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# Chromatography

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# INTRODUCTION

## ANALYTICAL TECHNIQUES

Analytical chemistry involves separating, identifying and determining the relative amount of the components in a sample of matter.

### TYPES

**QUALITATIVE** : “What is there”

**QUANTITATIVE** : “ How much is there”

### **Methods of Quantitative Analysis**

- Chemical Methods
- Instrumental Methods
- Microbiological Methods
- Biological Methods

### INSTRUMENTAL METHODS

#### **PHYSICAL PROPERTY MEASURED METHOD**

Absorption of radiation  
Emission of radiation  
Rotation of Radiation  
Electrical Potential  
Electrical Conductance  
Thermal Properties

#### **INSTRUMENTAL**

Spectrophotometry (UV, Visible)  
Flourimetry, Flamephotometry  
Polarimetry  
Potentiometry  
conductometry  
Differential Thermal Analysis

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- Chromatography is the separation of a mixture into individual components using a stationary phase and a mobile phase.
  
  - The chromatographic method of separation, in general involves the following steps:-
    1. Adsorption or retention of a substance or substances on the stationary phase.
    2. Separation of adsorbed substances by the mobile phase.
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1. Recovery of the separated substances by a continuous flow of the mobile phase, the method is called elution.
  2. Qualitative and quantitative analysis of the eluted substances.
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- Chromatography is a separation technique based on the differences in the rate at which the components of a mixture move through a porous medium (called stationary phase) under the influence of solvent or gas (called moving phase).
  - The mobile phase passes over the stationary phase & transports components of the mixture at different speeds in the direction of flow of the mobile phase.
  - The separation of the components is the results of the differential affinity of the components for the mobile phase & a stationary phase.
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- The stationary phase may be a solid or a liquid supported on a solid or a gel, & may be packed in a column, spread as a thin layer or distributed as a film.
  - The mobile phase may be gaseous or liquid.
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- Chromatographic methods present the most useful and powerful technique.
  - This technique was first invented by M. Twett, a botanist in 1906 in Warsaw.
  - Because of the rapidity & effectiveness, chromatography is used in all the fields particularly in chemistry, biology, medicine, dyes, forensic departments & clinical studies with many advantages over other methods.
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# ADVANTAGES:

- The advantages of chromatographic methods are that they are relatively “gentle” and the decomposition of the substances doesn’t occur. This is important especially for labile type of substances, or mainly for substances from the biological origin.
  - The separation can be carried out on macro or semi micro scale, i.e. a very small quantity of mixture is required for analysis.
  - Chromatographic techniques are simple, rapid & required simple apparatus.
  - Further, complex mixtures can be handled with comparative ease.
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# Classification:

- On the basis of nature of stationary & mobile phase –
    1. Gas – liquid Chromatography
    2. Gas – Solid Chromatography
    3. Solid –Liquid Chromatography, e.g. – Column Chromatography, TLC, HPLC.
    4. Liquid – Liquid Chromatography, e.g. – Paper partition Chromatography, Column Chromatography.
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■ On the basis of Principles of Separation –  
Adsorption Chromatography –

- When a mixture of compounds (adsorbate) dissolved in the mobile phase (eluent) moves through a column of stationary phase (adsorbent), they travel according to their relative affinities towards the stationary phase.
- The compound which has more affinity towards stationary phase travels slower & the compound which has lesser affinity towards stationary phase travel faster.
- Hence the compounds are separated.
- No two compounds have the same affinity for a combination of stationary phase, mobile phase & other conditions

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E.g. – Where adsorption is the principle of separation –

- a.) Gas – Solid Chromatography
  - b.) TLC
  - c.) Column Chromatography
  - d.) HPLC.
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## Partition Chromatography –

- When two immiscible liquids are present, a mixture of solutes will be distributed according to their partition coefficients.
- When a mixture of compounds are dissolved in the mobile phase and passed through a column of liquid stationary phase, the component which is more soluble in the stationary phase travels slower.
- The component which is more soluble in the mobile phase travels faster.
- Thus the components are separated because of the differences in their partition coefficients.
- No two components have the same partition coefficient for a particular combination of stationary phase, mobile phase & other conditions.

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- The stationary phase as such cannot be a liquid.
  - Hence a solid support is used over which a thin film or coating of liquid is made which acts as a stationary phase.
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E.g. – Where partition is the principle of separation –

- a.) Gas – Liquid Chromatography
  - b.) Paper Partition Chromatography
  - c.) Column Partition Chromatography
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## **On the Basis of modes of Chromatography**

- They are based on the polarity of the stationary phase & mobile phase used.
  - Types –
    1. Normal phase
    2. Reversed phase
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