

Ion Chromatography

Introduction

Ion Chromatography (IC) was introduced in 1975 by Small, Stevens and Baumann as a new analytical method. Within a short period of time, ion chromatography developed from a new detection scheme for a few selected inorganic anions and cations to a versatile analytical technique for ionic species in general. For a sensitive detection of ions via their electrical conductance, the effluent from the separator column was passed through a “suppressor” column. This suppressor column chemically reduces the background conductance of the eluent, while at the same time increasing the electrical conductance of the analyte ions.

In 1979, Fritz et al. described an alternative separation and detection scheme for inorganic anions, in which the separator column is directly coupled to the conductivity cell. As a prerequisite for this chromatographic setup, ion-exchange resins with low capacities have to be employed so that eluents with low ionic strengths can be used. In addition, the eluent ions should exhibit low equivalent conductances, thus enabling sensitive detection of the sample components.

At the end of the 1970s, ion chromatographic techniques were used to analyze organic ions for the first time. The requirement for a quantitative analysis of organic acids brought about an ion chromatographic method based on the ion-exclusion process, which was first described by Wheaton and Bauman in 1953.

In the last twenty years witnessed the development of separator columns with high efficiencies, which resulted in a significant reduction of analysis time. In addition, separation methods based on the ion-pair process were introduced as an alternative to ion-exchange chromatography, since they allow the separation and determination of both anions and cations.

The scope of ion chromatography was considerably enlarged by newly designed electrochemical and spectroscopic detectors. A milestone of this development was the introduction of a pulsed amperometric detector in 1983, allowing a very sensitive detection of carbohydrates.

A growing number of applications using post-column derivatization in combination with photometric detection opened the field of heavy and transition metal analysis for ion chromatography, thus providing a powerful extension to conventional atomic spectroscopy methods.

These developments make ion chromatography an integral part of both modern inorganic and organic analysis.

Figure 1 shows a typical chromatogram, which means the conductivity as a function of time.

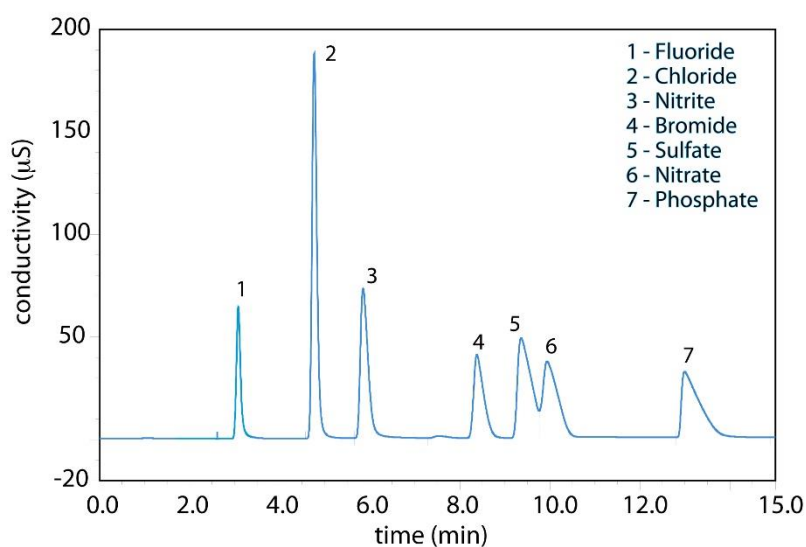


Figure 1. A typical ion chromatogram

Types of Ion Chromatography

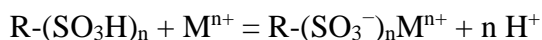
Modern ion chromatography as part of liquid chromatography is based on three different separation mechanisms, which also provide the basis for the nomenclature in use.

1. Ion-Exchange Chromatography (High Performance Ion Chromatography, HPIC)

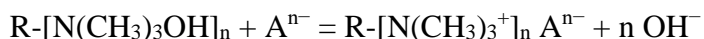
This separation method is based on an ion-exchange process occurring between the mobile phase and ion-exchange groups bonded to the support material. In ions with high polarizability, additional non-ionic adsorption processes contribute to the separation mechanism. The stationary phase consists of a polystyrene resin copolymerized with divinylbenzene and modified with ion-exchange groups. Ion-exchange chromatography is used for the separation of both organic and inorganic anions and cations, respectively. Separation of anions is

accomplished with quaternary ammonium groups attached to the polymer, whereas sulfonate groups are used as ion-exchange sites for the separation of cations.

Ion change during cation exchanging:



Ion change during anion exchanging:



2. Ion-Exclusion Chromatography (high Performance Ion Chromatography Exclusion, HPICE)

The separation mechanism in ion-exclusion chromatography is governed by Donnan exclusion, steric exclusion and sorption processes. A totally sulfonated polystyrene/divinylbenzene-based cation exchange material with high capacity is employed as the stationary phase. Ion-exclusion chromatography is particularly useful for the separation of weak inorganic and organic acids from those acids which are completely dissociated at the eluent pH. All acids with high acid strengths are not retained and elute unresolved within the void volume. In combination with suitable detection systems, this separation method is also useful for determining amino acids, aldehydes and alcohols.

3. Ion-Pair Chromatography (Mobile Phase Ion Chromatography, MPIC)

The dominating separation mechanism in ion-pair chromatography is adsorption. The stationary phase consists of a neutral porous divinylbenzene resin of low polarity and high specific surface area. Alternatively, chemically bonded silica phases of the octyl or octadecyl type with an even lower polarity can be used. The selectivity of the separator column is determined solely by the mobile phase. Besides an organic modifier, an ion-pair reagent is added to the eluent (water, aqueous buffer solution, etc.) depending on the chemical nature of the analytes. Ion-pair chromatography is particularly suited for the separation of surface-active anions and cations as well as transition metal complexes.

The Ion Chromatographic System

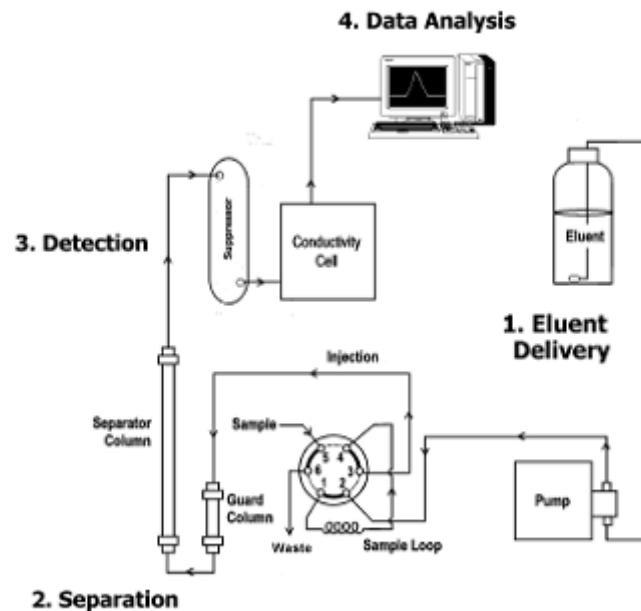


Figure 2: The general structure of the ion chromatograph

The basic components of an ion chromatograph are shown schematically in Figure 2. It resembles the setup of conventional HPLC systems.

A pump delivers the mobile phase through the chromatographic system. In general, either single-piston or dual-piston pumps are employed. A pulse-free flow of the eluent is necessary for the sensitive UV-Vis and amperometric detectors. Therefore, pulse dampeners are used with single-piston pumps and electronic circuitry with dual-piston pumps.

The sample is injected into the system via a valve injector. A three-way valve is required, with two ports being connected to the sample loop. The sample loading is carried out at atmospheric pressure. After switching the injection valve, the sample is transported to the separator by the mobile phase. Typical injection volumes are between 10 μL and 100 μL .

The most important part of all chromatographic systems is the separator column. The choice of a suitable stationary phase and chromatographic conditions determine the quality of the analysis. The column tubes are manufactured from inert material such as epoxy resins. In general, separation is achieved at room temperature. Elevated temperatures are required only in very few cases such as the analysis of carbohydrates or long-chain fatty acids.

The analytes are detected and quantified by a detection system. The performance of any detector is examined according to the following criteria:

- sensitivity
- linearity
- resolution
- noise (detection limit)

The most commonly employed detector is ion chromatography is the conductivity detector, which is used with or without suppressor system. The main function of the suppressor system as part of the detection unit is to chemically reduce the high background conductivity of the electrolytes in the eluent and to convert the sample ions into a more conductive form. In addition to conductivity detectors, UV-Vis, amperometric and fluorescence detectors are used.

Stationary Phases

In contrast to the silica-based column packings used in classic HPLC, organic polymers are predominantly employed as support material in ion chromatography. These materials show a much higher stability toward extreme pH conditions. While silica-based HPLC columns can only be used within a pH range between 2 and 8, ion exchangers based on organic polymers are also stable in the alkaline region. Nonetheless, a couple of silica-based ion exchangers have recently been developed which exhibit a much higher chromatographic efficiency in comparison to organic polymers. However, the stationary phases used in anion exchange chromatography not only differ in the type of support material, they can also be classified according to their different pore sizes and ion-exchange capacities.

1. Polymer-based Anion Exchangers

Styrene/divinylbenzene copolymers, polymethacrylate and polyvinyl resins are the most important organic compounds that were tested for their suitability as substrate materials in the manufacturing process for polymer-based anion exchangers. Styrene/divinylbenzene copolymers are the most widely used substrate materials. Since they are stable in the pH range between 0 to 14, eluents with extreme pH values may be used. The copolymerization of styrene with divinylbenzene is necessary in order to obtain the required stability of the resin. Upon

adding divinylbenzene to styrene, the two functional groups of divinylbenzene crosslink two polystyrene chains with each other (see *Figure 3*.)

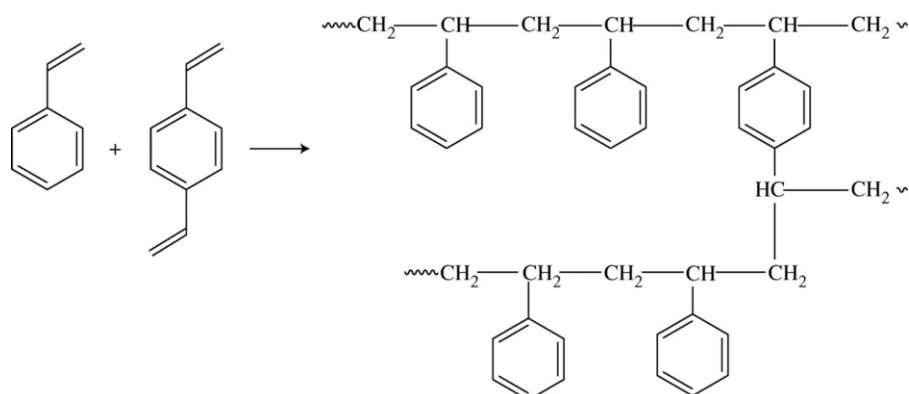


Figure 3. The copolymerization of styrene and divinylbenzene

2. Latex-Agglomerated Anion Exchangers

A special type of pellicular anion exchangers was first introduced in 1975 by Small et al. in their introductory paper on ion chromatography. These stationary phases, which are called latex-based anion exchangers, have been further developed by Dionex.

Latex-based anion exchangers are comprised of a surface-sulfonated polystyrene/divinylbenzene substrate with particle diameters between 5 μm and 25 μm and fully aminated porous polymer beads of high capacity, which are called latex particles. The latter have a much smaller diameter (about 0.1 μm) and are agglomerated to the surface by both electrostatic and van-der-Waals interactions. The stationary phase features three chemically distinct regions:

- an inert and mechanically stable substrate
- a thin coating of sulfonic acid groups which covers the substrate
- an outer layer of latex beads, which carry the actual anion exchange groups

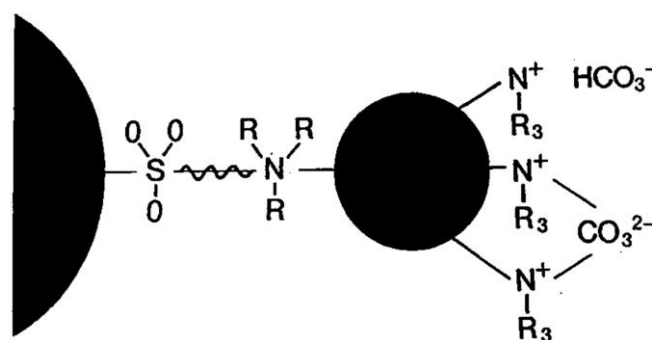


Figure 4. Structure of a latex-based anion exchange resin

Although the latex polymer exhibits a very high exchange capacity due to its complete amination, the small size of the beads finally results in a low anion exchange capacity. The surface sulfonation of the substrate prevents the diffusion of inorganic species into the inner part of the stationary phase. This complex system offers several advantages compared with other column packings such as silica-based anion exchangers and directly aminated resins:

- the inner substrate provides mechanical stability and a moderate backpressure
- fast exchange processes and thus a high chromatographic efficiency of the separator column are ensured by the small size of the latex beads
- swelling and shrinkage are considerably reduced due to the surface functionalization

Latex-agglomerated anion exchangers are chemically very stable. Even sodium hydroxide at concentrations of 4 mol/L is unable to cleave the ionic bond between the substrate particle and the latex bead.

3. Silica-based Anion Exchangers

Parallel to the development of organic polymers as anion exchange substrates, a number of silica-based anion exchangers were introduced. The development of low capacity exchange materials was in the foreground in order to be able to dispense the suppressor system using eluents with low background conductances.

In contrast to organic polymers, silica-based substrates have the advantages of higher chromatographic efficiency and greater mechanical stability. In general, no swelling and shrinkage problems are encountered, even if the ionic form of the ion exchanger is changed or an organic solvent is added to the eluent. Column temperatures up to 80°C also have no adverse effect on the stationary phase. Although the chromatographic efficiency of these stationary phases is very high, they may only be used within a narrow pH range (pH = 2 – 7).

4. Macrocyclic Stationary Phases

Non-charged macrocyclic compounds are also suitable for separating anions. The characteristic feature of macrocyclic compounds such as crown ethers, cryptans, and calixarenes (see Figure 5) is that they can selectively bind metal ions. They can be used for separation of cations for ligand shrinkage, where metals with different diameters suffer differently on the column. Using alkali hydroxide (LiOH, NaOH, KOH) anion as eluent, can also be separated from one macrocyclic stationary phase by an ion exchange mechanism since while the metal ion of the mobile phase forms a complex with the macrocyclic compound, positive-charged anion exchange functional groups are formed on the column which separates the sample anions.

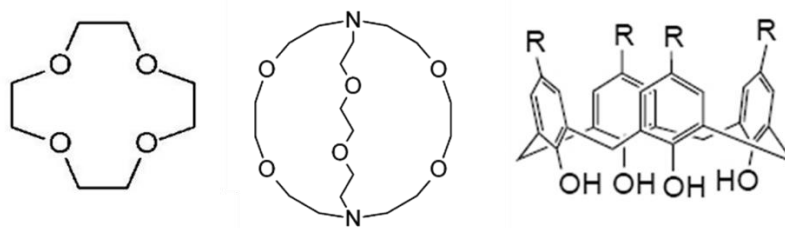


Figure 5. Crown ether, cryptan and calixarene.

5. Alumina Phases

In addition to silica (SiO_2)_x, alumina (Al_2O_3)_x is amongst the most common adsorbents in liquid solid chromatography. Although highly efficient separator columns were developed in the past with spherical beads of small diameter, this separation material is only of minor importance since the introduction of chemically bonded reversed phases on the basis of silica in HPLC.

Like many other metal oxides, alumina exhibits typical ion-exchange properties. It is also mechanically and thermally stable, and exhibits only slight swelling and shrinkage phenomena in aqueous media. Nonetheless, alumina was seldom used in the past as substrate for ion exchangers due to the low ion-exchange capacity and the inadequate stability against strong acids and bases.

Mobile phases

Mobile phases used in ion chromatography are generally organic solvent buffers. There are several aspects to be taken into consideration when selecting the appropriate eluent such as the pH of the mobile phase, buffer capacity, elution strength, affinity for complexation, counterion quality and concentration, and it is important that the mobile phase is compatible with the detection method.

For the determination of anions multiprotic weak acids are added to the mobile phase, when cation is separated, multiprotic weak bases are added to the mobile phase. Increasing the pH of the moving phase increases the number of negative charges due to deprotonation. This also means the increasing interaction of buffer anion in the moving phase on the surface of the stationary phase, so that the ions to be separated are displaced from the ion exchange site.

During determining cations, we add multiprotic weak bases into the mobile phase. The buffer capacity of the mobile phase is the highest around the pK_a of the weak acid of the buffer. Working at different pH, the buffer capacity of the mobile phase is small. The pH of the samples are generally not the same as the mobile phase. Thus we need to choose the buffer components so that the maximum buffer capacity is within the measurement conditions. If the concentration of buffer is increased, this reduces the sample binding potential. In addition, the complexing characteristic of the mobile phase is particularly important if we want to solve the separation of metal ions. To control retention and selectivity, methanol, ethanol, butanol, glycerol and acetonitrile are added to the buffer. These organic solvents are adsorbed on the surface of the stationary phase.

The type of eluent used in anion chromatography is mainly determined by the detection mode. The most commonly used detection mode is conductivity detection in the determination of organic and inorganic ions.

Selection of the appropriate eluent in spectrophotometric or amperometric detection is considerably simpler. In the former case, mainly alkali salts of phosphoric acid, sulfuric acid and perchloric acid can be successfully used, due to their high light transmittance in the UV range. In the case of amperometric detection, chlorine, chlorate and perchlorate salts of alkali metals, as well as alkali hydroxides and carbonates are used as mobile phase.

Chemical Suppression

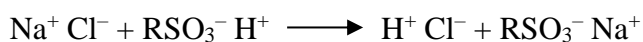
Suppression plays a key role in the analysis of anions and organic acids using ion-exchange chromatography and conductivity detection. Suppressor is a device placed between the column and the detector, and acts to reduce the background conductivity of the eluent and enhance the conductivity of the analytes. For anion analysis, the suppressor is a high capacity cation exchange membrane or resin in the acid form. It removes cations from the eluent and replaces them with H⁺.

Suppression

- decreases the background conductivity of the eluent
- minimizes baseline noise
- transforms analytes in free anions with protons as counterions (which involves a remarkable increase in the conductivity signal)
- optimizes the signal-to-noise ratio
- increases the detection sensitivity of the measurement system

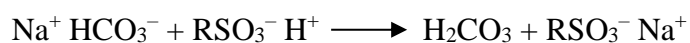
The suppressor module consists of three cartridges filled with cation exchanger material. The first cartridge is used for suppression. Simultaneously, the second cartridge is regenerated with diluted acid (e.g., sulfuric acid) and the third cartridge is rinsed with the eluate or water. Before each analysis, the suppressor is rotated 120° so that a freshly regenerated and rinsed cartridge is always available for suppression.

Analyte:



This example of an anion analysis includes a sodium analyte counterion. This ion is replaced with a proton with an equivalent conductivity that is five times higher. This significantly increases the conductivity of the sample solution and therefore also the signal strength. Salts from weakly dissociated acids (e.g., sodium carbonate/sodium hydrogen carbonate) are used as eluent.

Eluent:



The eluent counterions are also replaced with protons. The carbonic acid that is produced in this way is unstable and only weakly dissociated, meaning that lower background conductivity is measured. Depending on the eluent composition, background conductivity values of 10 to 20 $\mu\text{S}/\text{cm}$ are typical for chemical suppression.

Table 1.: Eluents commonly used for conductivity detection with chemical suppression of the background conductivity

Eluent	Eluent ion	Suppressor product	Elution strength
Na_2BO_4	BO_4^{2-}	H_3BO_3	very weak
NaOH	OH^-	H_2O	weak
NaHCO_3	HCO_3^-	$\text{CO}_2 + \text{H}_2\text{O}$	weak
$\text{NaHCO}_3/\text{Na}_2\text{CO}_3$	$\text{HCO}_3^-/\text{CO}_3^{2-}$	$\text{CO}_2 + \text{H}_2\text{O}$	medium strong
$\text{H}_2\text{NCH(R)COOH}/\text{NaOH}$	$\text{H}_2\text{NCH(R)COO}^-$	$\text{H}_3\text{N}^+\text{CH(R)COO}^-$	medium strong
$\text{RNHCH(R')SO}_3\text{H}/\text{NaOH}$	RNHCH(R')SO_3^-	$\text{RN}^+\text{H}_2\text{CH(R')SO}_3^-$	medium strong
Na_2CO_3	CO_3^{2-}	$\text{CO}_2 + \text{H}_2\text{O}$	strong

Detection

In ionchromatographic practice, conductivity detectors are most commonly used. The conductivity of the solutions is an additive property, depending on the quality of the ions (mobility) and the number of ions (concentration). In principle, the conductivity detector can be used for some non-aqueous eluents. The sensitivity of these detectors depends on the temperature; during the separation and detection the temperature must be kept strictly constant. For a suppressed system, the noise level must be below 4 nS / cm and the temperature range is generally 25 to 55 °C.

In addition, detectors with UV-Visible spectrophotometry are often used for detection. This is used in cases where the component is absorbed in the UV-Visible range. Examples include iodide, nitrite, nitrate, iodate or chromate ions. The detector is photodiode and the cell is a quartz cuvette. Deuterium and tungsten lamps are used as a source of light. In addition, a

diode array detector can be used, if the purpose is to simultaneously detect light absorption at different wavelengths.

We can detect fluorescent materials with fluorescence-based detectors. The principle of detection is that the components of the sample are excited by a given wavelength light and the components emit light and we can detect this light. For biological samples, this type of detection method is common.

Electrochemical detection may also be required in many cases. There are ions that can be electrochemically oxidized such as arsenide, azide, bromate, bromide, chloride, chlorate, cyanide, iodate, iodide, nitrite, nitrate, sulphide, sulphite, tetrathion, thiocyanate or thiosulfate ions. There are two types of electrochemical detectors: amperometric (measuring current through cell voltage) and voltametric detectors (measuring the current through the cell at intervals with varying voltages). This detection method may be relevant when the sample components can be introduced into an electrode reaction. Regardless of the type used, the three-electrodes (working electrode, auxiliary electrode and reference electrode) are used in all cases.

Other detectors:

- atom absorption (AAS)
- inductively coupled plasma atomic emission spectrometer (ICP)
- mass spectrometry (MS)

Laboratory practice

The first task of the laboratory practice is to perform multipoint calibration. Calibration solutions in the range of 0 to 15 ppm are measured for fluoride, chloride, chlorate, nitrite, nitrate and sulphate ions. Everyone then gets their own unknown sample, which contains one of the listed ions or even more ions. The purpose of this exercise is to qualitatively and quantitatively determine these unknown samples.

The lab note starts with a maximum half-page introduction, which explains what is ion chromatography with our own words, why we use it and what is most important about an ionchromatographic measurement. This must be ready for the beginning of the laboratory practice.

Questions:

1. What is ion chromatography?
2. What are the components of an ionchromatographic system?
3. How the ionchromatographic system works?
4. What kind of separation methods do you know?
5. What is the ion exchange chromatography?
6. What is suppressed ion chromatography? What is the benefit of this method?
7. List what solid phase phases are known? Characterize one with a few sentences!
8. What aspects should be considered regarding the selection of the mobile phase?
9. What kind of detection methods do you know? Describe them with 1-2 sentences