describes how the qualities of the eluent are affected by different parameters.

Table 1: *Influence by different eluent parameters on the retention in anion chromatography.*

Parameter	Effect on retention in anion IC
Ion strength	The eluting ability increases with eluent ionic strength. The selectivity among ions of equal charge is only marginal, whereas the selectivity between ions of different charge (mono- or polyvalent) is far more sensitive to changes in ion strength.
pH	The retention times for anions of weak acids increase when the pH of the eluent increases in the vicinity of the pK_a of the acid. This is due to the sample ion charge being controlled by the eluent pH – the more basic the eluent, the stronger the negative charge. In a suppressed system, the eluent pH is substantially higher than the pK_a's of most analytes, with the exception of, *e.g.*, acetate or phosphate ions.
Temperature	The ion exchange rate between the stationary and the mobile phase increases with increasing temperature. The viscosity of the eluent, and thereby the column backpressure, decreases and can give a better separation efficiency. The temperature can also affect the column selectivity.
Flow rate	The ions are eluted faster with high eluent flow rates, but faster elution will decrease the separation efficiency. The flow rate is also limited by the pressure durability of the separation column.
Buffer salt	The eluting power of the eluent, and also to some extent its selectivity, are affected by the anion of the eluent (through its pK_a value). A change of salt will normally also result in a change of the eluent pH.

Isocratic Elution and Gradient Elution

The most common type of elution is *isocratic*, *i.e.*, where the eluent has a constant concentration and composition during the entire run. Another possibility of elution is a *gradient*, i.e. where the eluent concentration is changed in a reproducible way in each run. The reason for using gradient elution is that ions with widely different retentions can be separated in the same run with good separation efficiency and reasonable retention times.

Hydroxide eluents are commonly used for gradient elution in anion chromatography. By gradually increasing the concentration of hydroxide ions, the eluting power of the eluent increases. As a result, ions with high retention stay at the top of the column almost without being eluted until the eluent concentration is sufficiently high. They then elute in sequence as sharp peaks. A pump that is capable of mixing the gradient must be used, and because the eluent ionic strength increases during the run, the suppressor system must work very effectively to minimize the baseline drift of the system. After each gradient run the column must be equilibrated with the start concentration of the eluent before the next sample can be injected.

Choice of Eluent

Several considerations govern the choice of eluent. The first factor is the kind of sample ions that will be separated, but the type of separation column is also important. The two most common eluents are based on hydroxide or carbonate as eluting anion. Their different qualities are described below.

Carbonate Eluents

The carbonate eluent has traditionally been used in anion analysis. The eluent is an aqueous solution of carbonate and hydrogen carbonate salts, and has the advantage that the total ionic strength as well as the proportions of the monovalent (HCO_3^-) and the divalent (CO_3^{2-}) ions can be varied to optimize the retention time and the selectivity between monovalent and multivalent sample ions.

Carbonic acid (H_2CO_3) is formed when the carbonate eluent passes through the suppressor. Despite the relatively high pK_a of carbonic acid, it still gives

some background conductivity after the suppressor. For typical carbonate eluents, the eluate pH is around 4 due to the carbonic acid, which produces a conductivity in the vicinity of 10-20 µS·cm^{-1}.

$$H_2CO_3 \leftrightarrows H^+ + HCO_3^- \qquad pK_{a1} = 6.36$$

At this pH, analytes that have been converted into strong acids (e.g. Cl^-, NO_3^- and SO_4^{2-}) will be fully dissociated, while the pH of the eluate controls the degree of dissociation of weak acids. Acetic acid is for example only about 20 % dissociated at this pH and because only the dissociated ions give rise to electrolytic conductivity, a major part is presented to the detector in a form that produces no signal, with lower sensitivity as a result.

The Hydroxide Eluent

The advantage of using a hydroxide eluent is that it is transformed into pure water in the suppressor reaction, and consequently gives a very low background conductivity.

To obtain a really low and stable background level, the hydroxide eluent has to be protected from air, since the carbon dioxide present in the air is easily absorbed by the basic eluent and converted into carbonate by the hydroxide ions. This contributes to an increase in the background conductivity and can give a slow baseline drift during the day.

By protecting the eluent with a carbon dioxide trap connected with the eluent bottle, the problem with carbon dioxide absorption is eliminated. To obtain a minimal background conductivity level, a new hydroxide eluent should be prepared every day.

Hydroxide eluents must be prepared in plastic bottles and protected from the CO_2 in the air!

The hydroxide eluent is strongly basic with a pH of around 12 and separation columns of some brands can be susceptible to damage by strongly alkaline eluents. It is thus important to check the column specifications before using a hydroxide eluent. Strong bases dissolve silicate from glassware, and the silicate contributes to a higher background level and can also cause other problems. Always use a plastic bottle for the eluent!

Because of the high pH of the hydroxide eluent, the retention order is different than with a carbonate eluent. Phosphate, *e.g.*, is eluted mainly as trivalent phosphate ion (PO_4^{3-}) and will elute after the sulfate ion (SO_4^{2-}).

Preparation of Eluent

The eluent must be prepared from freshly tapped, deionised laboratory water with low conductivity. Deionised water should not be stored, neither in glass vessels, nor in plastic bottles, since the conductivity increases fast with an accompanying deterioration in eluent quality.

The sodium hydroxide that is used should be of the purest quality. In order to obtain as low background conductivity as possible, the hydroxide eluents should be prepared from carbonate-free 50 weight-% NaOH or KOH.

Hydroxide eluents are very corrosive and must not be spilled on the skin or in the eyes. Use eye protection and other safety equipment.

The table below shows the molecular weights of some common salts that are used for preparation of eluents.

Table 2: *Molecular weights of some common eluent salts.*

Substance	Mw (g/mol)
$NaHCO_3$ (water free)	84.0
Na_2CO_3 (water free)	106.0
NaOH (use at least 99+ % purity)	40.0
H_2SO_4 (concentrated acid ≈ 18 M)	98.1
Na_2CO_3 x 10 H_2O	138.0

Degassing

The eluent should be degassed every day to avoid air in the pumps and to lower the noise in the detector. The best and easiest way is if the degassing can take place directly in the eluent bottle, to avoid the risk of contamination from other vessels. The eluent is best degassed by purging it with helium while stirring with a stirring bar. Do not touch the stirring bar with your hands, because that will contaminate the eluent with salts and cause a higher background. Use a gas sparging unit with a

plastic filter to avoid metal ion contamination of the eluent.

Helium gas has the advantage that it has a very low solubility in water. Since this is not the case, *e.g.*, with nitrogen gas, He should be used in spite of its higher price. When the eluent is degassed with He from a gas cylinder, there is a risk of drawing back eluent into the pressure regulator if the main tap is switched off. The eluent bottle should therefore be equipped with a vent valve to avoid back-suction.

A vacuum pump or a water suction pump can also be used for degassing, but this is less efficient.

The eluent should be degassed daily and the best technique is helium purging.

Eluent Filtration

The eluent is usually filtered through a filter with 0.45 µm pores. This prolongs the life of the column and protects the eluent pump from being damaged by particular matter.

Avoid cellulose filters, as they often release ions, and as they are not stable at high pH. It is better to use a filter made of chemically inert material such as polyvinylidenedifluoride (PVDF) or polytetrafluoroethylene (PTFE). Note that these filters are inherently hydrophobic and cannot be used for filtration of aqueous eluents unless they have been hydrophilised. These filters must thus be specified as "hydrophilic" when ordered. Always wash the filter with eluent prior to use, since a detergent has been added to accomplish the "hydrophilization".

The eluent must be filtered to avoid having the column inlet filter clogged.

Recipes for some common IC eluents

All solutions should be diluted to 2000 ml with deionised water having a conductivity < 0.1 µS·cm^{-1}. A useful tip for carbonate eluents is to prepare a stock solution with ten times the strength of the final eluent. New eluents can then be easily and quickly prepared by 1+9 dilutions. This is, however, not a good approach for hydroxide eluents.

Weak carbonate eluent

- 0.286 g $NaHCO_3$ (1.70 mM)
- 0.382 g Na_2CO_3 (1.80 mM)

Standard carbonate eluent

- 0.403 g $NaHCO_3$ (2.40 mM)
- 0.636 g Na_2CO_3 (3.00 mM)

Standard hydroxide eluent

- 1.600 g 50 % (w/w) NaOH (10.0 mM)

Starting Up the System

If you leave the system running with a low eluent flow even during the night, you can start injecting practically at once in the morning. If not, it will take at least half an hour before the system has been fully equilibrated and the background is completely stable. The SeQuant CARS™ regenerating system can be left on during the night even if the eluent flow has been stopped, as this allows the background to stabilize even faster.

Change of Eluent

There is less risk of getting air in the pump if you alternate between two eluent bottles. Rinse the bottle not presently used with deionised water and allow it to dry. Preferably use a plastic pipette to measure stock solution or add weighed amounts of salt and dilute with water to the desired volume.

The water should be taken directly from the purification/deionisation apparatus. If this has a built-in particle filter with < 0.5 µm pore size the eluent does not need further filtration, only degassing.

Decrease the eluent flow stepwise before shutting off the pump. Move the cover of the eluent bottle to the bottle with the new eluent. The shifting of tubing should be done at the same level as the pump. By this procedure, it is normally possible to avoid introduction of air into the system. To be absolutely sure, the purge vent of the eluent pump can be opened, or if there is no purge vent, the fitting on top of the precolumn can be disconnected. Start the eluent pump at a low flow rate and wait for any air to be pumped out. Close the purge vent (or connect the precolumn and the column) and increase the eluent flow stepwise to the operational flow rate. If there is still air in the pump head, the flow will be below the setting and also fluctuat-

ing. This results in longer retention times and unstable baselines in the chromatograms. It can be necessary to draw the air out at the pump outlet (preferably at the purge vent exit) with a syringe. The air bubble then usually expands and exits.

Empty the used eluent bottle, rinse with deionised water and leave to dry. Wait until the background is stable before you start injecting samples.

Salt Deposits

It is important to keep the system in good working order. Salt deposited around a fitting indicates a leak. If such leaks are discovered, the parts must be disconnected and cleaned carefully to avoid corrosion and system malfunction. Make sure the fitting is sealed when reassembled. Tighten the ferrule by hand and then 1/8 turn (no more!) at a time until it seals. It is often necessary to replace the ferrule to obtain a tight seal. Excessive tightening is a certain way of ruining the inner surfaces of the seal, which will typically require the replacement of a far more expensive part than a leaking ferrule.

The Eluent Pump

Usually an ordinary one or two piston HPLC pump is used to pump the eluent. An inert pump is often best for ion chromatography. This means that the parts of the pump head, and the inlet and outlet valves that are in contact with the eluent are made of PEEK (poly[ether ether ketone]) or titanium.

Unless properly passivated, stainless steel releases metals ions to the eluent, and shortens the lifetime of separation column and suppressor. Disturbance from the steel in the metal pump can be eliminated by inserting a metal chelating column upstream the injector.

A thin eluent film acts as lubricant for the moving parts inside the pump. Water has lower lubricating effect than organic solvents, and pumps designed for HPLC quite often wear out the piston seals with IC eluents. When different materials are available, seals made from ultra high molecular weight polyethene (UHMWPE) usually have the longest life.

If the pump is to be left unused for some time, it should be rinsed with deionised water, possibly containing a few percent of ethanol. Otherwise salt crystals can precipitate on the piston and result in scratches on the piston the next time the pump is started. Notice that the suppressor is not resistant to organic solvents. It must thus be disconnected from the pump before the ethanol or (other organic solvents) are discharged from the pump. This usually applies to the separation column as well.

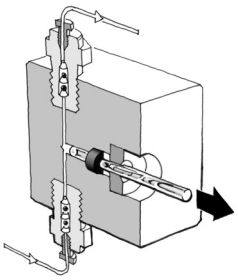

Figure 2: Typical pump head with inlet and outlet valves in an LC pump. Notice that when the pump draws eluent, the outlet valve (upper) closes while the inlet valve (lower) is open. When the piston changes direction, the valves change to the opposite.

A fluctuating flow (evident from an unstable baseline) can also be due to malfunctioning pump valves that need to be cleaned. Disconnect the pump from the rest of the system and pump pure water followed by 50-100 mL of 2-propanol directly to waste at the maximum flow rate of the pump. The cleaning action of the viscous 2-propanol is often sufficient to restore the function of the valve. Rinse the pump thoroughly with water, then with eluent *before* the column is reconnected. If the flow still fluctuates, the pump head has to be disassembled and the valves have to be washed in an ultrasonic bath. If this does not yield constant flow, the valves must be replaced. Part of the maintenance is also change the piston seal and clean the piston with 2-propanol. Bear in mind that it is easy to scratch or break the sapphire piston when disassembling the pump chamber. Study the operating instructions for the pump carefully *before* the work is begun.

In order to work properly an HPLC pump needs regular maintenance at least once every year.

The Injector

Introduction of exact sample volumes to the separation column is made by the injection valve. It has two positions, "LOAD" and "INJECT". In the *load* position, the sample loop can be filled with sample solution, while the eluent is being bypassed to the column. When the injector is turned to the *inject* position, the eluent will pass through the loop and transfer the sample to the column.

By varying the injection loop volume, the amount of sample introduced is varied. Injection volumes from 20 to 100 µl are common in standard IC systems. For a given volume least band broadening is given by a long tube with a small inner diameter.

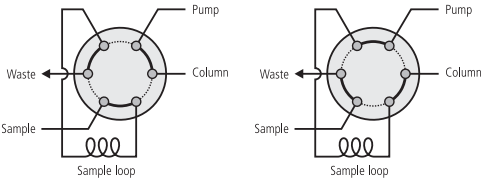

Figure 3: Schematic drawing of a six port injection valve, to the left in "LOAD" position and to the right in "INJECT" position.

In a tube with a laminar flow, the dispersion is proportional to the square root of the length and the square of the tube radius, respectively. This is why a tube with small inner diameter gives less band broadening. The choice of tube dimension is often a compromise, since long and narrow tubes yield a higher back pressure. For the same reason, it is important to choose small inner diameter interconnecting tubing in the system – we recommend the shortest possible length of 0.17 or 0.25 mm i.d. PEEK tubing in an IC system.

Avoid injecting excessively large sample volumes because the column is easily overloaded. Extreme cases of overloading result in peaks with a flattened apex. If an integrator is used, lower degrees of overloading can be detected by a decreasing ratio between peak height and peak area when the concentration of the standards increases.

When an autoinjector is used for injection, it may be necessary to adjust the rinse volumes used by the injector to ascertain that the needle and tubing are completely rinsed between samples. Also, the sample vials may need extra careful washing with deionised water before filling. Avoid rubber covers on the vials or as gasket in syringes. With plastic materials like PTFE and polypropene there is less contamination risk. Make sure that the filling time is sufficient for the injection loop to be washed out and completely filled with sample before injecting.

Manual injections are sometimes preferable. If injecting manually, it is usually advantageous to draw the sample through the injection loop by suction, instead of filling a syringe with the sample and then injecting it into the injection loop. If the sample is drawn by suction, the sample solution will only be in contact with the PTFE (or PEEK) tube from the sample vial to the injection loop, and there is less risk of contamination. It is important not to apply too fast suction, since air bubbles can form in the injection loop due to underpressure. Draw slowly at least 10 times the volume of the injection loop, so that the loop is thoroughly washed and filled. Switch the injector to "INJECT" position *before* removing the syringe to avoid loss of sample to waste through siphoning. Avoid using plastic syringes with rubber gaskets for analytical purposes, since rubber is known to be notorious in releasing spurious substances, including ions.

The Separation Column

The purpose of the separation column is obviously to separate the sample ions. There are different types of columns, all having in common that the packing material is furnished with charged functional groups – termed ion exchange groups – that perform the actual separation. In anion chromatography these ion exchange groups are positively charged and consist of quaternary ammonium compounds (R_4-N^+), where one of the substituents R is a carbon chain covalently attached to the support material (the packing material).

The other substituent groups R can be of different types, e.g., methyl or 2-hydroxyethyl. The sample ions will have different binding constants in their dynamic interaction with the ion exchange groups, and are thus separated as they pass through the column. Quaternary ammonium ion exchangers with hydrophilic substituent groups show a relatively strong interaction with hydroxide ions, and because of this columns with hydroxyalkyl substitution are used mainly for hydroxide eluents.

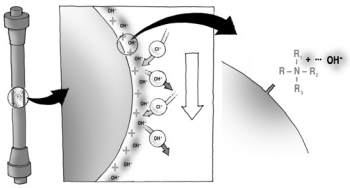

Figure 4: The separation process in an anion chromatography column. Sample ions bind to the charged groups of the stationary phase with different binding constants and are eluted by the eluent ion.

Choice of Separation Column

A column with anion exchange function is used for anion chromatography and the separation column choice is controlled by the analytes to be separated. There are many brands of such columns, and all common brands have quaternary ammonium groups with a permanent cationic charge.

When choosing a separation column, first consider the *capacity* and the *selectivity*. The *efficiency* of the column is also important.

Ion Exchange Capacity

The *capacity* of a column is determined by the number of ion exchange groups and is given as milliequivalents per gram dry, or per milliliter wet, ion exchanger, alternatively for the whole column. Unfortunately, many manufacturers ignore specifying the capacity, but it is very important since it determines the retention time as well the eluent strength required to elute ions on the column. If the capacity is too high, it will require an unnecessarily strong eluent, whereas if the capacity is too low, the dynamic range becomes small because the column is easily overloaded. Columns made for non-suppressed ion chromatography are generally not suitable for a system with suppressor, as they usually have a very low capacity.

Column usage can also affect the column capacity. Naturally occurring anions with multiple charge, *e.g.*, humic substances, have a tendency of getting irreversibly absorbed onto the column. The ion exchange groups are then blocked and the actual capacity is lowered. The column can also alter its polarity to some extent, due to highly charged organic ions partly binding to the anion exchange groups. The result will be shorter retention times, worse separation of the sample anions, and sometimes even retention for transition metal ions that form complexes with bound substances.

The capacity factor (k') of an ion describes the relative velocity of the ion through the column and is calculated according to:

$$k_A' = \frac{\left(t_R - t_0\right)}{t_0} \qquad [1]$$

where t_R is the sample ion retention time and t_0 the time required for the void volume to be pumped through the system.

Separation Selectivity

The *selectivity* of the column is due to several factors, *e.g.*, the support material and the chemical structure of the ion exchange group. The length and chemical composition of the spacers that link the ion exchange groups covalently to the support material are also of importance. This kind of information is usually not available from the column manufacturers.

The selectivity is thus due to an interaction of several characteristics of the column and the properties of the ions to be separated. These are:

1. The *charge* of the analyte ions (monovalent or multivalent).
2. The *hydrated radii* of the sample ions and the degree of crosslinking of the support material .
3. The ability of the analyte ions to either contribute to, or disrupt the *hydrogen bonding structure* of the surrounding water.

The selectivity factor (α) of two analyte ions A and B eluting close to each other is the measure of how well these ions are separated, and is calculated according to:

$$\alpha = \frac{k_B'}{k_A'} \qquad [2]$$

where k_A' and k_B' are the capacity factors for the first and the last eluted ion, respectively.

Separation Efficiency

In an efficient column, all ions of a certain kind are eluted together in a small volume. The peak has a

small *bandwidth* and is of course both higher and more resolved than a peak that elutes in a larger eluent volume. In order to compare columns, the retention time of the peak must also considered. Separation efficiency if commonly expressed as the number of theoretical plates, expressed as:

$$N = 5.54\left(\frac{t_R}{w_{½}}\right)^2 \qquad [3]$$

where $w_{1/2}$ is the peak width at half the peak height.

The factor determining the column *efficiency* is the number of "interaction instances" that the analyte ion will experience in relation to the separation material during its passage through the column. The number of interactions is termed "plates", according to a terminology borrowed from theory of distillation. High plate numbers are achieved when the sample ions only have to be transported short distances through the eluent to reach the particles of the packing material. This means that columns packed with small particles will achieve the best separation. If the particles become too small, the back-pressure along the column can get so high that the particle bed collapses. The lower practical limit for conventional columns packed with polymer particles is about 5 µm in diameter. The separation efficiency cannot be further increased by decreased particle size because of the relatively slow kinetics of the ion exchange (*i.e.*, the resistance to ion exchange).

High quality columns have stable particles almost perfectly packed in hexagonal close-packing, while low quality columns have particles that are poorly packed, or so fragile that they can be destroyed during a run. Then channels with less flow resistance can form through the packed bed, making parts of the sample zone travel faster through the column. As a result the separation efficiency is decreased and the peaks are broadened. If the particles disintegrate the back-pressure will increase, because the outlet filter of the column becomes clogged. As a consequence the column bed compresses, leaving an empty volume at the top of the column. Such settling increases the void volume and seriously affects the separation efficiency.

Decreased Efficiency with a Large Void

The void volume, often also referred to as the dead volume, is the total volume of liquid between the injector and the detector. The size of the void volume determines the time (t_0) it takes for a compound that is not retained on the separation material to reach the detector:

$$t_0 = \frac{\text{void volume}}{\text{flow rate}} \qquad [4]$$

The total void volume (V_{void} or V_0) is the sum of the volume inside and outside the column:

$$V_0 = V_{void} = V_{column} + V_{extra} \qquad [5]$$

The column void volume (V_{column}) is the volume between the individual particles that together form the packing material of the column. The eluent flows through this volume. For a fixed column size (diameter x length) the column void volume is practically constant and independent of the diameter of the particles in the packing material. The uniformity of the packing determines the extent of the band broadening.

An important source of band broadening is the volume outside the column (V_{extra}), *i.e.*, the volume in the injection loop, the interconnecting tubing, the suppressor, and the detector.

All tubing used for interconnecting the various components of the system should be *short* and have a *small* inner diameter, to minimize the band broadening and preserve the efficiency obtained in the column. There is a practical limit to how small the tubing can be, as the back pressure increases with a decreased inner diameter, and there is an increased risk of unintentional clogging.

The injection loop also contributes to the band broadening. This is why small injection volumes should be used and injection loops with large inner diameter should be avoided. Larger injection volumes can be used for samples with particularly low ion strength, but if more than 0.5 ml has to be injected, it is better to concentrate the sample *on-line* on a special enrichment column (→ Sample Enrichment on page 18).

In SeQuant's suppressor SAMS™, the flow path has been optimized, and the suppressor only gives a minor contribution to the band broadening.

Some detectors contain unnecessarily long tubing for thermostatting the eluent before it enters the flow cell, and this tubing contributes to the band broadening. Keep a watch of this if you are going to buy a new detector. Also note in isocratic runs, the volume between the pump and the injector is of no importance to the band broadening, but it is nonetheless impractical to use excess tubing since it will then take a long time to change eluents, and the eluent composition is distorted if gradient elution is used.

Minimize the void volume by keeping all flow paths as short as possible!

The table below shows the relation between the volume of the tubing and the inner diameter of a 10 cm tube of the most common dimensions.

Table 3: *Volume of a 10 cm tubing segment of some of the more common dimensions.*

Tube dimension	Volume (µL)
0.13 mm ID	1.3
0.17 mm ID	2.3
0.25 mm ID	4.9
0.50 mm ID	20
0.75 mm ID	44

Column Categories

There are two main types of ion exchange columns for ion chromatography, characterized by the support material being either inorganic silica or an organic polymer.

Silica-Based Columns

Columns based in silica supports cannot be used in suppressed anion chromatography because of the high pH of these eluents. Common carbonate eluents have a pH around 10, whereas hydroxide eluents have a pH close to 12.

Columns based on silica are unable to withstand a pH above ≈ 7.5 in the long term, and will simply dissolve in alkaline eluents. Silica-based columns are *meant for unsuppressed anion chromatography only* and will not be dealt with any further.

Polymer-Based Columns

There are many types of polymer-based columns. They can tolerate a far more basic conditions than silica-based columns and most of them can also be used with hydroxide eluents. The ion exchange group must however be hydrophilic to increase the selectivity of the column for hydroxide ions and to allow the use of reasonable eluent concentrations. You should always check the specifications of the column before using a hydroxide eluent. Columns where the ion exchange groups are attached to a polymer support based on styrene/divinylbenzene (S-DVB) copolymers usually have lower efficiency than other types of polymer based columns. By treating the S-DVB surface in different ways to obtain a more hydrophilic surface functionality, very efficient columns can be obtained, and these types of columns are common, as are columns based on methacrylate polymers.

A polymer-based separation column is the only option for suppressed anion chromatography!

Column Care

The separation column can be attacked by microorganisms, especially if it is stored with normal IC-eluents for a longer period. If the column will not be used for a long time, it should be washed with an eluent stronger than usual, followed by water. Plug the column and store in a refrigerator, but *not* below the freezing point. Some columns can have other storage recommendations. Always check the column specifications and follow the instructions provided there before leaving the it for a longer period, *e.g.*, during vacations.

A column that starts showing signs of bad separation, evident by lower efficiency (broader peaks), shorter retention times and deviations in the calibration curves, may have lost capacity due to strong binding of multivalent ions to the ion exchange groups. The capacity can often be restored, at least partly, by pumping 100 mM sodium hydroxide at a low flow rate (< 0.5 mL/min) in the reverse flow direction. It is important to disconnect the detector and the suppressor during this procedure, or they will be destroyed by excess pressure or debris released from the column.

The Pre-Column

A pre-column is usually placed upstream the separation column. The packing material is commonly of the same kind as the separation column, and the purpose of a disposable precolumn is scavenge dirt and multivalent ions that would otherwise be accumulated on the expensive separation column. If the samples contain large amounts of polyvalent metal ions, a metal chelating precolumn can be installed to trap these before entering the system.

Use a precolumn to prevent dirty samples from destroying the separation column.

High Separation Column Back-Pressure

If a high back-pressure develops over the column, it is either because the material has settled in the column or, more often, because "fines" (*i.e.*, parts coming loose from the packing material) clog the column outlet filter. One sign of a settled column is when analytes that previously eluted as symmetric peaks start tailing. If settled column material is suspected, the column can be carefully opened at the inlet end and checked for any unfilled volume under the filter. At the top of the column there is always a small volume without any material, but if this void exceeds ≈ 1 % of the total column length, the column should be filled up with clean packing material taken from the *bottom* of an old column with a spatula. If this is not done there will be band broadening from the void volume. The result will be lower chromatographic resolution and reduced sensitivity.

Few IC-columns can tolerate organic solvents or repeated large changes in the ionic strength of the eluent.

It is often less expensive to buy a new column than to waste time on "rescue actions". Preventive care is always motivated.

If the column material has settled, the column was either badly packed or it has been mistreated. Note that most ion chromatography columns will be destroyed by organic solvents. Their durability to osmotic shocks, caused by abrupt changes between strong and weak salt solutions, is also limited.

The Suppressor

The suppressor has a central role in the IC system, where it performs the double functions of lowering the background and increasing the useful signal.

Why is a Suppressor Used?

Detection in ion chromatography is almost always carried out by electrolytic conductivity, since this property is shared by all ions. The eluent contains a relatively high amount of salt and by that it also has a very high conductivity.

To be able to detect differences in the conductivity of the eluate, arising from the sample ions that are present at much lower concentration than the eluent, the amount of dissolved ions in the eluent has to be decreased considerably after the column. In other words, the conductivity of the eluent has to be *suppressed* and that is the purpose of the suppressor. A beneficial "by-effect" of suppression is that it also increases the analyte ion signals.

Electronic noise from the detector causes only a minor part of the total noise in an IC-system. Most of the noise is due to hydraulic or chemical causes such as pump flow rate fluctuations and variations in temperature.

The suppressor decreases the eluent background conductivity and increases the sensitivity for the sample ions.

The electronic noise from the detector is constant, irrespective of whether a suppressor is used or not. The noise originating from temperature variations is however much higher if no suppressor is used, because the background conductivity of the eluate is high without suppressor, and the amplitude of the noise depends on the temperature dependence of the ion conductivity (≈ 2 %/°C). Consequently, *the limit of detection is substantially worse in non-suppressed ion chromatography*.

Variations of the eluent flow also result in a higher noise in a system without suppressor.

In unsuppressed IC various "system peaks" will appear due to differences in the molar conductivities of the sample ion and the eluent ion. For example, this can result in negative peaks if hydroxide eluent is used, whereas strong eluents can result in positive peaks that elute late in the chromatogram and have to be waited for, before the next injection can be made.

One way to decrease the background of a system without suppressor, is to reduce the strength of the eluent and thereby its conductivity. To make the ions elute at the same retention time, the capacity of the column has to be decreased. This limits the total amount of ions that can be injected before the separation column becomes overloaded, and consequently the dynamic range is smaller than in a system with suppressor.

SeQuant manufactures and markets a membrane suppressor (SAMS™), which operates according to the classically used ion exchange principle. Other types on the market are electrolytic or packed suppressors, or devices operating according to a combination of these principles. Packed suppressors have the disadvantage of being consumed, so they have to be regenerated off-line at regular intervals. Some drift of retention time can be observed during the consumption of their proton charge. This might lead to a partial co-elution of the fluoride peak or a "water dip" a carbonate eluents are used. One way to reduce this problem is to alternate between two or more column suppressors in an automatic system. Such alternation may yield different background levels, reducing the precision and accuracy, unless the devices are well matched and the regeneration and washing is automated. Unless carefully designed, systems based on packed suppressors are also characterized by a larger band-broadening and a lower separation efficiency than systems with membrane suppressors.

The Membrane Suppressor

In the membrane suppressor the eluent is neutralized by continuous flow ion exchange through an ion exchange membrane, whereby the background conductivity is reduced.

The eluent flows on the inside of a membrane tube, while the regenerant, an acid with a concentration around 5-50 mM, flows on the outside in the opposite direction.

During the separation of anions with an alkaline eluent a cation exchange takes place through the membrane, *i.e.*, Na^+ or K^+ ions from the eluent are replaced by H^+ from the acid (the regenerant). The transport through the membrane is conducted by covalently bound sulfonic acid groups ($R\text{-}SO_3^-$) in the membrane, which selectively transport the cations. An electrostatic repulsion caused by *Donnan exclusion* prevents the anions in the eluate (*i.e.*, the sample ions, and OH^- or HCO_3^-/CO_3^{2-} from the eluent), as well as in the regenerant anion, from passing through the membrane.

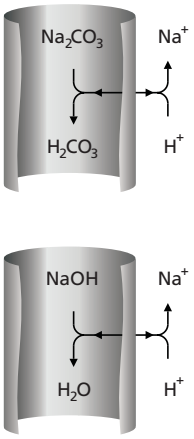

Figure 5: Schematic drawing of the ion exchange process in a membrane-based anion suppressor.

However, real ion exchange membranes are non-ideal and a small part of the regenerant (< 0.1 %) can therefore pass the membrane, if an inorganic acid like, *e.g.*, sulfuric acid is used as regenerant. To obtain an extremely stable background and to avoid changing acid in the regenerant channel, SeQuant provides CARS™, a continuous regeneration system. This system consists of a pump and a large cartridge with an ultra-pure ion exchanger. The regenerant solution contains a high molecular weight strong organic acid (ULB™), with extremely low "forbidden" ion transport across the suppressor membrane, to ensure a very low and stable background. When the acid has been consumed in the suppressor, it is pumped back to the cartridge to be regenerated, and then pumped to the suppressor again. This results in a very high capacity of the suppressor system.

CARS™ also minimizes baseline drift in gradient elution, and thus enables separation of ions with greatly different characteristics in a single run.

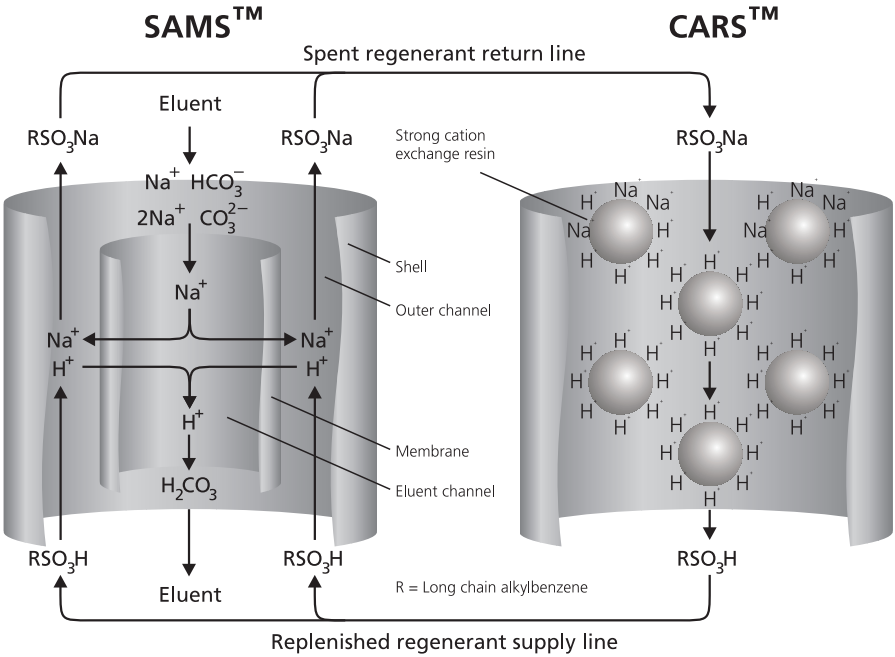

Figure 6: Complete ion exchange scheme in a SeQuant SAMS™ connected to SeQuant CARS™ for continuous regeneration.

Installation of the Suppressor

Bear in mind that the suppressor is an important part of the separation system. The tube connecting the column with the suppressor must be short and of a small inner diameter. Otherwise there will be an unnecessary large dead volume, which will give band-broadening and lower separation efficiency. The tube connecting the suppressor to the detector should also be minimized, but the inner diameter should not be smaller than 0.25 mm to prevent an excessively high back-pressure, which can damage the suppressor. To protect the suppressor from extreme pressures, it is possible to attach a pressure guard, which can be ordered from SeQuant. This also works as a security vent if the detector waste tube is accidentally pinched.

Testing the Suppressor Function

A possible *leak* in the suppressor membrane can be detected by shutting off the flow in the outer channel (the regenerant cannel), while continuing to pump eluent through the inner channel. If solution exits the regenerant channel, this means that the suppressor membrane has been ruptured. The suppressor must then be replaced with a new one.

The *efficiency* of the suppressor can be tested if the system is run with a stable baseline without injecting any samples. Collect a few millilitres of eluate from the outlet of the inner channel and measure the conductivity followed by the pH, *in that order*. A carbonate eluent containing 2.4 mM Na_2CO_3 and 3.0 mM $NaHCO_3$ usually has a background conductivity of 15-20 $\mu S \cdot cm^{-1}$ and a pH close to 4. A hydroxide eluent containing 10 mM NaOH has a background conductivity of 1-4 $\mu S \cdot cm^{-1}$ and a pH between 5 and 6, which is difficult to measure since the solution is unbuffered and lacks a ionic strength required to measure the pH reliably. The background conductivity with hydroxide eluents in CARS™ systems is often even lower. These figures require that the eluents have been prepared from high quality deionised water.

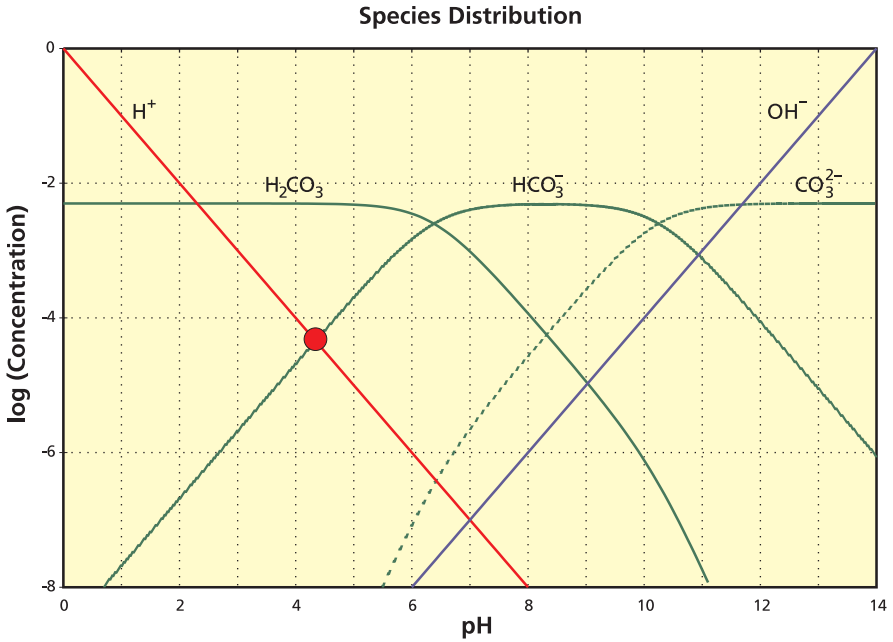

Figure 7: The distribution among carbonate species in a 5 mM carbonate eluent at different pH. The dot in the drawing marks the point of intersection for HCO_3^- and H^+, where the pH of the eluate is expected if the suppressor membrane behaves ideally.

How to Pinpoint a Deteriorated Suppressor?

If the suppressor has been operated according to the instructions, the main reason for deterioration is simply ageing, caused by accumulation of metal ions or other cationic substances that occupy the groups intended for the cross-transportation of eluent ions and protons. The reduced function will give an increasing background and baseline noise.

The binding of sample anions to metal ions stuck in the suppressor can also cause signal reduction, broadening of the peaks and other strange phenomena for anions with metal binding ability such as fluoride, sulfate, and phosphate. Escalating deviations from calibration linearity is an early sign of deteriorating suppressor function.

Unfortunately, a deteriorated separation column can give rise to more or less the same symptoms. The suppressor system should therefore always be checked when the column is replaced, and the linearity of the standard curves and the separation efficiency should be documented.

If you are using the CARS™ continuous regenerant system, you should make sure that the cartridge has not been consumed, before performing other trouble-shooting. Also check that the flow in the regenerant channel is normal.

A high background level and less linear calibration can be caused by a worn-out suppressor.

If a reduced suppressor function is discovered in a system where the regeneration is functioning properly, the suppressor has probably accumulated metal ions. You can then try to remove these ions by ***disconnecting the suppressor from the system*** and thereafter carefully injecting 50-100 mL of a solution containing 0.1 M magnesium sulfate, 0.1 M disodium EDTA and 0.2 M sodium hydroxide, into the regenerant channel.

Keep adding this solution until the liquid that exits from the regenerant channel outlet has a pH > 11. Then fill the inner channel with the same solution. Rinse both channels with water after about 24 h and the suppressor can then be reinstalled. Allow a

substantial time for the signal to stabilise, since the ion exchange resin in the suppressor outer channel added to stabilise the baseline acts as a reservoir of ions that has to be replaced by H^+.

If the function of the suppressor is still unsatisfactory, it must be replaced with a new unit.

The Detector

After the sample ions have been separated and suppressed, they have to be identified and quantified. The detector is therefore connected to a recorder or another kind of evaluation system.

The conductivity detector is by far the most common detector type used in anion chromatography, since electrolytic conductivity is a universal property shared by all ions. In some cases a UV-VIS detector is used, but this mostly found in systems without suppressor. The disadvantage of UV-VIS detection is that only a few inorganic ions absorb light. Azide, chlorite and nitrite are examples of such ions. Other ions must be detected indirectly.

The Conductivity Detector

In a conductivity detector the eluate from the suppressor passes through a flow cell with two (in a few designs four) electrodes, between which an AC potential is applied. When the sample ions enter the cell, the capability of the solution to conduct electrons increases. The increase in current is proportional to the increase in conductivity, which is in turn a linear function of the ion concentration[1].

The distance between the electrodes is usually represented by **d** and the electrode area by **A**. The ratio **d/A** is then termed the *cell constant* **K** of the detector and has the unit cm^{-1}. The conductance **G** between the electrodes is continuously measured and is dependent on $|\mathbf{z}_i|$, *i.e.*, the ion charge magnitude, the ion concentration $\mathbf{c}_i$, and the electric mobility $\mathbf{u}_i$ of the ions in the cell.

The conductivity is specific for every ion and linearly dependent on the concentration in "solutions of infinite dilution". In solutions with a total concentration of ions exceeding millimolar levels, the ions influence each other and the conductivity is no longer linearly dependent on the concentration.

The conductivity κ is an intrinsic property of the solution and can be calculated from the conductance and the cell constant. Thus, when a sample ion passes the detector the conductivity increases and a peak is obtained on the chromatogram.

Conductance (unit S or Ω^{-1}):

$$G = \frac{1}{R} \qquad [6]$$

Electrolytic conductivity (unit $S \cdot cm^{-1}$):

$$\kappa = K\,G = \frac{d}{A}\,G \qquad [7]$$

Molar conductance (unit $S \cdot cm^2 \cdot mol^{-1}$):

$$\Lambda = \frac{\kappa}{c} = \lambda_+ + \lambda_- \qquad [8]$$

where λ is the molar ionic equivalent conductivity of the positive and the negative ions in a salt, respectively, expressed in $S \cdot cm^2 \cdot mol^{-1}$, and **c** is the concentration in mol/litre (mol/1000 cm^{-3}), and R is the resistance in Ω. (Note that tabulated values usually give the ionic conductivity per equivalent, *i.e.*, per charge, with the unit $\Omega^{-1} \cdot cm^2 \cdot eq^{-1}$.)

Why are Calibration Curves in IC Non-Linear?

Fundamental limits of the technique is the reason why the calibration curves in ion chromatography are not completely linear. For anions of weak acids like acetate and F^-, this is quite easy to explain. Remember that the suppressor has converted all anions to their acids; for the ions in our example this is to acetic acid (HAc) and hydrofluoric acid (HF), respectively. When these acids are diluted in water they are only partly dissociated into Ac^- and F^-, respectively.

The rest exist as uncharged acid, not contributing to the ionic conductivity. When the concentration of the acid increases (as it does when an eluting peak grows or when the sample concentration increases), the pH of the solution decreases, which makes it more difficult for the acid to dissociate. Therefore, the dissociated fraction decreases with increasing concentration, which explains the non-linearity at high concentrations.

[1] *A linear dependence of conductivity with concentration is valid for the low concentrations of ions found in ion chromatography, as long as the dissociation is complete (see below).*

Table 4: *Molar ion conductivity for ions λ_o at 25 °C*

Ion		λ_0 $S \cdot cm^2 \cdot mol^{-1}$
Hydroxide	OH^-	198.6
Fluoride	F^-	55.4
Chloride	Cl^-	76.3
Nitrate	NO_3^-	71.4
Sulfate	SO_4^{2-}	160
Phosphate	PO_4^{3-}	207
Chlorite	ClO_2^-	52
Chlorate	ClO_3^-	64.6
Hydrogen carbonate	HCO_3^-	44.5
Carbonate	CO_3^{2-}	144
Hydronium	H^+	349.8
Lithium	Li^+	38.7
Sodium	Na^+	50.1
Potassium	K^+	73.5
Ammonium	NH_4^+	73.5
Magnesium	Mg^{2+}	106.1
Calcium	Ca^{2+}	119.0
Zink	Zn^{2+}	105.6

Table 5: *pK_1-values in water at 25 °C*

Acid	Formula	pK_1
Hydrochloric acid	HCl	< 0
Sulfuric acid	H_2SO_4	< 0
Nitric acid	HNO_3	< 0
Chloric acid	$HClO_3$	< 0
Oxalic acid	$H_2C_2O_4$	1.19
Chlorous acid	$HClO_2$	1.94
Phosphoric acid	H_3PO_4	2.15
Nitrous acid	HNO_2	3.14
Hydrofluoric acid	HF	3.18
Acetic acid	CH_3COOH	4.74
Carbonic acid	H_2CO_3	6.36
Hydrogen sulfide	H_2S	6.97
Hydrogen cyanide	HCN	9.21

Anions of strong acids are, on the contrary, always completely dissociated and their calibration curves are therefore far more linear in suppressed anion chromatography. Chloride, nitrate and sulfate are examples of such anions.

Even anions of strong acids can show non-linear concentration dependences, especially if weak acids are used as eluents, or if an inappropriate acid is used in the regeneration reaction. This is due to the suppressor reaction being non-quantitative (about 0.1% of the eluent ions are not removed across the membrane) and also due to a minute leakage of "forbidden ions" through the suppressor membrane (also ~0.1%). For standard IC-eluents the suppressor has been optimised to make the effects balance each other, i.e., the fraction of unsuppressed eluent is "titrated" by the leakage of "forbidden ions", with an optimally low background and maximum linearity as a result.

Non-linear calibration curves appear if one of these two processes are disturbed, i.e., *if an eluent much stronger or weaker than normal is used, alternatively if an unsuitable acid and/or an acid concentration too high is used in the regenerant. If the eluent is very weak (less than 1 mM cations) or is pumped at a low flow rate (less than 0.5 mL/min) the "forbidden" influx of acid from the regenerant channel can dominate, resulting in an eluate* more acidic *than it should be. However, if the eluent is very strong (more than 20 mM cations) or is pumped at a high flow rate (more than 1.5 mL/min) the efficiency in the suppressor (measured as the fraction of the ions that are exchanged) might decrease and the fraction of eluent cations that pass the suppressor will increase. This gives a* more basic eluate *after the suppressor than expected from the eluent composition.*

This effect is seen only when performing trace analysis, when deviations from linearity arise at low concentrations of sample ions. Then the calibration curve has an intercept below zero. *By optimising the conditions, so that the suppressor works at a balanced stoichiometry, the concentration of residual cations in the eluate is minimised and more linear calibration curves are obtained. Similarly, the forbidden ion leakage is controlled by choosing a regenerant acid with a low tendency of passing the membrane,* e.g., *the ULB™ regenerant solution, as supplied with the SeQuant CARS™ system. A good diagnostic test is to measure an exact pH of the eluate after suppression, to which ≈1 mM KCl has been added to increase the ion strength.*

Resetting the Detector

In ion chromatography there is always some background conductivity, that produces a stable output voltage. Even though it is important to monitor the background level, it is usually more convenient to remove this signal by using *autozero*. It is wise to set the output signal to zero on the detector, *not* on the recorder or integrator, because large part of the signal range is otherwise occupied by the background signal.

Calibrating the Cell Constant

The cell constant of the detector has to be calibrated when absolute measurements need to be made. When relative measurements are performed this is of less importance, but to be able to decide if the system is working properly, the values reported by the detector should not be *completely* wrong.

The cell constant is determined with standards of known conductivity that are pumped through the detector. Usually 1 mM KCl is used, which should give a conductivity of 149.8 $\mu S \cdot cm^{-1}$ at 25 °C. In a completely dry detector cell, the conductivity signal should always be zero. Consult your detector manual for information on how to calibrate if the displayed conductivity is not accurate.

When performing absolute measurements it is important to calibrate the detection cell!

The Importance of Temperature Control

The conductivity is dependent on the temperature and the molar ion conductivity at the temperature **T** can be related to tabled values at 25 °C, using the formula:

$$\lambda_T = \lambda_{25} e^{k(25-T)} \qquad [9]$$

where the temperature coefficient **k** is a constant for a given ion in a given solvent, usually about 2 % per °C for an anion in water at 25 °C.

Due to the temperature sensitivity, it is clear that the background conductivity will be more stable if the temperature of the detector cell is controlled. Detectors are usually constructed to measure the conductivity and the temperature simultaneously in the detector cell, and the output signal will be the conductivity that the detector response would correspond to at a standard temperature, typically 25 °C. A detector with this feature is *temperature compensated*. Rapid temperature changes caused by draught or direct sunlight should still be avoided, since it can give substantial contributions to the overall noise and drift of the detector signal. As mentioned earlier, it is not a good idea to use a long tube between the suppressor and the detector to thermally equilibrate the eluate, as the band-broadening can ruin the separation efficiency.

It is also advantageous to maintain the column at a constant temperature, since the efficiency of the separation process is temperature dependent.

A controlled detector cell temperature gives a more stable background level.

Sample Pretreatment

The need to use a sample pretreatment procedure is largely determined by the sample matrix, *i.e.*, the sample constituents in addition to the analyte ions. Filtration is often sufficient, but the choice of filter is important, considering the contamination risk.

Choose a hydrophilic filter with 0.45 µm pore size, made from either poly(tetrafluoroethene) [PTFE], poly(vinylidene difluoride) [PVDF]. Filters based on cellulose are generally unsuitable. Reduce the risk of contamination by rinsing each filter with deionized water or sample before use. New filter batches must be validated before use.

All samples should be filtrated before injection!

Water Samples

Most drinking water samples may be directly injected, or need only to be filtered before injection.

Natural waters usually contain particles that call for a filtration, and many raw water samples are also rich in humic substances and often contain multivalent compounds. Such compounds can get irreversibly stuck onto the separation column and cause damage after only a few injections. In order to remove humic substances, the sample can be filtered by cross-flow ultrafiltration in a special filter holder. Another way is to bind the humic substances to a disposable column for solid phase extraction and then elute the sample ions.

To extend the column life, humic substances must be removed from raw water samples!

Surface water usually contain higher amounts of organic material than well-water. The amount can also vary with the season.

Special Sample Matrices

SeQuant's application team can also assist in the development of specially adapted solutions to problematic samples.

Multivalent anions can be removed using a solid phase extraction procedure. The sample is eluted

with a relatively weak eluent through an extraction column, where the multivalent ions are trapped.

High amounts of **transitional metal ions** can be removed from the sample by a solid phase extraction with a cation exchanger. An alternative, on-line solution is to include a chelating column in the chromatography system. This will provide a continuous protection of the separation column and the suppressor.

Polymeric material with anion properties can be removed by gel filtration or adsorbtion on an anion exchanger.

Samples containing **strong bases at high concentration**, *e.g.*, from alkaline fusions or in the alkali industry, have to be conditioned to reduce the ion strength. The best and easiest way to perform this is in a membrane based equipment.

Trace Analysis with IC

To obtain the lowest detection limit possible when performing trace analysis, it is necessary to take special action during the sample pretreatment. It is important to keep the equipment in good shape to avoid contamination.

The choice of material for storage vessels and tubing that come into contact with the sample is important. Suitable materials are polytetrafluoroethylene (PTFE), polypropylene (PP), polystyrene (PS), or *high density* polyethene (HDPE), although some qualities also of these plastics may leak ions. Glass has ion exchange properties which become very evident in trace analysis, and because of this glass vessels are not suitable. Polyvinylchloride (PVC) should absolutely be avoided.

It is equally important to ascertain that the supply of deionized water used to prepare standards, and for sample dilutions and eluents, is of the highest quality possible. Low conductivity (< 0.1 $\mu S \cdot cm^{-1}$) does not guarantee that the water is free from organic material or macromolecular ions with low equivalent conductivity. The water purification equipment must therfore be properly maintained.

Contamination control is extremely important in trace analysis.

Limit of Detection

In order to determine the limit of detection of a method, the background signal has to be known. It is determined by repeated injections of a blank, usually deionised water treated in exactly the same way as the samples. The source of high blank signals *must* be found before reliable trace analysis can be performed. A notorious source of contamination is filters (→ *Filtration* on page 6). Distilled water is less suitable than deionised water, since it usually contains detectable amounts of chloride and sulfate.

In the absence of a background signal, the limit of detection is often given as the concentration that gives a signal three times the amplitude of the noise. The noise is measured "peak to peak" on the baseline, during the time in the chromatogram when the sample peak is expected. It is important that the system is in good shape, to keep the noise level low. The most common source of high noise is leakage in the pump, a pulsed flow, or air in the detector cell. The problem tends to aggravate if the eluent that has been chosen produces a high background conductivity after the suppressor.

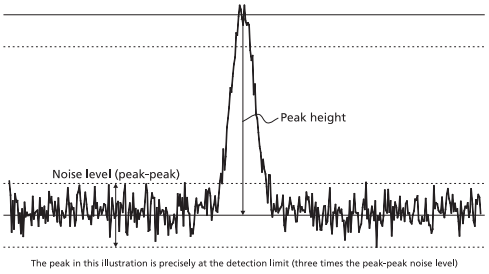

Figure 8: Determination of the limit of detection with a noisy baseline, as it can be when analysing trace levels. If the limit of detection is set to three times the peak-peak noise, the "peak" in the figure is actually exactly at the limit of detection.

Sample Enrichment

In some cases it can prove necessary to enrich the ions being studied. For trace analysis of samples with a low ionic strength, *on-line* enrichment is a preferred technique. A small enrichment column, packed with the same material as the separation column, is placed in the position of the injection loop in the sample injector. A large volume of the

water sample (up to several hundreds of milliliters if the sample has a very low ion strength) is then pumped in, whereby the sample ions are trapped on the enrichment column. When the injector is turned to the inject position, the eluent will elute the enriched ions to the analytical column.

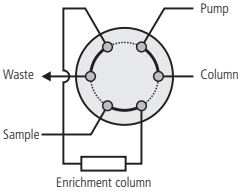

Figure 9: An enrichment column connected in the position of the injection loop in the injection valve. The sample should be loaded onto the enrichment column using a separate calibrated pump to obtain a reproducible enrichment volume.

A prerequisite for using precolumn enrichment, is that the sample itself is unable to elute ions from the enrichment column. Another limitation is that not only the ions of interest are enriched, but also ions that can co-elute or otherwise interfere with the determination of analytes. It is thus important that an enrichment method is validated for every sample matrix, *e.g.*, by standard addition.

Standard Operation Procedures

All serious quality assurance routines demand that methods used for chemical analysis are validated, and have a detailed description of the operation, called a *Standard Operation Procedure* (SOP).

It is important to distinguish between a *technique* and a *method*. Ion chromatography is a technique that can be part of a method for determination of ions in samples of a specified nature – alone or combined with other techniques (*e.g.*, solid phase extraction). A SOP should clearly identify the analysis it concerns (analysed ions and the type of samples), the nature of the equipment to be used, how samples should be processed, how eluents are prepared, etc. Even if the method description is thorough and followed very carefully, this does not guarantee that the ion chromatographic system has exactly the same properties and performance, as when the method was developed. This can for example be due to deviations of the purity of the chemicals, wear of equipment and so on. Because of this, it is a good habit to perform a *System Suitability Test* before starting the analysis.

System Suitability Test

To ensure a satisfactory quality all methods should encompass a *System Suitability Test*, *i.e.*, a suite of tests designed to ascertain that the present system is suitable for the method.

During development of the method, the system is repeatedly subjected to various tests, whereby test strategies are established and added to the method description, along with "norm values" and acceptable deviations from these values. Below are some suggestions for tests, suitable for analysis methods based on ion chromatography.

By regularly performing the tests below, it is not only possible to determine if the system is robust, but also if it is providing a stable performance over time. By *keeping a careful record of the results and plotting them as a function of time*, malfunctions resulting in faulty results can be discovered early. The SOP should give the limits to how much the measured parameters are allowed to vary before action is to be taken. When the instrument starts to produce responses outside these limits, it does not pass the *System Suitability Test*, and must be checked before it can be used in the method.

- **Drift and noise**
  After starting the pump with fresh eluent and while running the suppressor system, the noise and drift in the detector is recorded for 30 minutes. Depending on the choice of eluent, the level of noise and drift will vary. During the method development "typical" values and their limits for both drift and noise are established. With a suppressor, limits for "allowed" noise can be for example $< 0.05\ \mu S \cdot cm^{-1}$ and for the "permissible" drift $< 0.1\ \mu S \cdot cm^{-1} \cdot h^{-1}$.
- **Background**
  State a "normal" background conductivity and how high it can be allowed to be. High background levels are accompanied by higher noise, which the previous test should reflect. The water dip can provide an indication of the background level, because by rule of thumb *a large water dip indicates a high background conductivity*. Bear in mind that the size of the water dip is also affected by the ion strength of the injected sample, and of course by the selected range on the detector as well.

- **Precision**
  Repeated injections (at least three; preferably more) of a suitable standard solution, and an evaluation of peak heights or peak areas will show the precision of the system. Depending on the existing demands for the results of the analysis, the precision limit can be set at different levels, but usually the limit is set at a few percent relative standard deviation.
- **Resolution between peaks**
  The resolution between two peaks that elute closely, can also be tested. *It is especially important to have a good resolution if the first peak is much larger than the second.* If the peaks coelute, the eluent composition must be changed and the flow rate may have to be decreased. The resolution R_S is calculated as:

$$R_S = \frac{2[(t_R)_B - (t_R)_A]}{W_A + W_B}$$

  where the parameters $(t_R)_A$ and $(t_R)_B$ are the retention times of the first and the second eluting peak of the pair, respectively, whereas W_A and W_B are the width at the base of these two peaks. When $R_S > 1.5$ the peaks are baseline separated.
- **Separation between the water dip and F^-**
  In many methods it is important that the peak from F^- is completely separated from the water dip. Injection of a low concentration of fluoride gives a good indication. If the peak is not completely separated from the water dip, the integrator will fail to register parts of the peak, causing the reported concentration to be smaller than the injected amount. The calibration curve intercept will typically be negative.
  Fluoride is also one of the ions that are influenced by sample contaminations that stick to the column or suppressor. Metal ions in the samples are a source of many problems. They tend to precipitate in the basic eluent and then get trapped in the column or on the suppressor, where they can influence the retention of many anions that have an ability to bind metal ions.

Even metal ions that do not precipitate directly, *e.g.*, aluminium, can instead get strongly attached to the suppressor by ion exchange. This will cause the same effect and also result in a less efficient suppression.

Evaluation of Results

The sample concentrations can be determined by the aid of a calibration curve or from a response factor, *i.e.*, the integrator area or height units per concentration unit. It is possible to use either peak height or peak area for quantification, as long as the column does not get overloaded. Overloading is easily identified by plotting the peak height:peak area ratio versus the analyte concentration (should be constant), or by plotting the ratio between peak height and concentration versus the concentration (should be a linear relation).

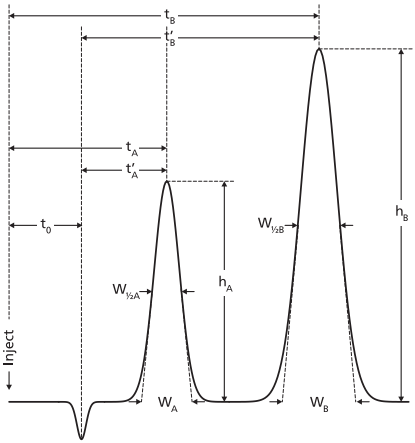

Figure 10: Chromatogram with measurable separation parameters indicated. See the text for an explanation of the symbols and their meaning.

At least three standard solutions of concentrations covering the entire analyte range and one blank solution should be injected before and after the samples every day. Note that there is a risk of introducing systematic errors by only injecting standards before and after the samples. It is therefore best to intersperse all the samples, blanks and standards in a random order.

A good SOP should finally give detailed accounts on how to evaluate chromatograms and convert these into analytical data.

Application Example

The chromatogram below shows an example of a separation of a mixture of anions using suppression by SeQuant SAMS™ suppressor continuously regenerated by a SeQuant CARS™ 4WD system.

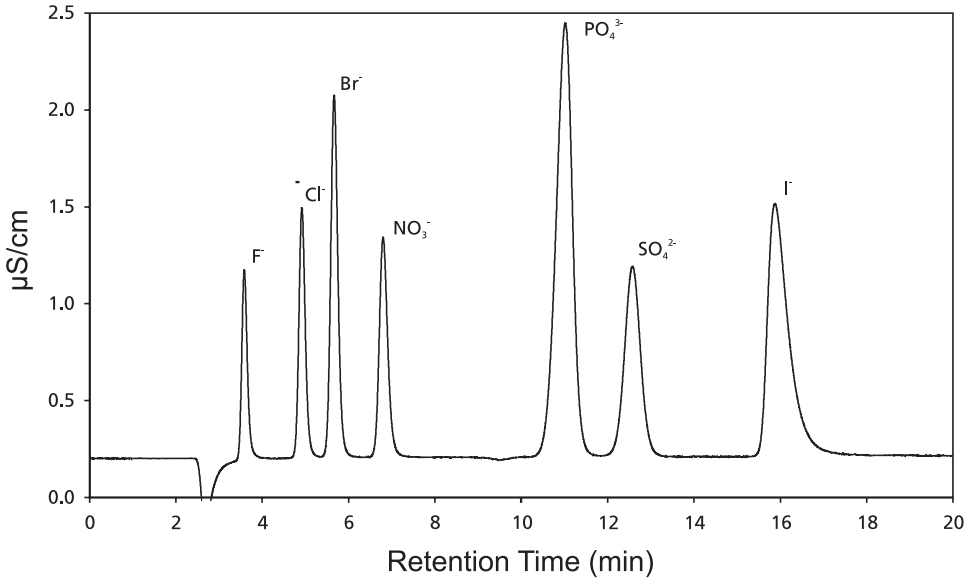

Figure 11: Separation of a mixture of anions. Sample: 1 ppm fluoride, 2 ppm chloride, 4 ppm bromide and nitrate, 32 ppm phosphate, 4 ppm sulfate and 20 ppm iodide, in water. Eluent: 1.7 mM $NaHCO_3$/1.8 mM Na_2CO_3; flow rate: 1 mL/min. Injection volume: 20 µL. Temperature: 27 °C. Column: Shodex anion exchange IC SI-90 4E (250 x 4 mm). Chromatography system: Shimadzu IC system with system controller, autoinjector, pump, degasser, column oven and conductivity detector (P/N: SCL-10Asp, SIL-10A, LC-10ADvp, DGU-14A, CTO-10ACvp, CDD-10Avp). Background suppression using SeQuant suppressor SAMS™ (P/N: 1125-100) and SeQuant regenerant system CARS™ 4WD (P/N: 7820-401), all installed in the column oven of the chromatography system.

If you have further questions on the use of our products, please do not hesitate to contact us!

Troubleshooting Scheme for Anion Chromatography Systems

Symptom	Possible source of error	Action
• High background conductivity, but a relatively low noise.	• The eluent has been contaminated with an anion of a strong acid.	• Check the electrolytic conductivity of the deionised water used to prepare the eluent and standards, and for dilution of the samples. • Prepare a new eluent (→ page 5). Change also the stock solution.
• High background conductivity in combination with high noise.	• The suppressor reaction does not work. • High backpressure in the detector. • The membrane of the suppressor is worn out or is inhibited by metal ions or hydrophobic cations.	• Check that the regenerant solution flows in a closed loop, as it should. • Check the detector by manually flushing water through the cell. Clean if necessary. • Wash the membrane with an alkaline magnesium EDTA solution (→ page 15). If this do not restore the full function of the suppressor, it is worn out and needs to be replaced with a new unit.
• High (often regular) noise with normal background level.	• Trapped air or malfunctioning valves in the eluent pump.	• Remove air from the pump and carefully degas the eluent (→ page 5). • Rinse with isopropanol (→ page 7). Change the pump valves if these are worn out.
• The sensitivity for anions of weak acids has decreased.	• Incomplete suppression. • The pH after the suppressor is too low.	• Check the regenerant flow – thereafter check if the cartridge lifetime is exceeded. • Check the pH of the eluate after the suppressor (→ page 14) and compare to the SOP (→ page 20).
• The sulfate or fluoride peak has decreased height and broadened, while other peaks are as usual.	• The suppressor or the separator column is contaminated by metal ions.	• Wash the suppessor membrane with an alkaline magnesium EDTA solution (→ page 15). If the problem remains the suppressor needs to be changed. • Run a clean-up procedure on the separator column. Follow the manufacturers instructions. Disconnect the column from the system before starting the clean-up.
• The baseline is drifting.	• The system has not stabilised yet. • Leakage in the flow system, temperature variations, debris on the column filters.	• Wait until the baseline has stabilised (Note: This can take a relatively long time). • Choose the simplest action first. Try also shutting off all pumps one by one. The injector can be a tricky source off error (→ page 8).
• Negative peaks.	• High background. • The cables between the detector and the recorder/integrator are connected with opposite polarity (signal to ground & vv).	• Check the regenerant flow, and if the regenerant cartridge has been consumed. • Make sure that – and + out from the detector connects to the corresponding terminals on the recorder/integrator. Possibly switch the cords between – and +.
• High backpressure.	• The column inlet filter is clogged. • The injector needs service or is placed in a position between *Load* and *Inject*.	• Disconnect the column. Compare with normal backpressure in SOP. • If the pressure remains when the column is disconnected the injector is the source. Check the position of the handle and perform service if necessary (→ page 8).

Notes

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