Chromatography and its applications

Reference

Introduction to chromatography theory and practice

Instrumental methods of chemical analysis by H.Kaur

INTRODUCTION

There are many methods which have been used to obtain and identify the substances in the high state of purity
Those methods worked quite satisfactorily in many cases but great amount of difficulty was realised with the compounds which had the composition of the individual components of very similar physical and chemical properties. Chromatography plays a very important and significant role in solving all such problems. The complicated separations are achieved very rapidly and efficiently by chromatography. This method is fruitful and common by this time chromatography has been used in almost every type of compounds and fields. Medicine, biology, art and painting and even intelligence department have used this method to their greatest advantage.

History

- Chromatography (Greek : Khromatos – Colour and graphos wtiting) a relatively new separation technique , was discovered by Dr.Michael Tswett (1906) in Warsaw for the separation of complex mixtures by the process of adsorption .The earliest work in the field of chromatography dates back to 1855 when Friedrich Runges obtained a coloured chromatogram by impregnating a filter paper with ferric sulphate, drying and adding a drop of potassium ferrocyanide solution

CLASSIFICATION OF CHROMATOGRAPHIC METHOD

- **Paper chromatography:** substances are applied as a small spot on filter paper dipped in an organic solvent. The mixtures are partitioned between paper(stationary phase) organic solvent (mobile phase).

- **Partition column chromatography:** The column is packed with a porous solid of high surface area The components of a mixture are separated by passing an organic solvent(mobile phase)through the column.

- **Ion exchange chromatography:** Ionised compounds are separated in aqueous solutions differences in affinity for ionised compounds

- **Thin layer chromatography:** The adsorbent (stationary phase) is spread over a glass plate in a thin film of even thickness. The solvent(mobile phase) moves up the plate by capillary action and thus affects separation .

HIGH PERFORMANCE LIQUIQ CHROMATOGRAPHY

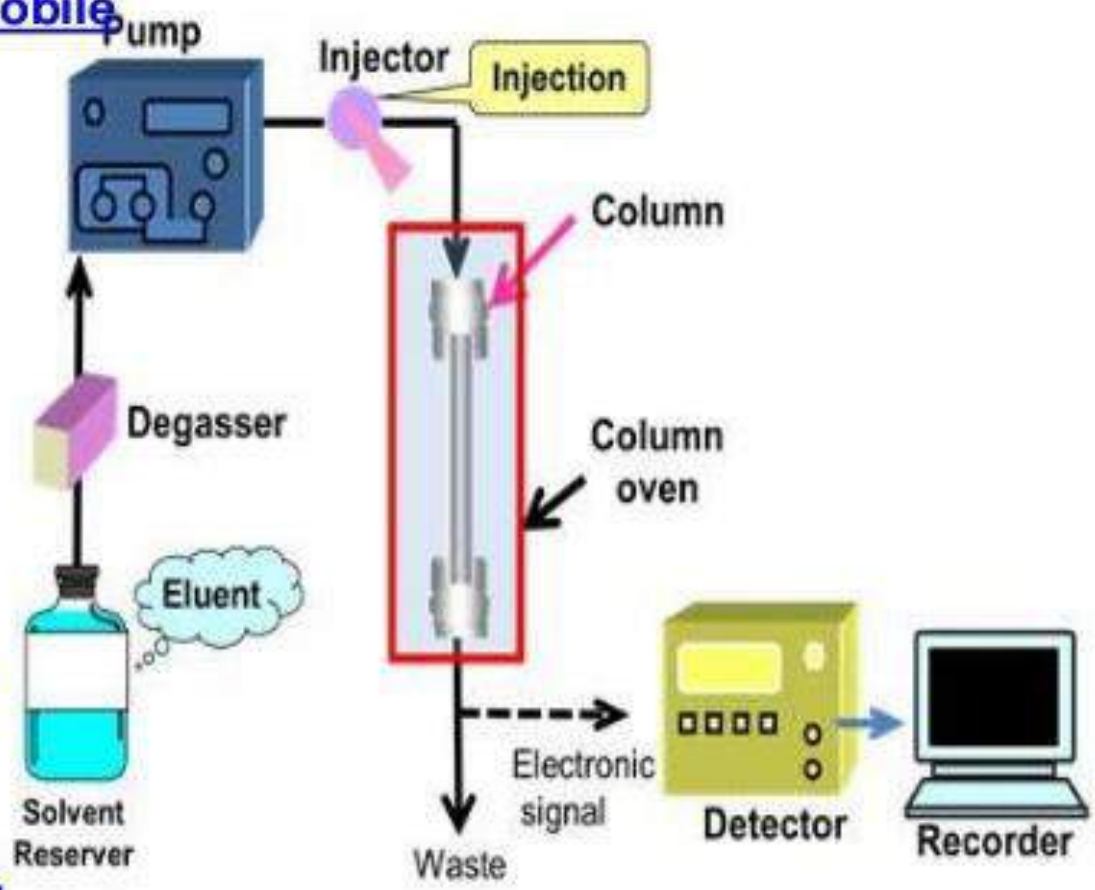
ALL forms of liquid chromatography (LC) are differential migration.LC covers a variety of separation techniques, such as liquid-solid, liquid-liquid, ion-exchange and exclusion chromatography. The technique of high pressure liquid chromatography or high speed liquid chromatography, later termed high performance chromatography (HPLC) attained greater significance in separation science. The technique of HPLC was developed by **Csaba Horvath(1964) Kirkland and Huber in 1969.** The first mixture to be separated Horvath group were nucleic acid components associated with thyroid function.

CHARACTERISTIC FEATURES OF HPLC

- High resolving power and speedy separation.
- Accurate quantitative measurement
- Repetitive and reproducible analysis using the same column
- HPLC is able to separate macromolecules and ionic species
- It can determine multiple components in a single analysis

Typical HPLC system consists of followings

- Solvent reservoirs (mobile Phase reservoirs)
- Pump
- Injector
- Column
- Detector
- Recorder
- Pulse Damper
- Degasser
- Column Heater



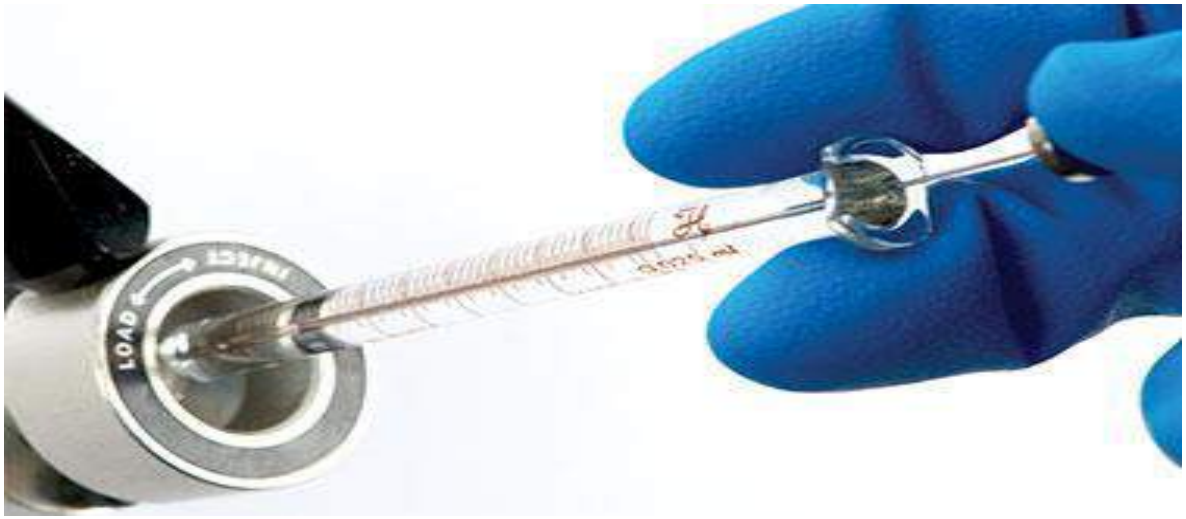
INSTRUMENTATION FOR HPLC

- **Solvent Resorvoir** : HPLC is usually a mixture of polar and non-polar liquid components whose respective concentrations are varied depending on the composition of the sample.
- **Pump** : A pump aspirates the solvent resorvoir and forces it through the system's column and detecter.

a) **Gas Displacement pump** These are pumps offer non pulsating flow but have limited solvent capacity

b) **Pneumatic pump** which is contained in a collapsible container and placed in a vessel.These pumps are inexpensive and pulse free but depend on solvent viscosity and column back pressure

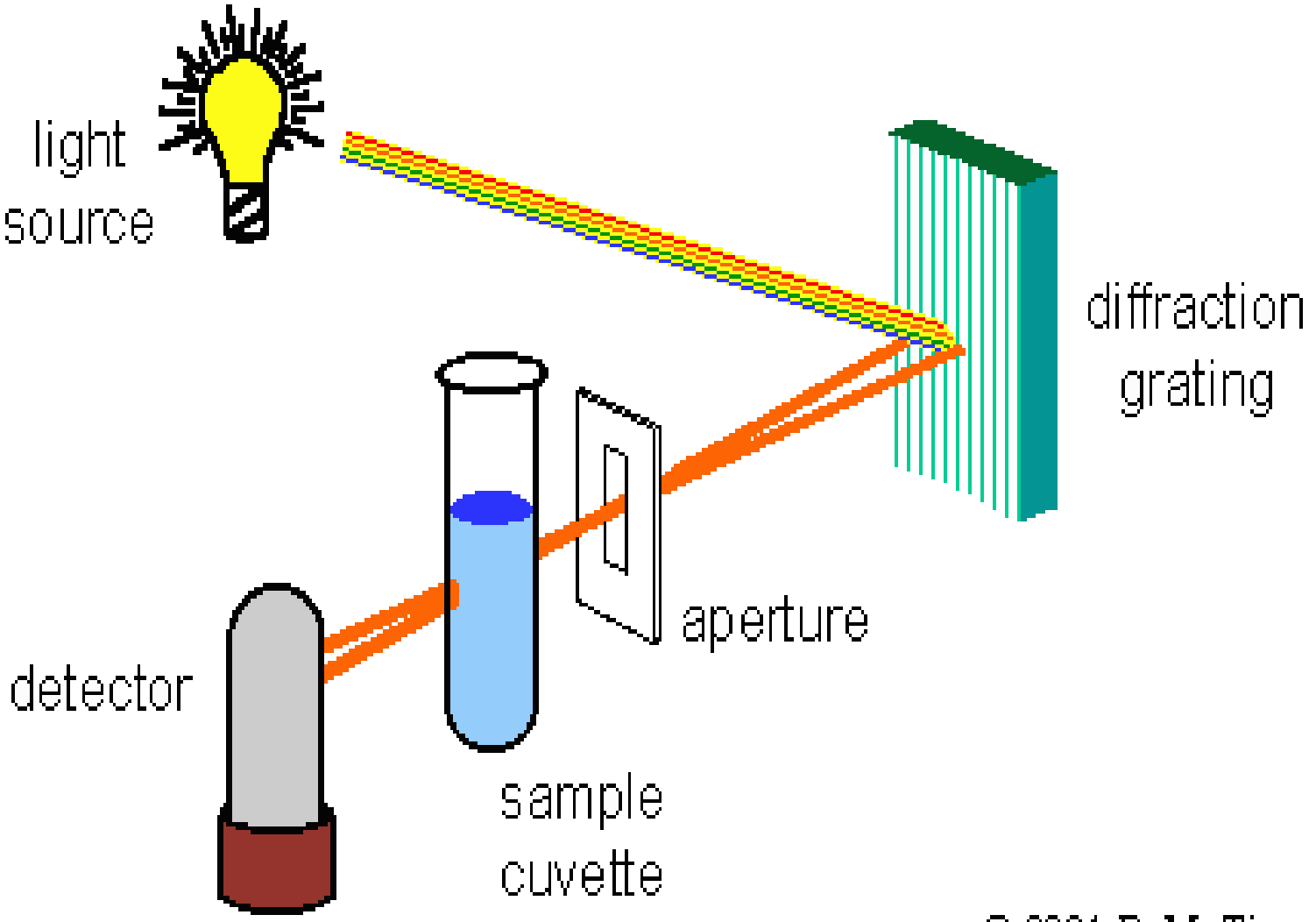
C) **Syringe pump** work on the principle of solvent displacement by a piston mechanically driven at constant rate. These pumps generate pluse less flow with high pressure



D) **Reciprocating pump** provide accurately controlled flow rates of 1-15 cm3 min -1 against a column back pressure

- **Columns** : Columns are usually made of polished stainless steel, are between 50 and 300 mm long and have an internal diameter of between 2 and 5 mm. They are commonly filled with a stationary phase with a particle size of 3–10 µm. Columns with internal diameters of less than 2 mm are often referred to as microbore columns.

- **Detector** : The HPLC detector, located at the end of the column detect the analytes as they elute from the chromatographic column. Commonly used detectors are UV-spectroscopy, fluorescence, mass-spectrometric and electrochemical detectors.


light
source
diffraction
grating
aperture
detector
sample
cuvette
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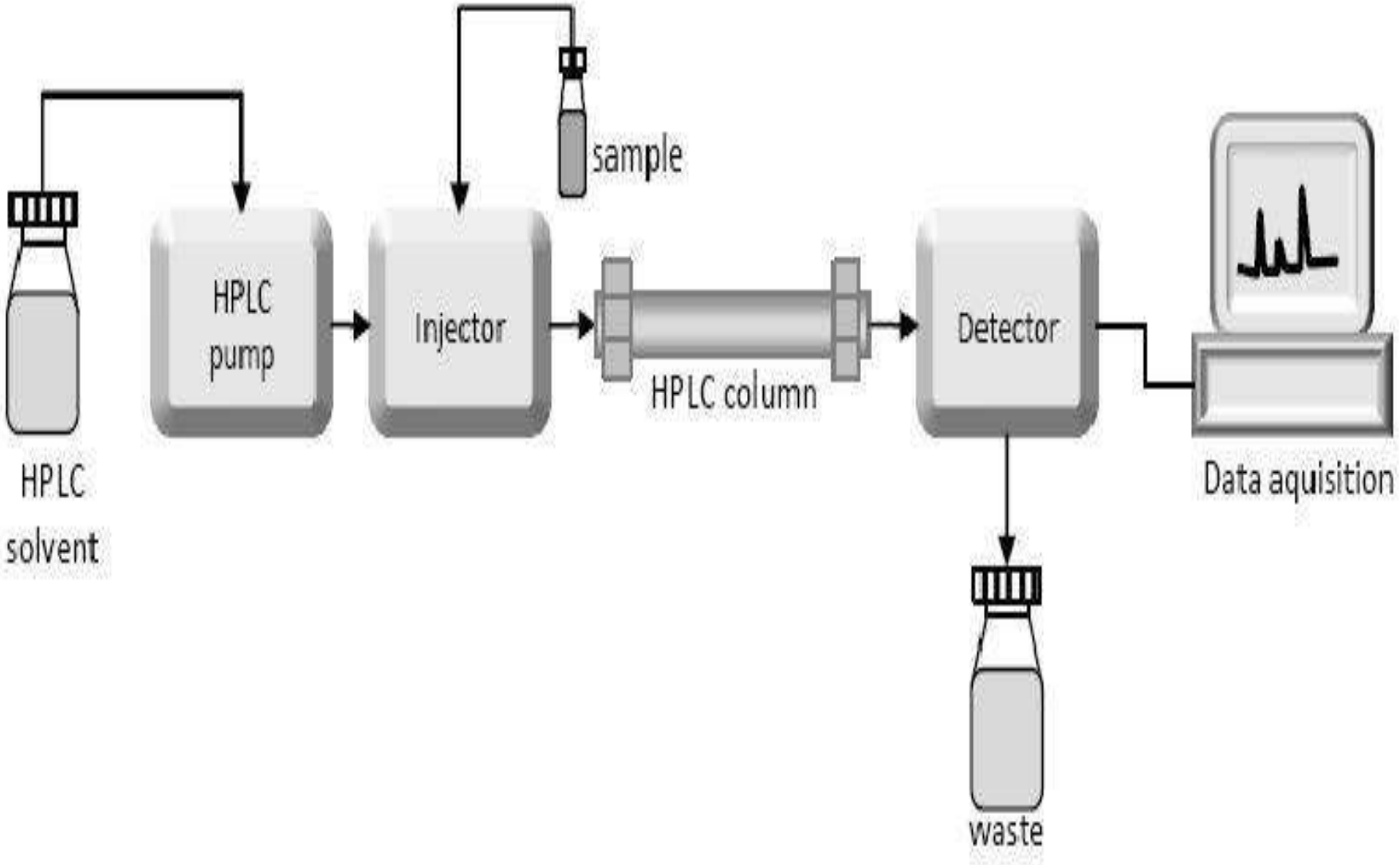
Types of HPLC detectors

Name	Advantage	Disadvantage
UV-Vis	Works w/all molecules	Non-specific; complex samples; absorption wavelength
DAD	Works for all wavelengths	High LOD
Fluorescence	Very specific; low LOD	Not everything fluoresces
IR	Works w/all molecules	Many solvents IR active
Refractive Index	Works w/*nearly* all molecules	Temperature sensitive; high LOD
Scattering	Uniform response; 5ng/25 μL LOD	Non-specific; interference from solvent
Electrochemical	Commercially available	Non-specific; high LOD
Mass Spec	Low LOD; analyte identification	Ability to ionize analyte

- **Data Collection Devices** : Signals from the detector may be collected on chart recorders to process, store and reprocess chromatographic data. The computer integrates the response of the detector to each component and places it into a chromatograph that is easy to read and interpret.
- **Recorder**

  A recorder, now more precisely termed a potentiometric recorder, is a device that draws the chromatogram that results from a chromatographic process onto chart paper and provides a visual representation of the separation that has been achieved. Most modern chromatographs no longer employ recorders to present the chromatogram and the results are automatically processed by a computer and presented on the computer monitor or printed out as required.


sample
HPLC pump
Injector
HPLC column
Detector
Data aquisition
HPLC solvent
waste

Effect of the temperature

There are two other significant effects of separation under the elevated temperature.

- Stabilization of the column and absence of local temperature fluctuations due to the solvent friction lead to the more uniform adsorption-desorption process.

- Another effect is the increase of the column efficiency. At the elevated temperature viscosity of liquids decrease and the diffusion coefficient increaseoptimum efficiency.

Applications of hplc

- In inorganic chemistry:

(i)The chromatographic separation of anions can be effectively carried out by using ion pair chromatography

(ii)For the separation of cations ,sulphonated inert polymer resins have been used.

- In organic chemistry:

(i) Separationof lipids:Lipids range from hydrocarbons and wax esters to highly polar sugar of phosphoric acid conataining glycol and phospholipids.The polar head group interact with a polar stationary phase

- **Pharmaceutical Applications**
  1. To control drug stability.
  2. Tablet dissolution study of pharmaceutical dosages form.
  3. Pharmaceutical quality control.
- **Environmental Applications**
  1. Detection of phenolic compounds in drinking water.
  2. Bio-monitoring of pollutants.
- **Applications in Forensics**
  1. Quantification of drugs in biological samples.
  2. Identification of steroids in blood, urine etc.
  3. Forensic analysis of textile dyes.
  4. Determination of cocaine and other drugs of abuse in blood, urine etc.

- **Food and Flavour**

  1. Measurement of Quality of soft drinks and water.
  2. Sugar analysis in fruit juices.
  3. Analysis of polycyclic compounds in vegetables.
  4. Preservative analysis.

- **Applications in Clinical Tests**

  1. Urine analysis, antibiotics analysis in blood.
  2. Analysis of bilirubin, biliverdin in hepatic disorders.
  3. Detection of endogenous Neuropeptides in extracellular fluid of brain etc.