Ion Chromatography: Working Principle and Applications

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1.   Introduction

Ion Chromatography is a relatively new method for analyzing anions, cations and polar substances and is based on the equilibration of solute ions between the solvent, acting as the Mobile Phase and charged ion-exchange sites on a solid surface acting as the Stationary Phase. The technique is being used extensively in the modern chemical analysis laboratories. The development of new detection methods and advances in separation materials continues to expand the application of ion chromatography. It can be used to reliably quantify substances throughout a wide range of concentrations. A whole variety of ions can be analyzed in a single determination. Complete automation of ion chromatography is easy and help save time and reduce costs. Due to its reliability and robustness, ion chromatography is used in many different fields of application.

2.   Structural Definitions

The **Mobile Phase** is usually an aqueous solution of salts of weak or strong Bronstead acids and bases.

The **Stationary Phase** is usually a "bed" of either organic plastic beads or inorganic particles. In both cases, the surfaces of the Solid Phase carry fixed charged functional groups of weak or strong Bronstead acids or bases.

The **organic plastic Stationary Phase**, often called a "resin bed", consists of small, amorphous non-crystalline particles of styrene and divinylbenzene (1%-16%) copolymers. This material can be used over a pH range 2-12, for small atomic or molecular ions (M.W.<500) which can penetrate the small pores of the resin. The resin can be made with "dissociable functional groups" fixed to the surface. These groups can be designed to act as either Bronstead acids or bases and react with water to give either negatively or positively charged groups respectively.

The **inorganic particulate Stationary Phase** usually consists of small, amorphous non-crystalline particles of "silica gel" or "alumina". Silica gel is chemically acidic and acts as a cation exchange medium over a pH range pH 2-8. Alumina is chemically basic and acts primarily as an anion exchange medium over the pH range 6 to 11. Alumino-silicate materials called "zeolites" can be used over almost the full pH range and exchange cations or anions as needed.

3. Functional Definitions

Retention is based on the attraction between solute ions and charged sites on the stationary phase. The **Retention Time** is a function of Stationary Phase retention. The stronger the retention, of an anion/cation exchanger Stationary Phase, the longer the Retention Time on the IC column.

For ions of the same radius. Retention depends *directly on ion charge*. In general, the greater the ion charge, the greater the attraction for ion exchange sites. Typically retention increases from monovalent, to divalent to trivalent ion charge.

For ions of equal charge, retention also depends *directly on ion size.* The larger the ion radius, the more polarizable the ion, and the more strongly it is attracted to ion exchange sites.

The **Elution Order** depends on the **Selectivity** of a Stationary Phase. Strong versus weak exchanger resins can produce quite different elution orders. The elution order of halides is F^-, Cl^-, Br^- and I^-, which corresponds to increasing size of these ions.

The **Eluent Ionic Strength** is defined by the eluent buffer concentration at a fixed pH. Since buffer ions replace analyte ions on the Stationary Phase, the higher the Ionic Strength, the shorter their retention times. Since buffers are prepared with polyvalent weak acids, the ionic strength varies with pH according to the buffer pK_A;

$$HPO_4^{2-}(aq) + H^+(aq) \leftrightarrows H_2PO_4^-(aq)$$

divalent   monovalent
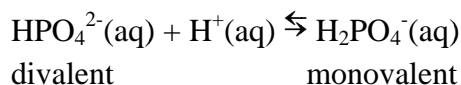
Thus the retention time of analyte ions can be changed with pH.

4. Applications

The various important fields of application for ion chromatography include:

1) The routine investigation of aqueous systems such as drinking water, rivers, effluents and rain water
2) For the analysis of ions in chemical products, foods, cosmetics and pharmaceuticals
3) Ultra-trace analysis such as in the semi-conductor and power industry.

eluent   pump   injector   column   detector
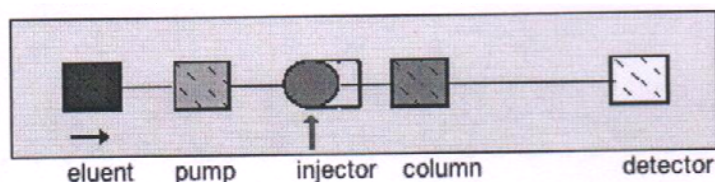
Schematic of an Ion Chromatography System

The above schematic represents a non-suppressed ion chromatography system. The sample is introduced onto the system via a sample loop on the injector. When in the inject position the sample is pumped with the eluent onto the column and the sample ions are then attracted to the charged stationary phase of the column. The charged eluent elutes the retained ions which then go through the detector (which is most commonly conductivity) and are depicted as peaks on a chromatogram.

## 2.   Three Main Modes of Ion Chromatography Columns

The different modes of chromatography (anion exchange, cation exchange and ion exclusion) simply relate to the different types of columns used to achieve the separation of the ions. The eluent mode of detection - however unless stated the following is all based on conductivity detection.

2.1   Ion exchange

Ion exchange chromatography (IC) is based on a stoichiometric chemical reaction between ions in a solution and the oppositely charges groups functional groups on the column resin. In the simplest case in cation chromatography these are sulfonic acid groups or carboxylic acid groups and in anion chromatography quarternary ammonium groups.

2.2   Anion exchange

Anion exchange chromatography forms the largest group of IC methods mainly because there are few alternatives with such simplicity, sensitivity or selectivity - particularly for sulfate. Anion exchange can exist with or without suppression and of the two, suppressed methods are the most widely used. Eluents for suppressed chemistries tend to be either carbonate based which give greater flexibility or hydroxide based.
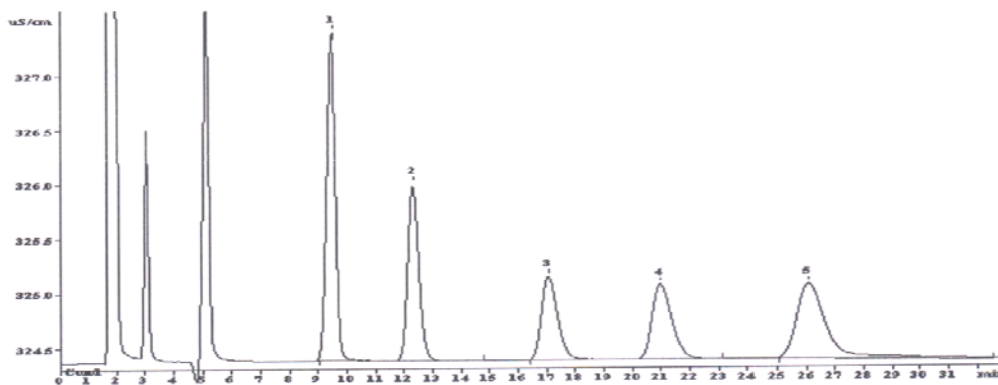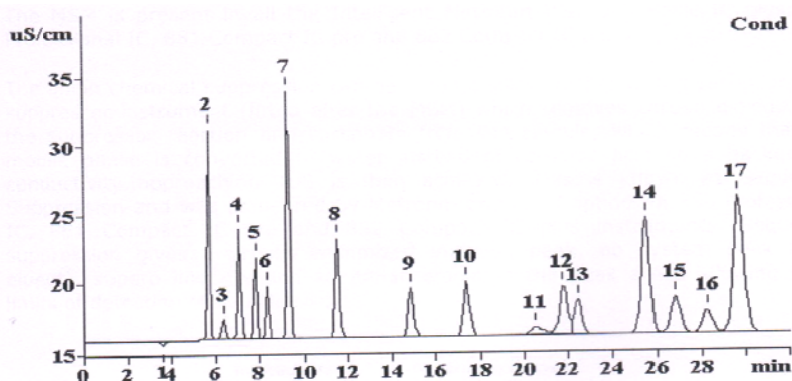

Chromatogram Showing an Anion Exchange Separation Followed by Direct Conductivity Detection (Non-Suppressed)

It is sometimes a benefit to work non-suppressed for example when analyzing weak acids like cyanide or borate that would not be seen if a suppressor is used.
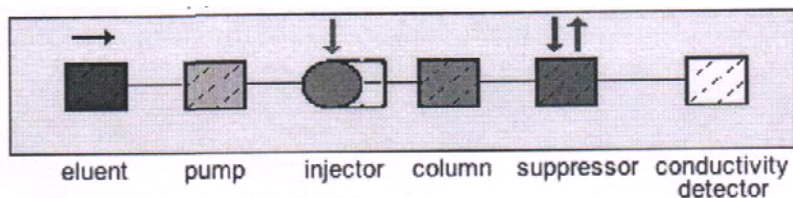
Suppression in Ion Chromatography

Often, a device called a suppressor is used and is placed between the column and detector as shown above. When suppression is used the detector is almost certainly conductivity. The chromatogram below shows a sample with a suppressor unit placed between the column and detector. The greatest achievement of suppression is to increase the sensitivity of the anion, however at the same time the background conductivity of the eluent is greatly reduced. The same suppressor units can also be used to increase the sensitivity of organic acids using a technique known as inverse suppression.

**Chromatogram Showing an Anion Exchange Separation Followed by Suppression and then Conductivity Detection**

The suppressor used in anion chromatography is simply a cation exchanger and its job is to remove cations and replace them with an H^+. So a sodium carbonate eluent (~800uS) would be converted to carbonic acid (~18uS) by the suppressor and the analyte, for example NaCl (~126uS without suppression) would become HCl (~426uS with suppression). The suppressor converts the analyte to the free acid form and the background is reduced whilst the sample signal is enhanced.

The Metrohm MSM (Metrohm Suppressor Module) contains 3 separate suppressor units. At any one time, one will be in-line with the eluent and conductivity detector, one will be in-line with dilute sulfuric acid (replacing the removed cations with H+) and the third is washed with water. The benefits of this technique are lack of baseline noise and a ruggedness that is reflected in the fact that the MSM comes with a ten year warranty and is not adversely affected by the transition metals or amines which can be a problem for other manufacturers.
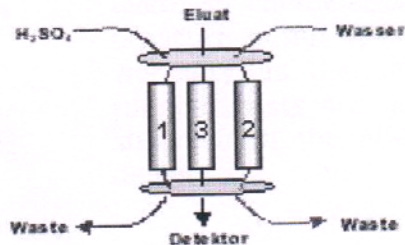
Schematic of Metrohm Suppressor Module

The anion chemical suppression can be taken a stage further with a secondary CO_2 suppressor instrument (fitted after the MSM) which removes carbon dioxide from the suppressor reaction and carbonate from the sample, which means that the mobile phase is converted to water instead of carbonic acid so a background conductivity approaching 1uS is then achieved. This is known as Sequential Suppression. Sequential suppression gives a greatly minimized injection peak, no system peak (from eluent), superb linearity and an enhancement in the peak areas allowing lower limits of detection to be achieved.
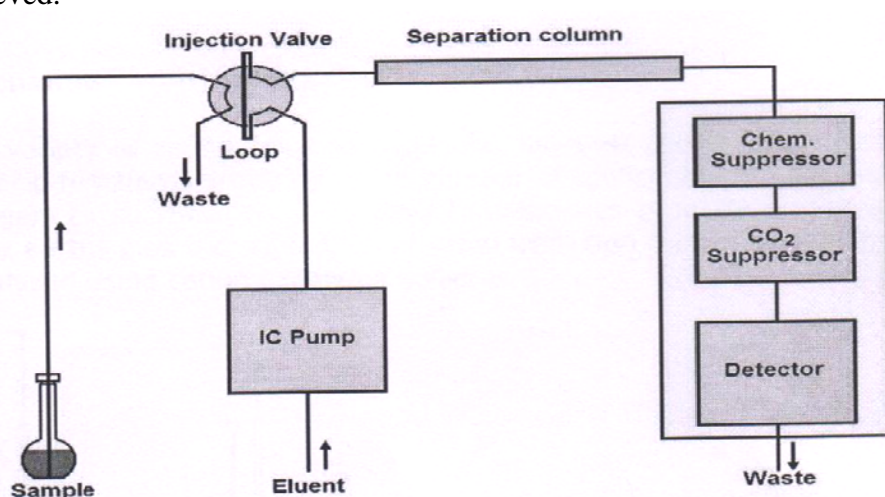
Gradient chemistry can be realized using the MSM-HC (high capacity) and uses the same cation exchanger as the MSM but in a larger form. The MSM-HC can be used in combination with a low pressure or high pressure pump system. Both the MSM and MSM-HC have a compact design.
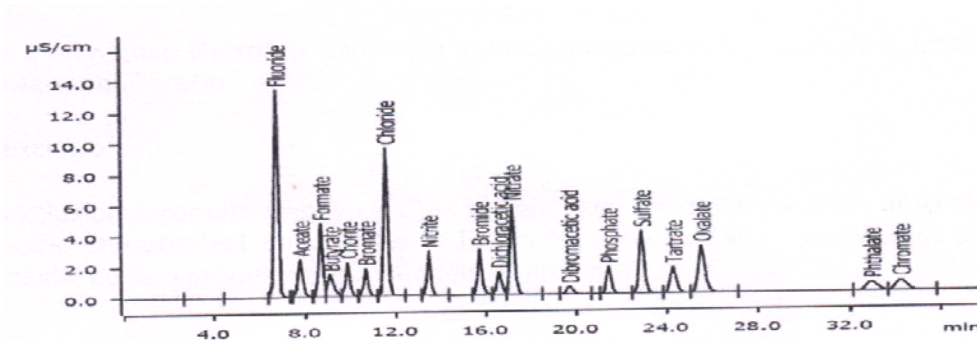
Chromatogram of a Carbonate Gradient Using the MSM-HC

2.3   Cation Exchange

There is a variety of cation colums available; however the modern ones contain carboxylic acid functional groups. A large number of applications for silica-gel based ion exchangers exist. These columns allow simultaneous separation of alkali metals and alkaline earths plus the separation of some transition metals. Small amines can also be analyzed using cation exchange columns.
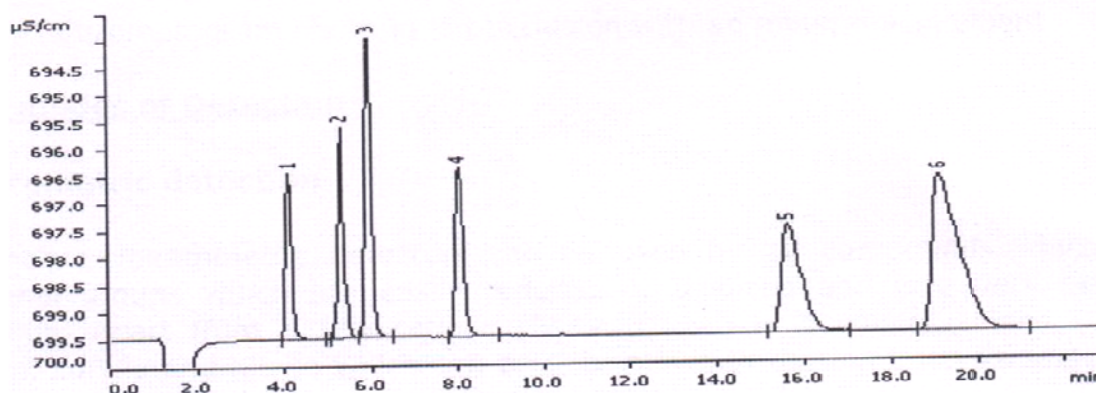

Chromatogram of Group I/II Metals with Nitric Acid/Dipicolinic Acid Eluents

The eluents used for non-suppresed cations exchange are week acids with the complexing agent such as dipicolinic acid, the concentration of which can effect the elution of calcium and heavy metals such as iron, zinc and cobalt. This can be used to great effect to change the selectivity of the separation.

Cations can become less sensitive when suppressed and so are analysed with direct conductivity detection. It makes sense also because cation suppressors can be notoriously unreliable and add to the costs of running the instrument. The stability and efficiency of the Metrohm conductivity detector coupled with a very low pump noise mean that Metrohm can work non-suppressed easily with fast instrument hardware equilibration time.

2.4   Ion exclusion

Ion exclusion chromatography (IEC) is mainly used for the separation of weak acids or bases. The greatest importance of IEC is for the analysis of weak acids such as carboxylic acids, carbohydrates, phenols or amino acids.
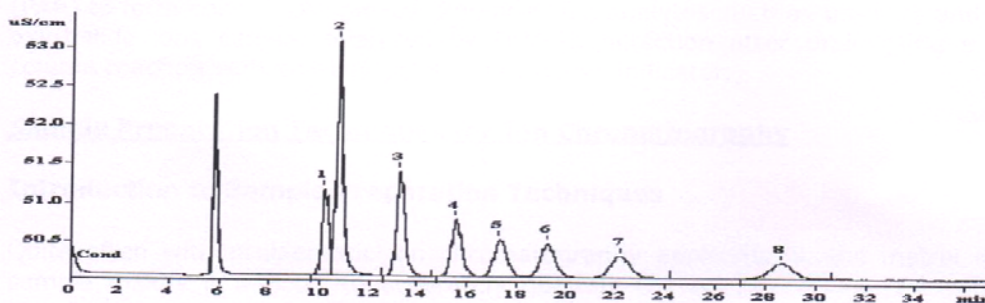

Chromatogram Showing Ion Exclusion with an Mineral Acid Eluent

3.   Other Modes of Detection

3.1   Amperometric Detection

In principle voltammetric detectors can be used for all compounds which have functional groups which are easily reduced or oxidized and is a very sensitive technique. Apart from a few cations (Fe^{3+}, CO^{2+}) it is chiefly anions such as cyanide, sulfide and nitrite which can be determined in the ion analysis sector. The most important applications lie however in the analysis of sugars by anion chromatography and in clinical analysis using a form of amperometric detection know as Pulsed Amperometric Detection (PAD).

3.2   Photometric Detection
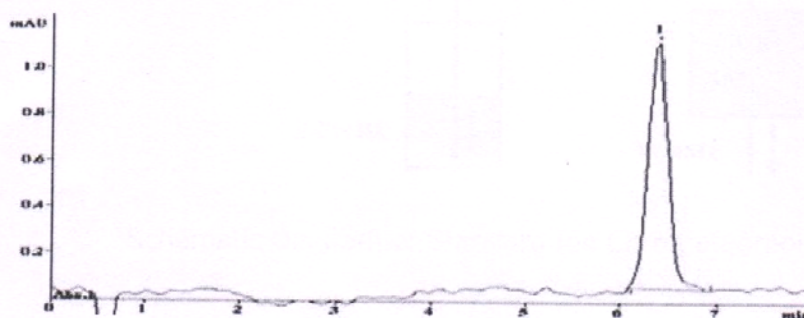
Chromatogram Showing Analysis of 1 PPM Chromate

Because of its extremely wide range of application photometric or UV/VIS detection is the most important detection method used in HPLC, as many organic

molecules contain chromophore groups, or can have one introduced or added, which are able to absorb in the UV or VIS spectrum. In the field of inorganic ion analysis UV/VIS detection plays a smaller role. While of the simple anions only analytes such as nitrate, bromide or iodide absorb, important analytes such as fluoride, sulfate or phosphate can only be measured indirectly. Many cations do not absorb at all, but multivalent and transitional metals in particular can be converted in a post-column derivatization with chelating agents formers such as 4-(2-pyridylazo)-resorcinol (PAR) to form colored complexes. Redox-active analytes such as bromate and other oxy-halide ions can be analyzed by UV/VIS detection after undergoing a post-column reaction with an electrochemically active indicator.

4.   Sample Preparation Techniques for Ion Chromatography

Quite often with problematic ion chromatography applications, the matrix of the sample makes it difficult to accurately quantify the species of interest with the standard ion chromatography set-up and some form of sample preparation then becomes necessary.
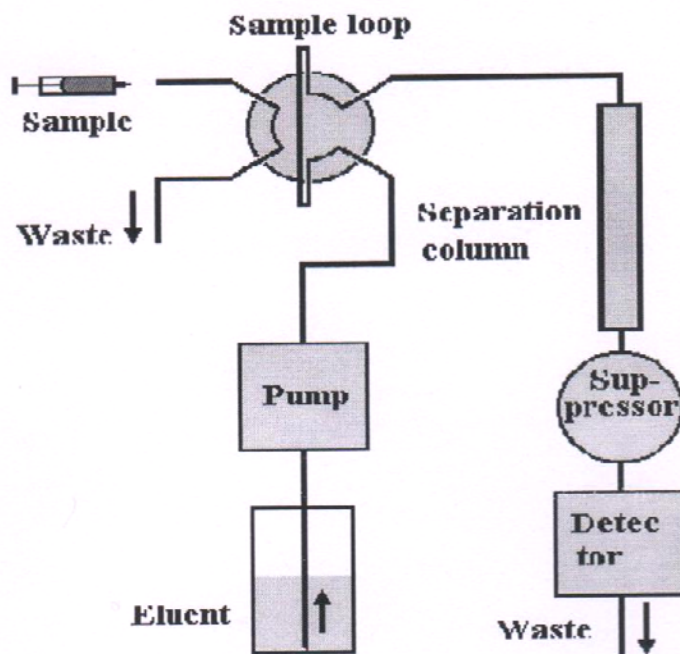
Schematic Diagram of Standard Ion Chromatography Set-up

The sample preparation may be as straightforward as simply diluting the sample with deionised water or can involve injection of the sample through a solid phase extraction cartridge to remove the interference. In the case of more difficult forms of sample matrices it may be necessary to add additional dedicated sample preparation modules to the standard ion chromatography configuration.

4.1   Dilution of the Sample

Dilution of the sample is performed when the concentration of the analytes of interest either exceed the working capacity of the separation column chosen, or there are sample matrix effects that can often be minimized by a dilution usually with water but eluent can also be used.

4.2   Filtration of the Sample

It is recommended to filter all samples prior to injection with 0.45mm filters to ensure that any particulate material from the samples don't make their way onto the injection valve or the analytical column where they can cause blockages and considerably reduce the lifetime of the column(s).

4.3   Solid Phase Extraction Cartridges

Passage of the sample through one or more solid phase extraction cartridges prior to injection will often retain selectively certain species within the homogeneous sample. Quite often the retained species are substances that would interfere with the chromatography had they not been previously removed. There are a number of different cartridges whose suitability depends upon the type of chemistry undertaken.

For anion analysis, the sample can be treated with a cation exchanger in the H+ form that removes divalent cations that can mask any fast eluting anions. This type of exchange cartridge removes carbonate/bicarbonate and is also useful for the removal of cations from samples being determined by ion exclusion chromatography. Another option is the use of a cation exchanger in the Ag+ form for the removal of any halides present in the sample.

Similarly for cation analysis, one can employ an anion exchanger in the OH- form to remove any interfering anions present in the sample. Another common type is the non-polar exchange cartridge (reversed phase) that often utilizes C18 groups to remove organic substances that would otherwise interfere with the chromatography.

4.4   Digestion of the Sample

If digestion techniques are to be employed then analyte content should be changed as little as possible and any organic matter present should ideally be destroyed completely. One can obtain analytical inaccuracies due to an exaggerated blank value as a result of the chemicals used during the digestion. Different types of digestion include wet, microwave and UV, the suitability of each depends on both the sample matrix and the analytes of interest being determined.

4.5   Instrumental Sample Preparation Modules

Often with more complex sample matrices, one has to add additional dedicated sample preparation modules to the standard ion chromatography configuration. There are a number of different instrument options available within the Metrohm range depending on the type of sample treatment required prior to analysis. Metrohm has actually been a pioneer of inline sample preparation modules with the release of the 754 IC Dialysis Unit as long ago as 1997, since then the technology has been optimized and considerably improved so that today the Metrohm IC portfolio contains many different sample preparation instruments to automate and improve analysis times and throughput of difficult sample matrices such as emulsions or diary products.
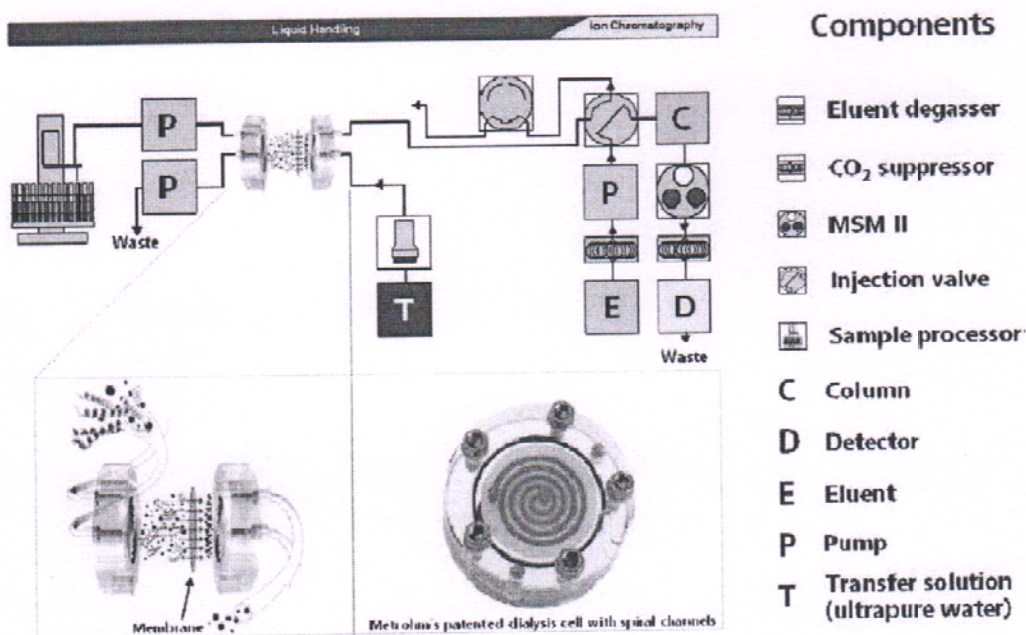

4.6 Dialysis

Dialysis is based on the selective diffusion of molecules or ions from one liquid (donor or sample solution) to another (acceptor solution) via a membrane. The driving force for the transfer is the concentration gradient across the membrane. Contrary to dynamic dialysis, where two solutions continuously pass through the dialysis module, at least one solution is temporarily stopped until the concentration in the acceptor solution is the same as that in the donor solution. This patented stopped-flow procedure takes between 10 and 14 minutes and can be directly coupled to an IC setup.

As the dialysis is performed during the recording of the previous samples' chromatogram, the overall analysis time is not prolonged. Whereas in the conventional setup 2 two channel peristaltic pumps transport the sample and the acceptor solution to and from the dialysis cell, in compact dialysis Dosinos (accurate liquid pipette) doses ultra pure water through the acceptor compartment of the cell. The stopped-flow status is achieved by stopping the Dosino and blocking the outlet capillary of the cell by feeding it

through the valve of the sample processor. The reported dialysis recovery rates have been found to be in excess of 98% using the patented stopped flow method.

4.7   Ultra-Filtration

The aim of sample filtration is to protect the separation columns from contamination and blockage from particulates that may be present in the sample. The ultra-filtration kit in combination with the 858 Advanced Sample Processor ensures automatic inline filtration with sample injection. It is eminently suitable for those samples with a light to medium load such as surface waters and digestion solutions.
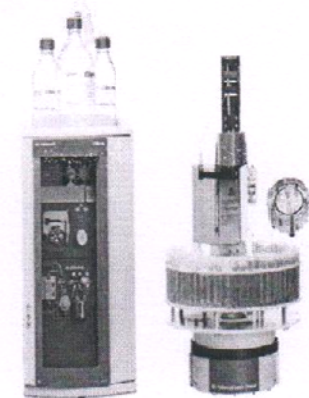

The samples are placed onto the sample carousel before being processed automatically. Sample filtration and introduction to the injection valve is achieved by means of an integrated double channel peristaltic pump meaning that it is possible to aspirate slightly viscous samples.

The sample is conveyed by one channel of the pump through the ultra-filtration cell passing the membrane. At the same time the filtrate is aspirated off from the rear of this membrane and transferred to the sample loop by the second channel of the pump. Only a small fraction of the sample is removed as filtrate so the contaminants remain mainly in the sample stream preventing the regenerated cellulose membrane from becoming blocked too quickly.

## 5.   Metrohm Sample Preparation Module

The 850 Professional IC Anion – MCS – Prep 3 is the professional ion chromatography system for the determination of anions with simultaneous Metrohm inline neutralization or Metrohm inline cation removal for analysis of for example alkaline solutions. In the bottom segment of the 850 Professional IC system there is an additional anion sample preparation module «MSM XL» and a bidirectional dual channel peristaltic pump. Both these components are used for Metrohm Inline Sample Preparation (MISP).

The modules consists of a reactor block that houses the cation exchangers with a control unit that contains a two channel peristaltic pump that conveys the regenerant and rinse solutions.
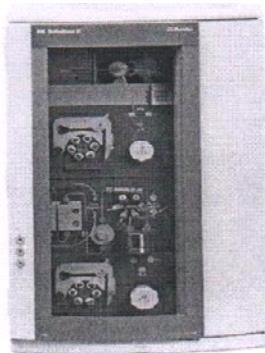
The matrix elimination occurs inline whilst the regeneration and rinsing of the packed bed suppressor inside the <<MSM XL>> occur simultaneously offline. A fresh suppressor channel is used for each new analysis and because the rinse and regeneration occurs after each determination, the capacity is practically unlimited.

The sample solution is transferred to the sample preparation module from an auto sampler via a loop injection and rinsed with deionised water. The sample cations are exchanged against protons (H+). If sodium hydroxide constitutes the sample matrix, water is formed by neutralization. The sample solution then passes onto the pre-concentration column where the trace anions to be determined are retained and then eluted by the eluent flowing in a counter flow direction. The analyte anions are then separated on the analytical column before quantification using chemical suppression with conductivity detection.
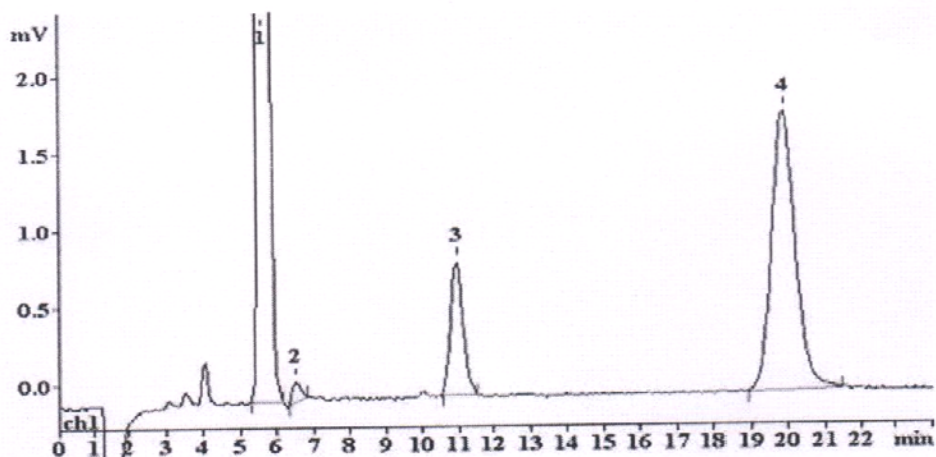
**Chromatogram Showing Matrix Elimination on a
Sample of Caustic Soda**