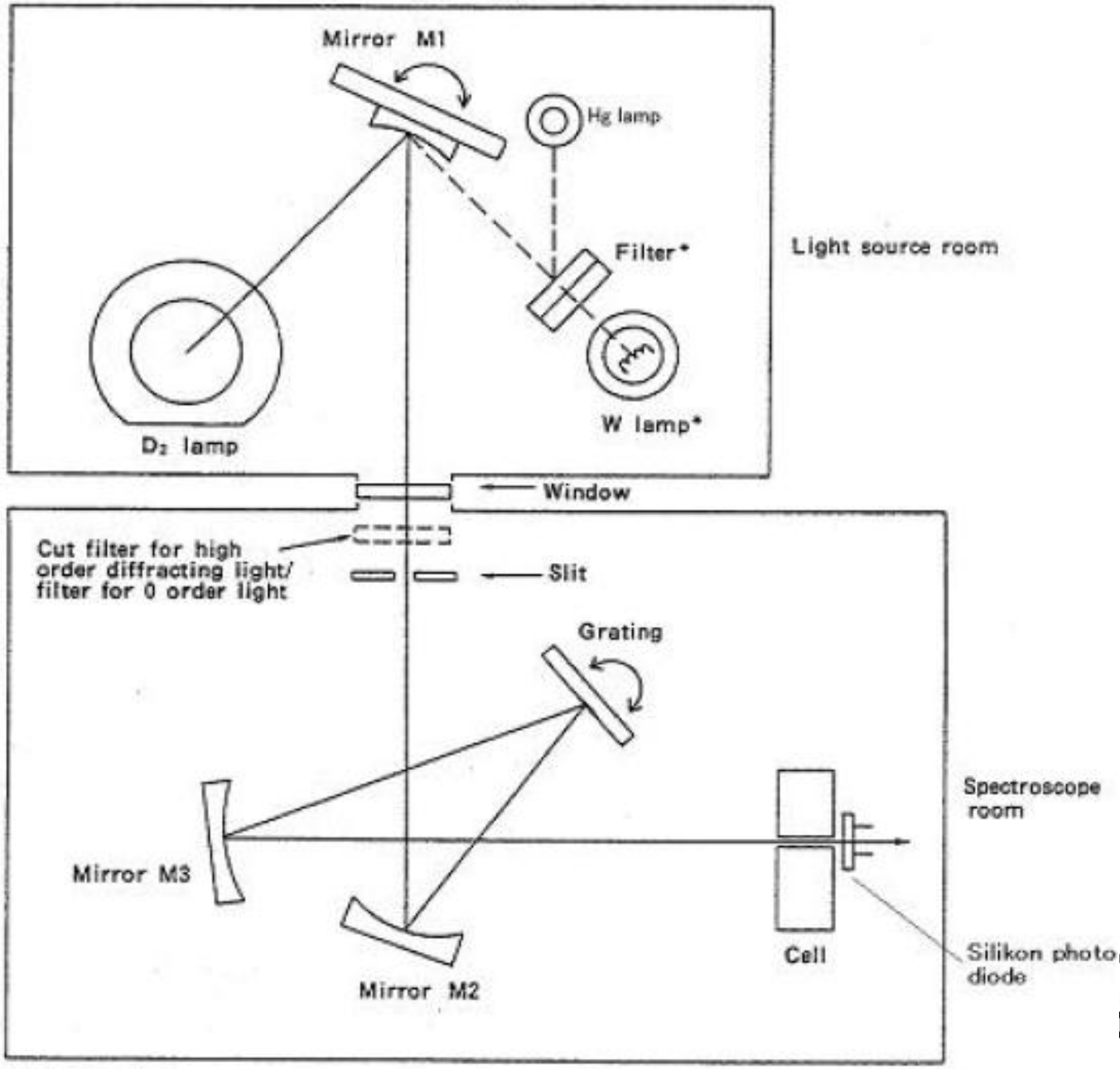
Detectors for HPLC

- UV-VIS Ultraviolet / Visible detector
- PDA Photodiode Array detector
- RF Fluorescence detector
- CDD Conductivity detector
- RID Refractive Index detector
- ECD Electrochemical detector
- ELSD Evaporative light scattering detector
- MS Mass spectrometer detector

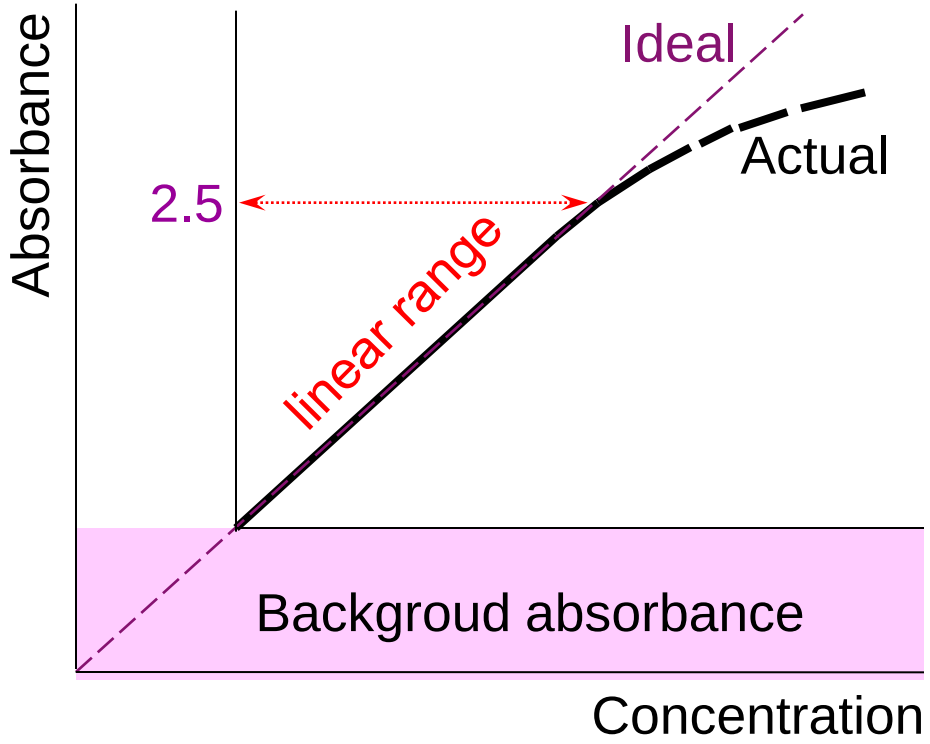
Ultraviolet / Visible Detector (1)



Ultraviolet / Visible Detector (2)

Lambert-Beer's Law

$$A = \varepsilon C L = - \log (E_{out} / E_{in})$$



A : absorbance

ε : molar absorptivity

C : analyte concentration

L : path length of the flow cell

E : energy

Ultraviolet / Visible Detector (4)

Advantage:

- **Sensitivity is high**
- **Relative robust to temperature and flow rate change**
- **Compatible with gradient elution**

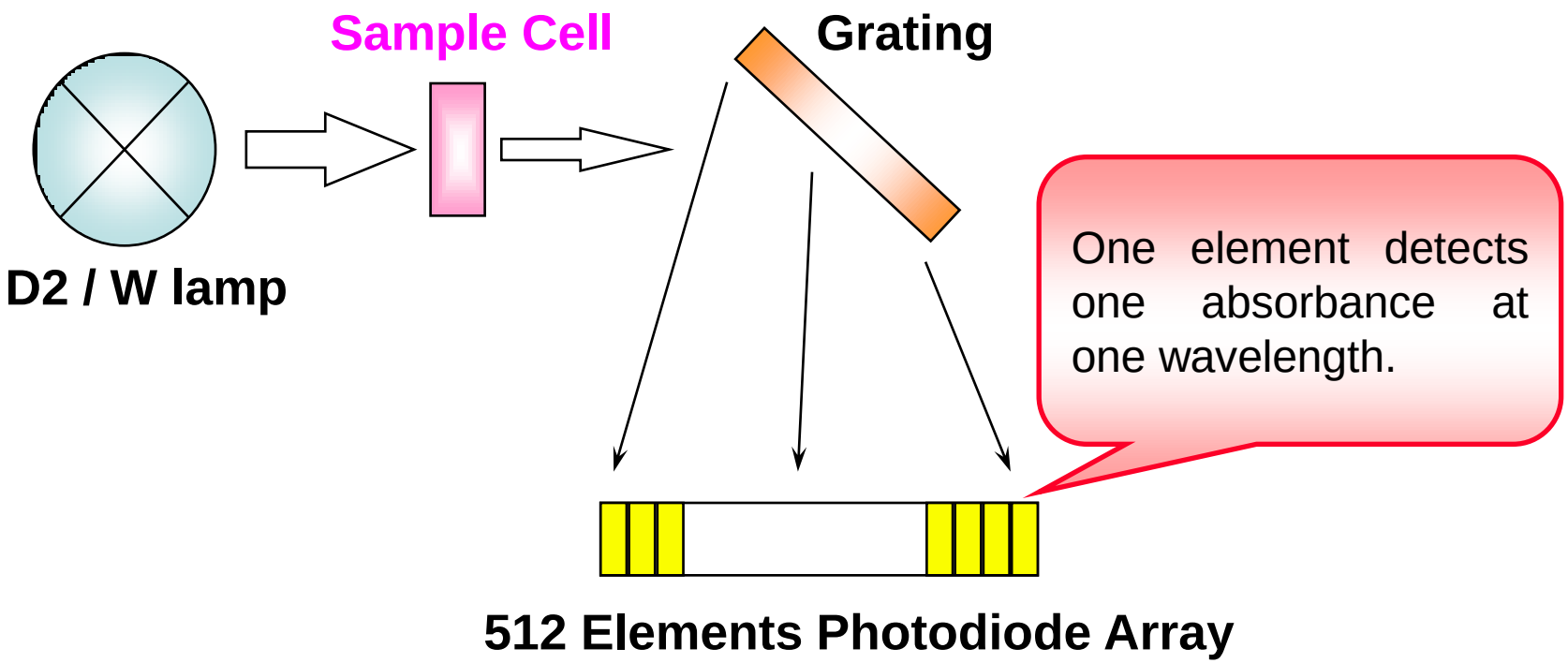
Disadvantage:

- **Only compounds with UV or visible absorption could be detected.**

Additional Functions

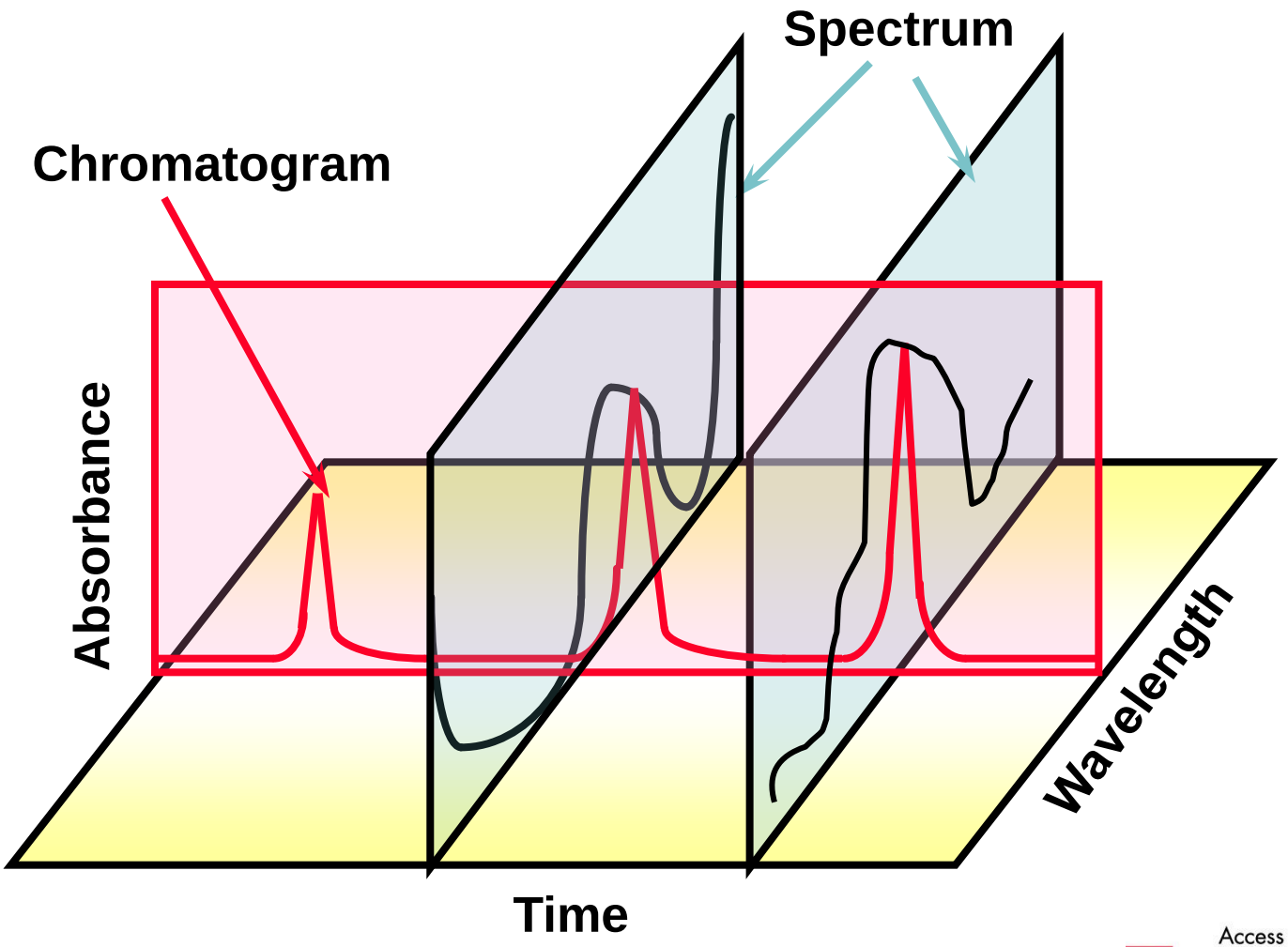
- **Dual Wavelength mode**
- **Wavelength Time Program mode**
- **Wavelength Scan mode**

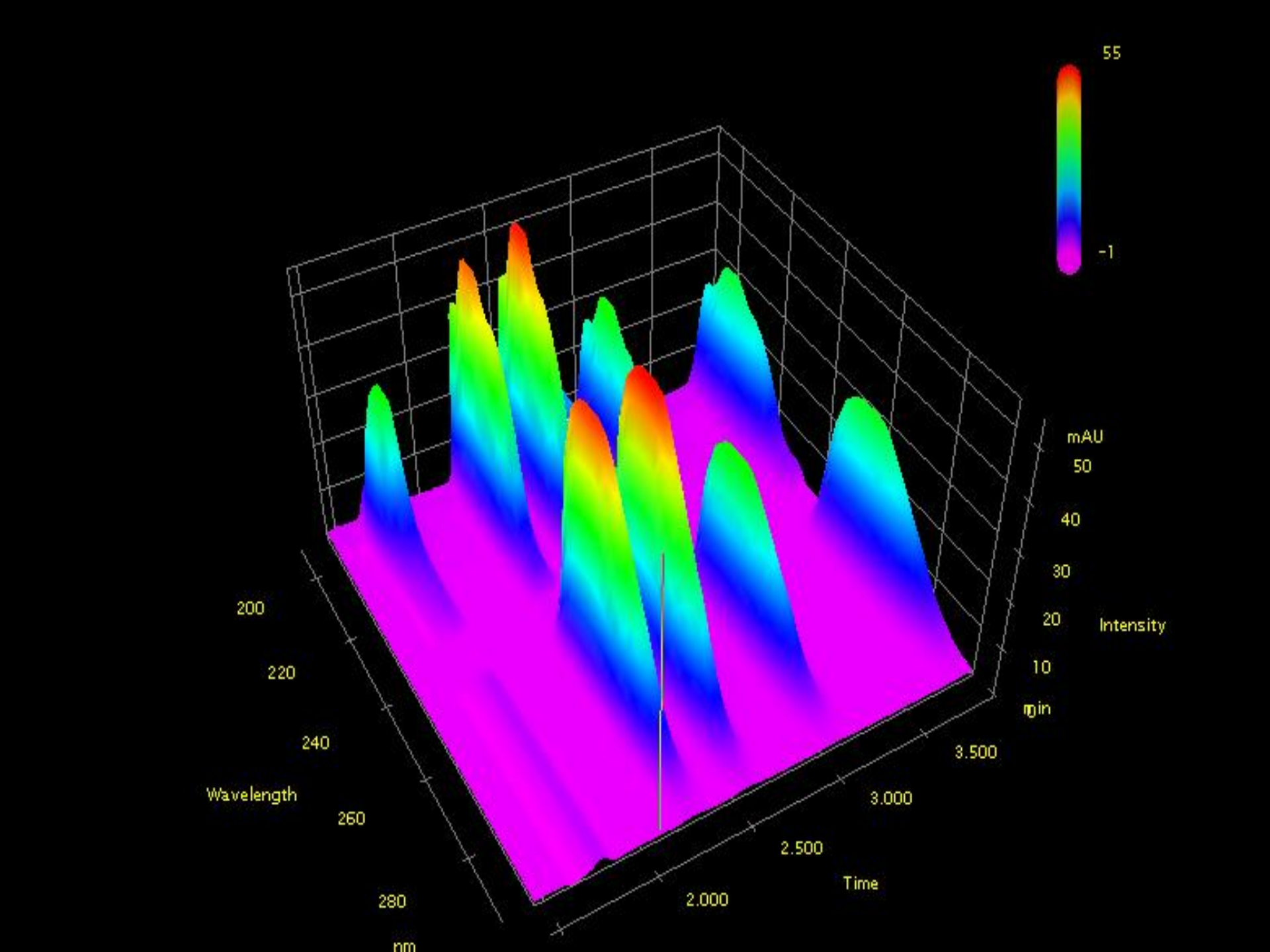
Photodiode Array Detector (1)



Photodiode Array Detector

3-D Data




55
-1
mAU
50
40
30
20
10
0
Intensity
200
220
240
260
280
nm
Wavelength
2.000
2.500
3.000
3.500
min
Time

PDA Detector

Advantages:

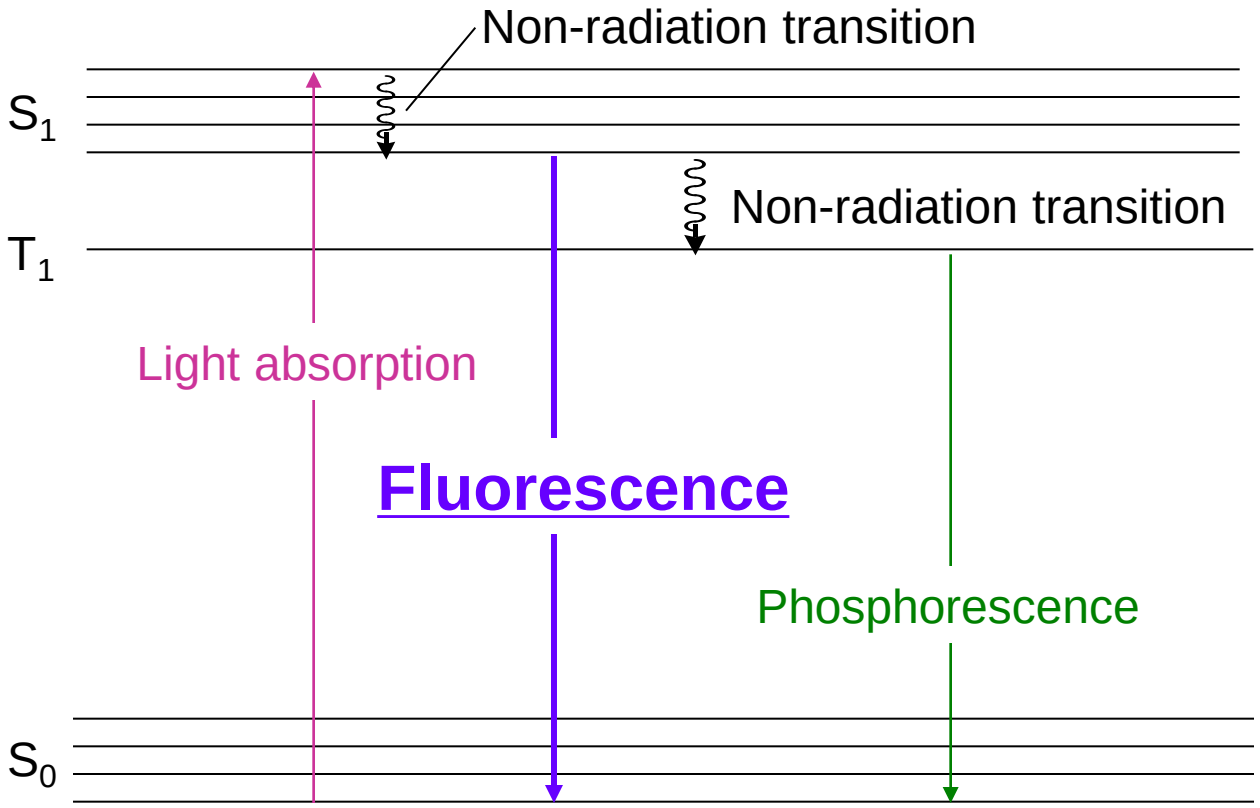
- PDA Detector could analyze a sample simultaneously at many different wavelengths.
- UV Visible spectra are useful for compound identification, checking peak purity, as well as finding the optimum absorbance for the compounds.
- UV Visible spectra of many compounds could be stored in the spectrum libraries, which are useful for compound identification.
- Relatively robust to temperature and flow rate fluctuations
- Compatible with gradient elution.

Disadvantages:

- Slightly less sensitive than UV-Visible detector.

Fluorescence of Compounds

Fluorescence is a type of luminescence in which the light energy is released in the form of a photon in nanoseconds to microseconds



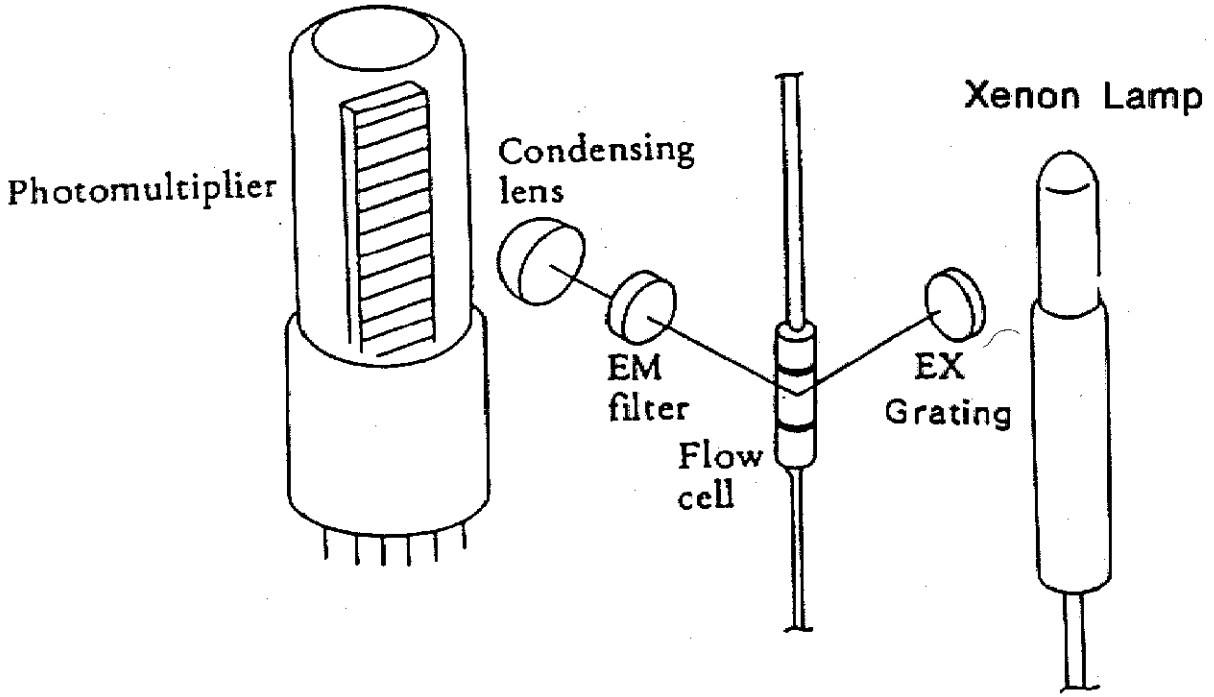
Relationship Between

Fluorescence Intensity & Concentration

$$F = 2.3\,\Phi_f\, I_0 \varepsilon b\, c$$

F	:Relative fluorescence intensity
Φ_f	:Quantum efficiency
I_0	:Intensity of incident radiation
ε	:Molar absorptivity
b	:Pathlength of flow cell
c	:Concentration

Fluorescence Detector



Fluorescence Detector

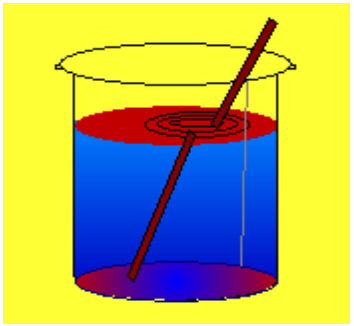
Advantage

- Sensitivity is higher than UV-Vis detector
- Selectivity is high because relatively few compounds fluorescence
- Compatible with gradient elution

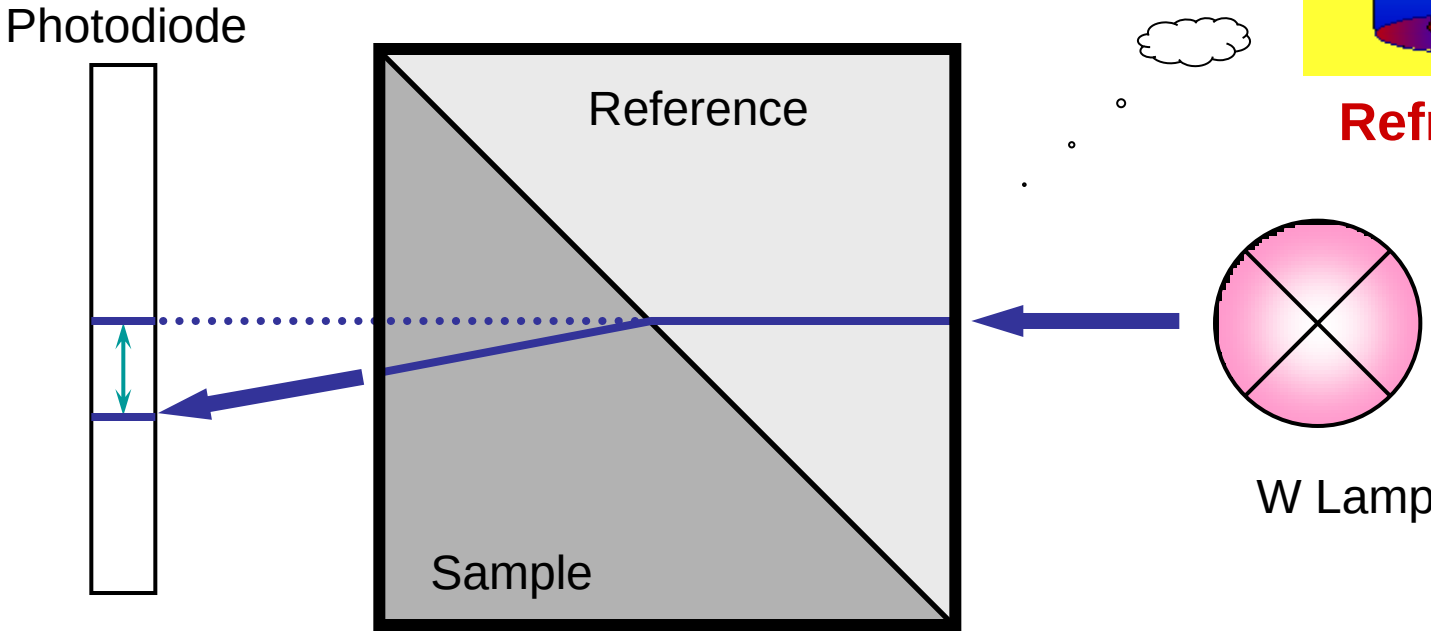
Disadvanage

- Difficult to predict fluorescence
- Greatly affected by environment
  - Solvent
  - pH
  - Temperature
  - Viscosity
  - Ionic strength
  - Dissolved gas

Refractive Index Detector (1)



Refraction



Refractive Index Detector (3)

Advantage

Responds to nearly all solutes

Unaffected by flow rate

Disadvantage

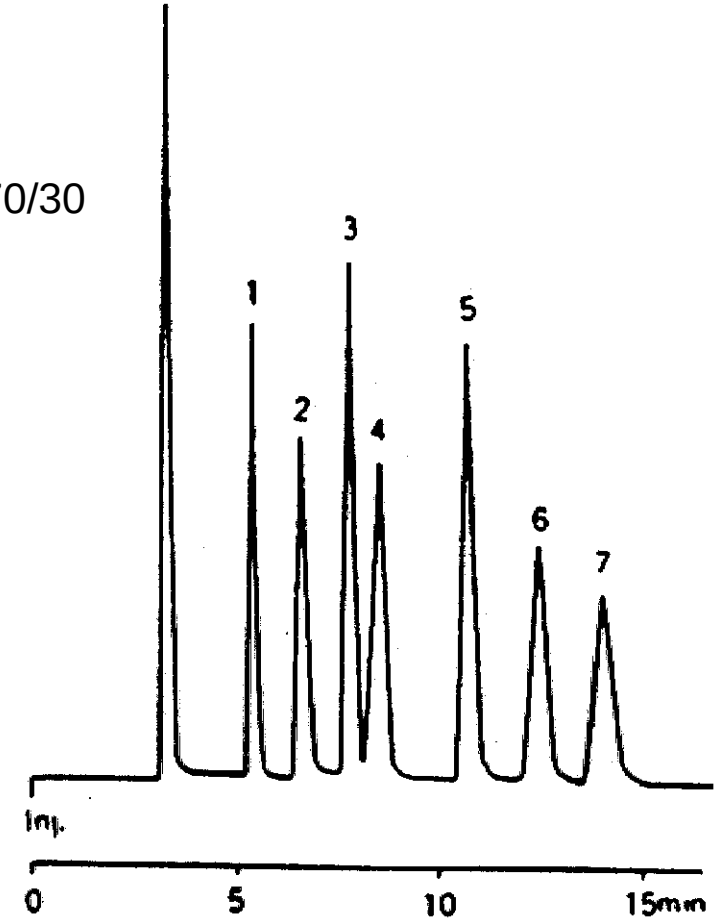
Not as sensitive as most other types of detectors

Could not be used with gradient elution

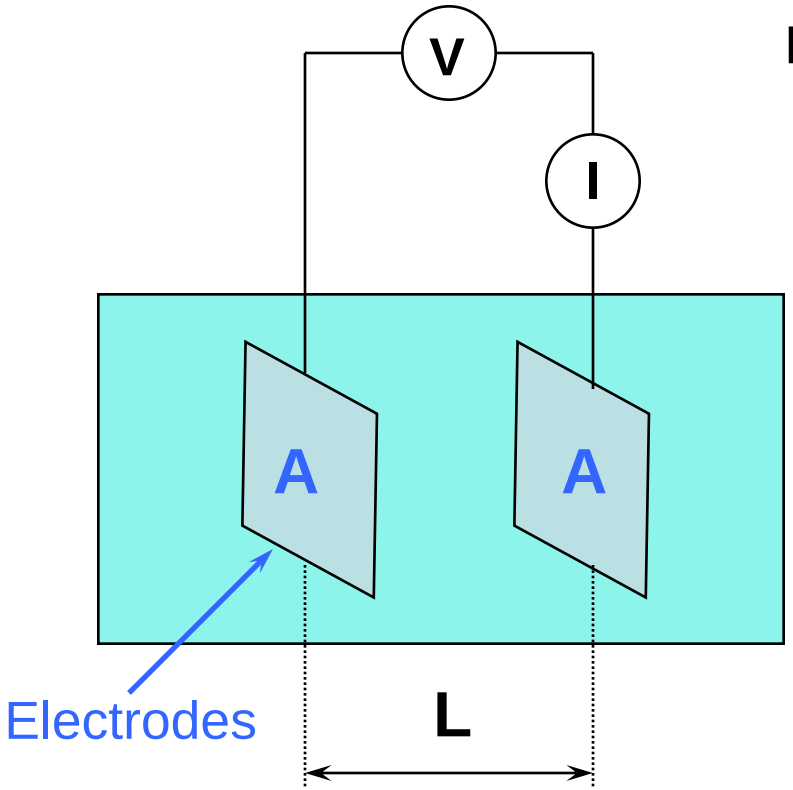
Refractive Index Detector (4)

Application Example

- Analytical Conditions
  - Column : Shim-pack CLC-NH2
  - Mobile phase : Acetonitrile / water = 70/30
  - Flow rate : 1.0 mL/min
  - Temperature : Ambient
- Peaks
  1. Glycerol
  2. Xylose
  3. Fructose
  4. Glucose
  5. Sucrose
  6. Manose
  7. Lactose

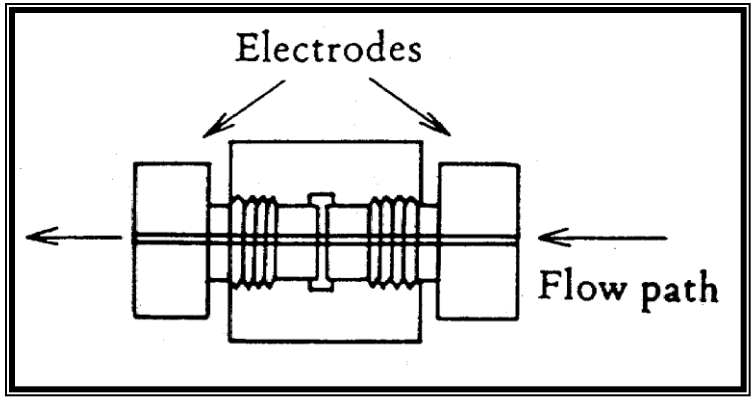


Conductivity Detector Principle



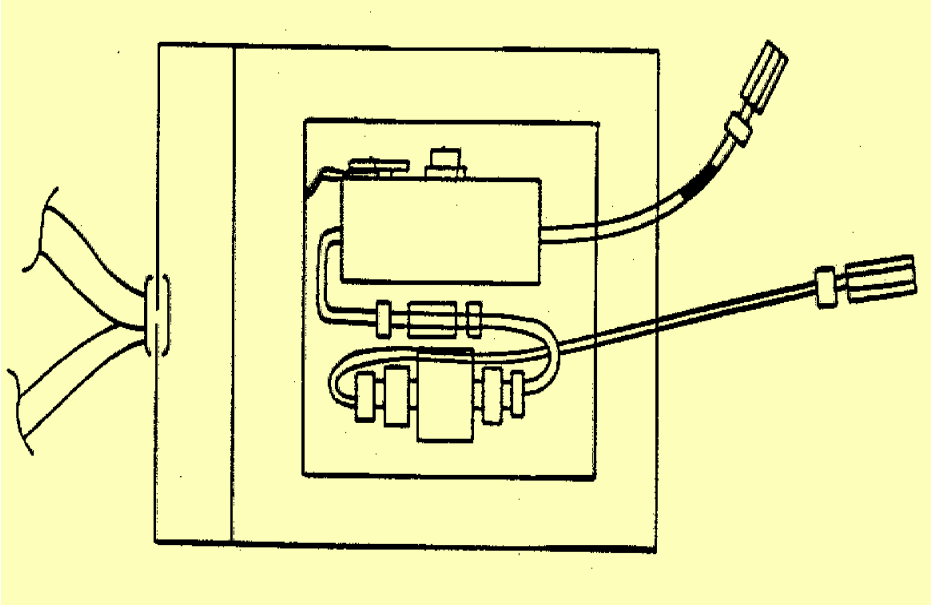
K (conductivity) = I [A] / E [V]
=A [cm²] / L [cm] * k
(k : specific conductivity)

$$k = (I/E)*(L/A)$$



Temperature Control of Conductivity Detector

- **Conductivity is very affected by temperature.**
- **Must keep the cell in the temperature control devise.**



Conductivity Detector

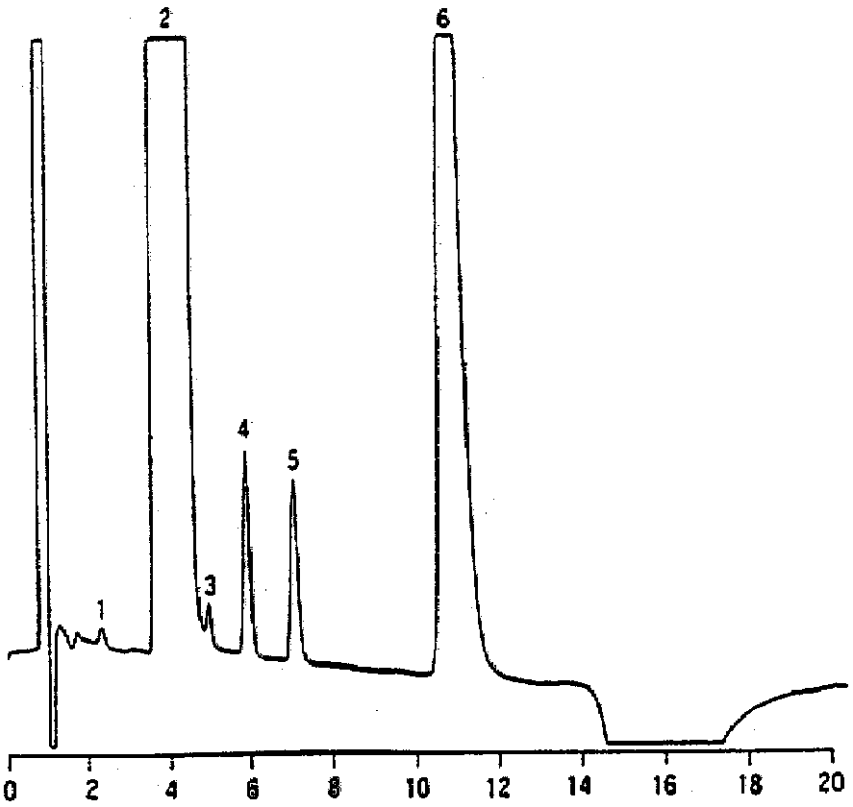
Advantages:

- Respond to ionic compounds and suitable for ion chromatography.
- High sensitivity for low concentration range

Disadvantages:

- Sensitive to the fluctuations in the solvent flow and mobile phase composition
- Not compatible with gradient elution.

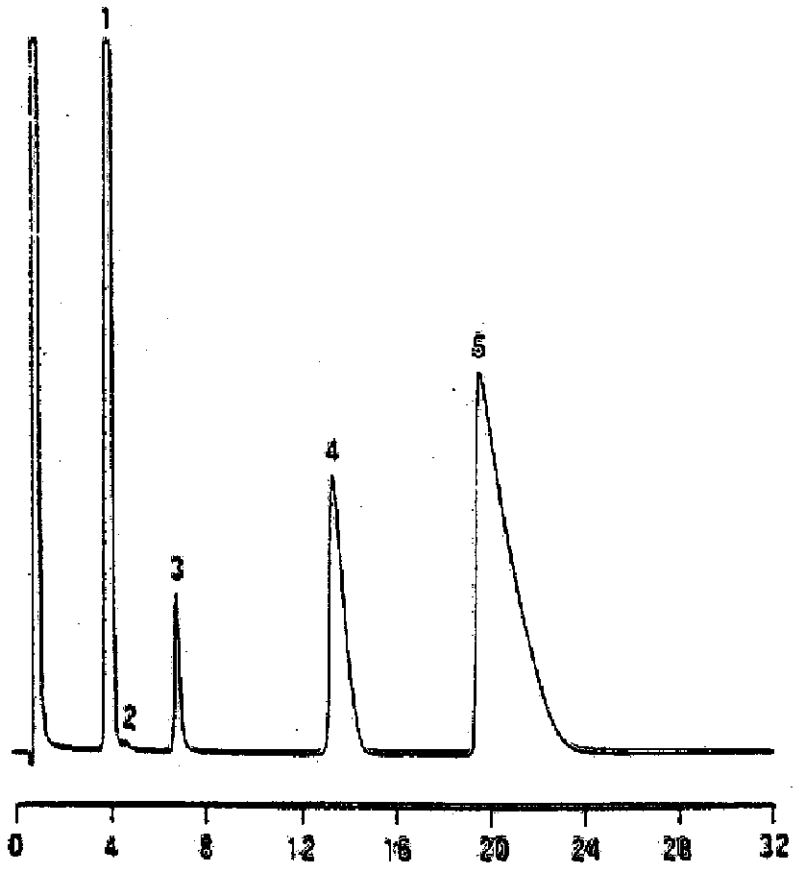
Application Example (Anions)



- Analytical Conditions
  - Column : Shim-pack IC-A3
  - Mobile phase :
    8.0 mM p-hydroxybenzoic acid
    3.2 mM Bis-Tris *
  - Flow rate : 1.5 mL/min
  - Temperature : 40ºC
  - Injection Volume : 100 µL
- Peaks
  - 1. F^- (1.4 ppm)
  - 2. Cl^- (10200 ppm)
  - 3. NO_2^- (10 ppm)
  - 4. Br^- (43 ppm)
  - 5. NO_3^- (44 ppm)
  - 6. SO_4^{2-} (431 ppm)

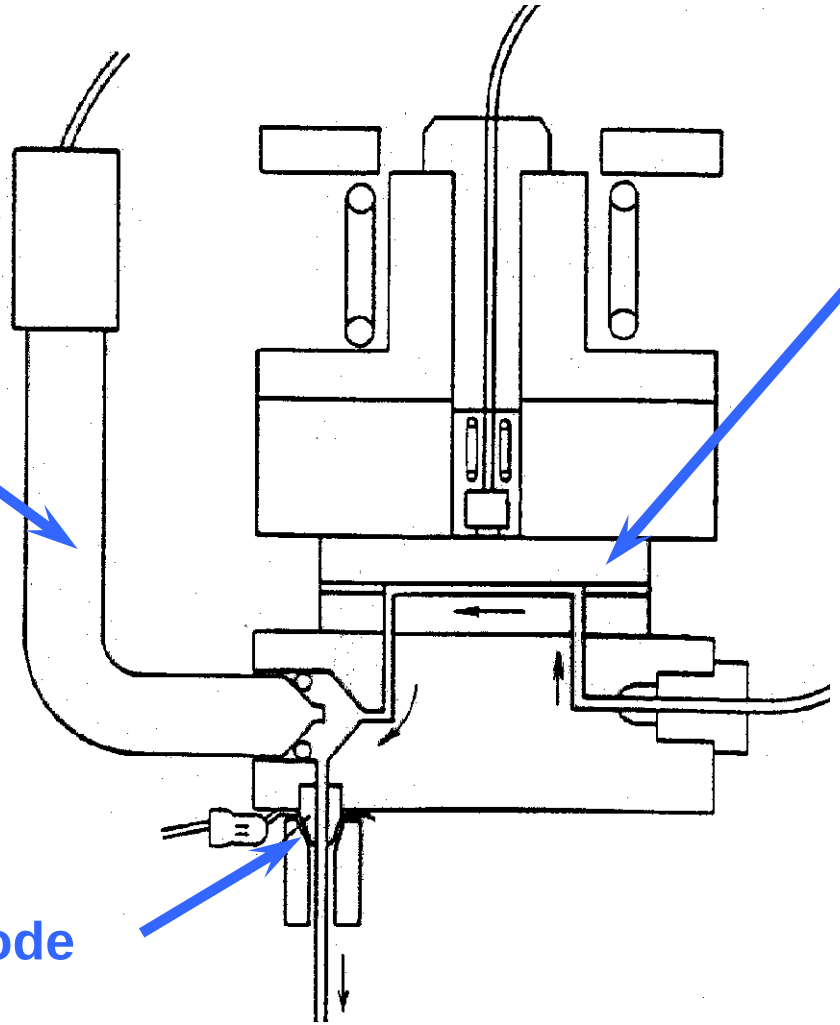
Bis-Tris : bis (2-hydroxyethyl) iminotris (hydroxymethyl) methane

Application Example (Cations)



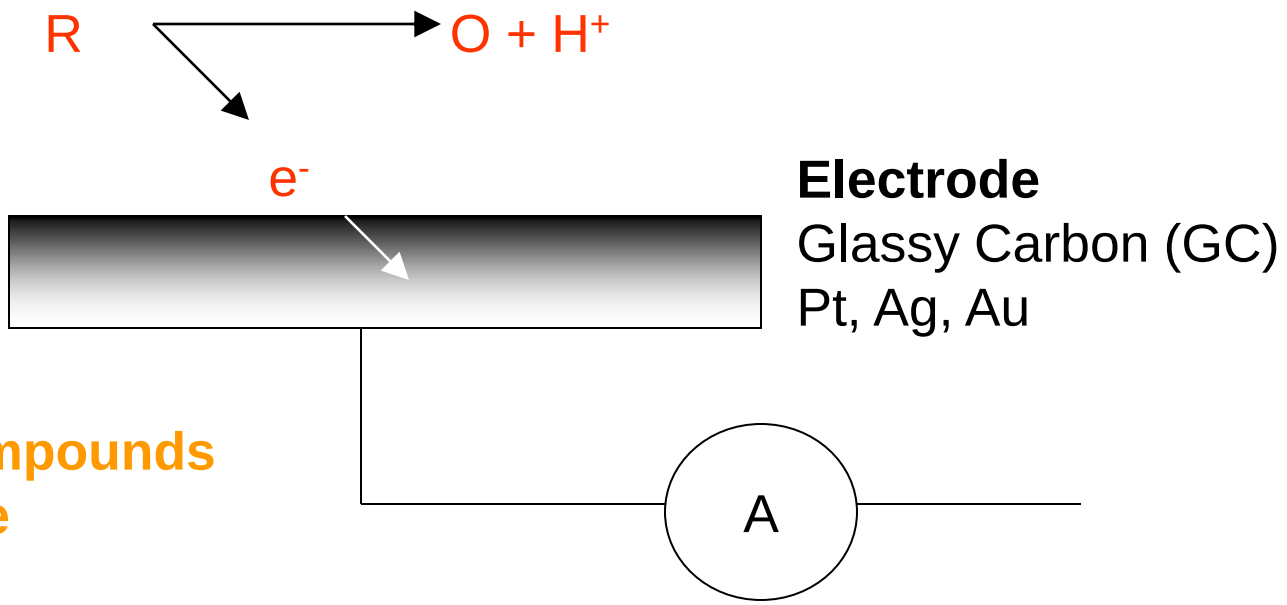
- Analytical Conditions
  - Column : Shim-pack IC-C3
  - Mobile phase : 2.0 mM Oxalic Acid
  - Flow rate : 1.0 mL/min
  - Temperature : 40°C
  - Injection volume : 100µL
- Peaks
  - 1. Na^+ (8.25 ppm)
  - 2. NH_4^+ (0.01 ppm)
  - 3. K+ (1.66 ppm)
  - 4. Mg^{2+} (2.22 ppm)
  - 5. Ca^{2+} (11.85 ppm)

Electrochemical Detector



Principle of ECD Detection

Electrochemical detector responds to compounds that can be oxidized or reduced, such as phenols, aromatic amines, ketones, aldehydes.



[Applications]
GC : phenol compounds general use
Pt : H_2O_2
Ag : halogen ion
Au : sugar analysis

Electrochemical Detector

Advantages:

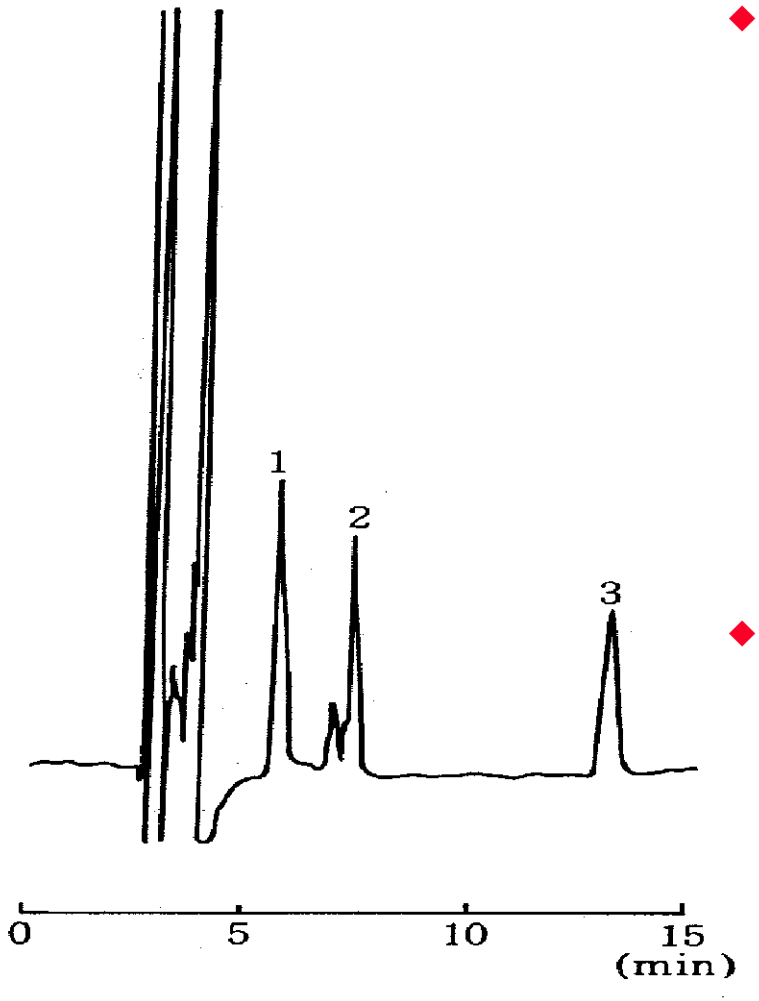
- Selective as relatively few compounds are electro-active.
- Excellent sensitivity for low concentration range.

Disadvantages:

- Sensitive to temperature and flow rate fluctuations
- Not compatible with gradient elution.
- Aqueous or other polar solvents containing dissolved electrolytes are required and they must be rigorously free from oxygen.

Application Example

(catecolamines)



- Analytical Conditions
  - Column : Shim-pack CLC-ODS
  - Mobile phase :
    80 mM phosphate buffer (pH=2.7)
    100 mM NaNO3, 200 mg/l SOS
    5 mg/l EDTA, 4 % acetonitrile
  - Flow rate : 1.0 mL/min
  - Applied Potential : + 0.8 V
  - Temperature : 40 C
  - Injection volume : 10 uL
- Peaks
  - 1. Noradrenalin (5 ppb)
  - 2. Adrenalin (5 ppb)
  - 3. Dopamine (5 ppb)

Evaporative Light Scattering Detector

Shimadzu ELSD-LT

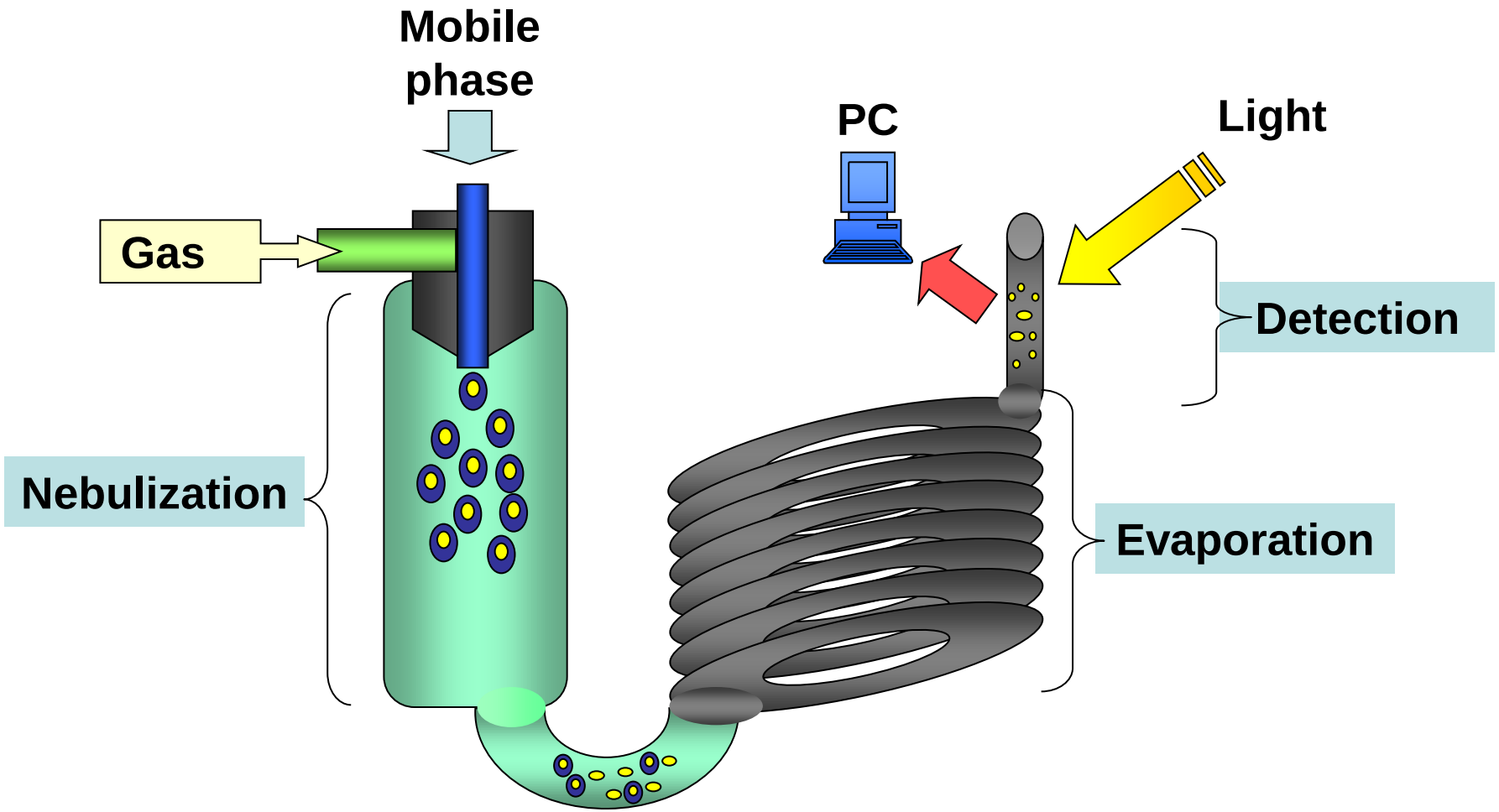
Detection Pinciple

Three steps

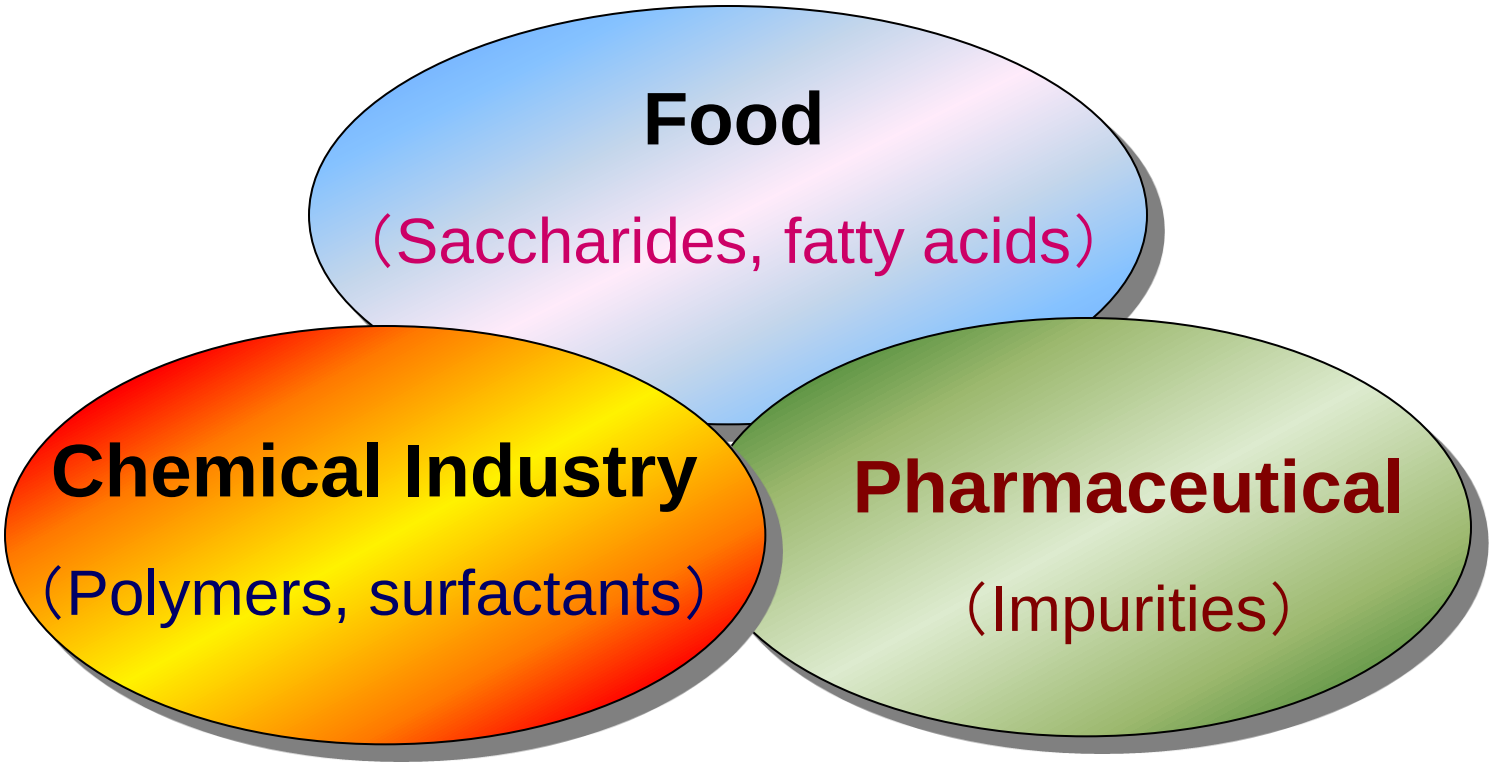
- Nebulization
- Evaporation
- Detection

ELSD responds to compound that is less volatile than that of the mobile phase

Evaporative Light Scattering Detector



Applications of ELSD



Mobile Phase & Nebulizing Gas

Mobile Phase

- **Water**
- **Methanol**
- **Acetonitrile**
- **THF**
- **etc**

Nebulizing Gas

- **Nitrogen**
- **Compressed air**
- **etc**

Evaporative Light Scattering Detector

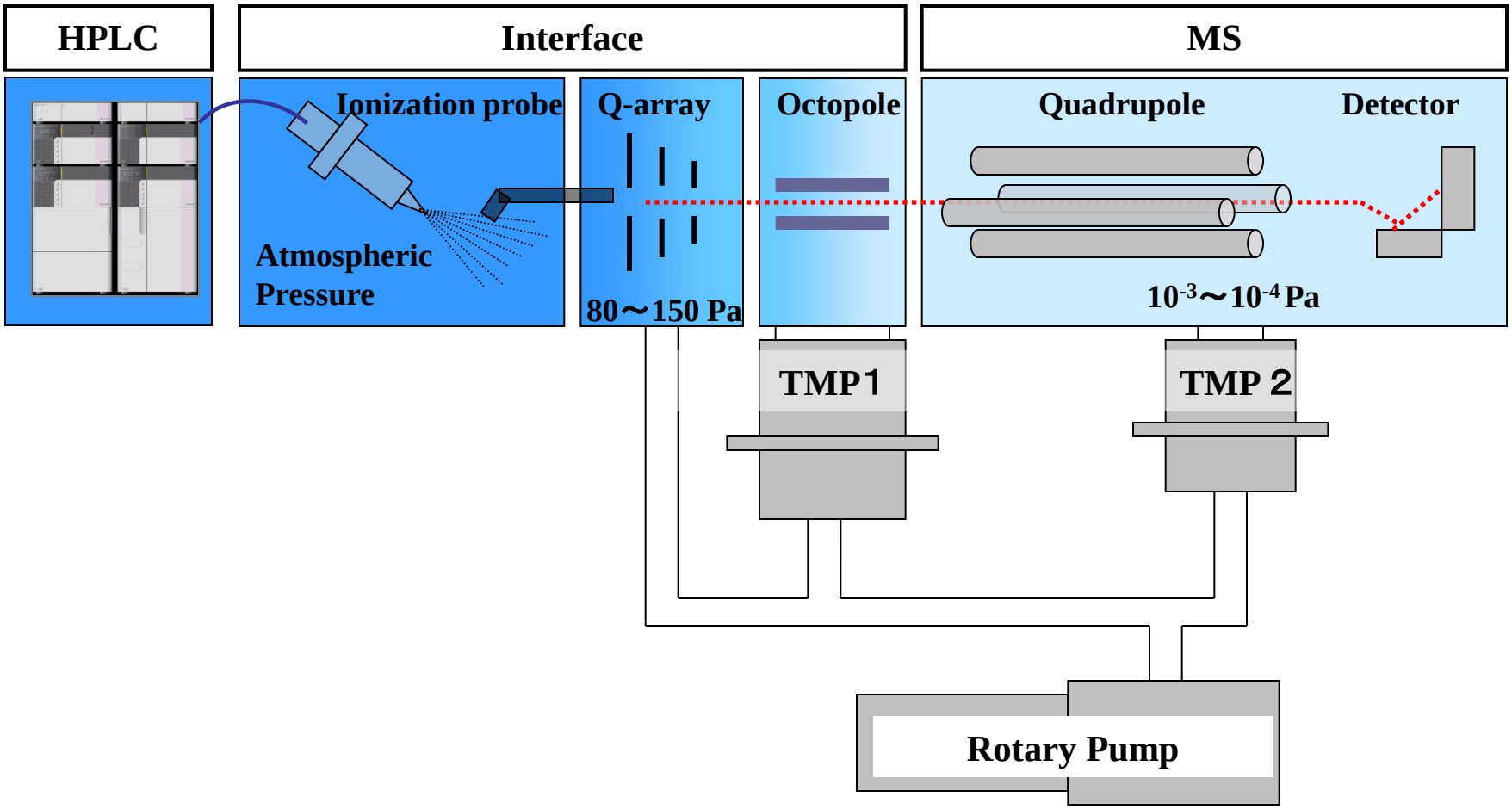
Advantages:

- Most compounds can be detected (universal detector)
- Compatible with gradient elution

Disadvantages:

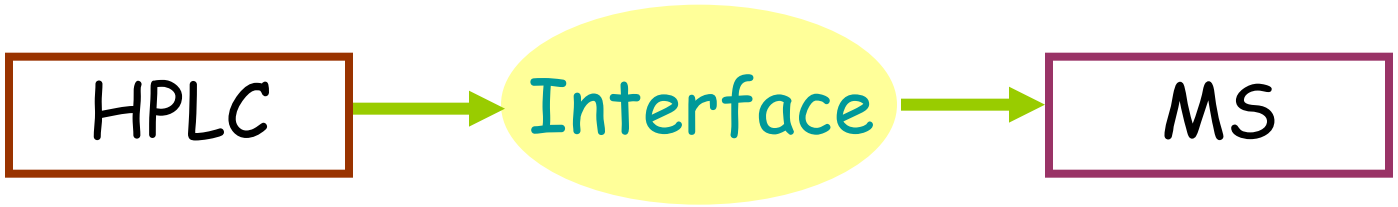
- Mobile phase must be volatile.
- Nebulizing gas is required.

Single Quadrupole LC/MS System



Interface of LC-MS

Key Technology



- Aqueous/organic solvent with buffers
- Non-volatile compounds

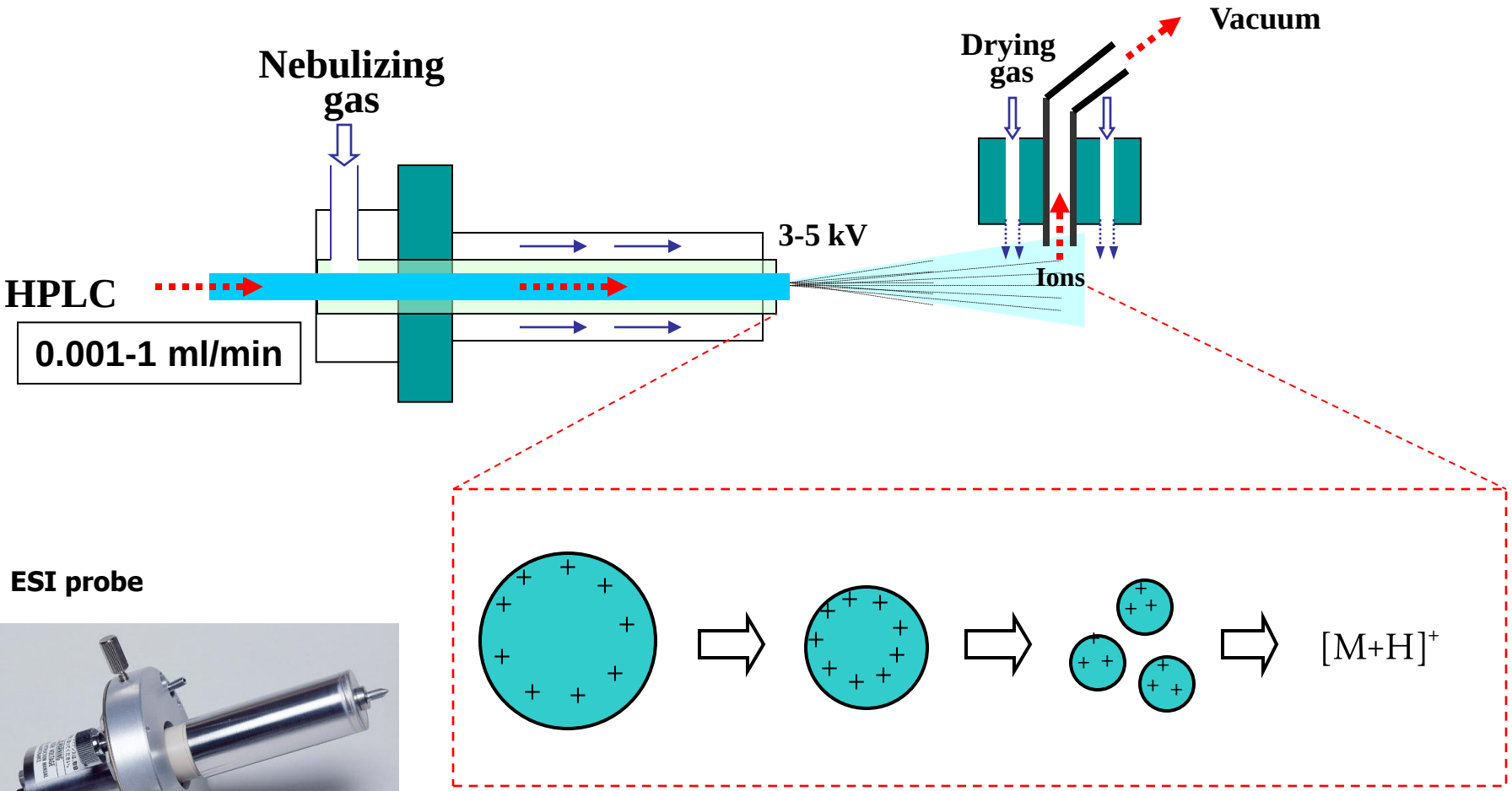
- To Remove solvent
- To Ionize analyte molecules

- High vacuum
- Analyze ions, m/z

- Research on interfacing HPLC to MS began in the 1970s; API (atmospheric pressure ionization) sources were commercialized in 1987.
- API interfaces: electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI)

Principles of ESI

Electro Spray Ionization



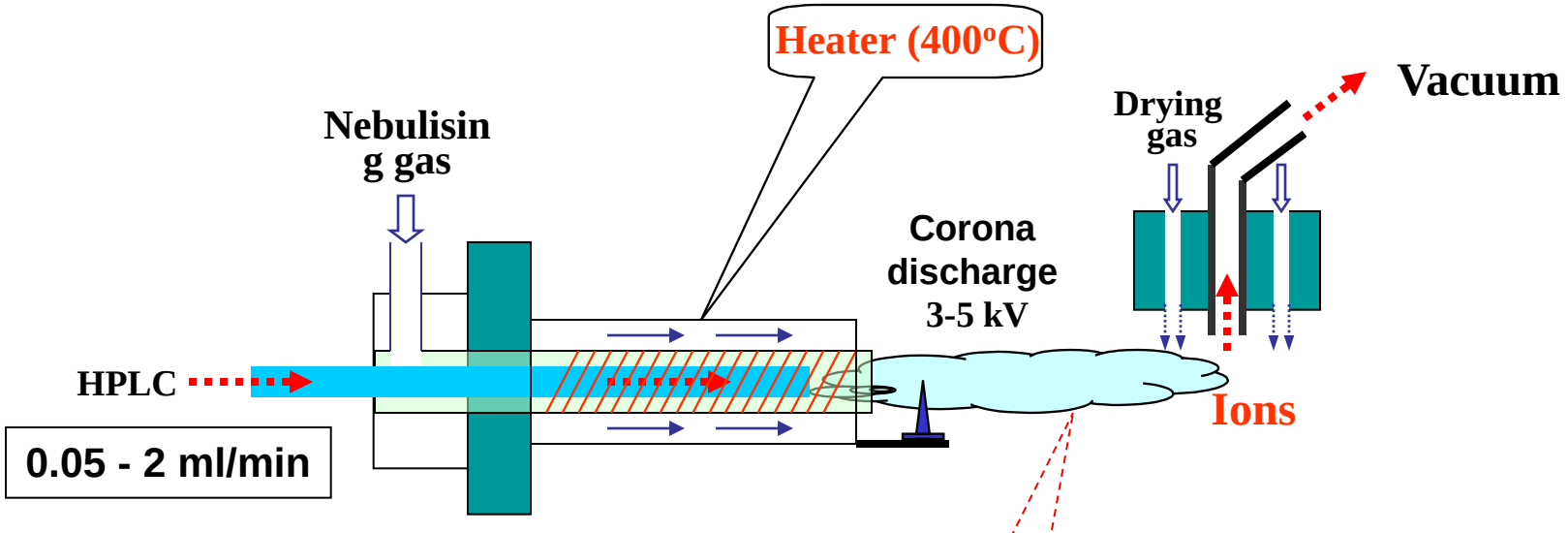
ESI probe

- Ionization in liquid phase
- Ionization at room temperature

Access to your success
SHIMADZU

Principles of APCI

Atmospheric Pressure Chemical Ionization



ESI probe

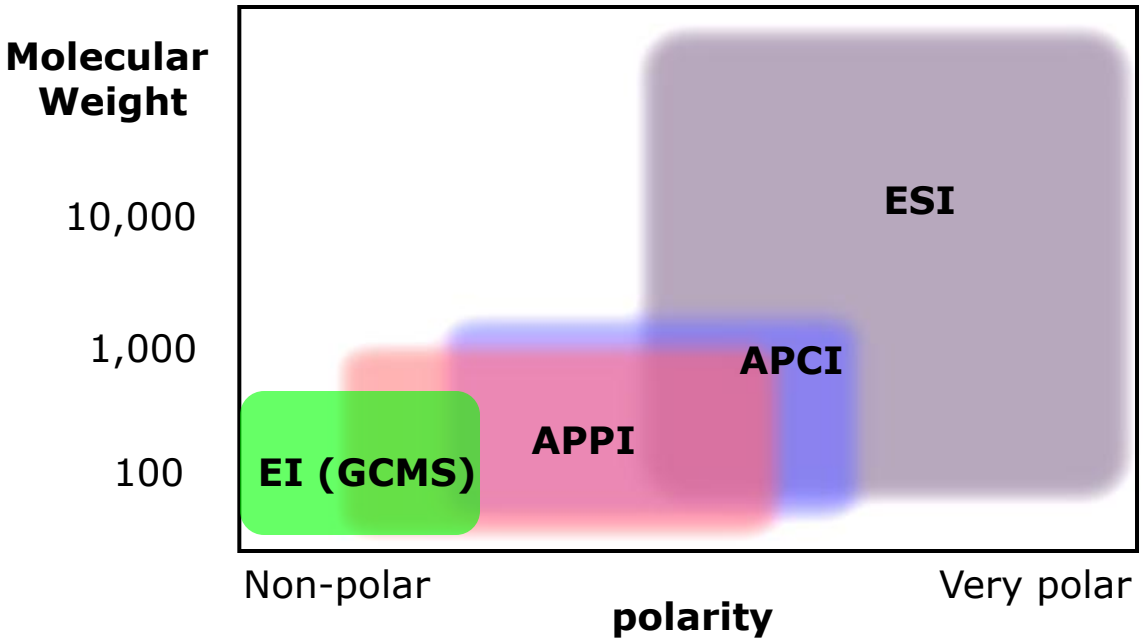
Discharge to form primary ion:

$$N_2 \rightarrow N_2^+$$

Gas phase ion – molecule reaction with charge or proton transfer

- Evaporate LC elute into gas phase by a heater (400°C)
- Ionization in the gas phase by discharge, ion-molecule reaction

Ionization diagram

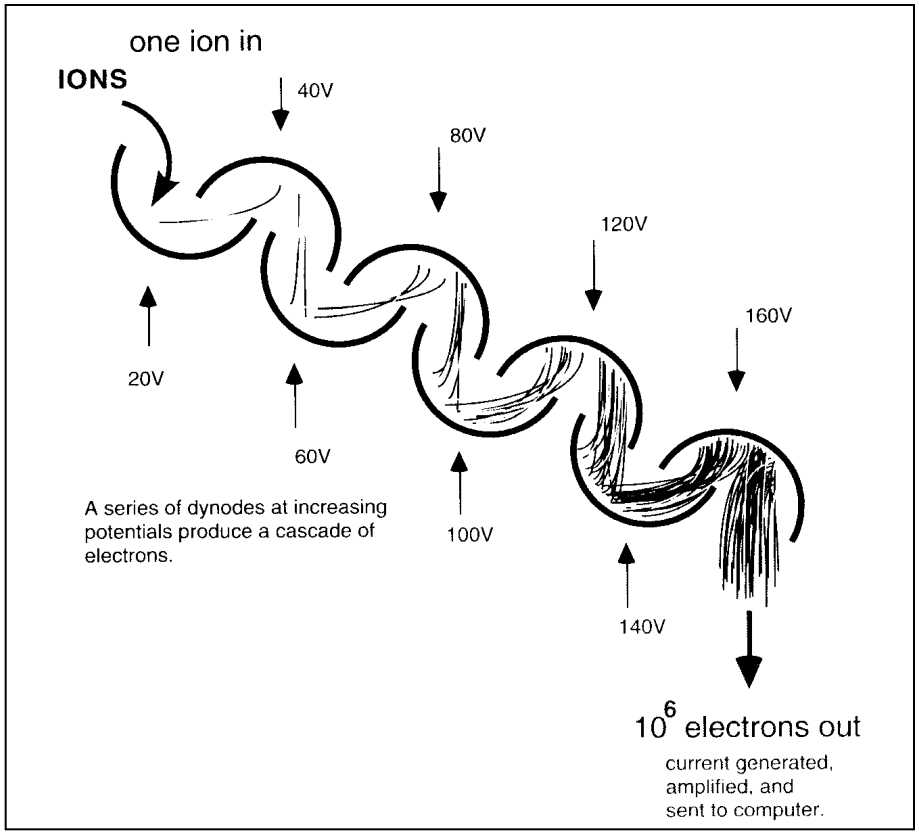


- **ESI** has been most widely used in various LC-MS systems. More reference data are available from open literature.
- **APCI** is chosen when its ionization effect is significantly better than ESI in certain analysis. “It is difficult to generalize which class of compounds can be ionized by which probe, because there are many exceptions.” *(Britt E. Erickson, Today’s Chemist Feb 2001)*
- **APPI** is chosen only when ESI and APCI could not ionize target compounds effectively.

Ion Detector

Electron Multiplier

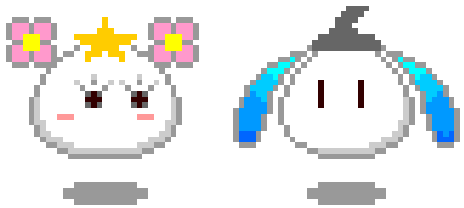
1. A series of dynodes maintained at ever-increasing potentials
2. Ions strike the dynode surface, resulting in the emission of electrons.
3. these secondary electron are then attracted to the next dynode where more secondary electrons are generated
4. ultimately resulting in a cascade of electrons



Ionization of Compounds in MS Detector

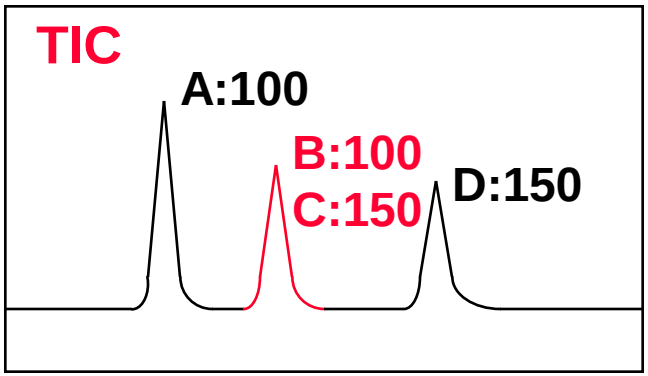
- **ESI**
  - **drugs and their metabolites**
  - **peptides**
  - **proteins**
  - **many kinds of natural product**
    (-OH, -NH_2,-COOH, SO_2, PO_3 etc.)

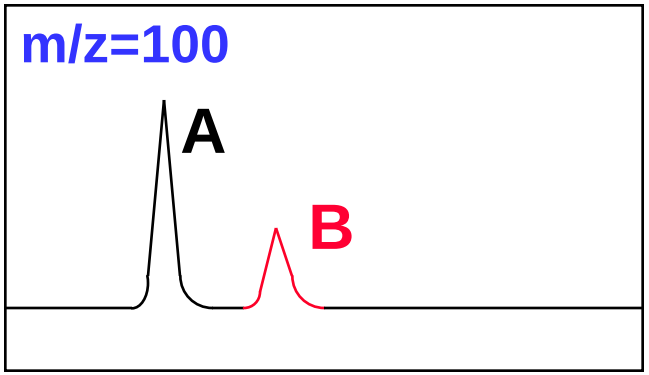
- **APCI**
  - **pesticides**
  - **steroids**
  - **drugs**

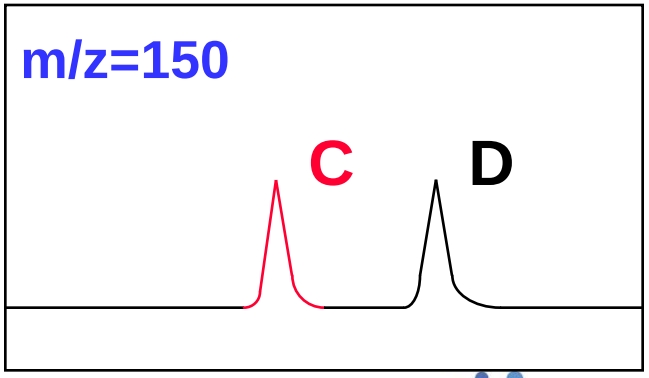


What kind of benefits LC/MS users can get ?

- **Determination of MW**
- **Qualitative capability**
- **Selective quantitative capability**
- **High sensitivity**







Selection of Detectors

Detectors	Type of compounds can be detected
UV-Vis & PDA	Compounds with chromophores, such as aromatic rings or multiple alternating double bonds.
RF	Fluorescent compounds, usually with fused rings or highly conjugated planar system.
CDD	Charged compounds, such as inorganic ions and organic acid.
ECD	For easily oxidized compounds like quinones or amines.
RID & ELSD	For compounds that do not show characteristics usable by the other detectors, eg. polymers, sccharides.

Comparison of Detectors

Detectors	Gradient Compatibility
UV-Vis & PDA*	Yes
Fluorescence (RF)	Yes
Refractive Index (RID)	No
Conductivity (CDD)	No
Electrochemical (ECD)	No
Evaporative Light Scattering (ELSD)	Yes
MS	Yes

*** The sensitivity of PDA Detector is slightly less than UV-Vis Detector**

Thank You!