

# *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.

# Introduction

Ion exchange chromatography may be defined as the reversible exchange of ions in the solution with ions electrostatically bound to some sort of insoluble matrix or a stationary phase. •

This technique is extremely useful in the separation of charge compounds like proteins differing by only one charged amino acid. In ion exchange chromatography technique one can choose whether to bind the substance of interest and allow the contamination to pass through the column and vice versa. •

This technique has been developing since 19th century which was firstly used for purifying the drinking water. distinct principle of ion exchange chromatography is chromatography performed in the column •

# Principle

- Ion exchange chromatography relies on the attraction between oppositely charged stationary phase, known as an ion exchanger, and analyte.
- The ion exchanger consists of an inert support medium coupled covalently to positive (anion exchanger) or negative (cation exchanger) functional groups.
- To these covalently bound functional groups the oppositely charged ions are bounded (mobile counter ion), which will be exchanged with like charge ions in the sample having charge magnitude more than the ions bounded to the matrix.
- Thus if anion exchange chromatography is performed, negatively charged sample components will interact more with the stationary phase and will be exchanged for like charged ions already bounded to the matrix

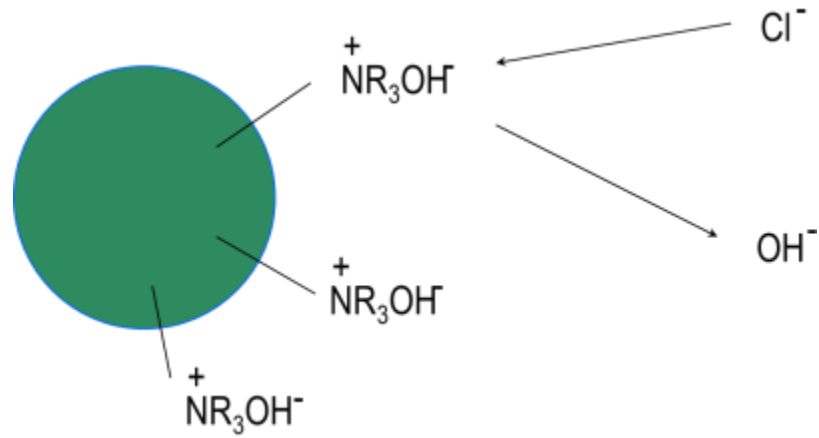
# Principles

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.

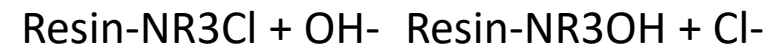
## Ion exchange mechanism, stationary phases and eluents

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.

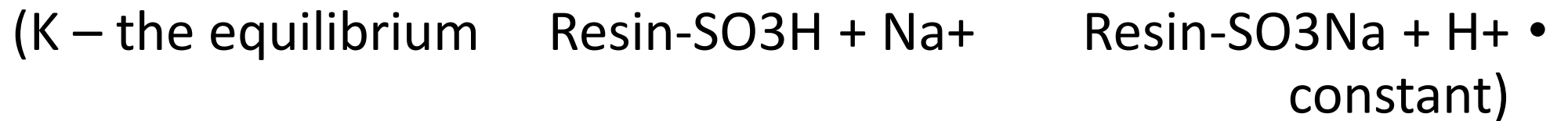
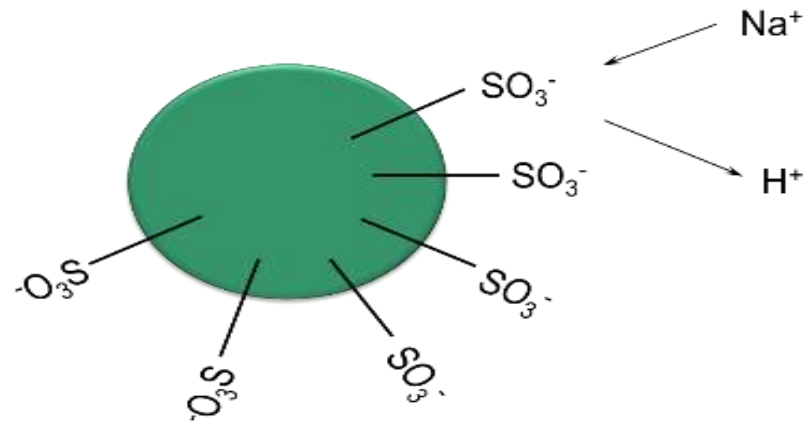
# The anion exchange process



(K – the equilibrium constant)



# The cation exchange process



The separation of anions is determined by their different affinities to the stationary phase. Quantitative expression of this equilibrium process is the selectivity coefficient,  $k_{X/OH}$

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

$[X^-]_{M,S}$  – 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:  $OH^- < F^- < ClO_3^- < BrO_3^- < HCOO^- < IO_3^- < CH_3COO^- < H_2PO_4^- < HCO_3^- < Cl^- < CN^- < NO_2^- < Br^- < NO_3^- < HPO_4^{2-} < SO_3^{2-} < SO_4^{2-} < C_2O_4^{2-} < S_2O_3^{2-} < SCN^- < ClO_4^-$

The coefficients for cations separated on the strong cation exchanger can be are ordered as follows:  $Li^+ < H^+ < Na^+ < NH_4^+ < K^+ < Rb^+ < Cs^+ < Ag^+ < Mg^{2+} < Zn^{2+} < Co^{2+} < Cu^{2+} < Cd^{2+} < Ni^{2+} < Ca^{2+} < Sr^{2+} < Pb^{2+} < Ba^{2+} < Al^{3+}$



# 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 below.

Anion exchangers	Cation exchangers
Quaternary amine $-N(CH_3)_3^+ OH^-$	Sulfonic acid $-SO_3^- H^+$
Quaternary amine $-N(CH_3)_2(EtOH)^+ OH^-$	Carboxylic acid $-COO^- H^+$
Tertiary amine $-NH(CH_3)_2 OH$	Phosphonic acid $PO_3^- H^+$
Secondary amine $-NH_2(CH_3)_2^+ OH^-$	Phosphinic acid $HPO_2^- H^+$
Primary amine $-NH_3^+ OH^-$	Phenolic $-O^- H^+$

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 acidic 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<sup>-</sup> 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-NH<sub>2</sub> for example, requires pH sufficiently low enough to protonate the amine group into Resin-NH<sub>3+</sub>. 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 divinyl benzene, 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  $2 < \text{pH} < 7$ . 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 ( $\text{HCO}_3^-$ ) and divalent ( $\text{CO}_3^{2-}$ ) 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 ( $\text{H}_2\text{CO}_3$ ) 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.

# Supression in ion chromatography

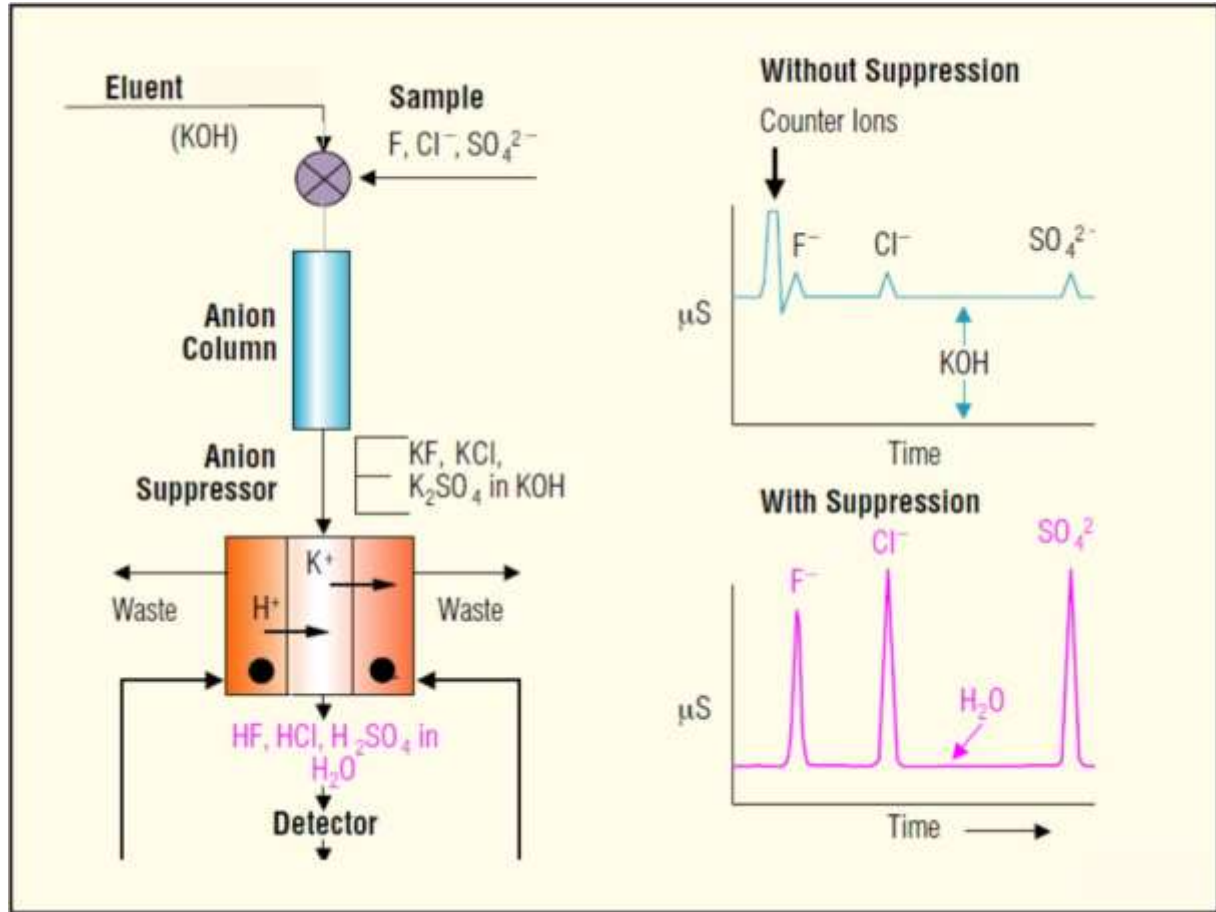
The suppressor has a central role in the IC system, where it performs the double functions of lowering the background and increasing the useful signal. Detection in IC is usually carried out by electrolytic conductivity, since this property is shared by all ions. The eluent contains a relatively high 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  $\text{Cl}^-$  ion as a solute eluted by an eluent that composes of  $\text{NaHCO}_3$ .

The eluent reaction in the suppressor is:

$\text{Resin-Na}^+ + \text{H}_2\text{CO}_3$  and the reaction of the analyte in the suppressor is:  $\text{Resin-H}^+ + \text{Na}^+ \text{HCO}_3^-$

$\text{Resin-Na}^+ + \text{HCl} \quad \text{Resin-H}^+ + \text{Na}^+ \text{Cl}^-$



The combined result of these two processes is that the mobile phase's •  
conductance is reduced greatly ( $\text{H}_2\text{CO}_3$ ) whilst the conductance of the sample  
ions is enhanced by the replacement of sodium ions ( $50 \text{ S}\cdot\text{cm}^2/\text{equiv.}$ ) with  
hydronium ions ( $350 \text{ S}\cdot\text{cm}^2/\text{eq}$ ). 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  $\text{OH}^-$  form, which provides  
hydroxyl ions to the stream. A simple example would be the separation of  $\text{Na}^+$   
ions using  $\text{HCl}$  in the mobile phase. The processes of suppression are:



The eluent is converted into water whilst the conductance of the sample band is •  
increased due to replacement of the  $\text{Cl}^-$  ions ( $76 \text{ S}\cdot\text{cm}^2/\text{eq}$ ) by  $\text{OH}^-$  ions ( $198 \text{ S}\cdot\text{cm}^2/\text{eq}$ ).

# Advantages

Cost effective •

Re-usable •

Easily collectable • •

Low maintenance cost •

Efficient technique •

Quick separation [fppt.com](http://fppt.com) •



# Applications

Softening of hard water •

Demineralization of water •

To analyze base composition of nucleic acid • •

To concentrate the metal ions in the sample •

To measure the additives in food and drug sample •

To separate protein mixtures •