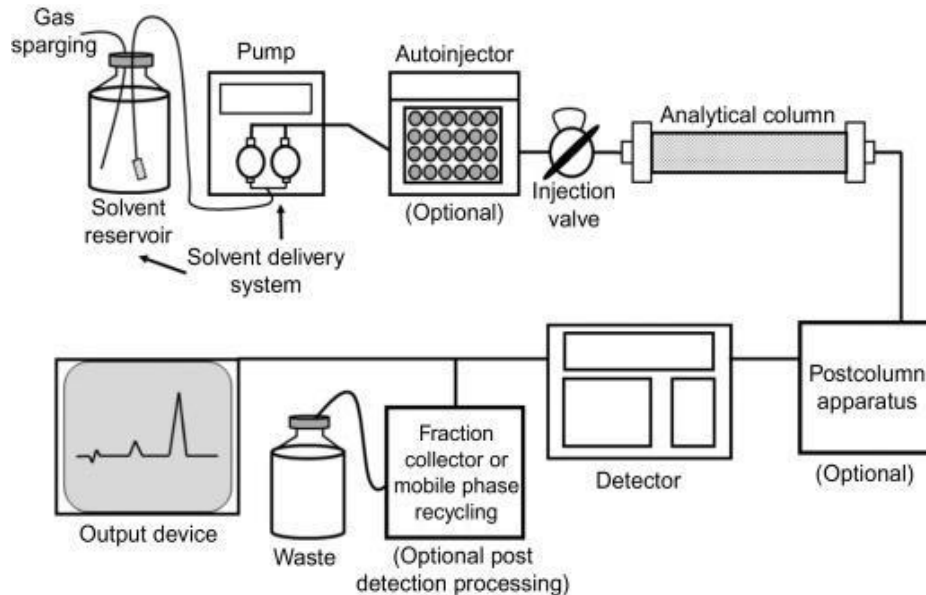


INSTRUMENTATION



1) PUMP:-

The role of the pump is to force a liquid (called the mobile phase) through the liquid chromatography at specific flow rate expressed in (ml/min). Normal flow rate in HPLC are in the 1-2 ml/min range.

During the chromatographic experiment, a pump can deliver a constant mobile phase composition or an increasing mobile phase composition. Best for the analysis of complex samples.

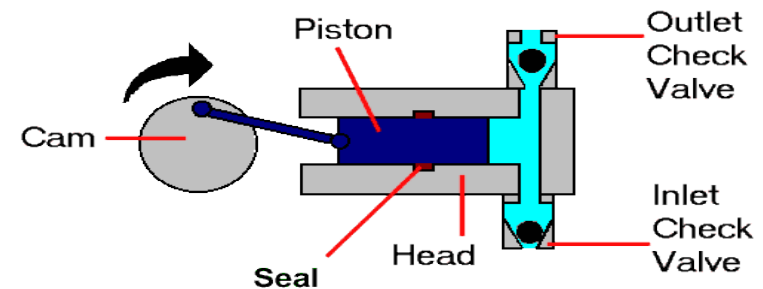
TYPES OF PUMPS :-

- 1] Constant flow reciprocating pump.
- 2] Syringe type pump .
- 3] Pneumatic pump.

• CONSTANT FLOW RECIPROCATING PUMP :-

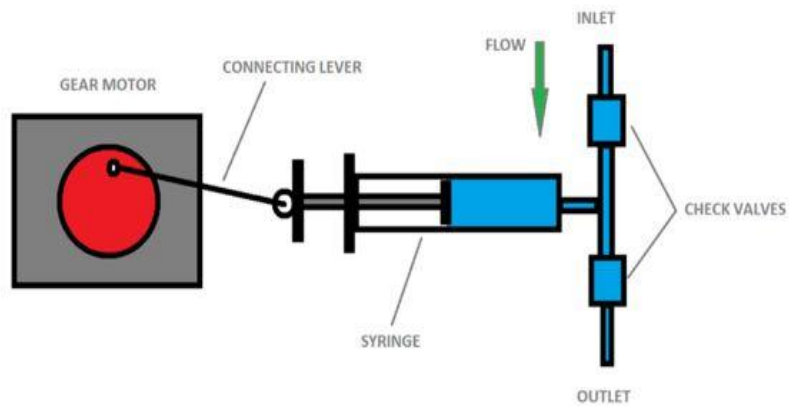
The pump reciprocating describes any continuously repeated backward and forward motion.

Solvent is sucked during back stroke and gets delivered to the column in forward stroke.



- **SYRINGE TYPE PUMP:-**

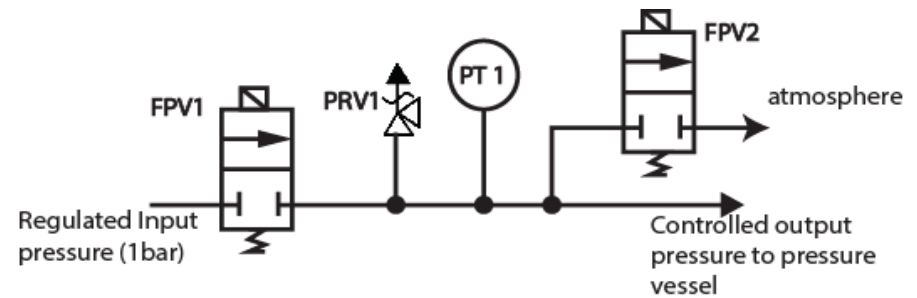
- Constant of large syringe chamber.
- Suitable for small bore column



- **PNEUMATIC PUMP:-**

Gas is used to pressurize the mobile phase present in a collapsible solvent.

Pneumatics are a branch of technology that use the force of compressed gases to generate mechanical effect. It is use to compressed air to create force that



the use to move fluid through a piping system.

- 2) **MIXING UNIT:-**

Mixing unit is used to mix solvents in different proportions and pass through the column.

There are two types of mixing units :

- 1) Low pressure mixing chamber which uses helium for degassing solvents
- 2) High pressure mixing chamber does not require helium for degassing solvents

- 3) **SOLVENT DEGASSING:-**

Several gaseous are soluble in organic solvent. When solvent are pumped under high pressure, gas bubbles are formed which will interfere with the separation process, steady base line and shape of the peak. This

can be done by using following technique:

- 1) Vacuum filtration
- 2) Helium purging
- 3) Ultrasonification

4) INJECTOR:-

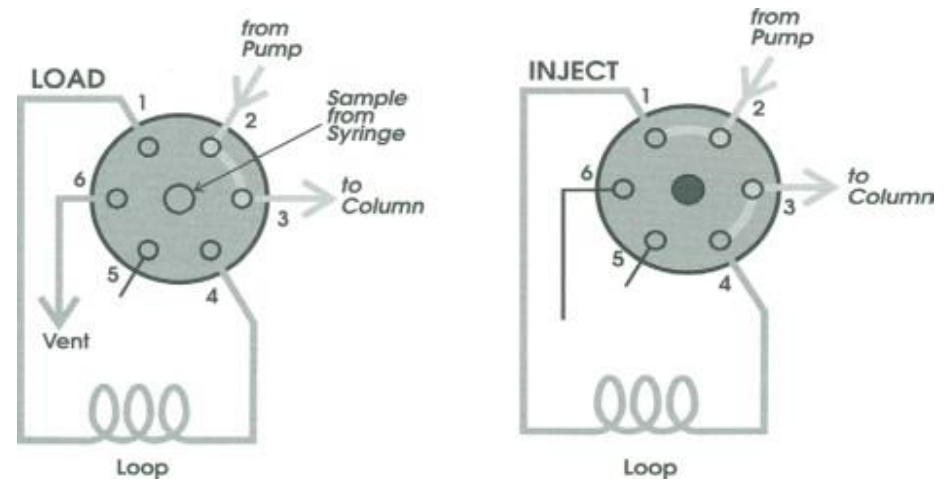
The injector serves to introduce the liquid sample into the slow stream of the mobile phase.

- Typical sample volumes are 5-20 microlitres.
- The injector must also be able to withstand the high pressure of the liquid system.
- An auto sampler is an automatic version for when the user has many samples to analyze or when manual injection is not practical.

Types of injector:

- 1) Septum injectors.
- 2) Stop flow.
- 3) Rheodyne injectors.

A sample injector is a device used in conjunction with injecting sample into high performance liquid chromatography.



5) COLUMN:-

- It is the heart of the chromatograph column length: varies from 5cm to 30cm.
- Column diameter: ranges from 2mm to 50mm.
- Particle size: from 1µ to 20µ
- Particle nature: spherical, uniform sized, porous material.

6) DETECTORS:-

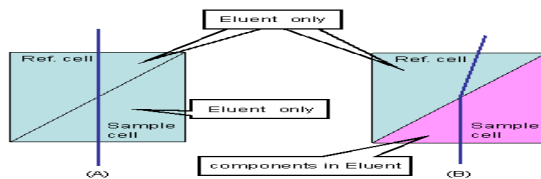
- Uv detection.
- Refractive index detector.
- Flourimetric detector.
- Conductivity detector.
- Ampherometric detector

1) UV DETECTION :-

The UV, VIS, PDA detector are categorized as absorbance detector. They provide good sensitivity for light absorbance compounds.

2) REFRACTIVE INDEX DETECTOR:-

IR detector measures changed in reflex index. A glass cell is divided into two chambers



3) FLOURIMETRIC DETECTOR:-

ELSD provide good sensitivity for non volatile analytes at ng level.

4) CONDECTIVTY DETECTOR :-

solution containing ionic components will conduct electricity. Conductivity detector measure electronic structure and measure value is directly proportional to the concentration of ions present in solution.

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MENTOR:- Mrs.A.V. Gaikwad

DEPARTMENT:- Instrumental method of analysis

SUBJECT:- High Performance Liquid chromatography (HPLC) Instrumentation

CLASS :- Final year B pharm.

REFERENCED BOOK :- Dr. A. V. KASTURE
G. R. CHATWAL.

