HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC):-

High performance liquid chromatography or HPLC is also called High pressure liquid chromatography. It is a technique that is used to separate, identify and quantify each component in a mixture.

→ It involves the pumps to pass a pressurised liquid solvent containing the sample mixture through a column filled with a solid adsorbent material.

→ Each component in a sample interacts slightly differently with the adsorbent material causing different flow rates for different components, leading to the separation of components as they flow out of the column.

→ HPLC can be used for medical (Eg:- detecting viral level in the blood), research (Eg:- Separating the components of a complex biological sample) and manufacturing (Eg:- During production of pharmaceuticals and biological products).

→ The columns used in HPLC are filled with an adsorbent which is usually granular. Solid particles of silica, polymers etc, which are 2-50 μm in size.

→ The pressurized liquid is typically a mixture of solvents (eg: water, Acetonitrile or methanol etc) and is referred to as Mobile phase.

* Components of HPLC :-

HPLC includes following components:-

(1.) Column :-

→ Column used for HPLC are made up of Stainless steel and are made to withstand a pressure upto 5.5×10^7 pascals.

→ Typical Column dimensions are 2-4 mm in diameter and 5-50 cm in length. Preparative Columns of diameter upto 25 mm are also available Commercially.

→ Porous plugs of stainless steel or teflon are used in the ends of the Column to retain the packing material.

→ HPLC Columns are packed with a specific packing material which can be :-

1.) Pellicular material :-

These are porous particles ~~Cot~~ Coated onto an inert solid core such as glass bead of about 40 μm in diameter.

2) Microporous Material:-

In these the micropores ranify throug the particles of 5-10 μm in diameter.

3) Bonded Material:-

In this the Stationary phase is Chemically bonded to an inert support such as silica.

4) Selection of Column:-

The HPLC technique was initially developed as Liquid-Liquid Chromatography methods. But there arose difficulties in maintaining the Stationary phase. Therefore different types of packing materials can be used in column for HPLC. Based on this the chromatography can be:-

(1) Adsorption Chromatography.

→ In this Adsorbents such as silica and Alumina as pellicular or microporous form are used with a range of particle size.

2) Partition Chromatography:-

In this the Stationary phase (packing material coated on to the inert microporous or pellicular support

3) Normal phase liquid Chromatography:-

In this the Stationary phase is a polar Compound such as Alkyl nitrile or Alkyl-amine and the mobile phase is a non-polar Solvent such as hexane. For Reversed phase Liquid chromatography the Stationary phase is non-polar compound such as Octasilane (OS) or Octadecylsilane (ODS) and the mobile phase is a polar Solvent such as water, Acetonitrile or water, methanol..

4) Ion exchange Chromatography:-

This involves the electrostatic interaction between charged Solute and a charged Stationary phase. Separation is achieved by differential affinities of charged solutes towards oppositely charged ionic groups in the Stationary phase. In this process, cross-linked microporous polystyrene resindues are widely used as packing material.

[2] Solvent-delivery System:-

It includes a pump which delivers the appropriate Solvent from a reservoir at a pre-selected rate. When the Composition of Solvent or, mobile phase is kept Constant (a single Solvent system), it is called Isocratic elution mode, and when varied (a mixture of Solvents) then it is called Gradient-elution mode,

during the chromatographic analysis.

The solvent reservoir is associated with a high pressure pumping system, which are capable of outputs of 5×10^7 Pa. There must be a flow capacity of 10 to 100 c.c./minute.

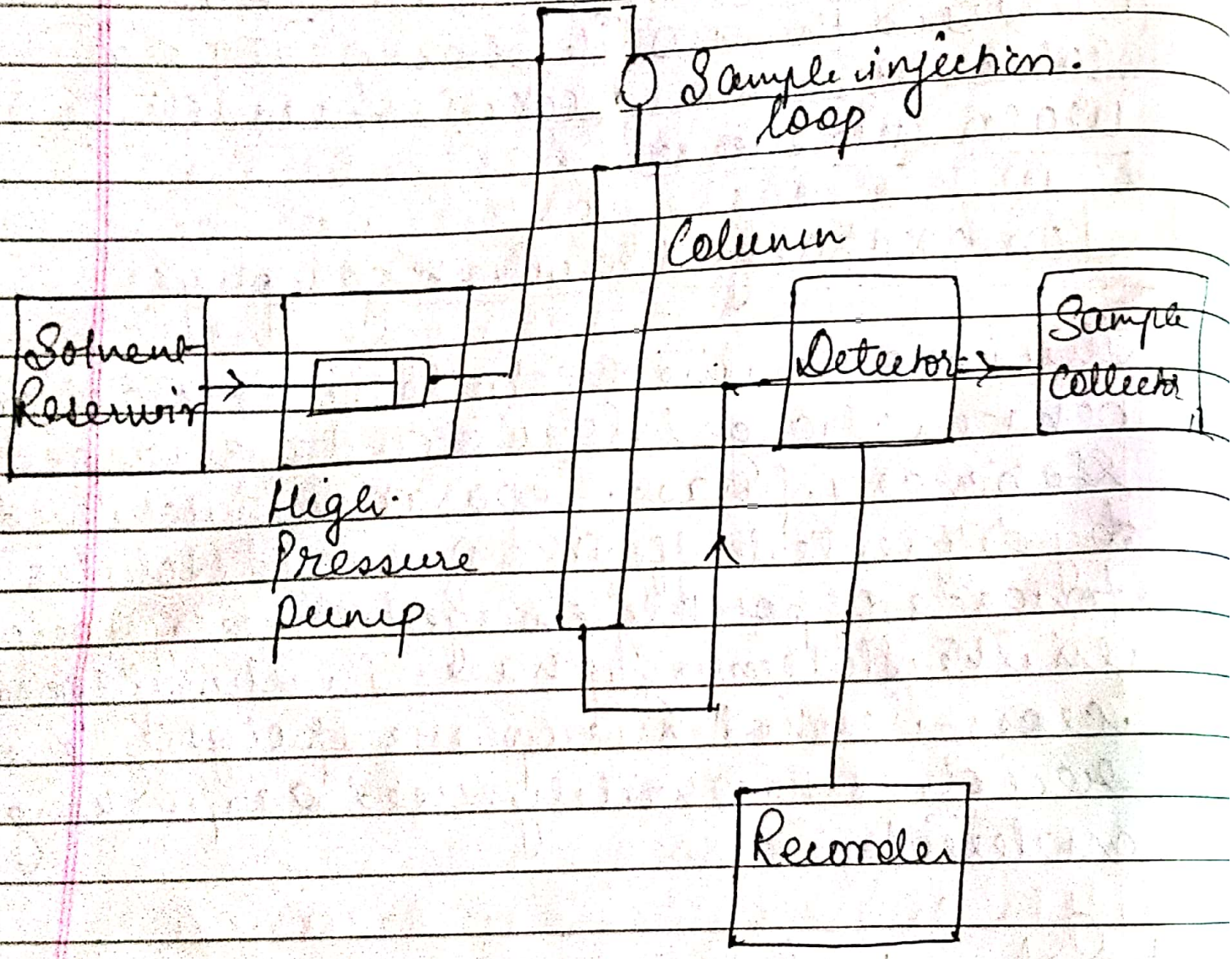

Fig:- Components of an HPLC System

3) DETECTORS:-

Since the HPLC system requires very small amount of sample hence a detector of very high sensitivity is required. Most commonly, the detector is a variable wavelength detector based upon ultraviolet visible spectrophotometry. These detectors have the facility to record the complete absorption spectrum of each analyte, thereby also aiding identification. Such detectors are capable of measuring absorbance to 190nm wavelength and have the sensitivity upto 0.001 absorbance units for full scale deflection (AUFS).

Fluorescent samples can be detected by fluorescent detectors.

Certain HPLC systems use Electrochemical detectors, which can be of two types:-

1. Amperometric and Coulometric. Such detectors are useful in assay of catecholamines, vitamins and antioxidants.

HPLC can also be coupled with mass spectrometer or NMR Spectroscopy to give the structural information about the analytes.

* Applications of HPLC :-

→ About all types of biological molecules can be assayed or purified using HPLC.

→ Separation of proteins can be made using fast protein liquid chromatography (FPLC).

→ This technique enables complex mixtures such as trypsin-digests of proteins and cellular supernatants of microbes to be applied directly.