

# LECTURE # 4

## Detectors used in HPLC:

### CLASSIFICATION OF HPLC DETECTORS

#### ☐ UV/VIS

- Fixed Wavelength
- Variable Wavelength
- Diode array (PDA)

#### ☐ Refractive index

- Deflection Detector
- Refractive Detector (Fresnel refractometer)

#### ☐ Fluorescence Detector

#### ☐ Electrochemical Detector

#### ☐ Conductivity Detector

#### ☐ Evaporative light scattering (ELSD)

#### ☐ Mass detector (LC-MS)

#### ☐ IR detector

#### ☐ Optical rotation detector (chiral detectors)

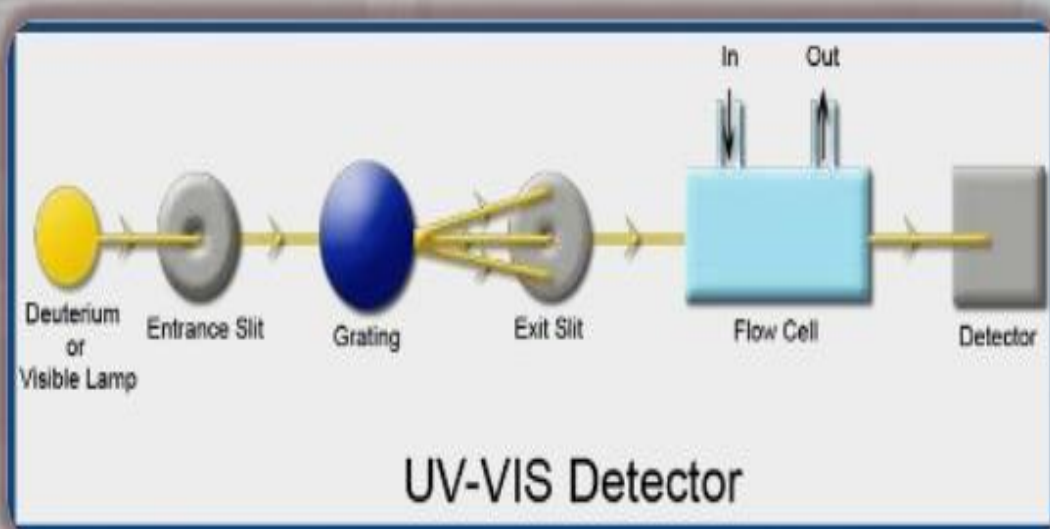
# IDEAL PROPERTIES OF A DETECTOR

The detectors used in HPLC should have following ideal properties:

- ☐ High sensitivity.
- ☐ Good stability and reproducibility.
- ☐ A linear response to solute.
- ☐ Negligible base line noise.
- ☐ Should be inexpensive.
- ☐ Capable of providing information on the identity of the solute.
- ☐ A short response time independent of flow-rate.
- ☐ High reliability and ease of operation.
- ☐ The detector should be non-destructive.
- ☐ Responses independent of mobile phase composition.
- ☐ A temperature range from room temperature to at least 400 °C

# ULTRAVIOLET/VISIBLE SPECTROSCOPIC DETECTORS

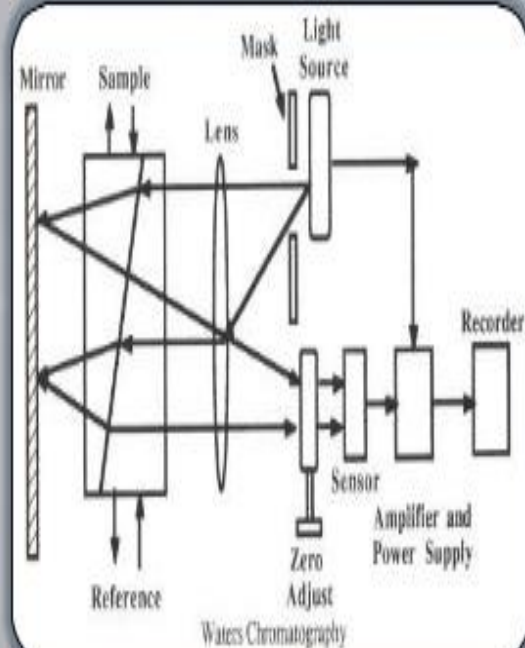
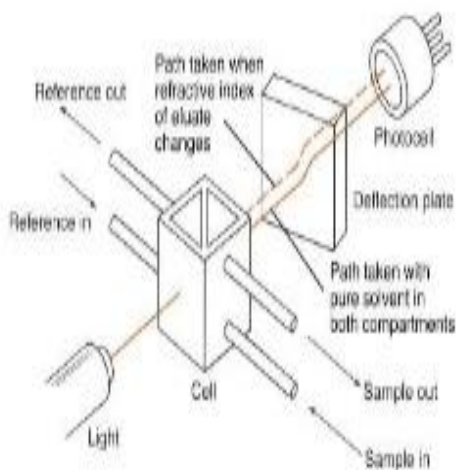
- Measures the ability of solutes to absorb light at a particular wavelength(s) in the ultraviolet (UV) or visible (Vis) wavelength range.
- When light of a certain wavelength is directed at a flow cell, the substance inside the flow cell absorbs the light. As a result, the intensity of the light that leaves the flow cell is less than that of the light that enters it. An absorbance detector measures the extent to which the light intensity decreases (i.e., the absorbance).



# REFRACTIVE INDEX DETECTOR

- Measures the overall ability of the mobile phase and its solutes to refract or bend light.
- Refractive index detector measures the molecule's ability to deflect light in a flowing mobile phase in a flow cell relative to a static mobile phase contained in a reference cell.
- The amount of defection is proportional to the concentration of the solute in the mobile phase.

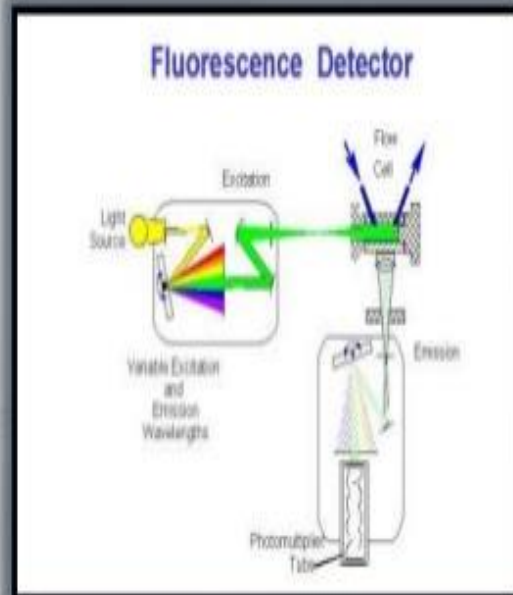
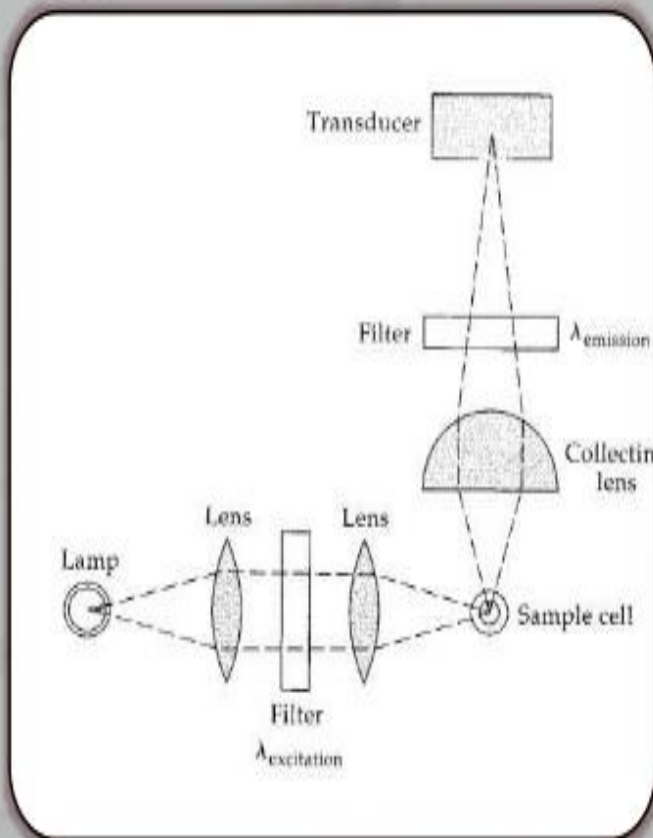
Refractive Index Detector.





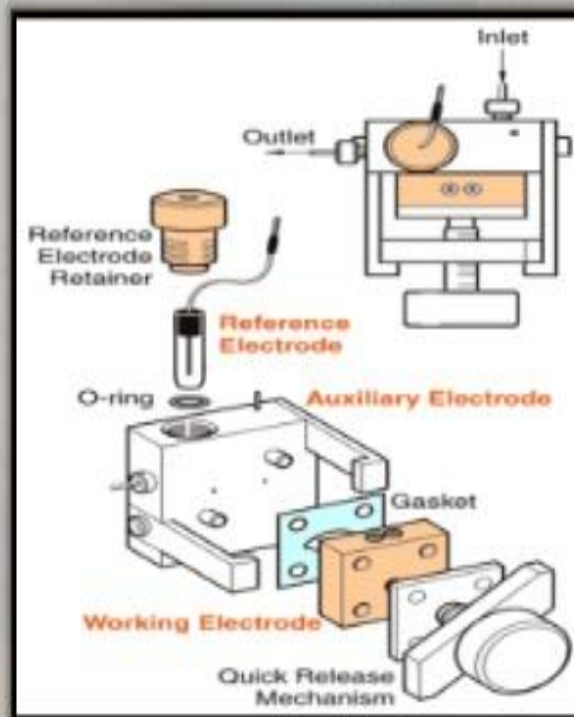
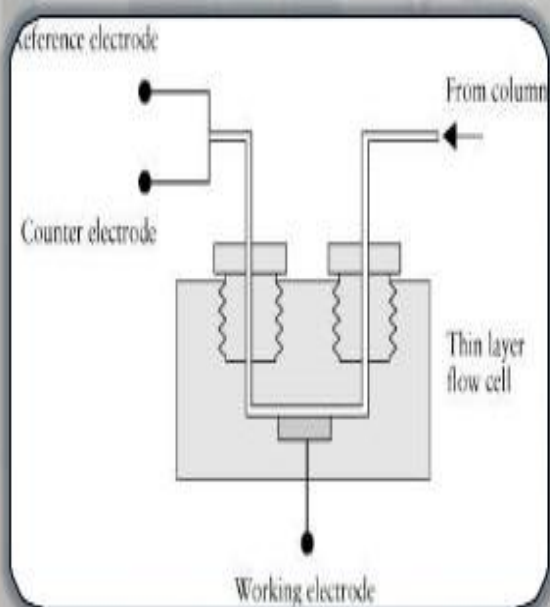
# FLUORESCENCE DETECTOR

- Fluorescence detectors for HPLC are similar in design to the fluorometers and spectro-fluorometers. The fluorescence detector is a near-ideal detector *for those solutes that exhibit molecular fluorescence*.
- Their sensitivity depends on the fluorescence properties of the components in the elute.



# ELECTROCHEMICAL DETECTORS

- It is based on the measurement of the current resulting from an oxidation/reduction reaction of the analyte at a suitable electrode.
- The level of current is directly proportional to the analyte concentration.
- Three electrodes are employed which are:
  - ❖ Working electrode
  - ❖ Auxiliary electrode
  - ❖ Reference electrode



# CHIRAL DETECTORS

- Chiral detectors are used for detection of optically active compounds such as amino acids, sugars, terpenes and other compounds containing an asymmetric carbon.
- There are two chiral detection techniques, polarimetry or optical rotary dispersion (ORD) and circular dichroism (CD).
- ORD detectors are based on differences in refractive index and CD detectors differentiate enantiomers by measuring differences between the absorption of light and left-handed circularly polarized light due to existence of a chiral chromophores.

