MODULE-3

INSTRUMENTAL METHODS

Thermal analysis

Thermal analysisis the dynamic relationship between temperature and mass change or heat change.They are divided in to two,

1. Thermogravimetric analysis

2. Differential thermal analysis

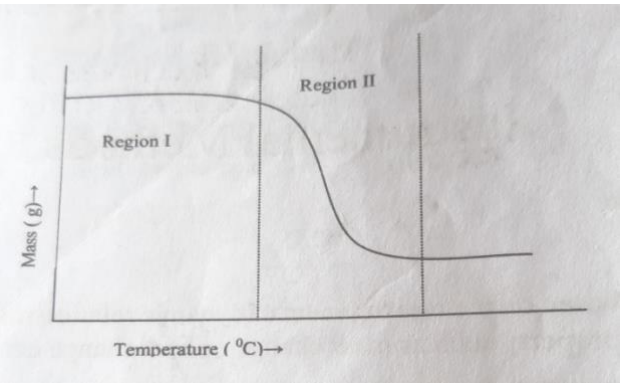
1. Thermogravimetric analysis (TGA)

The mass of substance is monitored as a function of temperature.

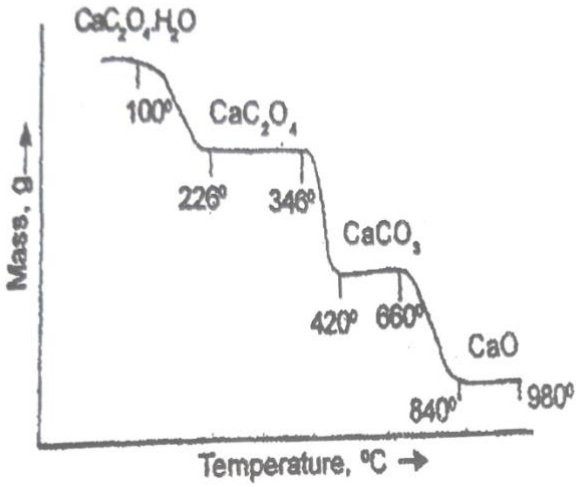
Principle:-

- The sample is heated from room temperature to temperature as high as 1200^0C in a controlled atmosphere.
- The temperature increases, the sample may undergo physical or chemical changes which will be accompanied by mass loss.
- The measurement is normally carried out in a air or inert atmosphere such as nitrogen, helium and argon etc.
- The weight is record as a function of increasing temperature.
- The graph obtained in TGA is called Thermogram.
- Thermogram is a plot of mass verses temperature.
- Stage I- indicate no mass change.ie material is thermally stable
- Stage II-indicate mass changes or weight loss. This can due toDehydration, Decomposition or Evaporations

Thermogram



TGA OF CALCIUM OXALATE

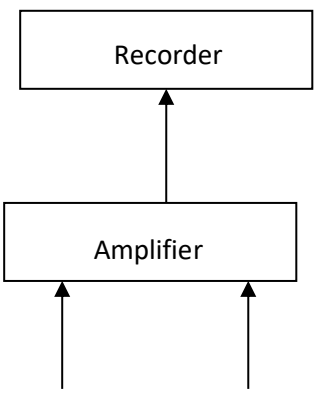


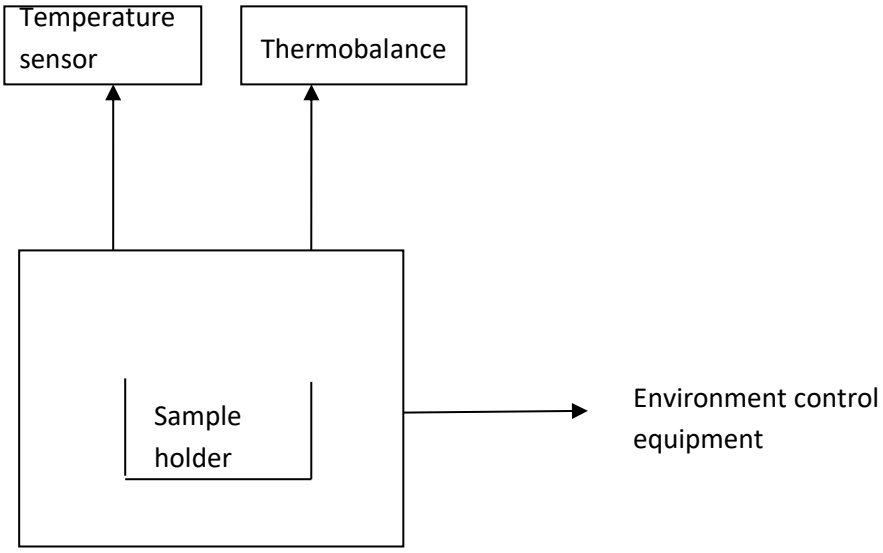
- Hydrated calcium oxalate is stable up to 100^0 C.
- Removal of water starts at100^0 C and gets completed at 226^0C.
- The horizontal portion ranging from 226 to 346^0C shows the thermal stability of anhydrous calcium oxalate.
- Above 346^0C the decomposition of anhydrous calcium oxalate takes place.
  $CaC_2O_4 \rightarrow CaCO_3 + CO$
- The $CaCO_3$ formed is stable up to 660 ^{0}C. Above this temperature decomposition of calcium carbonate takes place.
  $CaCO_3 \longrightarrow CaO + CO_2$

Instrumentation

The main components of TGA apparatus are the following,

- Sample holder
- Furnace with temperature programming facility
- Environment control equipment
- Temperature sensor
- Amplifier
- Recorder





Furnace

Procedure:

- Sample is taken in a sample holder.
- Sample holder is surrounded by a furnace.
- Environment control equipment provides suitable atmosphere.
- Sample holder is attached to temperature sensor and thermobalance.
- Temperature sensor recorded the sample temperature.
- Thermobalance recorded the mass of the sample.
- The signals are amplified and recorded.
- Thermogram is obtained.

Applications

1. To determine moisture and volatile content on materials.
2. Estimated lifetime of a product.
3. To understand composition of material.
4. To find thermal stability of a material.
5. To detect purity and identification of the compound .

Limitation

- It cannot not be used to find out, transition from one phase to another like solid to liquid.
- It cannot be used to study fusion reaction, crystalline transition, and solid state reactions.

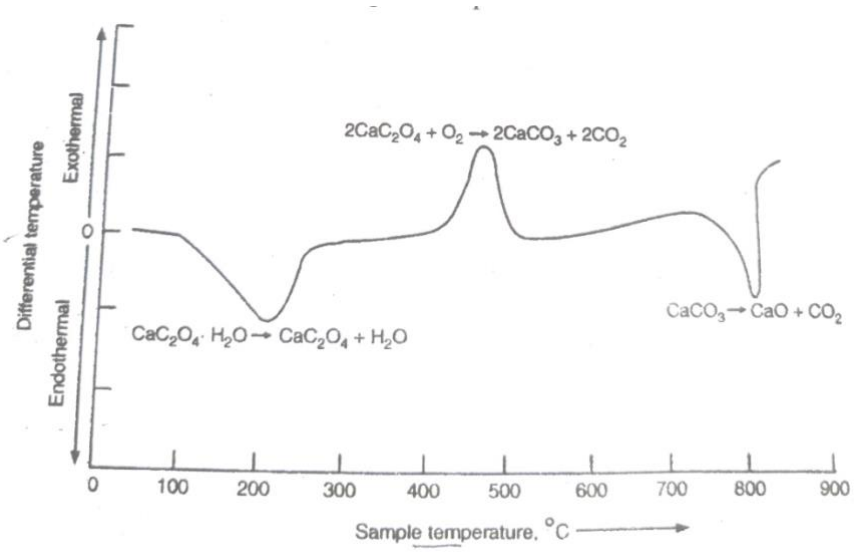
2. Differential Thermogram (DTA)

It is thermo analytical method in which heat change is monitored as afunction of temperature.

Principle:

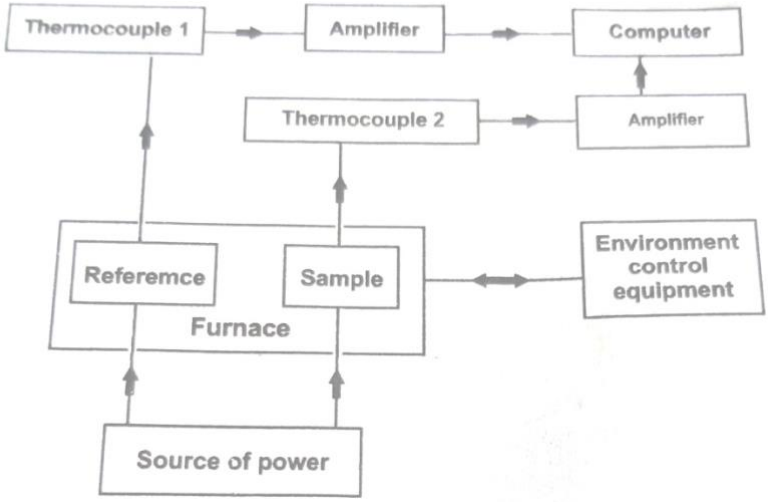
- Sample and reference material are heated under identical condition at constant rate.
- As the temperature increases ,heat change is noted
- The graph obtained in DTA is called differential thermogram
- Differential thermogram is a plot of T(temperature of the sample) verses ΔT(Difference in temperature between sample and reference)
- Heat changes in the sample either exothermic or endothermic
- Exothermic changes are represented by upward peak and endothermic represented by downward peak.
- The area under DTA peak can be calculated to give enthalpy change of the process.

DTA of Calcium Oxalate



Instrumentation

- Sample holder
- Furnace with temperature programming facility
- Temperature sensor1
- Temperature sensor2
- Amplifier
- Recorder



Procedure:

- Sample and reference are heated under identical condition
- Temperature sensor 1 measure the temperature of sample and
- Temperature sensor 2measure the difference in temperature of sample and reference.
- The signal is amplified and recorded.
- Differential thermogram is obtained.

Application

- To determine enthalpy change of the process.
- To distinguish endothermic and exothermic heat changes.
- Study of decomposition temperature of inorganic solids.
- To give information such as melting point and transition temperature.
- Enthalpy change of processes can be estimated by counting the peak.

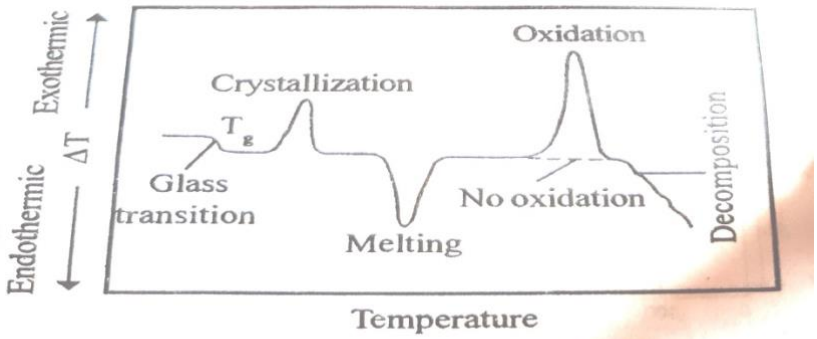


Fig.3.6 DTA of a polymer

CHROMATOGRAPHY

Chromatography is the method used for separation,purification and identification of a mixture of substance.All chromatographic technique has two phases.

1.Stationary phase-it is a fixed phase (solid or liquid supported on inert solid)

2.Mobile phase-it is a moving phase (liquid or gas)

Classification

Based on the mechanism of separation

1. Adsorption chromatography-the stationary phase is solidand mobile phase may be aliquid or gas. The separation occurs due to the adsorbing capacity of component.Eg:- Column chromatography, thin layer chromatography
2. Partition chromatography – In partition chromatography, stationary phase is liquid supported on inert solid. Mobile phase may be liquid or gas. The separation is caused by the partitioning of components between the stationary phase and mobile phase.

Based on the mobile phase

1. Liquid chromatography – Mobile phase must be liquid. Stationary phase may be solid or liquid supported on inert solid. Eg: HPLC

2. Gas chromatography – Mobile phase must be gas. Stationary phase must be solid or liquid supported on inert solid. Eg: Gas chromatography.

Column chromatography

Column chromatography is one of the most useful methods for the separation and purification of both solids and liquids.

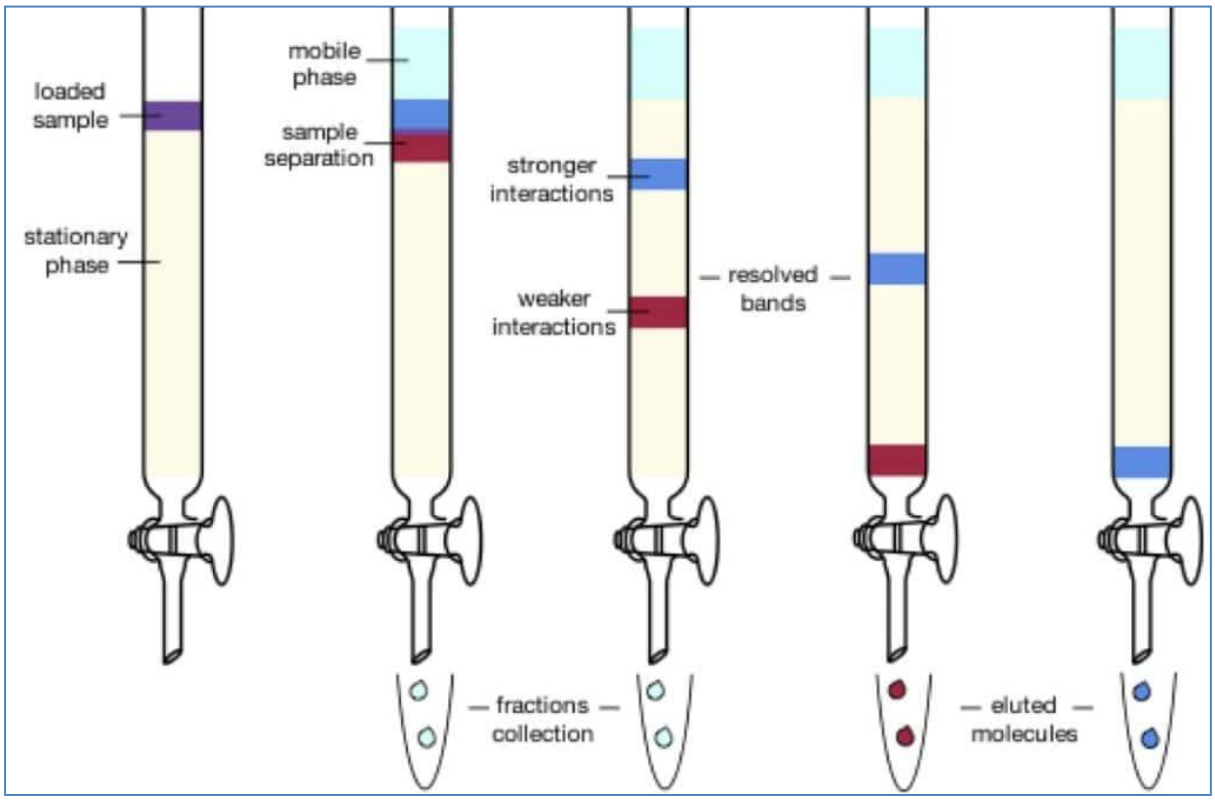
Principle:

- Adsorption is the principle of column chromatography. So the stationary phase is solid and mobile phase is liquid or gas.
- Separation based on the absorbing capacity of components.

Procedure:

- Cylindrical glass tube filled with silica powder act as a stationary phase.
- Bottom of the glass tube contain porous disc.
- Suitable solvent selected act as a mobile phase.
  (Eg: Hexane)

- Mixture to be separated (A+B) is dissolved in a mobile phase or solvent.
- It is introduced at the top of the column and allowed to pass through the column.
- The components of the mixture are adsorbed at different regions depending on their ability of adsorption.
- The components with greater adsorption power will be adsorbed at the top and other will be adsorbed at the bottom.
- The component separated as a band in a column is called chromatogram.
- After the formation of chromatogram, elusion process takes place.
  Elusion means dissolving out of the component from adsorbent using mobile phase or solvent. The solvent used for elusion is called elueant.



Applications:

- Separation of organic compounds.
- Identification of products.
- Concentration of solute from their dilute solutions.
- Purify natural compound mixtures like alkaloids, glycosides etc.
- Separation of organic compounds from plant materials.

Thin layer chromatography (TLC)

TLC is a chromatographic separation method used to separate mixtures, to check the purity of a mixture and to monitor the progress of a reaction.

Principle:

- Adsorption is the principle of column chromatography. So the stationary phase is solid and mobile phase is liquid or gas.
- Separation based on the absorbing capacity of components.

Procedure:

- Preparation of TLC plate – It is prepared by taking glass plate or aluminium sheet is coated with powder silica. Then it is dried and heated. This TLC plate act as a stationary phase.

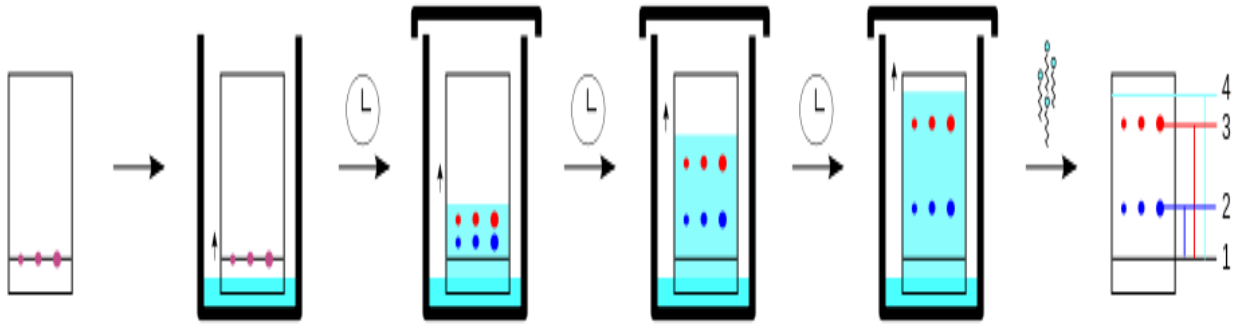
- Spotting the sample of TLC plate –
    a. Take the TLC plate and then draw a line 1cm above from the bottom. Next the sample is dissolved in a mobile phase or solvent.
    b. Using capillary tube, sample is spotted on the line.
    Then TLC plate is placed in a cylindrical glass jar with a cover glass.
    c. Glass jar contains more amount of mobile phase.
    Carefully noted bottom end of the plate touches the solvent.
    Then glass jar is covered.
    d. Then the solvent rises up, as the solvent passes this spot, it carries the component at different rate, resulting in the separation of components.
    e. The solvent reached near the top of the plate.
    TLC plate taken from the jar.
    f. Spots are marked and calculate R_f value.
    R_f is **retention factor** value.

$$(R_f) = \frac{distancetravelledbysamplespot}{distancetravelledbysolventfront}$$

You can identify different components by comparing its standard R_f values.

Note: - The compounds are not coloured. The following visualization techniques are used.

- Iodine vapours.
- UV lamp.
- 2% H_2SO_4 spray.
- 10% NaOH spray.
- Ninhydrin spray.



Applications:

- To check the purity of the sample.
- To determine the appropriate solvent for a column chromatographic separation.
- To monitor the progress of a chemical reaction, by observing appearance of a product or the disappearance of the reactant.
- To monitor the column chromatographic separation.

High Performance/ Pressure Liquid Chromatography (HPLC)

- HPLC is used for the separation of **non-volatile**, high molecular weight organic compounds and natural products. Eg: Terpenoids, cholesterol, polypeptides.
- It is a revised column chromatographic technique and the process is fasted by introducing pressure system.
- Main advantages are (i) high speed separation, (ii) two or more solvent can be used.

Principle:

- Mobile phase must be liquid.
- Based on stationary phase, principle of chromatography may be adsorption or partition.
- If the stationary phase is solid, principle is adsorption.
- If the stationary phase is liquid supported on inert solid, principle is partition.

Instrumentation:

The main components of HPLC are,

(i) Solvent reservoir – to collect the different solvent.

(ii) Pressure pump – to apply the pressure of the order 400 – 1000 atm.

(iii) Regulator – adjust the flow rate.

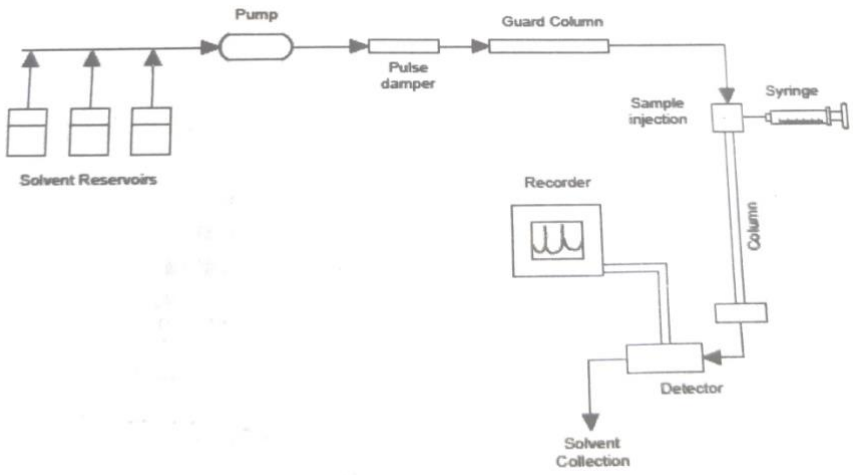
(iv) Guard column – to remove impurities from the solvent or mobile phase. Therefore it increases the life of the analytical column.

(v) Sample injector.

(vi) Analytical column – packed with appropriate material in the range 3-10 gm. It Is found that efficiency is improved as the particle size is reduced to achieve reasonable flow rate pressure pumb are used. the flow rate of 1-10ml/min can achieve with a pressure at 5000psi,coloums is made to smooth stainless steel about length13 cm, diameter of 1 cm.

(vii) Detector- two types detectors are used in HPLC. They are (i) bulk property detector – to detect the bulk properties of the mobile phase. Eg: refractive index, density, dielectric constant etc.

(ii) solute property detector – detects the properties of solute, such as absorbance, fluorescence etc.



Procedure:

- Mobile phase is pumped at required pressure and flow rate adjusted with regulator.
- Non-volatile samples are injected.
- Then passes through the analytical column – here the distribution of the components takes place at different rate.
- Elusion can be done in two ways.
  (i) Isocratic elusion – a single solvent is used.

(ii) Gradient elusion - two or more solvent is used. It is more used because it gives better separation in less time.

- The components that emerge out of the column are detected and recorded.

Applications of HPLC

(i) Separation of non-volatile organic compound

(ii) Used for the separation of polypeptide.

(iii) More amounts of components can be separated as compared with gas chromatography.

Gas chromatography

It is used for the separation of gaseous mixture of volatile organic compounds.

Principle:

- Mobile phase must be gas.
- Based on stationary phase, principle of chromatography may be adsorption or partition.
- If the stationary phase is solid, principle is adsorption.
- If the stationary phase is liquid supported on inert solid, principle is partition.

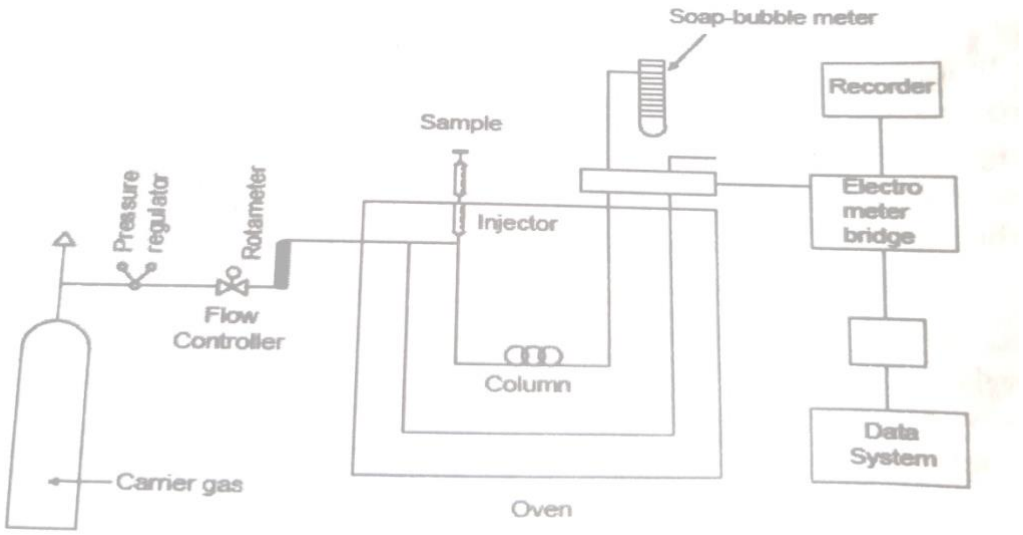
Comparison between gas solid chromatography (GSC) and gas liquid chromatography (GLC)

GSC	GLC
Mobile phase is gas.	Mobile phase is gas.
Stationary phase is solid.	Stationary phase is liquid supported on inert solid.
Principle is adsorption.	Principle is partition.
Thermally stable	Less stable above 300°C
Separation of permanent gases.	Separation of all volatile material except permanent gases.

Instrumentation:

The main components are

- Carrier gas – usually nitrogen or noble gases are used. Its Flow through the system carrying the sample in vapour state.
- Stationary phase- it should be low thermal stability and chemically inactive.
- Sample injector system-The carrier gas is connected to the sample port injector. The injection port is heated to rapid vaporisation but not allowing degradation of the solute.
- Column – The different component in the vaporised samples are separated from each other due to the different intreaction with column packing .Columns are placed solid powder or a liquid coating on inert solid. Columns are made of stainless Steel,Nickel ,copper etc.
- Detector – two types detectors are used.
  (i) Thermal conductivity detector (TCD) - Detection based on the thermal conductivity of the sample.
  (ii) Flame ionization detector (FID) - here the components and hydrogen gas is mixed, then burned, ions and electrons are produced. Detection based on the conducting power of these ions.



Procedure:

- Sample mixture is injected to the sample injector port where it gets vapourised. Then it is carried by the carrier gas into the analytical column.
- Components of the mixture get distributed with the two phases differently.
- Different components are carried at different rate so that they emerge at different time from the column.
- The time taken by each component is a characteristic property called **retention time (R_t),** used for identification.
- R_t factor of a component compared with their standard value.

R_t is the time taken by the solute or sample passes between sample injection port to detector.

Application

1.Chemical analysis of volatile organic compound.

2.Determination of ethyl alcohol content in blood.

3. It is used to test impurity of organic compounds. The presence of impurities will give addition peak.

4.Analysis of hydrocarbon fuels perfumes etc.

5. Identification of component mixture of organic compounds

Nano materials

The materials which are created from blocks of nanoparticles or they are defined as" a set of substance where at least one dimension is less than approximately 100 nanometres" Nano materials are defined as materials with at least one dimension in the size range from approximately 1 - 100 nanometres .

Nanomaterials are of interest because at this scale unique optical, magnetic, electrical and other properties emerge. this emergent properties have the potential of great impacts in electronics,medicine and other fields .Nano carbon such as fullerenes and carbon nanotubes are excellent examples of Nanomaterials. The properties of Nanomaterials are entirely different from bulk materials. The reasons for this are 1.Increased relative surface area .2.Quantum effects.

Classification of the Nanomaterials

I. Classification based on **dimension**

Classification of the nanostructured materials and Systems essentially depends on the number of dimensions which lie within the nano metric range(1-100 nm)

a). Zero dimension (0 -D):Here all the three dimensions are in the Nanometric range.

Eg. quantum dots

b).One dimension(1-D): Here one of the dimensions is outside the nanometric range and the other two are within the range.

Eg.Nano wires, nano tubes, nanofibers.

C). Two dimension (2-D):Here two of the dimensions are outside the nanometric range and one is within within the range.

Eg. Nano film ,Nano layers, Nano coating.

d). Three dimension (3-D):Here all the dimensions are outside the nanometric range

Eg. Bundles of nano wires and nano tubes.

II. Classification based **on materials**

a) Carbon based Nanomaterials:- These are defined as materials in which the nano component is pure carbon.

Eg. carbon nanotubes(CNT),wires , fullerenes .

b) Metal based Nanomaterials :-Metal based Nanomaterials are made of metallic nanoparticles like gold ,silver, metal oxides etc.

Eg.TiO_2, SiO_2 ,nano gold.

c)Nanocomposites:- Composite Nanomaterials contain a mixture of simple nanoparticles or compounds such as nanosized clays within a bulk material .The nanoparticles give better physical, mechanical or chemical properties to the bulk material.

d)Nanopolymers or Dendrimers :-

Dendrimers a nanosized polymers built from branch units. these are tree like molecules with defined cavities. They can be functionalized at the surface and can hide molecules in their cavities. A direct application of dendrimers is for drug delivery.

e) Biological Nanomaterials:-

These Nanomaterials are of biological origin and are used for nanotechnological applications. The important features of these particles i) self-assembly properties and ii) specific molecular recogmition .Example of DNA nanoparticle, nanostructured peptides. Various self-assembled peptides can be designed to release compounds under specific conditions and are used in drug delivery Systems.

Synthesis of nanoparticles

Chemical methods

I.**Hydrolysis**:-

Nanoparticles of metal oxides can be prepared by the hydrolysis of their alkoxide solutions under controlled conditions .Eg. silica (SiO_2),Titania(TiO_2),alumina (Al_2O_3) are prepared by this method.

$Ti(OR)_4 + 2H_2O \rightarrow TiO_2 + 4ROH$

This method can be divided into two;-

i)**Hydrothermal synthesis**;-The process involves heating a solution taken in a steel Bomb to a temperature between 100^o c and 1000 ^{o}c.On heating high pressure from 1 atm to 1000 atm is generated inside the bomb depending on temperature and solvent used.The solvent vapours under high pressure and temperature facilities the interaction of precursors during synthesis. If water is used as the solvent, the method is called hydrothermal synthesis.

The process can be used to prepare Nano articles of various geometry including thin films, bulk powders,single crystals and nano crystals. The morphology of the crystals (3D,2D,or 1D) Of the crystals formed is controlled by manipulating the solvent and temperature. For example MgO nanoparticles can be prepared by the hydrolysis of $MgCl_2$ solution by hydrothermal method.

$MgCl_2 + H_2O \rightarrow MgO + 2HCl$

ii)**Sol-gel method**

The sol-gel method is based on the phase transformation of a sol into a gel.A sol is a colloidal system of Nano solid particles dispersed in a liquid. A gal is a colloidal system in which liquid droplets are dispersed in solid nanoparticles. Hydrolysis of metallic alkoxides can give a sol at a suitable temperature and pH The sol contains many other impurities. In order to remove impurities so is transformed into a gel by changing the pH or other factors .The gel can be purified by filtration and washing with suitable solvents. The purified gel on drying give solid nanoparticles. For eg.Al_2O_3 nanoparticles are obtained by hydrolysis of aluminium oxide by sol-gel technique.

$Al(OR)_3 + 3H_2O \rightarrow Al(OH)_3 + 3ROH$

2.Reduction

Nanoparticles of gold and silver can be prepared by the reduction of their respective solutions using reducing agents, such as sodium borohydride(NaBH4),acerbic acid, glucose etc.along with a protective agent like tholil,glucose etc.This method can be of two types;- reduction using reducing agent and electro reduction.

$Ag^+ + e^- \rightarrow Ag$

a) **Reduction using reducing agents**

Silver nanoparticles can be prepared by this met6.60 ml 1mM $AgNO_3$ solution is taken in a beaker covered with a watch glass and heated in a hot plate with magnetic string on boiling the solution 60ml of 1mM of trisodium citrate is added dropwise, about 1drop per second. The beaker is then closed and kept for some till the colour of the solution changed to a light golden colour.Then it is allowed to cool.The solvent can be removed by freeze-drying.

b)**Electro reduction**

Copper nanoparticles have been prepared by electro reduction process using copper plating bath containing homogeneously codified $CuSO_4$ solution. The nanoparticles are formed as spongy black coloured layers of ball structures at the cathode. The spongy layers of copper can be easily separated to give fine particles.

Properties of nanoparticles

Physical properties

i)Crystal structure of nanoparticles is same as bull structure with different lattice parameter.

ii)The melting point of nanoparticles decreases with size.

Chemical properties

The chemical properties of nanoparticles are significantly different from those of bulk materials due to 1)Large fraction of surface atoms. 2)High surface energy, 3)spatial confinement

4)reduced imperfections

i)unusual mechanical, electrical, optical and magnetic prop6

ii)High creativity and catalytic act6.

iii)Nanoceramics are more ductile at high temperature.

Applications of Nanoparticles

1.Magnetic nanocompsites are used as ferrofluids for high density information storage and magnetic refrigeration.

2.Nanostructured metal oxide thin films are use as gas sensors (CO,CO_2,CH_4 and aromatic hydrocarbons).

3.Carbon nanotube based transistors are used for miniaturizing electronic devices.

4.A mixture of carbon nanotubes and fullerenes is used for making solar cells.

5.Nanoparticles can be used as catalysts.

6.Nano-cadmium telluride exhibit different colour depending upon its size.It can be used for dyeing fabrics which never fades.

7.Nanomaterials are used as targeted delivery, gene therapy, photo imaging, antioxidant activity etc.

8.nanoparticles are used in water treatment like removal of toxic pollutants, heavy metals, oli droplets, pesticides, insecticides etc.

SCANNING ELECTRONE MICROSCOPE

- SEM is scanning electrone microscope.
- It is a powerful tool used for the surface characterisation of materials.
- It is atype of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons.
- This examination can provide information about topography, morphology (shape and size of the particles), composition, and crystallographic information.

Principle

Fundamental principle of scanning electrone microscopy is the electrons interact with atoms in the sample, producing various signals that contain information about the surface topography and composition of the sample.

The accelerated electrons in aSEM carry significant amounts of kinetic energy, and this energy is dissipated as a variety of signals produced by electrone sample interactions.

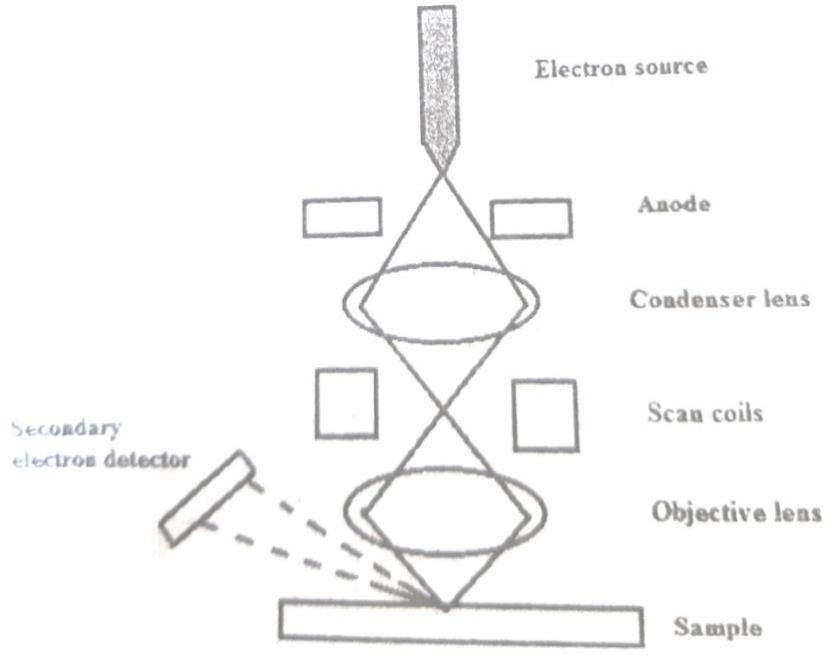
Back scatter electrons are incidental electrons reflected backwards, which provide composition data related to element and compound detection.diffracted back scatter electrons determine crystalline structures as well as the orientation of minerals and micro-fabrics. Rays emitted from beneath the sample surface, can provide element and mineral information.SEM produces black and white, three diamontional images.

Instrumentation

Consist of the following parts:-

i. Electron gun-it is the source of electron(eg-tungsten wire)
ii. Condenser lens- compresses the electrons to a narrow beam.
iii. Aperture- it controls the diameter of the electron beam.
iv. Objective lens- it focuses the electron beam to the sample.
v. Sample chamber-this chamber keeps the sample.
vi. Detector- to detect the signals.
vii. Amplifier-to amplify the signals.
viii. Display-to show the SEM image obtained.

The block diagram



Working

In this process abeam of energetic electrons are produced in an electron gun, and it passes down the column and on to a series of electromagnetic lenses.these lenses are tubes,wrapped in coil and referred to as solenoids.the coils are adjusted to focus the incident electrone beam on to the sample.

Advantages

- It gives 3D topographical images.
- Instrument is very fast and easy to operate.
- Data is available in digital form.
- Easy to prepare sample.
- Uses electrons to form image rather than light.

Applications

- As very essential research tool in life science, biology, medical, forensic science and metallurgy.
- To characterise nanowires and their gas sensing behaviour.
- For a speedy, accurate measurement of the composition of semiconductors.
- For criminal and other forensic investigation.
- In medical science to identify diseases, viruses and testing new vaccinations and medicines.
- SEM helps in the characterization of solid materials.
- It helps to identify crystalline structures.
- It can detect and analyze surface fractures, surface contamination and provide information in microstructures.