Course Outline

1. General informations on chemical analysis
2. Sample preparation
3. Metals analysis
4. **Soluble inorganic pollutants analysis**
5. Organic pollutants analysis
6. Quality assurance

4. Soluble inorganic pollutants analysis

- They gather:
  - Pollution by nitrogenous compounds:
    - nitrate (*nitrate*); nitrite (*nitrite*); ammonium (*ammonium*)
  - Pollution by phosphorous compounds:
    - phosphate (*phosphate*)
  - Pollution by mineral salts
    - sulphate (*sulfate*); chloride (*chlorure*), fluoride (*fluorure*)…

➡ **Water Soluble compounds**

4. Soluble inorganic pollutants analysis

- Ubiquitous in different environments: soil, water, air (particles)
  - Sources: Industries, daily life (laundry, dental health ..), breeding / spreading (fertilizer), road traffic ...

- Environmental impact:
  - Pollution of water and soils:
    - Major nutrients in the biosphere (nitrate, phosphate) → risk of eutrophication
    - Toxicity for aquatic fauna (nitrate/nitrite) or for human (nitrates, nitrites, fluoride)
  - Atmospheric pollution:
    - Toxicity of nitrates → blocking of hemoglobin prohibiting the transport of oxygen (also true for waters rich in nitrates)
    - Acid rains (Sulphates and nitrates)
    - Impact of sulphate particles on radiative budget

4. Soluble inorganic pollutants analysis

- **Aim**: Analysis of ion species (*cations* or *anions*) : sulphate, nitrate, chloride, ammonium, calcium, metals…
- **Techniques applicable to aqueous phases:**
    - Ion Exchange Chromatography *(Chromatographie ionique)*
    - UV/Vis Spectrometry *(Spectrométrie UV/Vis)*
    - Ion-Selective Electrodes *(Electrodes ioniques spécifiques)*

4. 1. Ion Chromatography

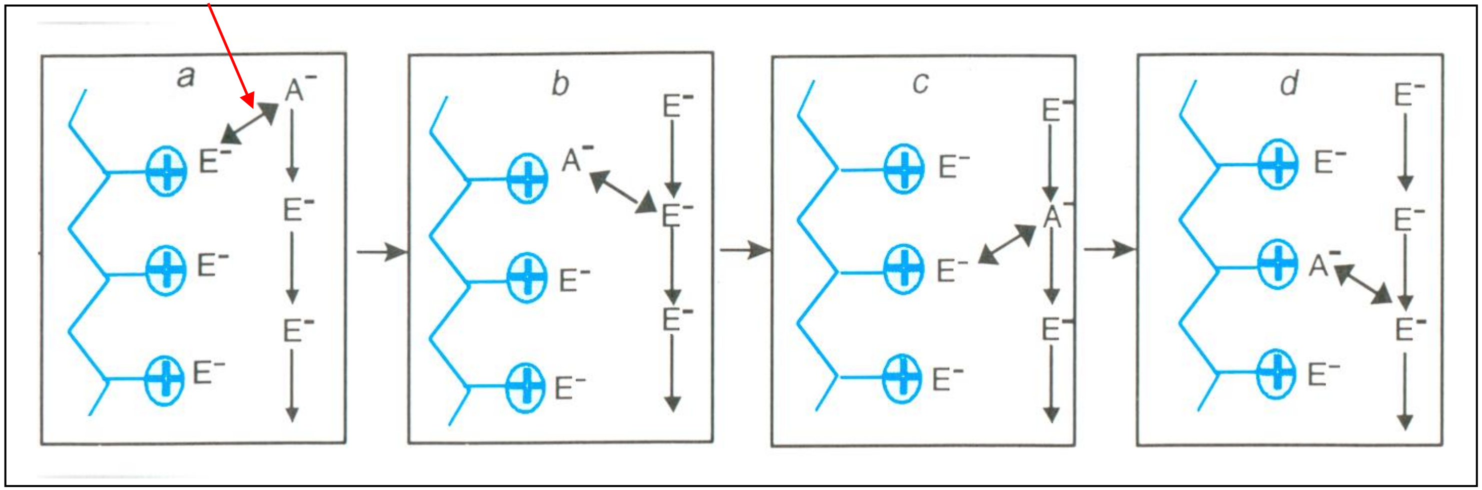
- Aim: Separate then analyse the different ion species (cations or anions)
- Principle : Affinity of species between the stationary phase (column) and the mobile phase (eluent)→ by ion exchange
- Applications:
  - Estimation of regulated species concentrations in drinking water, river, rain ...
  - Estimation of ionic species concentrations in soils, sludge, aerosols ... after extraction in an aqueous phase.

4.1. Ion Chromatography: Theory

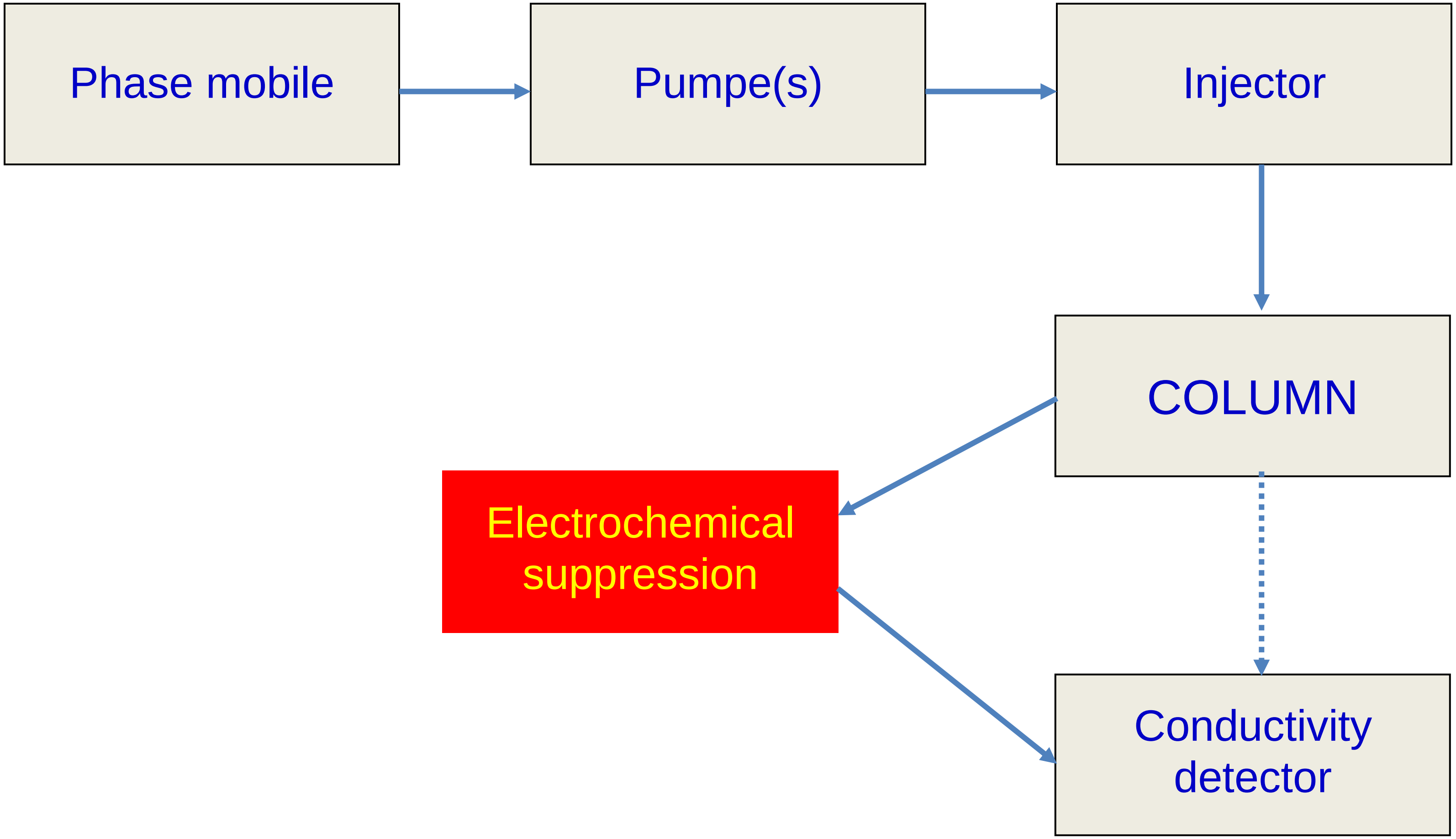
Ion exchange governed by the equilibrium equation which determines the distribution of the species between the mobile phase and the stationary phase.

Ex : Anion exchange

Ion bonding



4.1. Ion Chromatography: Instrumentation



Phase mobile

Pumpe(s)

Injector

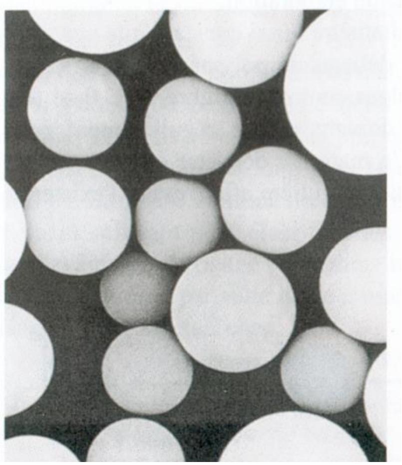
COLUMN

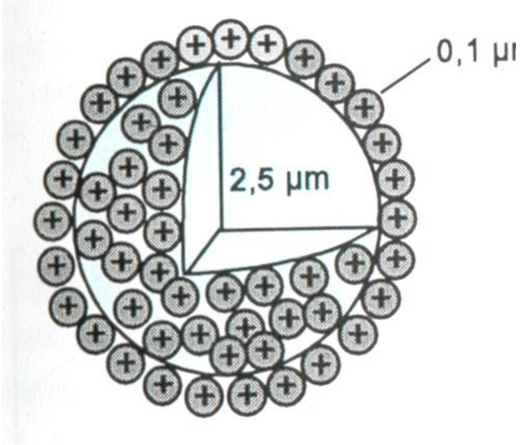
Electrochemical suppression

Conductivity detector

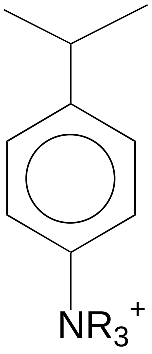
4.1. Ion chromatography: Stationary Phases

- Stationary phase with charge surface (opposite charge to the sample ions)
- 2 supports:
  - **bonded silica gel** (85% of cases): hard material, stable in almost all organic solvents but low capacity of adsorption
  - **Polymer gels (polystyrene):** low selectivity but high capacity of adsorption (vs silica)
- 2 types of bonding:
  - Cation exchange : sulfonate (SO_3^-) or carboxylate ($–COO^-$)
  - Anion exchange: quaternary ammonium ($R-NR_3^+$)

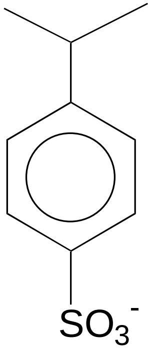




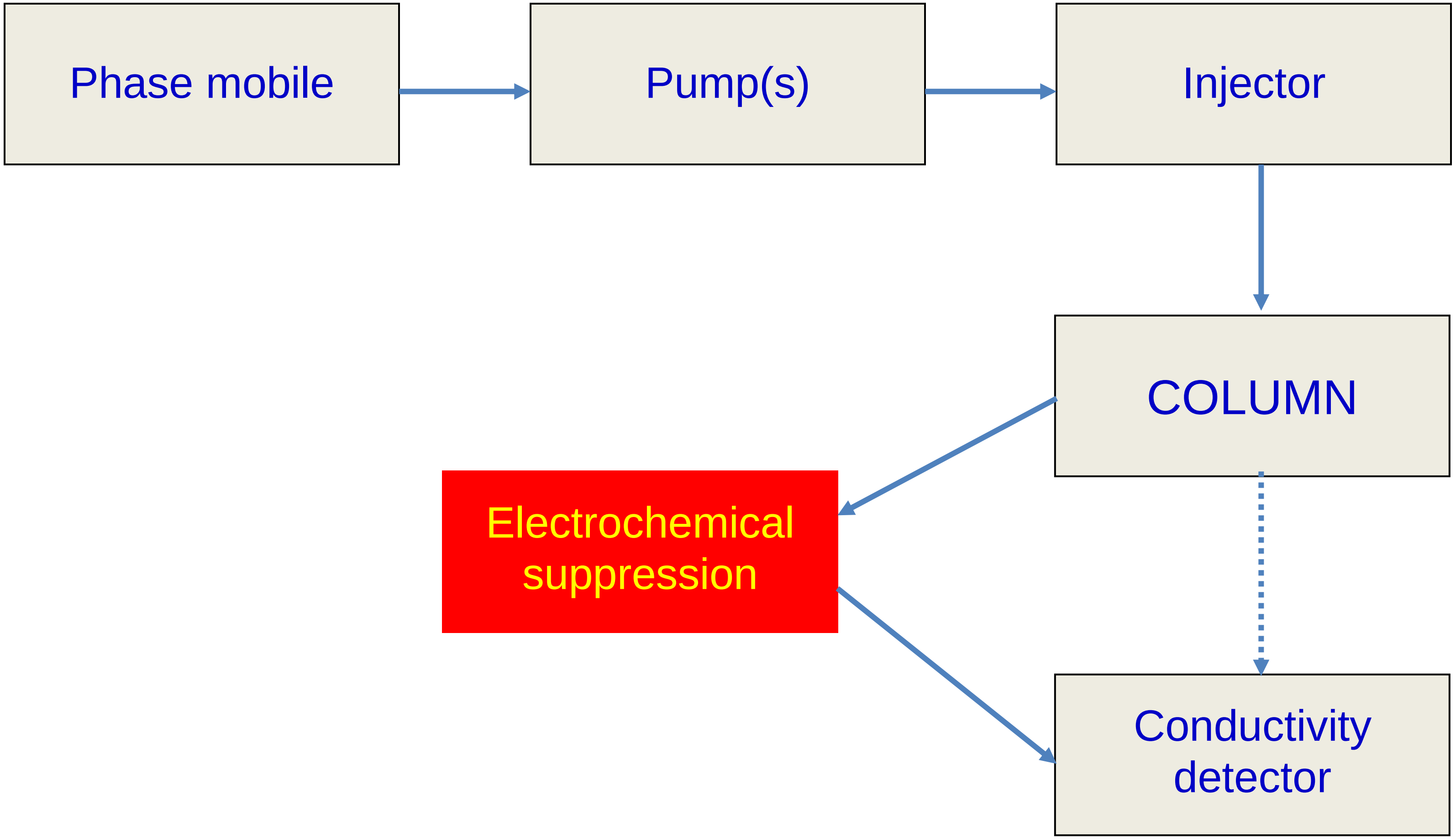
Echangeur A^-



Échangeur C^+



4.1. Ion Chromatography: Instrumentation



Phase mobile

Pump(s)

Injector

COLUMN

Electrochemical suppression

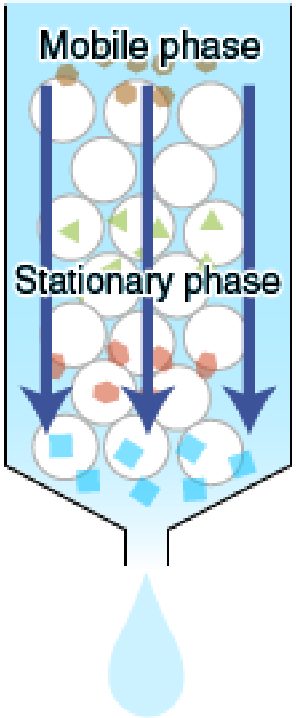
Conductivity detector

4.1. Ion Chromatography: Mobile phases

🟦 Mobile phase

○ Stationary phase

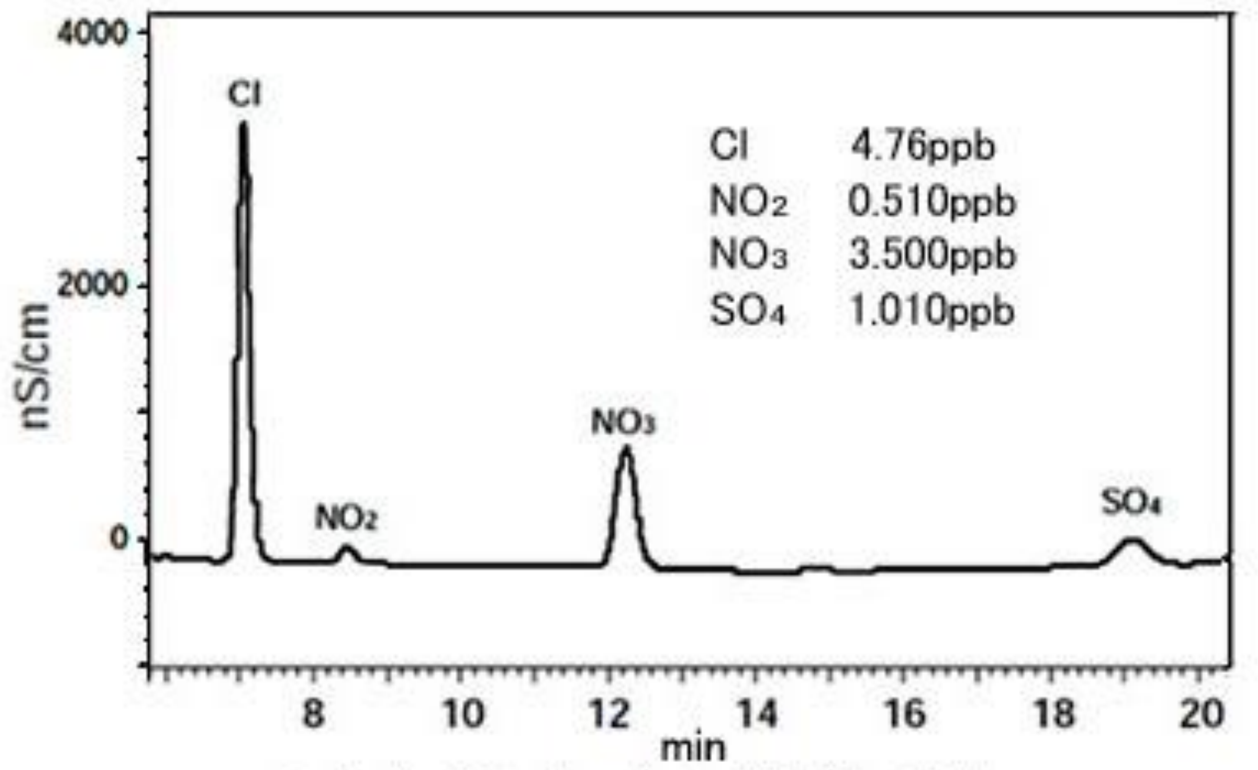
Sample



- The mobile phase is generally a more or less concentrated aqueous buffer.
- The retention of the analytes depends on the concentration and the charge of the dissolved ions in the eluent.
- Ability to use an elution gradient for complex samples containing variable ionic strength components
  - e.g. Start with a very dilute mobile phase with gradual introduction of a stronger concentration component.

4. 1. Ion chromatography:

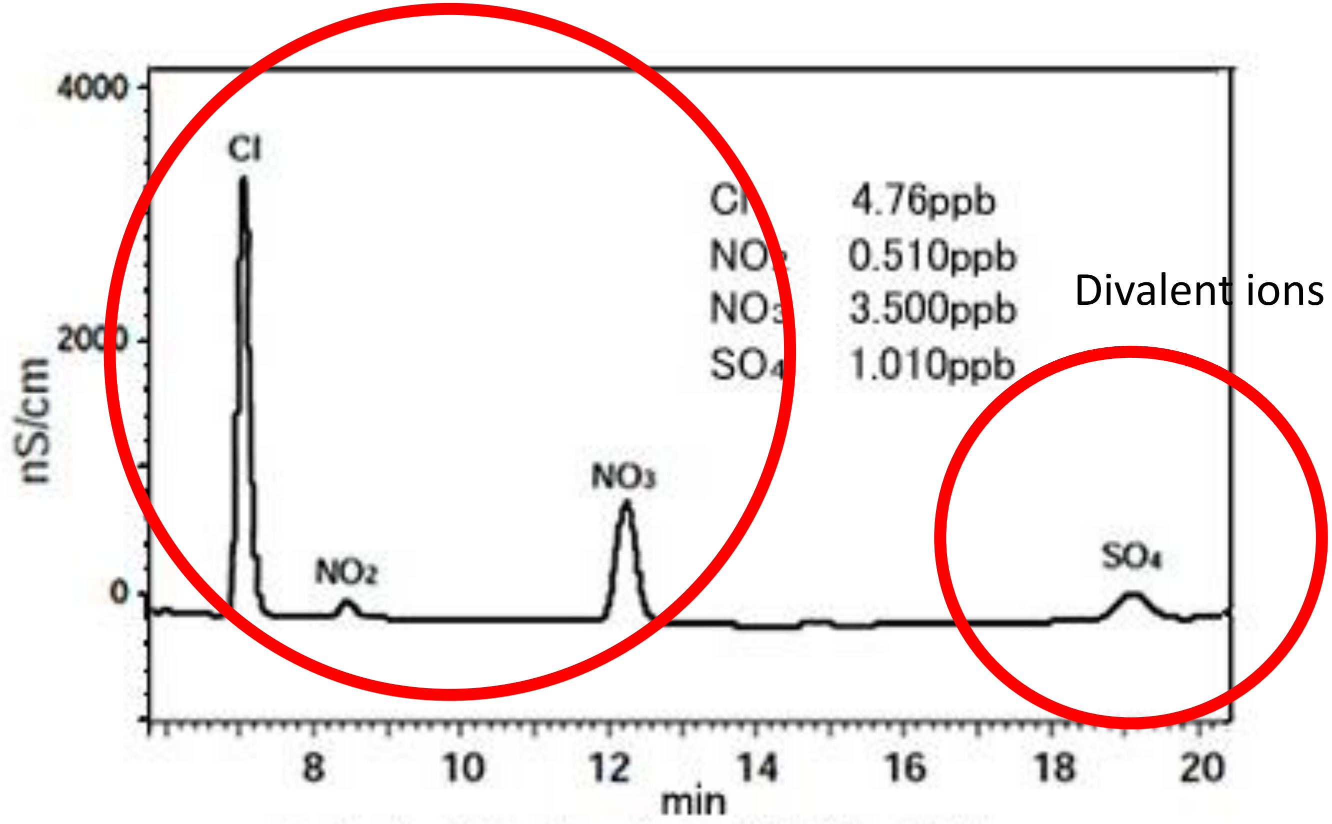
- Retention time?



Analysis of Semiconductor Washing Water

4. 1. Ion chromatography:

- <u>Retention time?</u>

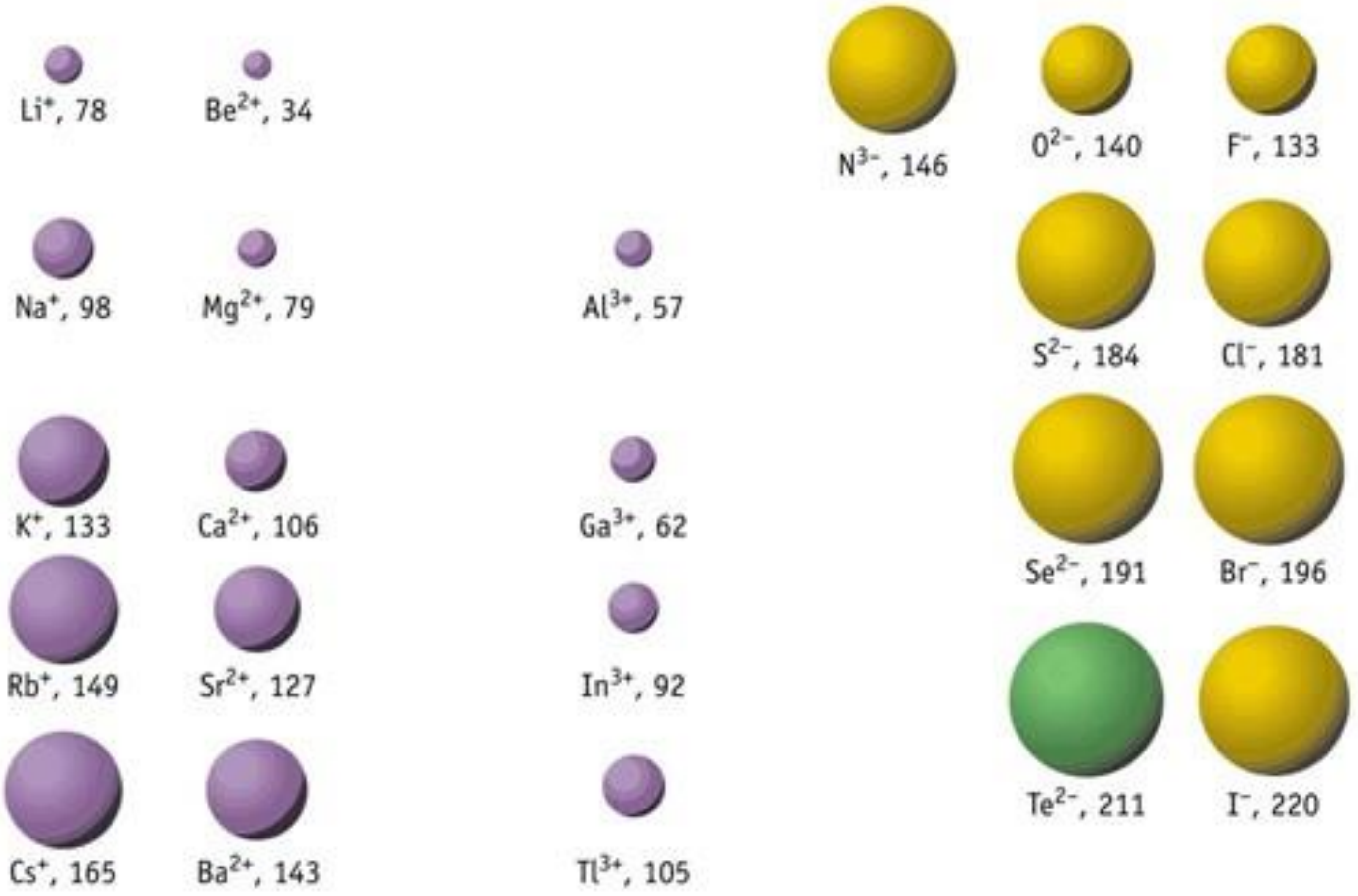


Singly charged ions = monovalent ions

Divalent ions

Analysis of Semiconductor Washing Water

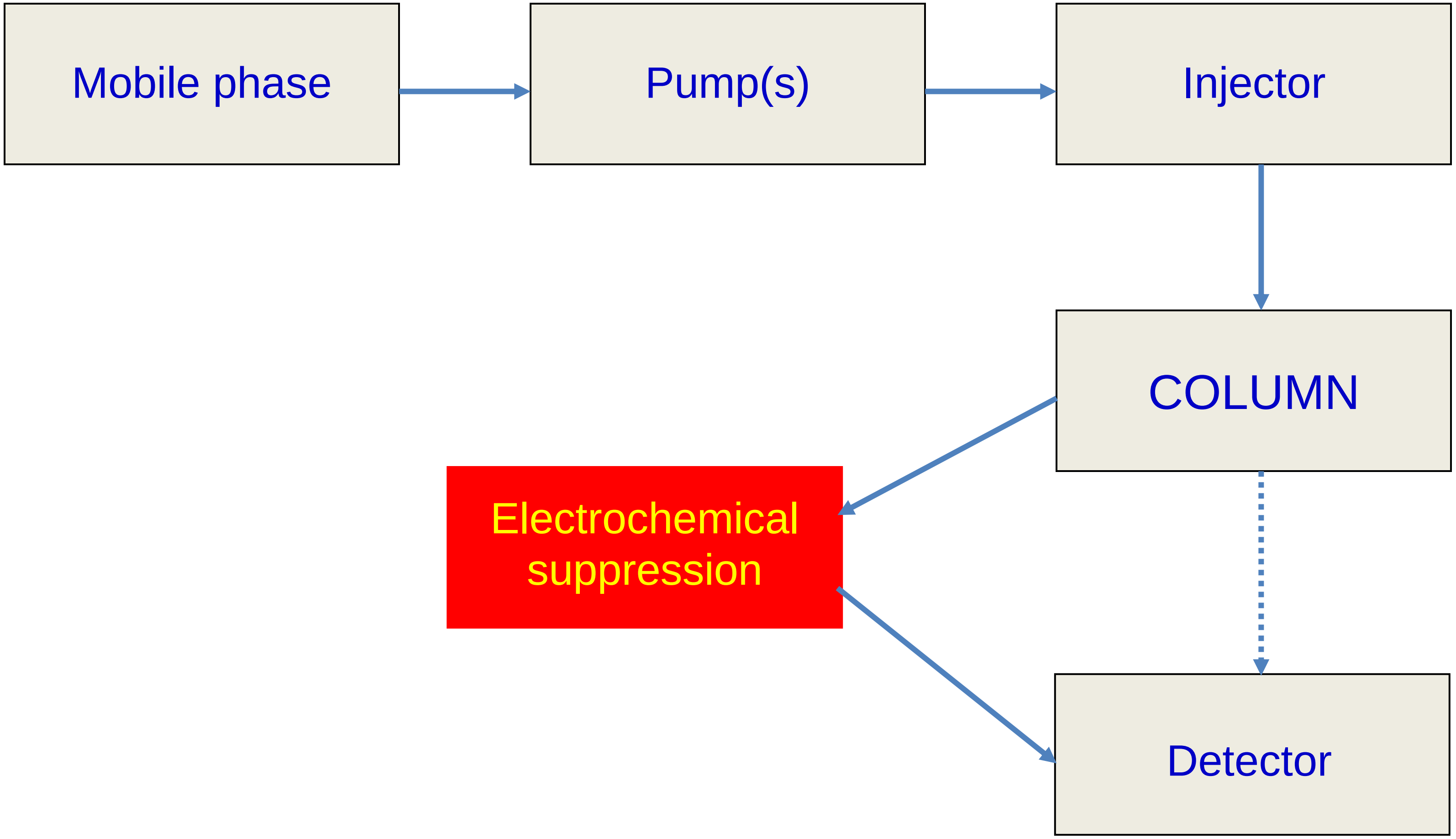
Ionic radius



© 2003 Thomson - Brooks/Cole

The greater the ionic radius, the longer the retention time

4.1. Ion Chromatography: Instrumentation



1. Conductivity detector
2. UV/Vis detector

4.1. Ion Chromatography: detectors

Detection by conductivity

- Theoretical aspects
  - The conductivity of an electrolyte solution is a measure of its ability to conduct electricity (Unity = Siemens.cm^2)
  - For a solution,

$$k = \Sigma(c_i \times |z_i| \times \Lambda_{0i})$$

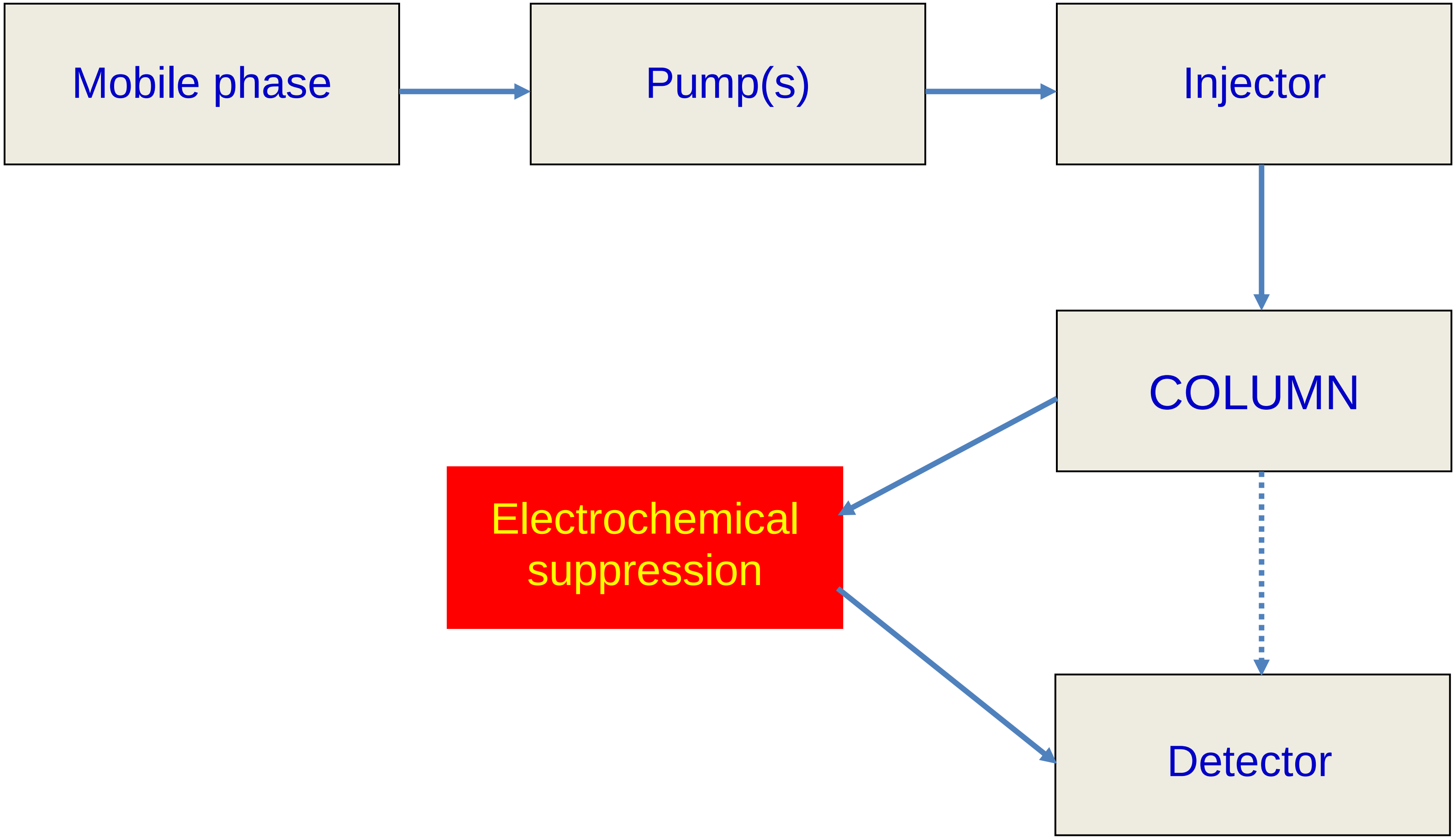
Plus C ↑, plus k ↑

- with Λ_0 = ion molar conductivity (S.cm^2.mol^{-1}) at 25°C

Some ion molar conductivities

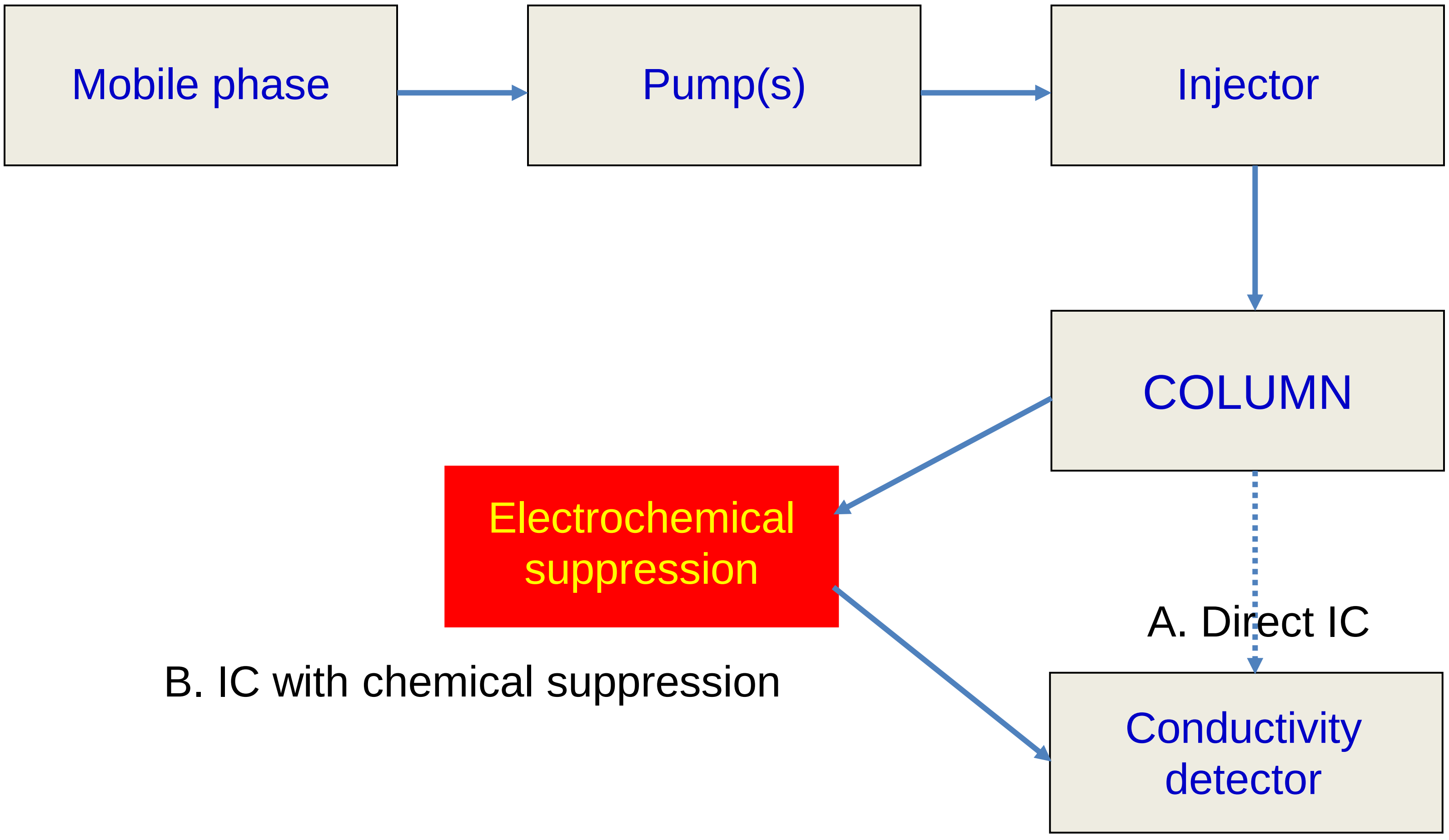
Anions	***Λ_0 (S.cm^2.mol^{-1})***	***Cations***	***Λ_0 (S.cm^2.mol^{-1})***
OH^-	198	H^+	350
F^-	54	Li^+	39
Cl^-	76	Na^+	50
Br^-	78	K^+	74
I^-	77	NH_4^+	73
NO_2^-	72	½ Mg^{2+}	53
NO_3^-	71	½ Ca^{2+}	60
HCO_3^-	45	½ Sr^{2+}	59
$H_2PO_4^-$	33	½ Ba^{2+}	64
SO_4^{2-}	16	½ Zn^{2+}	53
Acétate	41	½ Hg^{2+}	53
Propionate	38	½ Cu^{2+}	55
Salicylate	30	½ Pb^{2+}	71

4.1. Ion Chromatography: Instrumentation



1. Conductivity detector
2. UV/Vis detector

4.1. Ion Chromatography: Instrumentation



Mobile phase

Pump(s)

Injector

COLUMN

Electrochemical suppression

A. Direct IC

B. IC with chemical suppression

Conductivity detector

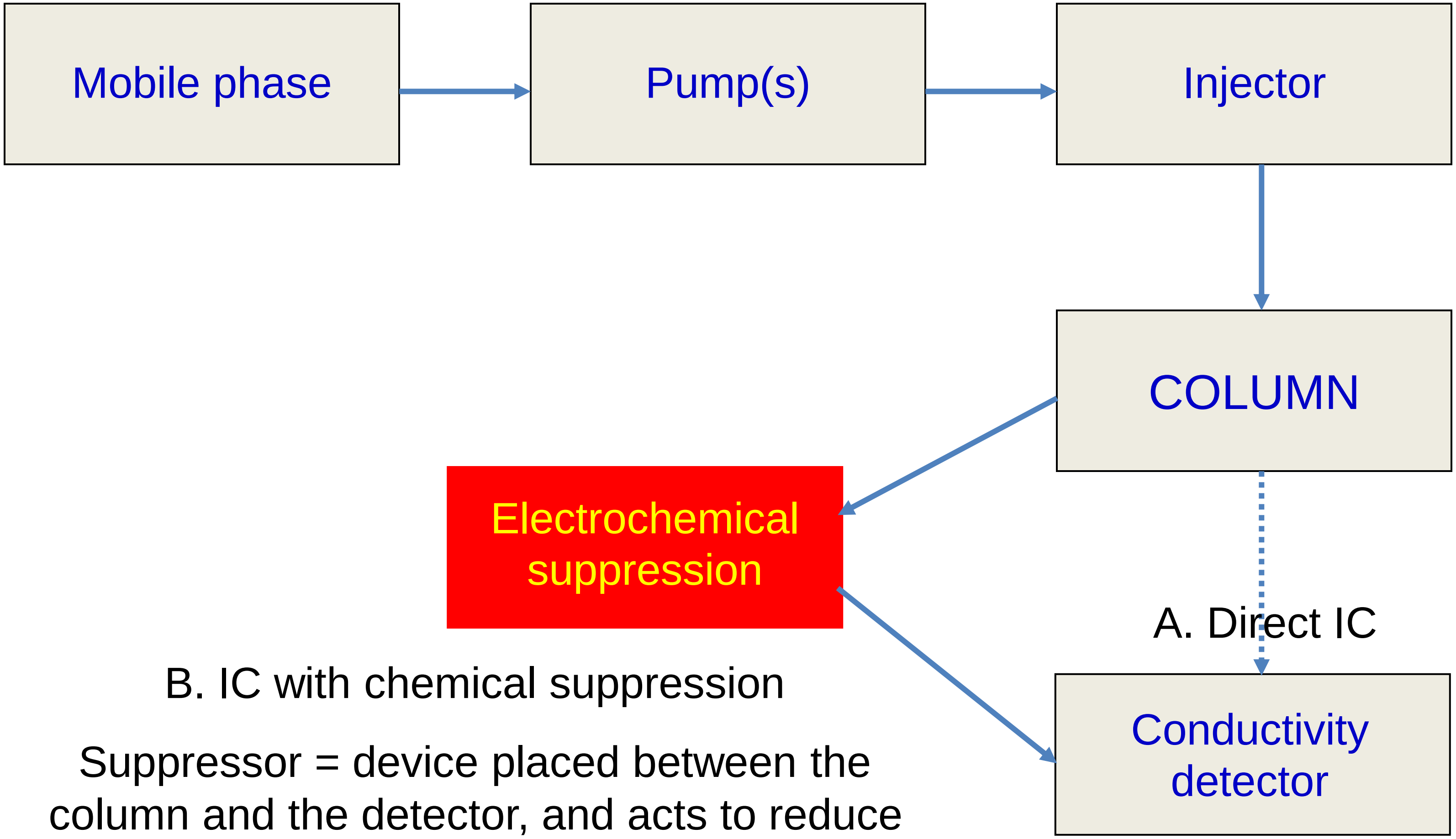
4.1. Ion Chromatography: detectors

A. Direct Ion chromatography

- Total conductivity = electrolyte conductivity - eluent conductivity

- If high background conductivity (eluant conductivity) → problem of sensitivity for low and high concentrations
  - Ideally: Eluant with a low conductivity but a high affinity with solid phase.
- Conductivity depends on the temperature → during the separation and detection the temperature must be kept strictly constant (22 to 55°C)
  - Temperature regulated system ($\Delta T \leq 0.01$°C)

4.1. Ion Chromatography: Instrumentation



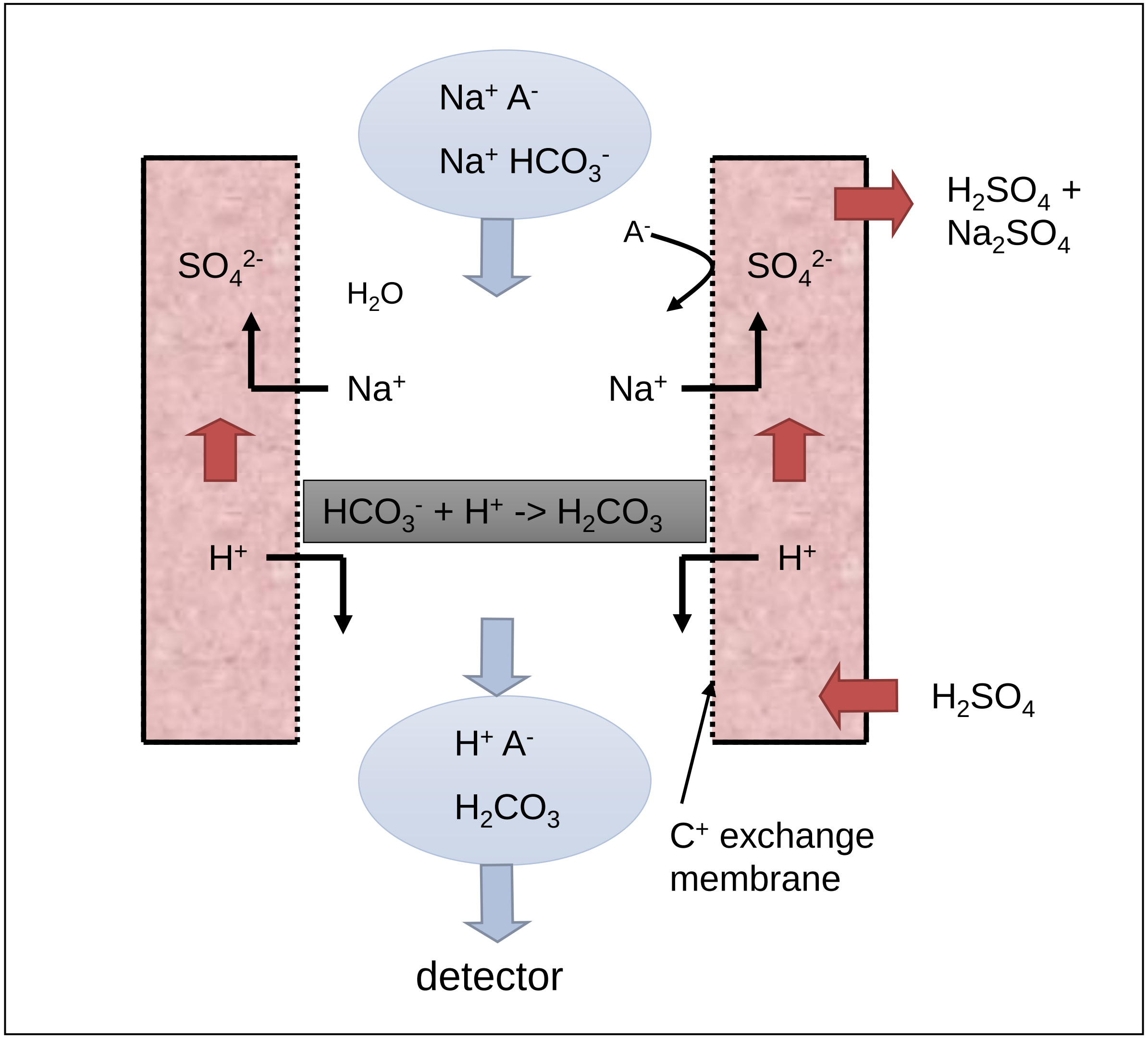
B. IC with chemical suppression

Suppressor = 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.

4.1. Ion Chromatography: detectors

B) Ion chromatography with chemical suppression

Suppressor with cation exchange membrane (for anions analysis)



4.1. Ion Chromatography: detectors

B. Ion chromatography with chemical suppression

- For eluent:

$$Na^+ \;+\; HCO_3^- \xrightarrow[- Na^+]{+ H^+} H_2CO_3$$

$\Rightarrow$ H_2CO_3 (weak acid) partially dissociated → low conductivity

- For studied electrolytes:

$$Na^+ \;+\; Cl^- \xrightarrow[- Na^+]{+ H^+} HCl \rightarrow H^+ + Cl^-$$

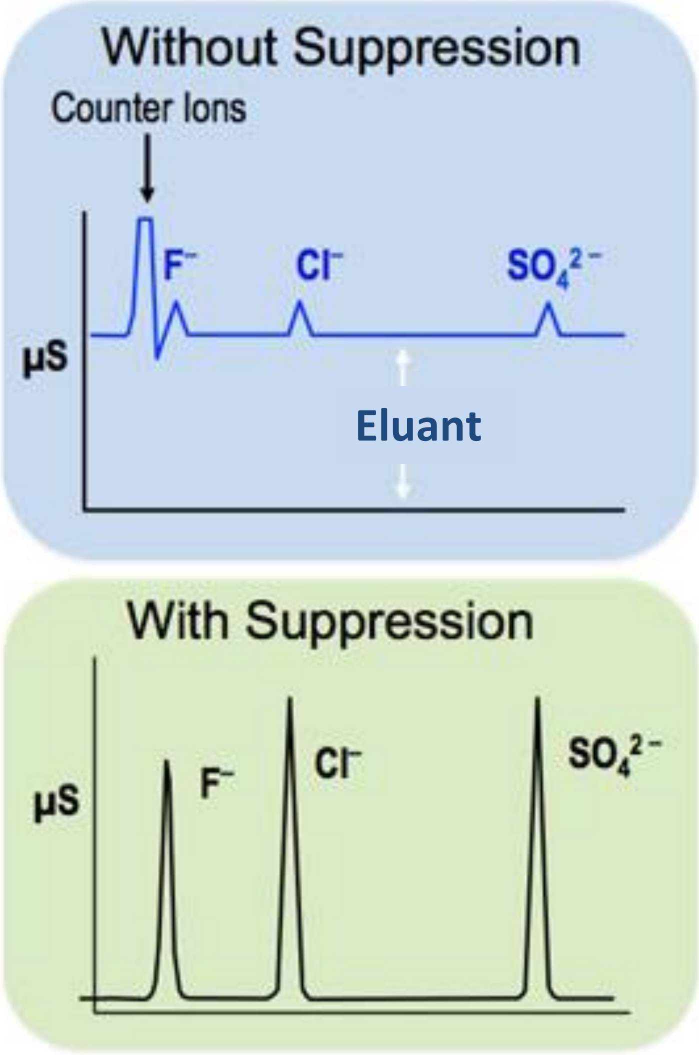
$\Rightarrow$ HCl (strong acid), completly dissociated → highly conductive acid form to enhance its conductive response

Signal to mesure is $\Lambda_0^-(Cl^-)$ on a low background conductivity

4.1. Ion Chromatography: detectors

B) Ion chromatography with chemical suppression

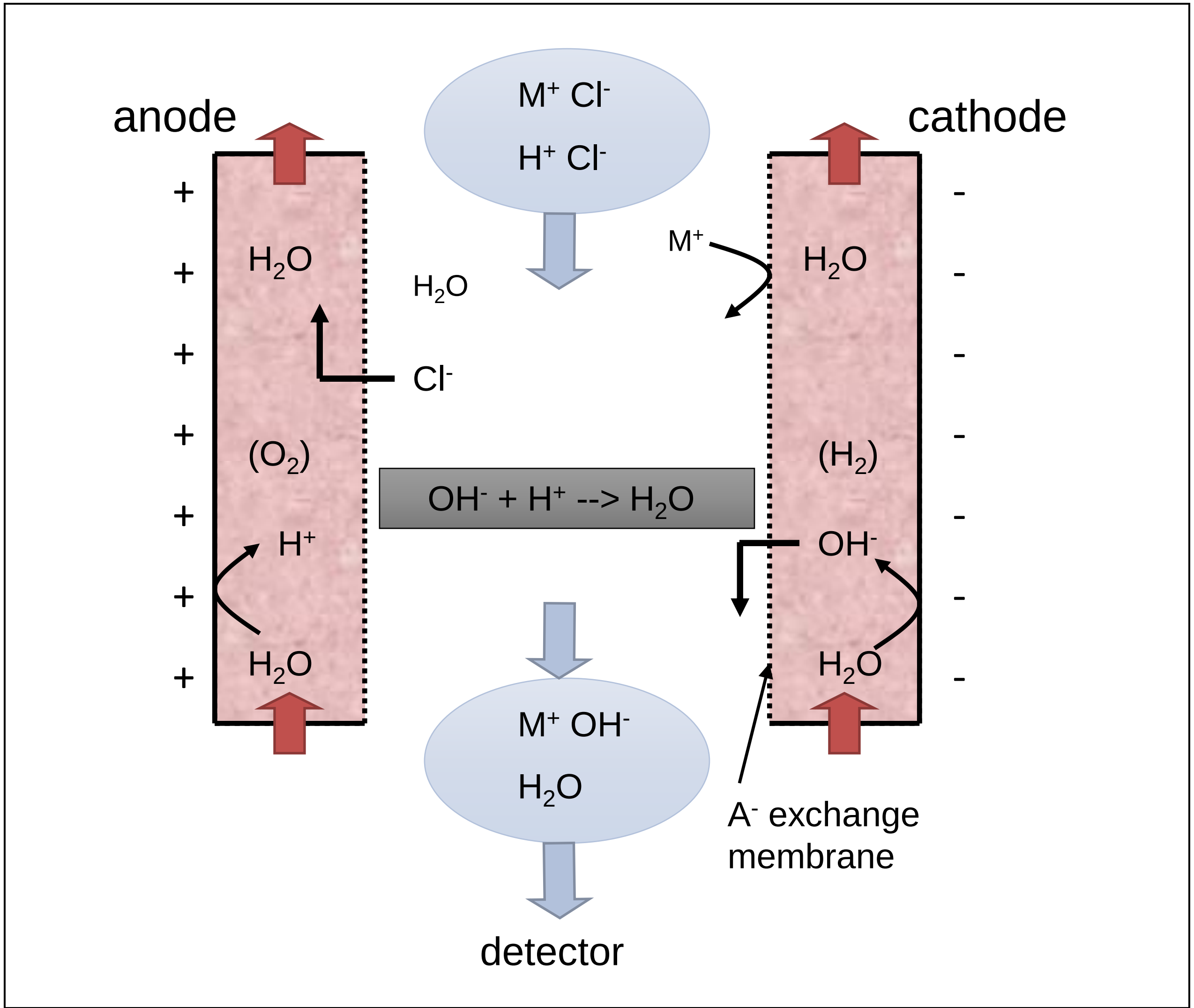
Suppressor with cation
exchange membrane
(for anions analysis)



4. 1. Ion Chromatography: detectors

B) Ion chromatography with chemical suppression

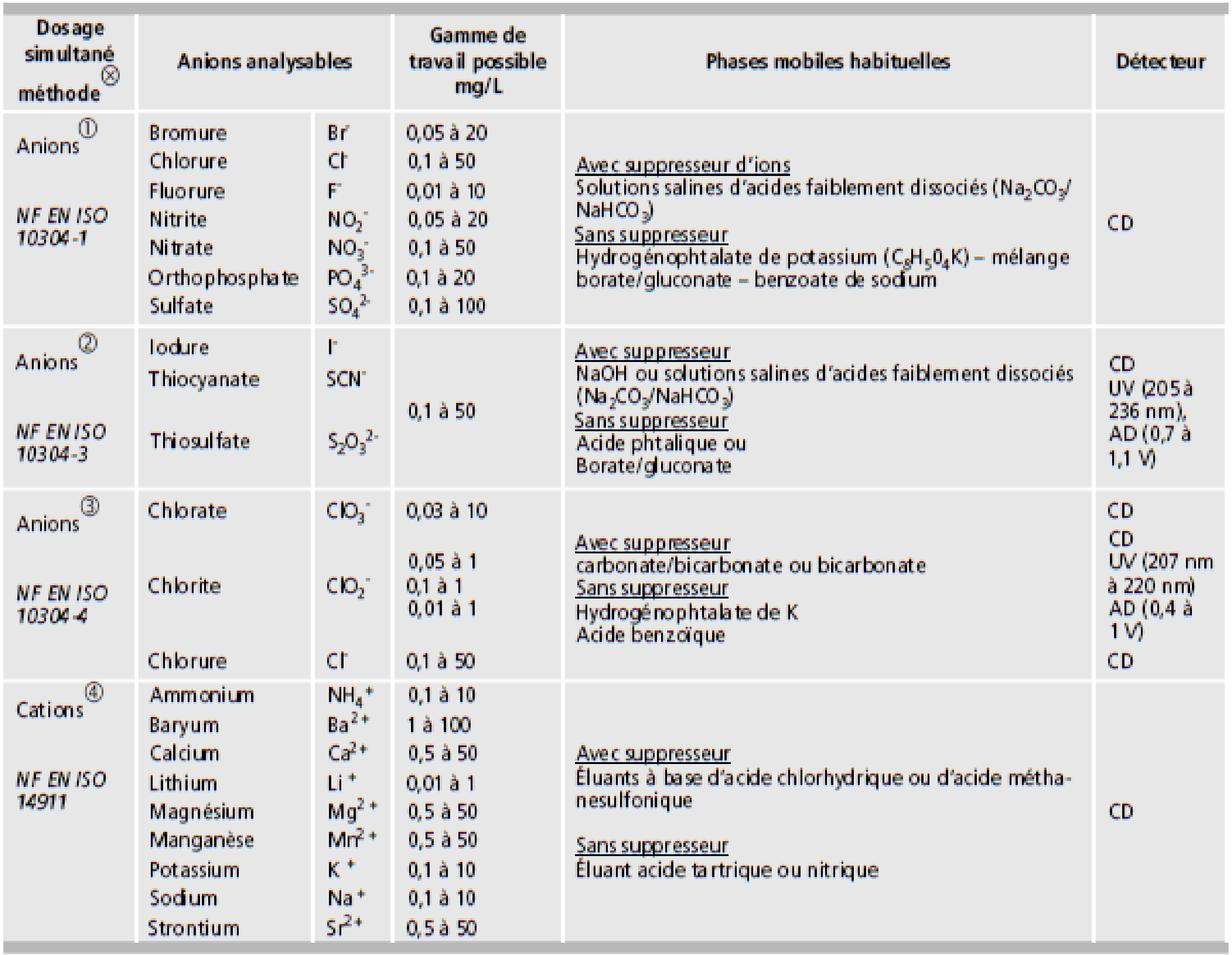
Suppressor with anion exchange membrane autorégénérée (for cation analysis)



4. 1. Ion chromatography: Applications

- Applications:
  - Estimations of regulated species

Qualité de l'eau : Dosage des anions/cations dissous par chromatographie des ions en phase liquide (NF-EN ISO 10304 et 14911)



Dosage simultané méthode ⊗	Anions analysables		Gamme de travail possible mg/L	Phases mobiles habituelles	Détecteur
Anions ① *NF EN ISO 10304-1*	Bromure	Br^-	0,05 à 20	Avec suppresseur d'ions Solutions salines d'acides faiblement dissociés (Na_2CO_3/$NaHCO_3$) Sans suppresseur Hydrogénophtalate de potassium ($C_8H_5O_4K$) – mélange borate/gluconate – benzoate de sodium	CD
	Chlorure	Cl^-	0,1 à 50		
	Fluorure	F^-	0,01 à 10		
	Nitrite	NO_2^-	0,05 à 20		
	Nitrate	NO_3^-	0,1 à 50		
	Orthophosphate	PO_4^{3-}	0,1 à 20		
	Sulfate	SO_4^{2-}	0,1 à 100		
Anions ② *NF EN ISO 10304-3*	Iodure	I^-	0,1 à 50	Avec suppresseur NaOH ou solutions salines d'acides faiblement dissociés (Na_2CO_3/$NaHCO_3$) Sans suppresseur Acide phtalique ou Borate/gluconate	CD UV (205 à 236 nm), AD (0,7 à 1,1 V)
	Thiocyanate	SCN^-			
	Thiosulfate	$S_2O_3^{2-}$			
Anions ③ *NF EN ISO 10304-4*	Chlorate	ClO_3^-	0,03 à 10	Avec suppresseur carbonate/bicarbonate ou bicarbonate Sans suppresseur Hydrogénophtalate de K Acide benzoïque	CD
	Chlorite	ClO_2^-	0,05 à 1 0,1 à 1 0,01 à 1		CD UV (207 nm à 220 nm) AD (0,4 à 1 V)
	Chlorure	Cl^-	0,1 à 50		CD
Cations ④ *NF EN ISO 14911*	Ammonium	NH_4^+	0,1 à 10	Avec suppresseur Éluants à base d'acide chlorhydrique ou d'acide méthanesulfonique Sans suppresseur Éluant acide tartrique ou nitrique	CD
	Baryum	Ba^{2+}	1 à 100		
	Calcium	Ca^{2+}	0,5 à 50		
	Lithium	Li^+	0,01 à 1		
	Magnésium	Mg^{2+}	0,5 à 50		
	Manganèse	Mn^{2+}	0,5 à 50		
	Potassium	K^+	0,1 à 10		
	Sodium	Na^+	0,1 à 10		
	Strontium	Sr^{2+}	0,5 à 50		

Detected ions by IC with conductivity detector

Fluoride
Formate
Acetate
Propionate
Iodate
Chloride
Organophosphates
Hypophosphite
Chloroacetate
Bromate
Chroride
Glycolate
Pyruvate
Nitrite
Dichloroacetate
Phosphite
Phosphate
Selenite
Succinate
Bromide
Malonate
Chlorate
Nitrate
Maleate
Itaconate
Tartarate
Sulfate
Sulfite
Dioxytartarate
Ascorbate
Trichloroacetate
Fumarate
Arsenate
Oxalate
Fluoroborate
Selenate
Thiosulfate
Tungstate
Molybdate
Chromate

IC can also be used to detect organic species:

In particular, carboxylic acids (R-COOH) under their carboxylate form (acetate, formate…)

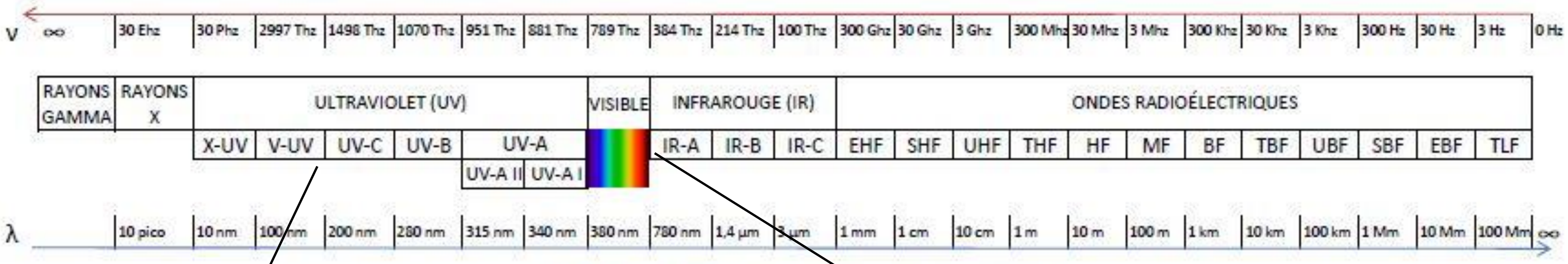
4. Inorganic pollutants analysis

- **Aim** : Ionic species analysis (*cations* or *anions*) : sulphate, nitrate, chloride, ammonium, calcium, metals…

- **Techniques for aqueous phases :**
  - Ion chromatography
  - UV/Vis Spectrometry
  - Ion-Selective Electrodes

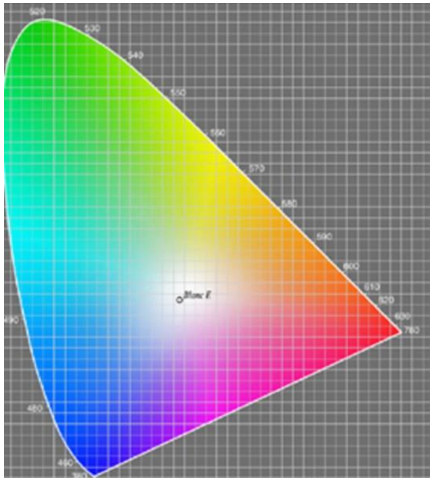
4. 2. UV/Vis Spectrometry

- Theoretical aspects



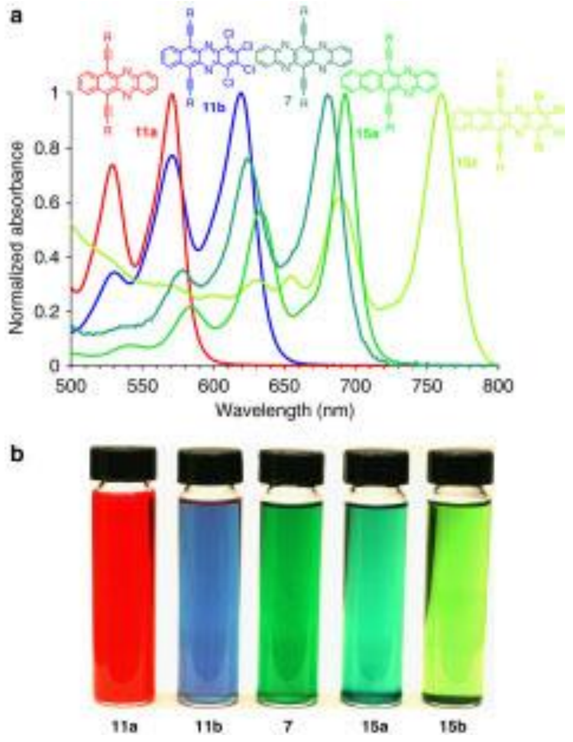
THÉORIE, DOMAINES DU SPECTRE ÉLECTROMAGNÉTIQUE

Range of measurement



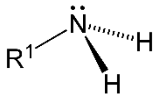
4. 2. UV/Vis Spectrometry

- The measurement by UV-Vis spectrometry concerns in particular:
  - Some colored substances that absorb in the visible spectrum → colorimetry
    - For already absorbing species
    - Either on species that reacted with a reagent to form a chromophore complex → derivatisation

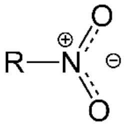


4. 2. UV/Vis Spectrometry

- The measurement by UV-Vis spectrometry concerns in particular:
  - Some colored substances that absorb in the visible spectrum → colorimetry
    - For already absorbing species
    - Either on species that reacted with a reagent to form a chromophore complex → derivatisation
  - Some substances that absorb in the UV spectrum

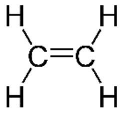


Amine primaire

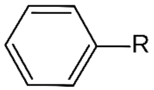


Groupement nitro

Functionnal groups	***λ_{max} en nm***
Amino	195
Aldéhyde	210
Carboxyle	200
Ester	205
Ethylène	190
Cétone	195
Nitro	310
Phényle	200



Ethylène

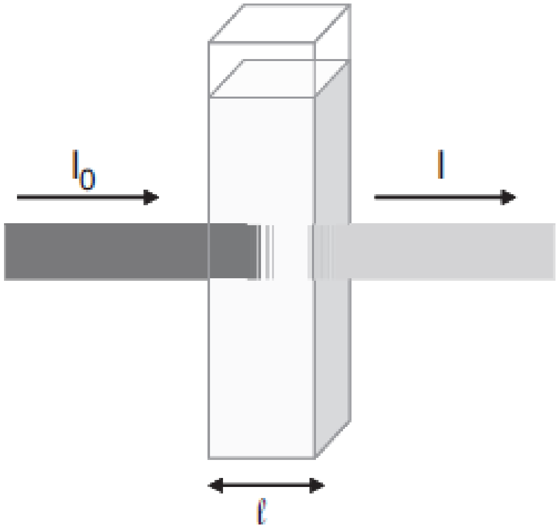


Groupement nitro

4. 2. UV/Vis Spectrometry

- UV-vis spectrometry principle:

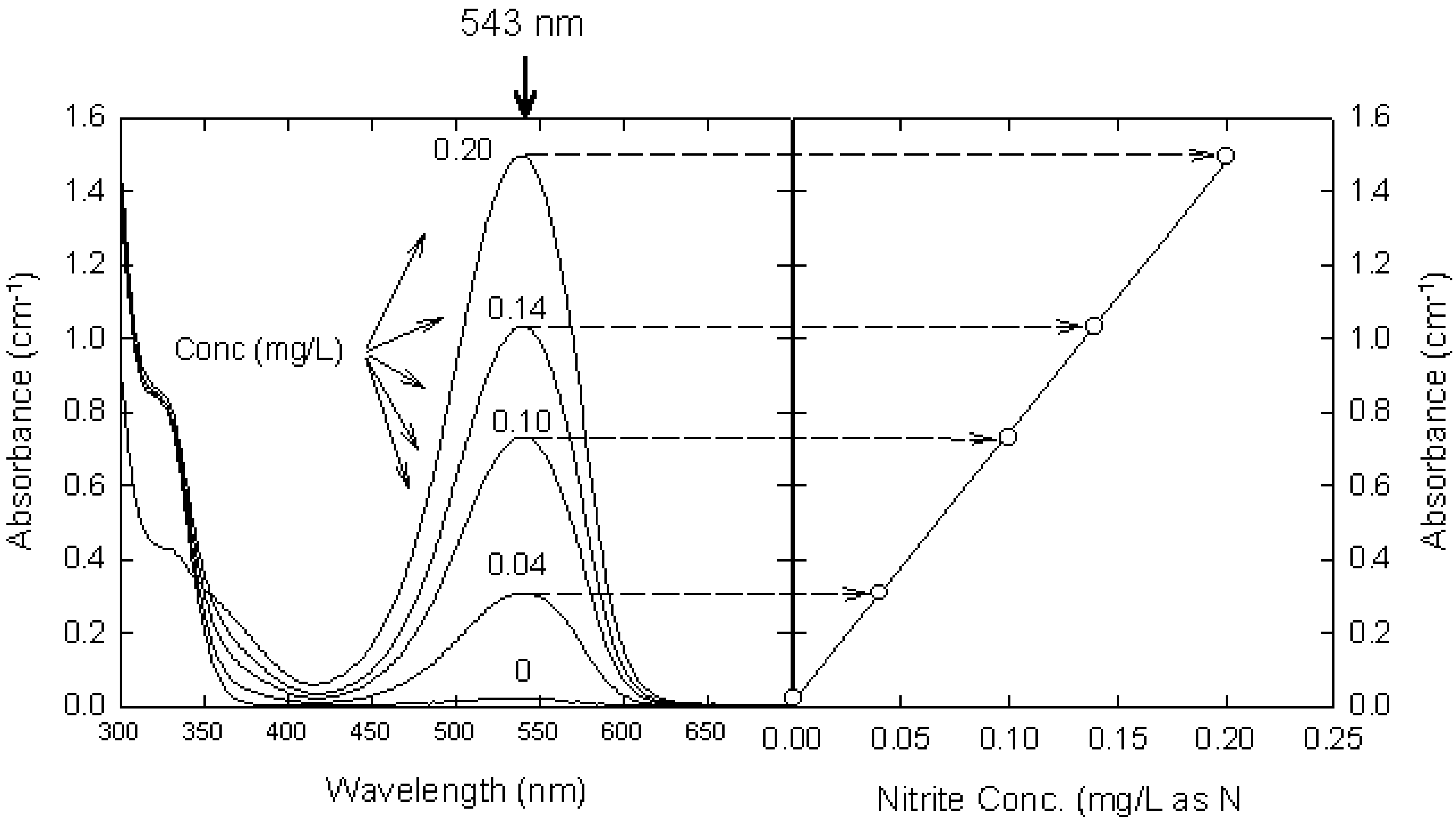
Beer-Lambert law: $\boxed{A = \log (I_0/I) = \varepsilon_\lambda L c}$



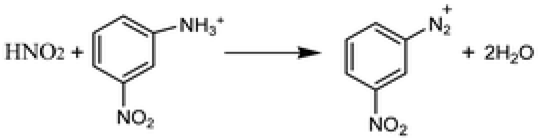
where

- A is the measured absorbance
- I_0 is the initial light intensity
- I is the light intensity after it passes through the sample
- ε_λ wavelength-dependent molar absorptivity coefficient ($mol^{-1}.L.cm^{-1}$)
- L is the path length (cm)
- c is the analyte concentration ($mol.L^{-1}$).

4. 2. UV/Vis Spectrometry

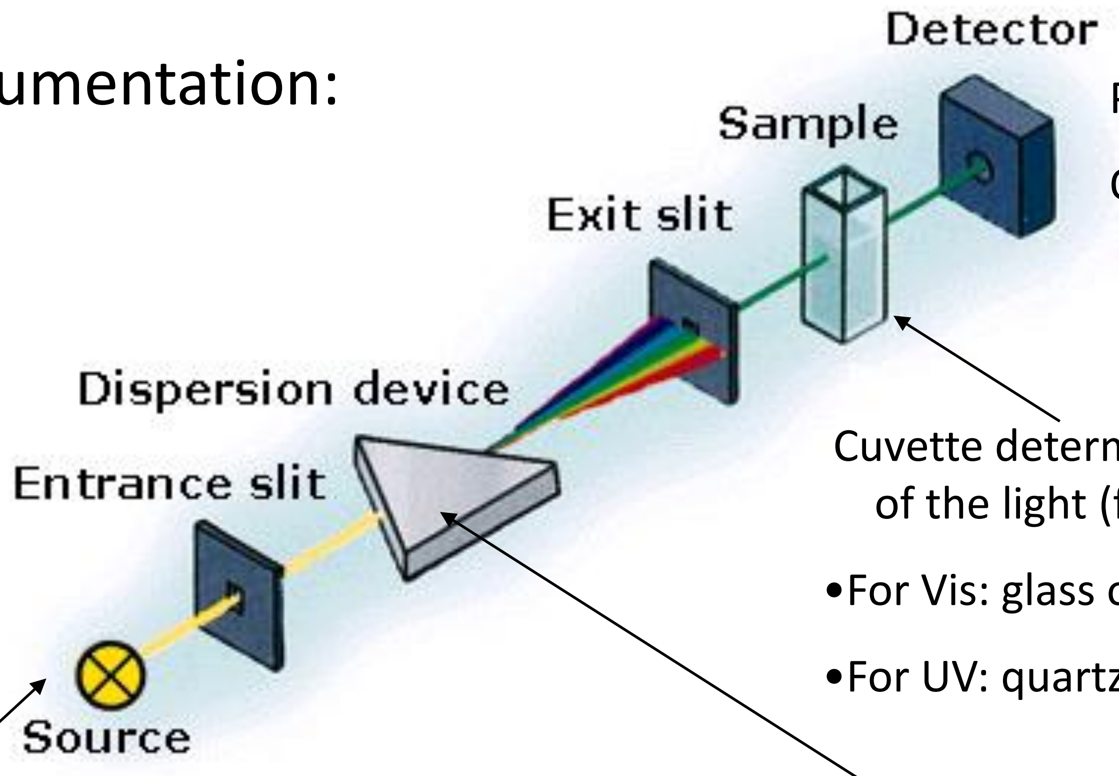


Absorption spectrum of nitrite after derivatization by diazotisation→ formation of a fuschia complex



4. 2. UV/Vis Spectrometry

- Instrumentation:



Photomultiplier tube

Or CCD (solid detector)

Cuvette determines the path length of the light (from 0.5 to 10 cm):

- For Vis: glass or plastic
- For UV: quartz

A beam with a varied wavelength

- For Vis: Tungsten filament (300-2500 nm: lampe à incandescence à filament de Tungstène
- For UV: deuterium arc lamp (190–400 nm)
- For both: Xenon arc lamp (arc à xenon: 160nm to 2000nm)

Monochromator (or prism): To isolate the absorption wavelength of the compound of interest and obtain a parallel beam

4. 2. UV/Vis Spectrometry

- Instrumentation:

4. 2. UV/Vis Spectrometry

- Applications:
  - Ammonium measurements:
    - Colorimetric measurement at 410 nm by derivatization with Nessler reagent in alkaline medium:

$$NH_4^+ + OH^- \rightarrow NH_3 \quad \text{then}$$

$$NH_3 + 2HgI_4^- + 3OH^- \rightarrow \underbrace{HOHgNHHgI}_{\text{Yellow-orange complex}} + 7I^- + 2H_2O$$

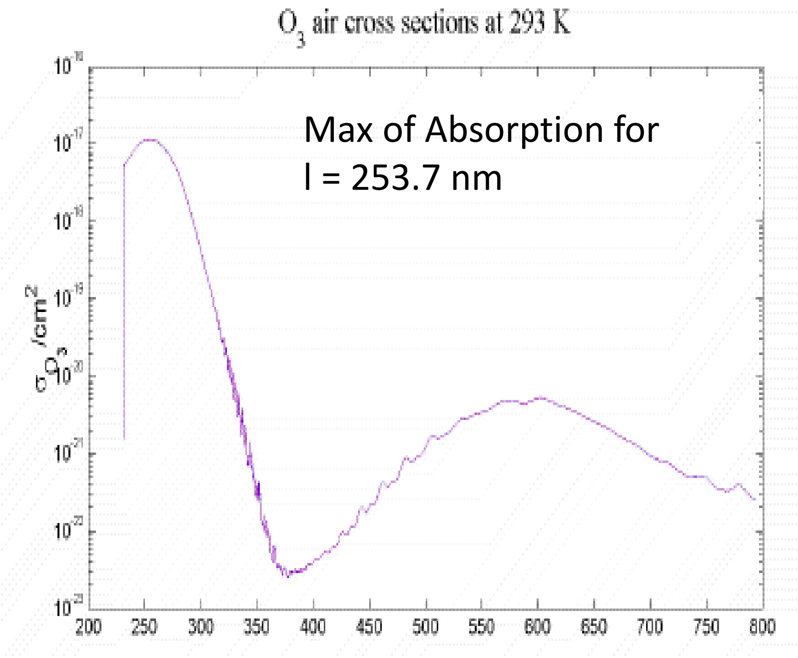
(not adapted for sea water because Cl- is an interferent)

  - Measurements of nitrate, phosphate, silicium…
  - Measurements of gaseous ozone:
    - Analyzer based on 253.7 nm UV spectrometry measurement

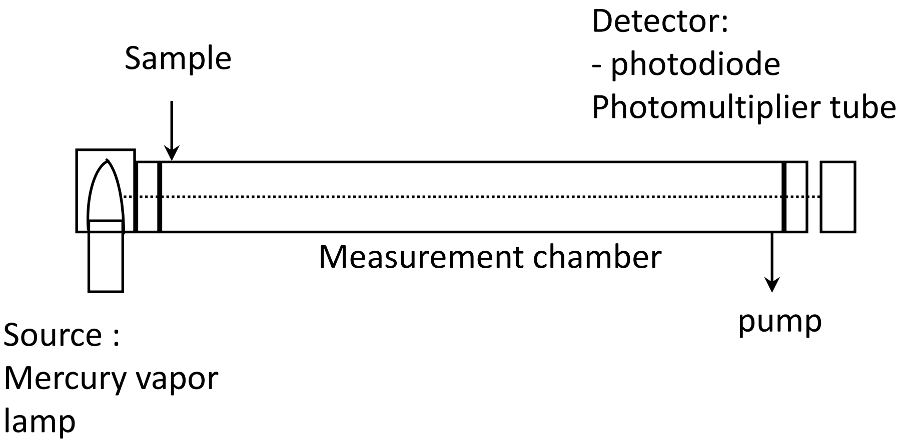
4. 2. UV/Vis Spectrometry

Example: Analyzer of ozone

Ozone has a UV-visible **absorption** spectrum:



Max of Absorption for
l = 253.7 nm



Sample

Detector:
- photodiode
Photomultiplier tube

Measurement chamber

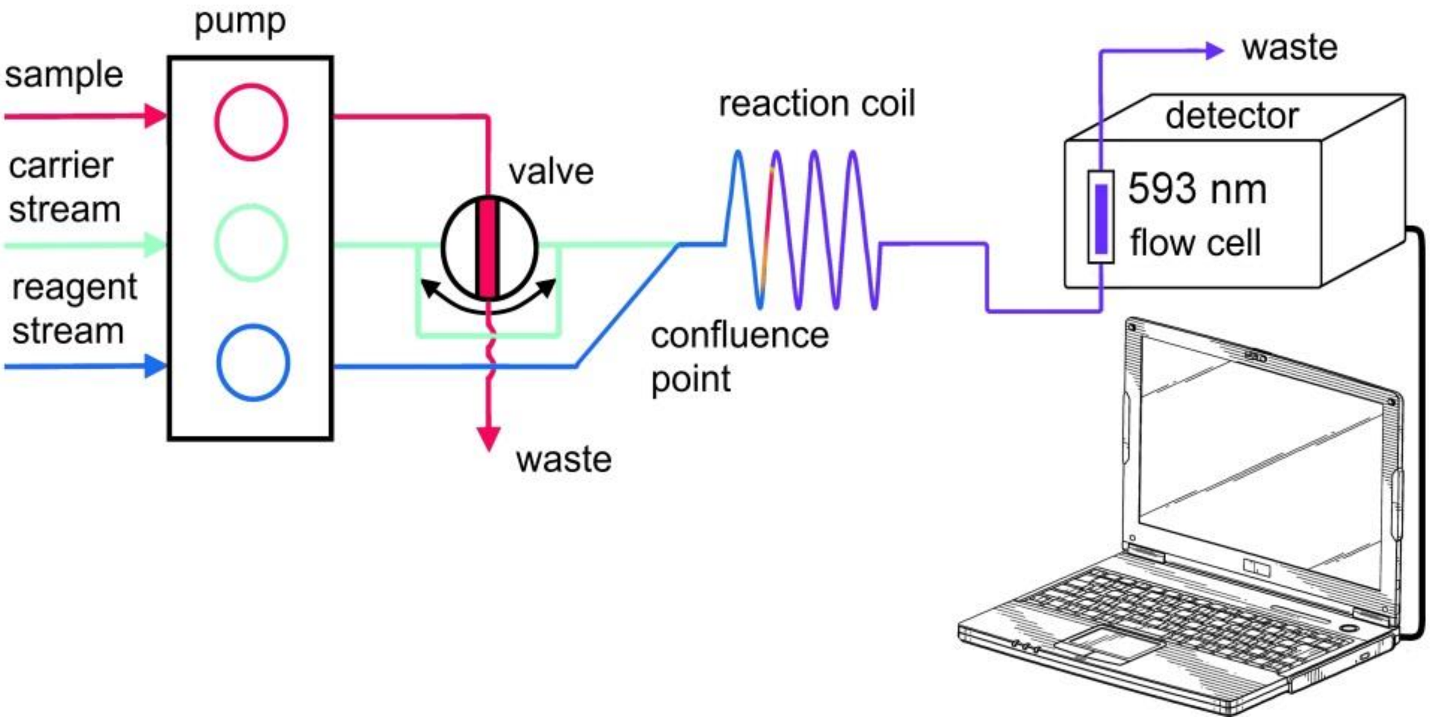
pump

Source :
Mercury vapor
lamp

Continuous measurement of
atmospheric ozone concentrations

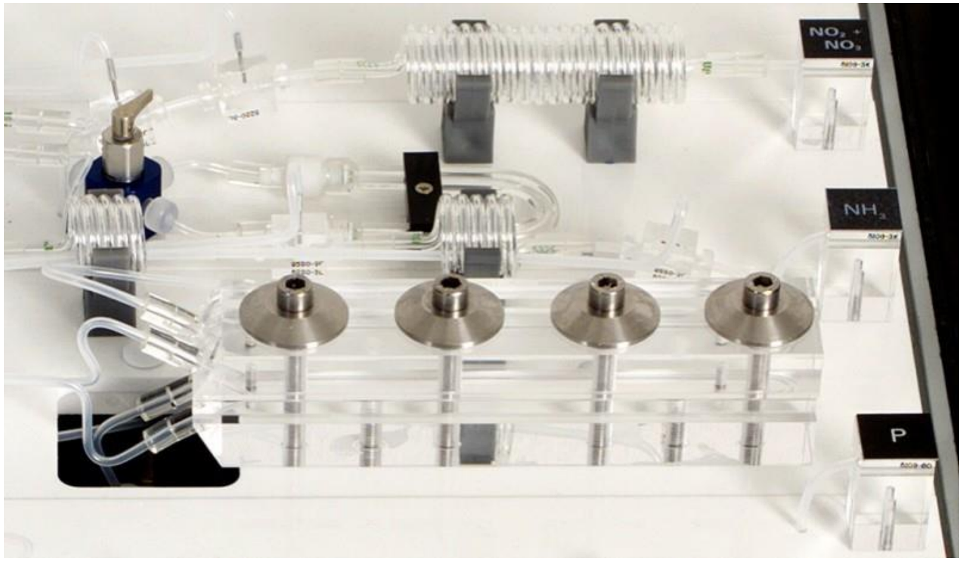
4. 2. UV/Vis Spectrometry

- More advanced techniques:
  - For continuous measurement →Flow analysis (Technicon method (from the manufacturer's name)):
    - <u>Flow Injection Analysis </u>(FIA, *Analyse avec injection en flux continu*): wherein the sample is injected into a moving fluid and moves continuously, without segmentation.



4. 2. Spectrométrie UV-Vis

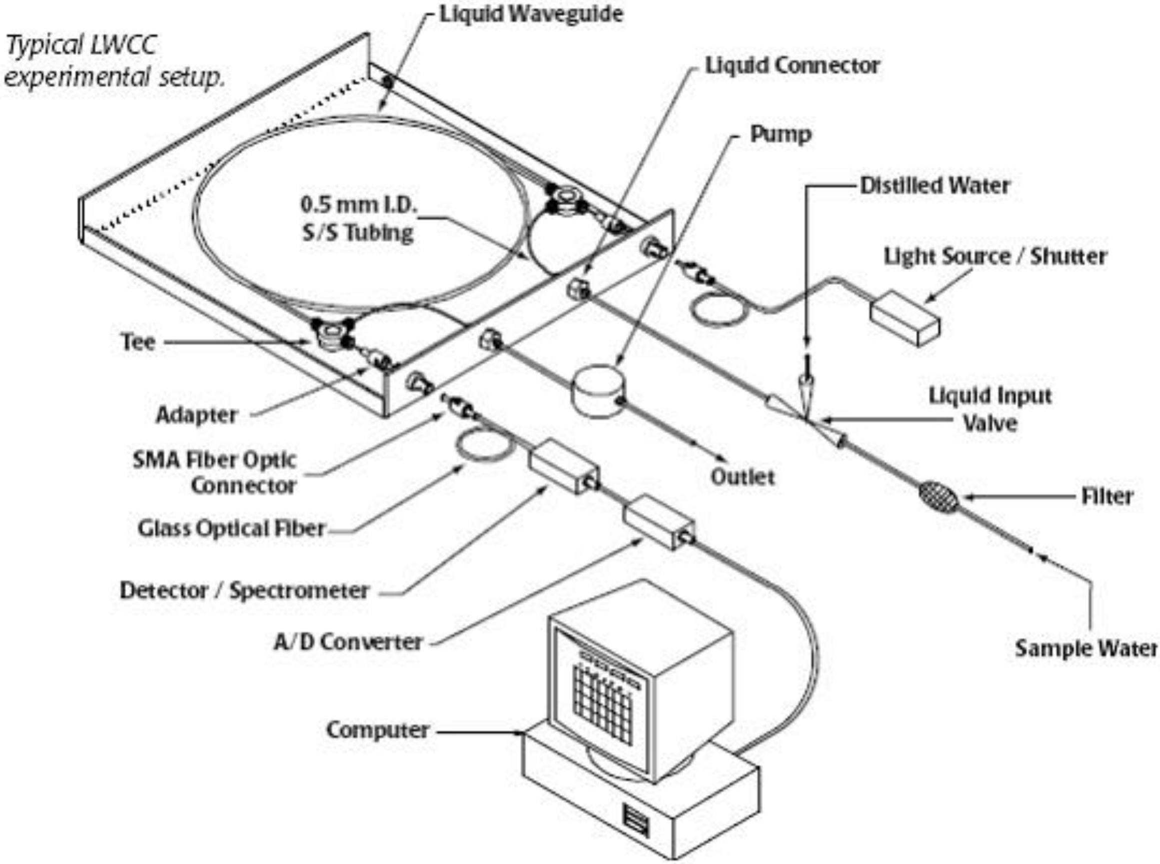
- More advanced techniques:
  - For continuous measurement → Flow analysis (Technicon method (from the manufacturer's name)):
    - Continuous Flow Analysis (CFA, *Analyse en flux continu*) or Segmented Flow Analysis (SFA, analyse en flux segmenté) or Sequencial Flow Analysis (SFA, *analyse par injection séquentielle*), in which the continuous flow is segmented by air bubbles or other segmentation gas → sample differentiation.



4. 2. UV/Vis Spectrometry

- Les techniques plus perfectionnées:
  - Pour les concentrations traces → Guide d'ondes (*LWCC : liquid waveguide capillary cell*): permet des chemins optiques allant de 0.5 à 5 m de 230-730 nm.

Utilisable pour de petits volumes (200µL)

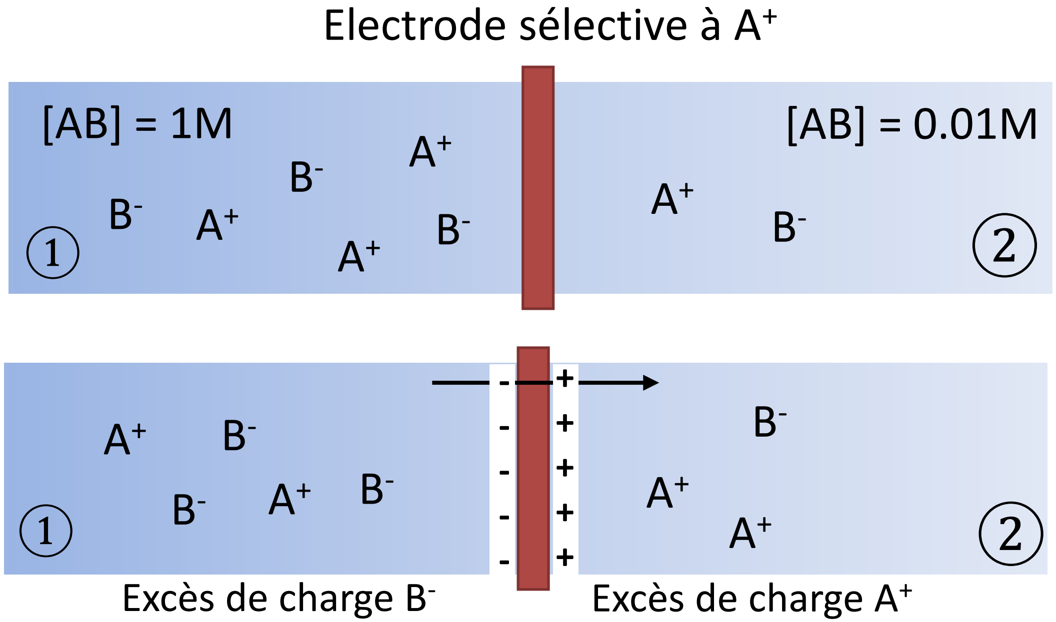

Typical LWCC experimental setup.

4. Inorganic pollutants analysis

- **Aim** : Ionic species analysis (*cations* or *anions*) : sulphate, nitrate, chloride, ammonium, calcium, metals…
- **Techniques for aqueous phases :**
  - Ion chromatography
  - UV/Vis Spectrometry
  - Ion-Selective Electrodes

4.3. Ion-selective electrodes (I.S.E)

- **Principle**:
  - ISE consists of a thin “specific” membrane across which only the intended ion can be transported:



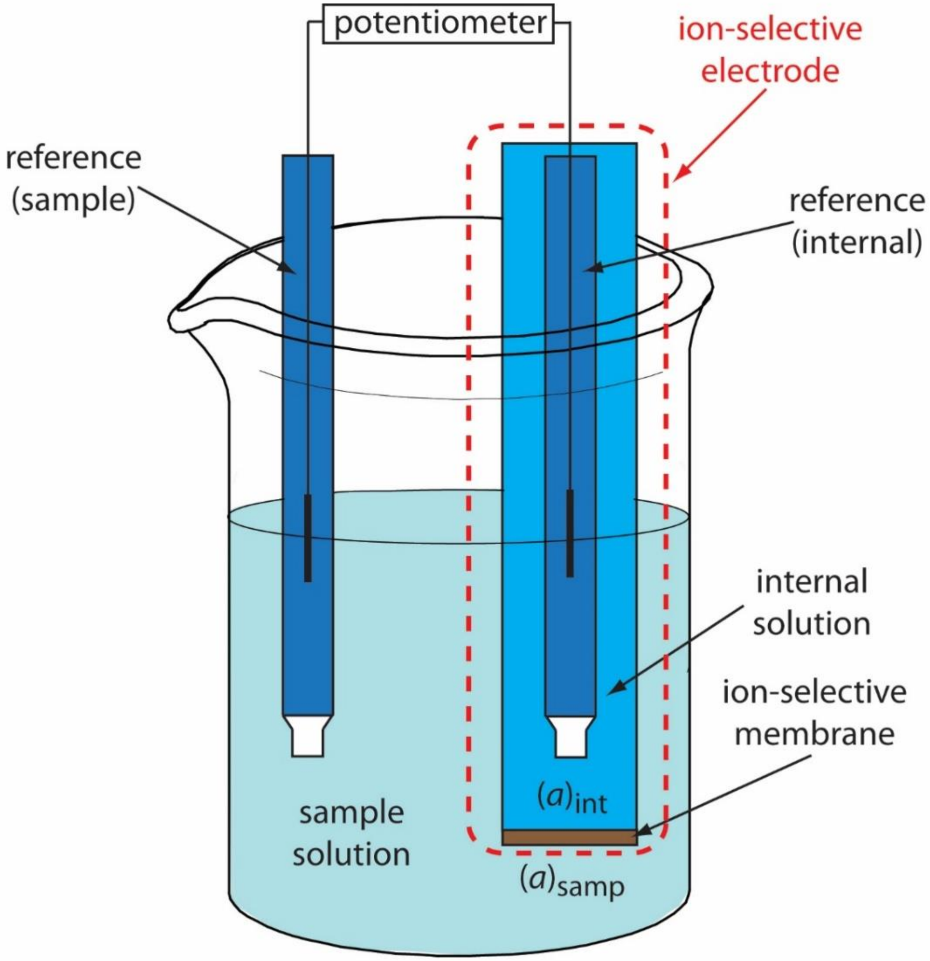
The value of membrane potential is described by the Nerst equation:

$$Em = E° - \frac{0.059}{z}\log(\frac{a_{1)}}{a_2})$$

4.3. Ion-selective electrodes (I.S.E)

- **Principle**:
  - If the membrane separates a sample and an internal reference solution:

$$Em = E° - \frac{0.059}{z}\log(\frac{a_{ech)}}{a_{int}})$$



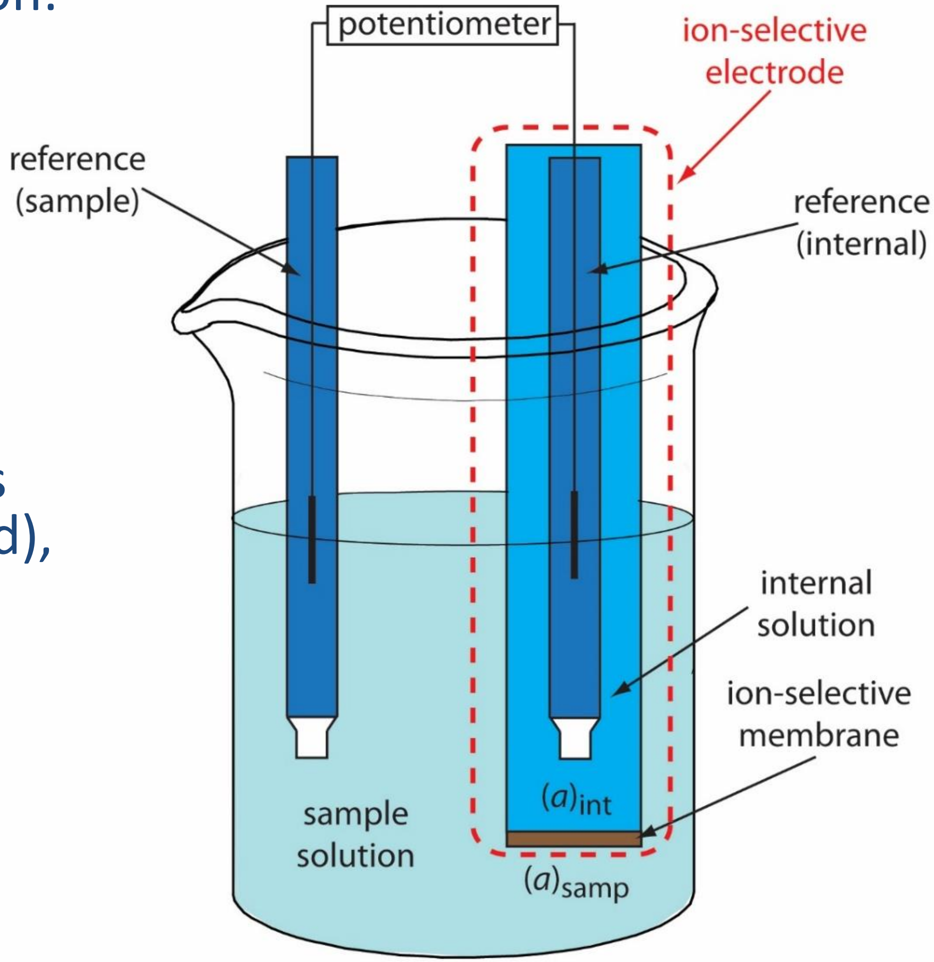
4.3. Ion-selective electrodes (I.S.E)

- **Principle**:
  - If the membrane separates a sample and an internal reference solution:

$$Em = E° - \frac{0.059}{z}\log(\frac{a_{ech)}}{a_{int}})$$

  - If we consider that the activity is fixed (because very concentrated), then

$$Em = cste - \frac{0.059}{z}\log(a_{ech})$$



4. 3. Ion-selective electrodes (I.S.E)

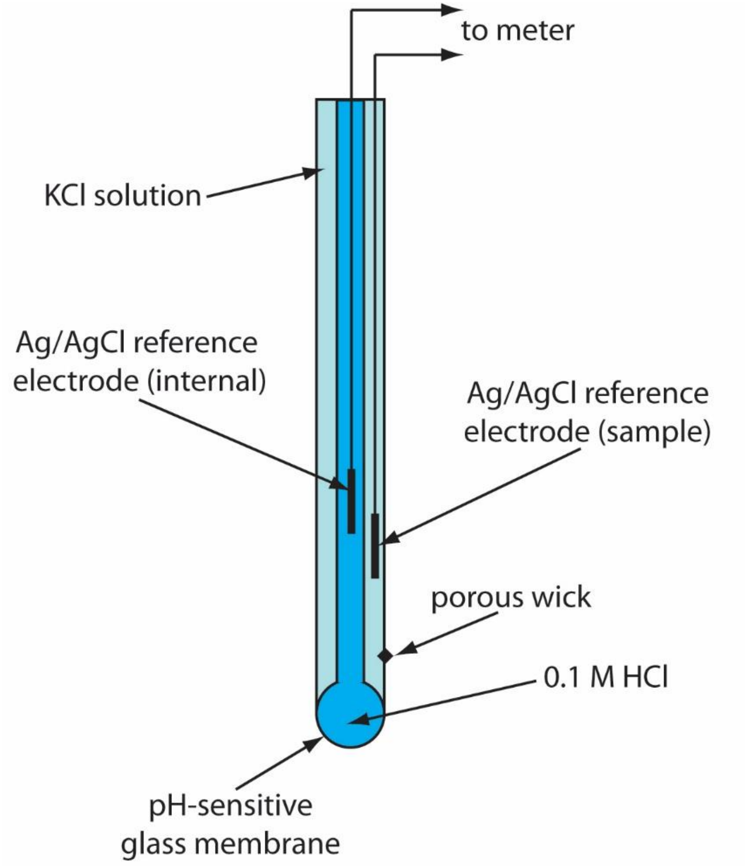
- Ionic Strength Adjustment Buffer (Tampon ionique, ISAB or ISA):

$$a = \gamma[c]$$

  - It is used to adjust the ionic strength of the solution to be analyzed so that a concentration is measured and not an activity.
  - An ionic strength buffer, ISA, is a high ionic strength solution that does not interfere with the sample and equals the ionic strength of the standards and samples.
  - For each electrode, commercial providers give the ISA buffer to use

4. 3. Ion-selective electrodes (I.S.E)

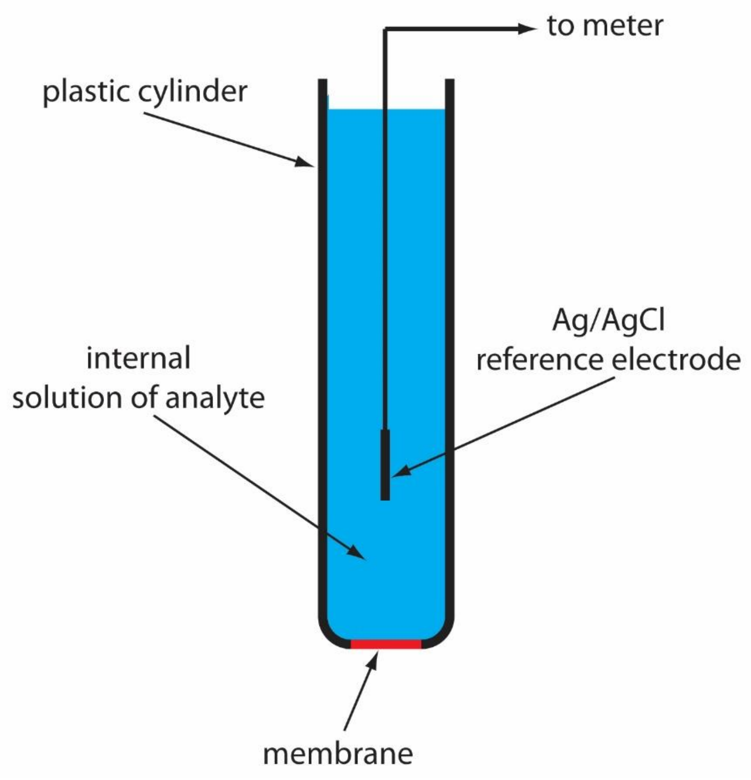
- In general the two electrodes are combined
- The different types of electrodes:
  - Glass electrodes (*Electrode de verre ou électrode pH*)



- Glass membranes are selective to cations
- For pH electrode, it's selective for H^+, but also for: Li^+, K^+, Rb^+, Cs^+, NH_4^+, Ag^+

4. 3. Ion-selective electrodes (I.S.E)

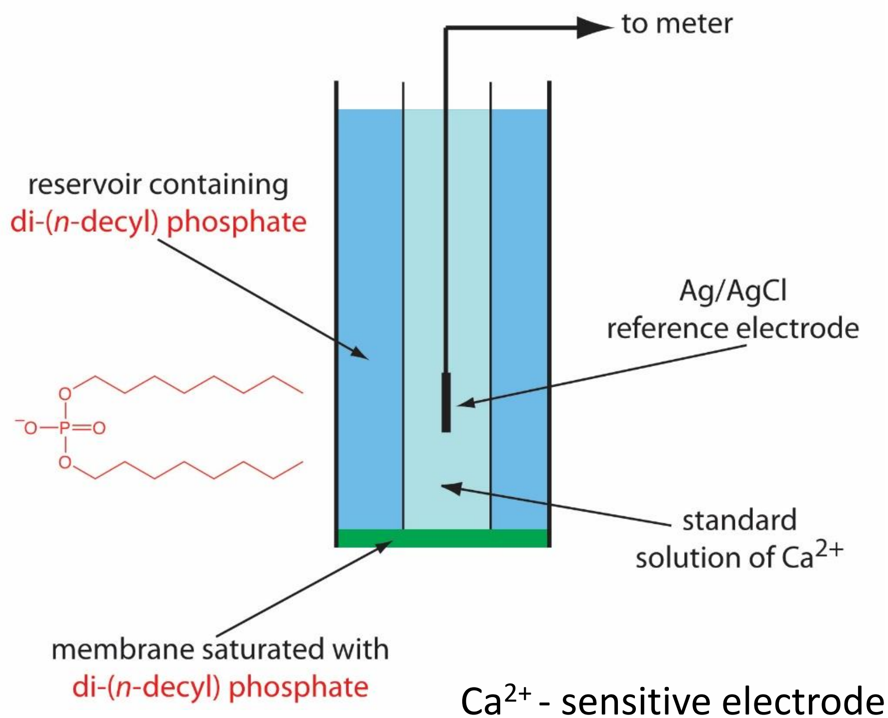
- In general the two electrodes are combined
- The different types of electrodes:
  - Glass electrodes (*Electrode de verre ou électrode pH*)
  - Solid-state ISE (Electrode avec membrane à corps solide)



- Membrane in a crystalline material of inorganic salt (s) consisting of the analyte
- The most common is the fluoride electrode (F-) with a LaF_3 crystal

4. 3. Ion-selective electrodes (I.S.E)

- In general the two electrodes are combined
- The different types of electrodes:
  - Glass electrodes *(Electrode de verre ou électrode pH)*
  - Solid-state ISE *(Electrode avec membrane à corps solide)*
  - Liquid-based ISE *(Electrode à membrane en polymère synthétique)*

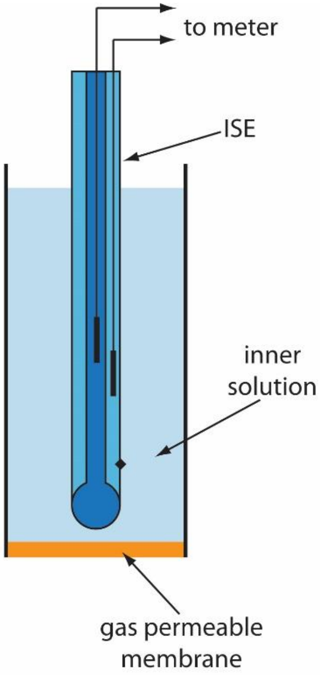


Hydrophobic membrane containing an organic liquid that makes the selectivity → organic complex anion or cation exchanger

Ca^{2+} - sensitive electrode

4. 3. Ion-selective electrodes (I.S.E)

- In general the two electrodes are combined
- The different types of electrodes:
  - Glass electrodes *(Electrode de verre ou électrode pH)*
  - Solid-state ISE *(Electrode avec membrane à corps solide)*
  - Liquid-based ISE *(Electrode à membrane en polymère synthétique)*
  - Gas-sensing electrode (*Electrode sensible aux gaz*)



- Permeable gas membrane, then potential measured by internal ISE which measures the dissolved gas or the reactive species:

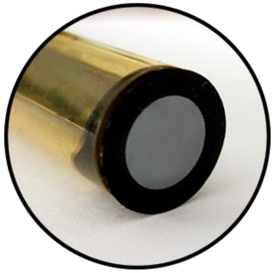
$\mathbf{CO_2(\mathit{aq})} + 2H_2O(\mathit{l})\text{f} \leftrightharpoons HCO_3^-(\mathit{aq}) + \mathbf{H_3O^+(\mathit{aq})}$

$\mathbf{NH_3(\mathit{aq})} + H_2O(\mathit{l})\text{f} \leftrightharpoons \mathbf{NH_4^+(\mathit{aq})} + OH^-(\mathit{aq})$

With pH-senstive glass electrode

4. 3. Ion-selective electrodes (I.S.E)

- Specificities of the method:
  - No effect of turbidity, the presence of suspended matter.
  - Very fast response (some seconds)
  - Limit to a pH range depending on the membrane
  - If the species to be measured is not "free", i.e. if it is complexed with other species or absorbed, it can not be measured → Sample preparation required
  - Interference problem: Other ions can disrupt the measurement → Sample preparation needed

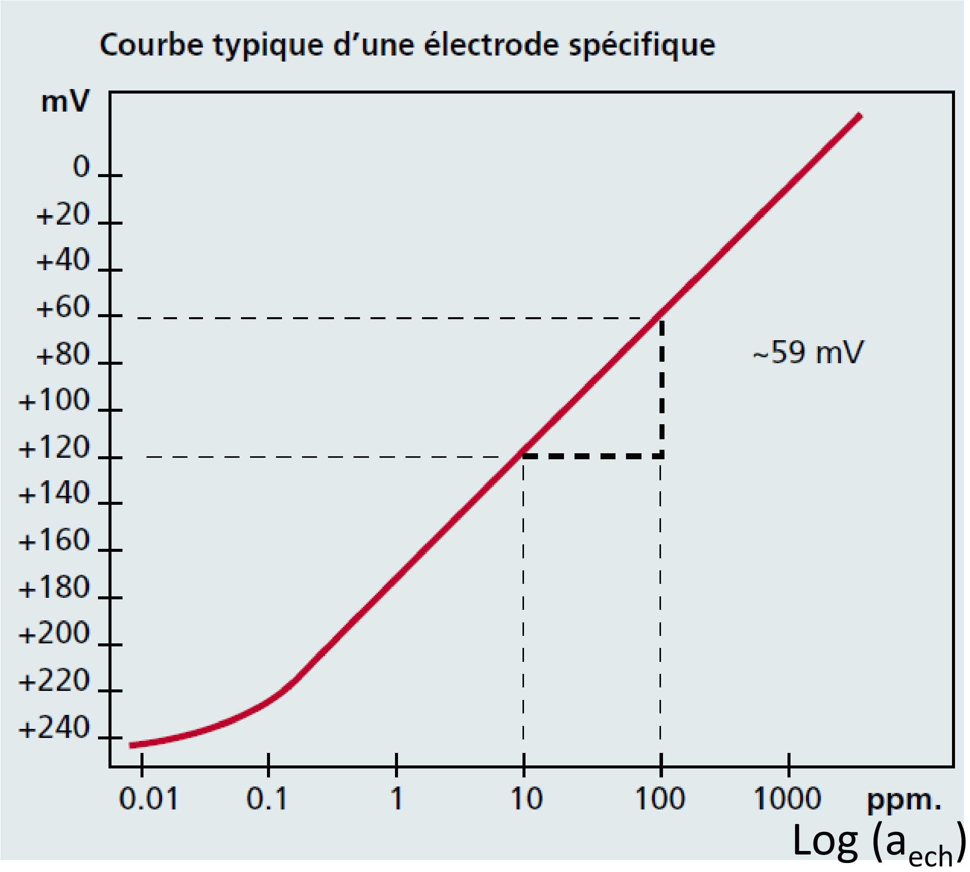

4. 3. Ion-selective electrodes (I.S.E)

- Specificity of the method:

Ion electrode	Membrane	Concentration range/mol dm^{-3}	Major interferences
K^+	Valinomycin	10^{-6}–1	Cs^+, NH_4^+
Na^+	Glass	10^{-6}–sat.	Ag^+, H^+, Li^+
F^-	LaF_3	10^{-6}–sat.	OH^-, H^+
Cl^-	$Ag_2S/AgCl$	10^{-5}–1	Br^-, I^-, CN^-, S^{2-}
Br^-	$Ag_2S/AgBr$	10^{-6}–1	I^-, CN^-, S^{2-}
I^-	Ag_2S/AgI	10^{-7}–1	CN^-, S^{2-}
CN^-	Ag_2S/AgI	10^{-6}–10^{-2}	I^-, S^{2-}
Cd^{2+}	CdS/Ag_2S	10^{-7}–1	Ag^+, Hg^{2+}, Cu^{2+}
Pb^{2+}	PbS/Ag_2S	10^{-7}–1	Ag^+, Hg^{2+}, Cu^{2+}
Cu^{2+}	CuS/Ag_2S	10^{-8}–sat.	Ag^+, Hg^{2+}, S^{2-}
Ca^{2+}	$(RO)_2PO^-/(RO)_3PO$	10^{-5}–10^{-1}	Zn^{2+}, Fe^{2+}, Pb^{2+}, Cu^{2+}
$Ca^{2+}+Mg^{2+}$ (hardness)	$(RO)_2PO\$/ROH$	10^{-7}–1	Cu^{2+} Zn^{2+} Fe^{2+}, Ni^{2+}, Pb^{2+}
NO_3^-	R_4N^+/ether	10^{-5}–1	ClO_4^-, ClO_4^-, I^-, Br^-

4. 3. Ion-selective electrodes (I.S.E)

- Signal: Potential (mV) is increasing with decreasing concentration



$$Em = cste - \frac{0.059}{z}\log(a_{ech})$$

Pente

- Pour z = 1 (ex. NH_4^+) → ~-59 mV
- Pour z = 2 (ex. Ca^{2+}) → ~-29.5 mV

4. Soluble inorganic pollutants analysis

Multi-ions

Mono-ion

Ion chromatography	**UV-Vis Spectrometry**	**ISE**
Expensive	Cheap	Cheap
In laboratory	Field	Field or laboratory
Sensitive	Fast and low volume	Continuous measurements

All methods are used depending on the situation

Course Outline

1. General informations on chemical analysis
2. Sample preparation
3. Metals analysis
4. Soluble inorganic pollutants analysis
5. **Organic pollutants analysis**
6. Quality assurance

5. Organic pollutants analysis

- Several types of pollutants regulated or under recommendation :
  - Carbonyl compounds (aldehydes et ketones): formaldehyde, benzaldehyde.. → VOC (Volatile Organic compounds)
  - Monocyclic hydrocarbons: BTEX (Benzene, Toluene, Ethyl-benzene, Xylene)
  - Persistent organic pollutants (POP) are bioaccumulative, toxic and mobile compounds :
    - Plant protection chemicals: herbicides, pesticides and fungicides (organochlorine compounds (DDT), triazines, carbamates, etc.)
    - Polychlorinated biphenyls (PCBs): industrial sources (oils, lubricants, adhesives, etc.): Completely banned for 20 years, but not biodegradable
    - PAHs (Polycyclic Aromatic Hydrocarbons), dioxins, furans
    - Medicines and hormones (pill) + cosmetics

- Ubiquitous in different compartment: soil, water, air (particles/gas)
  - Sources: Industry (combustion/solvant), agriculture, road traffic…

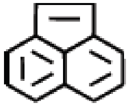
- Environmental impacts:
  - In water: High turbidity → risk of eutrophication
  - Toxicity to animals and humans: irritants, carcinogens, ...
  - Key role in atmospheric and aqueous chemistry

5. Analyse des polluants organiques

PAH: both in the volatized or particulate forms:

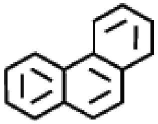
Naphtalène
($C_{10}H_8$)

Acénaphtène
($C_{12}H_{10}$)



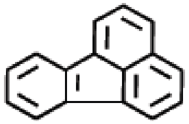
Acénaphtylène
($C_{12}H_8$)

Fluorène
($C_{13}H_{10}$)

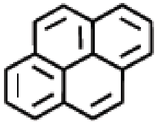


Phénanthrène
($C_{14}H_{10}$)

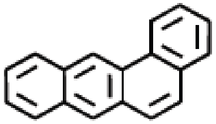
Anthracène
($C_{14}H_{10}$)



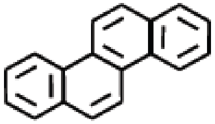
Fluoranthène
($C_{16}H_{10}$)



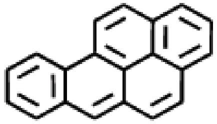
Pyrène
($C_{16}H_{10}$)



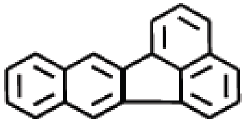
Benzo(a)anthracène
($C_{18}H_{12}$)



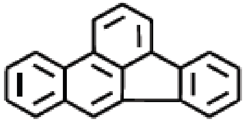
Chrysène
($C_{18}H_{12}$)



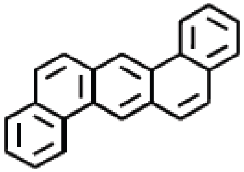
Benzo(a)pyrène
($C_{20}H_{12}$)



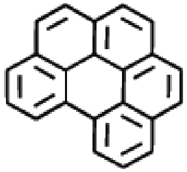
Benzo(k)fluoranthène
($C_{20}H_{12}$)



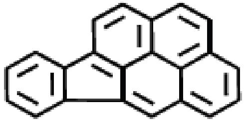
Benzo(b)fluoranthène
($C_{20}H_{12}$)



Dibenz(a,h)anthracène
($C_{22}H_{14}$)



Benzo(ghi)pérylène
($C_{21}H_{16}$)



Indéno(1,2,3-cd)pyrène
($C_{22}H_{12}$)

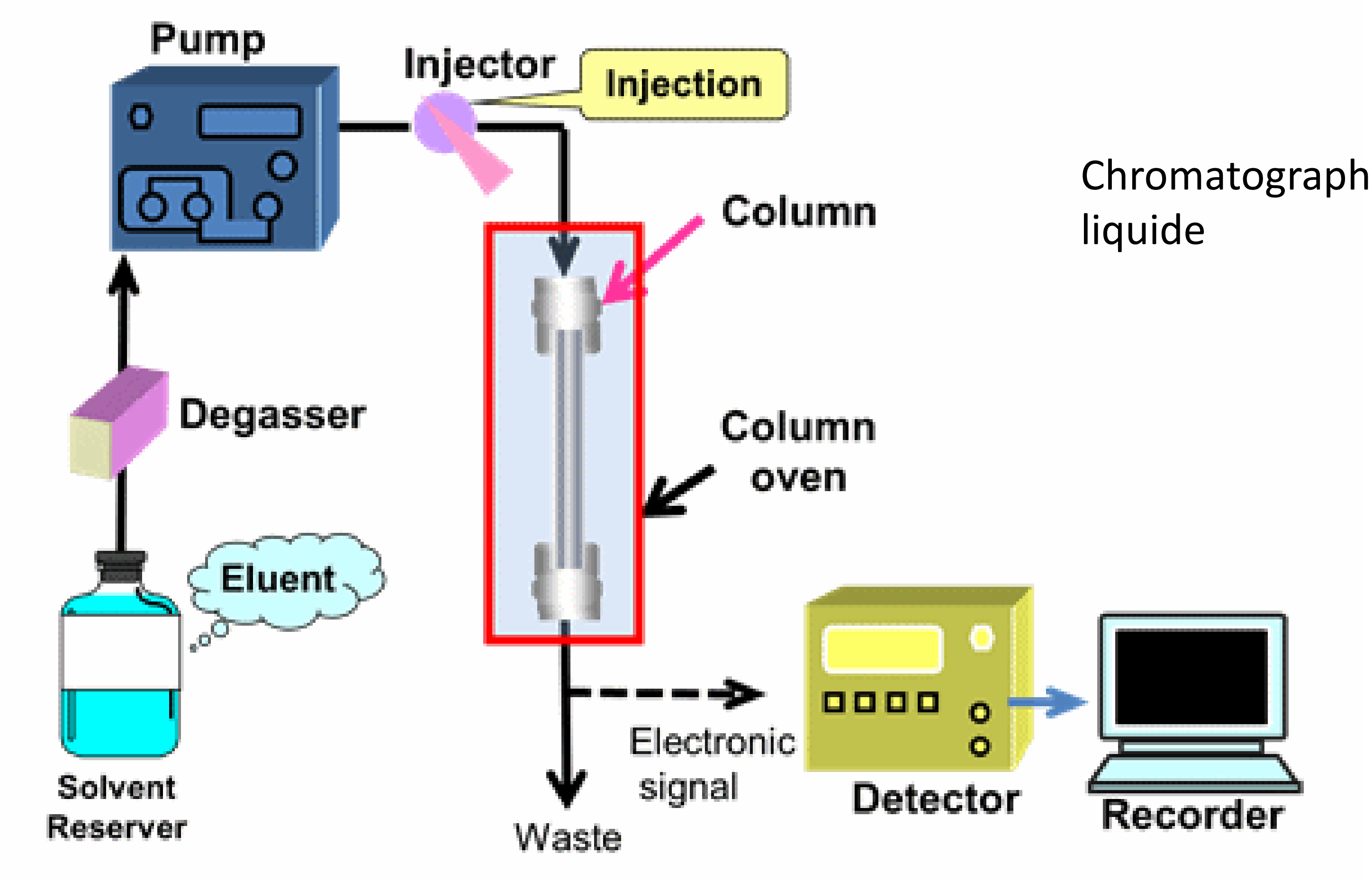
5. Analyse des polluants organiques

- **Aim**: Determination of organic species or identification of their constituent functions

- **Techniques applicable to liquid/gaseous phases:**
  - HPLC: High Performance Liquid Chromatography (*Chromatographie Liquide Haute Performance*)
    - LC-MS: Liquid Chromatography-Mass Spectrometry *(Couplage Chromatographie liquide avec Spectrométrie de Masse)*
  - GC: Gaseous Chromatography *(CPG: Chromatographie en Phase Gazeuse)*
    - GC-MS: Gaseous Chromatography-Mass Spectrometry *(Couplage Chromatographie Gazeuse et Spectrométrie de Masse)*
  - Spectrofluorimetry *(Spectrofluorimétrie)*

5. 1. HPLC

- Principle: High pressure chromatography with separation based on the polarity of the molecules



Chromatographie liquide

5. 1. HPLC

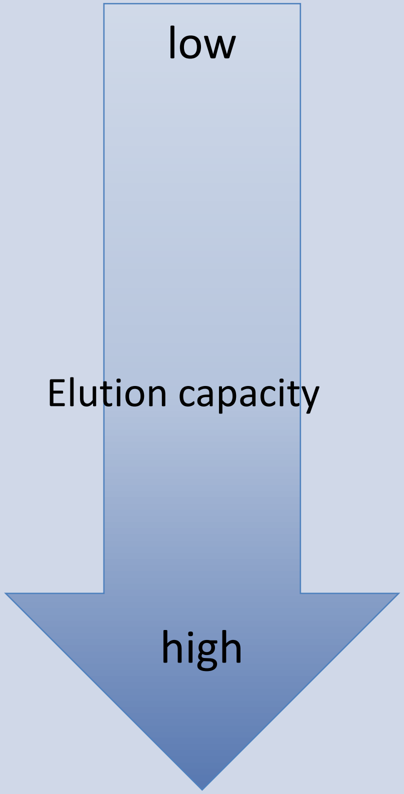
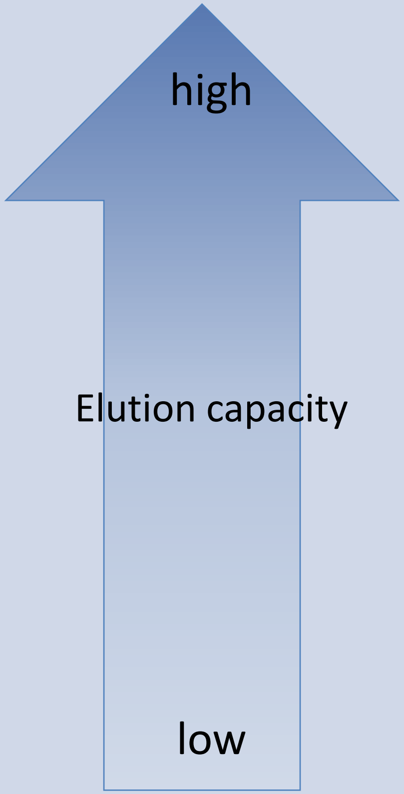
- 2 retention/elution mechanisms :

⚠ **Polar molecules attract each other and vice versa!**

  - *Reversed phase*: extraction of low polar or apolar analytes (hydrocarbons, PAHs, low polar pesticides, etc.)
    - Polar liquid sample: typically aqueous matrix
    - Solid phase= low polar phase (bonded Si-C18, C8, XAD)
    - Eluant = low polar solvant or less molar than water
  - Normal phase: extraction of polar analytes (polar pesticides)
    - Liquid sample with low polarity (oil, hexane,…)
    - Solid phase= polar phase (bonded Si –CN, $-NH_2$ et –diol, silice, alumine, florisil)
    - Eluant = polar solvant or more polar than the sample

5. 1. HPLC

- Eluant option:

normal phase	Solvants classés par polarité croissante	Reverse phase
 low Elution capacity high	Hexane Toluene Trichloromethane Dichlorométhane Éther Acétate d’éthyle Acetonitrile Methanol Water	 high Elution capacity low

5. 1. HPLC

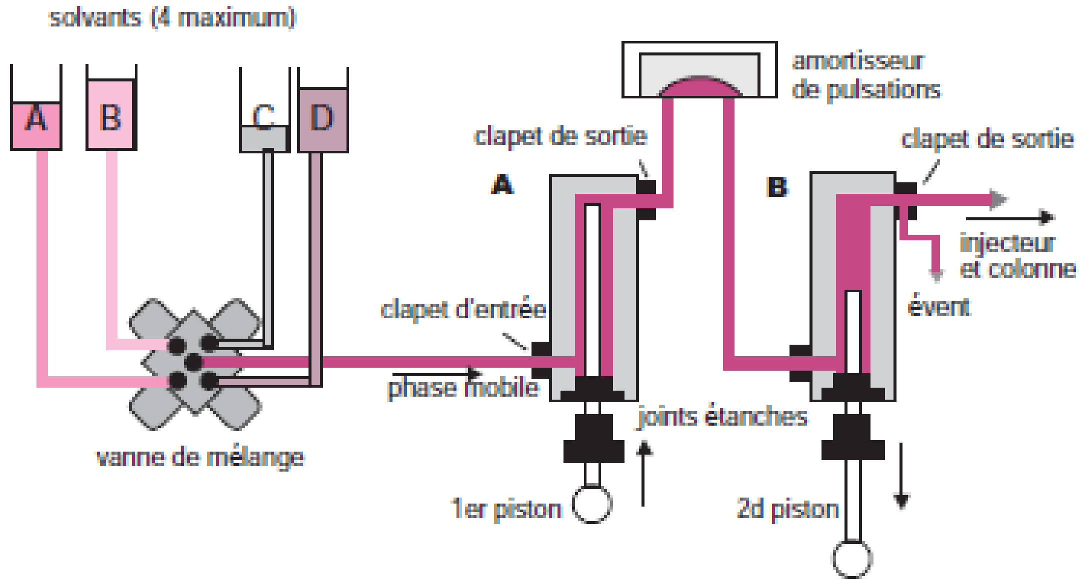
- Mobile phase
- Solvent or a mixture of solvents that must be of analytical purity
  - Associated with a deaerator (dégazeur)

5. 1. HPLC

- Solid phase (column)
  - Small metal tube, always in stainless steel to resist pressure and organic solvents
  - Often associated with a guard column to protect the column

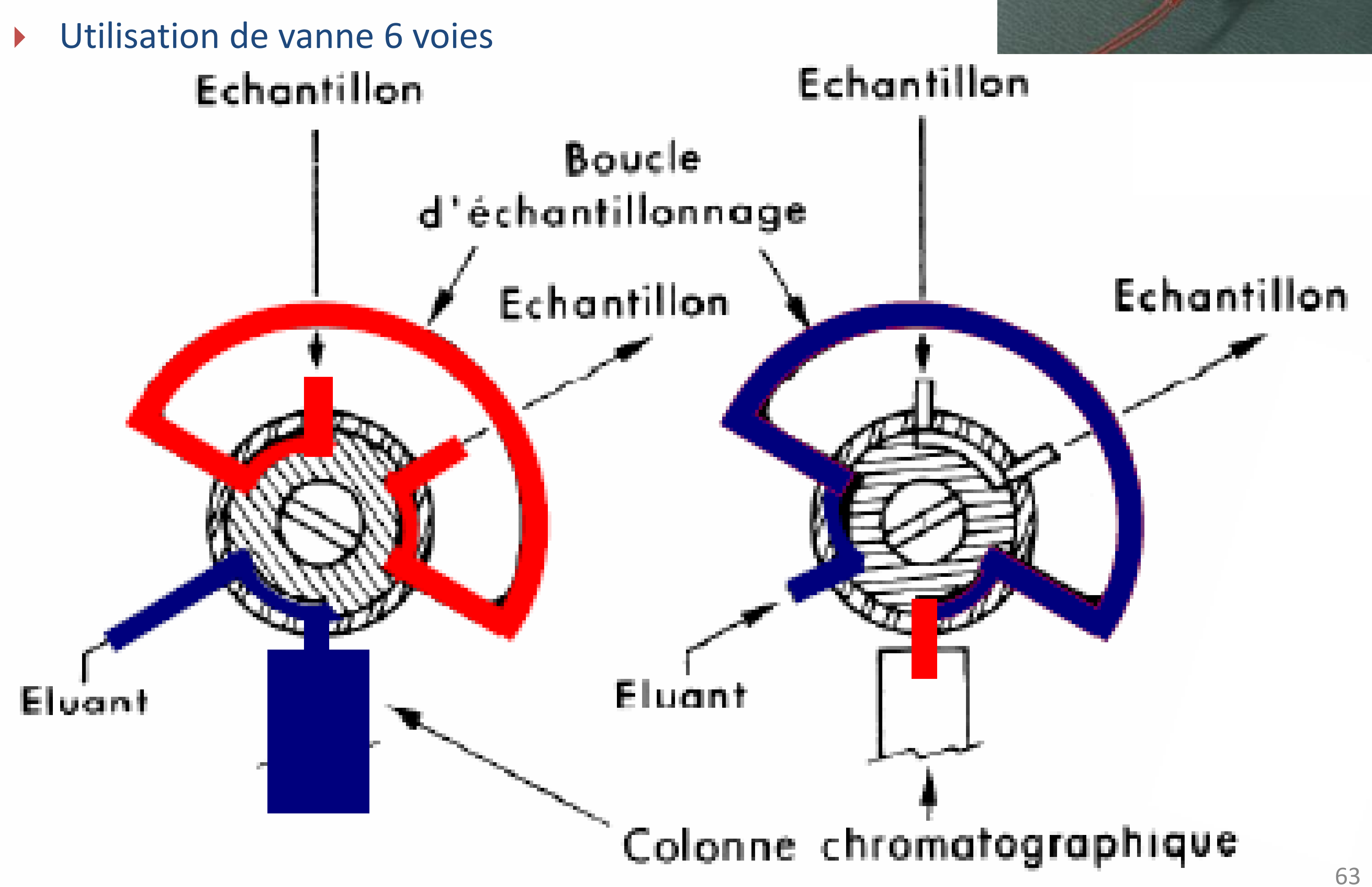
5. 1. HPLC

- Appareillage: pompes
- Utilisation de pompe à piston
  - Sert à faire passer la phase mobile rapidement dans la colonne
  - Débit non pulsé et stable
  - Pression importante au niveau de l’injecteur
  - Possibilité de faire varier la composition de la phase mobile



5. 1. HPLC

- Appareillage: injecteur
  - Utilisation de vanne 6 voies



5. 1. HPLC

- Appareillage: Détecteur
- Détecteur à absorbance dans l’U.V. et le visible
  - Loi de Beer-Lambert
  - Son principal inconvénient est qu’il ne détecte que les composés qui ont une absorbance appréciable à la longueur utilisée → dérivatisation nécessaire

- Détecteur à indice de réfraction
  - Mesure en continu la différence d’indice de réfraction entre la phase mobile et l’effluent de la colonne
  - Son principal inconvénient est sa faible sensibilité.

- Détecteur à fluorescence
  - Les composés fluorescents réémettent sous forme de radiations lumineuses une fraction plus ou moins grande du rayonnement de la source auquel ils sont soumis.
  - Intensité de fluorescence = f(concentration de la substance)

5. 1. HPLC

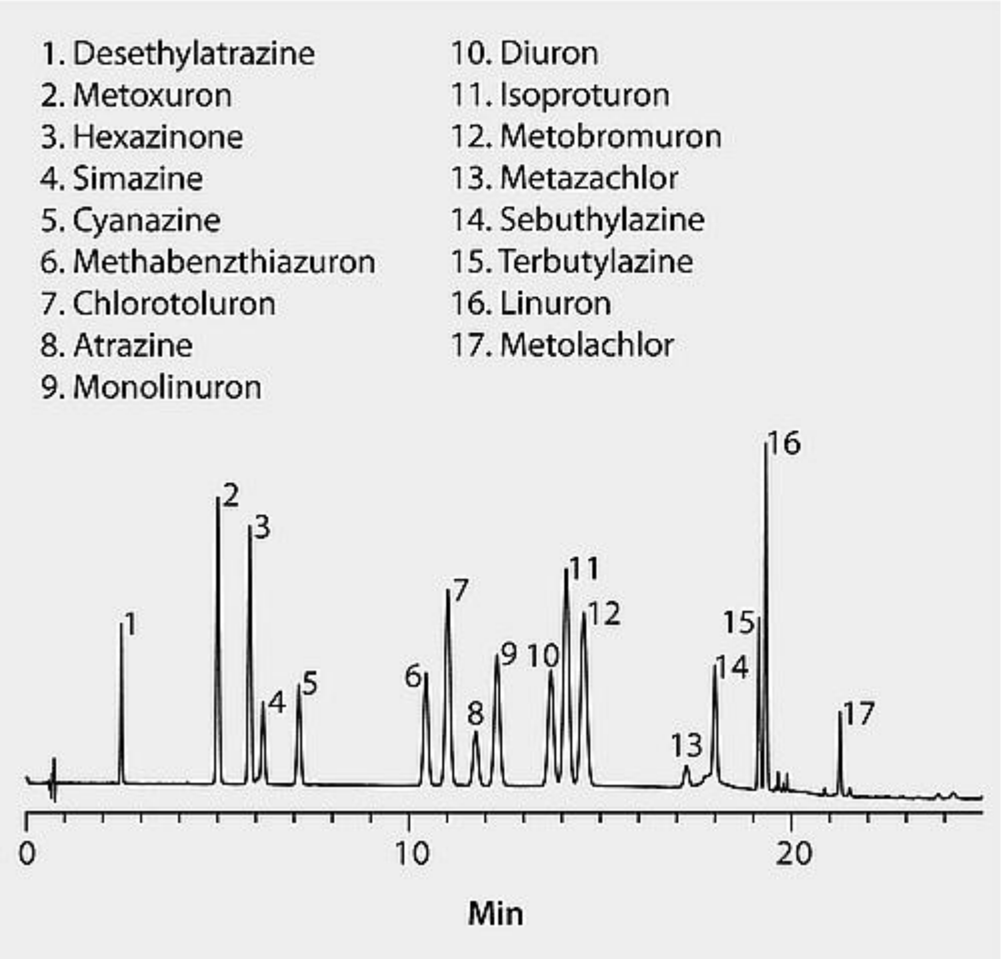
- Applications:

Exemple: Séparation des herbicides de l’eau

-Colonne C18

- Phase mobile: acetate d’ammonium (pH 6.8) /acetonitrile

- Détecteur UV 240 nm



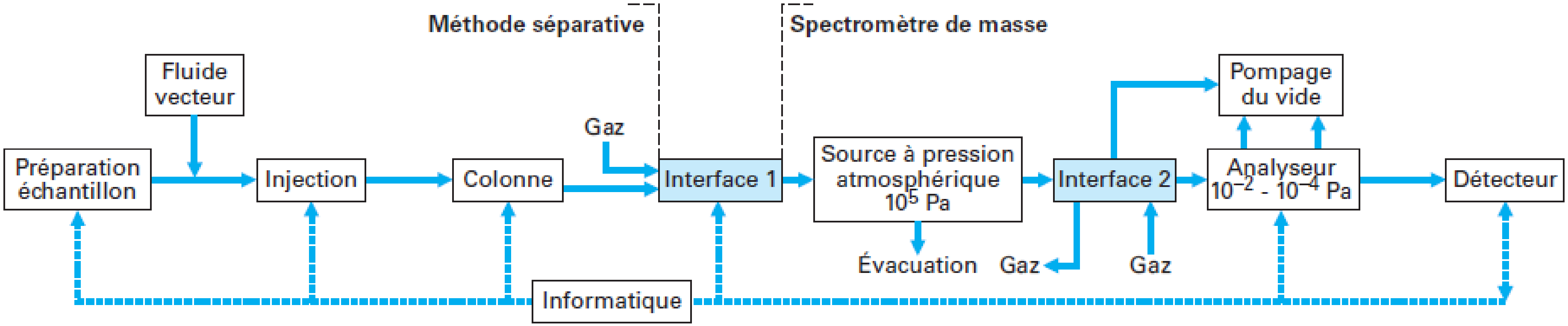
5. 1. Couplage LC-MS

- But: Identification de la structure de la molécule

- Principe:
  - LC: Séparation des molécules organiques
  - MS: Identification de leur structure
    - Volatiliser: Séparer les molécules les unes des autres: on passe de l'état de matière condensée à un état gazeux.
    - Ioniser: Transformer les molécules en ions
    - Mesurer les rapports m/z: La masse moléculaire est calculée à partir du rapport masse (m)/nb de charges (z)

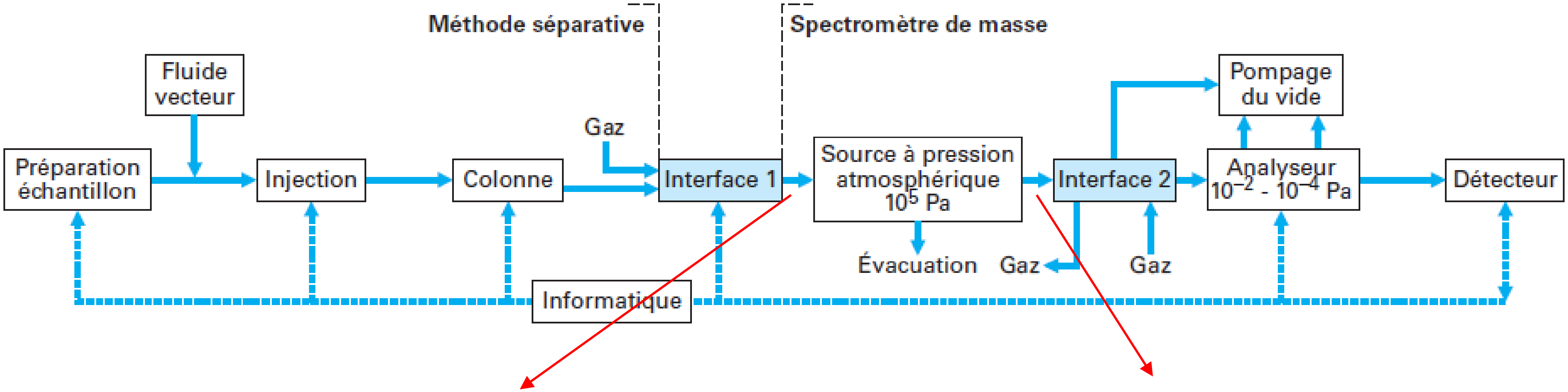
5. 1. Couplage LC-MS

- LC-MS: Appareillage
  - Plusieurs problèmes à résoudre:
    - Le solvant est introduit dans le MS en quantités bien plus importantes que les produits à analyser donc possibilité d'encrassement → élimination du solvant
    - Passage d'un échantillon liquide à pression ambiante en sortie de LC à des ions sous vide dans le MS → méthodologie avec double interface



5. 1. Couplage LC-MS

- LC-MS: Appareillage

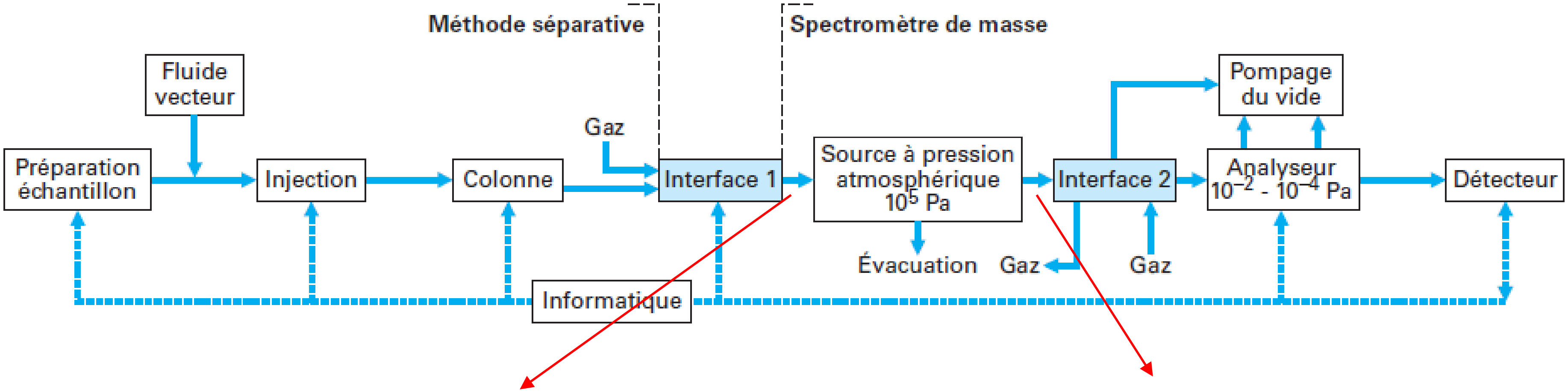


Nébuliseur
+ Source d'ions à partir des solutés de l'éluant

Zone d'échantillonnage vers le vide
+ optique de guidage des ions

5. 1. Couplage LC-MS

- LC-MS: Appareillage



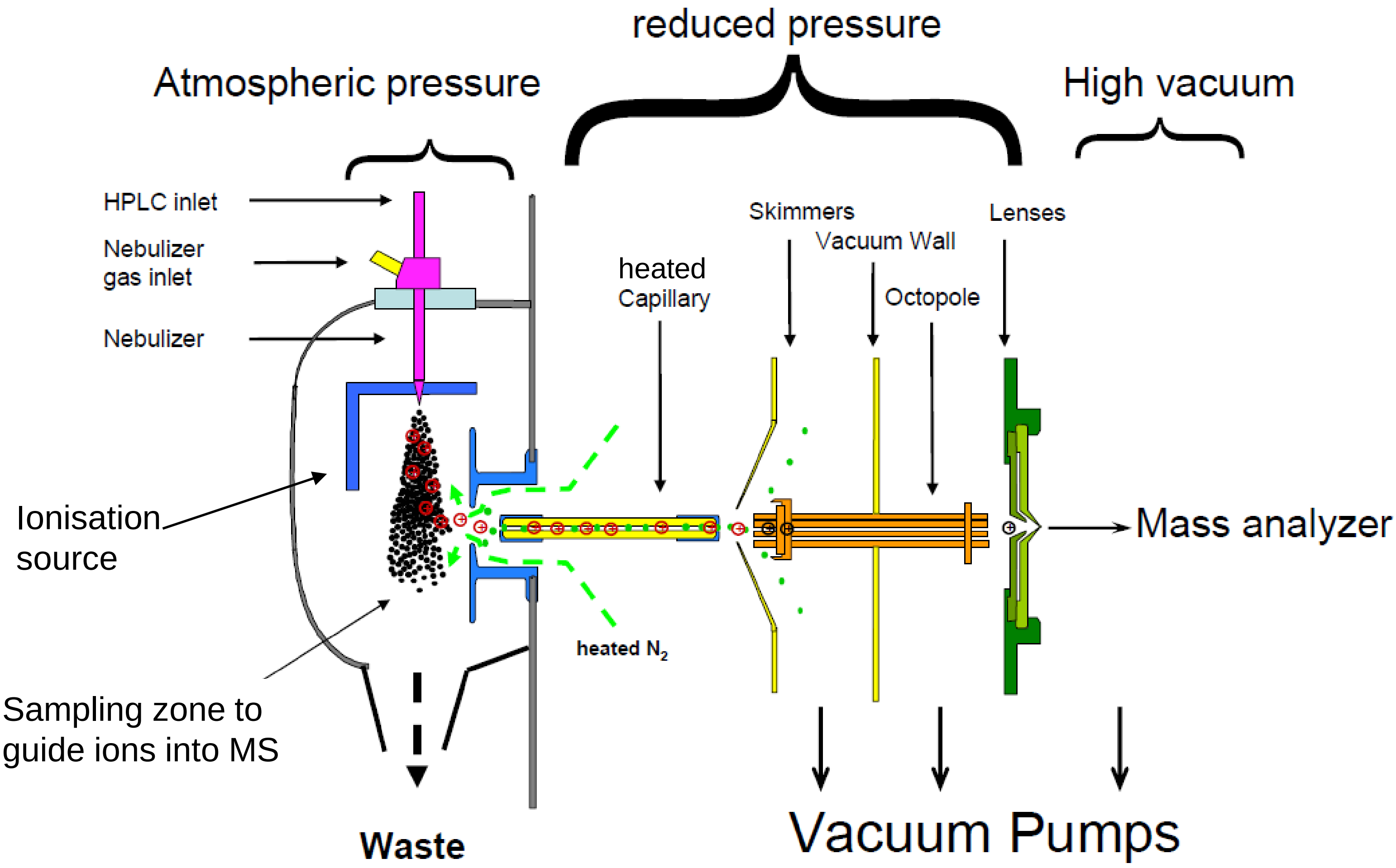
Nébuliseur
+ Source d'ions à partir des solutés de l'éluant

Zone d'échantillonnage vers le vide
+ optique de guidage des ions

Chambre d'ions à pression atmosphérique
(API: *Atmospheric Pressure ionisation*)

5. 1. Couplage LC-MS

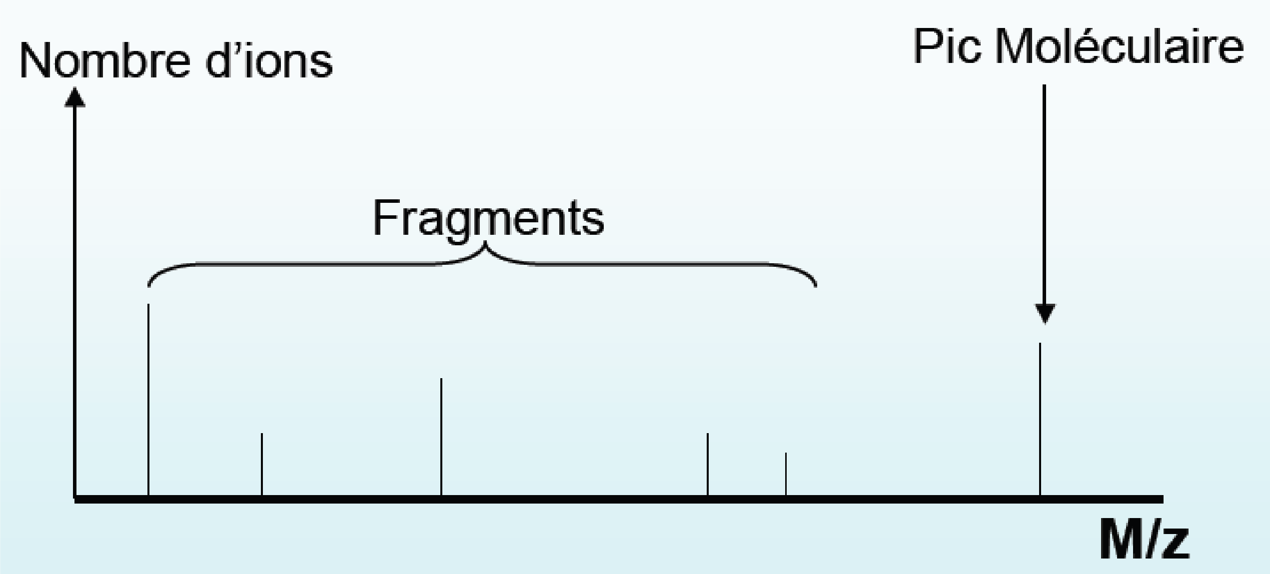
- MS: Appareillage: Chambre API



courtesy of Agilent

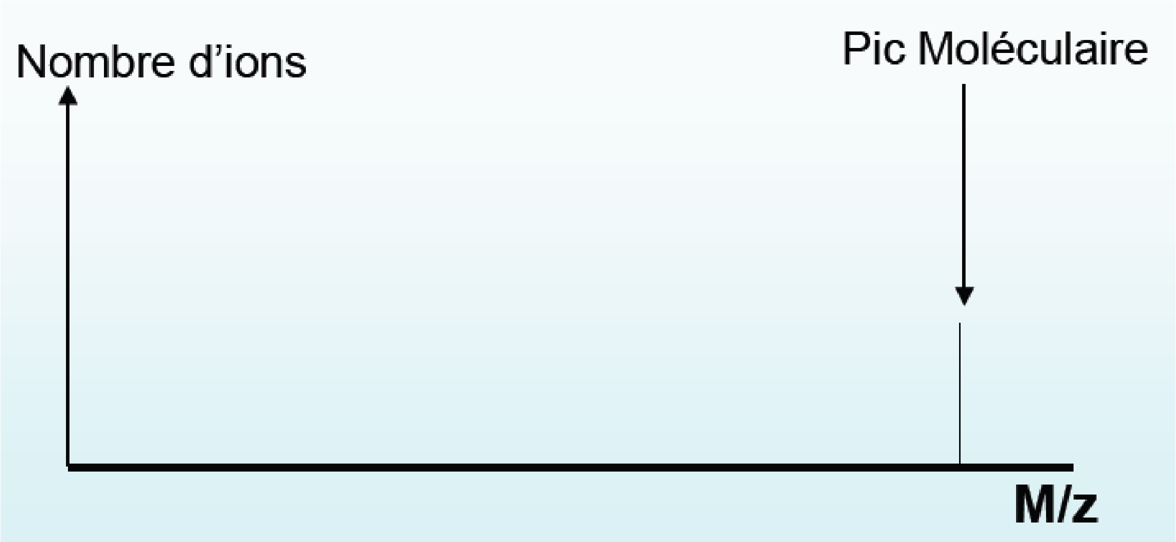
5. 1. Couplage LC-MS

- MS: Appareillage: Source d'ionisation:
- Ionisation peut être positive ou négative
- Les « ionisations dures » génèrent des ions moléculaires, à nombre impair d'électrons, qui se fragmentent beaucoup et parfois même totalement.



5. 1. Couplage LC-MS

- MS: Appareillage: Source d’ionisation:
- Ionisation peut être positive ou négative
- Les « ionisations dures » génèrent des ions moléculaires, à nombre impair d’électrons, qui se fragmentent beaucoup et parfois même totalement.
- Les « ionisations douces » génèrent des ions moléculaires à nombre pair d’électrons, qui sont relativement stables et qui ont des durées de vie suffisantes pour traverser l’analyseur, arriver jusqu’au détecteur, et donc être mesurés.



5. 1. Couplage LC-MS

Sources d’ionisation

- Collision atomique
- Collision électronique
- Collision photonique

10-100 eV

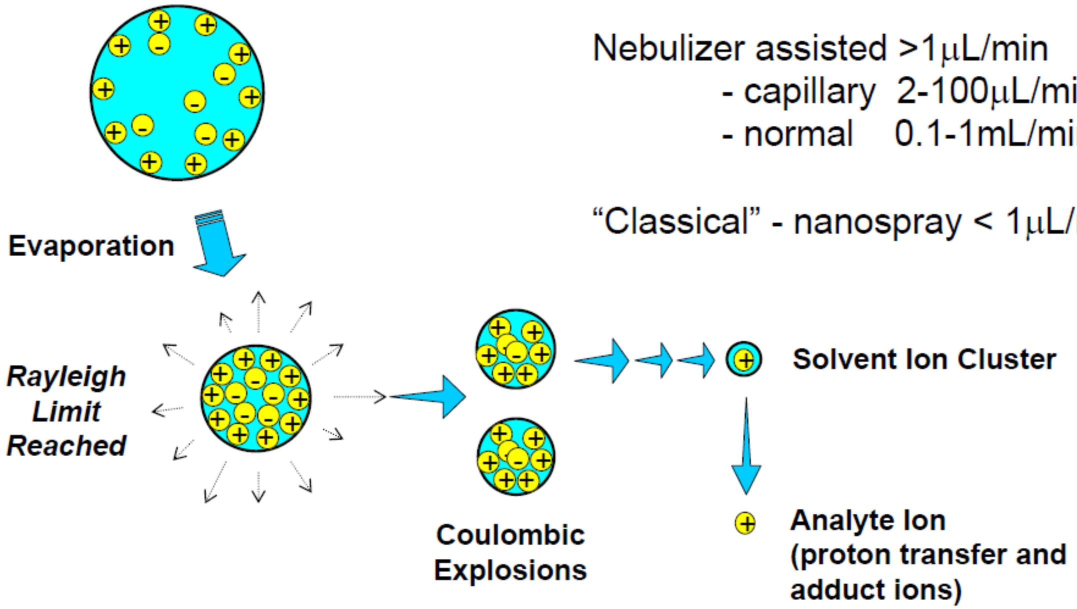
3 eV

1.5 eV

5. 1. Couplage LC-MS

- MS: Appareillage: Les sources d'ionisation dans l'API
  - Source ESI (Electrospray):
    - Ionisation dure
    - Pour des molécules déjà chargées! Ce n'est pas vraiment une source d'ions mais un extracteurs d'ions

Charged Droplets containing ions in solution ⟶ **Analyte Ions in the gas phase - both +ve and -ve**

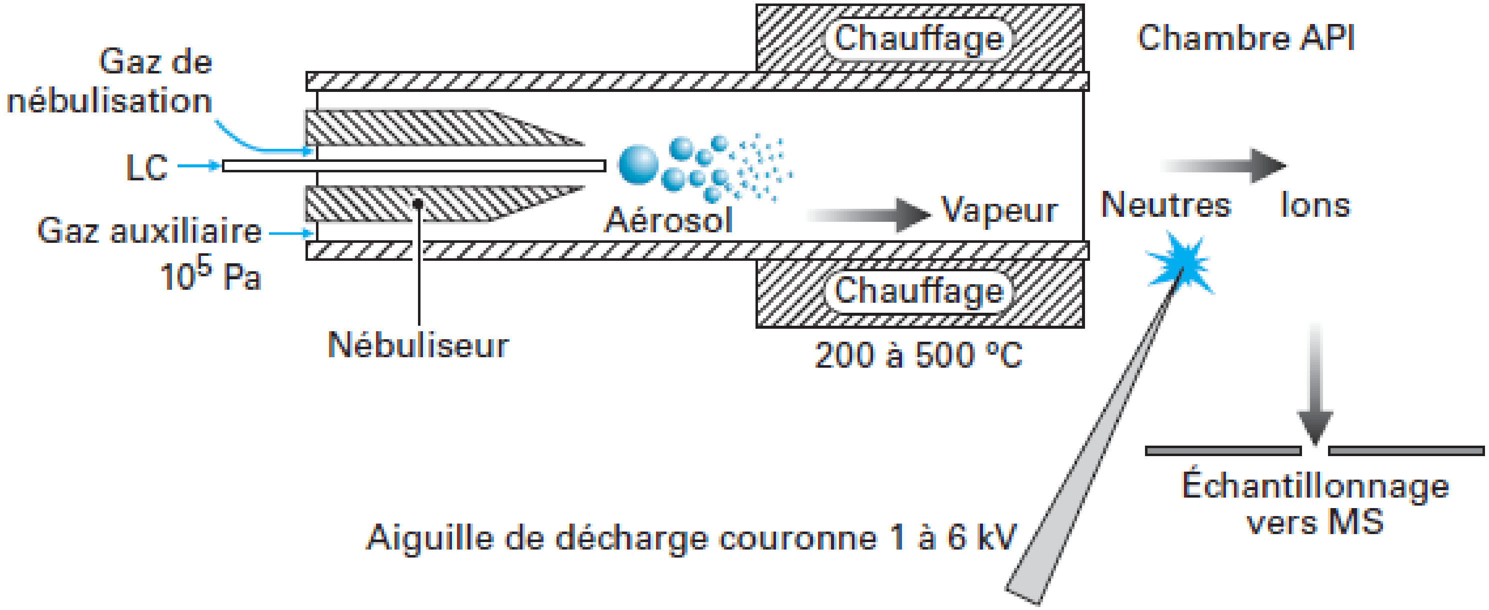


Nebulizer assisted >1μL/min
- capillary 2-100μL/min
- normal 0.1-1mL/min

"Classical" - nanospray < 1μL/min

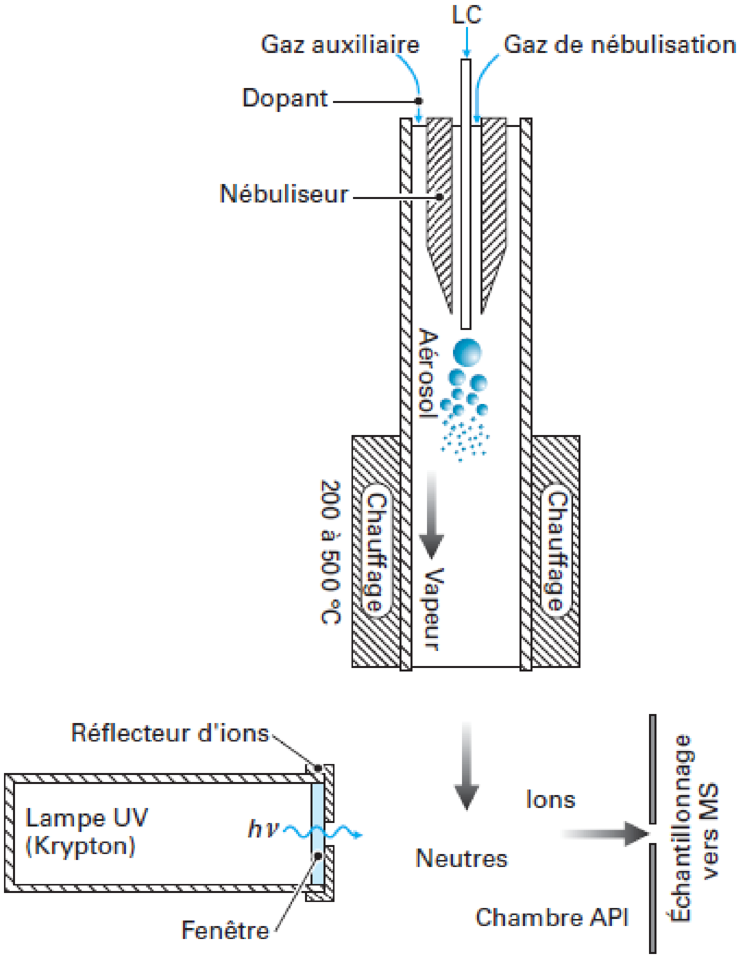
5. 1. Couplage LC-MS

- MS: Appareillage: Les sources d'ionisation dans l'API
  - Source APCI (Atmospheric Pressure Chemical Ionisation):
    - Ionisation plutôt dure
    - Nébuliseur chauffé
    - Pointe d'électrode émettant des électrons ionisants par décharge couronne (effet corona)



5. 1. Couplage LC-MS

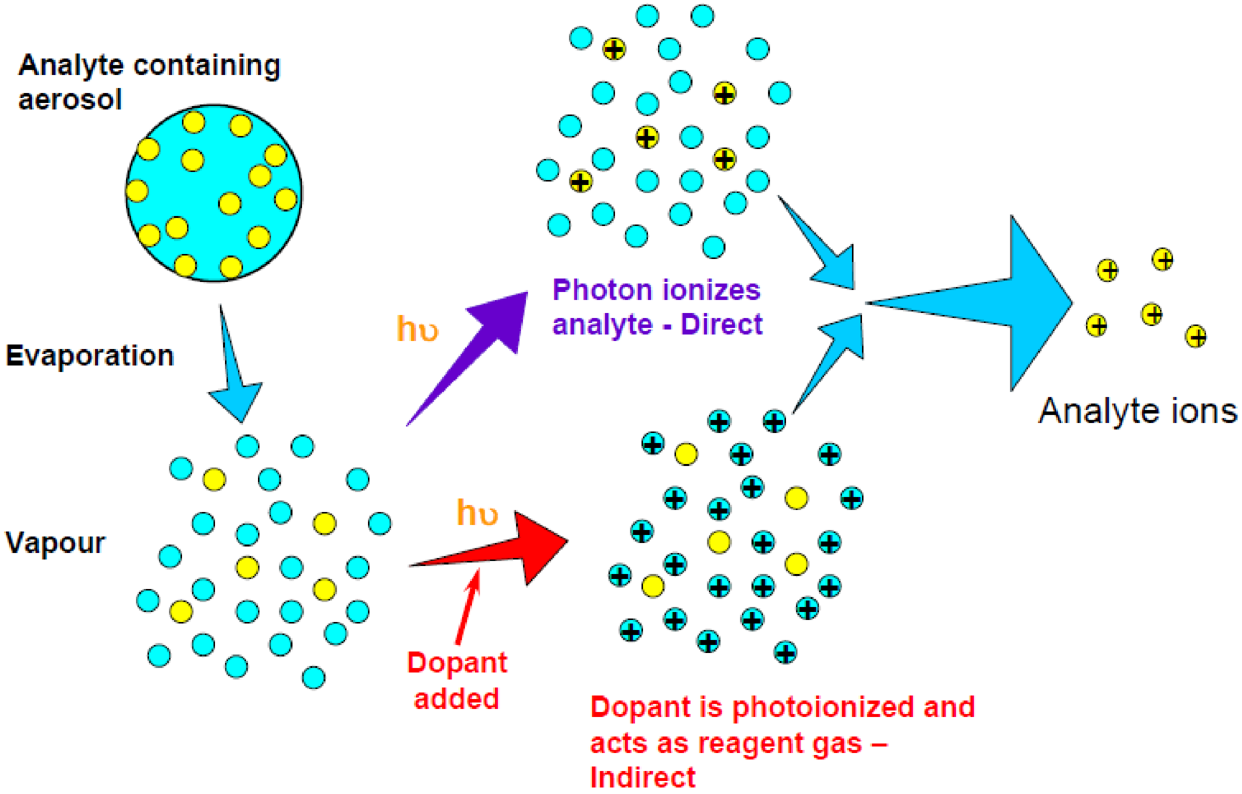
- MS: Appareillage: Les sources d’ionisation dans l’API
  - Source APPI (Atmospheric Pressure PhotoIonisation):



- Ionisation plutôt douce
- Nébuliseur chauffé
- Photons UV émis par une lampe à décharge sous vide

5. 1. Couplage LC-MS

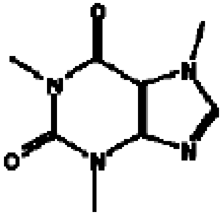
- MS: Appareillage: Les sources d'ionisation dans l'API
  - Source APPI (Atmospheric Pressure PhotoIonisation):
    - Ionisation plutôt douce
    - Nébuliseur chauffé (350-500°C)
    - Photons UV émis par une lampe à décharge sous vide



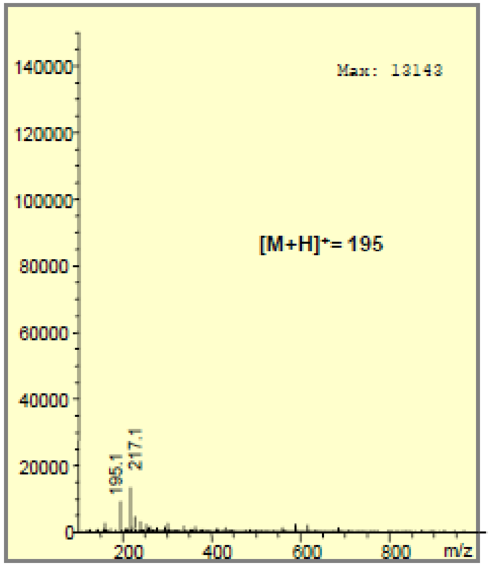
5. 1. Couplage LC-MS

- MS: Appareillage: Les sources d'ionisation dans l'API
  - Potentiel de fragmentation:

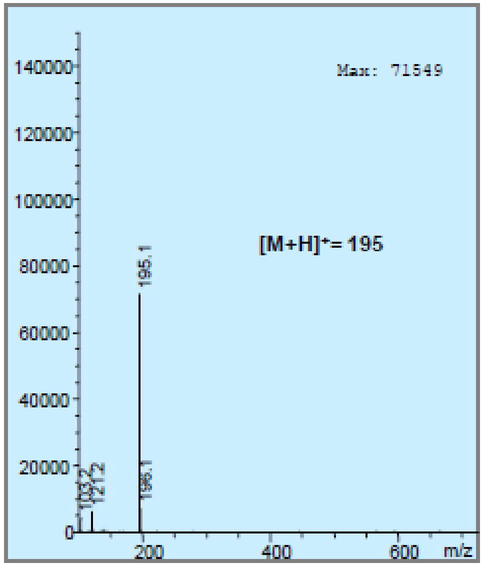
Caffeine



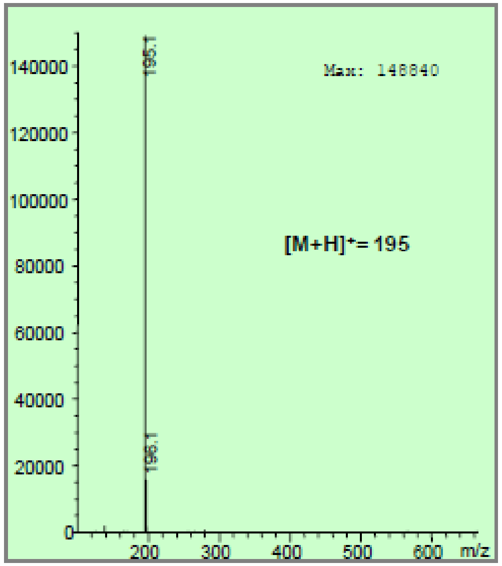
ESI



APCI

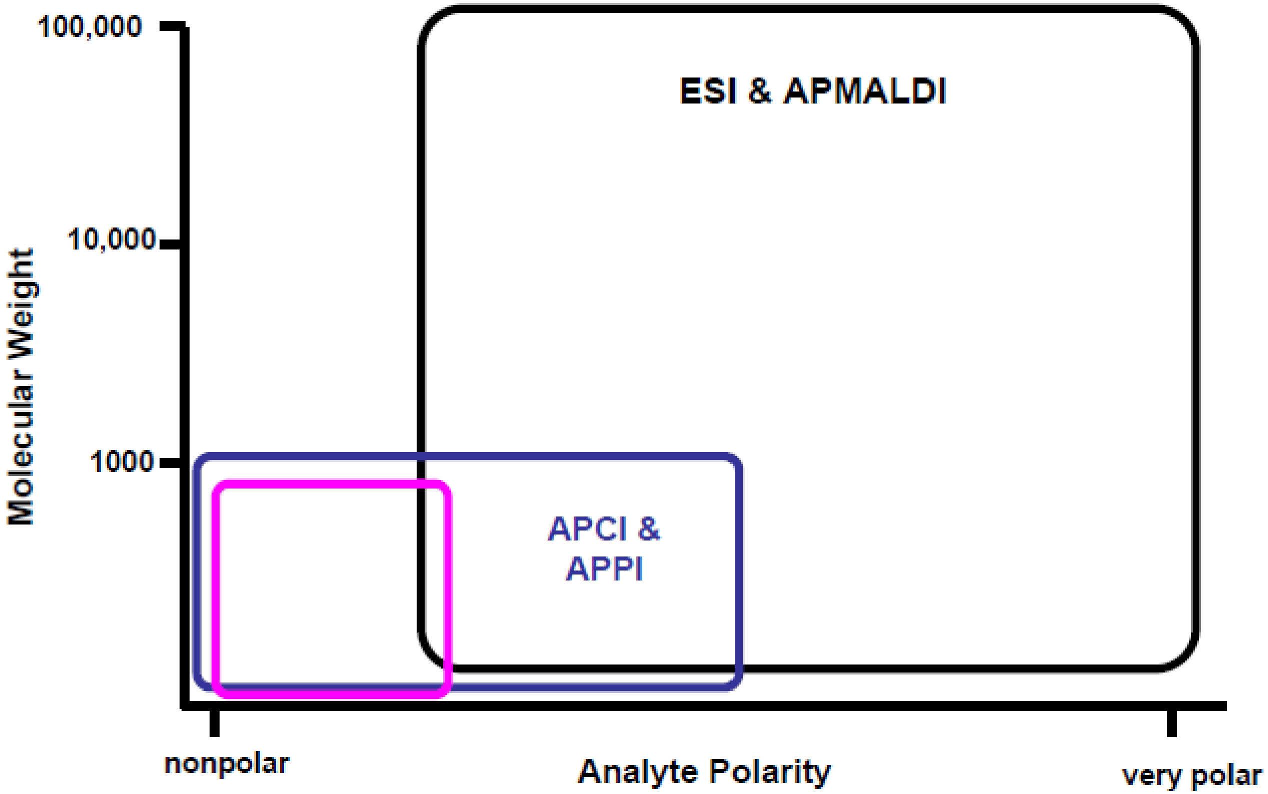


APPI



5. 1. Couplage LC-MS

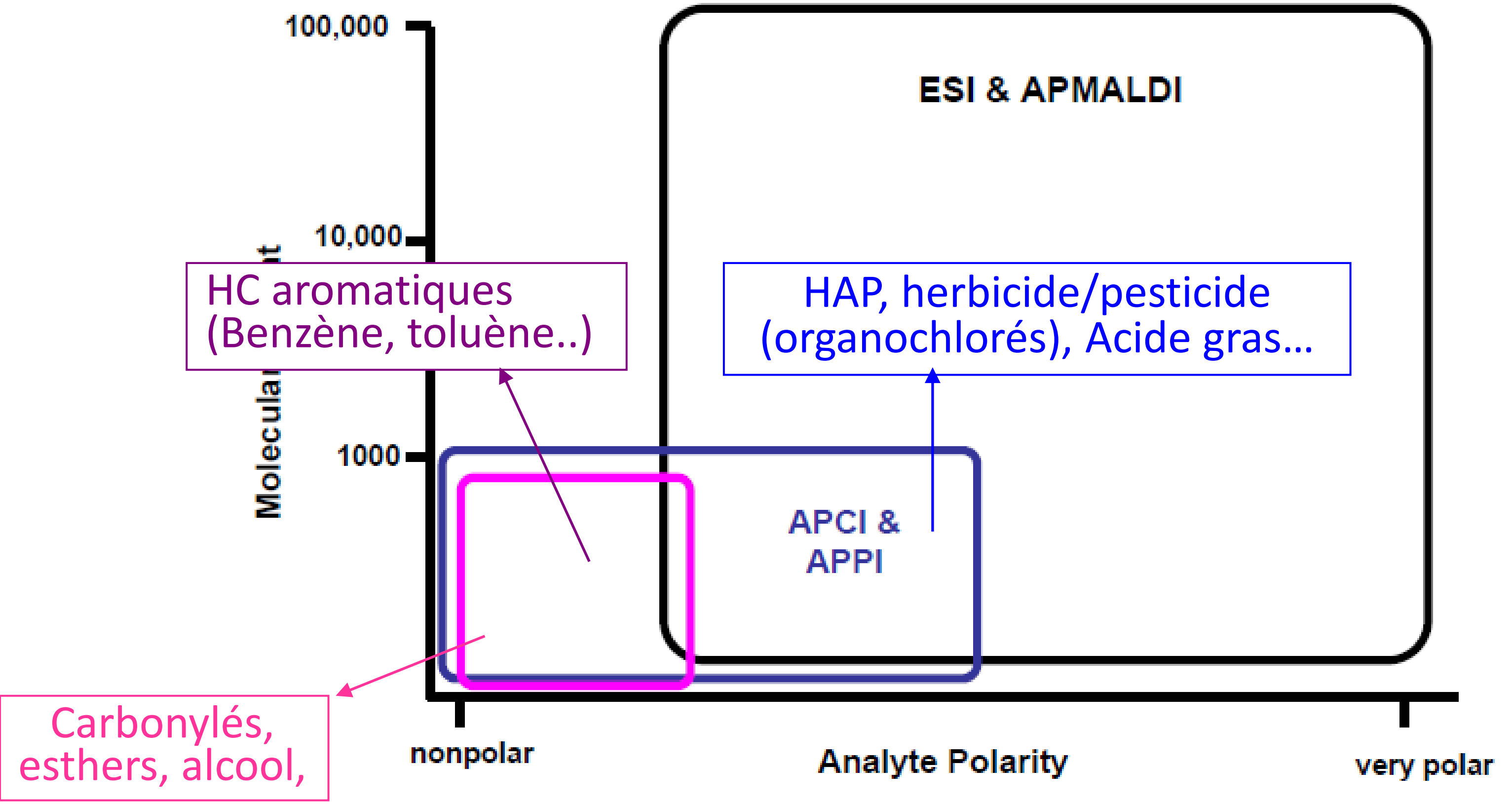
- MS: Appareillage: Les sources d’ionisation dans l’API



ESI: Electrospray Ionization & APMALDI
APCI: Atmospheric Pressure Chemical Ionization
APPI: Atmospheric Pressure Photo Ionization
EI, CI, GC-MS

5. 1. Couplage LC-MS

- MS: Appareillage: Les sources d’ionisation dans l’API



HC aromatiques
(Benzène, toluène..)

HAP, herbicide/pesticide
(organochlorés), Acide gras…

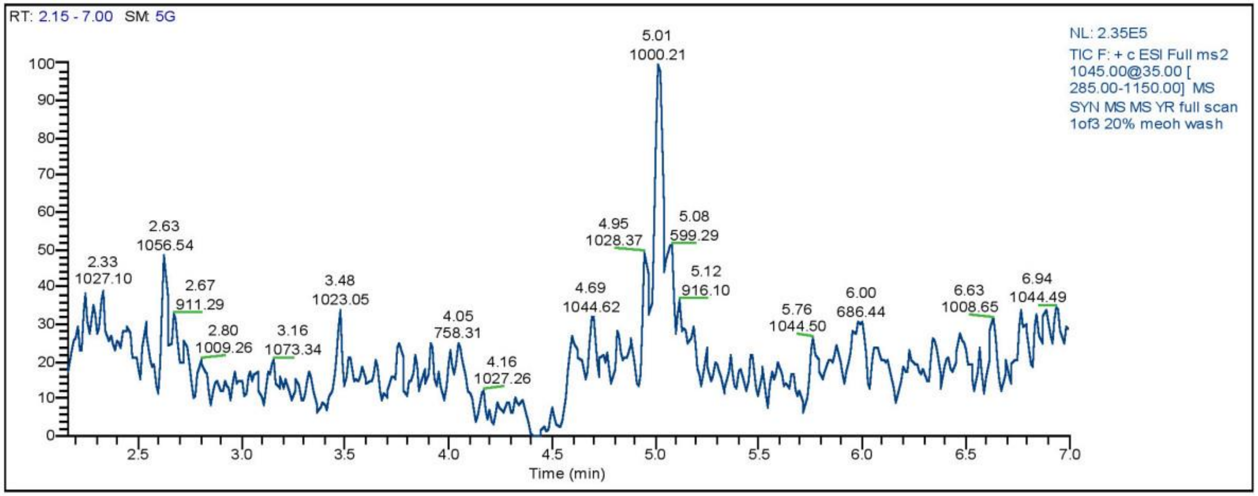
Carbonylés,
esthers, alcool,

ESI: Electrospray Ionization & APMALDI
APCI: Atmospheric Pressure Chemical Ionization
APPI: Atmospheric Pressure Photo Ionization
EI, CI, GC-MS

5. 1. LC-MS

- LC-MS: signals?

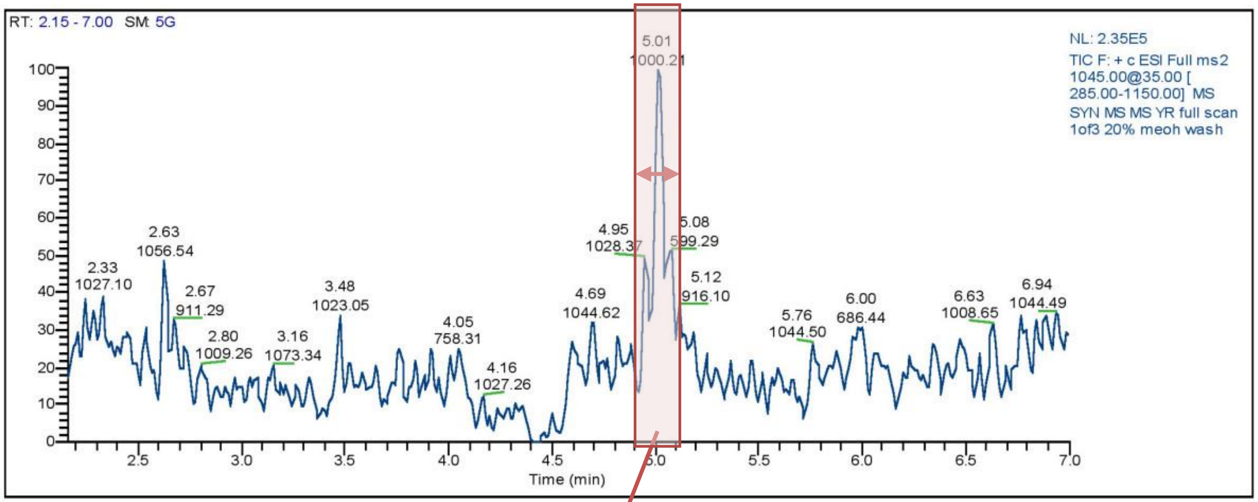
Chromatogram



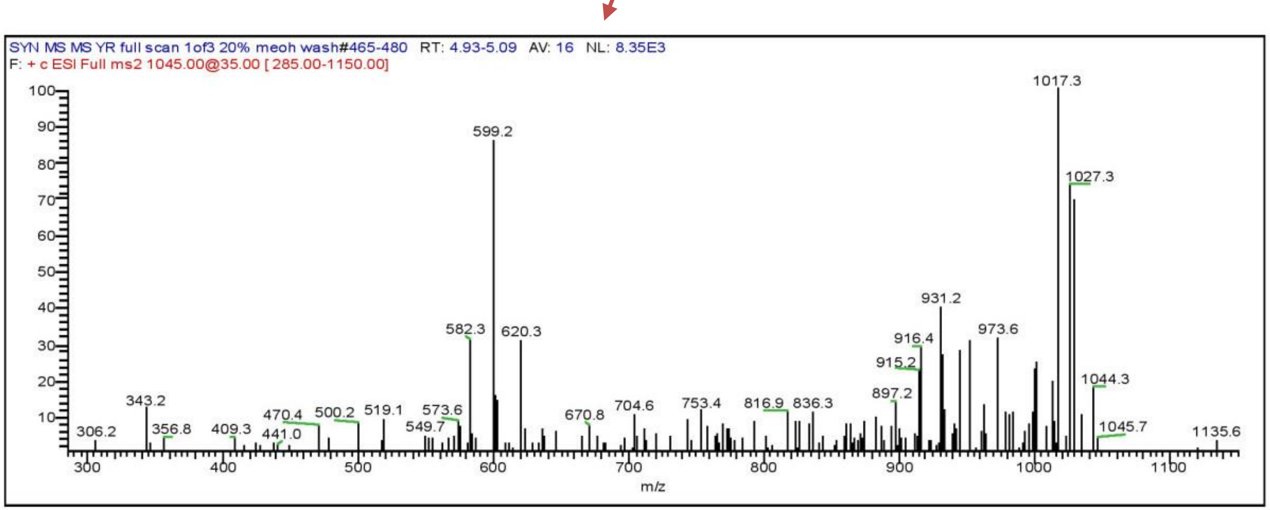
5. 1. LC-MS

- LC-MS: signals?

Chromatogram

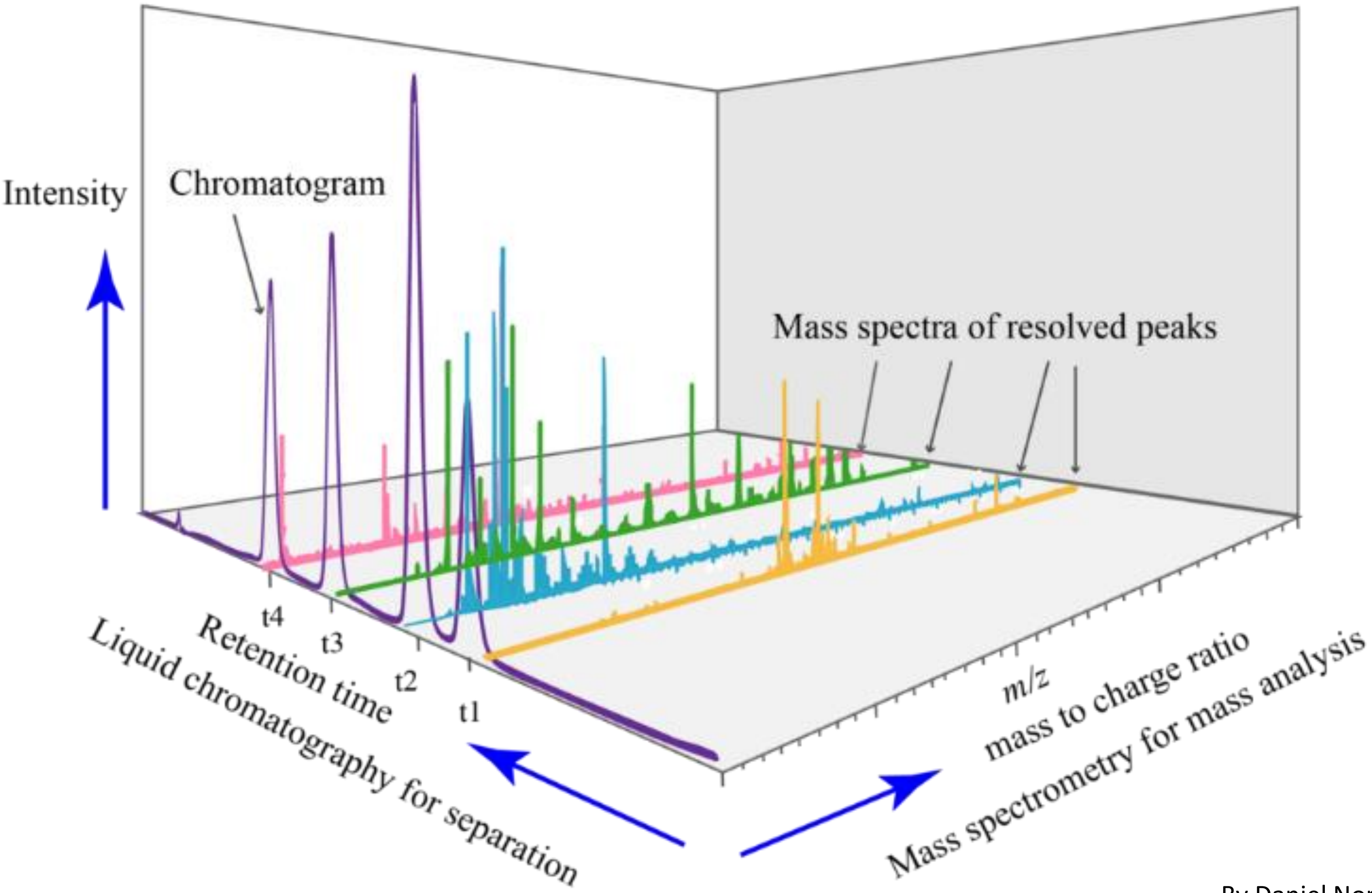


Mass spectrum



5. 1. LC-MS

- LC-MS: signals?

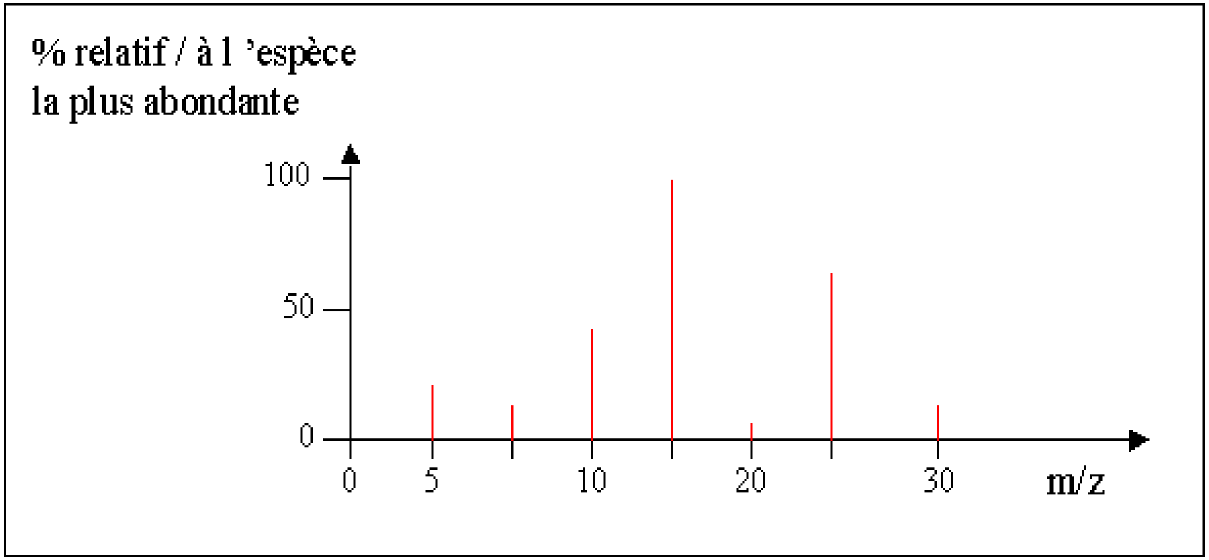


By Daniel Norena-Caro

5. 1. LC-MS

- LC-MS: signals?

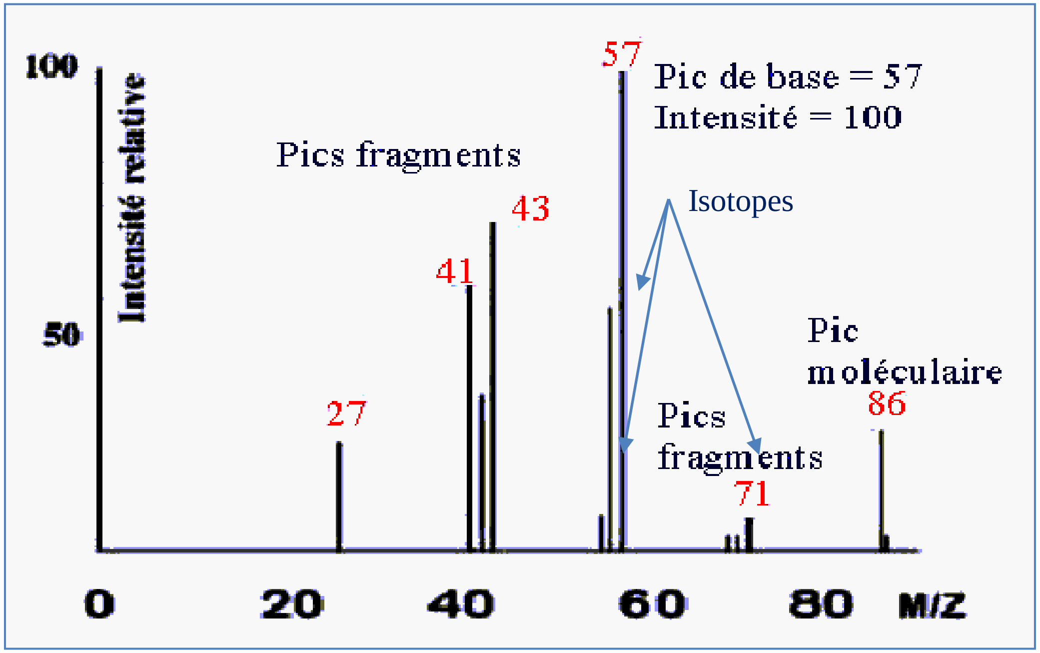
Intensity ↔ relative abundance of ions



Intensity of the most intense peak of the spectrum: arbitrarily set at 100

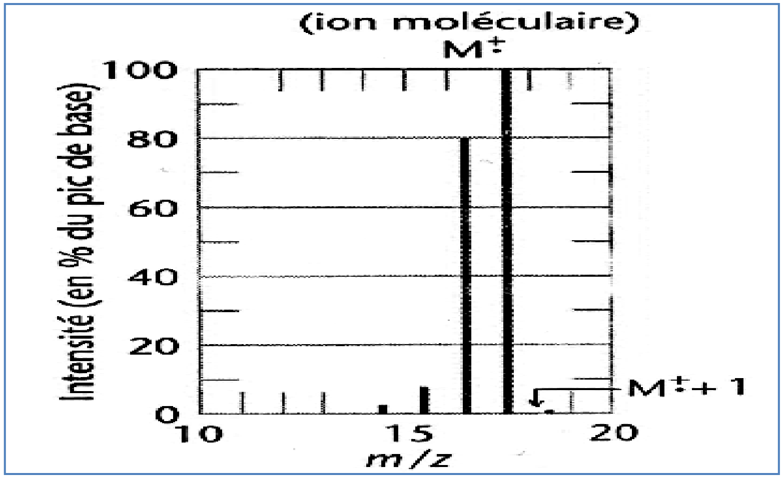
5. 1. LC-MS

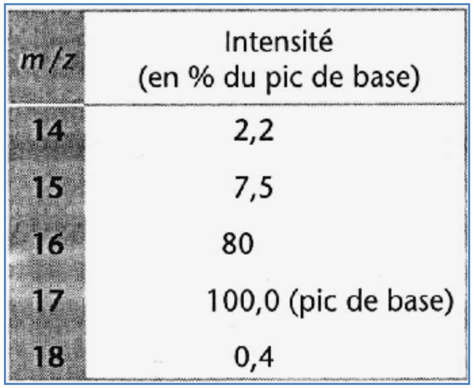
- LC-MS: signals?
  - Base peak: the most intense peak→ ion the most abundant
  - Molecular ion peak (parent peak) → corresponds to the molar mass
  - Fragment peaks
  - Isotopes (M+1, M+2 peaks)



5. 1. LC-MS

- LC-MS: signals?
  - Ex: Ammonia (NH_3) for positive ion mode

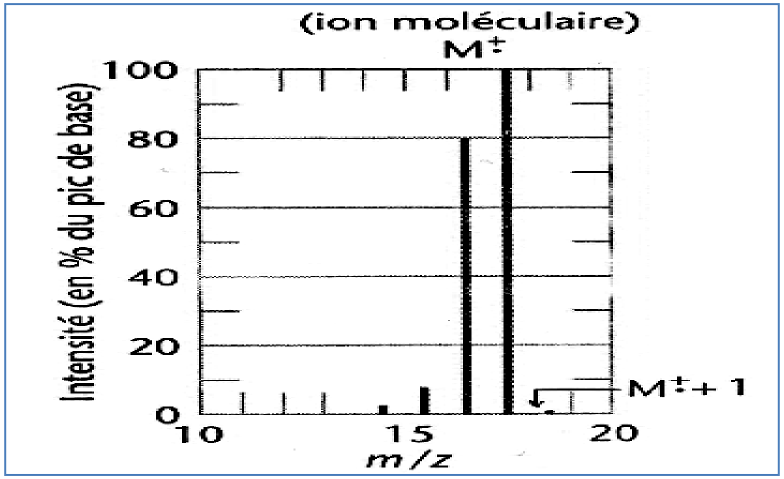


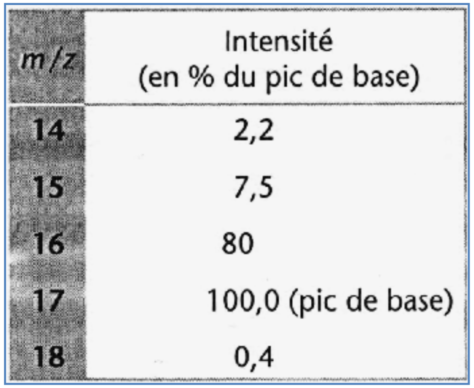


m/z	Intensité (en % du pic de base)
14	2,2
15	7,5
16	80
17	100,0 (pic de base)
18	0,4

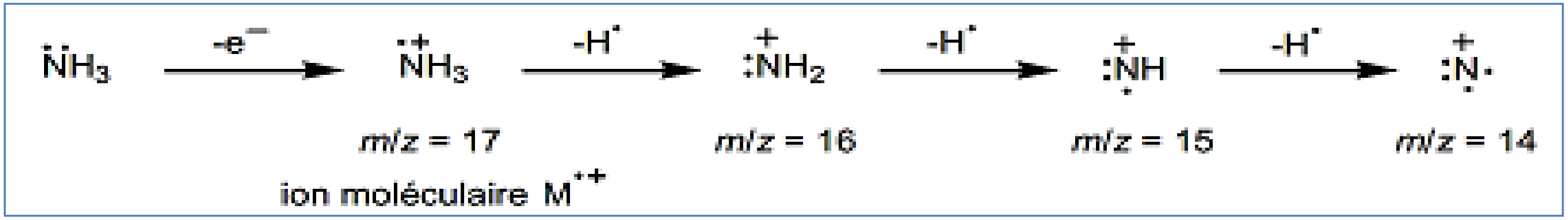
5. 1. LC-MS

- LC-MS: signals?
  - Ex: Ammonia (NH_3) for positive ion mode





m/z	Intensité (en % du pic de base)
14	2,2
15	7,5
16	80
17	100,0 (pic de base)
18	0,4

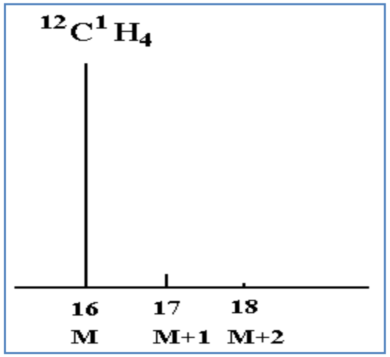


- Molecular ion peak m/z = 17
- peaks m/z = 14, 15 et 16 : fragment peaks
- Base peak m/z = 17 ⇨ molecular ion peak NH_3^+

5. 1. LC-MS

LC-MS: Principe du Pic moléculaire

Ion molecular mass= sum of the masses of the most abundant isotopes (^{12}C,^{1}H,^{16}O,..) of the elements present in the molecule ± 1.



- Methane $^{12}C^{1}H_4$: molecular ion at m/z = 16

+ $^{13}C^{1}H_4$ (m/z = 17) + $^{12}C^{2}H^{1}H_3$ (m/z = 18)

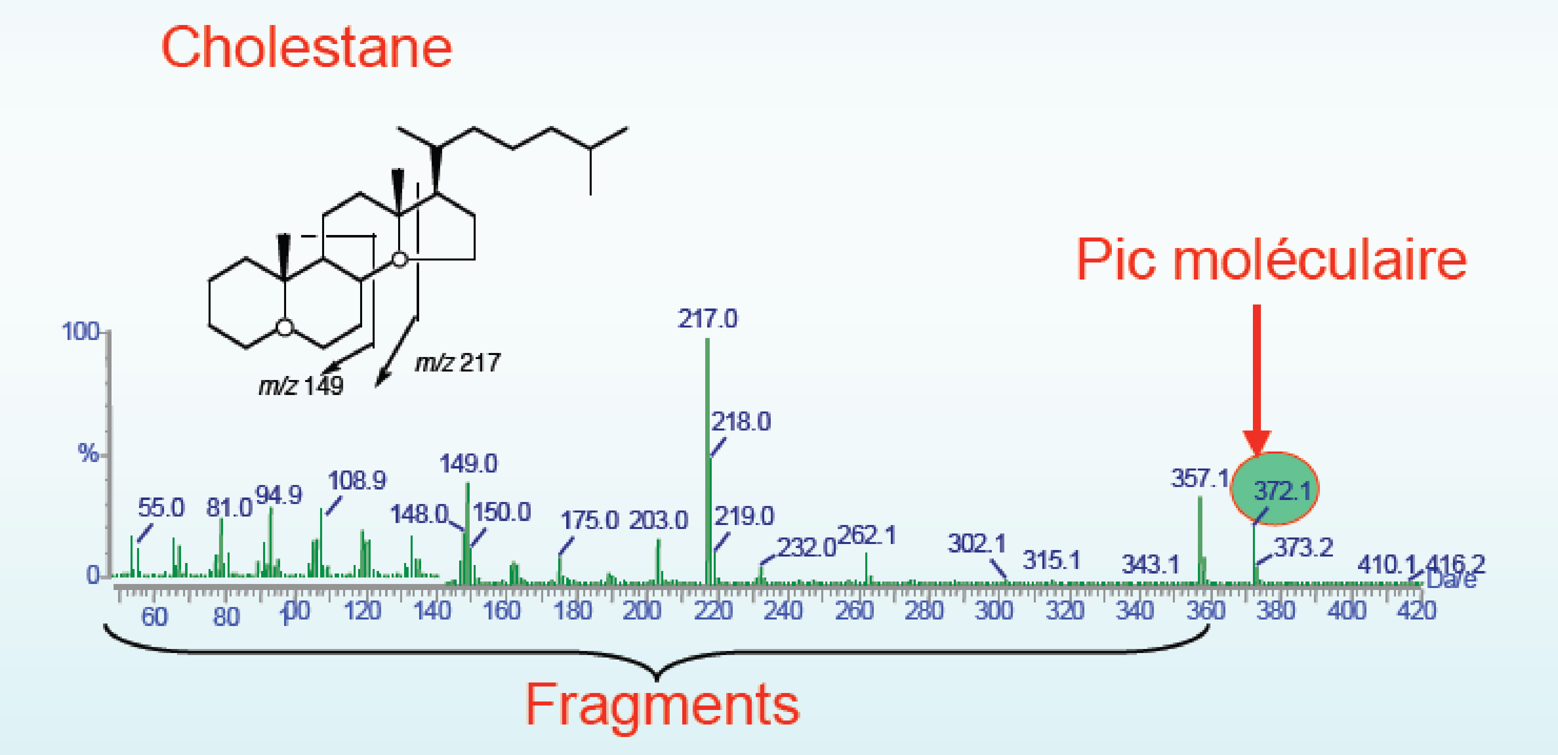
- Benzamide C_7H_7NO

Molecular ion $M^{+\cdot}$ m/z = 121

7	**x**	**^{12}C**	**=**	**84**
7	**x**	**^{1}H**	**=**	**7**
1	**x**	**^{14}N**	**=**	**14**
1	**x**	**^{16}O**	**=**	**16**
		M	**=**	**121**

5. 1. LC-MS

- LC-MS
  - Molecular ion peak→ allows to estimate the molar mass, the isotopes
  - Fragment peaks → allows to inform about the functions present in the molecule→ structure



5. 1. LC-MS

- Fragmentation:

alcohols, ethers, linear or branched chain alkanes:

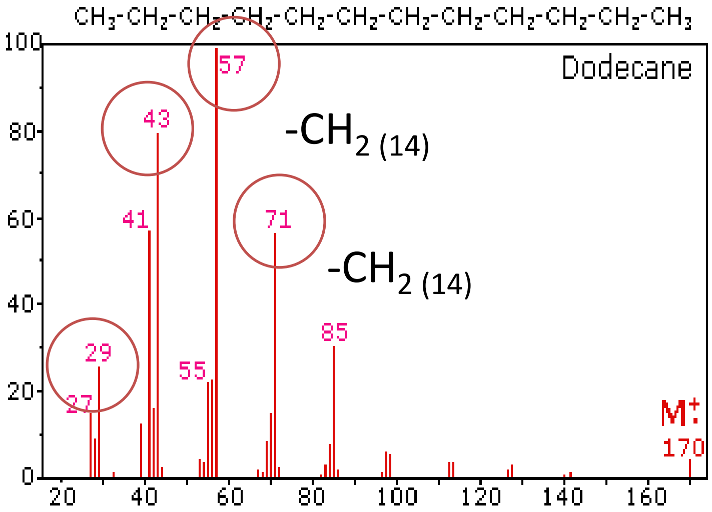
⇨ great tendency to fragmentation

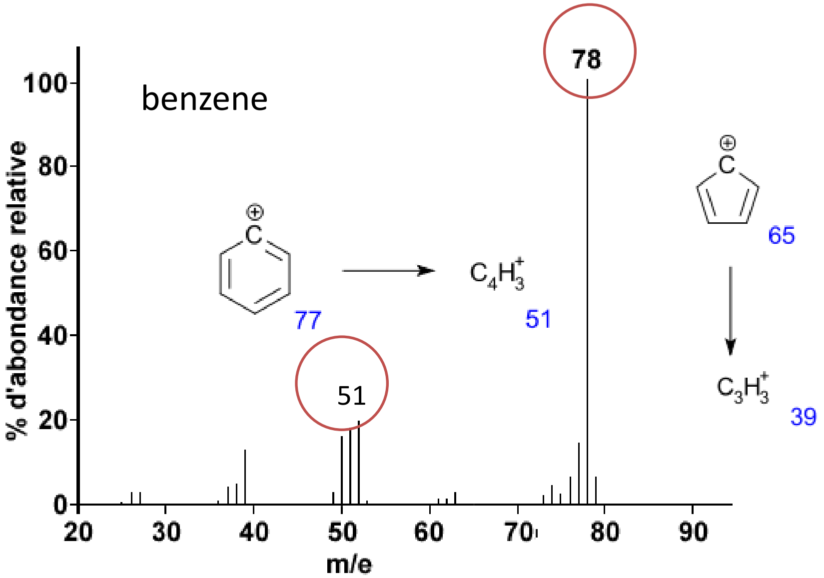
⇨ Low molecular ion peak

Aromatic rings, other π conjugated systems and cycloalkanes :

⇨ double bonds, ring and especially aromatic ring stabilize the molecular ion

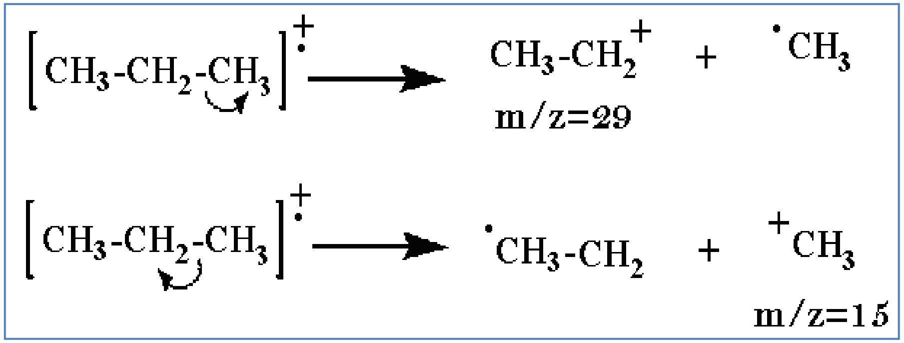
⇨ greater probability of molecular ion peak appearance





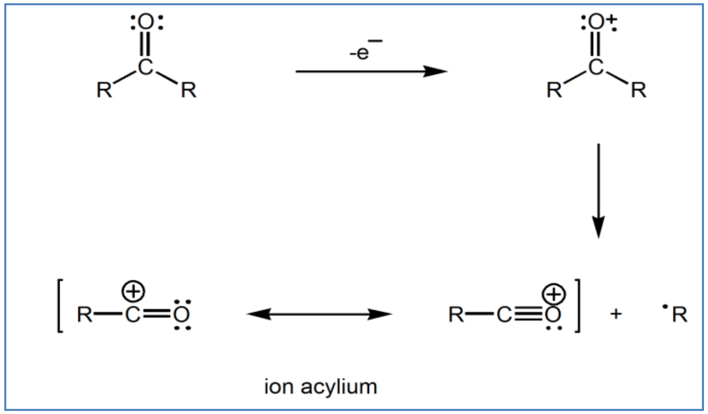
5. 1. LC-MS

- Fragmentation:
  - Simple break =



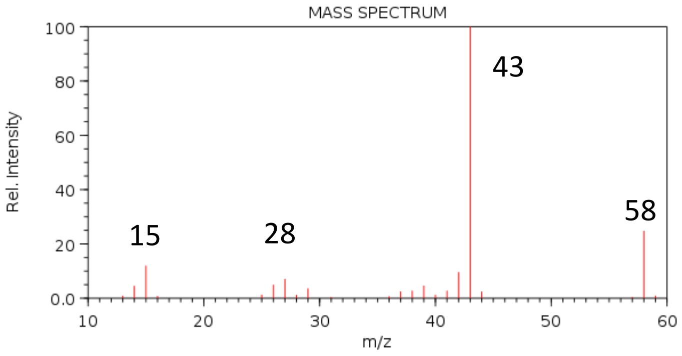
Exemple du propane

→ Formation of a more stable cations
→ peak at m/z = 29 more intense than at m/z = 15



Exemple des cétones

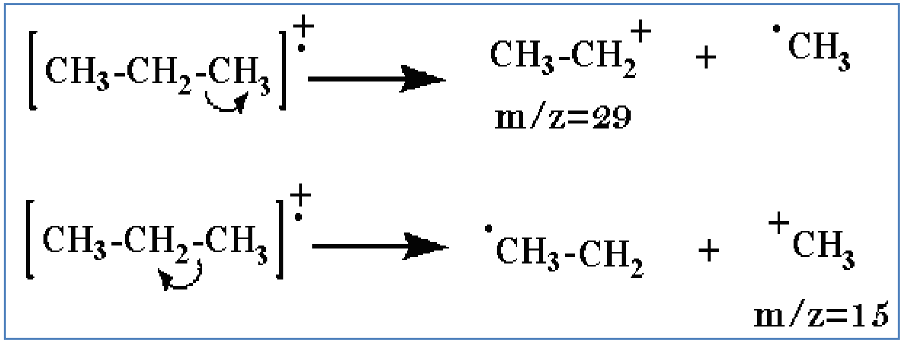
→ Formation of stable acylium ion so 4 fragments: RCO^+, $R'CO^+$, R^+, R'^+



NIST Chemistry WebBook (http://webbook.nist.gov/chemistry)

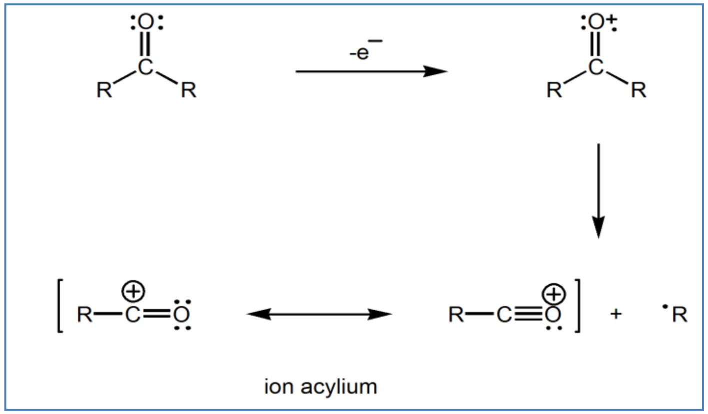
5. 1. LC-MS

- Fragmentation:
  - Simple break =



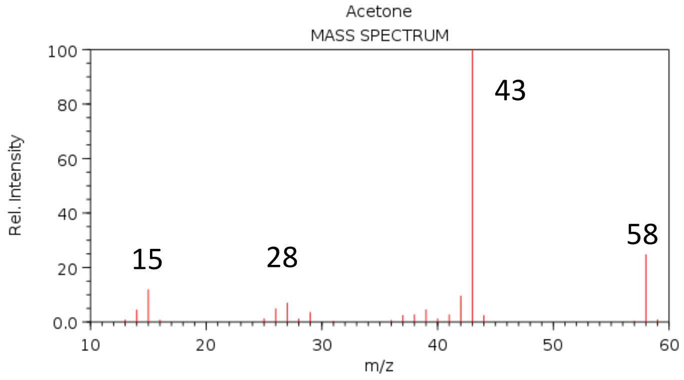
Exemple du propane

→ Formation of a more stable cations
→ peak at m/z = 29 more intense than at m/z = 15



Exemple des cétones

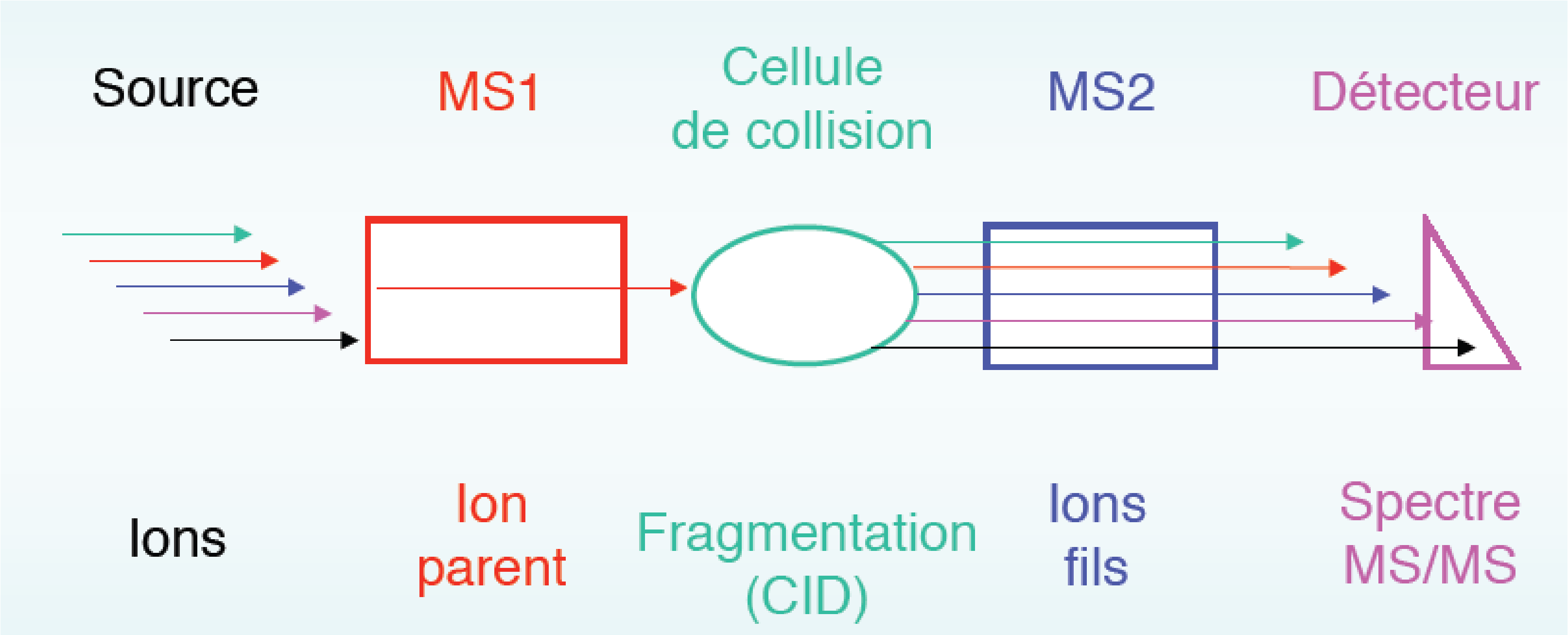
→ Formation of stable acylium ion so 4 fragments: RCO^+, $R'CO^+$, R^+, R'^+



NIST Chemistry WebBook (http://webbook.nist.gov/chemistry)

5. 1. LC-MS

- Combination LC-MS-MS (LC tandem MS)
  - Best identification



Source

MS1

Cellule de collision

MS2

Détecteur

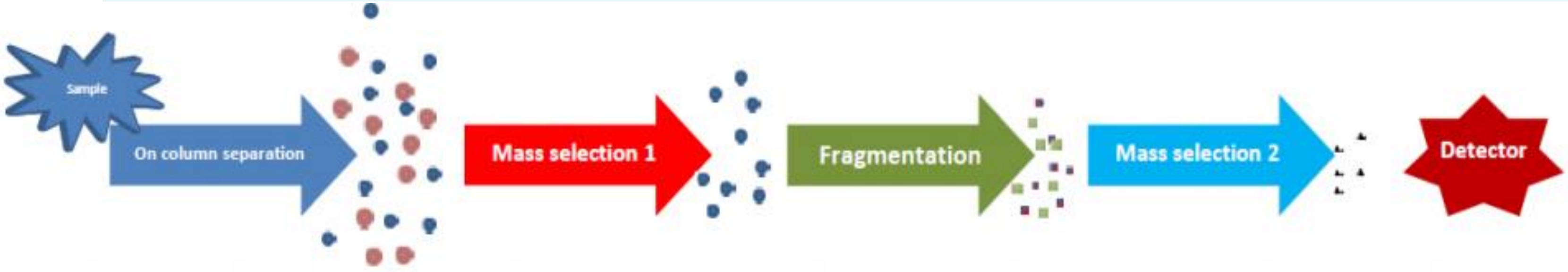
Ions

Ion parent

Fragmentation (CID)

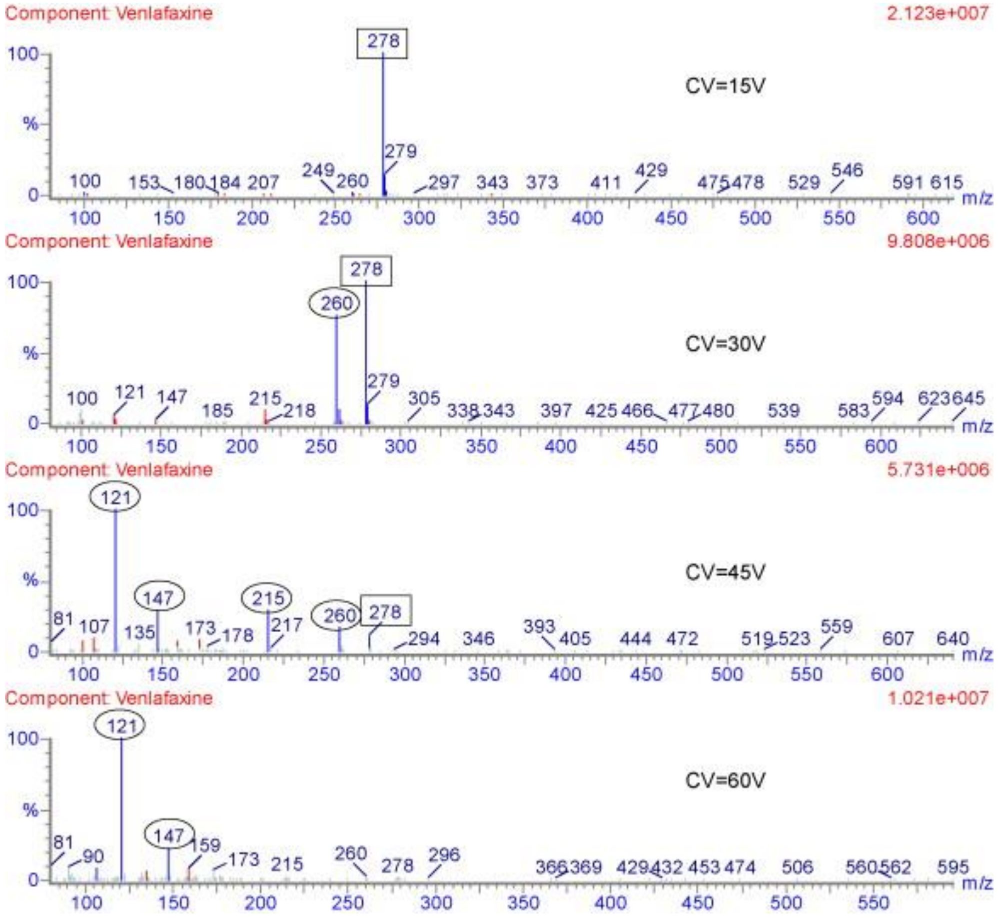
Ions fils

Spectre MS/MS



5. 2. Couplage LC-MS

- Quel signal?
  - Identification par comparaison avec une bibliothèque de données

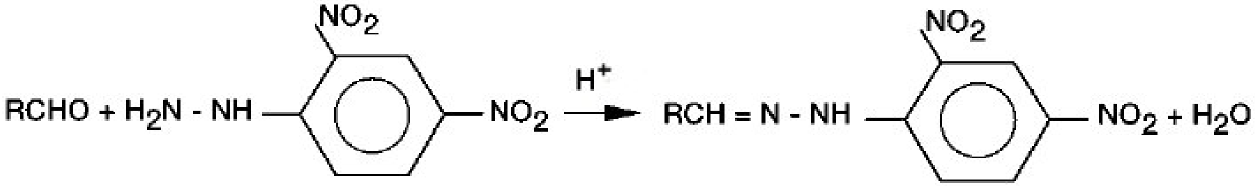


ESI-LC-MS: Spectres de masse de la venlafaxine enregistrés à différentes tension de cône (CV) : Est encadré le pic moléculaire de la venlafaxine (MH+) et encerclé les différents fragments caractéristiques de la molécule.

Cassagnes et al., IBS, 2008

5. 1. HPLC

- Applications:
  - NF X43-264 (2011): Prélèvement et dosage d'aldéhydes par pompage sur supports imprégnés de DNPH et dosage par chromatographie en phase liquide CLPH
    - Avec dérivatisation à la DNPH (Dinotrophenylhydrazine) sur silicagel



  - NF T 90-123 / NF EN ISO 11369 : Qualité de l'eau. Dosage de certains agents de traitement des plantes: Méthode par HPLC avec détection UV après extraction solide-liquide.
  - NF X 43-025: Air Ambiant: Analyse des Hydrocarbures Aromatiques Polycycliques par HPLC avec fluorimètre après mise en solution.
  - Norme XP T90-223 (2013) : Qualité de l'eau - Dosage de certains résidus médicamenteux dans la fraction dissoute des eaux - Méthode par extraction en phase solide et analyse par chromatographie en phase liquide couplée à la spectrométrie de masse en tandem (LC-MS/MS)

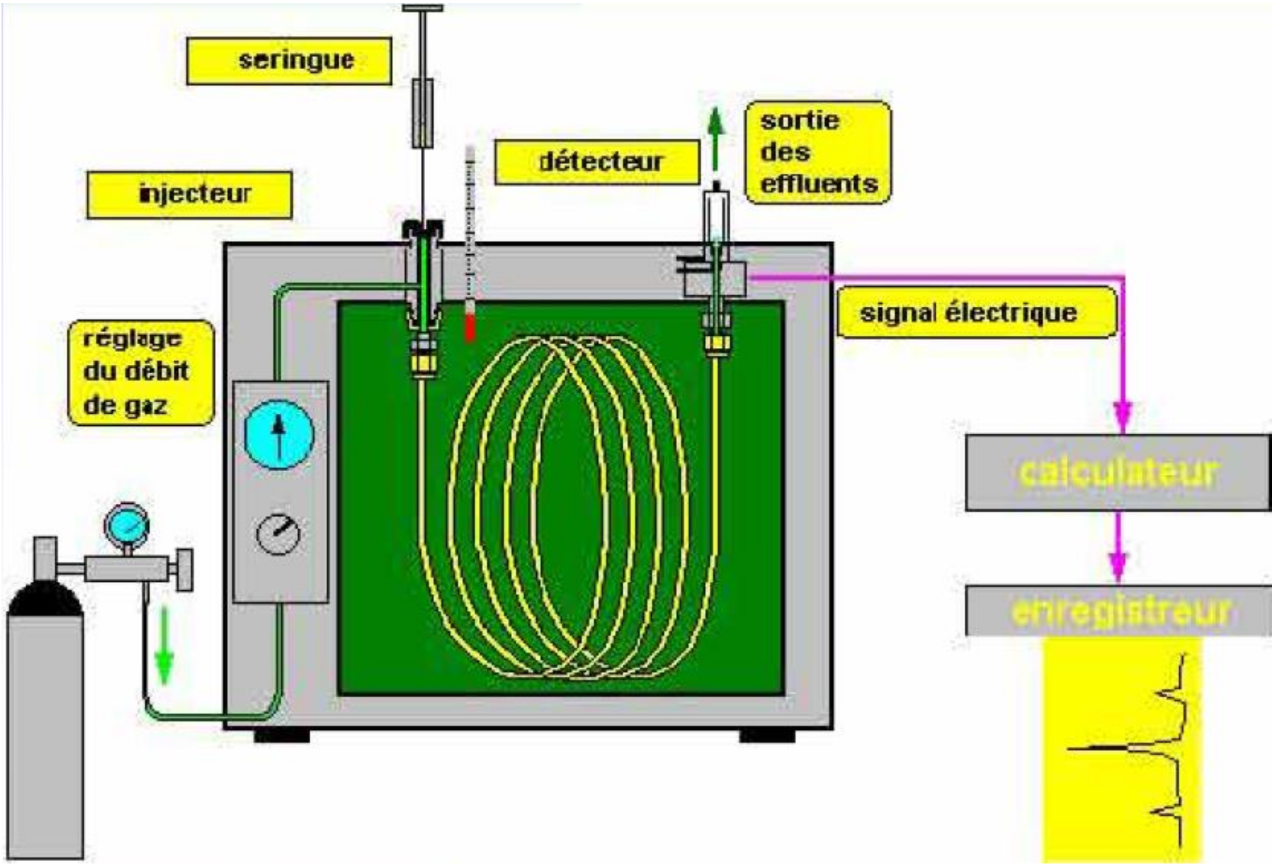
5. Organic pollutant analyses

- **Aim**: Determination of organic species or identification of main functions which they are composed

- **Techniques for liquid phase:**
  - HPLC: High Performance Liquid Chromatography
    - Combination LC-MS: Liquid Chromatography-Mass Spectrometry
  - GC: Gaseous Chromatography
    - Combination GC-MS: Gaseous Chromatography-Mass Spectrometry
  - Spectrofluorimetry

5. 2. Gas Chromatography

- Principle: Chromatography with separation based on volatility and polarity of molecules
  - **Separation of gaseous compounds** ⇒ need to volatilise the coumpounds in the liquid or solid phases

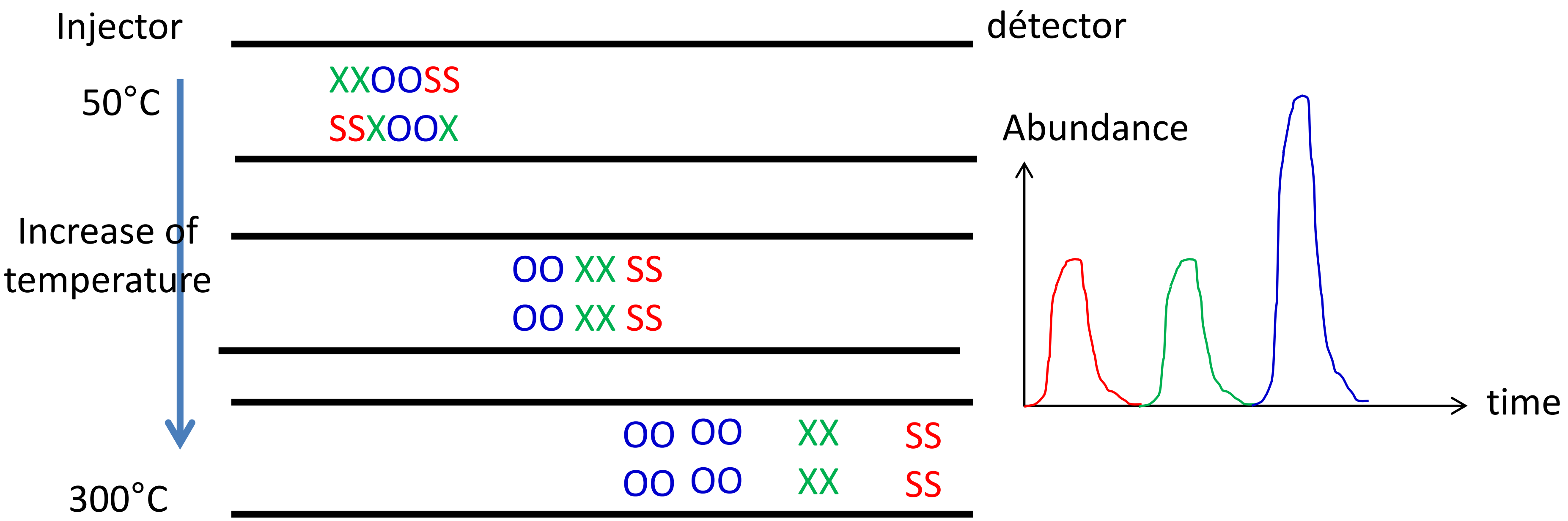


5. 2. Gas Chromatography

- Principle: Chromatography with separation based on volatility and polarity of molecules
  - **Separation of gaseous compounds** ⇒ need to volatilise the coumpounds in the liquid or solid phases

5. 2. Gas Chromatography

- Principle of separation:

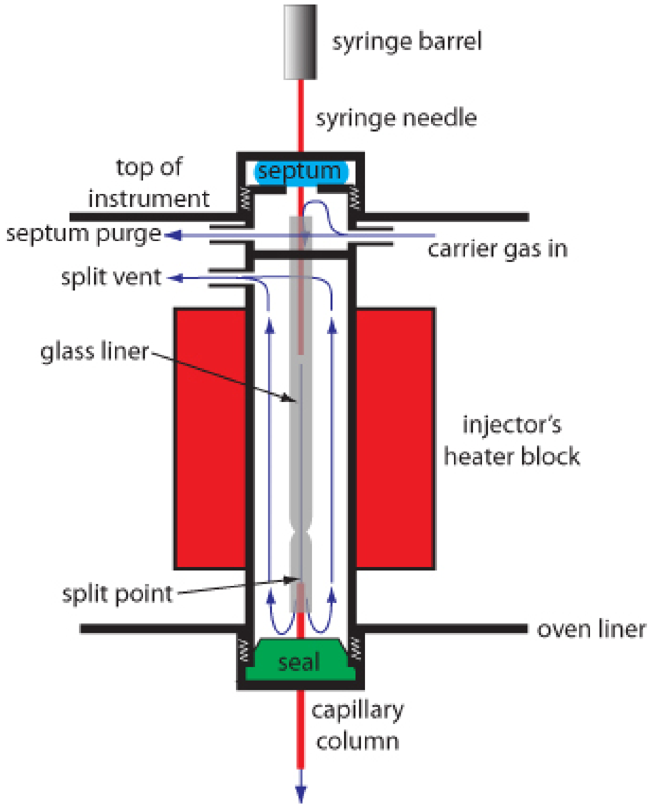


Here: Separation from polarity and thermal gradient
Other possibility: thermostatic column and separation from polarity

- Derivatisation to increase the volatility of certain species which have a low volatility or which are thermally instable such as polar species containing: -COOH; -OH; $-NH_2$; $-SO_3H$; -SH

5. 2. Chomatographie en phase Gazeuse CPG

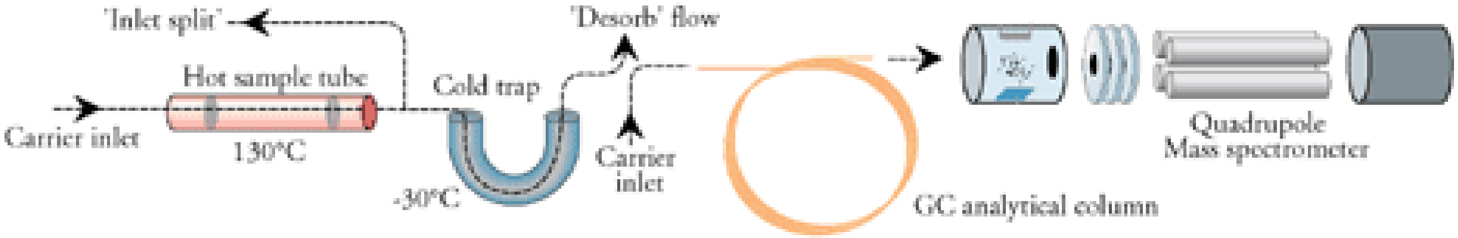
- Instrumentation:
  - **Carrier gas** : helium, hydrogen, nitrogen, argon
  - **Injector**:

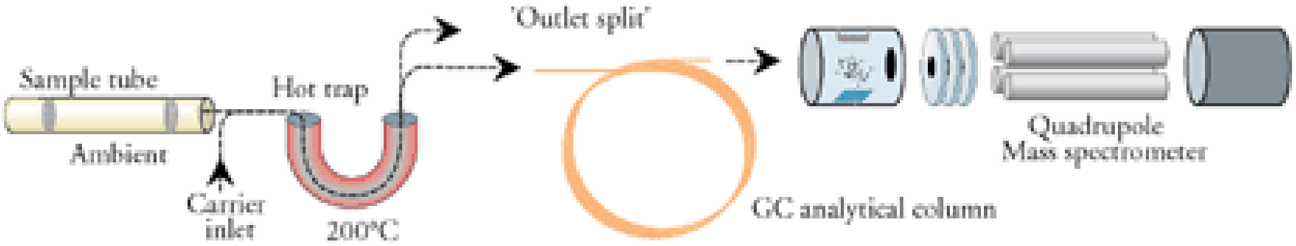


- For the liquid samples, it makes it possible to vaporize and entrain at the top of the column the sample mixed with the carrier gas.
- **Split** injector (injection of part of the sample)/**Splitless** injector (injection of the entire sample)
- Case **thermal desorber** for cartridges with gaseous absorbant.

5. 2. Chomatographie en phase Gazeuse CPG

- Appareillage:
  - **Carrier gas**: helium, hydrogen, nitrogen, argon
  - **Injector**: Analytical Thermal Desorber (ATD)



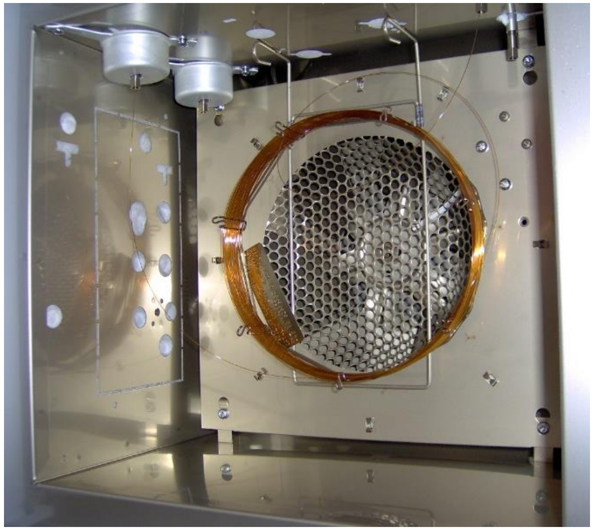


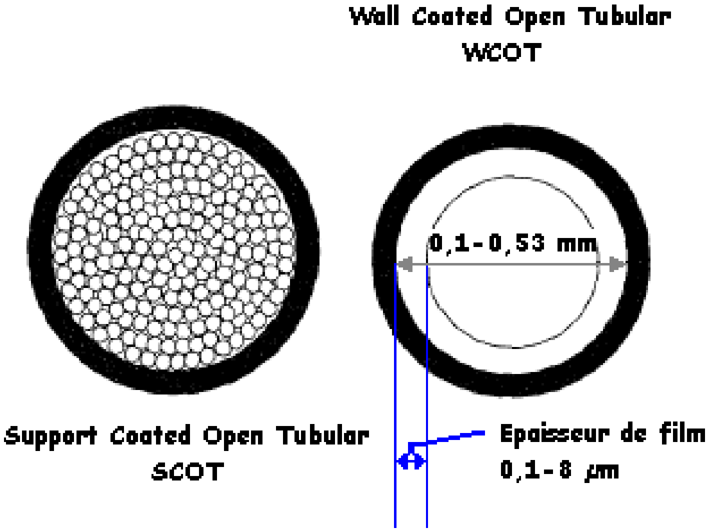
5. 2. Chomatographie en phase Gazeuse CPG

- Instrumentation: column = capillary columns
  - fused silica impregnated externally with polyimide of internal diameter less than 0.53 mm and 25 to 100 m in length
  - 2 main types:

SCOT : Support Coated Open Tubular : where the stationary phase is an impregnated solid

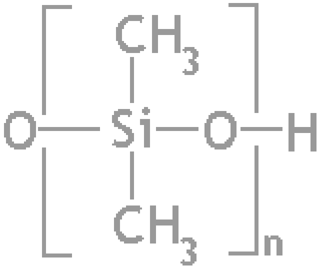
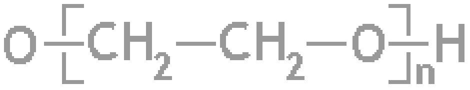
WCOT : Wall Coated Open Tubular: where wall covered with a liquid film grafted or not





5. 2. Chomatographie en phase Gazeuse CPG

- Instrumentation: stationary phase
  - According to the grafting, it can be polar to apolar
  - The most common grapfting are:

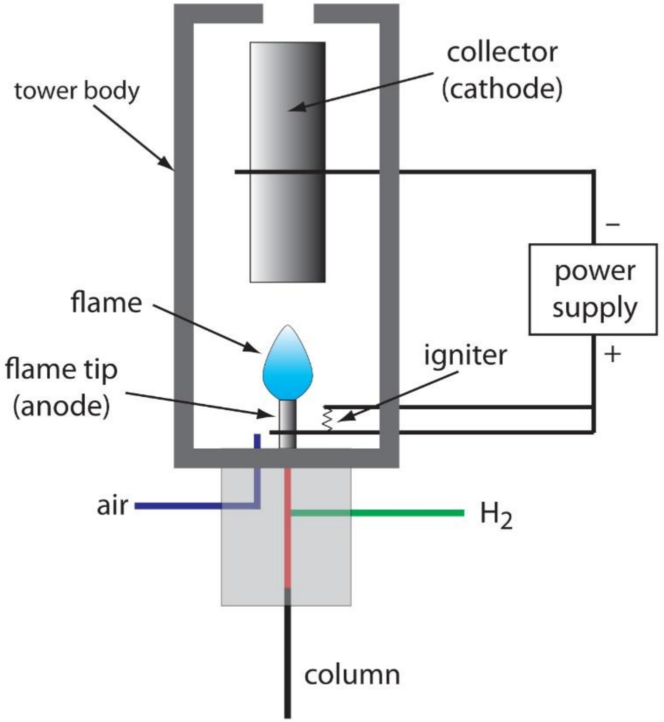
 $O-\left[\underset{CH_3}{\overset{CH_3}{Si}}-O\right]_n-H$	 $O-\left[CH_2-CH_2-O\right]_n-H$
Crossbond dimethyl polysiloxane Non-polar - resistant	Crossbond polyethylene glycol Polar - fragile

5. 2. Chomatographie en phase Gazeuse CPG

- Instrumentation: Main detectors
  - FID : Flame Ionization Detector (détecteur à ionisation de flamme) : the most frequent detector for organic compounds analysis
  - ECD: Electron Capture Detector (détecteur à capture d'électrons) : Selective detector for electro-negative (halogenated hydrocarbons: PCB's, organochlorine pesticides, herbicides)
  - Mass spectrometry (GC – MS) : provides information of molecular structure

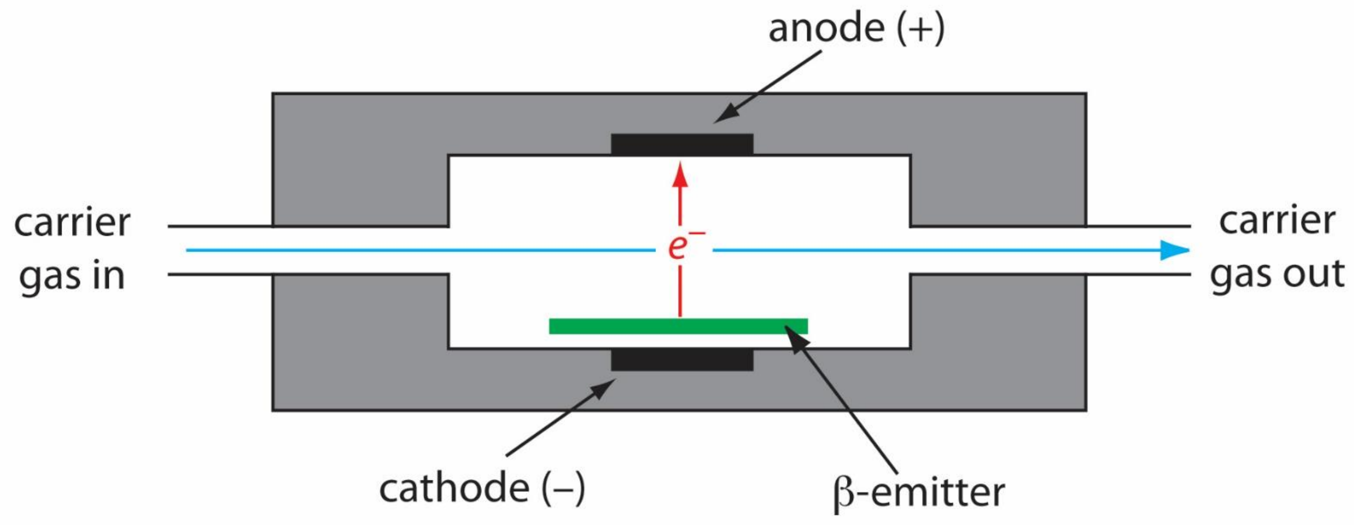
5. 2. Chomatographie en phase Gazeuse CPG

- Instrumentation: Main detectors
  - FID: Flame Ionization Detector
    - The sample gas is introduced into a hydrogen flame inside the FID. Any hydrocarbons in the sample will produce ions when they are burnt. Ions are detected using a metal collector which is biased with a high DC voltage. The current across this collector is thus proportional to the rate of ionisation which in turn depends upon the concentration of HC in the sample gas.



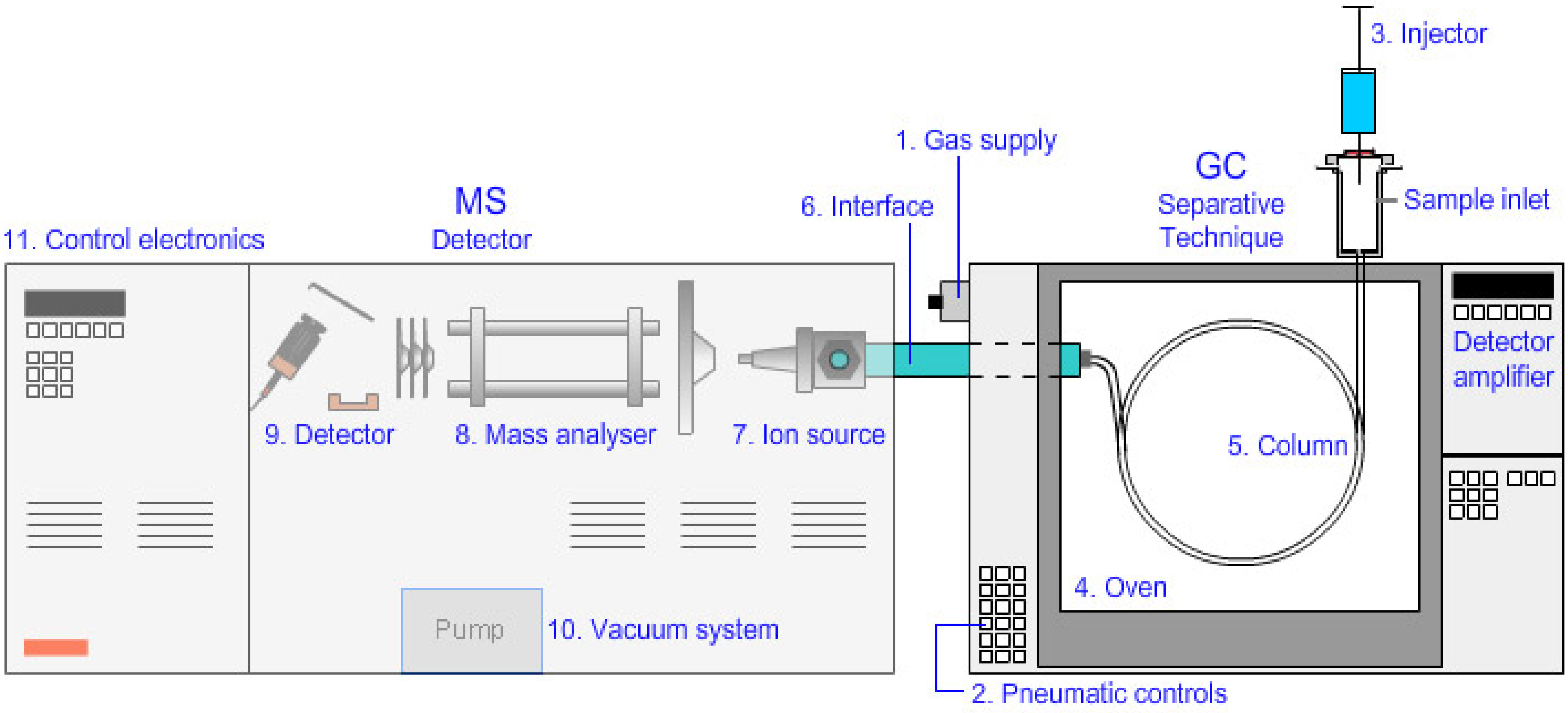
5. 2. Chomatographie en phase Gazeuse CPG

- Appareillage: Principaux détecteurs
  - ECD: Electron Capture Detector
    - A (β-ray) radio active source (^{63}N) which can ionize the carrier gas is located in the detector. A current is produced between two electrodes in the detector supplied with a potential difference and this is monitored as a continuous background current. When there are electro-negative components present in the carrier gas, the background current is reduced because these components capture electrons



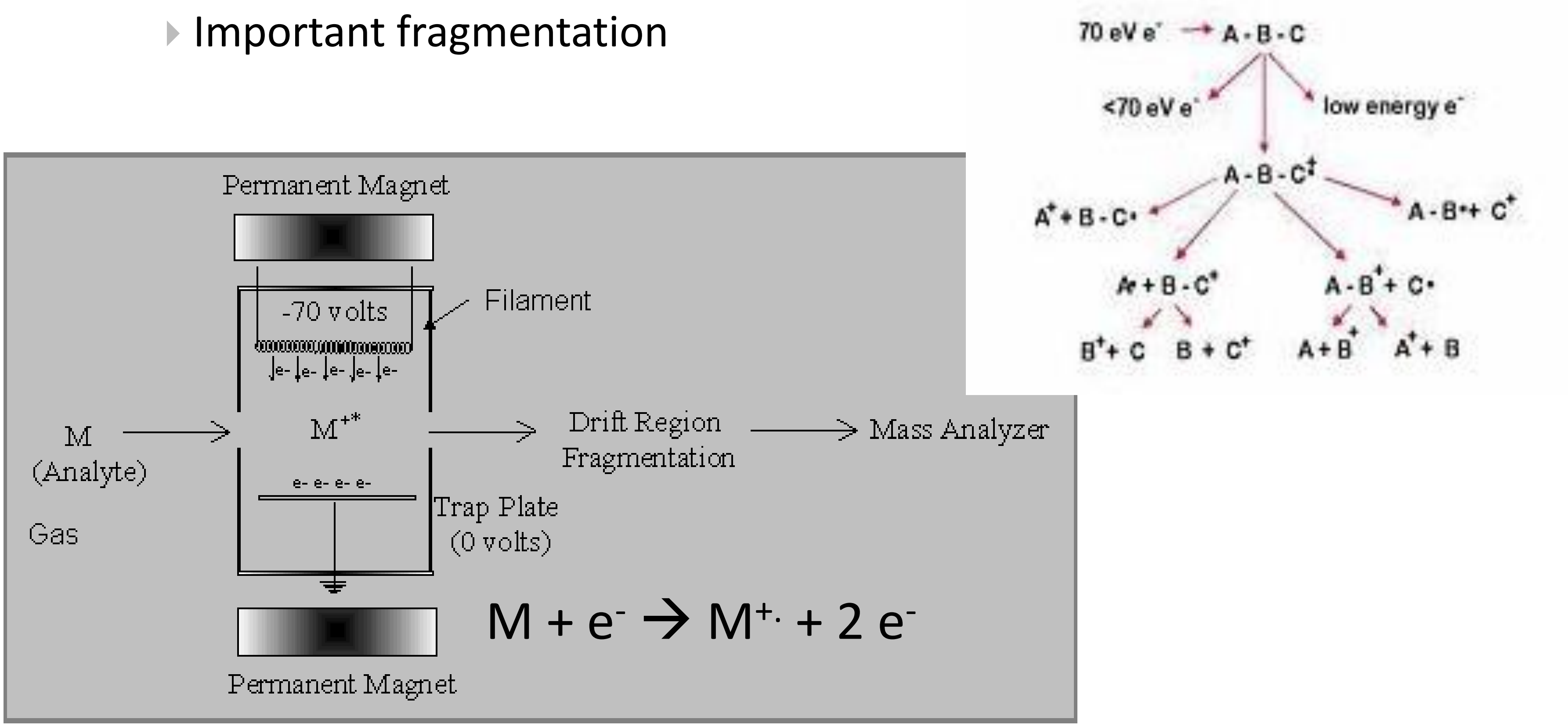
5. 2. GC-MS

- Instrumentation
  - A simplest combination since the sample is already in gaseous phase



5. 2. GC-MS

- Main difference with LC-MS: Ionisation source
  - *EI: Electronic Ionisation* (Ionisation électronique)
    - Formation of positif ions
    - Important fragmentation



$M + e^- \rightarrow M^{+\cdot} + 2\,e^-$

Molecular ion M

5. 2. GC-MS

- Main difference with LC-MS: Ionisation source
  - *CI: Chemical Ionisation (*Ionisation Chimique)
    - Formation of positive or negative ions
    - Using a reactive gas to ionise and hence soft fragmentation Passage (methane, ammoniac…)

$$CH_4 + e^- \longrightarrow CH_4^{+\bullet} + 2e^-$$

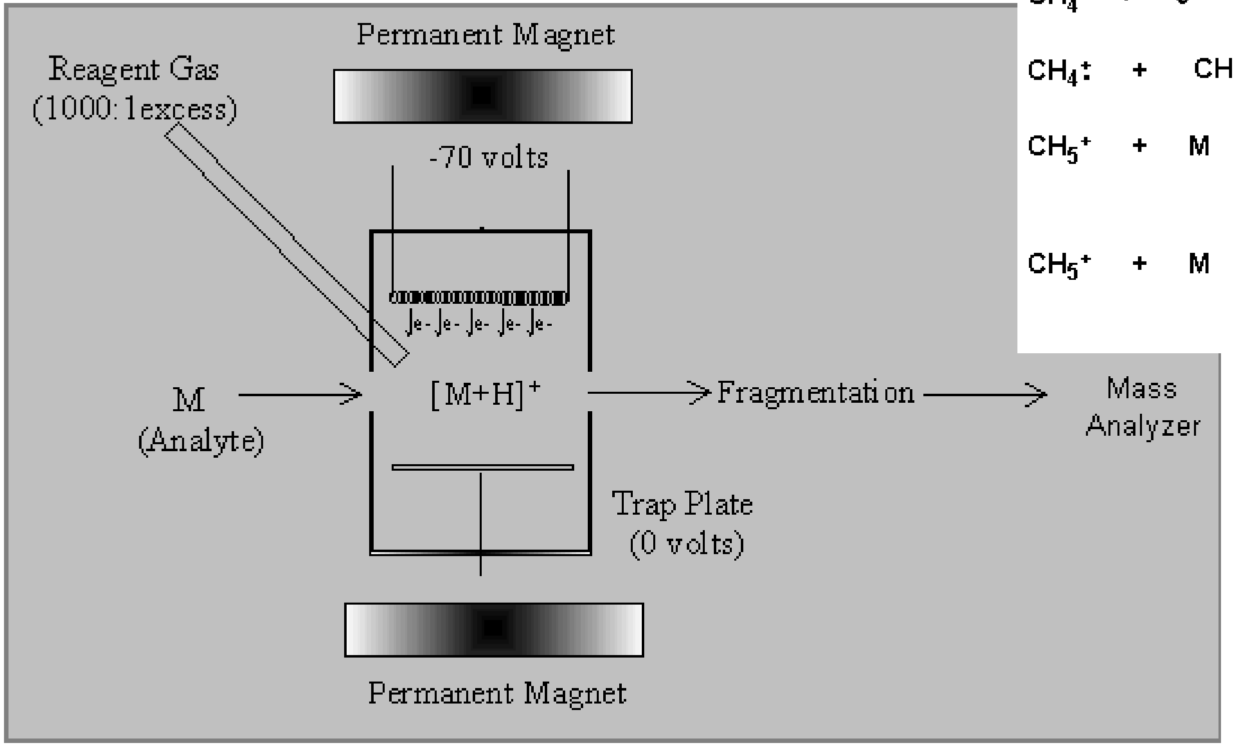
$$CH_4^{+\bullet} + CH_4 \longrightarrow CH_5^+ + CH_3^\bullet$$

$$CH_5^+ + M \longrightarrow MH^+ + CH_4$$

ion moléculaire à M+1

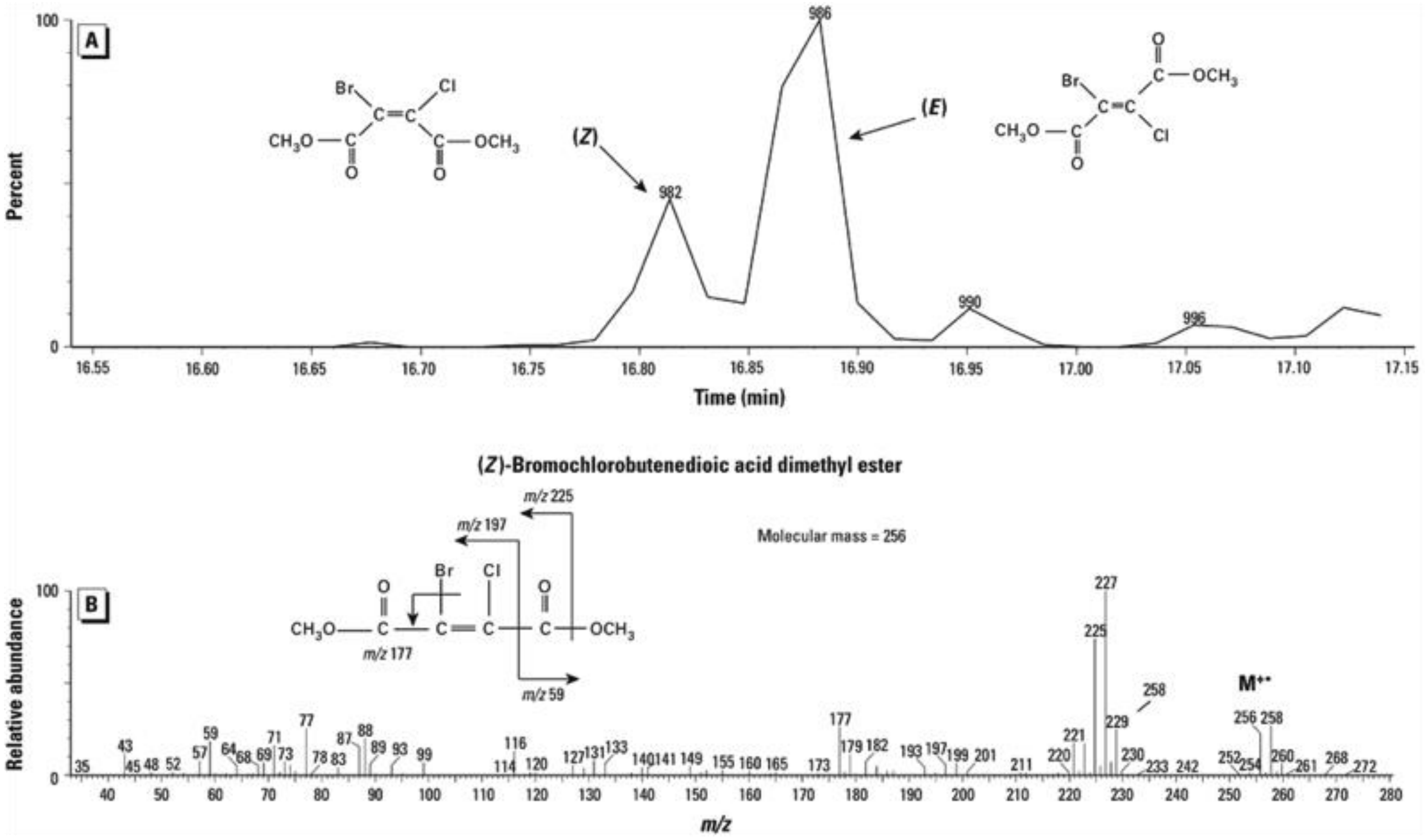
$$CH_5^+ + M \longrightarrow MCH_4^{+\bullet} + H^\bullet$$

ion moléculaire à M+16



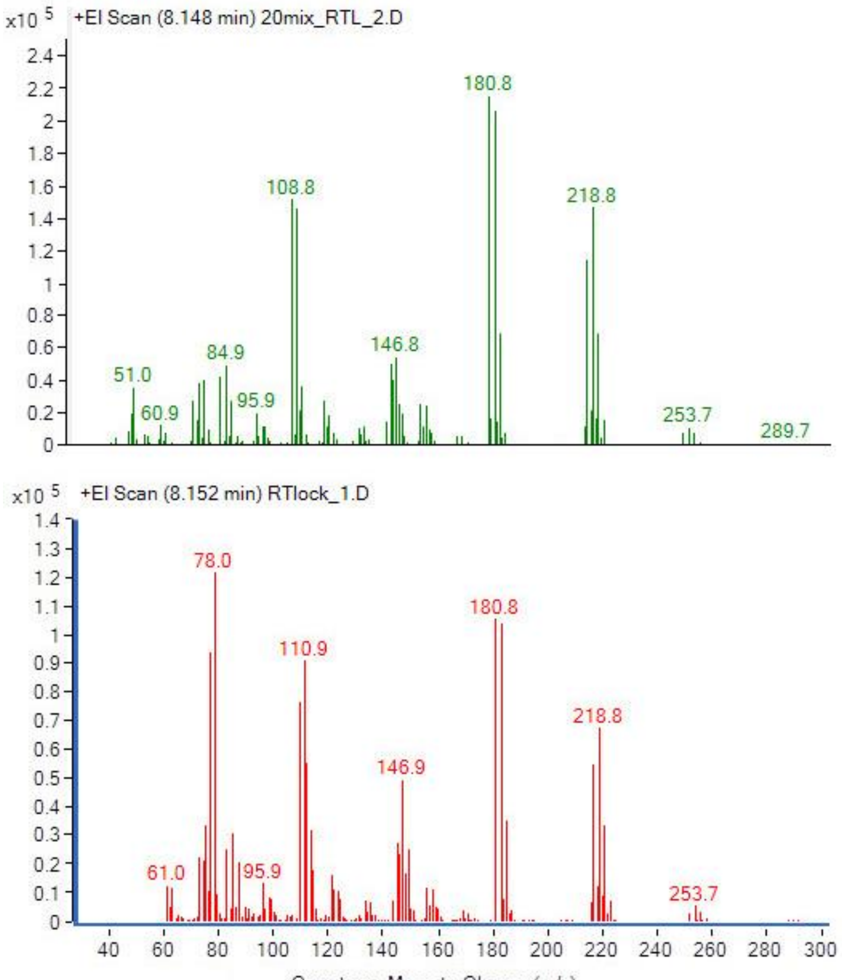
5. 2. GC-MS

- What is the signal?
  - Double spectrum: chromatogram + mass spectrum:



5. 2. Couplage CPG-MS

- What is the signal?
  - Identification by comparing with a database



5. 1. GC-MS

- Applications:
  - **NF T 90-120 / NF EN ISO 6468** : Dosage des pesticides organochlorés et des polychlorobiphényles (PCB): Méthode par chromatographie en phase gazeuse après extraction liquide-liquide
  - **NF ISO 18287 Août 2006 :** Qualité du sol - Dosage des hydrocarbures aromatiques polycycliques (HAP) - Méthode par chromatographie en phase gazeuse avec détection par spectrométrie de masse (CG-SM)
  - **NF ISO 16000 -2005**: Air intérieur – dosage des COV dans l'air intérieur des locaux par échantillonnage actif sur le sorbant Tenax TA, désorption thermique et chromatographie en phase gazeuse utilisant MS/FID

5. Organic pollutant analyses

- **Aim**: Determination of organic species or identification of main functions which they are composed

- **Techniques for liquid phase:**
  - HPLC: High Performance Liquid Chromatography
    - Combination LC-MS: Liquid Chromatography-Mass Spectrometry
  - GC: Gaseous Chromatography
    - Combination GC-MS: Gaseous Chromatography-Mass Spectrometry
  - Spectrofluorimetry

5. 3. Spectrofluorimetry

- Aim: Identification of molecular functional groups/molecular structure → analysis of fluorescence

- Principle: Molecular fluorescence
  - Photoluminescence is light emission from any form of matter after the absorption of photons. We can distinguish:
    - Phosphorescence: long time (slow process of relaxation)
    - Fluorescence: some seconds (fast process)

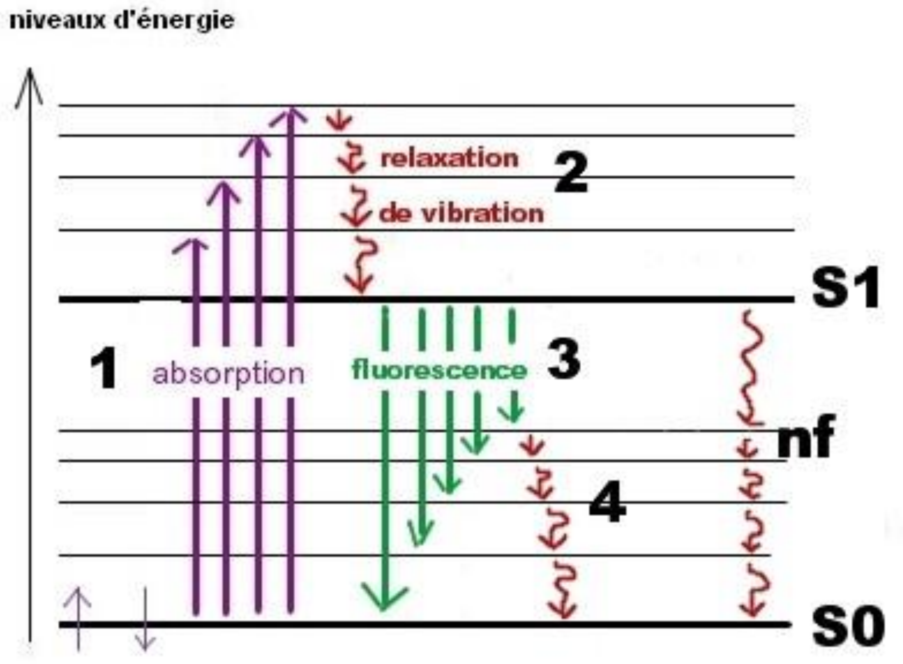
  - Fluorimetry = experimental technique by which the molecular fluorescence is measured from visible to UV

⚠ *It is different of X-Ray spectrometry for atomic emission in the X-ray spectrum*

5. 3. Spectrofluorimetry

- Aim: Identification of molecular functional groups/molecular structure → analysis of fluorescence

- Principle: Molecular fluorescence (photoluminescence)



$h\nu_{absorption} > h\nu_{fluorescence}$

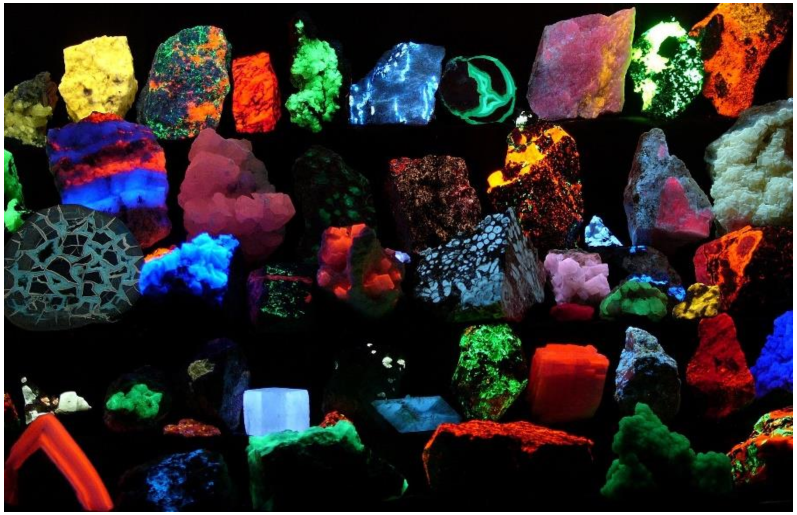
$\lambda_{absorption} < \lambda_{fluorescence}$

In UV-Vis spectrometry, the absorption of light is measured,

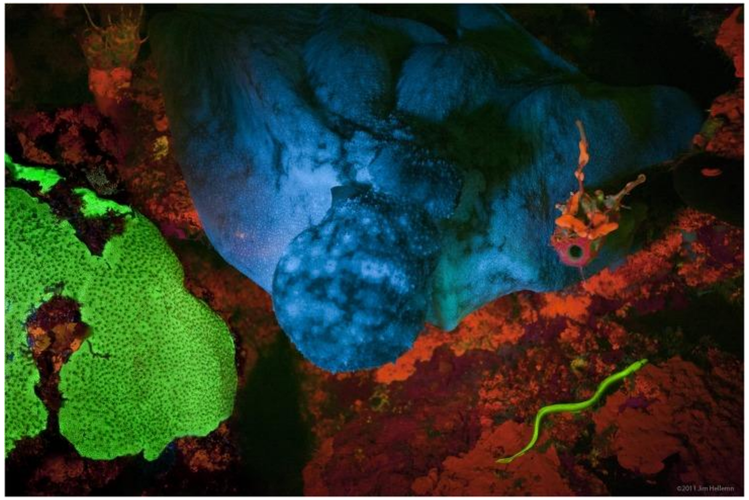
Here, the emission of light during the return to the ground state is measured→ rare phenomenon (generally no emission during the transition from the excited state to the ground state)

Compounds that can re-emit light = fluorochromes

5. 3. Spectrofluorimetry



Fluorescence of minerals under UV light



biofluorescence

Natural fluorescent chemical compounds are rare.

Fluorescence may be expected generally in:

1- Aromatic molecules that contain conjugated double bonds

2- Polycyclic aromatic compounds (with great number of π electrons)

3- Substituents strongly affect on the fluorescence; substituents such as NH_2 and OH groups enhance the fluorescence, while electron with drawing group such as NO_2, Cl^-, Br^-, I^- and COOH groups decrease the fluorescence

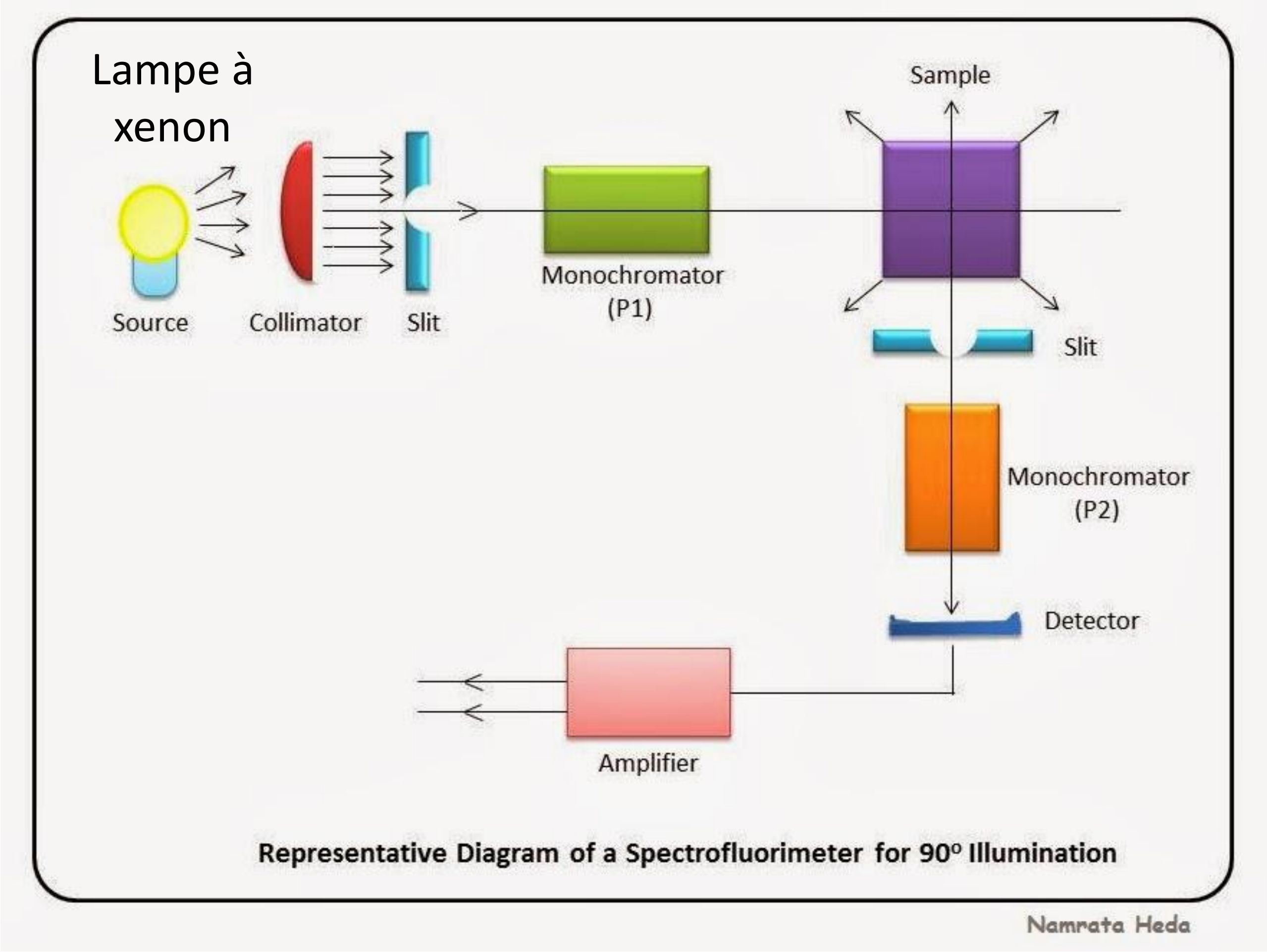
5. 3. Spectrofluorimétrie

- **Aim**: Determination of organic species or identification of main functions which they are composed

- Principle: Molecular fluorescence
  - Relation concentration/fluorescence intensity :
    - For low absorption ($A < 0.02$: <u>trace conc.)</u>: so $F = K \times C$
      - ☐ Avec K, specific constant for studied molecule and C, concentration
    - For high absorption, so $F = I_0 \phi$
      - ☐ I_0 : intensity of incident radiation
      - ☐ ϕ quantum yield of the fluorescence (number of emitted photons/number of absorbed photons)
      - ☐ That is the fluorescence is independent of concentration, and proportional to the intensity of incident radiation only
  - Adapted method for trace analysis: Fluorescent substances may be determined at conc. up to 1000 times lower than those required for absorption spectrophotometry.

5. 3. Spectrofluorimétrie

- Instrumentation:



Representative Diagram of a Spectrofluorimeter for 90º Illumination

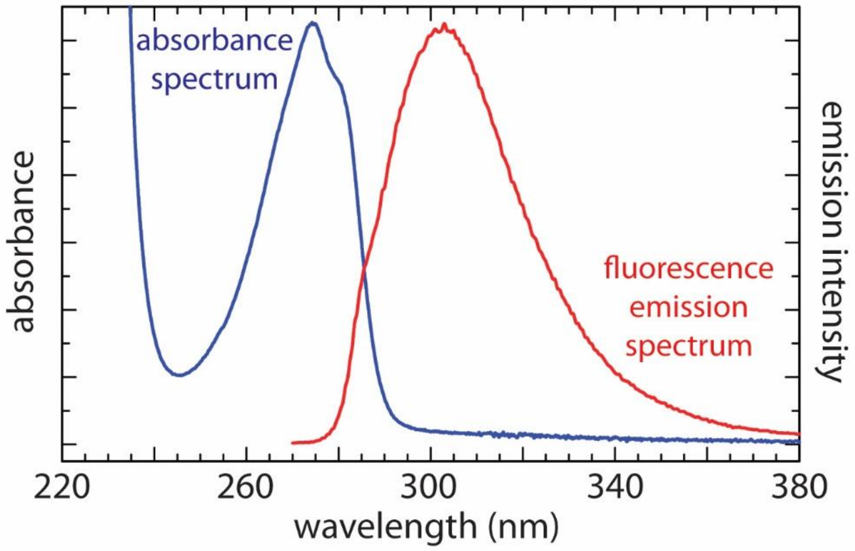
Fluorescence in all the directions

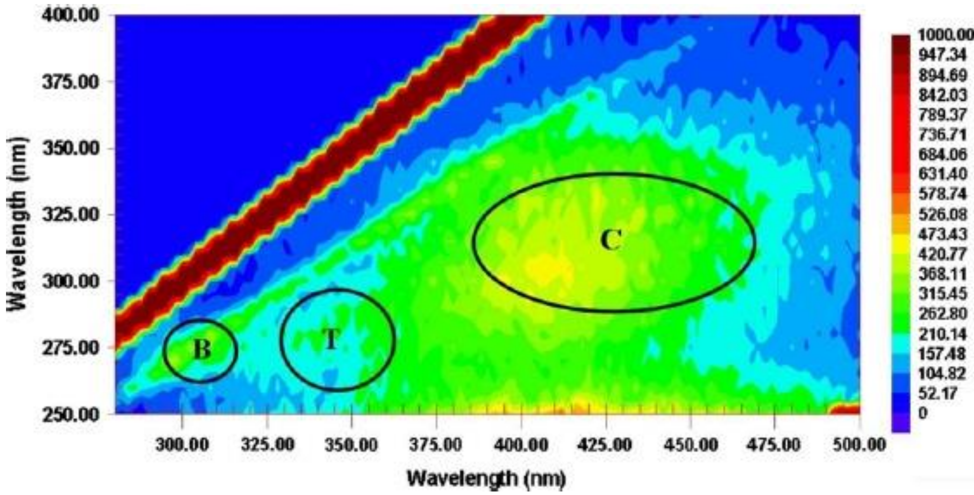
⬇

Measurement at 90° to limit interference by incident radiation

5. 3. Spectrofluorimetry

- Signals? 2 fluorescent spectra:
  - Emission Spectrum: When a monochromator source of constant light intensity is used to irradiate a sample, the emission fluorescence is analysed at different wavelength
  - Excitation Spectrum: the intensity of the fluorescence is plotted as a function of the wavelength of the radiation used to excite the fluorescence





5. 3. Spectrofluorimetry

- Instrumentation: 2 methods:
  - Fluorimetry: measurement for one wavelength: so identification of fluorescent compound at this wavelength
  - Spectrofluorimetry: measurement on the spetrum, so identification of all the fluorescent compounds
    - Measurement of the fluorescence intensity at variable wavelengths of excitation and emission, and production of fluorescence spectra

5. 3. Spectrofluorimétrie

- Applications
  - HAP measurements in water and soils… → in combination with HPLC
  - Estimation of dissolved organic matter in the surface waters (river and sea water), notably organic macromolecules

    →DOM (*Dissolved Organic Matter*)
    + CDOM (*Chromophoric (or Colored) Dissolved Organic Matter*)

  - Mesurament of chlorophyl (a) in seawater