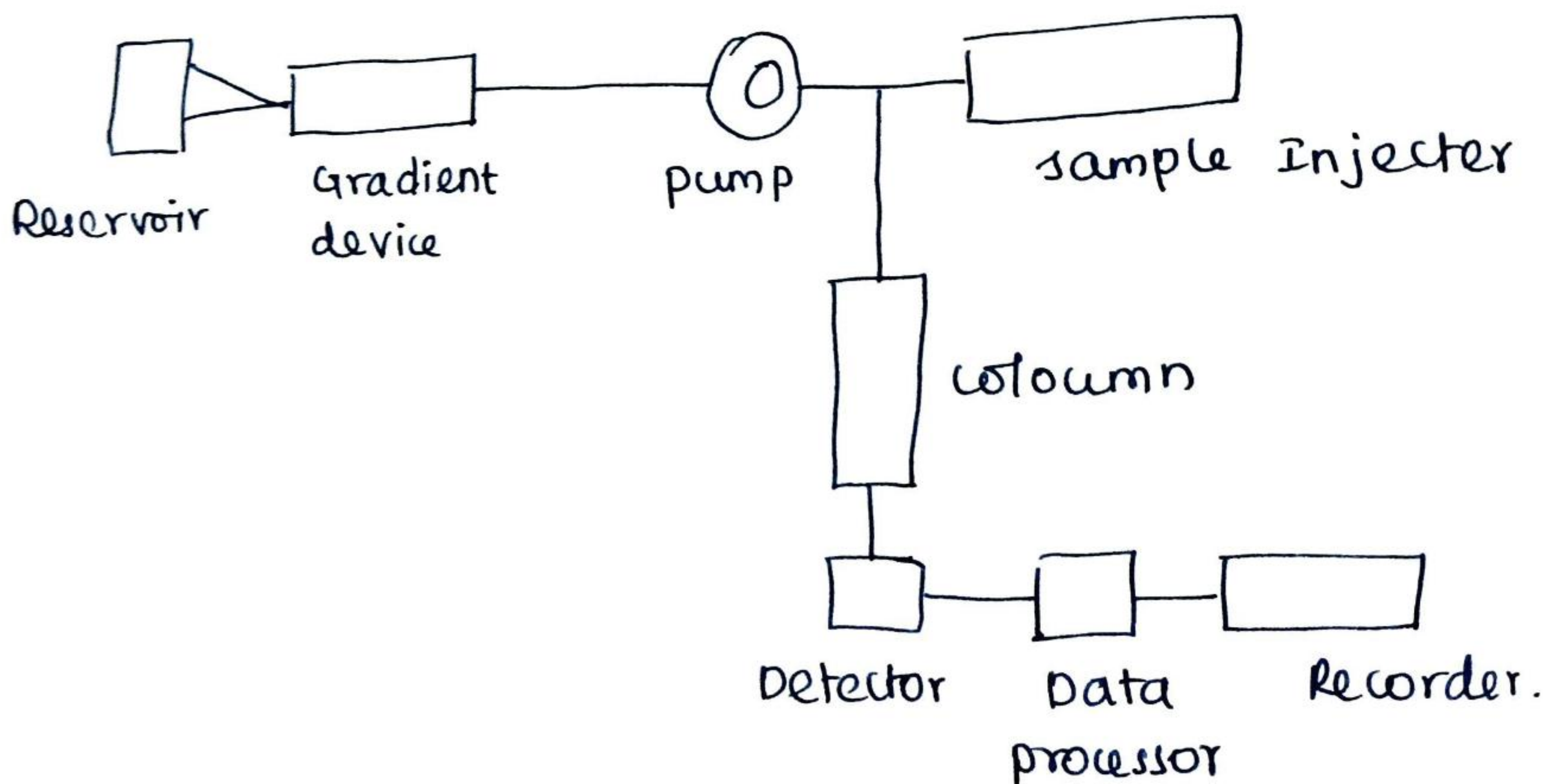


HPLC chromatography :

- (i) It is called as "High performance Liquid chromatography".
- (ii) It is used to tightly packed column containing small particle of the stationary phase.
- (iii) It will provide the Large surface area.
- (iv) It is similar to "gas chromatography".
- (v) Column packed with small particles.
- (vi) The flow rate of the column would be slow.
- (vii) To overcome this problem to put 6000 psi pressure is applied on the HPLC chromatography column.
- (viii) This tech is known as "High pressure liquid chromatography".



(ix) Several separation mech can be employed (i.e)
(i) adsorption (ii) partition and
(iii) ion exchange and size exclusion.

(x) The partition mech is most widely used in this method.

(xi) The adsorption of HPLC separation for isomeric nitro aniline ^{carried out by} on silica column.

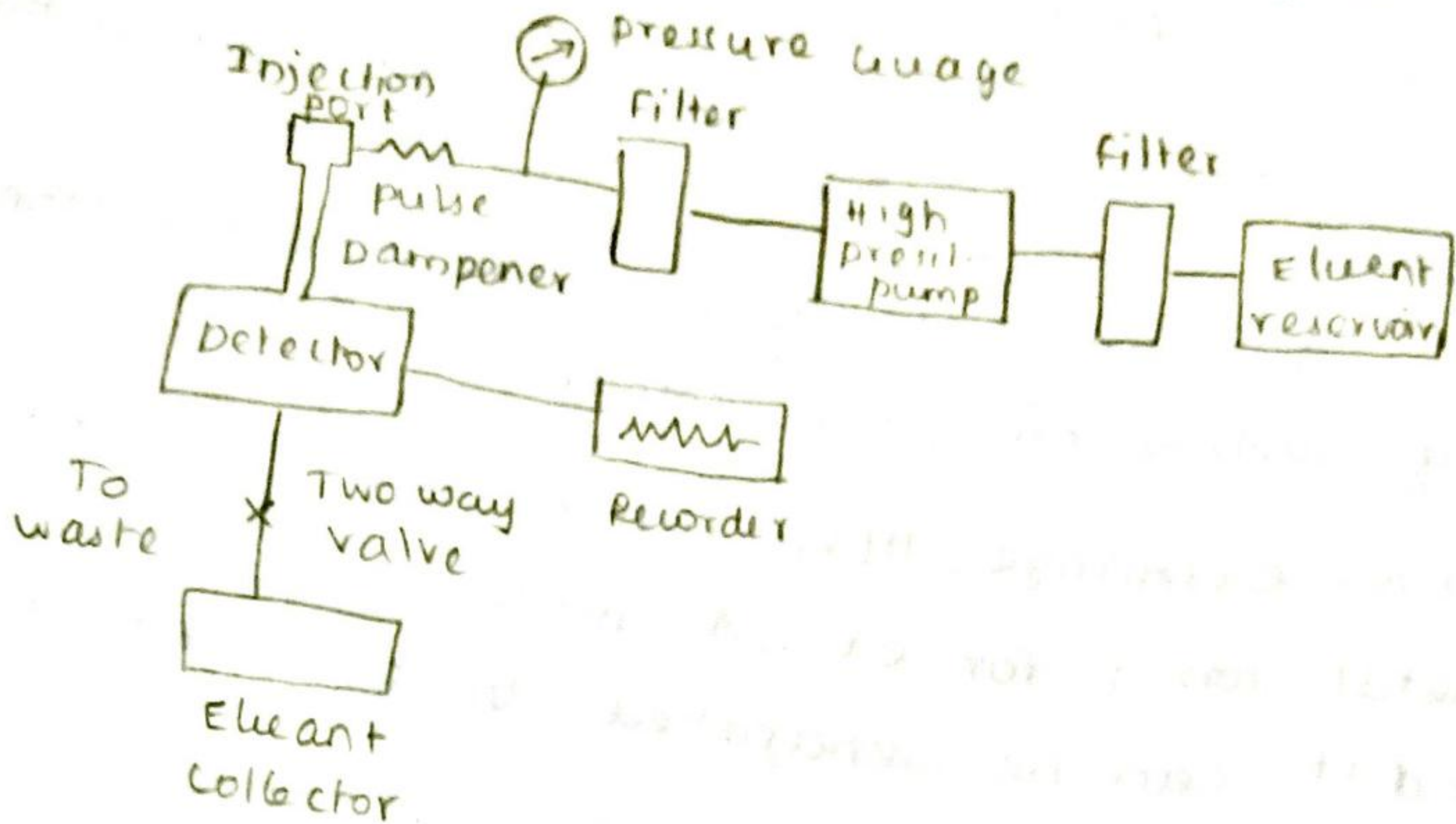
(xii) Ion-exchange HPLC can be used for separating metal ion ; for ex : A mixture of Ca^{2+} , Zn^{2+} Cd^{2+} can be separated to this method.



A - solvent
B - para
C - meta
D - ortho

HPLC chromatography :

Instrumentation : A schematic diagram of typical HPLC.



- (i) A solvent reservoir and mixing system
- (ii) high pressure pump
- (iii) a sample inlet pump
- (iv) a column
- (v) a detector and recording unit.

1. The column :

- (i) The column used for HPLC are generally made of stainless steel and are manufactured so that they can withstand pressure of upto 5.5×10^7 Pa (8000 psi)
- (ii) straight column of 20 to 50 cm in length and 1 to 4 mm diameter are generally used.

- (iii) The best columns are precision bored with an internal mirror which allows efficient packing of the column.
- (iv) porous plugs of stainless steel (or) teflon are used in the ends of the column to retain the packing material.
- (v) The plugs must be homogenous to ensure uniform flow of solvent through the column.
- (vi) It is important in some separation involving liquid partition and ion-exchange that the column temp. is thermostatically controlled during the analysis.

2. column packing :

Three forms of column packing material are available based on a rigid solid structure.

There are :

(i) microporous supports :

It is used for 5 to 10 μm in diameter.

(ii) pellicular :

It is used for porous particles are coated onto an inert solid core such as a glass bead to about 40 μm in diameter.

(iii) bonded phases :

(i) If stationary phase is chemically bonded onto an inert support.

- (ii) for adsorption chromatography, adsorbent such as "silica" (or) "alumina" are available as micro porous forms with a range of particle size.
- (iii) "pellicular" system generally have a high efficiency but low sample capacity. so "micro porous" supports are preferred.
- (iv) All form of HPLC column packing are characterised by their regular spherical shape.
- (v) These small sphere pack most efficiently and give good flow rate.
- (vi) In liquid-liquid partition system, the stationary phase may be coated onto the inert support. Both "microporous" and "pellicular" supports are used.
- (vii) one disadvantage of supports coated with liquid phase it is easily washed out, so over come this problem, we can take liquid phase that is covalently bonded to supporting material which may be "silica" (or) "silicone polymer".
- (viii) The advantage of ^{silicone polymer} bonded phase, it is not eluted the developing solvent. these are "chemically", "hydrolytically" and "thermally" stable.
- (ix) In-normal phase liquid-liquid chromatography the stationary phase is a "polar cpd" such as "nitrile (or) "alkylamine" derivatives, and the mobile phase is "non-polar cpd" such as "hexane".

(x) for "reverse phase chromatography", the stationary phase is a non-polar (pd) such as a (g) (or) (l) hydrocarbon and the mobile phase is a polar solvent such as H₂O / acetonitrile, (or) water / methanol mixture.

3. Column packing

(i) Several methods are using column packing, and the method used are depending on the nature of the packing material and dimensions of the particle.

(ii) Rigid solid and hard gel should be packed as densely, but without fracturing the particles during the packing process.

(iii) The most widely used technique for column packing is "high pressure slurring tech".

(iv) Hard gels are packed, it is necessary for them to be allowed to swell first in the solvent to be used in the chromatographic process before packing under pressure.

soft gels are ^{can't be} packed under pressure and have to be allowed to pack from a slurry in the column under gravitational sedimentation only.

Chromatography solvent :

(i) Isocratic separation may be used for "single solvent". (or) 2 (or) more solvents mixed in fixed position.

(ii) All solvents used in HPLC systems must be specially purified since traces of impurities

can affect the column and interfere with the detection system.

5. pumping system :

(i) constant pressure pump :

It produce a pulseless flow through the column.

(ii) constant displacement pumps :

It maintain a constant flow rate through the column irrespective of changing conditions within the column.

6. Detector system :

(i) Most commonly the detector is variable wavelength, ultra-violet, visible spectrophotometer, a fluorimeter, a refractive index monitor or an electrochemical detector.

(ii) A recent development has been the interfacing of HPLC to a "mass spectrometer".

(iii) for the detection of anions in ion-chromatography, a conductivity detected by eluant suppression is used.

7. Applications :

(i) Reverse-phase partition HPLC is particularly useful for the separation of polar compounds such as drugs and their metabolites, peptides, vitamins, polyphenols and steroids.

(ii) The tech is particularly widely used in clinical and pharmaceutical work as it is possible to apply biological fluids such as serum and urine directly to the column, preferably using a guard column.

(iii) The separation of some highly polar cpds such as amino acids, organic acids and the catecholamines