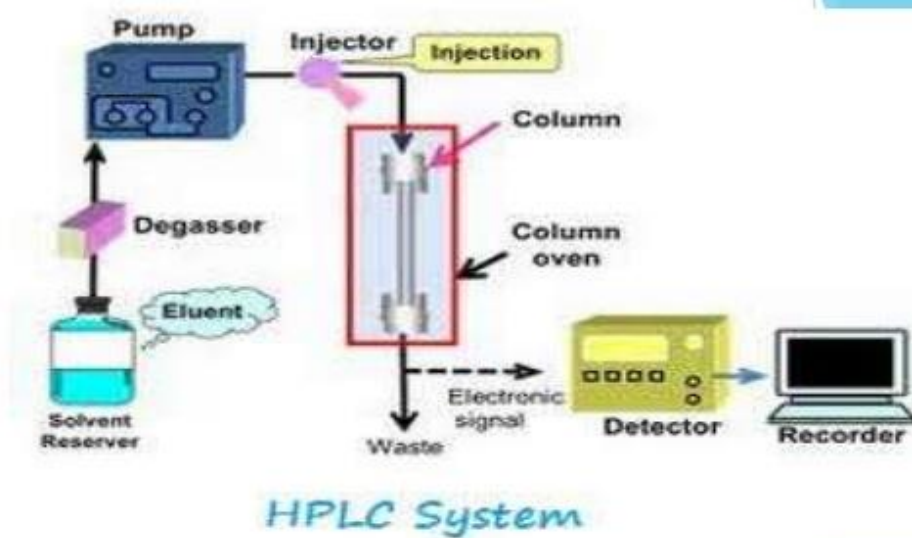


LECTURE # 3

Instrumentation of HPLC

The basic HPLC instrumentation consists of

- Solvent reservoir,
- pump(s),
- injector (manual or automatic injection),
- column(s),
- detectors,
- data handling device.



The pump(s) deliver solvents (mobile phase) within a given flow range, related to the size of the columns and choice of the detector.

The sample is introduced at the inlet of the column often by an auto-injection, the components are separated in the column and eluted by a connecting tubing into the detector, and signals are transferred from detector to a PC where the data handling takes place. With the proper software, all the steps can be completely controlled by the PC. If one column is not sufficient, another can be added. The first column can also be a short guard column protecting the analytical column.

Solvent reservoir:

An HPLC system begins with the solvent reservoir, which contains the solvent used to carry the sample through the system, the solvent should be filtered with an inlet solvent filter to remove any particles that could potentially damage the system's sensitive components.

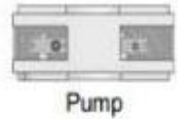
Chromatographic dependent methods:

These are

- Elution method of development
- Gradient elution method of development
- Frontal method of development
- Displacement method

In HPLC, according to the mode of elution , we use isocratic separation or gradient separation.

Gradient vs. Isocratic Conditions



Isocratic

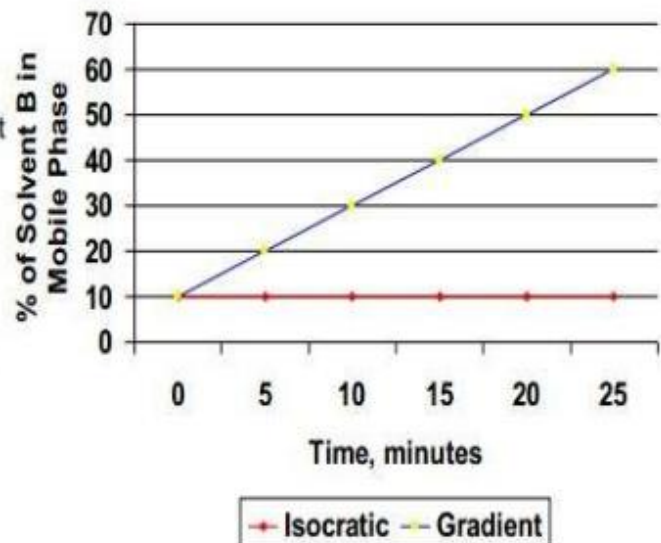
mobile phase solvent composition remains **constant** with time

- Best for **simple separations**
- Often used in **quality control applications** that support and are in close proximity to a manufacturing process

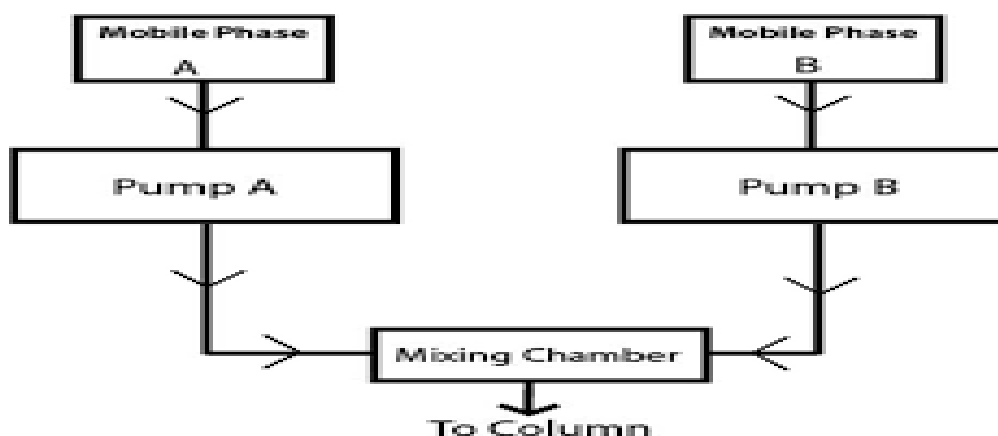
Gradient

mobile phase solvent ("B") composition **increases** with time

- Best for the analysis of **complex samples**
- Often used in **method development** for unknown mixtures
- Linear gradients are most popular (for example, the "gradient" shown at right)



Solvent Gradient elution:

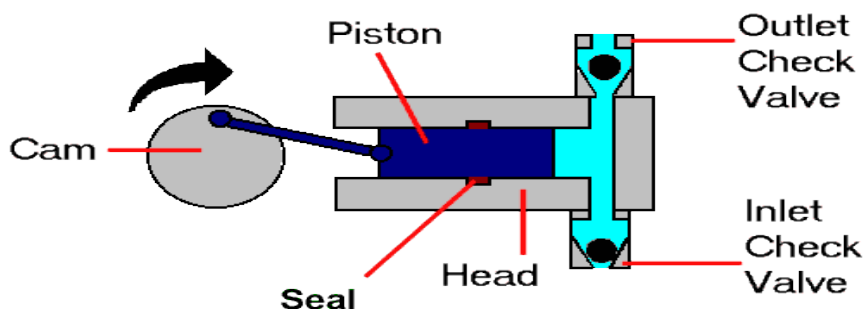


The most commonly used programming technique in LC is solvent-gradient elution, where the composition of the mobile phase changes during the run by mixing two or more components. In solvent gradient elution, a weaker mobile phase is used in the initial part of the elution to provide adequate retention of weakly retained compounds, while the elution strength increases and consequently the retention of analytes gradually decreases during the gradient run, which results in improved resolution of weakly retained compounds and shorter retention times of the strongly retained one.

PUMPS in HPLC:

Pressure pump consist of

- Inlet pressure pump
- Outlet pressure pump



Pump head

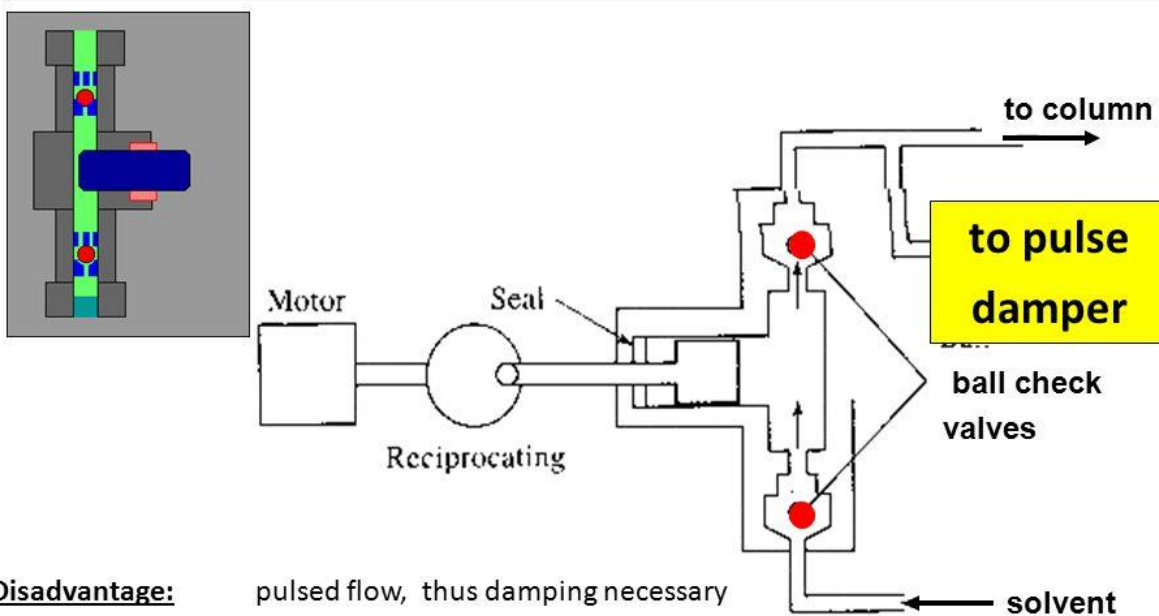
Both type of pressure pump in HPLC have both system of inlet and outlet pressure system.

- They are made up of stainless steel.
- Length is usually 10-50 cm.
- Diameter is 5-10 mm.
- The inner lining of pump is coated with acid-base salt resistance material.
- 750 psi pressure is given and can overcome back pressure and enhanced forward flow.
- Back pressure occurs due to the particle small size.

Mainly three types of pumps are used in HPLC

- Constant flow reciprocating pump
- Syringe type pump
- Pneumatic pump

2.1. Reciprocating Pump (in 90% of systems)



Disadvantage:

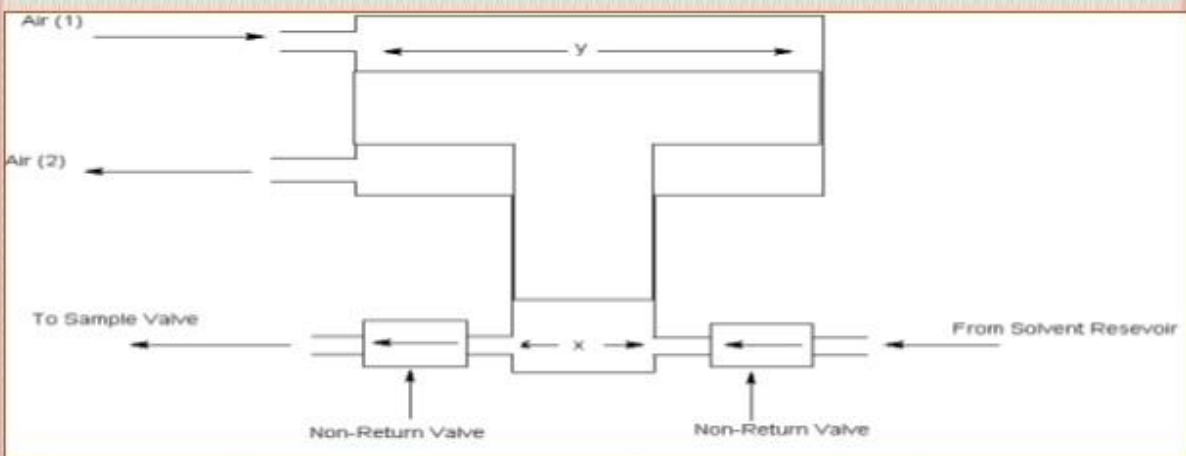
pulsed flow, thus damping necessary

Advantages:

small internal volume (35 – 400 μL), high output pressure (10,000 psi)
adaptability for gradient elution, constant flow rates (largely independent of column back-pressure and solvent viscosity).

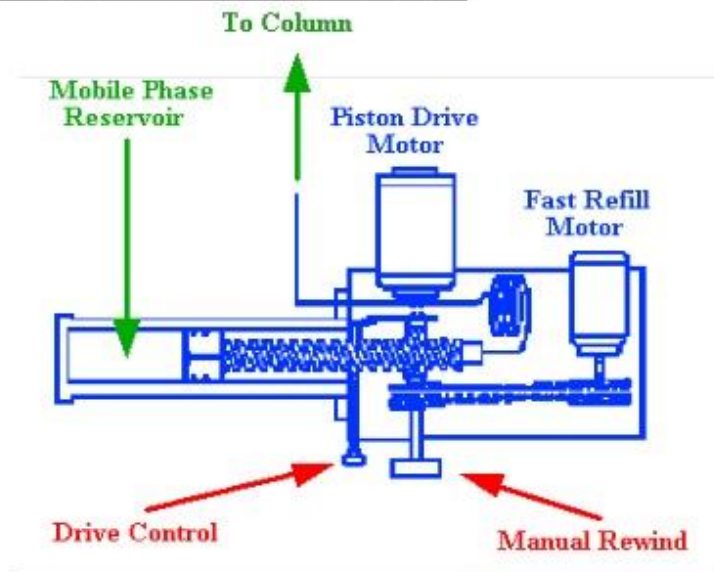
PNEUMATIC PUMPS

- In this pumps, the mobile phase is driven through the column with the use of pressure produced from a gas cylinder.
- It has limited capacity of solvent
- Due to solvent viscosity back pressure may develop.



MECHANICAL PUMPS

1. DISPLACEMENT PUMPS



Syringe or displacement type of pump:

- Consists of large syringe like chamber
- Suitable for small bore column

Sample introduction:

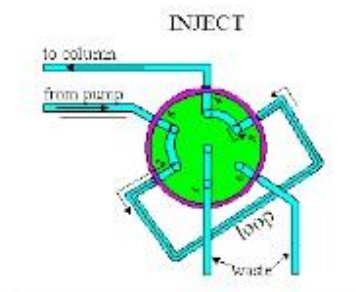
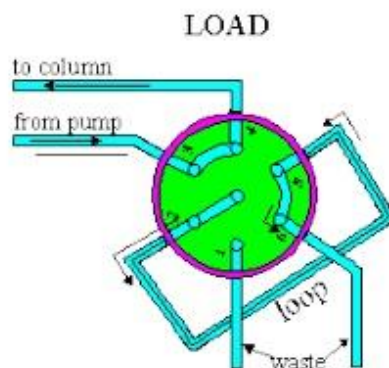
Injection Port

- The sample introduction device such as **injector** to introduce the sample in a flow of mobile phase at high pressure.
- It is not possible to use direct syringe injection on column like GC as the inlet pressure in LC is too high.
- The valve injection through fixed or variable loop is a common way of introducing the sample.

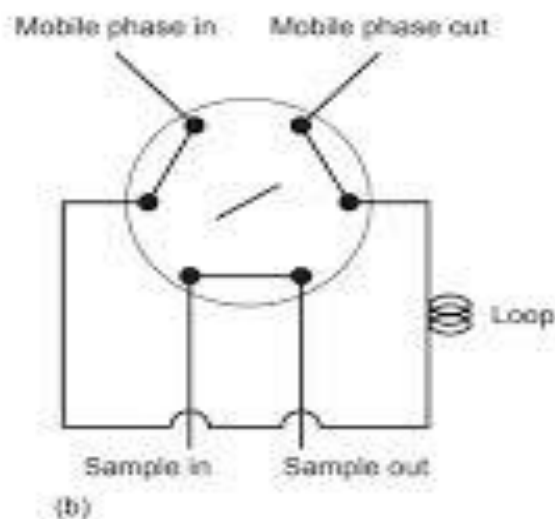
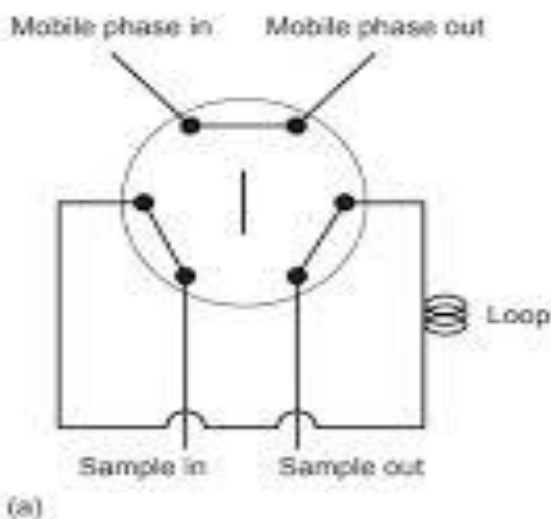
The **Rheodyne valve** is the mostly used device. The loop can be partially or fully filled. There are both the types of injectors available.

The advantage of partial filling is the possibility of using small amount of sample, when there is scarcity of sample.

The precision of the injection is 1% RSD



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Columns:

It is the heart of chromatography

Column length: varies from 5 cm to 30 cm,

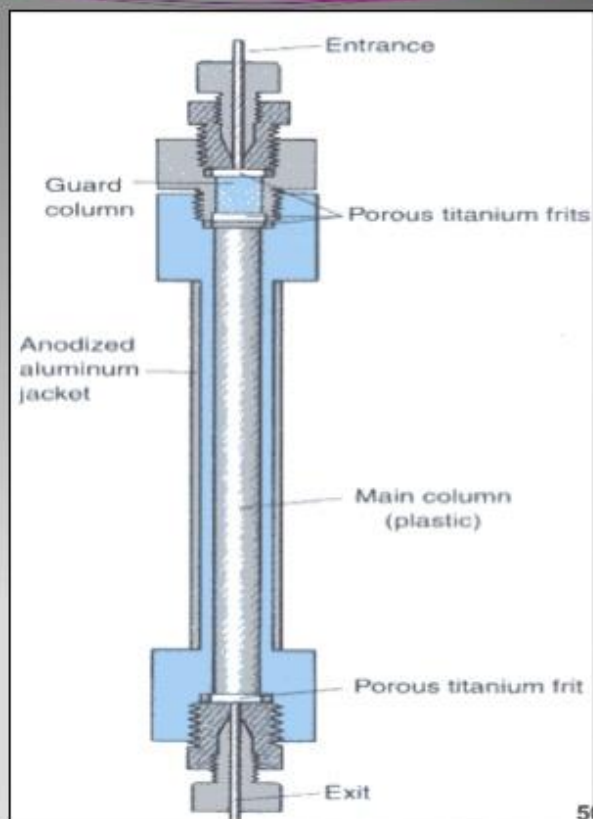
Column diameter: ranges from 2 mm to 50 mm.

Particle size: from 1μ to 20μ .

Particle nature: spherical, uniform sized, porous materials are used.

❖ COLUMN:

- There are various columns that can be used in HPLC method.
- They are as follows:
 - Guard Column
 - Derivatizing Column
 - Capillary Column
 - Fast column
 - Analytical Column
 - Preparatory Column



Material of construction for the tubing:

- Stainless steel (the most popular, gives high pressure capabilities).
- Glass (mostly for biomolecules).

- PEEK (poly ether ethyl ketone) polymer (biocompatibility and chemical inert to most solvent).

Packing material:

- Packing material is prepared from silica particle, alumina particle and ion-exchange resin.
- Porous plug of stainless steel or Teflon are used in the end of the columns to retain the packing material.

