

# Affinity Chromatography

**Mr. Sagar Kishor Savale**

[Department of Pharmacy (Pharmaceutics)]

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[avengersagar16@gmail.com](mailto:avengersagar16@gmail.com)

## 1. Introduction

### 1.1 Chromatography

- Applies the principles of the “fractional” separation procedures
- Non-instrumental analysis which partitions components between two phases usually a mobile phase and a stationary phase, based on the difference in the components physical properties
- Can separate complex mixtures composed of many very similar components.
- Chromatography is often coupled with analytical instruments to complete analysis.
- A single chromatographic analysis can isolate, identify , and quantitate multiple components of mixtures

### 1.2 History of Chromatography

- 1903 – Tswett, a Russian botanist coined the term chromatography. He passed plant tissue extracts through a chalk column to separate pigments by differential adsorption chromatography
- 1915 R.M Willstatter, German Chemist win Nobel Prize for similar experiment

- 1922 L.S Palmer, American scientist used Tswett's techniques on various natural products
- 1931 Richard Kuhn used chromatography to separate isomers of polyene pigments; this is the first known acceptance of chromatographic methods
- 1938 Thin Layer chromatography by Russian scientist N.A Izmailov and M.S Shraiber
- 1941 Liquid-Liquid partition chromatography developed by Archer John, Porter Martin and Richard Laurence Millington Syngé
- 1944 Paper Chromatography one of the most important methods in the development of biotechnology
- 1945 Gas Chromatography 1<sup>st</sup> analytical gas-solid (adsorption) chromatography developed by Fritz Prior
- 1950 Gas Liquid Chromatography by Martin and Anthony James; Martin won the Nobel Prize in 1952
- 1966 HPLC named by Csaba Horvath, but didn't become a popular method until 1970s
- 1950s Ion-Exchange chromatography declassified this technique
- 1970s Ion Chromatography was developed by Hamish Small and co-workers at the Dow Chemical company
- 1930s Affinity Chromatography was developed for the study of enzymes and other proteins

### 1.3 Principles of Chromatography

**Chromatography is used when there is a difference in the retention times of different components**

### 1.4 Two types of phases

- 1) Stationary phase
- 2) Mobile phases

### 1.5 Properties of Chromatographic Properties

- 1) Immiscible stationary and mobile phases
- 2) An arrangement where a mixture is deposited at one end of the  
Stationary phase
- 3) Flow of the mobile phase towards the other end of the stationary  
Phase
- 4) Different rates of partitioning for each component
- 5) Means for visualizing the separation of each component

### 1.6 Techniques

- 1) **Ion Exchange Chromatography (IEC)** - separates biomolecules based on their net surface charge
- 2) **Ion Chromatography (IC)** - more general form of IEC allows separation of ions and polar molecules based on the charge properties of the molecules
- 3) **Affinity Chromatography (AC)** - is the purification of a biomolecule with respect to the specific binding of that biomolecule due to the chemical structure

- 4) **Gas Chromatography (GC)** - is a technique used to separate organic molecules that are volatile
- 5) **Gas-Liquid Chromatography (GLC)**

### 1.7 Terminology

- **Elution** - washing of the mixture
- **Eluent** - additional solvents used for elution
- **Effluent** - exiting fluid stream
- **Residence** - time spent on column
- **Stationary Phase** - Column packaging material
- **Mobile Phase** - fluid carrying the mixture of analytes

### 2. History of Affinity Chromatography

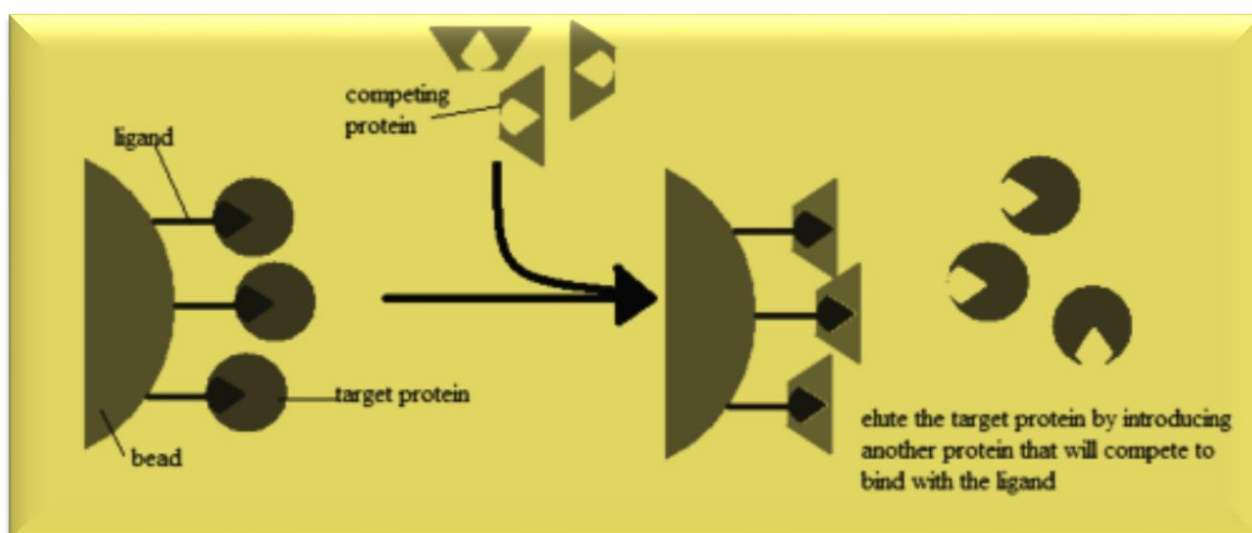
- ◆ 1930s, first developed by A.Wilhelm Tiselius-a swedish biochemist, won the Nobel Prize in 1948
- ◆ Used to study enzymes and other proteins
- ◆ Relies on the affinity of various biochemical compounds with specific properties

### 2.1 Affinity Chromatography

- Affinity Chromatography is essentially a sample purification technique, used primarily for biological molecules such as proteins.
- It is a method of separating a mixture of proteins or nucleic acids (molecules) by specific interactions of those molecules with a component known as a ligand, which is immobilized on a support. If a solution of, say, a mixture of proteins is passed over

(through) the column, one of the proteins binds to the ligand on the basis of specificity and high affinity (they fit together like a lock and key).

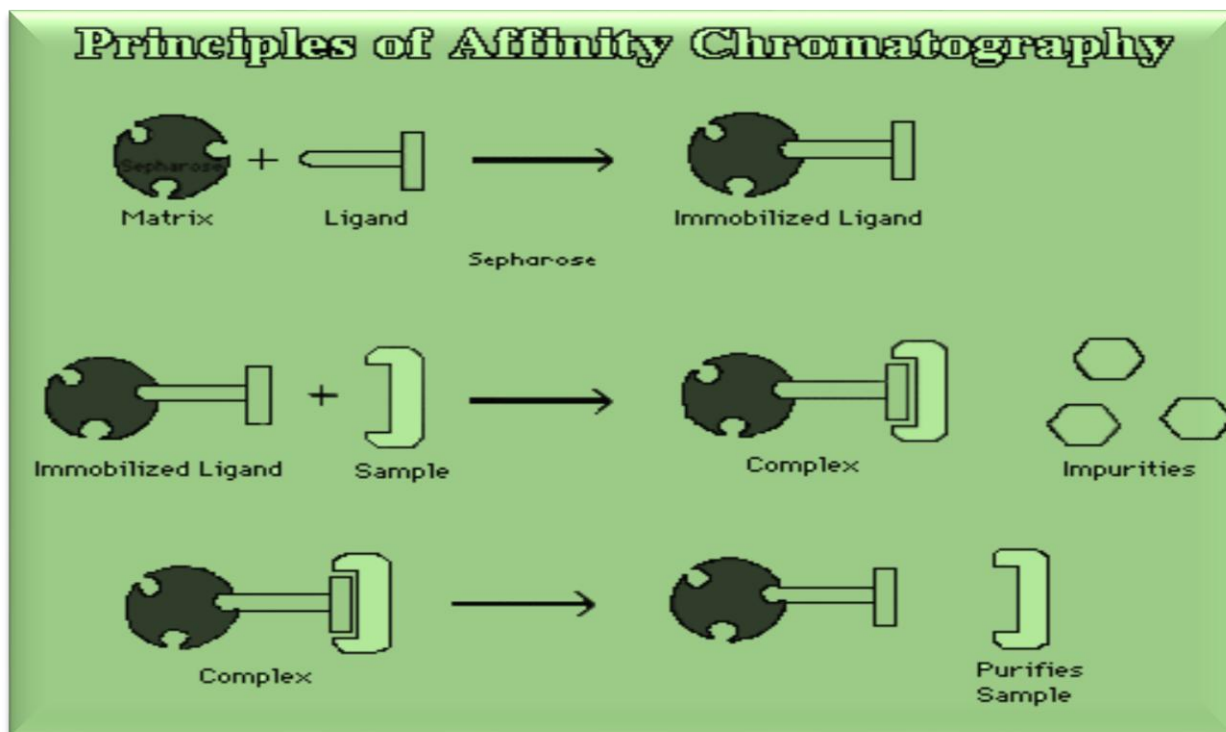
- The other proteins in the solution wash through the column because they were not able to bind to the ligand.



## 2.2 Principle

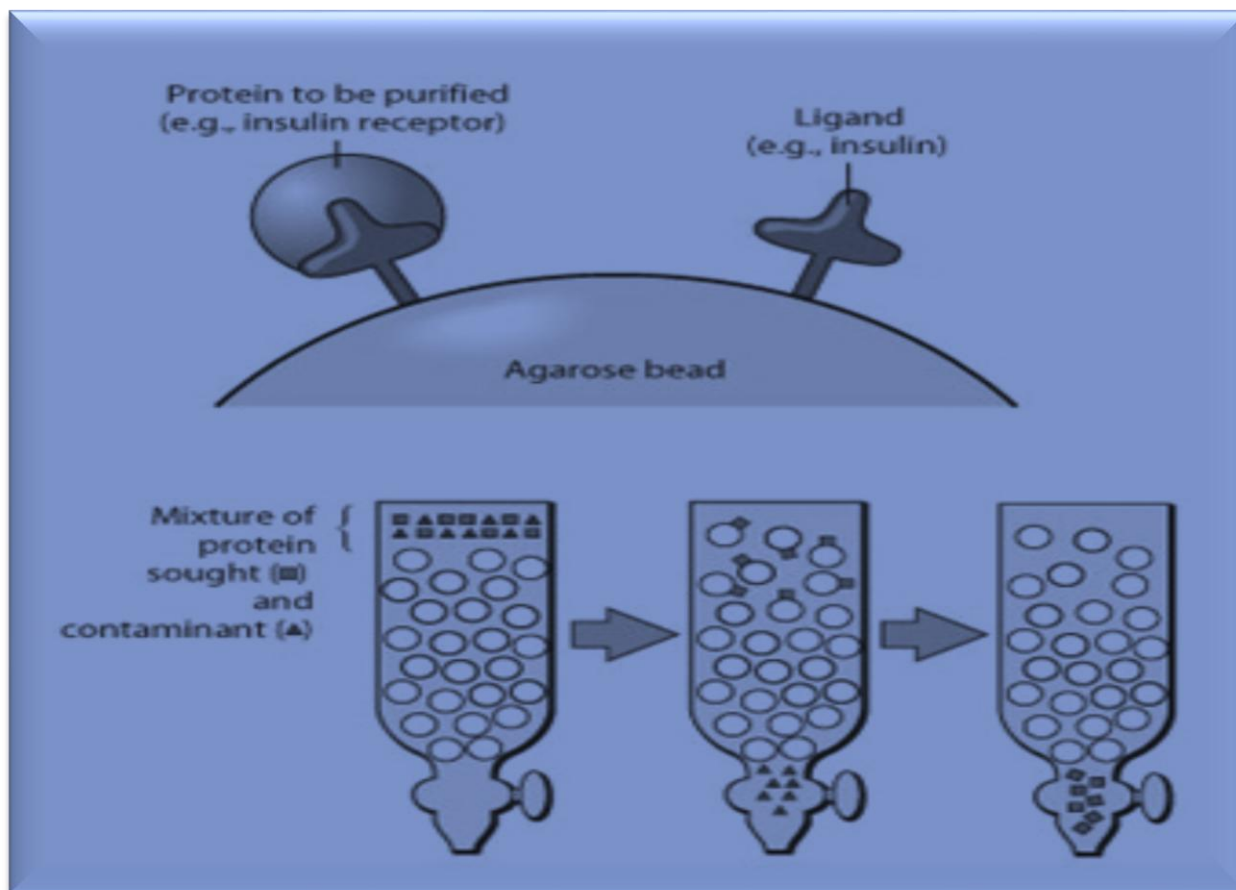
- Affinity chromatography is one of the most diverse and powerful chromatographic methods for purification of a specific molecule or a group of molecules from complex mixtures
- It is based on highly specific biological interactions between two molecules such as interactions between enzyme and substrate, receptor and ligand, or antibody and antigen.
- These interactions which are typically reversible are used for purification by placing one of the interacting molecules referred to as affinity ligand onto a solid matrix to create a stationary phase while a target molecule is in the mobile phase.

- Many of the commonly used ligands coupled to affinity matrices are now commercially available and are ready to use.



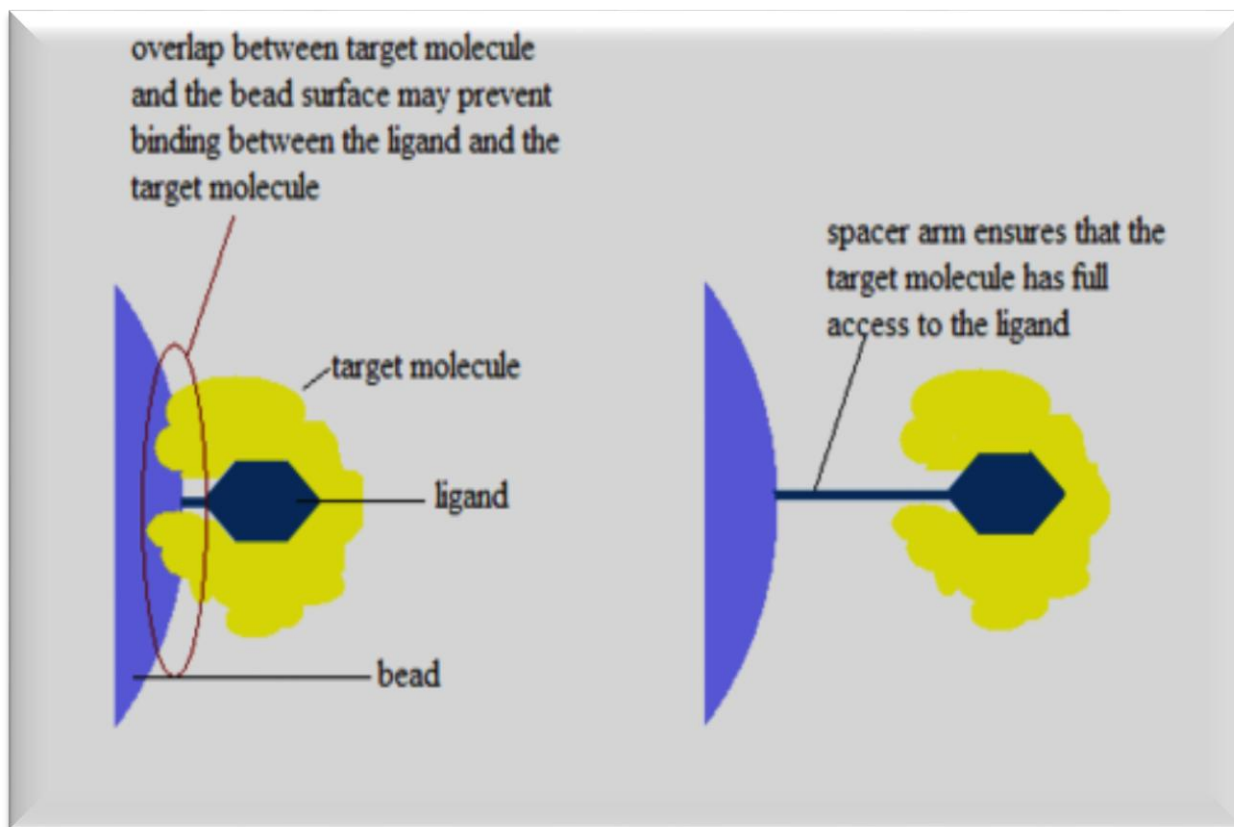
### 2.3 Chromatographic Media

- A matrix in its use here is a substance, usually in bead form to which a specific ligand is covalently bound.
- In order to for the matrix to be effective it must have certain characters:
- It must be insoluble in solvents and buffers employed in the process
- It must be chemically and mechanically stable...
- It must be easily coupled to a ligand or spacer arm onto which the ligand can be attached.
- It must exhibit good flow properties and have a relatively large surface area for attachment



## 2.4 Immobilized Ligand

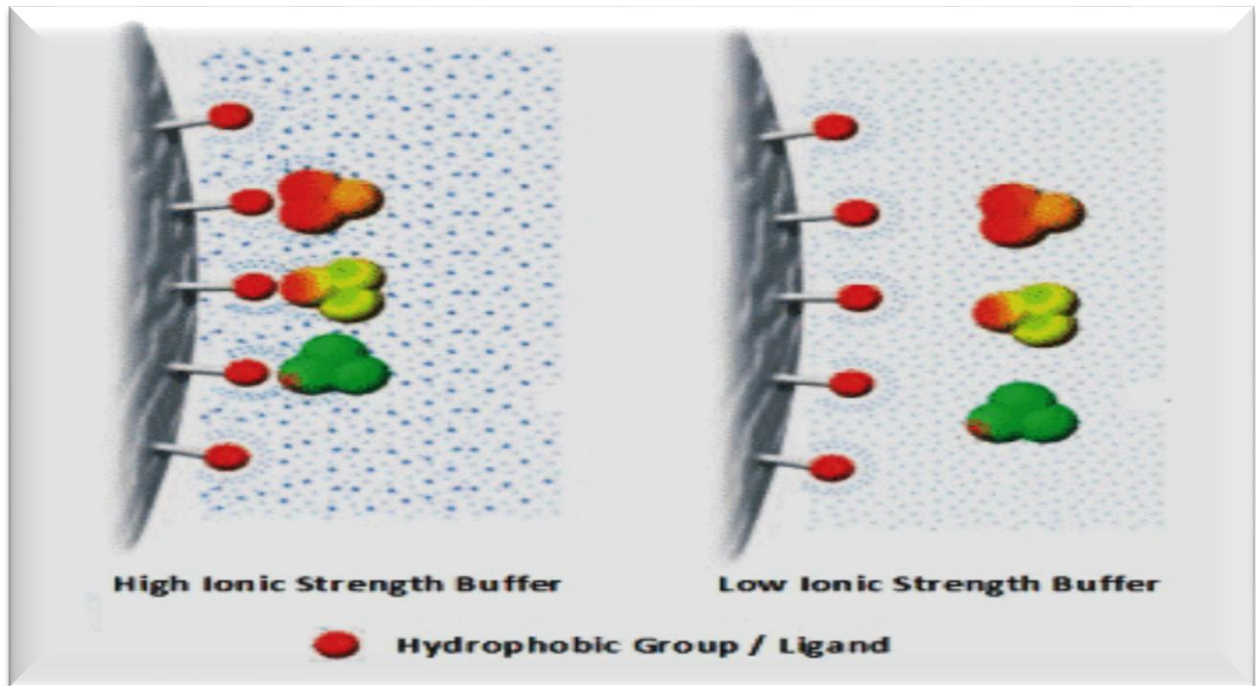
- The ligand can be selected only after the nature of the macromolecule to be isolated is known.
- When a hormone receptor protein is to be purified by affinity chromatography, the hormone itself is an ideal candidate for the ligand.
- For antibody isolation, an antigen or hapten may be used as ligand.
- If an enzyme is to be purified, a substrate analog, inhibitor, cofactor, or effector may be used as an immobilized ligand.



### 2.5 Attachment of Ligand to Matrix

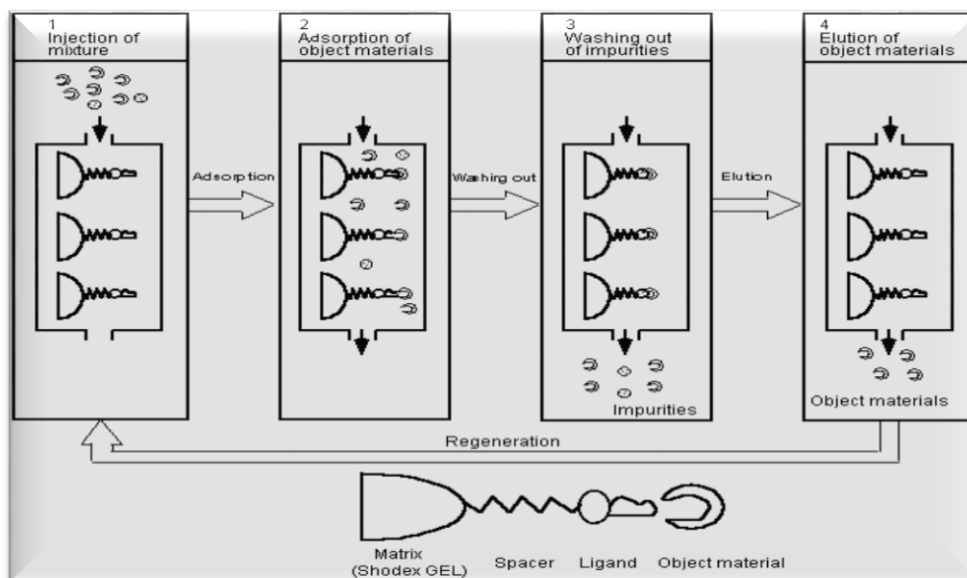
- Several procedures have been developed for the covalent attachment of the ligand to the stationary phase. All procedures for gel modification proceed in two separate chemical steps:
- Activation of the functional groups on the matrix and
- Joining of the ligand to the functional group on the matrix.
- **A wide variety of activated gels is now commercially available. The most widely used are described in the following:**





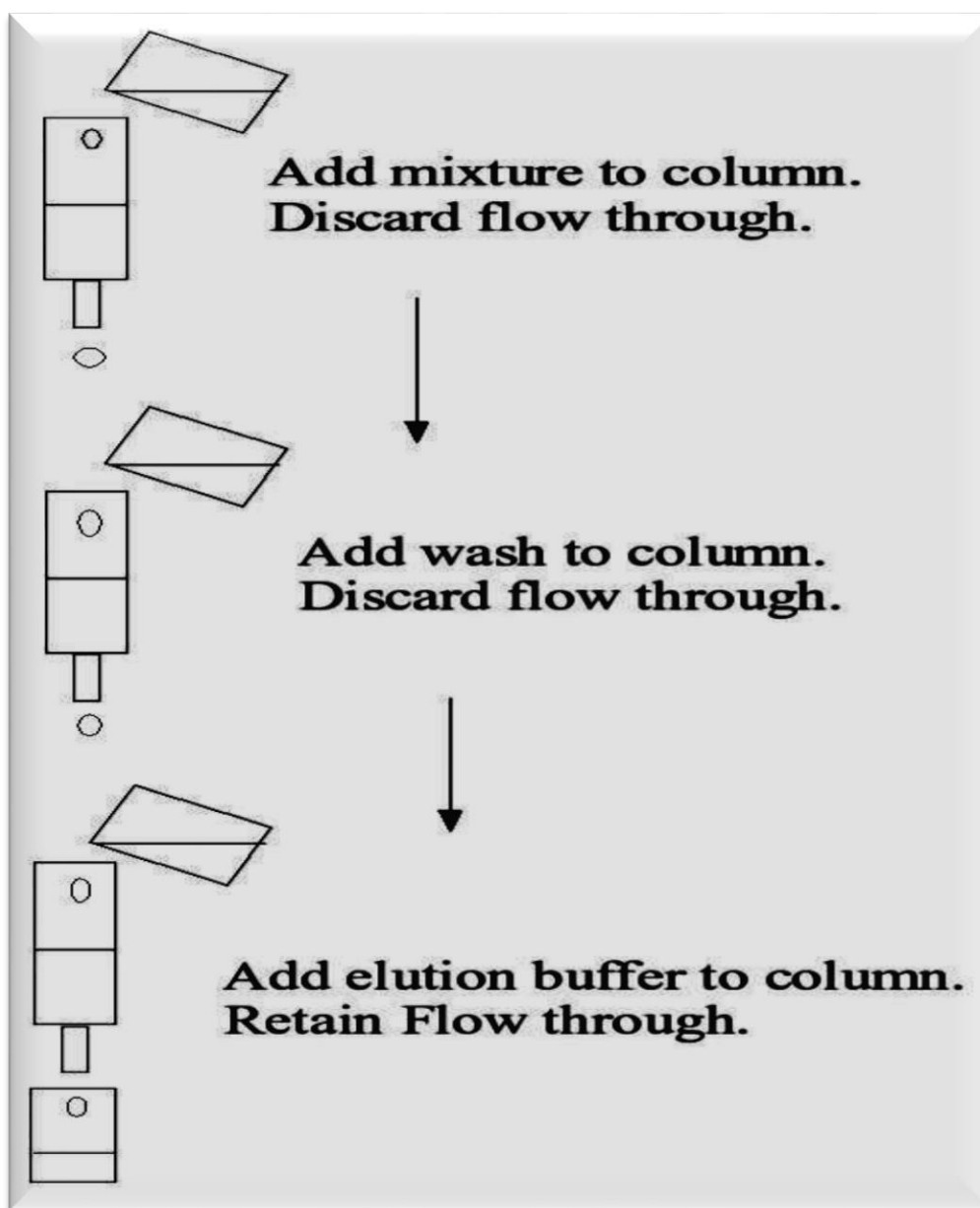
### 3. Specificity is based on three aspect of affinity

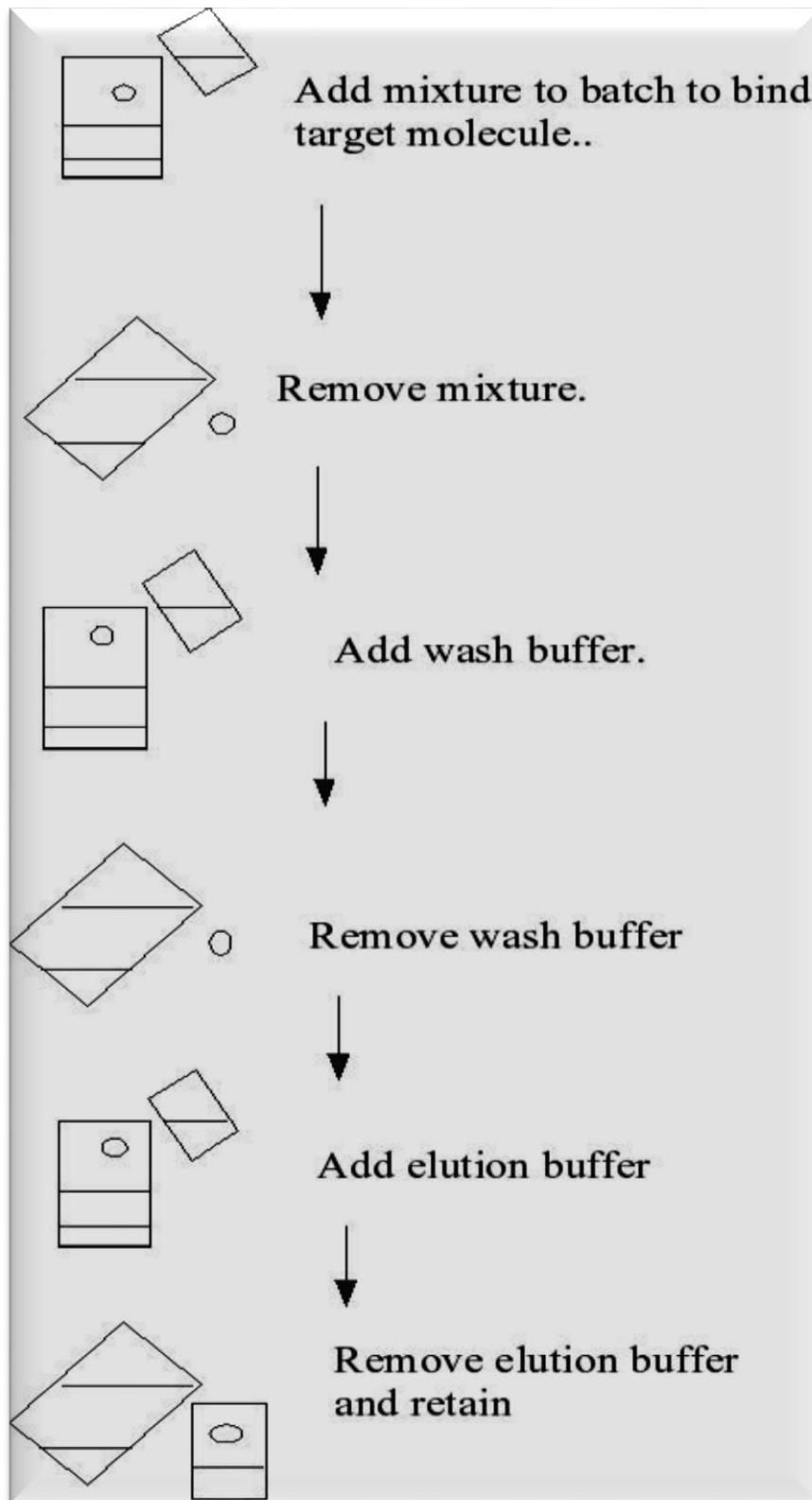
- 1) **Matrix:** for ligand attachment.
- 2) **Spacer arm:** used to bind ligand to matrix.
- 3) **Ligand:** molecule that binds reversibly to a specific target molecule(site of interaction)



#### 4. Examples

- Antigen  $\longrightarrow$  Antibody
- Antibody  $\longrightarrow$  Antigen
- Substrate  $\longrightarrow$  Enzyme
- DNA  $\longrightarrow$  Histon
- Hormone  $\longrightarrow$  Binding Protein/Receptor





## 5. Used of Affinity Chromatography

- ◆ Purify and concentrate a substance from a mixture into a buffering solution
- ◆ Reduce the amount of a substance in a mixture
- ◆ Discern what biological compounds bind to a particular substance, such as drugs
- ◆ Purify and concentrate an enzyme solution

## 6. Advantages Of Affinity Chromatography

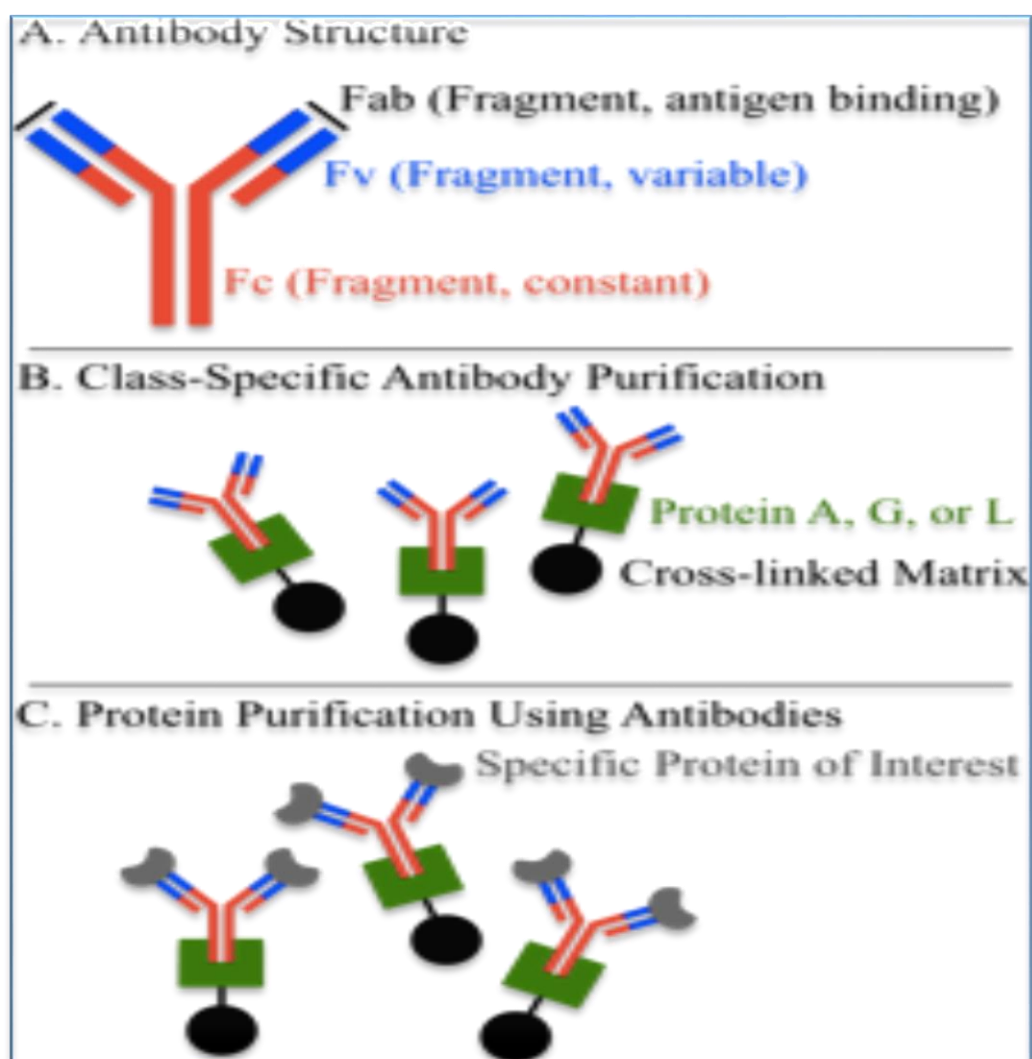
- 1) Extremely high specificity
- 2) High degrees of purity can be obtained
- 3) The process is very reproducible
- 4) The binding sites of biological molecules can be simply investigated

## 7. Disadvantages Of Affinity Chromatography

- 1) **Expensive ligands**
- 2) **Leakage of ligand**
- 3) **Degradation of the solid support**
- 4) **Limited lifetime**
- 5) **Non-specific adsorption**
- 6) **Relatively low productivity**

## 8. Applications

- 1) It is used for isolation and purification of all biological macromolecule.
- 2) It is used to purify nucleic acid, antibodies, and enzymes. Etc
- 3) To notice which biological compounds bind to a particular substance.
- 4) To reduce an amount of substance in a mixture
- 5) Used in Genetic Engineering - nucleic acid purification
- 6) Production of Vaccines - antibody purification from blood serum
- 7) And Basic Metabolic Research - protein or enzyme purification from cell free extracts



## 9. References

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- **Introductory Practical Biochemistry-S.K Swahney, Randhir Sing**
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- **bioWORLD, Concanavalin A - Specifically binds to mannosyl and glucosyl residues of polysaccharides and glycoproteins.**
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