Chromatographic Methods of Analysis

Section: 7 HPLC

Prof. Tarek A. Fayed

Liquid chromatography

Applied to the separation of any compound that is soluble in a liquid phase; biological compounds, synthetic or natural polymers, and inorganic compounds.

- Use large, non-rigid support material, particles size > 150 μm.
- Column; diameter 10 ~ 50 mm, column length L: 50 ~ 500 cm, flow rate F: < 1 ml /min.
- Gravity; Large H, small N.
- Poor system efficiencies and large plate heights.

Basic Principles of HPLC

- **HPLC High Performance Liquid Chromatography (Also called; High Pressure Liquid Chromatography)**
- **It is a form of liquid column chromatography used for separation, identification, and quantification of compounds in bio- and analytical chemistry.**
- **It represents, adsorption, ion exchange and gel chromatography in the form of column.**
- **It is really the automation of traditional liquid chromatography under conditions which provide enhanced resolution separations during shorter periods of time.**

Types of HPLC Separations

- **Normal Phase:**

Separation of polar components by using a polar bonded stationary phase (like silica or alumina) and non-polar solvents (like hexane).

- **Reversed Phase:**

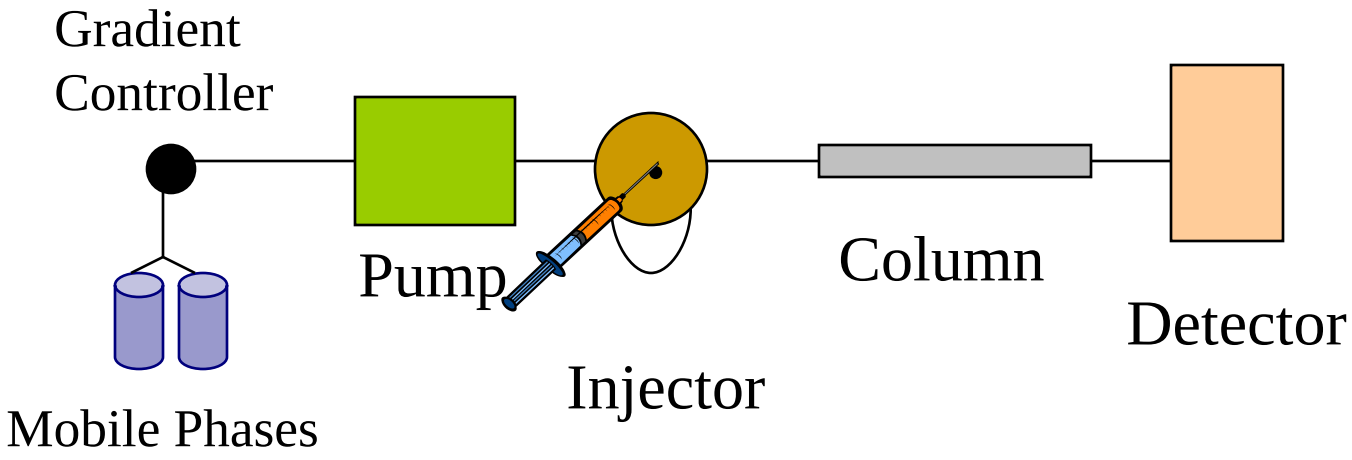
Separation of non-polar analytes by using a non-polar bonded stationary phase (like polystyrene) and a polar solvents (like methanol).

Property	Normal	Reversed-Phase
Packing Polarity	Polar	Non-Polar
Mobile Phase Polarity	Non-Polar	Polar
Elution Order	Non-Polar first	Polar First first
Increasing Polarity of Mobile Phase	Reduces t_R	Increases t_R

Requirements for HPLC

- **Column:**
  - **Stainless steel tubing for high pressure, or heavy-wall glass for low P (< 600 psi).**
  - **Analytical column: straight with length (5 ~ 25 cm), diameter (3 ~ 5 mm), particles size (3 ~5 µm).**
- **Temperature control: < 150 °C ± 0.1 °C.**
- **Column packing: silica, alumina, a polystyrene-divinylbenzene synthetic or an ion-exchange resin.**
- **Solvent Reservoirs (can be a single solvent or multi-solvents).**
- **Apply high pressure to force liquid through the beads faster (pressures up to 100 atm)**
- **Control flow rate (from 0.1 to 10 ml/min).**

Instrumentation



HPLC Chromatograph

Hewlett-Packard Series 1100 HPLC

Detectors

- UV detectors:
  - Single wavelength (filter).
  - Variable wavelength (monochromator).
  - Multiple wavelengths.
- Fluorescence
- Electrochemical (Amperometry, voltammetry, coulometry and conductormetry).
- Mass Spectrometric.
- Refractive index detector.

Advantages of HPLC

- High speed (separation in few minutes).
- High resolution.
- High sensitivity (separate samples up to ng or fg).
- Reproducibility of ± 1% (not so for LC).
- High accuracy.
- Automation.
- Separation of large number of components (upto 20 component can be separated).

Disadvantages of HPLC

- High cost.
- Complexity.
- Low sensitivity for some compounds.
- Irreversibly adsorbed compounds can not be detected.
- Co-elution difficult to be detect.

Applications of HPLC

- **Separation of typical non-volatile compounds as:**
  - **Pharmaceuticals like aspirin, ibuprofen, or acetaminophen.**
  - **Salts like sodium chloride and potassium phosphate.**
  - **Proteins like egg white or blood protein.**
  - **Organic chemicals like polymers (e.g. polystyrene, polyethylene).**
  - **Heavy hydrocarbons like asphalt or motor oil.**
  - **Many natural products such as ginseng, herbal medicines, plant extracts.**
- **Separation of thermally unstable compounds as:**
  - **Trinitrotoluene (TNT).**
  - **Enzymes .**
  - **Polychloronated hydrocarbons.**