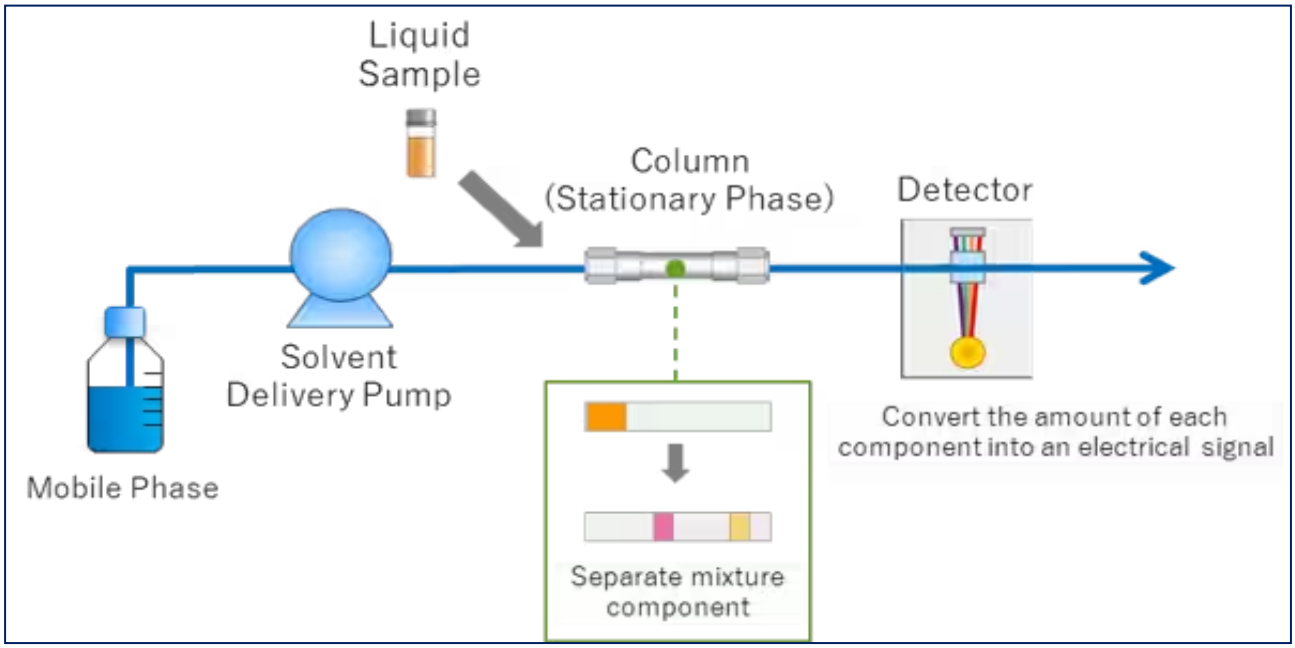
HPLC Detectors

HPLC is one of the most powerful techniques for separation and quantitative determination of the constituents in a mixture. It works on the principle of Affinity chromatography having two phases viz: stationary and mobile phase. The constituent with lower affinity for stationary phase travels faster and vice-versa. A detector in HPLC is placed at the end of the system. Its work is to analyse the solution which is eluting from the column.



Detectors

A detector in HPLC is placed at the end of the system. Its work is to analyse the solution which is eluting from the column. The concentration of individual component of the analyte is proportional to the electronic signal coming out of the component of the mixture.

Characteristics of the Ideal Detector

- Should have **adequate sensitivity.**
- It must produce **stable and reproducible signal**.
- It should have a **linear response to** the concentration of the analyte that extends over several orders of magnitude.
- A **short response time -** independent of **flow rate**
- **High reliability** and **ease of use**.
- It should be **non destructive**
- It should have **minimal internal volume** to reduce zone broadening
- It should be **compatible with liquid flow**.
- **Temperature variation** must not affect the response.
- It must be **independent of eluent composition** (gradient).

Types of Detectors

Detectors are broadly classified into two groups:

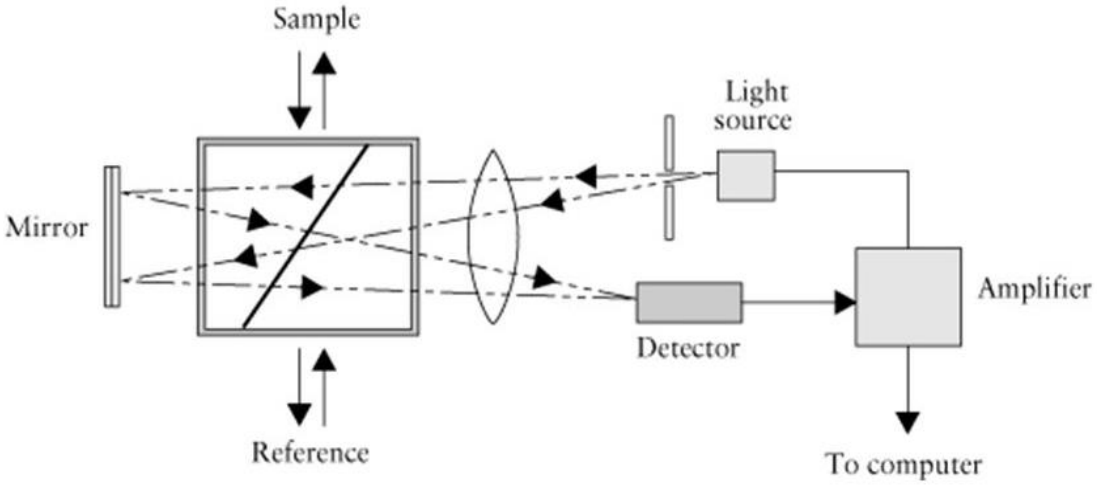
Bulk Property Detectors: Bulk property detectors are those that measure the changes in solute and mobile phase in combination. Such detectors show fluctuation in readings even with slight change in mobile phase combination. Examples are: refractive index and conductivity detectors. Due to poor sensitivity and limited range, they are used less despite being universally applicable. In general they are called as non selective detectors because they react to the bulk property of the analyte.

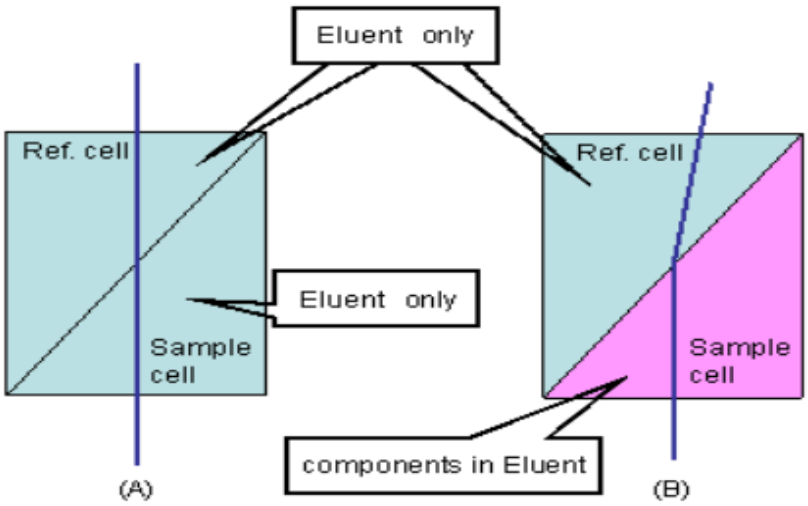
Solute Property Detectors: Solute property detectors are also called as selective detectors because they give response for a particular physical or chemical property of the analyte, being ideally independent of the mobile phase. Practically, it is not possible to achieve complete independence from mobile phase but the signal discrimination usually makes it sufficient to work with solvent changes as in gradient elution.

Bulk Property Detectors

Electrical Conductivity HPLC Detectors: These detectors senses all the ions, whether they are from a solute, or from the mobile phase. It measures the conductivity of mobile phase along with the solute which needs to be backed-off by suitable electronic adjustments. Thus it is a type of Electrical Conductivity Detector. The measured electronic resistance is directly proportional to the concentration of ions present in the solution.

Refractive Index HPLC detectors: They are also one of the bulk property detectors and are based on the change of the refractive index of the eluent from the column with respect to pure mobile phase. There are different types of Refractive index detectors: Christiansen effect detector, interferometer detector, thermal lens detector and the dielectric constant detector. They are mostly used for detection of non-ionic compounds that neither fluoresce nor absorb in the UV region. They face the drawback of being less sensitive, need of temperature control and less suitability to gradient elution.





- **Advantages:**

✓ Respond to nearly all solutes

✓ Reliable and unaffected by flow rate

- **Disadvantage :**

✓ Less sensitive

✓ Less suitability to gradient elution

✓ Need of temperature control

Electrochemical HPLC Detectors: They are termed as "Electrochemical detectors" for the reason being that they usually measure the current associated with the oxidation or reduction of solutes. They act as amperometer or coulometer in HPLC. They are classified as equilibrium and dynamic detectors. The suitability of these detectors depends on the volumetric characteristics of the solute molecules in the aqueous or organic mobile phase.

- **Advantages:**

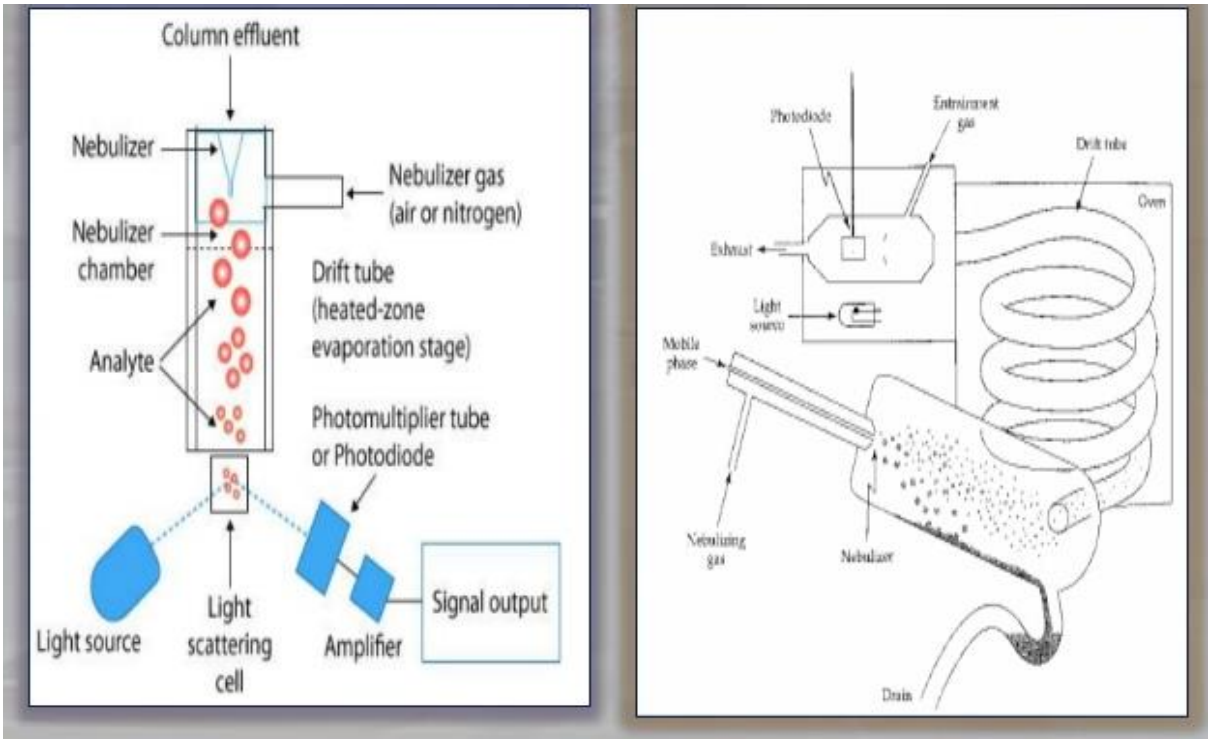
✓ High sensitivity, simplicity, convenience and widespread applicability.

- **Disadvantages:**

✓ Sensitive to changes in the flow rate or composition of the eluent

✓ Require working electrode, reference electrode and auxiliary electrode

Evaporating Light Scattering HPLC Detectors: Light scattering HPLC detectors are useful for large molecular weight molecules like surfactants, lipids and sugar. It measures the scattered light coming out of the eluent. Low angle laser light scattering detector and the multiple angle laser light scattering detectors are the two types of Light scattering detectors available. They are also called as Evaporative light scattering detector because in this the beam of light by particles of compound remaining after evaporation of the mobile phase. The importance of such type of detector is growing with time because it acts as universal detector and does not require a compound to have a chromophore for detection. They can be used with gradient elution.



- **Advantages :**

✓ Respond to almost all solutes.

✓ Can be used with gradient elution.

✓ Suitable for non volatile solutes

✓ More sensitive than RI detector with detection limits of 0.2ng/ml

- **Disadvantage :**

✓ Mobile phase should be volatile