4. Ion chromatography (IC)

4.1. Educational aims and objectives

Principles of ion chromatography method and instrumentation. Methods for ionic compounds analysis including ion chromatography and its subtypes. Retention mechanism in different ion chromatography methods. Stationary phases used in ion chromatography. The role of suppression in ion chromatography. Different detection types in ion chromatography. Defining distinguishing features of IC in comparison to classical HPLC methods with respect to types of columns and eluents, suppressors and detection methods.

4.2. Required knowledge

Questions to prepare
1. What are the main three types of modern ion chromatography and what is the principle of this classification?
2. Describe the separation mechanism in ion-exclusion chromatography.
3. Describe the separation mechanism in ion-pair chromatography.
4. Describe the separation mechanism in ion-exchange chromatography.
5. Define the main parts of the ion chromatography system and describe their functions.
6. Describe two main types of functional groups used in stationary phases in ion-exchange chromatography. Give at least three examples for each of them.
7. Describe two most common materials used as a support for stationary phases in ion-exchange chromatography. Compare them.
8. Describe the most common types of eluents used for the IC separation of anions? For which types of separations they are used and what are the advantages of them?
9. What is the role of suppression in ion chromatography method? Describe its mechanism.
10. Describe the principles of conductivity detection used in IC?

4.3. Theoretical principles

4.3.1. Introduction

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:
- Ion-Exchange Chromatography (known as High-Performance Ion Chromatography, HPIC),
- Ion-Exclusion Chromatography,
- Ion-Pair Chromatography.

\textit{Ion-Exchange Chromatography (IEC)}

Ion-exchange chromatography is these days the most common method within ion chromatography techniques and therefore is sometimes called High Performance Ion Chromatography (HPIC). Separation mechanism is based on an ion-exchange process occurring between the mobile phase and ion-exchange groups bonded to the support material. The stationary phase usually consists of a polymer resin modified with ion-exchange groups which can differ according to the group of analytes (anions or cations). As ion-exchange chromatography is the main object of this exercise, it will be discussed entirely in the further part of this script.

\textit{Ion-Exclusion Chromatography (IEC)}

The separation in ion-exclusion chromatography is determined by Donnan exclusion, steric exclusion, and sorption processes. A totally sulfonated polymer-based cation-exchange material with high capacity is employed as stationary phase. Porous ion exchange material works as a semipermeable membrane separating two aqueous phases, namely mobile phase from aqueous stationary phase occluded in pores of polymeric material. One of the most popular application of IEC is separation of weak organic and inorganic acids. In this case, a strong acid eluent facilitates protonation of weak organic acids. In the neutral form, these acids are not subject to Donnan exclusion and penetrate into the pores of negatively charged polymer resin. Separation is accomplished by differences in pKa, size, and hydrophobicity of
the acid anions. The Donnan exclusion mechanism causes stronger acid anions to elute before weaker acid anions according to increasing pKa; for example, acetate (pKa=4.56) elutes before propionate (pKa=4.67).

Fig. 1. Schematic representation of the ion-exclusion separation mechanism.

Ion-Pair Chromatography (IPC)

The main separation mechanism in Ion-Pair Chromatography is adsorption. The stationary phase consists of neutral porous divinylbenzene resin of low polarity and high specific surface area. Alternatively, the 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 depending on the chemical nature of the analytes. IPC method is particularly suited for the separation of surface-active anions and cations as well as transition metal complexes.

Ion-pairing chromatography (IPC) can be used for both positively and negatively charged analytes:

- negatively charged reagent can be used to retain positively charged analytes (ionic bases)
  \[ A^- + R^+ \rightleftharpoons A'R^+ \] (A^- - ionized acid; R^+ - ion-pairing reagent)

- positively charged reagent can be used to retain negatively charged analytes (ionic acids)
  \[ BH^+ + R^- \rightleftharpoons B'R^- \] (BH^+ - ionized basis; R^- - ion-pairing reagent)

Typical ion-pairing reagents include:
- alkylsulfonates (R-SO\(_3^-\)),
- tetraalkylammonium salts (R4N\(^+\)),
- strong carboxylic acids (trifluoroacetic acid, TFA; heptfluorobutyric acid, HBA).

Fig. 2. Schematic representation of the ion-pair separation mechanism; (a) bonded phase; (b) stationary phase; (c) ion-pair reagent in mobile phase; (d) ion-pair reagent adsorbed to Stationary phase; (e) analyte ion in mobile phase; (f) analyte retained on column by ion-pair mechanism.
4.3.2. The ion chromatography system
Because the ion chromatography technique is one of the liquid chromatography methods, the instrumentation does not differ significantly from those used in HPLC analysis. Major parts of the instrumentation are: pump, injector (manual injection valve or autoinjector/autosampler), column, suppressor (if suppression ion chromatography is performed) and detector.

Fig. 3. Schematic presentation of ion chromatography system with suppression and conductivity detector.

4.3.3. Ion exchange mechanism, stationary phases and eluents
In modern ion-exchange chromatography (IEC) the sorbents are mostly polymer resins with immobilized functional groups. Two main types of functional groups can be distinguished: anion exchangers and cation exchangers. Separation mechanism 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, also additional non-ionic adsorption processes contribute to the separation mechanism. The stationary phase consists of a resin modified with ion-exchange groups which can differ according to the group of analytes (anions or cations). Separation of anions is usually managed with quaternary ammonium groups attached to the resin, whereas sulfonate groups are used as ion-exchange sites for the separation of cations.

The anion exchange process

\[
\text{Resin-NR}_3\text{OH} + \text{Cl}^- \quad \xrightarrow{K} \quad \text{Resin-NR}_3\text{Cl} + \text{OH}^- \quad (K \text{ – the equilibrium constant})
\]

The cation exchange process

\[
\text{Resin-SO}_3\text{H} + \text{Na}^+ \quad \xrightarrow{K} \quad \text{Resin-SO}_3\text{Na} + \text{H}^+ \quad (K \text{ – the equilibrium constant})
\]
The separation of anions is determined by their different affinities to the stationary phase. Quantitative expression of this equilibrium process is the selectivity coefficient, \( k_{X/OH^-} \)

\[
k_{X/OH^-} = \frac{[X^-]_S \cdot [OH^-]_M}{[OH^-]_S \cdot [X^-]_M}
\]

\([X]_{M,S}\) – concentration of the analyte ions in mobile (M) and stationary phase (S), respectively
\([OH^-]_{M,S}\) – concentration of the hydroxide ions ion in mobile (M) and stationary phase (S), respectively

The selectivity coefficients (and retention times) for anions separated on the most popular, strong anion exchanger can be listed in the ascending following order: OH< F< ClO< BrO< HCOO< IO< CH₃COO< H₂PO< HCO< Cl< CN< NO₂< Br< NO₃< HPO₄²< SO₄²< SO₂³< C₂O₄²< S₂O₄²< SCN< ClO₄⁻

The coefficients for cations separated on the strong cation exchanger can be ordered as follows:

Li⁺< Na⁺< NH₄⁺< K⁺< Rb⁺< Cs⁺< Ag⁺< Mg²⁺< Zn²⁺< Co²⁺< Cu²⁺< Cd²⁺< Ni²⁺< Ca²⁺< Sr²⁺< Pb²⁺< Ba²⁺< Al³⁺

Stationary phases in ion-exchange chromatography

Ion-exchangers are characterized both by the nature of the matrix used as a support and the nature of the ionic functional groups on the surface. The main types of functional groups used in ion-exchange chromatography are listed below in the Table 1.

<table>
<thead>
<tr>
<th>Anion exchangers</th>
<th>Cation exchangers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quaternary amine</td>
<td>Sulfonic acid</td>
</tr>
<tr>
<td>-N(CH₃)₂⁺·OH</td>
<td>-SO₃²⁻ H⁺</td>
</tr>
<tr>
<td>Quaternary amine</td>
<td>Carboxylic acid</td>
</tr>
<tr>
<td>-N(CH₃)₂(EtOH)⁺·OH</td>
<td>-COO⁻ H⁺</td>
</tr>
<tr>
<td>Tertiary amine</td>
<td>Phosphonic acid</td>
</tr>
<tr>
<td>-NH(CH₃)₂⁺·OH</td>
<td>-PO₃²⁻ H⁺</td>
</tr>
<tr>
<td>Secondary amine</td>
<td>Phosphonic acid</td>
</tr>
<tr>
<td>-NH₂(CH₃)₂⁺·OH</td>
<td>-HPO₄²⁻ H⁺</td>
</tr>
<tr>
<td>Primary amine</td>
<td>Phenolic</td>
</tr>
<tr>
<td>-NH₃⁺·OH</td>
<td>-O⁻ H⁺</td>
</tr>
</tbody>
</table>

Cation-exchangers functional groups can function as such only when they are ionized, therefore they are classified into strong acid and weak acid types accordingly. The strong acidic functional groups are ionized over a wide pH range, in contrast to the weak acidic functional groups, which are ionized over a limited pH range. Sulfonic acid exchangers are strong acid types, whilst the remaining cation exchangers’ functional groups in Table 1 are weak. The weak acid functional group requires the use of pH higher than its pKa. For example, a carboxylic functional group such as Resin-COOH will be able to retain cation only in its Resin-COO⁻ form, which exists mainly at pH’s above its pKa.

Anion-exchangers are classified as strong base and weak base exchangers. Quaternary amine functional groups form strong anion-exchangers, whilst less substituted amines form weak base exchangers. The strong base will be positively charged over a wide pH range, therefore will be able to function as an anion-exchanger, in contrast to the weak anion-exchangers. A weak anion-exchangers such as Resin-NH₃⁺ for example, requires pH sufficiently low enough to protonate the amine group into Resin-NH₃⁺. Most of the ion-chromatography separations, using silica or polymeric ion exchangers perform on strong anion-exchanger (SAX) or strong cation exchangers (SCX).

The types of matrices used as support for stationary phases in ion chromatography can be divided to two main groups: silica-based and synthetic organic polymers (mostly styrene and divinylbenzene, PS-DVB). The most important advantage of silica-based stationary phases is the good chromatographic efficiency, stability and durability in high pressures. A serious drawback of the silica-based stationary phases is the limited pH range over which the columns can be operated, usually 2 < pH < 7. The prime advantage of polymer-based ion-exchangers is their tolerance towards eluents and samples with extreme pH values, between 0-14. However, the polymeric resins are subject to pressure limitations, because they are relatively soft materials, as a result, the column lengths and flow rates are limited.


**Eluents in ion-exchange chromatography**

In modern ion chromatography, as in other liquid chromatography techniques, 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, that also act as a buffer, providing stable pH. The most common type of elution in IC is an isocratic elution, where the eluent has a constant concentration and composition during the entire run. Another possibility is a gradient elution, where the eluent concentration is changed during the separation.

The two most common eluents for the separation of anions are based on carbonate or hydroxide as eluting anion. The carbonate 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 divalent (CO$_3^{2-}$) ions can be varied to optimize the retention time and the selectivity between monovalent and multivalent sample ions. However, the main drawback of carbonate eluent is fact that it still gives some background conductivity after the suppressor reaction, where carbonic acid (H$_2$CO$_3$) is formed. The carbonate eluent is usually used to perform isocratic separations. Hydroxide eluents are commonly used for gradient elution. 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.

**4.3.4. Supression in ion chromatography**

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. Detection in IC is usually carried out by electrolytic conductivity, since this property is shared by all ions. The eluent contains a relatively high amount of salts and by that it also has a very high conductivity, which needs to be suppressed. A device, called the suppressor, is inserted between the ion-exchange separator column and the detector. The suppressor modifies in fact both the mobile phase and the separated analytes coming out of the separator column, so that the mobile phase's conductance is reduced and that of the analytes is enhanced, hence detectability of the analytes is improved.

The most simple means to accomplish suppression of a carbonate eluent in anion-exchange chromatography is to pass it through a cation-exchange column in the hydrogen form. The most simple example for the function of a suppressor is the case of Cl$^-$ ion as a solute eluted by an eluent that composes of NaHCO$_3$.

The eluent reaction in the suppressor is:

\[
\text{Resin-H}^+ + \text{Na}^+ + \text{HCO}_3^- \rightleftharpoons \text{Resin-Na}^+ + \text{H}_2\text{CO}_3
\]

and the reaction of the analyte in the suppressor is:

\[
\text{Resin-H}^+ + \text{Na}^+ + \text{Cl}^- \rightleftharpoons \text{Resin-Na}^+ + \text{HCl}^-
\]

The combined result of these two processes is that the mobile phase's conductance is reduced greatly (H$_2$CO$_3$) whilst the conductance of the sample ions is enhanced by the replacement of sodium ions (50 S cm\(^2\)/equiv.) with hydronium ions (350 S cm\(^2\)/eq). The detectability of the analyte is therefore enhanced.

A similar procedure can be applied to cation-exchange chromatography, when the suppressor is an anion-exchange column in the OH$^-$ form, which provides hydroxyl ions to the stream. A simple example would be the separation of Na$^+$ ions using HCl in the mobile phase. The processes of suppression are:

\[
\text{Resin-OH}^- + \text{H}^+ + \text{Cl}^- \rightleftharpoons \text{Resin-Cl}^- + \text{H}_2\text{O}
\]
\[
\text{Resin-OH}^- + \text{Na}^+ + \text{Cl}^- \rightleftharpoons \text{Resin-Cl}^- + \text{Na}^+ + \text{OH}^-
\]

The eluent is converted into water whilst the conductance of the sample band is increased due to replacement of the Cl$^-$ ions (76 S cm\(^2\)/eq) by OH$^-$ ions (198 S cm\(^2\)/eq).
4.3.5. Conductivity detection in IC

In ion chromatography method, as in any chromatographic technique, the detector measures some physico-chemical property of the mobile phase/analyte as it elutes from the column. The response of the detector will change due to changes in the column’s effluent. The most common detection type currently used for IC separations is conductometry.

In the conductivity detector the eluate from the suppressor passes through a flow cell with two electrodes, between which an AC potential is applied. Sample ions entering the cell increases the capability of the solution to conduct electrons. Consequently, the increase in current is proportional to the increase in conductivity, which is in turn a linear function of the ion concentration.

The distance between the electrodes is usually represented by d and the electrode area by A. The ratio is then termed the cell constant K. The conductance G between the electrodes is continuously measured and is dependent on the ion charge magnitude Iz, the ion concentration ci, and the electric mobility ui of the ions in the cell.

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 peak is obtained in the chromatogram.
Conductance \([S \text{ or } \Omega^{-1}]\):
\[
G = \frac{1}{R}
\]

Electrolytic conductivity \([S/cm]\):
\[
\kappa = K G = \frac{d G}{A}
\]

Molar conductance \([S\cdot cm^2\cdot mol^{-1}]\):
\[
\Lambda = \frac{\kappa}{c} = \lambda_+ + \lambda_-
\]

where \(\lambda\) is the molar ionic equivalent conductivity of the positive and the negative ions in a salt, respectively, expressed in \(S\cdot cm^2\cdot mol^{-1}\); \(c\) is the concentration in mol/L; and \(R\) is the resistance in \(\Omega\).

<table>
<thead>
<tr>
<th>Anions</th>
<th></th>
<th>Cations</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>OH(^-)</td>
<td>198</td>
<td>H(^+)</td>
<td>350</td>
</tr>
<tr>
<td>F(^-)</td>
<td>54</td>
<td>Li(^+)</td>
<td>39</td>
</tr>
<tr>
<td>Cl(^-)</td>
<td>76</td>
<td>Na(^+)</td>
<td>50</td>
</tr>
<tr>
<td>Br(^-)</td>
<td>78</td>
<td>K(^+)</td>
<td>74</td>
</tr>
<tr>
<td>I(^-)</td>
<td>77</td>
<td>NH(_4^+)</td>
<td>73</td>
</tr>
<tr>
<td>NO(_2^-)</td>
<td>72</td>
<td>½Mg(^{2+})</td>
<td>53</td>
</tr>
<tr>
<td>NO(_3^-)</td>
<td>71</td>
<td>½Ca(^{2+})</td>
<td>60</td>
</tr>
<tr>
<td>HCO(_3^-)</td>
<td>45</td>
<td>Sr(^{2+})</td>
<td>59</td>
</tr>
<tr>
<td>½CO(_3^{2-})</td>
<td>72</td>
<td>½Ba(^{2+})</td>
<td>64</td>
</tr>
<tr>
<td>½SO(_4^{2-})</td>
<td>80</td>
<td>½Zn(^{2+})</td>
<td>53</td>
</tr>
</tbody>
</table>

Table 2. The molar ion conductivities at 25°C.

4.4. Qualitative and quantitative determination of inorganic anions in drinking water

4.4.1. Equipment, reagents and standards

**Equipment**
- Dionex DX-100 ion chromatography system with built-in isocratic pump, conductivity detector and injection valve with sample loop;
- Chromeleon 6.8 chromatography data system;
- Analytical column IonPac AS22 (4x250 mm) and guard column IonPac AG22 (4x50);
- Anion self-regenerating suppressor ASRS 300 (in version dedicated for columns 4 mm).

**Reagents and standards**
- Deionized water (type I grade), resistivity at 25°C: 18,2 MΩ-cm or better;
- IonPac AS22 column eluent concentrate, contains 0,45 mol/L sodium carbonate and 0,14 mol/L sodium bicarbonate (needs to be diluted 100X for the experiment);
- Combined seven anion standard, contains fluoride (20 mg/L); chloride (30 mg/L); nitrite (100 mg/L); bromide (100 mg/L); nitrate (100 mg/L); phosphate (150 mg/L); sulfate (150 mg/L).

4.4.2. Tasks and experimental procedure

**Task 1**: Determination of retention times of the anions
**Task 2**: Identification of anions in unknown sample
**Task 3**: Preparing the calibration curves
**Task 4**: Determination of unknown concentrations in drinking water samples
**Task 5**: Statistical analysis of acquired data