Affinity Chromatography

Principle -Affinity chromatography is a highly selective method used for the purification of specific molecules from complex mixtures based on their binding affinity to a specific ligand that is immobilized on the stationary phase. This technique is particularly useful for separating proteins, nucleic acids, and other biomolecules.

The principle relies on the specific interaction between a target molecule (e.g., a protein) and a ligand that is designed to specifically bind to it. The ligand is often a molecule that mimics the natural substrate, receptor, or antibody of the target molecule. The affinity of the interaction is high, so the target molecule will preferentially bind to the ligand, while other non-target molecules flow through the column.In this process:

1. The sample is loaded onto a column containing the immobilized ligand.
2. The target molecule binds to the ligand due to their high affinity.
3. Non-binding molecules are washed away, and the bound target is eluted by changing the conditions (e.g., altering pH, salt concentration, or using a competing ligand).

Components-

1. Stationary Phase (Column Matrix):
   - The stationary phase in affinity chromatography typically consists of a solid matrix such as agarose, silica, or Sepharose. The matrix is usually packed into a column where the separation occurs.
   - The stationary phase is functionalized with a specific ligand (e.g., an antibody, enzyme substrate, or metal ion) that will selectively bind to the target molecule. The choice of ligand depends on the nature of the molecule being purified.
2. Ligand (Immobilized on Stationary Phase):
   - The ligand is the key component in affinity chromatography. It is immobilized on the stationary phase, and its specific binding to the target molecule allows for selective capture.
   - Examples of ligands include antibodies for antigen-antibody binding, substrates or inhibitors for enzyme interactions, metal ions (such as Ni^{2+} or Co^{2+}) for affinity to histidine-tagged proteins, or aptamers for nucleic acids and proteins.
3. Mobile Phase (Elution Buffer):
   - The mobile phase is the buffer used to wash the sample through the column and to elute the bound target molecule.
   - The elution buffer may contain salt, pH modifiers, or competing ligands that disrupt the interaction between the ligand and the target molecule, allowing the target to be released and collected.

4. Sample:
   - The sample is the mixture that contains the target molecule to be purified. This sample is usually prepared from cell lysates, serum, culture media, or other biological fluids.