



Study on Detectors used in HPLC

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ABSTRACT

HPLC detectors are mostly used to detect the solute present in the eluent that comes from the HPLC column. Detectors are used in HPLC. It is the most important part of a HPLC. Some comparisons of all detectors are done on the basis of the sensitivity that particular solute. The article gives a deep insight on the various ideas regarding it. There are two general types of detectors: destructive and non-destructive. The destructive detectors perform continuous transformation of the column with measurement of some physical property of the resulting material. The non-destructive detectors measure directly some property of the column eluent and thus affords for the further analysis recovery

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Introduction

A chromatography detector is a device used in high performance liquid chromatography (HPLC) to detect components of the mixture being eluted off the chromatography column. The detector senses the presence of the individual components as they leave (elute) the column. The detectors convert a change in effluents into an electric signal that is recorded by data system.

Ideal Properties of A Detector

1. The detectors used in HPLC should have following ideal properties:
2. High sensitivity.
3. Good stability and reproducibility.
4. A linear response to solute.
5. Negligible base line noise.
6. Should be inexpensive.
7. Capable of providing information on the identity of the solute.
8. A short response time independent of flow-rate.
9. High reliability and ease of operation.
10. The detector should be non-destructive.
11. Responses independent of mobile phase composition.
12. A temperature range from room temperature to at least 400^o C

Detector Types

Detectors used depend upon the property of the compounds to be separated. Different detectors available are:

- a. Refractive index detectors
- b. U.V detectors
- c. Fluorescence detectors
- d. Electro chemical detectors
- e. Evaporative light scattering detectors
- f. IR detectors
- g. Photo diode array detector
- h. Conductivity detector
- i. Amperometric detector

Refractive index detectors:-

- Nearly universal but poor detection limit
- Passes visible light through 2 compartments, sample & reference.
- When the solvent composition are the same the light passed through the compartments the light beam that passes through is recorded as zero.
- When a solute is in the sample compartment, refractive index changes will shift the light beam from the detector.

Limit of detection (LOD) 10 ng of solute.

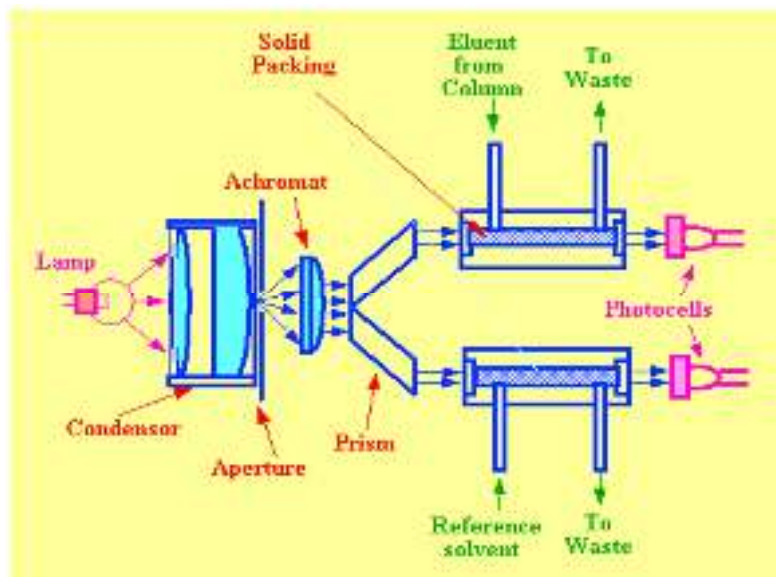


Fig. No: Diagram of Refractive index detectors

U.V detectors:-

- Based on electronic transitions within molecules.
- Most common type of detector for LC
- Fixed wavelength, Hg lamp 254 nm ($\pi \Rightarrow \pi^*$)
- Tunable wavelength, selectable for specific wavelengths, monochromators or filters. Still limited to single wavelengths.
- - 1 pg LOD
- Solvent limitations with UV-VIS abs. Detectors
- Z-shape, flow-through cell (V, 1 ~ 10 μ L and b, 2 ~ 10 mm)
- Spectrophotometer: more versatile.

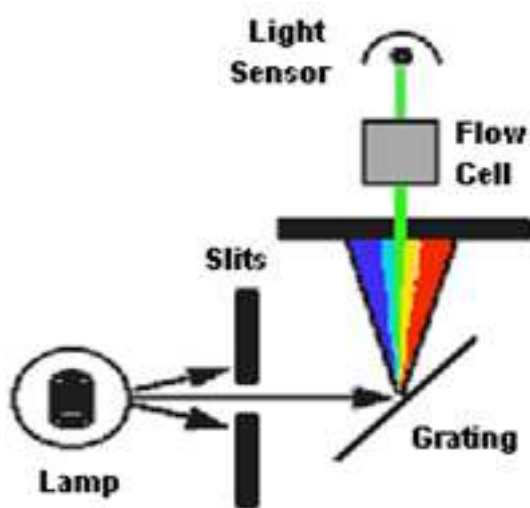
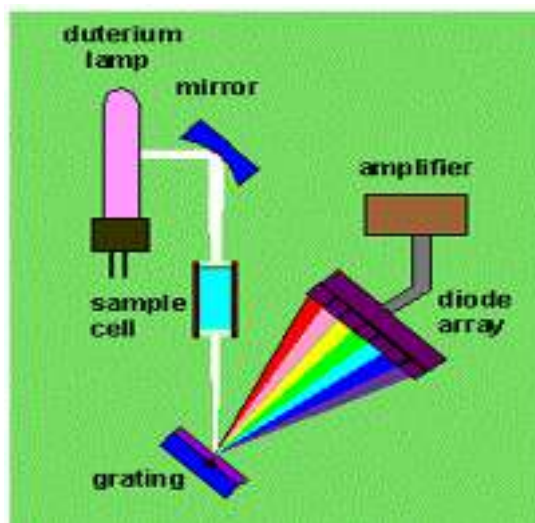


Fig. No.: Diagrams of U.V detectors.

Fluorescence detectors:-

- Review - based on emission of excited state molecules.
- Detector 90° from excitation axis.
- LOD 10 fg

- Hg or Xe lamp
- Fluorometer and spectrofluorometer
- Fluorescing species or fluorescent derivatives

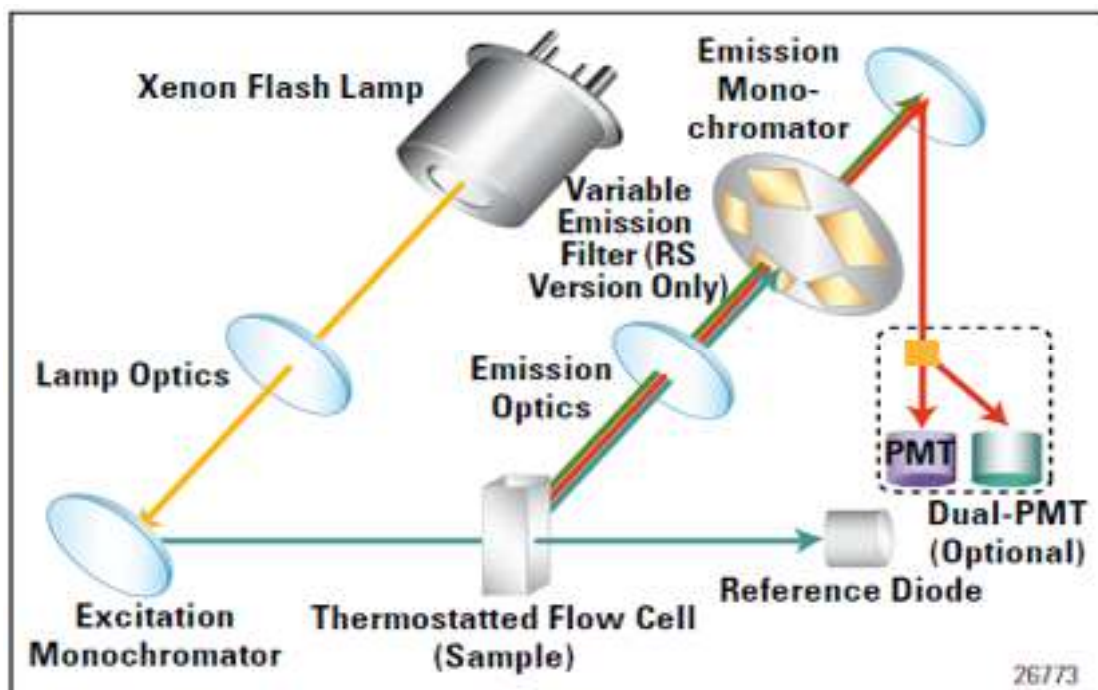


Fig. No: Diagram of Fluorescence detectors.

Electro chemical detectors:-

- Based on amperometric response of analyte to electrode usually held at constant potential.
- simplicity, convenience and wide-spreading application
- Thin-layer flow cell of Teflon : 50 μ m thick, 1 ~ 5 μ L volume
- Indicator E: Pt, Au, C

- Multi-electrode: simultaneous detection or sample purity indication
- Evaporative light scattering detectors:
 - Eluate is mixed with $N_2(g)$ and forms a fine mist.
 - Solvent (M.P.) evaporates leaving fine particles of analyte. The particles themselves are detected by light scattering.
 - Response is proportional to analyte mass.

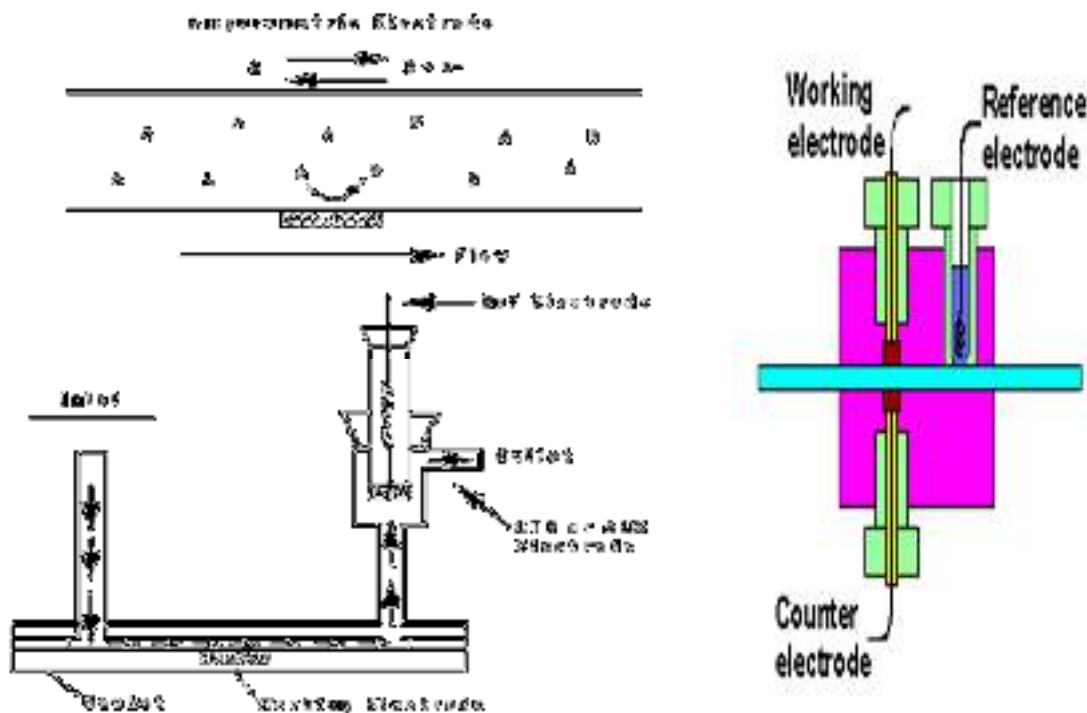


Fig. No.: Diagram Of Electro chemical detectors.

IR detectors:-

- filter instrument or FTIR
- Similar cell (V, 1.5 ~ 10 μ L and b, 0.2 ~ 1.0mm)
- Limit: no suitable solvent, special optics
- FT-IR allows for spectrum records of flowing systems analogous to the diode array system.
- Water/alcohols can be major interferences to solute detection
- LOD 100 ng

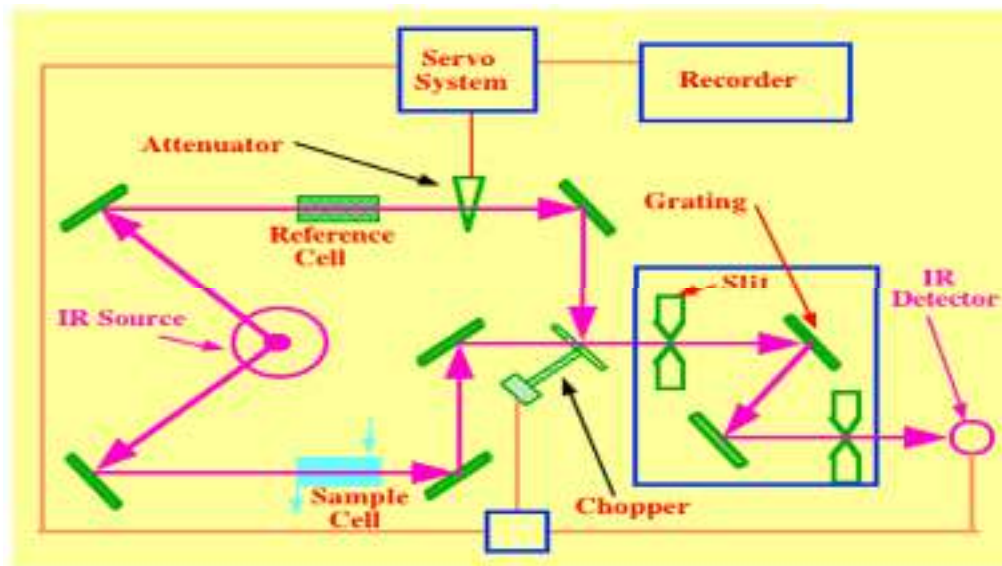


Fig. No.: Diagram of IR detector.

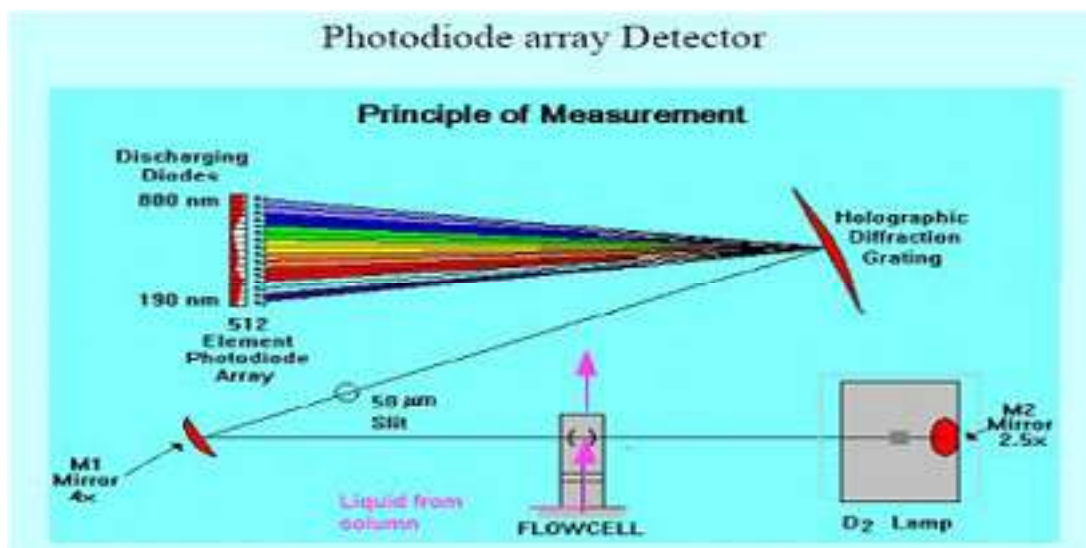


Fig Photo diode array detectors:-

It is latest one which similar to U.V detector. It operates from 190-600 nm thereby allowing for the recording of the entire spectrum of each solute as it passed through the diode array detector. The resulting spectra is a 3-D or three dimensional plot of Response vs Time vs Wave length.

Fig. No.16 : Diagram of Photo diode array detector

Conductivity detectors:-

Based upon the electrical conductivity, the response is recorded. This detectors is used when the sample has conducting ions like an-ions and cat-ions.

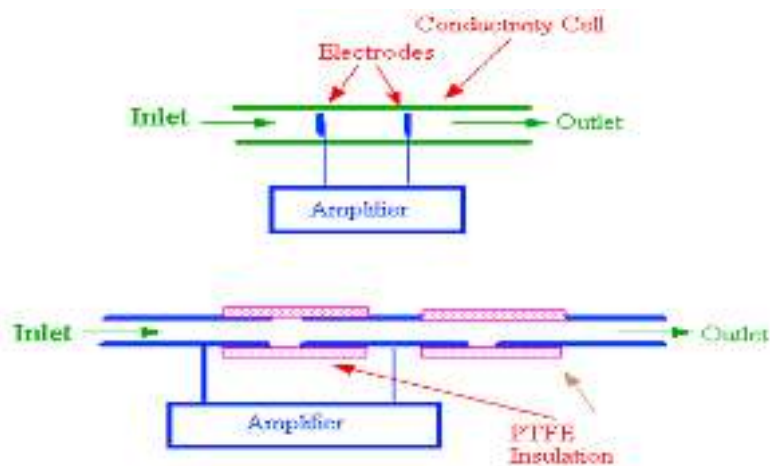


Fig. No: Diagram of Conductivity detector.

Amperometric detector:-

This detector is based on reduction or oxidation of the compounds when a potential is applied. The diffusion current recorded is proportional to the concentration of the compound eluted. This is applicable when compounds have functional groups. Which can be either oxidised or reduced? This is a highly sensitive detector.

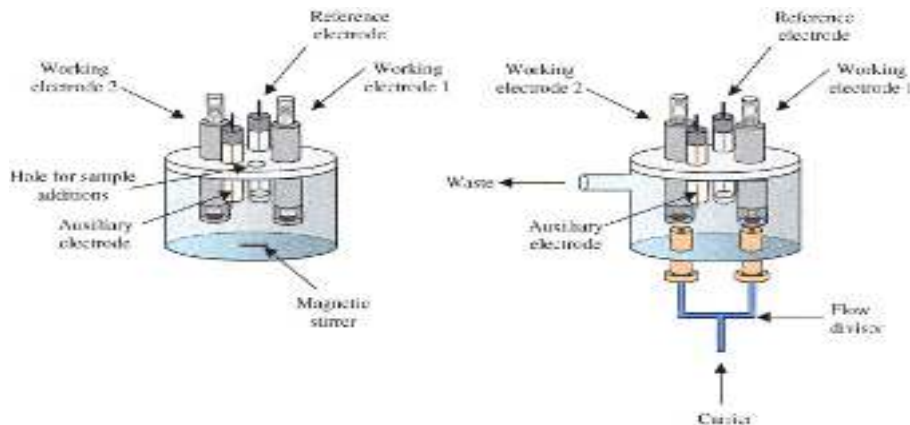


Fig. No.: Amperometric detector

Conclusion:

Detector is the key element which is present in any device that is used for the identification and estimation of any compound. It detects in a faster rate i.e., within some fractions of second. Hence a detector is considered as a brain of an instrument. Without the help of a detector, no one would be able to analyze any compound

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