"Capacity Building and Strengthening Institutional Arrangement"

Workshop: Analysis and sampling of water

GENERAL PRINCIPLES ON EQUIPMENT USED FOR ENVIRONMENTAL ANALYSIS (HPLC, IC, GC-MS, ICP-MS)

Ms. Elisa Calabretta

APAT

Agency for Environmental Protection and Technical Services

Summary

- Principles of chromatography
  - HPLC
  - IC
  - GC
- ICP-MS

WHAT IS CHROMATOGRAPHY?

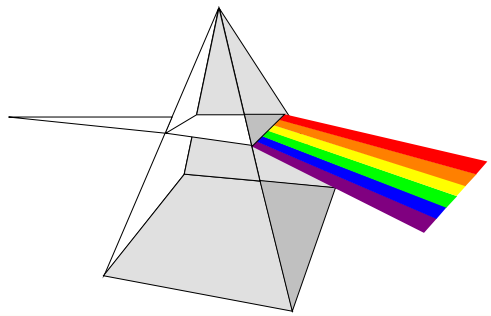
The chromatography is a separative technique. It is based on the different attitude of every molecule or ion to distribute into two different phases: the stationary phase and the mobile phase.

The stationary phase is “immobilized” while the mobile phase flows along the first one (a continuous extraction).

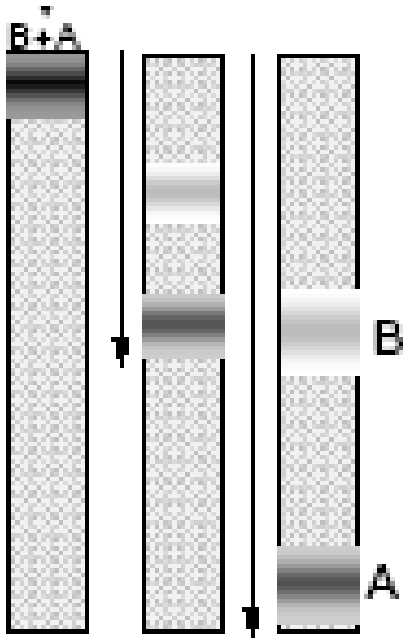
The word “chromatography” comes from the Greek (separation of the color).

***chroma* = color**

***graphein* = to write**



Chromatographic process



A and B at the top of the column

Adding the mobile phase to the column, A and B will “run” in a different way through it; so they will be separated and will come out from the column at different time called “retention time”

The law at the base of this process is:

K= Cm/Cs K, Distribution Coefficient

Cm is the concentration of A or B in the mobile phase

Cs is the concentration of A or B in the stationary

Classification of Chromatographic Techniques

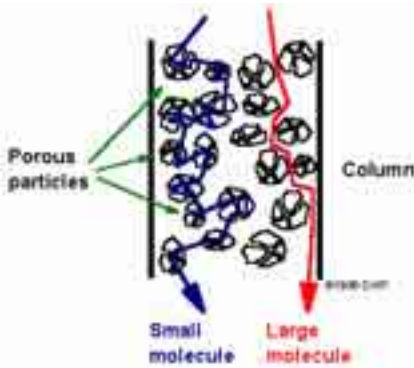
is based on the type of the stationary phase:

- *ADSORPTION CHROMATOGRAPHY:* when the stationary phase is solid with active sites capable to stabilize several secondary bonds with the molecules (dipole-dipole, hydrogen- bond).

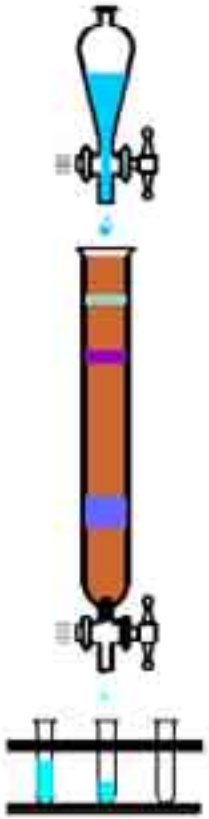
- *PARTITIONING CHROMATOGRAPHY:* when the stationary phase is liquid and immiscible with mobile phase; the substances partition between them.

- *ION CHROMATOGRAPHY:* when stationary phase is made up of macromolecules which have active charged groups; these groups are able to substitute their counter-ions with the molecule present in the mobile phase if charged themselves.

- *EXCLUSION CHROMATOGRAPHY:* when the stationary phase is a porous solid, characterized by pores of different size; the solute molecule are differently retained according to their size



LIQUID GRAVITATIONAL CHROMATOGRAPHY



- ✓ The sample is deposited at the top of the column
- ✓The separation of substances in the mix is performed via elution with suitable solvent or mixture of solvents
- ✓The flow is a gravity flow
- ✓Several fractions are collected and the analysis is performed with analytical techniques off-line

GRAVITATIONAL CHROMATOGRAPHY

Advantages

- Low cost
- Simple technique
- Suitable for preparative aims

Disadvantages

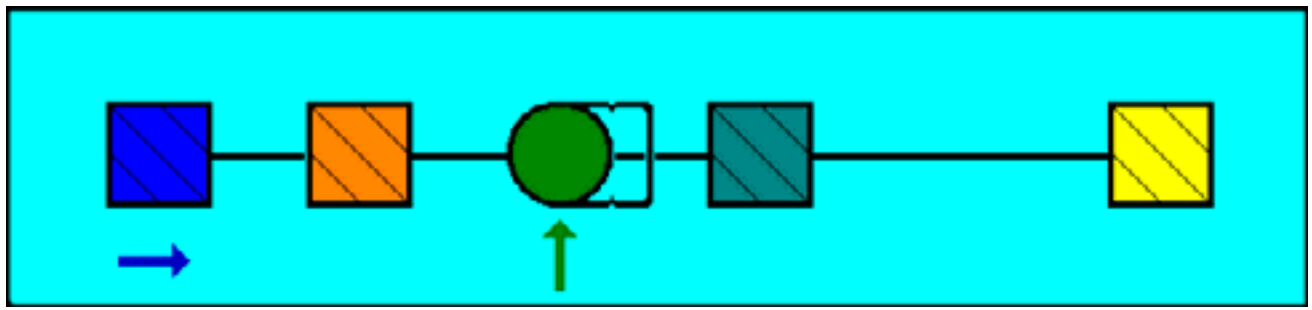
- Long time of analysis
- Low resolution
- Not suitable for quantitative analysis

HPLC - High Pressure Liquid Chromatography

The HPLC is certainly the most used separation technique:

- high sensibility and good LOD;
- accurate quantitative separations;
- possibility to separate non volatile or semi-volatile organic compounds;
- rapidity and precision in quantitative analyses;
- wide applicability to substance that are of primary importance in the industry and in the environmental analyses.

SCHEME OF AN HPLC SYSTEM



ELUANTS PUMP INJECTION SYSTEM COLUMN DETECTOR

HPLC INSTRUMENTATION

Eluants

In HPLC the eluants are collected in glass bottles of 500 mL or more. These containers are often equipped with a filtration or degassing system to remove dissolved gases (oxygen and nitrogen) that can interfere, causing bubbles in the detector.

Isocratic operation mode: the separation of the substances is performed with a constant eluant composition.

Gradient operation mode: during the analysis there is a continuous variation of eluant mixture.

Eluants

The mobile phase is usually a mixture of one or more solvents with these characteristics

1. *Physical properties*: high purity, low cost, UV transparence, not corrosive, low viscosity, low toxicity, not flammable, solvent the sample.
2. *Strength* : the strength is correlated to the polarity of the solvent, the water is a strong solvent in normal phase but weak in reversed phase.
3. *Selectivity*: it depends on interactions such as dipolar moment, induced dipole, hydrogen bond.

HPLC

Pumps

Pumps requirements:

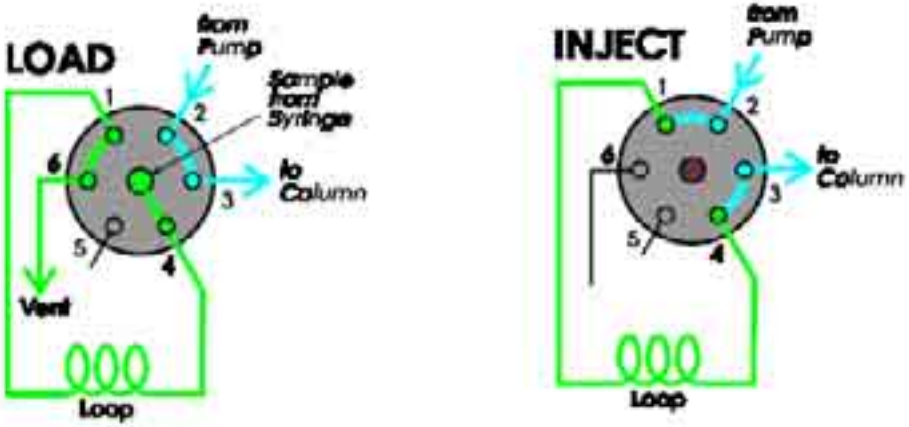
- capacity to create high Pressure up to 400 atm,
- flow exit without pulsations,
- flow rate range : 0,1-10 ml/min,
- check of the flow rate with a relative reproducibility 0,5%,
- resistant components to the corrosion.

HPLC

Injection system

The injection system can be manual or automatic.

The second system is preferable, because it ensures a better reproducibility in the injection.



Columns

They are generally built in stainless steel and are filled with silica, alumina or polymers particles. There are analytical columns and protection columns.

The protection columns retain the particulate or other interferences present in the solvents and so increase the medium life of the analytical columns.

	Analytical columns	
Length (cm)	from 10 to 30	from 3 to 7,5
d.i.(mm)	from 4 to 10	from 1 to 4,6
filling particles (μm)	3, 5 or 10	3 or 5

It is possible to thermostat the column in order to obtain better chromatograms even if it's not necessary.

Columns

If the stationary phase is liquid (partitioning chromatography) it is chemically bounded to the support via the silanolic groups.

Reversed phase: the stationary phase is non polar (hydrocarbon C8 or C18) and the mobile phase is relatively polar (water, methanol or acetonitrile); so less polar compounds are more kept in the column than the polar compounds that will go out first.

Normal phase: the stationary phase is polar (amino phase or diol phase) and the mobile phase used are hydrocarbon or isopropanol.

Normally the choice of the stationary phase is based on the principle: “like with like“. It means that the stationary phase is chosen with a similar polarity of that of the compounds of interest.

In the adsorption chromatography the stationary phases used are solids SiO_2 or Al_2O_3 .

Detectors

HPLC detectors measure in continuo a characteristic of the eluate from the column in a flow cell, situated at the column exit.

They can be distinguished:

- *Bulk property detectors* measure physical characteristics of the mobile phase (solute and solvent, for example the refractive index or the density)

- *Solute property detectors*, they measure only a property of the solute, for example the UV Absorption detector;

Detector characteristics in HPLC

- high sensibility
- wide range of linearity
- low Noise
- low drift (The base line moves away from the horizontal line)
- high efficiency of the cell. It is determined by the volume of the cell: a lower volume permit a better resolution of peaks of eluated compounds. It is measured in volume.

Detectors

Detector	Charactheristics
Absorbance (UV-Vis)	The most common detector for compounds that absorb. Good sensitivity (ng)
Diode array	The identification of the analyte is possible thanks to spectral data
Fluorescence	Specific detector with high sensitivity
Refractive index	Universal, it is used for compounds without chromophore,polymers organic acid
Mass Spectromery	Excellent sensitivity (fg) and specificity, univocal identification of the analytes
Conducibility	For ionic compounds
Electrochemical detector	For elettroactive compounds

The DAD detector permits the contemporaneous measurement of Absorbance at different values of wavelength, its use implies less time of analysis than with a simple UV detector.

Qualitative and Quantification analysis

Qualitative analysis: identification by comparison of retention time t_r of the sample with the one of a standard solution previously injected

Quantitative analysis: measuring the area of each peak that is proportional to the concentration of the analyte; the concentration is determined from the area of the peak by a calibration curve of the element obtained by injecting different standard solutions of known concentrations

- Internal standard
- standard additions method

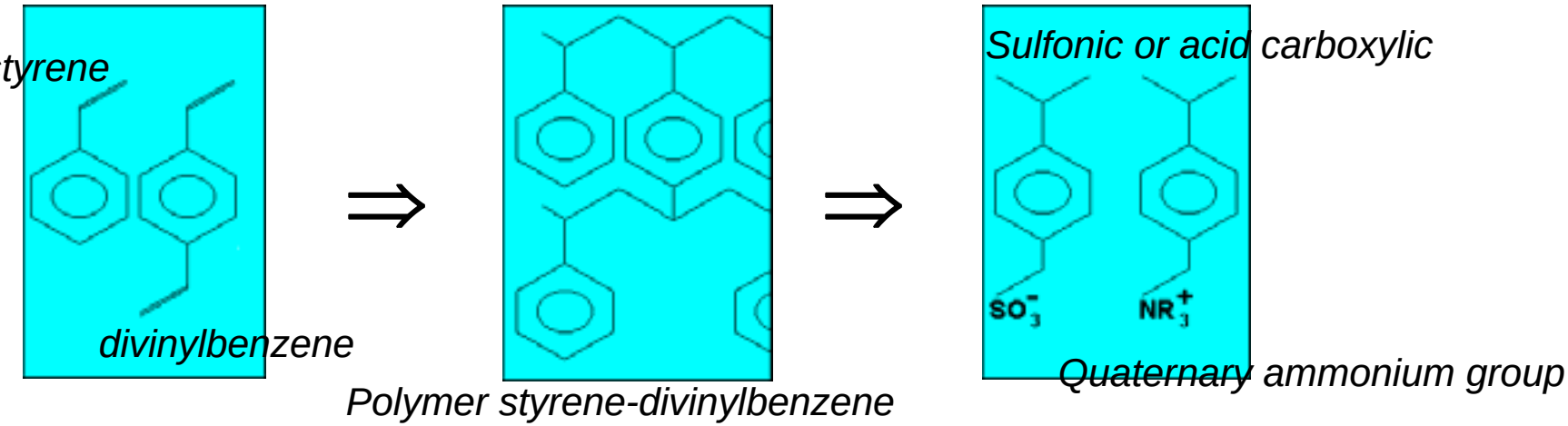
ION CHROMATOGRAPHY

Ion chromatography is a form of liquid chromatography that uses ion-exchange resins to separate atomic or molecular ions based on their interaction with the resin.

Its greatest utility is for the analysis of anions for which there are no other rapid analytical methods.

It is also commonly used for cationic and biochemical species such as alkaline earths and amino acids and proteins.

Stationary phase



The column packing consists in a resin with ion-exchange group bonded to inert polymeric particle.

The polymer is made up of styrene and divinylbenzene.

For anion separation the anionic exchange group is usually a quaternary ammonium group.

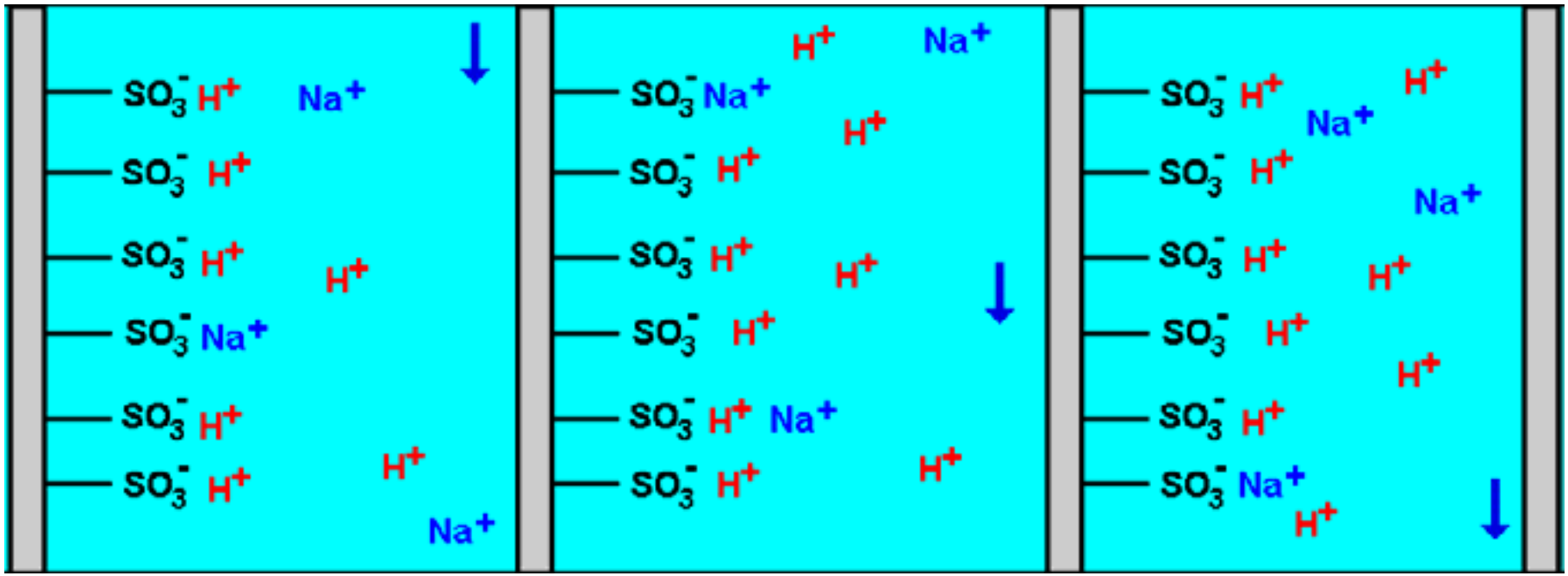
For cation separation the cationic exchange group is usually a sulfonic or carboxylic acid.

The cation separation is usually based on the equilibrium of the element with the sulfonic or carboxylic acid exchange group.

$$R\text{-}SO_3^- H^+(s) + M^{x+}(aq) \leftrightarrows R\text{-}SO_3^- M^{x+}(s) + H^+(aq),$$

Different cations have different values of Keq and are therefore retained on the column for different time.

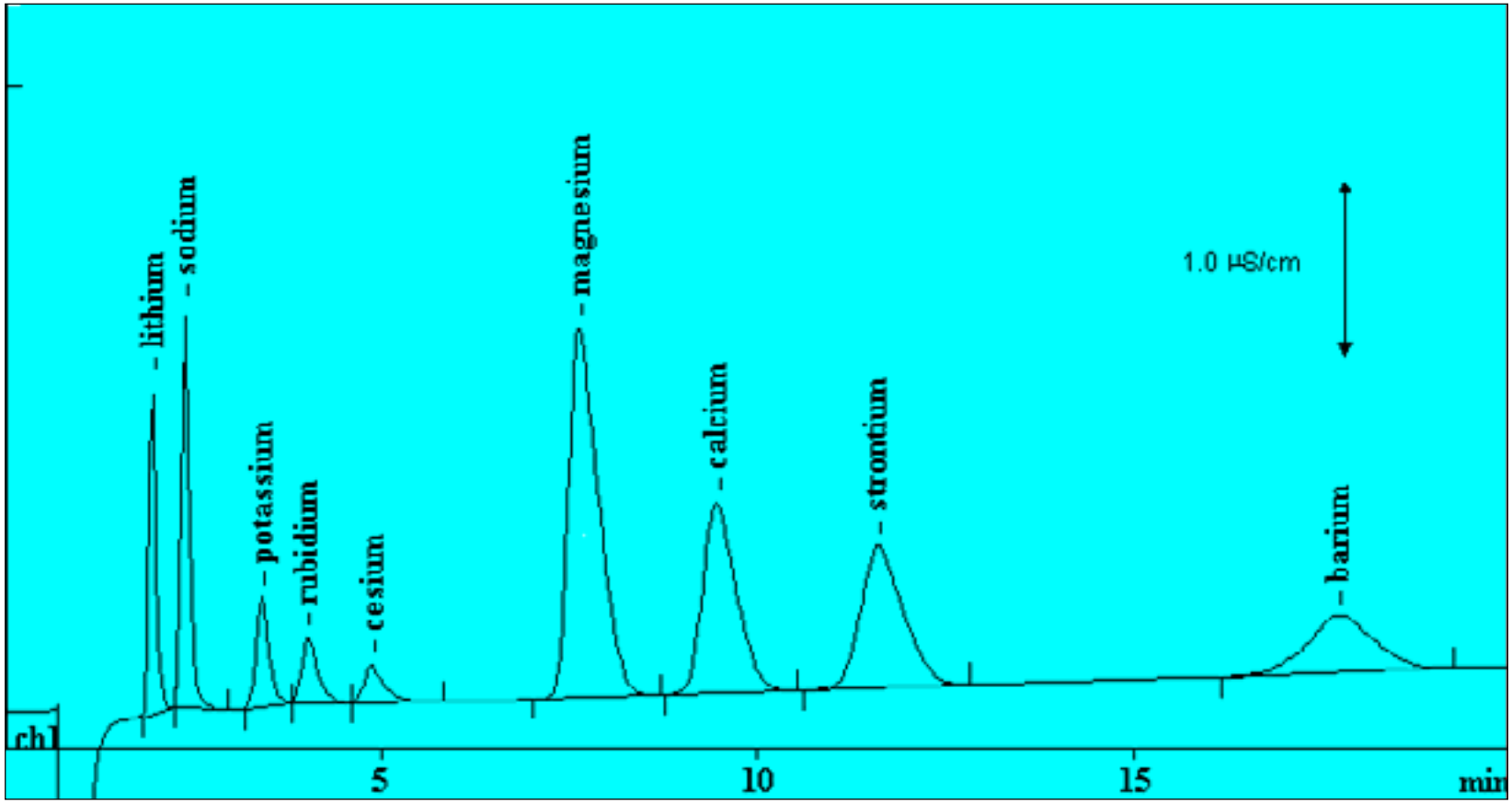
Cation separation mechanism



The mobile phase and the stationary phase compete for the analyte.

The mobile phases used are: tartaric acid, nitric acid, ossalyc acid/etylenediamine/acetone

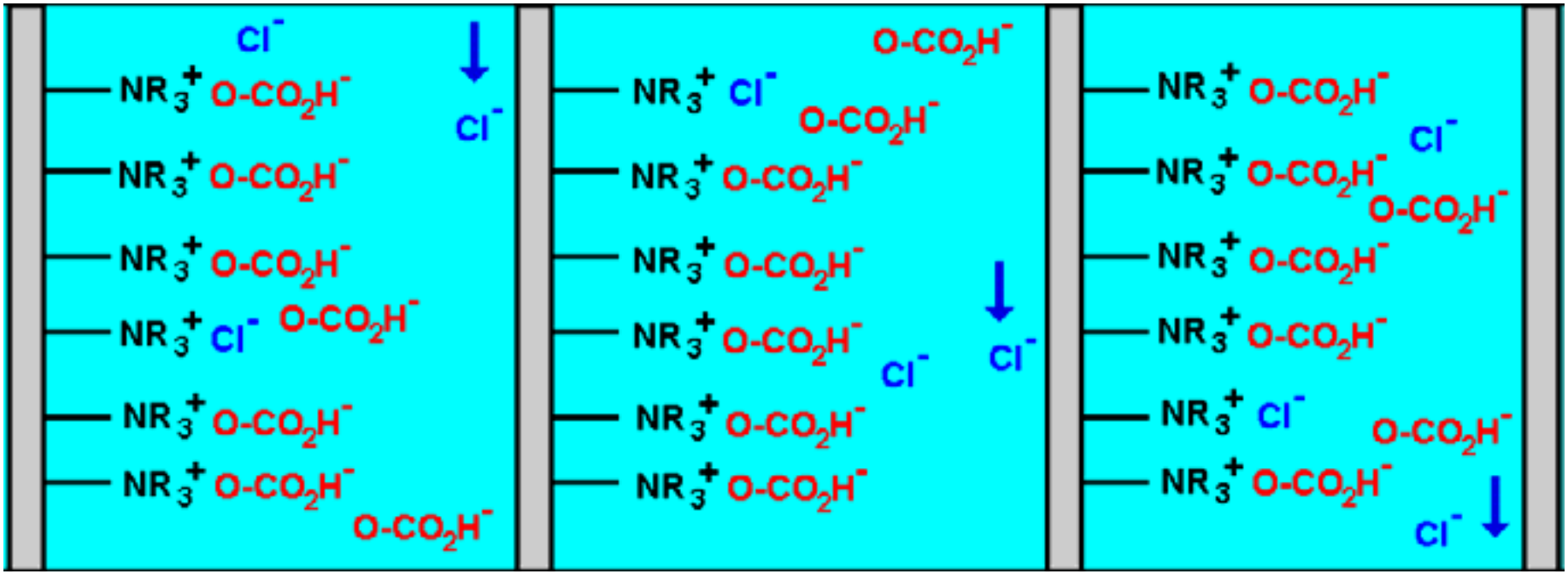
Typical cations chromatogram



Eluent: 5 mmol tartaric acid

Column: Metrosep Cation 1-2

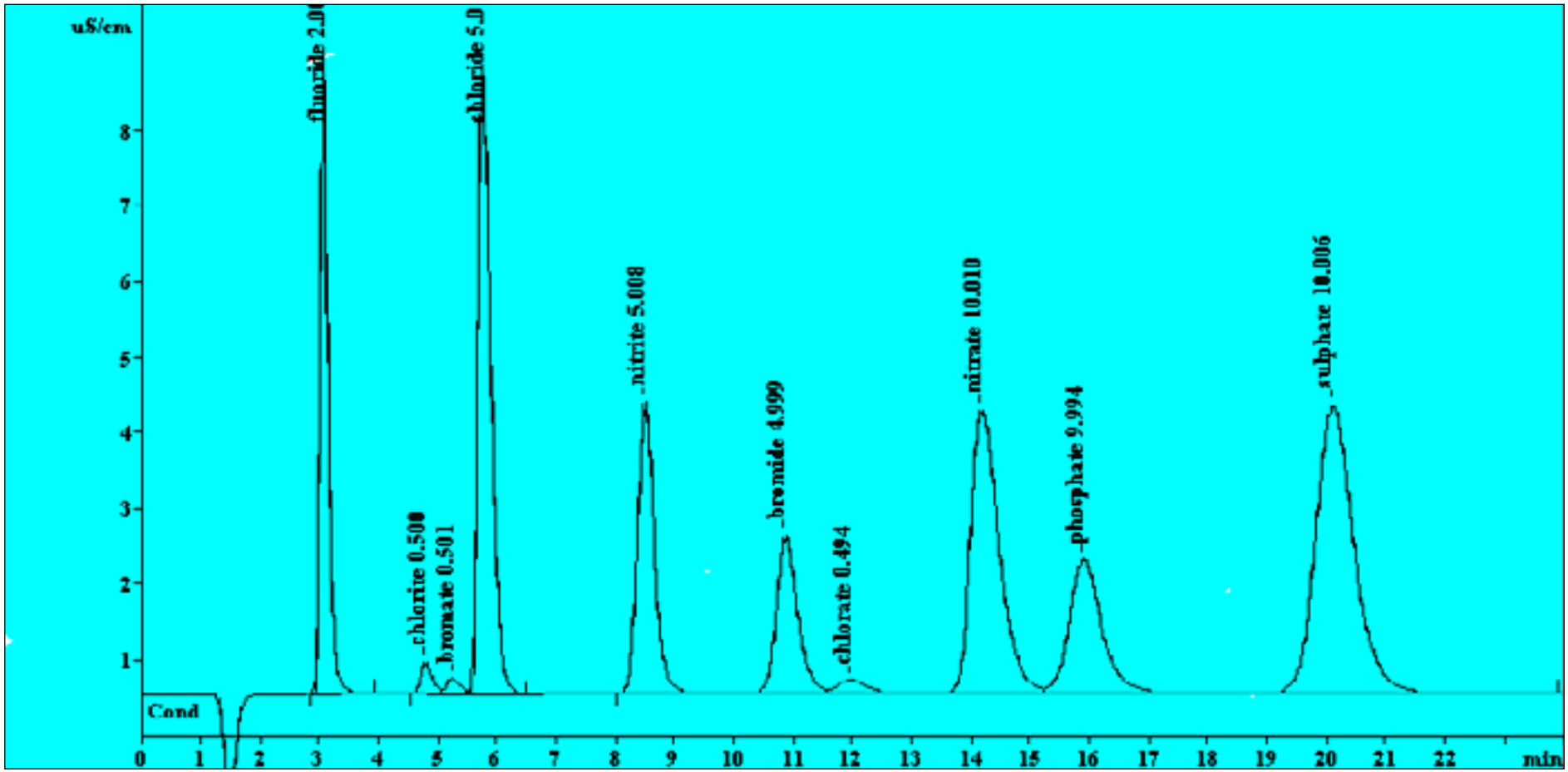
Anion separation mechanism



The mobile phase and the stationary phase compete for the analyte.

The mobile phases can be: ftalic acid, benzoic acid, carbonate, bicarbonate, KOH, NaOH, p-hydroxy benzoic acid.

Typical anions chromatogram



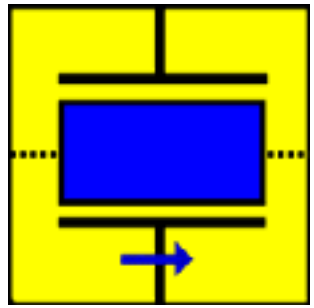
Eluents: $Na_2CO_3/NaHCO_3$ (1.3/2.0 mmol/L)

Column: Metrosep Dual 2

Ion detection

Detector used in ion chromatography are:

- conductivity detector
- UV/Vis detector
- amperometric detector



The detector most used is the conductivity detector.

It is very simple and sensitive.

$$\kappa = \frac{1}{R} * K_c$$

K conductivity(S/cm)
1/R conductance (S)
K_0 conductivity cell constant

$$\kappa = \sum (\Lambda_i * z_i * c_i)$$

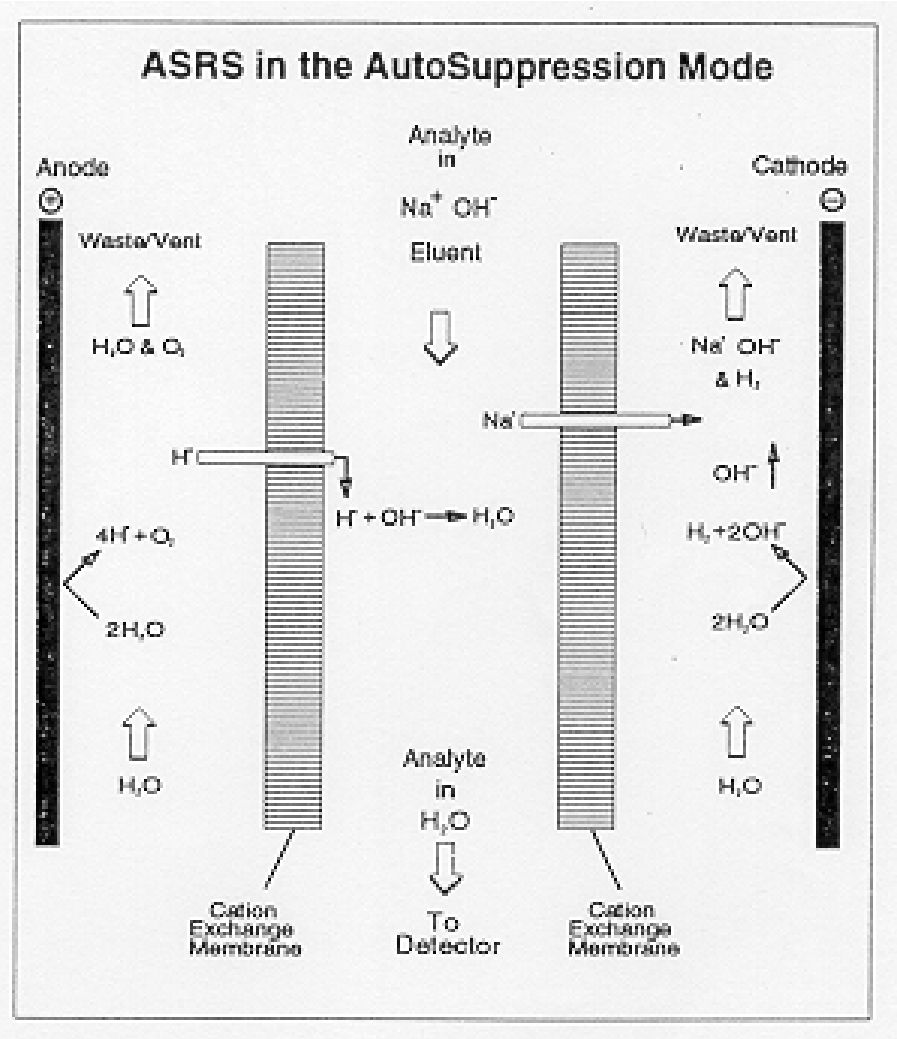
K conductivity (S/cm)
Λ Equivalent conductivity constant (Sxcm^2/ mol)
c concentration
z charge

But… the concentration of eluant is very high relative to that of the ions to separate. It is often difficult to detect the slight drops in highly conductive eluant as less conductive ions pass through the detection cell.

INTRODUCTION OF THE *SUPPRESSOR*

The conductivity suppression allows more sensitive detection of the conductivity difference between ions and the mobile phase.

The mobile phase ions are converted to a neutral form or removed with an eluant suppressor, which consists of an ion-exchange column or membrane.



Self regenerating suppressor showing anion exchange through membrane walls

Reference Standard

- EN ISO 10304-1: 1995 Water quality-Determination of dissolved fluoride, chloride, nitrite, orthophosphate, bromide, nitrate and sulfate ions, using liquid chromatography of ions- Part1: Method for water with low contamination (ISO 10304-1:1192)

- EN ISO 10304-2 :1996 Water quality- Determination of dissolved anions by liquid chromatography of ions- Part 2: determination of bromide, chlorine, nitrate, nitrite, orthophosphte and sulfate in waste water (ISO 10304-2: 1992)

- ISO 14911: 1998 Water quality- determination of dissolved Li^+, Na^+, $NH4^+$, K^+, Mn^{2+}, Ca^{2+}, Mg^{2+}, Sr^{2+} and Ba^{2+} using ion chromatography- Method for water and waste water

GAS CHROMATOGRAPHY

Introduction

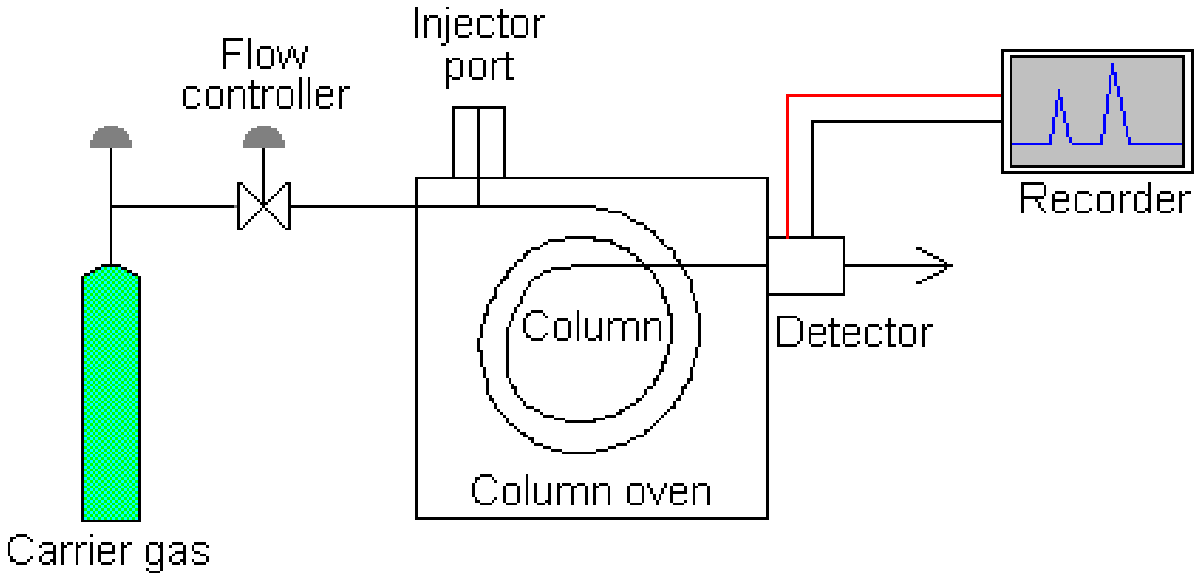
Gas chromatography is a widely used technique for environmental analysis. It is utilized for the determination of volatile and semivolatile organic compounds. The analytes are separated upon their affinity for the stationary phase.

This technique especially coupled to mass spectrometer detection allows determination of organic compounds in trace (units of ppb or ppt).

Gas chromatography- specifically gas-liquid chromatography- involves a sample being vaporized and injected onto the head of the chromatographic column. The sample is transported through the column by the flow of inert, gaseous mobile phase.

The column itself contains a liquid stationary phase which is adsorbed onto the surface of an inert solid.

INSTRUMENTAL COMPONENTS



Carrier gas chemically inert its function is only to transport the sample through the column (He, H_2)

Injection port the sample is injected via microsyringe in the injector port where there is a vaporisation chamber. The temperature of the sample port is about 50°C higher than boiling point of the least volatile compound.

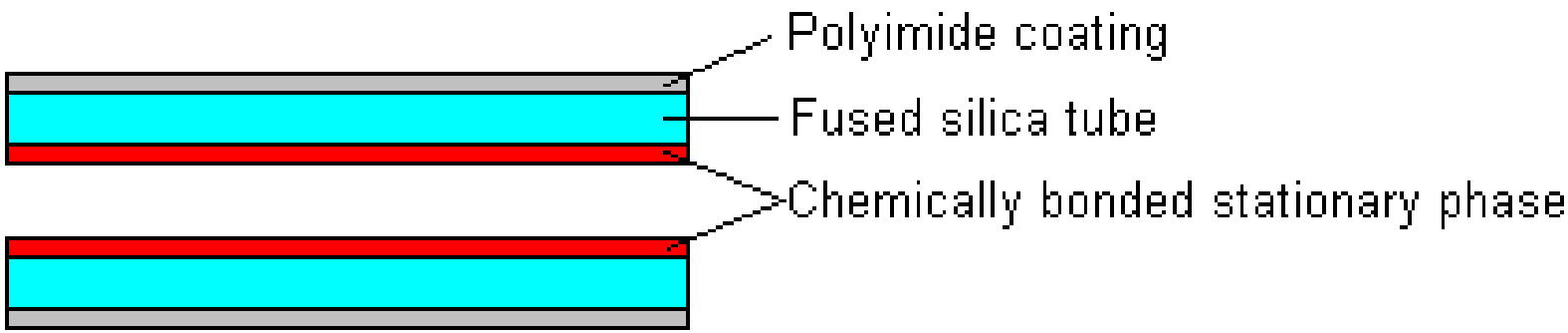
Column packed and capillary

Detector different types of detector : FID, ECD, MS

Columns

- *Packed columns* contains a finely divided, inert, solid support material (commonly based on diatomaceous earth) coated with liquid stationary phase length 1,5- 10m
- *Capillary columns* internal diameter of a few tenths mm
  - *WCOT Wall-Coated Open Tubular:* capillary tube whose walls are coated with liquid stationary phase
  - SCOT *Supported-Coated Open Tubular:* the inner wall of the capillary is lined with a thin layer of support material onto which the stationary phase has been adsorbed
  - *FSOT Fused Silica Open Tubular:* thinner walls than the glass capillary columns WCOT (strength, flexibility and low reactivity);

Cross section of a Fused Silica Open Tubular Column



Properties and characteristics of columns in Gas Chromatography

Type of column	FSOT	WCOT	SCOT	Impacked
length	10-100	10-100	10-100	1-6
d.i. (mm)	0,1-0,53	0,25-0,75	0,5	2-4
Efficiency (plate/m)	2000-4000	1000-4000	600-1200	500-1000
Dimension of sample, ng	10-75	10-1000	10-1000	1o-10^6
Chemical inertness	The best	----------	----------→	The worst
Flexibility	Yes	No	No	No

Type	Comercial names	Polarity	M.A.O.T. (Maximum Allowance Temperature ofOperation)
Hydrocarbon greases	Apiezon	Apolar	250-300 °C
Polydimethylsiloxane (O-Si-O)-R	SE-30, OV-1, OV-101,RSL-150, DC-200, HP-1, etc	Apolar	350 °C
Polyphenylmethylsiloxane R=CH_3 (95%), Ph (5%)	SE-52, OV-73, HP-5	Apolar	350 °C
Polyphenylmethylsiloxane R=CH_3 (80%), Ph (20%)	OV-7, AT-20, SPB-20	Medium polarity	350 °C
Polyphenylmethylsiloxane R=CH_3 (50%), Ph (50%)	OV-17, HP-17, AT-50	Medium polarity	350 °C
Poly(trifluoropropyl)metyl-siloxane R=CH_3 (65%), CF_3-C_2H_4(50%)	OV-202, OV-210, RSL-400	Polar	275°C
Polyethylene glycol	Superox-II, Carbowax, HP-20M, AT-WAX	Polar	250-275 °C

Choice of the stationary phase

The polarities of the compounds of interest dictate the choice of stationary phase, under the rule *"like dissolves like".*

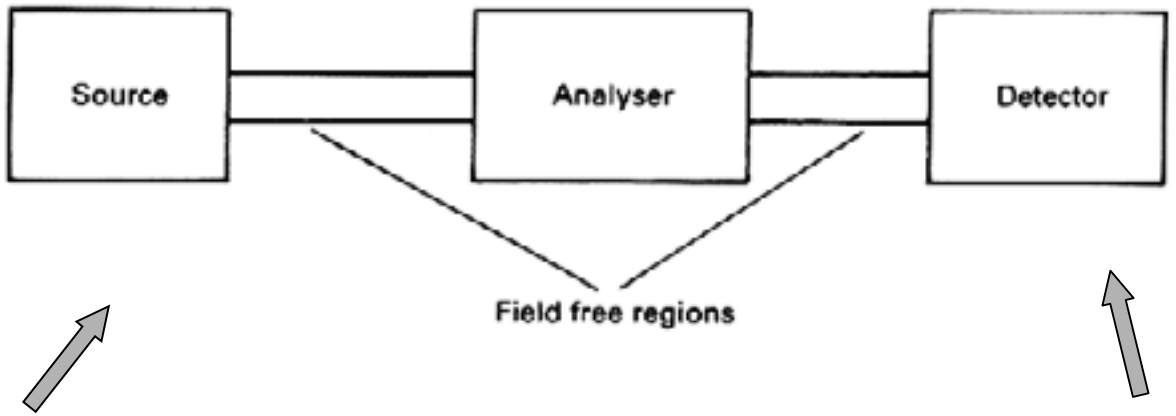
PCBs apolar compounds → Polydimethylsiloxane

Nitroaromatics → Poly(trifluoropropyl)metyl-siloxane

Detectors

Detector	Type	Support gases	Selectivity	Detectability	Dynamic range
Flame ionization (FID)	Mass flow	Hydrogen and air	Most organic compounds.	100 pg	10^7
Thermal conductivity (TCD)	Concentration	Reference	Universal	1 ng	10^7
Electron capture (ECD)	Concentration	Make-up	Halides, nitrates, nitriles, peroxides, anhydrides, organometallics	50 fg	10^5
Nitrogen-phosphorus	Mass flow	Hydrogen and air	Nitrogen, phosphorus	10 pg	10^6
Photo-ionization (PID)	Concentration	Make-up	Aliphatics, aromatics, ketones, esters, aldehydes, amines, heterocyclics, organosulphurs, some organometallics	2 pg	10^7

MS detector



Gas –phase Ionization Source

Ions are separeted according to their m/z

ions are detected

spectrum

Gas-phase Ionization

- *EI - Electron Impact ionization (hard)*

A beam of electrons passes through the gas-phase sample (Energy= 70eV).
An electron that collides with a neutral analyte molecule can knock off another electron resulting in a positively charged ion.

$CH_3OH + 1e^- \rightarrow CH_3OH^{+\cdot}$ *(molecular ion)*

$CH_3OH^{+\cdot} + 1e^- \rightarrow CH_2OH^{+}$ *(fragment ion)*

- *CI- Chemical Ionization (soft)*

It uses ion-molecule reaction to produce ions from the analyte. The chemical ionization process begins when a reagent gas (methane, isobutane or ammonia) is ionized by electron impact. These ions then react with molecula of the sample to product analyte ions. (soft ionization tecnique).

$R + e^- \rightarrow R^{+\cdot}$

$R^{+\cdot} + RH \rightarrow RH^{+} + R^{\cdot}$

$RH^{+} + S \rightarrow SH^{+} + R$

R=Reagent gas

S= Sample

Characteristics of the two types of Ionization

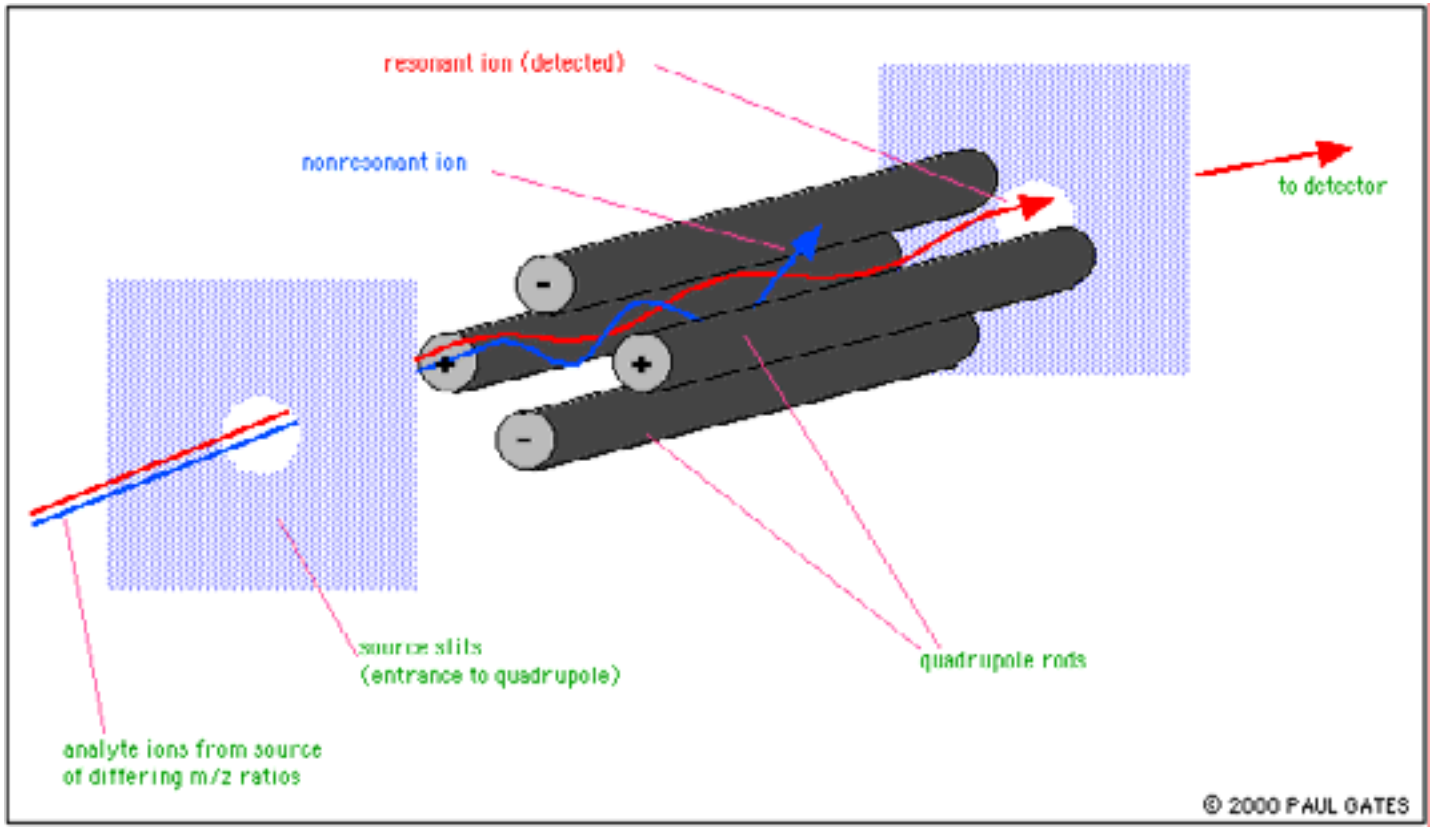
Ionization method	Typical analyses	Mass range	Method Highlights
Electron Impact (EI)	Relatively small, volatile	To 1,000 Dalton	Hard method versatile provides structure info
Chemical Ionization	Relatively small, volatile	To 1,000 Dalton	Soft method molecular ion peak $(M+H)^+$

Ions Analyzer

- ## Quadrupole Mass Analyzer

Molecular ions and fragment ions are accelerated by manipulation of the charged particles through the mass spectrometer. Uncharged molecules and fragments are pumped away. The quadrupole mass analyzer uses (+) and negative (-) voltages to control the path of the ions. Ions travel down the path based on their mass to charge ratio (m/z). Therefore a ion path will depend on its mass. Voltages, applied on the rods are scanned so that ever increasing masses can find a successful path trough the rods to the detector.

Quadrupole Mass Analyzer



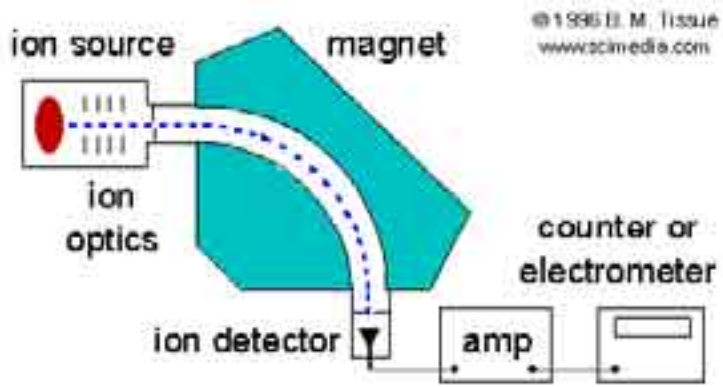
Ions Analyzer (2)

- *Magnetic Sector Mass Spectrometry (High Resolution)*

By varying the intensity of the magnetic field, ions are directed differently through the instrument. Only the ions that make it to the detector are recorded.

$$\text{kinetic energy} = \frac{m_i v^2}{2} = z_i V_a$$

$$\frac{m_i v^2}{r} = B z_i v \quad \rightarrow \quad \frac{m_i}{z_i} = \frac{B^2 r^2}{2 V_a}$$



This Magnetic Sector allows the determination of dioxins present in the environment in ultratrace.

Acquisition mode

- *Full Scan mode* record or transmit all ions in a particular range, individual ions can be extracted and used for qualitative work.
- *SIM Selected Ion Monitoring mode* only look at one or two ions, all other ions are lost , the selected ions are used for quantitative work (higher sensitivity than in full scan).

GC-MS analysis produces a Total Ion Chromatogram, that provides information similar to other GC detectors.

T_r and spectrum are used for qualitative analysis

Area of each peak is used for quantitative analysis

Standard Methods

- US-EPA 8041 Phenols (GC-FID or ECD)
- US-EPA 8061 A Phthalates (GC-ECD)
- US-EPA 8070A Amines (GC-NPD)
- US-EPA 8081A Chlorinated Pesticides (GC-ECD)
- US-EPA 8082 PCBs (GC-ECD)
- US-EPA 8100 PAH (GC-FID)

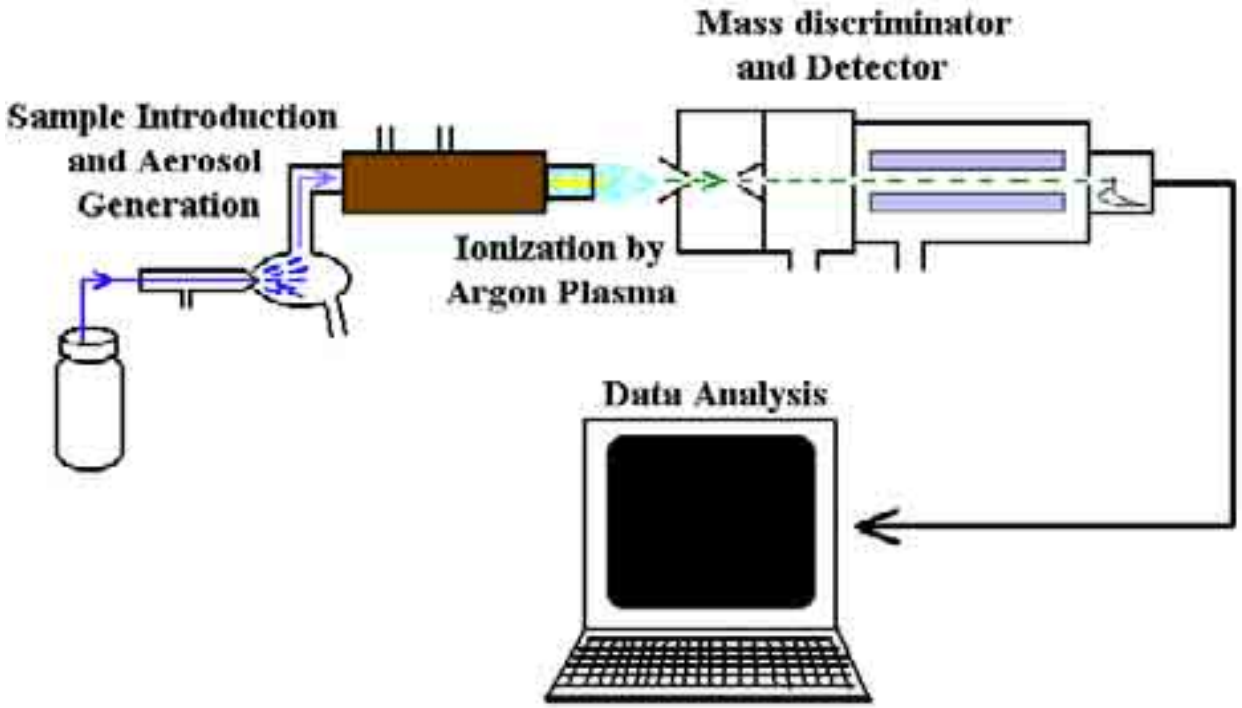
ICP-MS

Introduction

Inductively coupled plasma mass spectrometry is capable of trace mutielement analysis, often at the part per trillion level. ICP-MS finds application in a number of different fields including drinking water, waste water, natural water system, geology and soil science and medicine.

Samples are decomposed to elements and ionized in a high temperature plasma; then the elements of interest are determined by their mass to charge ratios.

Only liquid samples can be analysed by ICP-MS; soil and other solid sample must be dissolved by a digestion process first to be injected into the ICP-MS



Schematic of ICP-MS main process

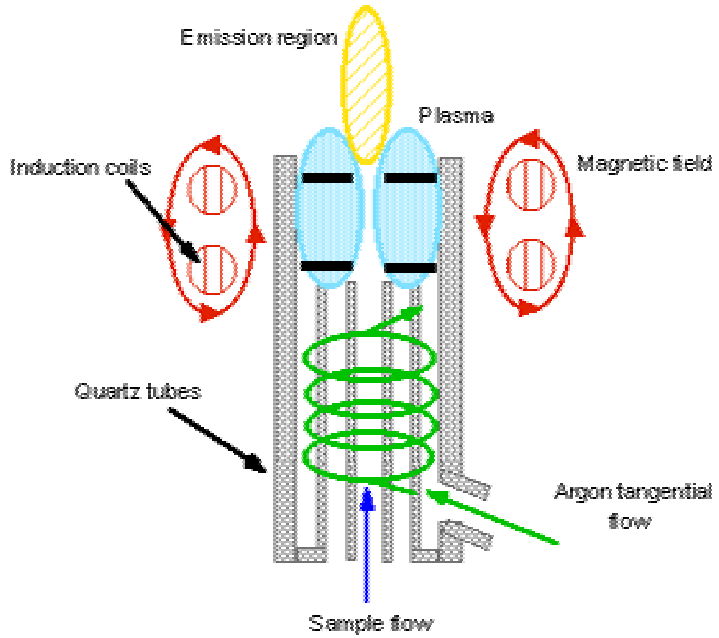
Sample introduction

• *aqueous sample* are introduced by a peristaltic pump into a nebulizer where the carrier gas Argon aspirates the sample with high velocity, forming a fine aerosol. The aerosol then passes into a spray chamber where larger droplets are removed via a drain. This process is necessary to produce droplets enough to be vaporized in the plasma torch.

• *soil sample* are introduced in ICP-MS by way of a laser ablation system (very expensive)

Ionization by plasma

Plasma is a gas in which atoms are present in a ionized state. The torch of an ICP consists of three concentric tubes. The torch is situated within a water-cooled coil of a radio frequency (r.f.) generator. As flowing gases are introduced into the torch, the r.f. field is activated and the gas in the coil region is made electrically conductive.



The plasma is maintained by inductive heating of the flowing gases.

The hot plasma removes any remaining solvent and causes sample atomization followed by ionization.

Mass filter and detection

The substances ionized in the torch are extracted via a special set of metal cones and ion-focusing elements into the mass spectrometer analyzer *(quadrupole)*

The most common type of ion detector is the *channeltron electron multiplier*. The ions are attracted to the interior cone surface, when they strike the surface additional secondary electrons are emitted which move further into the tube emitting additional secondary electrons

Qualitative and Quantification analysis

Qualitative analysis: identification by their mass to charge ratios

Quantitative analysis: measuring the signal (cps count per second) that is proportional to the concentration of the analyte; the concentration is determined from the signal by a calibration curve of the element obtained by analysing different standard solutions

- Internal standard (Rh)
- standard additions method

Advantages of ICP-MS

-Trace and ultra trace measurements of >70 elements-From Li to U

- Speed of multielement analysis (despite that AAS analysis)

- Flexibility to optimize for specific applications

- Determination of isotope ratio

- wide dynamic range (8 to 9 orders)

Element	Detection Limit (ppt)
U,Cs,Bi,	Less than 10
Ag, Be, Cd, Rb, Sn, Sb, Au	10-50
Ba, Pb, Se, Sr, Co, W, Mo, Mg	50-100
Cr, Cu, Mn	100-200
Zn,As,Ti	400-500
Li,P	1-3 ppb
Ca	Less than 20ppb

Disadvantages of ICP-MS

A disadvantage is due to the isobaric interferences correlated to the presence of polyatomic ions formed by argon gas and some environmental salt matrices;

For this reason hydrocloric acid cannot be used for digestion of the samples

Upgrade with Reaction/collision cell permits to eliminate this kind of interferences

Higher Cost

Reference Method

ASTM- D 5673-02 Elements in Water by Inductevely Coupled Plasma- Mass Spectrometry