Clinical Chemistry

Separation mechanisms

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A species of techniques have been used in clinical chemistry laboratory for sample testing

Most Fundamental Methods Include:

Electrophoresis
Chromatography
Spectrophotometry
Mass spectrometry
Fluorometry
Nephelometry
Turbidimetry
Biochip(Protein and DNA Chip/Array)
Biosensor

Separation mechanisms

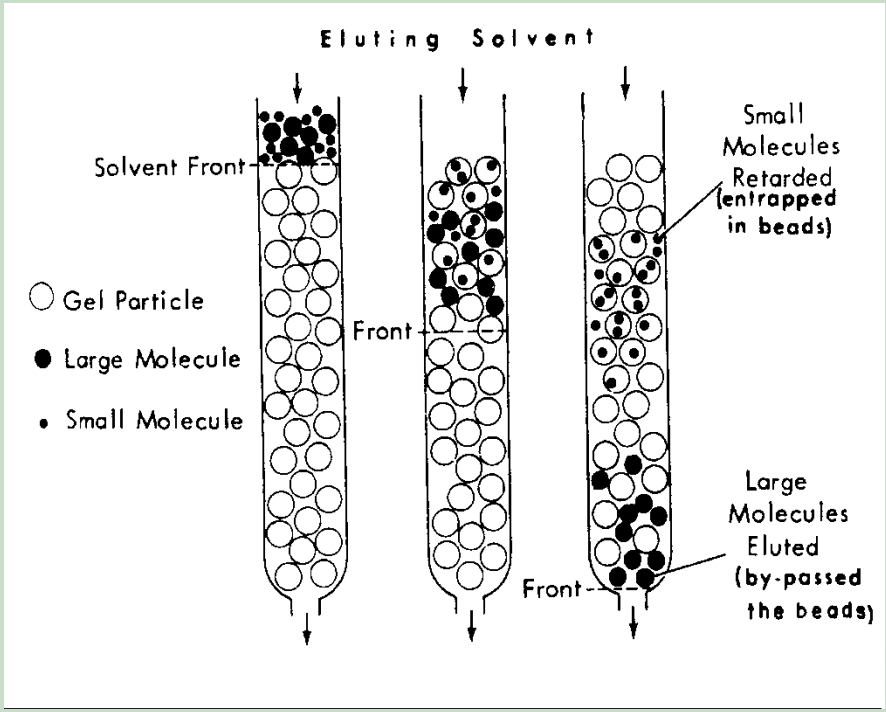
Adsorption, affinity, ion exchange, partition, and steric exclusion chromatography describe the predominant chemical or physical mechanisms used to separate solutes.

Gel-Filtration Chromatography

It is also known as steric exclusion chromatography ,gel-permeation, size exclusion, molecular exclusion, molecular sieve chromatography and separate solutes on the basis of their molecular size.

A variety of materials have been used as stationary phases including cross link dextran (Sephadex), polyacrylamide(Bio-Gel), agarose (Sepharose), etc.

Molecular size chromatography



Molecules too large to enter the pores remain exclusive in the mobile phase and rapidly elude from the column. Molecules that are intermediates in size (and small molecules) have access to various fractions of the pore volume and elude slowly.

In addition to preparative applications , gel-filtration chromatography has been used in the clinical laboratory to：

1. to determine molecular weights of macromolecules，
2. to remove low-molecular-weight salts or buffer ions from protein solutions.

Adsorption chromatography

Adsorption chromatography exploits the polarity , or the related tendency for hydrogen binding __of molecules in order to partition between a polar sorbent and a less polar solvent, or vice-versa, as the mobile phase moves through the stationary sorbent.

Affinity chromatography

The term affinity chromatography describes a number of separation mechanisms with interactions that occur between biochemical species (enzyme-substrate, hormone-receptor, or antigen-antibody complexes).

The stationary phase in affinity chromatography is prepared by immobilizing a ligandon particles of a support either directly or via a *spacer.*

Ion-exchange chromatography

In ion-exchange chromatography , solutes in a sample are separated by their difference in sign and magnitude of ionic charge.

In practice, ionic analytes are selectively eluted from ion-exchange resins by varying the pH and/or ionic strength of the mobile phase.

Cation-exchange resins contain covalent bound, negative charged functional groups, such as sulfonate ions, carboxylate ions or carboxy-methyl (CM) groups.

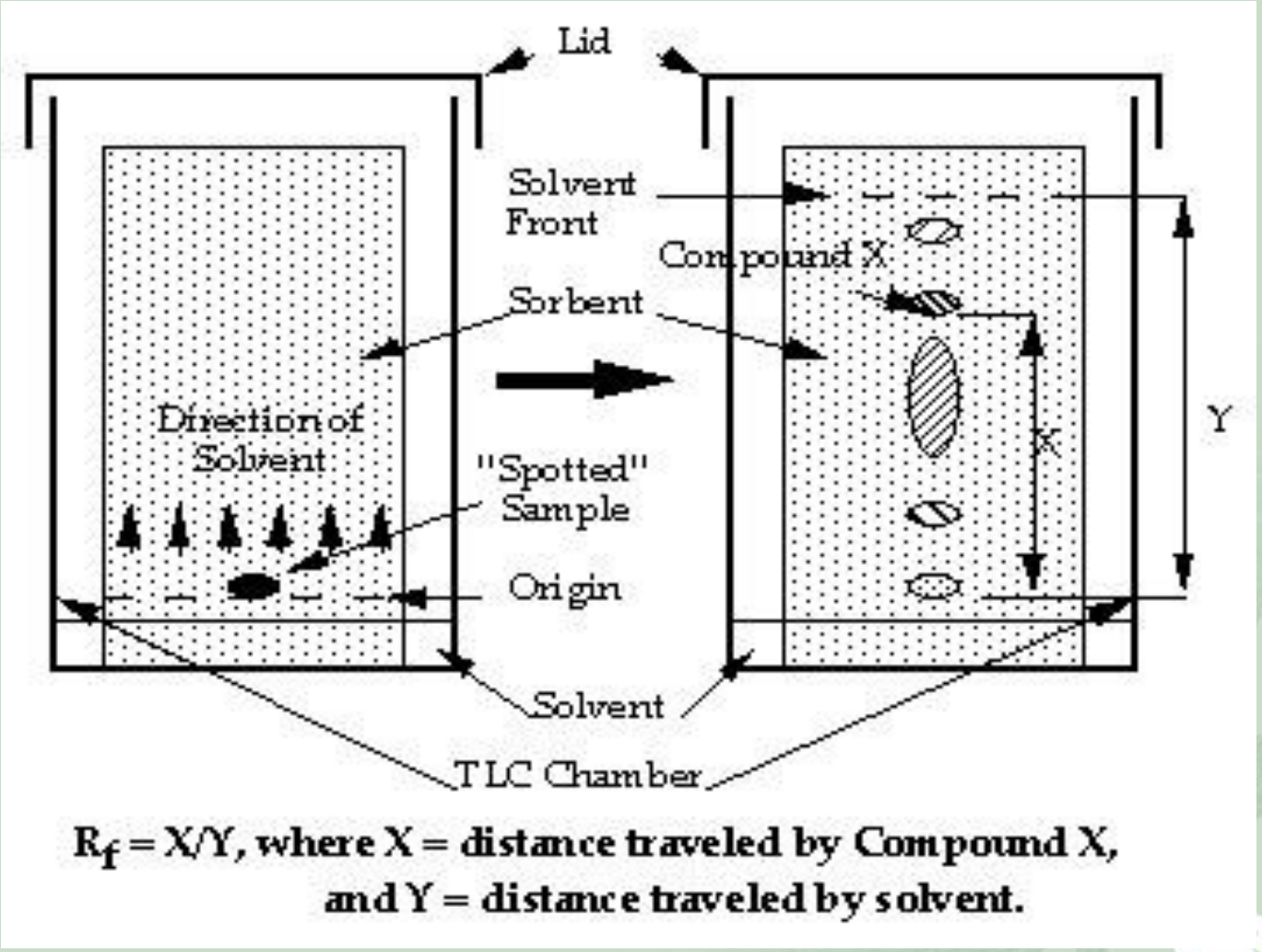
This technique is most useful for separation of organic and inorganic ions, amino acids, nucleotides, and proteins.

Anion-exchange resins are characterized by the presence of strong basic quaternary amines (triethylamino-ethyl groups) or weak basic groups (aminoethyl, diethylaminoethyl) which can bear a positive charge.

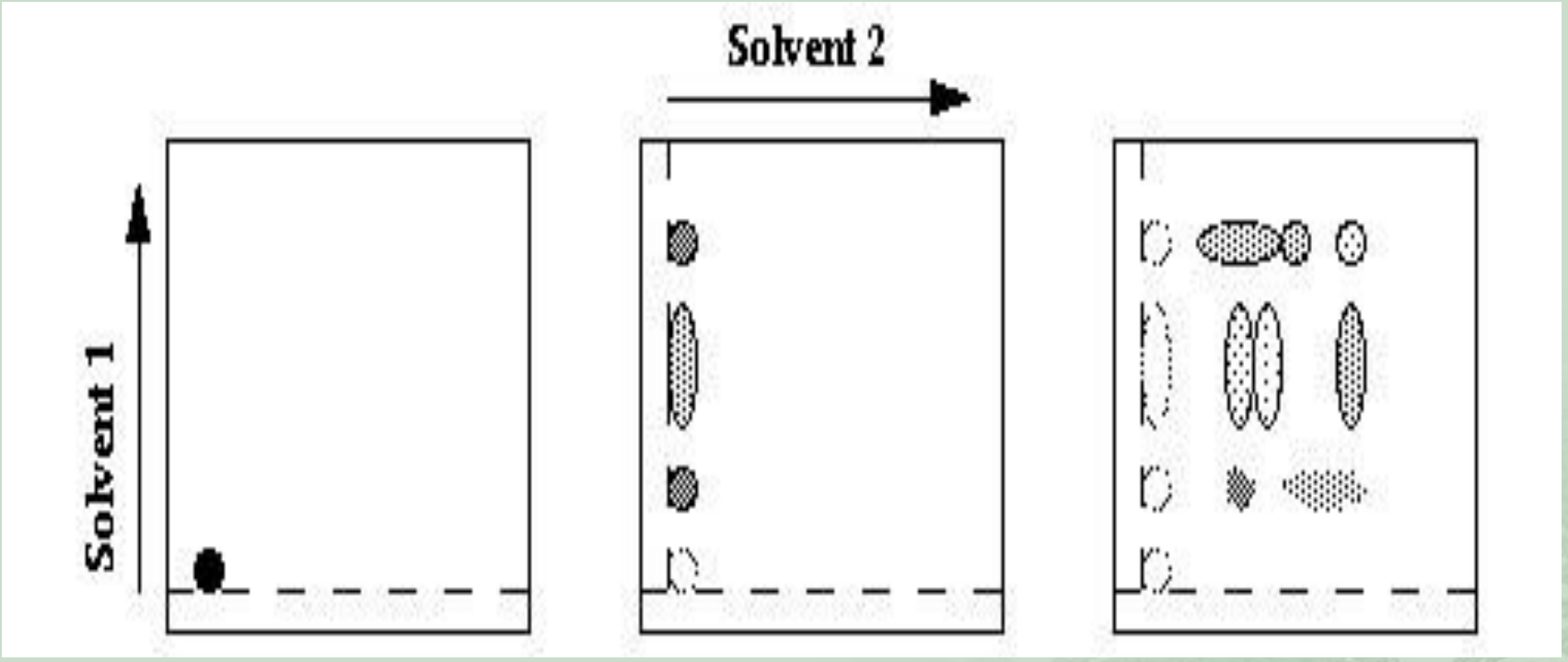
Ion-exchange chromatography is widely used to separate and remove inorganic ion from aqueous mixtures.

Partition chromatography

In partition chromatography (also called thin-layer chromatography) , a thin film of liquid is adsorbed onto the surfaces of support particles. Separation is based on differences in the relative Solubility of solute molecules in this film and the mobile phase.



Thin-layer chromatography, showing set-up and separation of constituents and the calculations for Rf value



FIG： Two-dimensional thin layer or paper chromatography

Gas chromatography (GC)

GC is a process by which a mixture is separated into its constituent components by forcing a gaseous mixture of it and mobile phase (carrier gas) through a column contain the stationary phase.

Separation of the solutes in the mixture is based on the relative differences in their vapor pressures and their interaction with the stationary phase.

A compound with a high vapor pressure will be eluted more rapidly than compounds with lower vapor pressures.

High-Performance Liquid Chromatography (HPLC)

In LC , separation is based on the distribution of the solutes between a liquid mobile phase and a stationary phase. When an efficient column is used in a liquid chromatograph, the technique is HPLC . Because column efficiency is inverse related to the particle size of the column packing, relative high pressure is required to pump liquid through an efficient column.

Different components of HPLC

A basic liquid chromatograph consists of a solvent reservoir , a pump to force the liquid mobile phase through the system; an injector for introducing an aliquot of sample into the column; a chromatographic column to separate the analytes being measured; an on-line detector to detect the separated analytes as they elute from the column; and a computer to control the system and process data.

Different components of HPLC

glycosylated hemoglobin