- Analytical Separation Techniques:
- The separation of various components
- in the sample is very necessary
- before doing qualitative or quantitative
- analysis.

     A no. of methods are available
- 4 separation, purification and sometimes,
- identification of different components.

     A list of these methods is
given below.

⇒ Evaporation.

⇒ Decantation.

⇒ Filtration.

⇒ Crystilization.

⇒ Sublimation.

⇒ Sublimation under rediced pressure.

⇒ Electrode deposistion.

⇒ Magnetic separation

⇒ Precipitation

⇒ Osmosis.

⇒ Electropsmosis

⇒ Dialysis .   ⇒ Electrodialysis

⇒ Electrophoresis ,movement.

Gel Electrophoresis, Capillary Elec....
Capillary zone " , Slab "
Free solution " , Capillary electrochromatography
Capillary isotachophoresis electrophoresis .
⇒ Solvent extraction method.
liquid - liquid extraction (LLE)
Solid - Phase (solid)" (SPE)
Liquid - Liquid microextration
⇒ Ultrasound extraction
⇒ Microwave assisted extraction.
⇒ Super critical fluid extraction.
⇒ Chromatography
Paper " , Thin Layer " (TLC), Column "
Gas " (GC) , HPLC (High performance liquid)
(UPL) ultra
Hydrodynamic chromatography.
Counter current " .
Fast Protein Liquid Chr... (FPLC)
Chiral " , Affinity "
Soap chromatography etc and many more

Chromatography:

Phase: State of matter (Solid, Liquid, Gas)

Stationary phase: Not moveable (Solid or liquid)

Mobile Phase: Moveable (Gas, Liquid)

Adsorption: Physical attachment of Gas or Liquid molecules on the surface of Solid by weak forces such as Hydrogen bonding, London dispersal forces, Dipole dipole forces etc.

Solubility: Dissolve of substances in Liquid.

Mixer of compounds get separated between stationary and mobile phase.

According to IUPAC

International Union of Pure and Applid Chem.

Chromatography is defined as.

" It is a physical method of separation in which mixture of substances is separated between two phases, one is stationary phase and the other is mobile phase which passes through or along the stationary phase and bring about separation "

The term chromatography was derived by Russian Botanist Mikhail Tswett in 1906 who separated plant pigment using calcium carbonate ($CaCO_3$) powder as stationary phase and petroleum ether as mobile phase. from 2 greek words chroma = colour and graphein = writing or pattern.

CaCO_3 ⊢————→

plant extract,
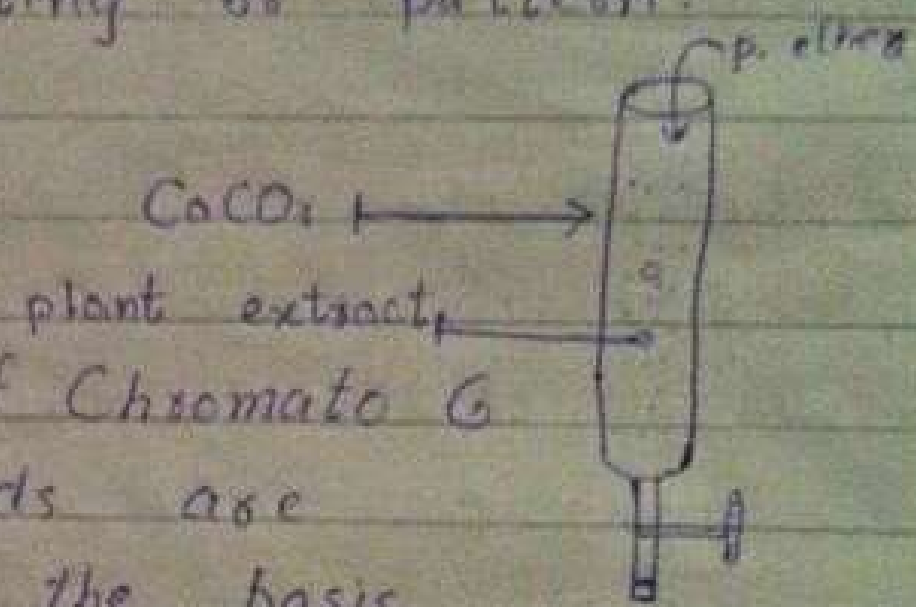
Classification of Chromato G Graphic Methods are classified on the basis of different features.

1. On the basis of Physical states of 2 phases.

i. Liquid - Liquid C.

It is a type of chromatography in which both phases are liquid. The liquid stationary phase is adsorbed as thin film on an inert support.

The separation is based upon difference in solubility of compound between the two liquid phases. examples include:

Paper chromatography, Partition TLC

ii- Liquid - Solid C.

It is a type of C.G in which stationary phase is solid and mobile phase is liquid.

The separation is based upon the differences in adsorption en stationary phase and desorption by the mobile phase.

Example include.

Column C.G, Adsorption Thin Layer C.G (TLC)

HPLC (High Performance Liquid

Affinity C.G, Ion Exchange C.G (IEC)

Gel G.G (SEC) Size exclusion G.G

Ion Pair C.G

iii- Gas - Liquid C. (GLC)

It is a type of c g in which st. phase is liquid and mobile phase is gas

iv. Gas-Solid C. (GSC)

2. On the basis of phenomenon of separation

i. Partition Chromatography

Partition is a phenomenon in which a substance is distributed b/w the two fluids (Liquid + Gas).

Analytical Techniques

Classification of Chromatographic methods.

B. On the basis of phenomenon of separation

1. Partition Chromatography

In this CG st. phase is liquid which is adsorbed as thin film on an inert support, and the mobile phase is either a liquid or a gas.

Examples include

- Paper E G
- Partition TLC
- Gas G.G (GC)
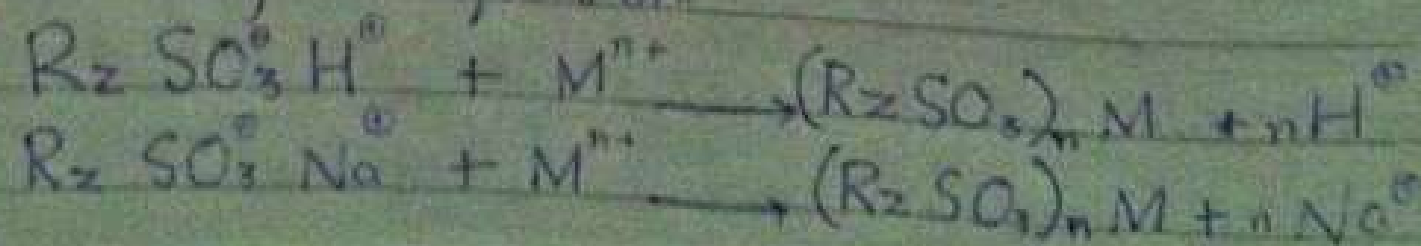
2. Adsorption Chromatography

Adsorption is a phenomenon

in which molecules of a substance are physical attached on a surface of a solid by weak forces. In this C.G st. phase is a solid while mobile phase is either a liquid or a gas.

Separation takes place on the basis of adsorption of analyte components over st. phase and their differential desorption by mobile phase examples include

- Column C.G   • Adsorption TLC
- Ion Exchange C.G • Affinity C.G
- Gel C.G (Size Exclusion Chromatography) etc

3 - Ion Exchange C.G

In this C.G exchange of ions takes place b/w the liquid mobile phase and solid st. phase, called ion - exchange resin. (ion exchanger)
This is explained by following equation

$$R_z SO_3 H^{\ominus} + M^{n+} \longrightarrow (R_z SO_3)_n M + nH^{\ominus}$$

$$R_z SO_3 Na^{\ominus} + M^{n+} \longrightarrow (R_z SO_3)_n M + n Na^{\ominus}$$

4. SEC or Gel C.G

In this C.G st. phase is a swollen gel, having pores in it structure and mobile phase is a liquid. When mobile phase passes through gel, the analyte molecules are separated on the basis of their sizes and shapes.

5. Affinity C.G

Affinity C.G has been largely used for purifying biological molecules, e.g amino acids, antibodies, antigen, enzymes, viruses, bacteria etc and is based upon the affinity of a particular host with a particular guest. for example In this C.G specific antigens are enzymes having affinity for particular atibodies, or enzymes or chemically bonded with some solid support such as silica, alumina.

Complex biological mixture is passed through the column whereby substrates or antibodies bound
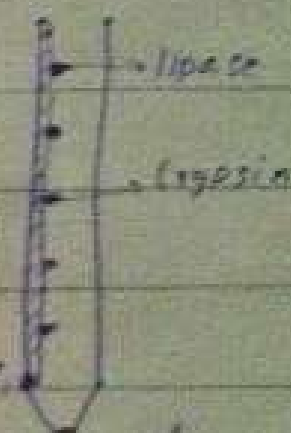
to their respective antigens or enzymes

C. On the basis of supporting medium

1. Paper chromatography.
Paper is made of cellulose fiber.

2. Column C.G (Stil or Glass)

3. Thin Laye C.G (TLC): Plate of glass □

① It is a type of Partition C.G in which paper act as a support for mobile phase

Paper is made up of cellulose fiber having hydroxyl group upon which water is bounded by hydrogen bonding. which act as stationary phase. and some organic solvent act as mobile phase

Used for polar

In some case the hydroxyl groups are replaced chemically by acyl groups. Now some organic solvent behaves as stationary phase and water

Column Adsorption C.C (cc)

Start of C.C

Column C.C can be studied in following headings.

1. Introduction,   2. Basic Principle
3. General Procedure, 4. Adsorbent for CC.
5. Eluting solvent for CC. 6. Applications.

1. It was first time demonstrated by a Russian Botanist Mikhail Twett in 1906 who separated plant pigments using a column filled with calcium carbonate $CaCO_3$ powder as st. phase. He poured plant extract over a vertical column of $CaCO_3$ and irrigated the column with petroleum ether. After elution with petroleum ether he observed different coloured band on the calcium carbonate column.

Hence, he derived
the term C.G i.e,
Chroma = Colour, Graphien
= Writing / Pattern

Later on these components were
identified as Chlorophyl A, B, Viola
xanthin, Xanthophyl and Caroten (bands)

$C_{55} H_{72} N_4 Mg$ (A) $C_{55} H_{70} N_4 Mg$ (B)

Hence Mikhail Tswett concluded that
the technique of C.G could be used
for the separation of coloured organic
compounds, especially those which decompose
on adding, by distillation or any
other thermal technique.

2. Basic Principle: (finely powder

St. phase is solid
which is adsorbant, while mobile
phase is gas or liquid

Based on difference of
adsorbance and desorbance of
compounds.

In column C.G, st. phase

is finely powdered solid called adsorbant and the mobile phase is either a liquid or a gas. The most common adsorbant include silica gel or alumina. The mixture of compounds to be separated is poured over the adsorbant and mobile phase is passed through the column. The compounds in the mixture get separated on the basis of differences in their adsorption over st. phase and desorption by the mobile phase.

Since adsorption is a surface phenomenon, the degree of separation depends upon the surface area of adsorbant

Other factors which affect separation are. (Short Question)

- Nature of adsorbant.
- Nature of eluting solvent.
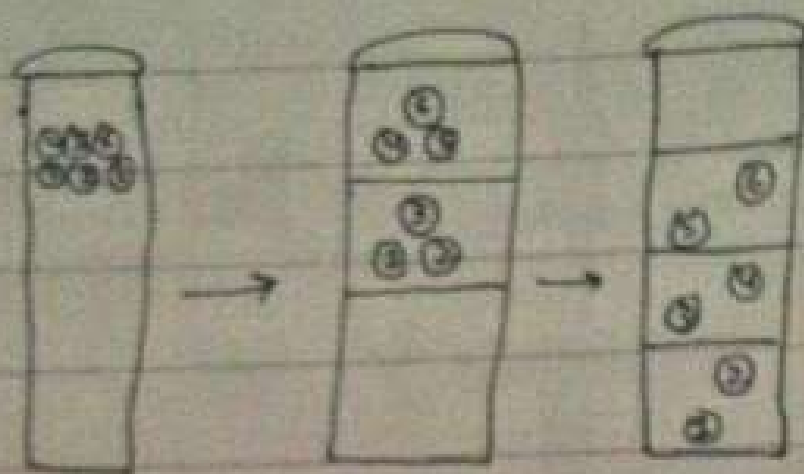- Duration of elution.
- Dimensions of column.

- Temperature.
- Surface are

3. Adsorbants for CC

29.09.2016   Analytical Chemistry
3. Adsorbants for Column Chromatography.

General Procedure:

         In column C.G the st. phase is placed as _slurry_ in cylindrical column i.e plugged with a glass wool or an inert porous disc at the bottom. The sample is dissolved in a minimum amount of a solvent and is applied to the column. After then, mobile phase is allowed to pass through the column.

         If appropriate eluting conditions are selected, the individual components in the mixture emerge at the bottom into the recieving beaker which is explained by above diagrams.

Adsorbants in C.C.G

A large number of adsorbants had been prepared, modified and applied to the column C.G. In general a good adsorbant must posses following characteristics.

- Uniform size in the range of 50-200 um.
- Spherical Shape
- Easily available and cheap.
- Must not react with mixture
- Must not be soluble in eluting solvent.
- Must posses polar sites (Polarity)

There are 3 types of adsorbants on the basis of their adsorbing power. strength.

A list of some adsorbants is given below.

Adsorbent	Absorbing Strength	Mixture Sep.
• Silica Gel	Strong	Steroids, Amino acids, proteins
• Alumina	"	Steroids, Alkaloids Esters

· Charcoal	Strong	Peptides, Carbohydro.
· $MgCO_3$	Moderate	~~$MgCO_3$~~ Porphyrins
· Ca PO_4	Moderate	Protein, Polynucleotides
· Cellulose	Weak	Proteins
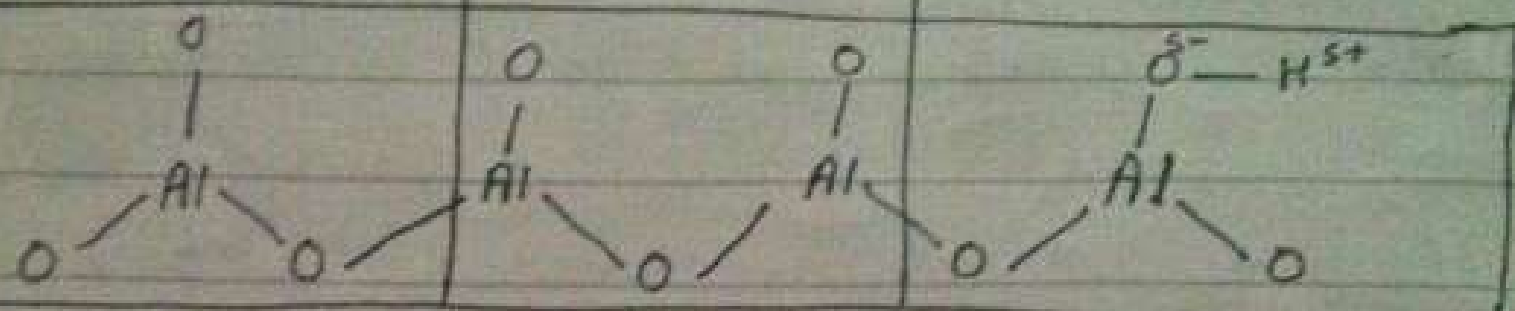
The two most common adsorbants are alumina and silica gel. The alum... contains polar sites (Al-OH, Al-O⁻), (Al^{n+})

Eluting Solvents for C.C.G

The choice of solvents depends upon two factors.

1 - Nature of mixture to be separated

2 - Nature of adsorbant used.

The solvent causes desorption of adsorbed components and results in the formation of their bands.

This desorption process mainly depends upon of polarity of solvent.

Polar solvent like H_2O, Ethanol

hold the solute particles strongly and cause poor separation.

Therefore it is more convinient to use a combination of two or more solvents of varying polarity.
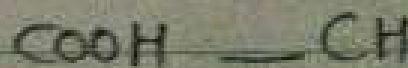
6. Applications:

Applications of column C.G

Column C.G has been used in,

- In the purification of various dyes e.g sudan red, Indigo, methylene blue, flourescene
- In the separation of urinary keto steroids.
- In the separation of plasma cortisol
- In the " " sterioisomers

$$CH - COOH$$
$$\|$$
$$CH - COOH$$

Malic Acid
Cis - type

$$CH - COOH$$
$$\|$$
$$COOH - CH$$

Fumaric acid
Trans type

- In testing the homogeneaty of coloured compounds. • In establishing the identity or non-identity of compounds.

30_08_2016   Analytical Technique.

Thin Layer Chromatography (TLC)

Binder : Plaster of Paris, Gypsum, Polyvinyl Alcohol

TLC can be discussed by following

- Introduction   • Basic Principle
- Basic Operations in TLC

⇨ Choice of st. phase.

⇨ Production of Thin Layer on plate/strip.

⇨ Choice of eluting solvent

⇨ Methods of development of chromatoplates.

⇨ Detection of spots on TLC Plates.

    ★ Physical Methods   ★ Chemical Methods

    ★ Biological   "

- Advantages of TLC
- Disadvantages / Limitations of TLC
- Applications of TLC.
- Introduction:

            TLC is a planner form of C.G that is largely used for

qualitative analysis of broad range of compounds, and may also be used for quantitative analysis. This technique was first time introduced by Ismailave and Shraiber in 1938, but it did not achieve much popularity as it enjoys today.

• <u>Basic Principle</u>:

In TLC the st. phase is a thin layer (0.1 to 0.3 mm) of finely powdered solid st. phase which is supported on a glass or aluminium plate or a plastic strip. This st. phase may be adsorbant, ion excha -nger, molecular sieve or it can serve as support for liquid film. This st. phase is adhered with the surface with the help of suitable binder such as gypsum $(CaSO_4 \cdot 2H_2O)$, Plaster of Paris $(CaSO_4)_2 \cdot H_2O$, Polyvinyl alcohol. The mobile phase comprises of a single liquid or a mixture of liquids. As mobile phase passes along the thin layer separation of components of a mixture takes place. This

separation of TLC plates involves a no. of phenomenon such as

- Adsorption
- Partition
- Ion Exchang
- Molecular Sieve
- Ion Pair
- Reverse Phase
- Basic Operation in TLC

⇒ Choice of Stationary Phase.

04-09-2016   Analytical Techniques.

★ Basic Operations in TLC

⇒ Choice of St. Phase:

⇒ Production of Thin Layer on Plates:

- Slurry ⟶ uniform layer by adapter

  5-6 hours at 105°-110°C.

Activation of Plate

In TLC a thin layer of adsorbent is prepared by spreading an ageous slurry of the finely prepared powdered solid on the clean surface of glass, plastic or aluminium strips. This solid can be adsorbant, an ion exchanger, a molecular sieve, or it can serve as support 4 (for) liquid film. Usually a binder such as gypsum, plaster of paris, poly vinyl alcohol is added to the slurry to help stationary phase to adhere onto the support. The slurry is spread on the plate as thin layer of 0.1-0.3 mm thickness with the help of spreading adapter. The solvent (water) is evaporated off and adsorbant is activated by placing the plates in an oven at 110°C for several hours.
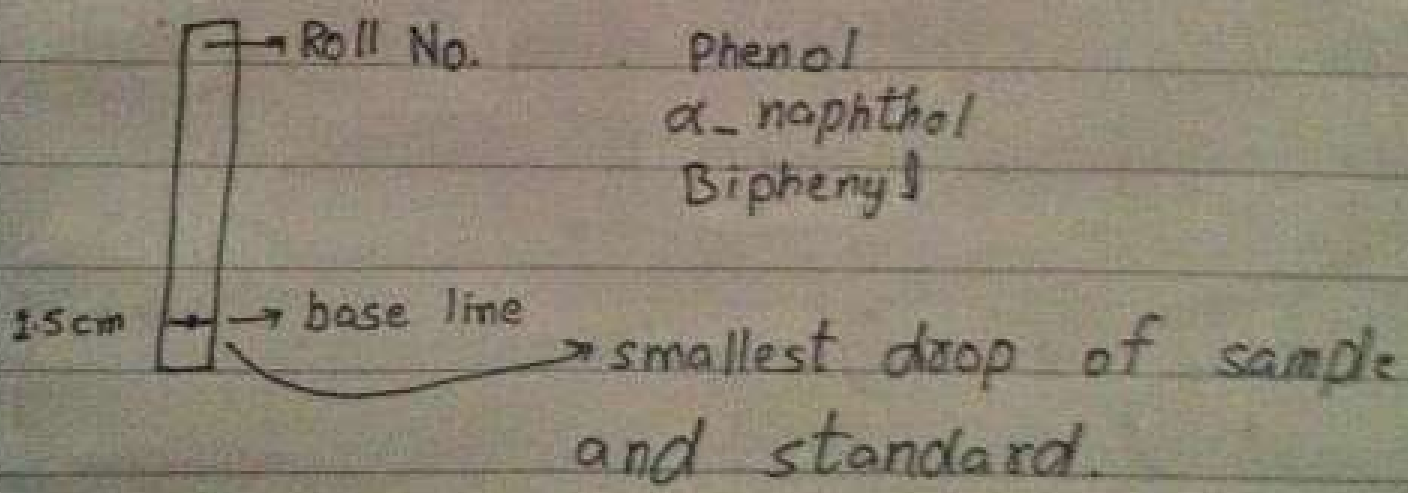
⇨ Choice of Eluting Solvent. (S.G)
    The solvents have arranged

in increasing order of their polarity. The resulting list is called eluotropic series which is shown below.

↗minium (less polar)

1. Petroleum ether.   2. Cyclohexane

3. CCl₄   4. Toulene

5. Benze   6. Chlosoform

7. Ether   8. Ethyl acetate

9. Acetone   10. Ethanol

11. Methanol   12. Water

13. Pyridine (maximum) highly polar.

increasing elating power ↓

⇒ Application of Sample on TLC Plate

→ Roll No.   Phenol
              α_naphthol
              Biphenyl

1.5cm → base line
        ↪ smallest drop of sample
           and standard.

First of all, about 0.1% solution of sample is prepared in a suitable solvent then a thin pencil line is drawn across the plate, a few centimeters from the bottom (2 +). Now the

sample (or sometime standards) is spotted on the base line for measurement of R_f values. The spot must be made as small as possible for maximum separation and minimum tailing. The spot is allowed to dry with a warm air blower e.g hair dryer, to evaporate the solvent.
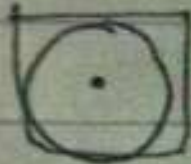
⇒ Development of Chromatograph: (TLC plate)
Asceding (bottom to top) development
Descending (top to bottom) *
Circular (Square plate) development ⊡
2 Dimensional development.

04-09-2016   Analytical Technique.

⇨ Development of TLC Plates:

After drying sample spot, the TLC Plate is placed in a jar (beaker) called chromato-graphic tank containing eluting solvent (s). After covering the jar with an air tight lid, the solvent travels up the plate via capillary action. This is called ascending dev. of plates, and it may take fifteen 15-45 minutes depending upon the comple-xity of mixture.

Other methods of dev, being include.

• Descending Dev.
• Radial or Circular Dev.

- Multiple development
- Two Dimensional Dev.
- Gradient Dev.
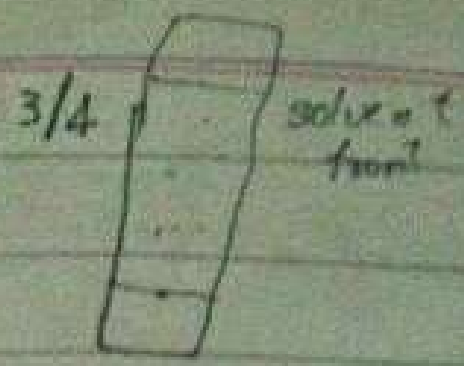
⇒ Detection/Location of Resolved Components on TLC Plate.

When the mobile phase travels the distance of 3/4 of the TLC Plate, The plate is removed from the tank and after marking solvent front the plate is allowed to dry. If the resolved components are coloured, they are readily located on the plate, but if they are colourless, their location can be determined either by physical methods, biological methods or chemical methods

1- Physical Method

i- UV Detection.

a- Most of the organic compounds
b exhibit absorption in short range
e UV Radiation at 254 nm, they are readily detected under UV Lamp.

b. Some organic compounds show the phenomenon of phosphoresence hence they are detected by the glow they pooduce /cause.

c. Mostly a flouresent dye is incorporated into the thin layer during its pooduction. Under UV lamp whole plate floresce except areas containing resolued components.

ii. Laser Detection:

The compounds which floresce under laser are dectected by this method.

iii. Auto Radiography:

The compound labelled with radioactive isotopes are dectected by either radiography or scanning method.

2. Enzymatic and Biological Methods:

Enzymatic methods are employed for detection of substrates. e.g. for the detection of amylase enzyme the developed plate is spoayed with

starch solution and is incubated for a suitable time at a specific temperature. After then, the plate is sprayed with iodine solution. The amylase appears as white area, the remaining plate appears blue.

The biological method of detection is called bioautography and is based upon growth inhibition. In this method the developed plate is sprayed with agar, seeded with test organism and is incubated at a suitable temperature. Microorganisms that are sensitive to the substance analyzed grow all over the plate except areas containing that substance.

Genome: Complete set of DNA including all the genes.
3 billion DNA base pair
1 million = 10 lac
10 million = 1 billion = 1 carore

06-09-2016   Analytical Techniques.
➡Location of Resolved Components: in TLC
1. Physical Methods.
2. Enzymatic and Biological Methods
3. Chemical Methods
• Locating Reagents./Visualizing Reagent
    The developed TLC Plates are sprayed with some reagent to give coloured pooducts from which the resolved components are visualized. These locating reagents are mostly in the solution foom but they may be gases or vapouss.
        Some of the most commonly used locating reagents are examplified in the following.

Locating Reagent	Compound Visualized
Ninhydrin	Amino acids, amines
Sodium rhodizonate	Calcium ions (Ca^{2+}), Mg^{2+} Sr^{2+}, Ba^{2+}
Rubeanic acid	Co^{+2}, Mn^{+2}, Zn^{+2}, Fe^{+2}
Ferric Chloride in Pota-slum Ferro Cyanide $[Fe^{+3}[Fe(CN)_6]]$ (FeCl₃)	Phenol
2,4- Di Nitro Phenyl Hydro-zine (DNPH)	Aldehydes, Ketones
Rhydamine - B	Lipids
Bromocresol Green and other indicator	Carboxylic acids
$HgNO_3$ Mercuric nitrates	(anti-epleptic drugs) Barbiturates

The 2 most commonly used locating reagents
are conc. H_2SO_4 and iodine which react
with almost all the organic compounds.

27_09_2016　Analytical Techniques.

Gas Chromatography: (GC)

He. N. O, F. Cl, Ne, Ar

GC can be discussed by following headings.

⇨ Introduction and Origin

⇨ Basic Principle of GC

⇨ Brief Theory of GC Separation

⇨ Instrumentation (Instruments and its Parts)

* Carrier Gas System

* The Injectors

* The Column

* The Oven (Maximume 386°C)

* The Detectors (GCMS　mass spectrometry)

* Data System

⇨ Advantages

⇨ Disadvantages

⇒ Applications of GC

⇒ Introduction and Origin:

The idea of development of GC as analytical tool was pioneered by AJP Martin and Richard L. Synge in 1941 but unfortunately little notice was taken of their suggestion. They continued their effort to bring the concept to reality in 1951 when they published their epic paper by separating and quantifying the 12 components in a C_1-C_5 fatty acid mixture. It is quiet surprising that T.A Burger in 1996 published a paper in a journal chromatographia and showed the separation of 970 components in a gasoline sample.

⇒ Basic Principle:

In GC, the components of a vapourized sample are separated as a consequence of being partitioned b/w a gaseous mobile phase and a solid or a liquid

st phase held in a column. In performing GC separation, the sample is vapourized and injected onto the head of a chromatographic column. Elution is brought about by the flow of an inert gas through the column. In contrast to other chromatographic techniques the mobile phase does not interact with analyte molecules, its only function is to transport the analyte molecules.

⇒ <u>Brief Theory of GC Separation:</u>

In GC, the sample is vapourized if it is not a gas by injecting into a heated post situated in front of column. This vapourized sample then enters into the column where stationary phase is present. On the basis of physical state of st. phase, GC is of 2 types.

- Gas Solid CG (GSC)
- Gas Liquid CG (GLC)

GSC has limited applicability b/c of semi-permanent adsorption of analyte components onto the solid surface which cause severe tailing

GLC has now become the widely used and most important technique which is probably used by all types of chemists. In GLC the sample is introduced as a gas at the head of a column. As a result the components having phynite solubili in the st. liquid phase distribute themselves b/w this phase and the mobile gas phase. Elution is then carried out by forcing an inert gas such as "He" or N_2. The rate of movement of various components along the column depends upon their tendency to dissolve in the st phase. Components having negligible solubility in st phase move rapidly through the column while