Liquid Chromatography

1. Introduction and Column Packing Material

2. Retention Mechanisms in Liquid Chromatography

3. Method Development

4. Column Preparation

5. General Instrumental aspects

6. Detectors

(Chapter 4 and 5 in The essence of chromatography)

Detectors in Liquid Chromatography

A. Refractive Index Detector
B. Absorption Detector (UV/Vis)
C. Fluorescence Detector
D. Conductivity Detector
E. Electrochemical Detector

These detectors differ from those used in GC in that most of them are nondestructive. This makes LC more attractive for purification or preparative-scale work that GC. LC does lack behind HC, however, in the fact that it does not currently have a good universals detector available for its use.

A. Refractive Index Detector

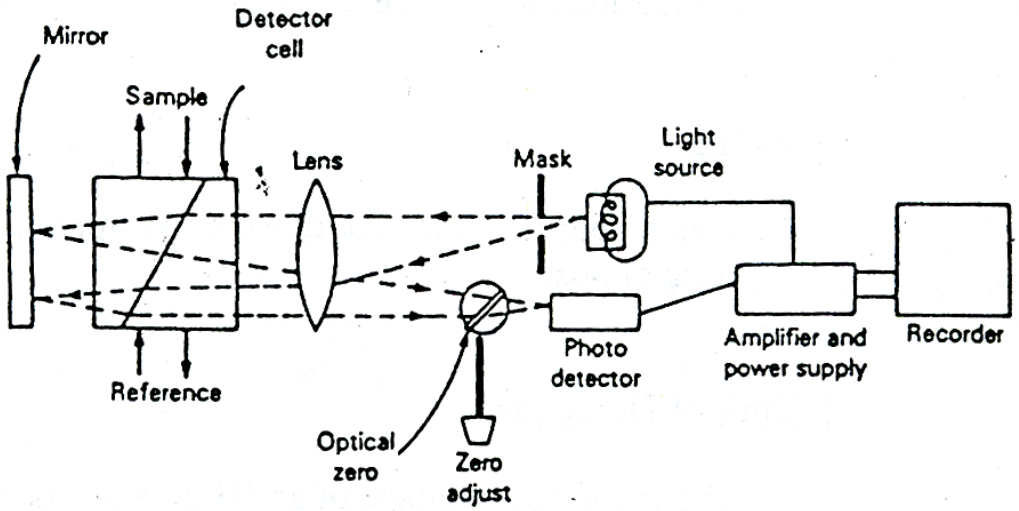
1. The RI detector is one of the few universal detector available in LC

2. Principle:

The RI detectors measure a bulk property of the mobile phase leaving the column: its ability to refract to bend light (i.e., its refractive index). This property changes as the composition of the mobile phase changes, such as when solutes from the column. By detecting this change, the presence of solutes can be detected.

3. Detector Design:

i. One of simplest of RI detectors is the deflection RI detector



ii. In this detector, light is created by a source and passed through flow-cells containing mobile phase eluting from the column (sample stream) and a reference stream (usually mobile phase with no solute in it). The light passing through these flow0cells is passed through a second time using a mirror and passed to a detector where its intensity is measured.

iii. When the refractive index of liquid in the sample and reference flow-cell are the same, little or no bending of light occurs at the interface between the low-cells. This allows the largest amount of light possible to reach the detector.

iv. As solute elute from the column, the refractive index of the liquid in the sample flow-cell will be different that that in the reference flow-cell and light will be bent as it passes between them. This changes the amount of light reaching the detector, producing a response.

4. Applications:

RI detector are universal applicable to the detection of any solute in LC. This makes them useful in preliminary work in LC where the nature or properties of a compound may not be known yet. They also the detector of choice for work with carbonhydrates or in the separation of polymer by size-exclusion chromatography.

Some disadvantages: (1) they do not have very good limits of detection, (2) they can not used with gradient elution, where the composition of the mobile phase is changing with time. (3) The temperature of the system must also be controlled to avoid baseline fluctuations with these detectors.

5. Sensitivity

The response of a RI detector is approximately the same for all compounds.

6. Limit of Detection: 10^{-5} to 10^{-6} M

7. Linearity/ Dynamic Range: The response of a RI is usually linear over a 10^4-fold range in concentration.

B. Absorbance Detector (UV/Vis)

1. The absorbance detector is the most common type of detector in LC.

2. Principle:

Absorbance detector measures the ability of solutes to absorb light at a particular wavelength range. This absorbance is described by the Beer-Lambert Law.

$$A = \varepsilon l c$$

Where: A = Absorbance of light at a given wavelength

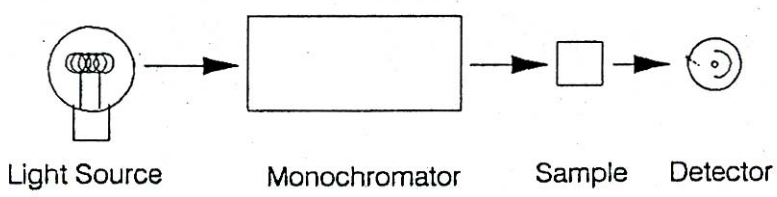
ε = **Molar absorption coefficient of the solute**

l = path length of the flow-cell

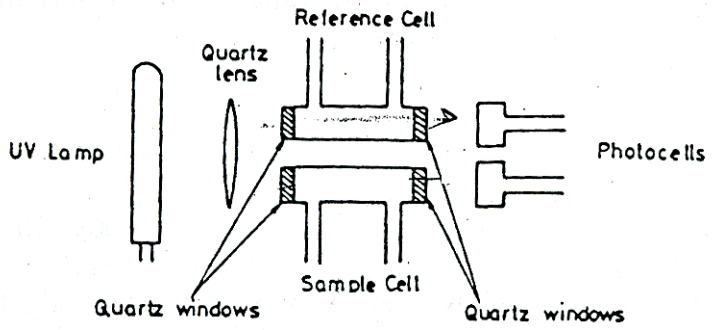
c = concentration of solute

3. Detector design:

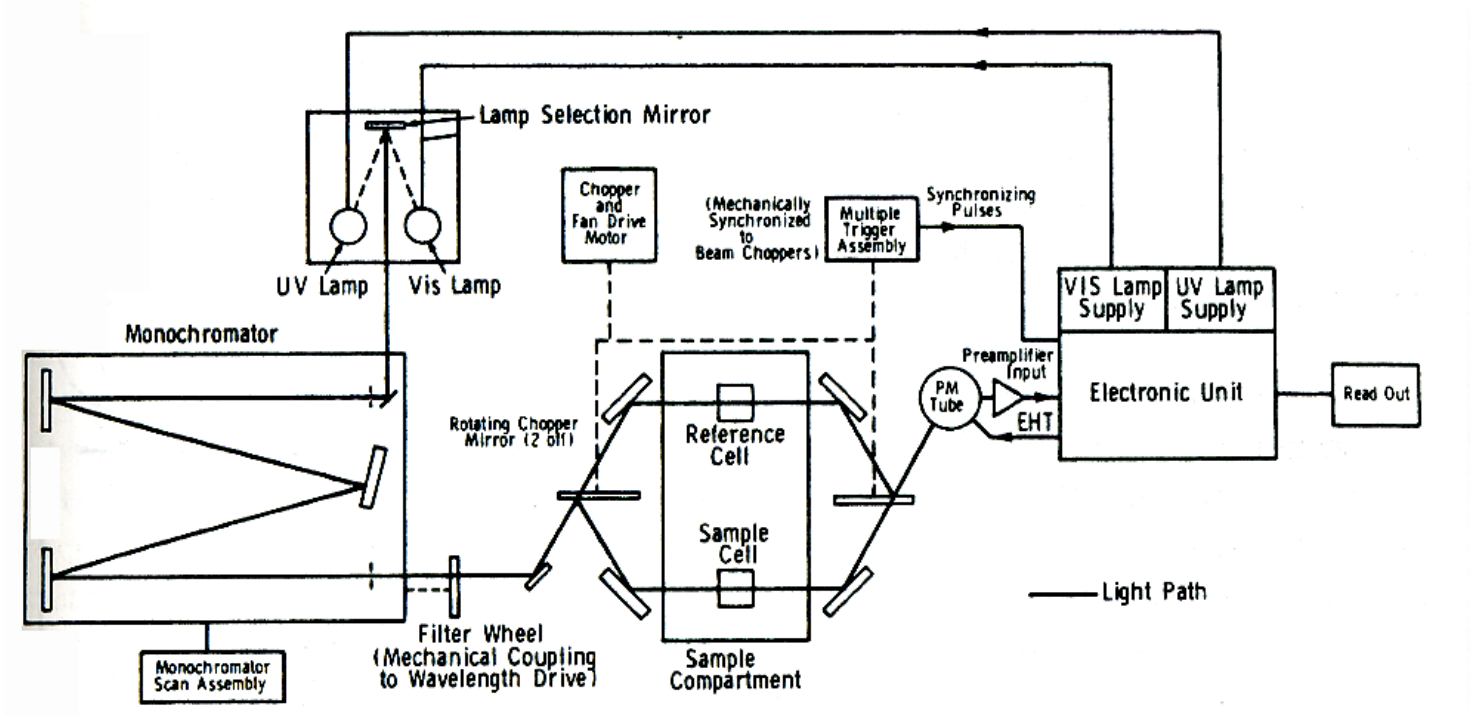
i. There are three types of UV-Vis absorbance detector: fixed wavelength detectors, variable and diode array detector. They are generally based on the following type of design:



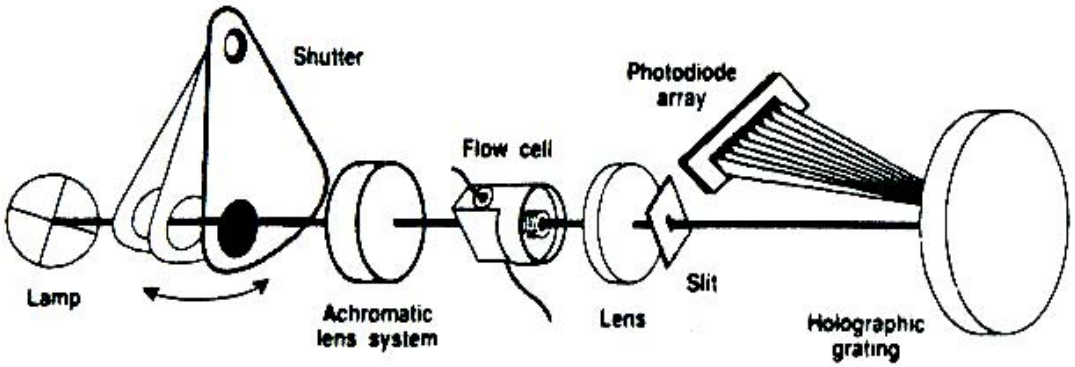
ii. In a fixed wavelength detector, absorbance of only one given wavelength is monitored by the system at all time. The wavelength is usually 254 nm. A fixed wavelength detector is the simplest and cheapest of types of detector, but is limited in terms of it flexibility and the types of compounds it can used to monitor.



iii. In a variable wavelength detector, a single wavelength is monitored At any given time, but any wavelength in a wide spectral range can be selected. The wavelengths that can be monitored can vary from 190 nm to 900 nm. The ability to use one instrument for more than one wavelength is achieved by adding in more advanced optics to the system.



iv. Photo diode array detectors operate by simultaneously monitoring absorbance of solutes at different wavelength. The result is that an entire spectrum of a solute can be taken in a minimum amount of time.



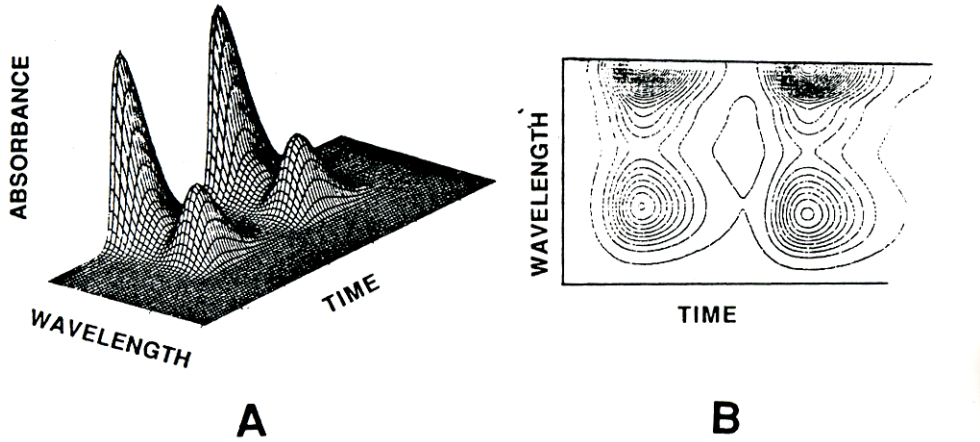


Figure 5.11. Examples of the display options for chromatographic and spectroscopic data obtained from a diode array detector. A three-dimensional isometric plot of absorbance, wavelength and time is shown in A. The same data plotted as a contour diagram is shown in B. (From ref. [119]; ©Elsevier).

Advantages of Diode Array Detectors

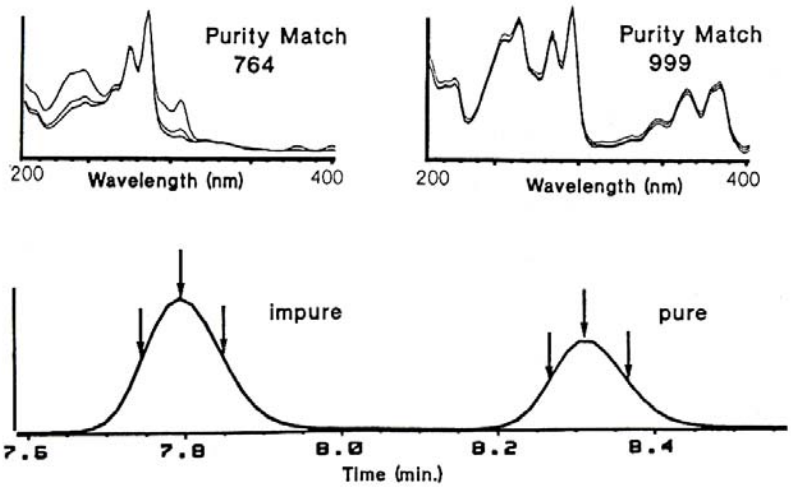
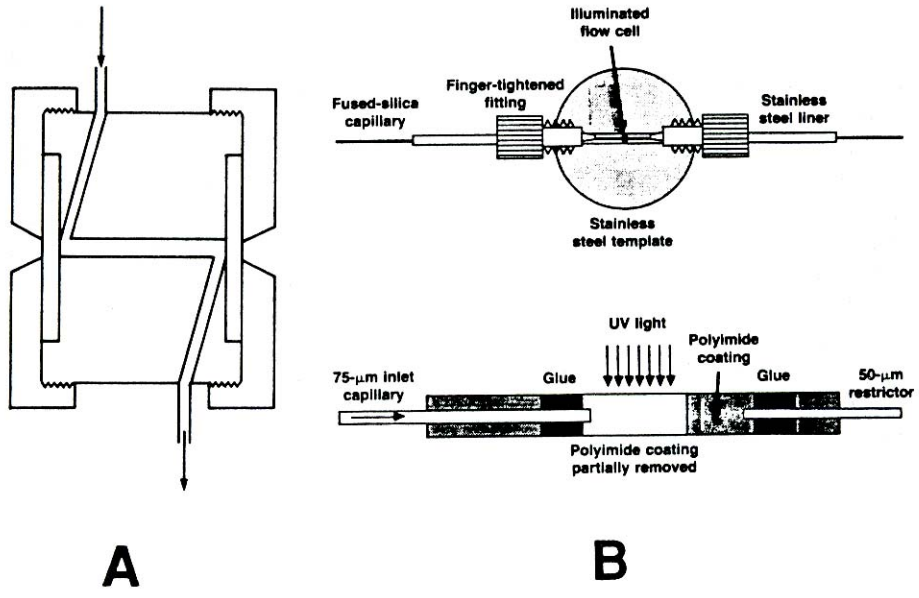


Figure 5.12. Example of peak purity determination by matching spectra recorded at different peak positions. (From ref. [107]; ©Marcel Dekker).

v. Design of the cell



4. Applications:

Absorbance detector can be used to detect any compound absorbing at the wavelength monitored. Absorbance detector can be sued with gradient elution.

5. Sensitivity:

The response of an absorbance detector depends on the molar absorption coefficient. The larger this value is, the larger the response of the detector

6. Limit of detector: 10^{-8} M

7. Linearity/Dynamic range: 10^{5}-fold range

C. Fluorescence Detector

1. **A fluorescence detector is an example of a selective detector, with limits of detection smaller than those by either RI or absorbance monitors.**

2. **Principle:**

$$F = I \Phi (1-e^{-\varepsilon l c}) = I \Phi \varepsilon l c \text{ (at low concentration)}$$

F = Fluorescence intensity
I = intensity of the excitation light
Φ= Fluorescence quantum yield
ε = Molar absorption coefficient of the solute
l = path length of the flow-cell
c = concentration of solute

c. Detector design

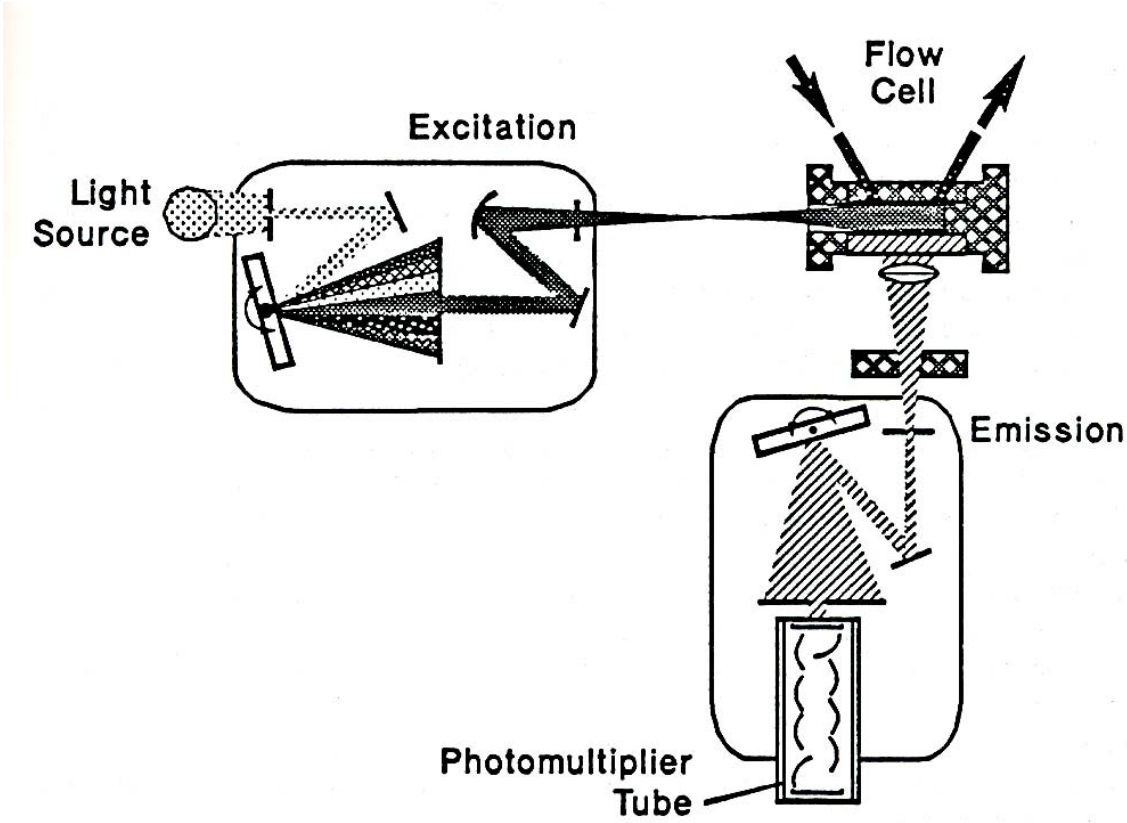
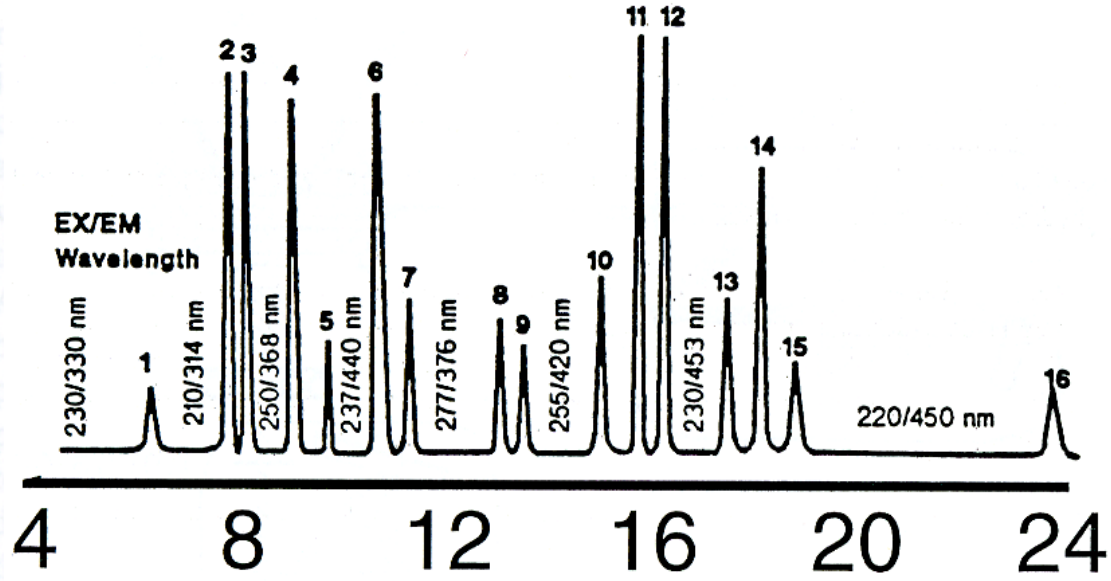


Figure 5.14. Schematic diagram of a fluorescence detector with rapid scanning monochromators for programmable selection of excitation and emission wavelengths.

d. Applications:

It can be used to detect any compound absorbing and emitting light At the given excitation and emission wavelength.



e. Sensitivity:

$$F = I \Phi (1-e^{-\varepsilon l c}) = I \Phi \varepsilon l c \text{ (at low concentration)}$$

f. Limit of detection: 10^{-10} M

g. Linearity/Dynamic Range: 10^3 to 10^4-fold

D. Conductivity Detector

1. A conductivity detector is an example of a 'universal' detector for ionic compound.

2. Principle:

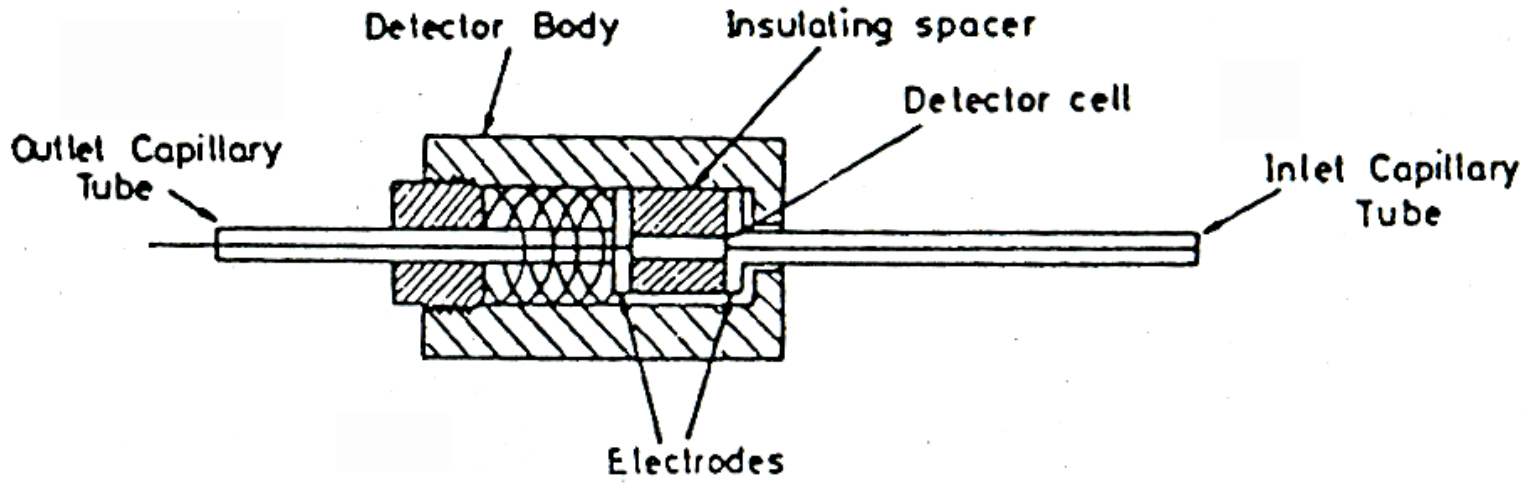
i. This detector measures the ability of a solution to conduct a current when placed in an electrical field. This ability depends on the number of ions or ionic compounds present in the solution.

ii. The relationship between the current, electric field and conductivity of the solution is shown as follows:

$$I = C\,E$$

I = Current
C = conductivity
E = electric field strength

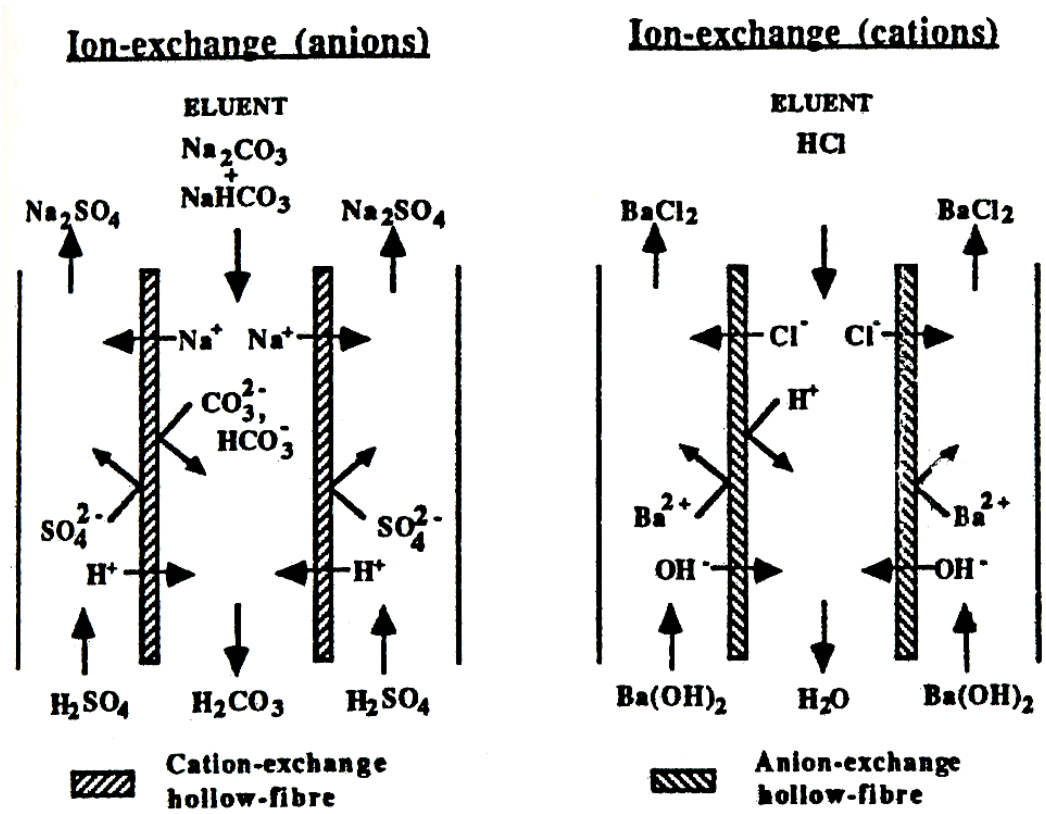
3. Detector Design



4. Applications: for any compound that is ionic or weakly ionic. It is widely used in ion chromatography.

5. Sensitivity:

The response of a conductivity detector depends on the charge and size of the compound of interest. Small, highly charged compounds tend to produce larger response that large, less charged compound.



6. Limit of Detection: 10^{-6} M

7. Linearity/Dynamic range: 10^4-fold

E. Electrochemical detector

1. It can be used to detect an compound which can undergo an electrochemical reaction

2. Principle:

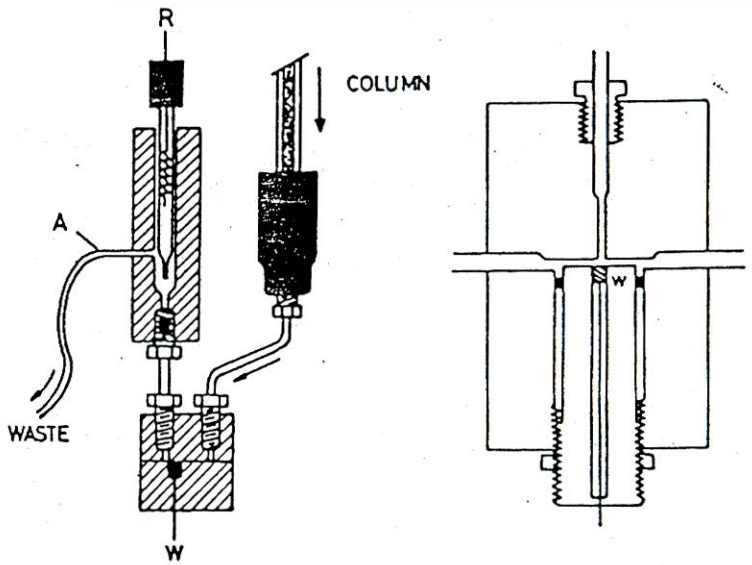
i. This detector measure the ability of a solute to undergo either oxidation (i.e., loss of electrons) or reduction (i.e. gain of electrons)

$$\text{Oxidation: } A \rightleftharpoons A^+ + e^-$$

$$\text{Reduction: } A + e^- \rightleftharpoons A^-$$

ii. One way in which such a reaction can be monitored is by measuring the change in current under a constant electric field. Another way is to measure the change in the electric field produced when a constant current is present.

3. Detector Design



4. Applications:
Electrochemical detectors can be used to detect any solute that can undergo oxidation or reduction.

Detection by reduction: aldehyes, kentones, nitriles, conjugated acids…

Detection by oxidation: phenols, peroxides, purines, diols…

5. Sensitivity: It depends on the extent of oxidation or reduction that occurs at given potential of the electrode.

6. Limit of detection: 10^{-11} M

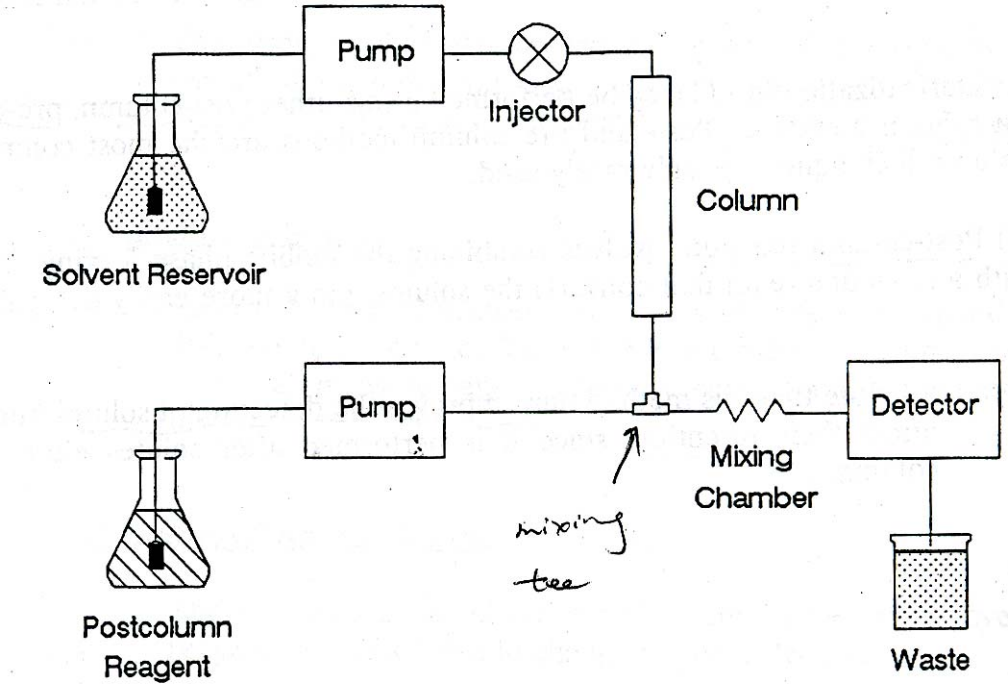
7. Linearity/Dynamic Range: 10^{-6}-fold

8. Disadvantages: destructive detector

F. Sample derivation Reactions

Sample derivation may be sued in LC as well for following reasons:
(a) To improve the response of the solute to certain types of detector
(b) To improve the separation of the solute from other samples.

1. Post-column reactions



Typical Post-column reactions

Compounds detectable	Reagents employed	Reaction conditions	Remarks
Amino acids	Fluorescamine *o*-Phthaldehyde Ninhydrin	Ambient Ambient 140°C	Fluorescence Fluorescence 440 and 570 nm
Acids	*o*-Nitrophenol	Ambient	432 nm
Carbonyls	2,4-DNP	3 min	430 nm
Carbohydrates (reducing)	Ce(IV) Ferricyanide Neocuproin	Variable — 97°, 10 min	Fluorescence Electrochemical —
Steroids (3-oxo-4-ene)	Iso-nicotinyl hydrazine	70°, 2 min	Fluorescence {360(I) and 450(II)}
Estrogens	Hydroquinone-H_2SO_4	120°, 15 min	Fluorescence {535(I) and 561(II)}
Guanidino compounds	9,10-Phenanthrene-quinone	60°, 2 min	Fluorescence {365(I) and 460(II)}
Catechol amines	Ethylenediamine/ hexacyanoferrate	75°, 5 min	Fluorescence {400(I) and 510(II)}
Thiols	5,5'-Dithio-(2-nitrobenzoic acid)	Ambient, fast	412 nm
Cannabinoids	Photolysis	Variable	Fluorescence
Nitrite(s), nitrosamides, nitrosocarbamates	Griess-reagent	Ambient, 3 min	550 nm

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