HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
IV Sem B.Sc Chemistry

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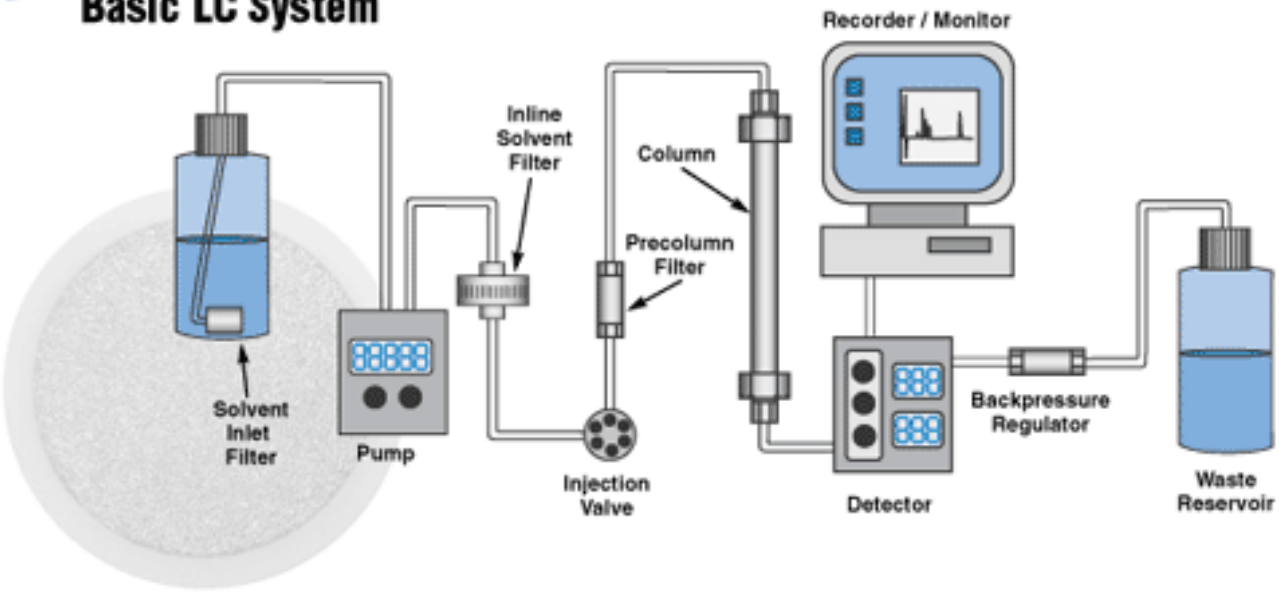
What is HPLC?

- Originally referred to as ***High-Pressure Liquid Chromatography***
- Now more commonly called ***High Performance Liquid Chromatography***
- HPLC is really the automation of traditional liquid chromatography under conditions which provide for enhanced separations during shorter periods of time, *utilizing very small particles, small column diameters, and very high fluid pressures.*

Schematic:

Basic LC System



- HPLC is a form of liquid chromatography used to separate compounds that are dissolved in solution.
- Compounds are separated by injecting a sample mixture onto the column. The different component in the mixture pass through the column at different rates due to differences in their partition behavior between the mobile phase and the stationary phase.

Components

1. Solvent
2. Solvent Reservoir
3. Pumps
4. Sample Injection System
5. Columns
6. Detectors
7. Data Processing
8. Waste

Solvents

- All solvents should be ‘HPLC’ grade.
- This is a type of reagent grade material.
- It has been filtered using a 0.2 μm filter.

Solvent degassing

- All solvents should be degassed prior to use.
- This reduces the chances of bubbles being formed in the column or detector. Oxygen present at high pressure can also cause a problem.
- Methods that can be used
- ! Displacement with a less soluble gas
- ! Applying a vacuum
- ! Heating the solvent.

Solvent reservoir

- Mobile phase
  - isocratic elution – solvent composition remains constant
  - gradient elution – composition of the solvent is changed continuously or in a series of steps
- To carry sample into the column

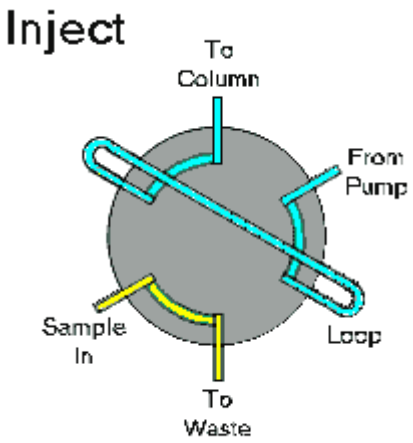
Pump

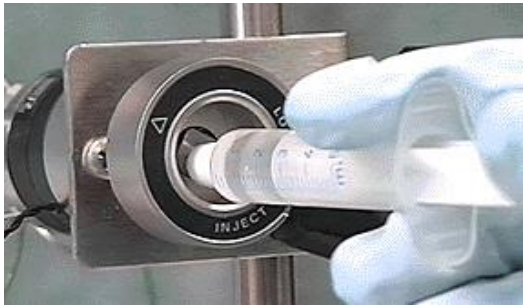
- To produce an appropriate pressure to push solvent into the sample.

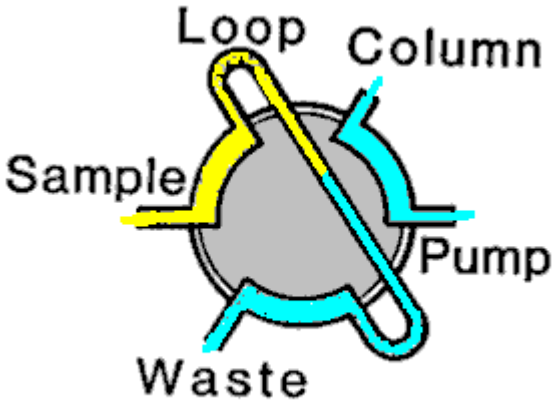
- A pump capable of pumping solvent up to a pressure of 6000 psi and at flows of up to 10 ml/min

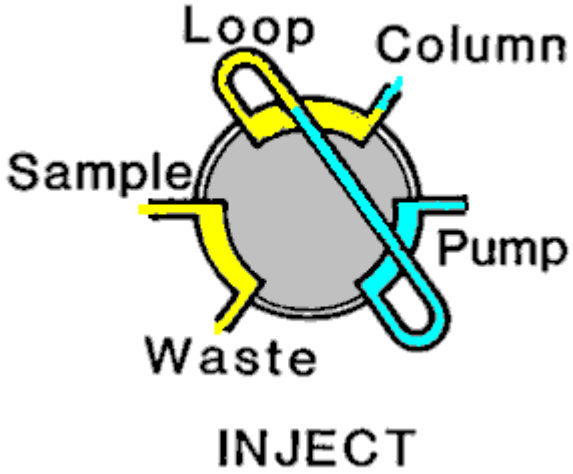
Sample injection system

- In order to introduce a sample onto the column for analysis, a special valve called the injector must be used to transfer the sample into the pressurized system


Inject
To
Column
From
Pump
Sample
In
Loop
To
Waste


INJECT


Loop
Column
Sample
Pump
Waste
LOAD


Loop
Column
Sample
Pump
Waste
INJECT

Column

- The heart of a HPLC system is the column.
- The column contains the particles that contains the stationary phase.
- The mobile phase is pumped through the column by a pump

Columns

- The column is one of the most important components of the HPLC chromatograph because the separation of the sample components is achieved when those components pass through the column. The High performance liquid chromatography apparatus is made out of stainless steel tubes with a diameter of 3 to 5mm and a length ranging from 10 to 30cm
- Normally, columns are filled with silica gel because its particle shape, surface properties, and pore structure help to get a good separation.

High performance partition chromatography

They can be sub divided into

Liquid – liquid PC: stationary phase is liquid that is held on the surface of the packing material by physical adsorption

Liquid – bonded phase PC : the stationary phase is an organic species that is attached to the surface of the packing material by chemical bonds

Normal and reversed phase chromatography

- There are a wide variety of stationary phases available for HPLC :
- **Normal Phase**.
  - Polar stationary phase and non-polar solvent.

    stationary phase-water, Tri ethylene glycol

    Mobile phase-hexane , i-propyl ether

Least polar analyte is eluted first

- **Reverse Phase.**
- - Non-polar stationary phase and a polar solvent.

  Stationary phase-hydrocarbon

  Mobile phase –water, acetonitrile, methanol, THF

Most polar component eluted first

Size exclusion

- In size exclusion the HPLC column is consisted of substances which have controlled pore sizes and is able to be filtered in an ordinarily phase according to its molecular size. Small molecules penetrate into the pores within the packing while larger molecules only partially penetrate the pores. The large molecules elute before the smaller molecules.

Ion exchange

- In this column type the sample components are separated based upon attractive ionic forces between molecules carrying charged groups of opposite charge to those charges on the stationary phase. Separations are made between a polar mobile liquid, usually water containing salts or small amounts of alcohols, and a stationary phase containing either acidic or basic fixed sites.

Detector systems

- **Bulk property -** measures an overall change in the mobile phase.
- **Solute property -** measures a solute specific property.

Properties of a good detector

- A detector must provide
- high sensitivity, low detection limits,
- linearity,
- Reproducibility.

- **UV/Vis detector**
- A solute property detector.
- Sample must exhibit absorption in UV/Vis range. Solvent must not absorb significantly at the measured wavelength.

Refractive index detector

- Bulk property detector - general purpose.
- Based on refraction of light as it passes from one media to another.
- Presence of a solute changes the refractive index of the solvent.

Heat of absorption detector

- A small amount of heat is released when a sample absorbs on a suitable surface.

Electrochemical detectors

- A number of properties have been evaluated
- Detector types
- ! ! Dielectic constant
- ! ! Amperometric
- ! ! Conductometric
- ! ! Polarographic
- ! ! Potentiometric