PRE-FORMULATION STUDY

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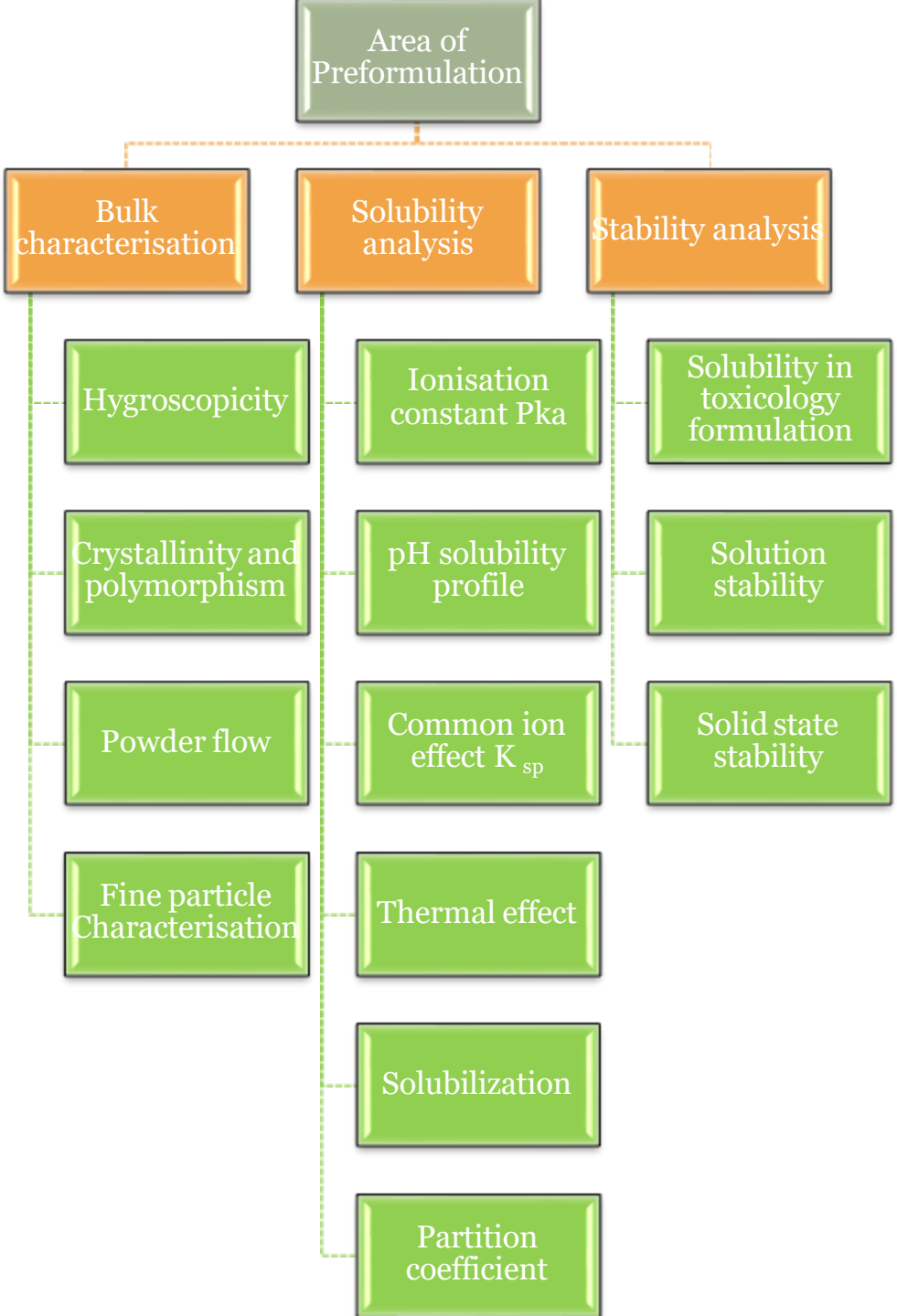
DEFINITION

- Preformulation testing is the first step in the rational development of dosage forms.
- It can be defined as an investigation of physical and chemical property of a drug substance alone and when combined with excipients.

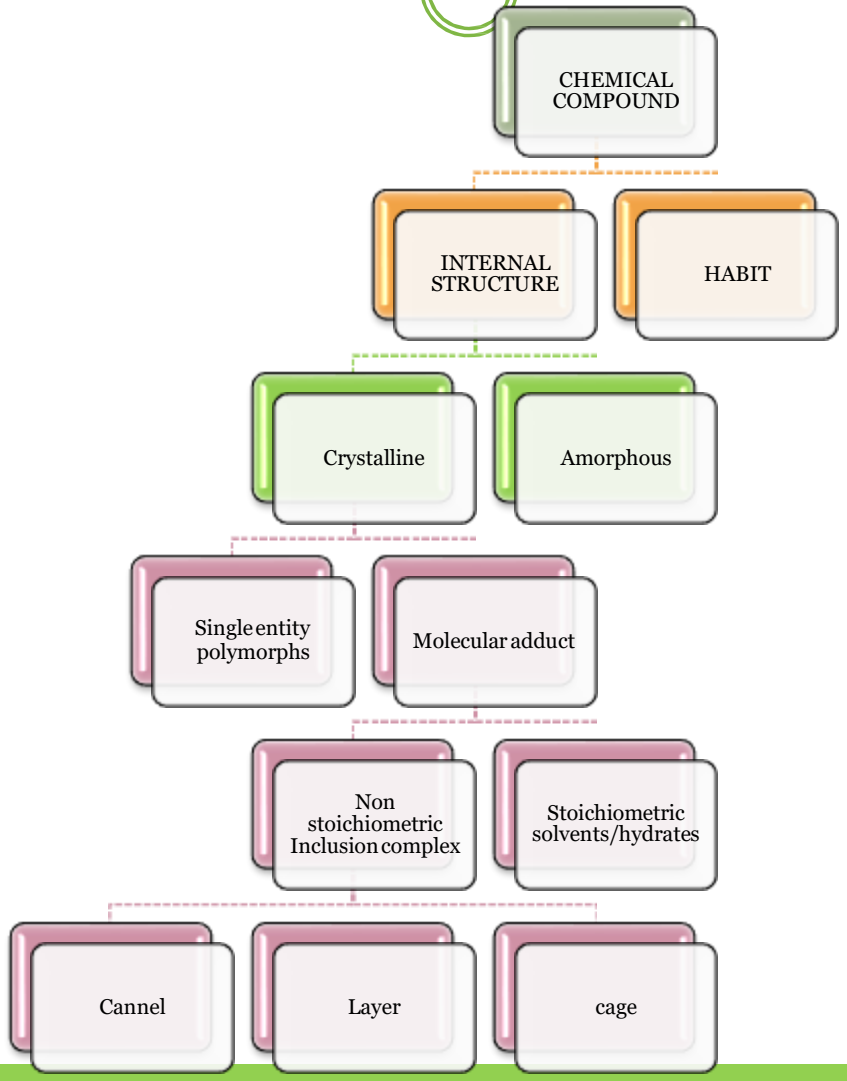
NEED....???

- Preliminary evaluation
- Molecular optimisation
- Suitability of Excipients
- Suitability of dosage foam


Area of Preformulation
Bulk characterisation
Solubility analysis
Stability analysis
Hygroscopicity
Crystallinity and polymorphism
Powder flow
Fine particle Characterisation
Ionisation constant Pka
pH solubility profile
Common ion effect K sp
Thermal effect
Solubilization
Partition coefficient
Solubility in toxicology formulation
Solution stability
Solid state stability

BULK CHARACTERIZATION

CRYSTALLINITY AND POLYMORPHISM



Crystalline state

- Crystal habit & internal structure of drug can affect bulk & physicochemical property of molecule.
- Crystal habit is description of outer appearance of crystal.
- Internal structure is molecular arrangement within the solid.
- Change with internal structure usually alters crystal habit.

  Eg. Conversion of sodium salt to its free acid form produce both change in internal structure & crystal habit.

HABITS

- A single internal-structure for a compound can have several different habits, depending on the environment for growing crystals.

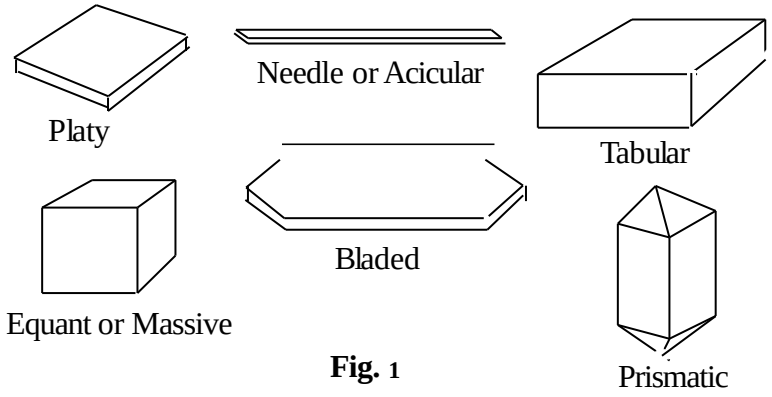


Fig. 1

DIFFERENCE BETWEEN CRYSTALLINE AND AMORPHOUS FORM

Crystalline forms	Amorphous forms
Have fixed internal structure.	Do not have any fixed internal structure.
More stable than its amorphous forms.	Less stable than its crystalline forms.
Has lesser solubility than its amorphous form.	Have greater solubility than its crystalline forms.
Lesser tendency to change its form during storage.	Tend to revert to more stable forms during storage.

POLYMORPHS

- It is the ability of the compound to crystallize as more than one distinct crystalline species with different internal lattice.
- Different crystalline forms are called polymorphs.
- Polymorphs are of 2 types
  1. Enatiotropic
  2. Monotropic

TYPES OF POLYMORPHS

- The polymorph which can be changed from one form into another by varying temp. or pressure is called as Enantiotropic polymorph.

  Eg. Sulfur.

- One polymorph which is unstable at all temp. & pressure is called as Monotropic polymorph.

  Eg. Glyceryl stearate.

- Polymorph differ from each other with respect to their physical property such as
  - Solubility
  - Melting point
  - Density
  - Hardness
  - Compression characteristic

Molecular adducts

- During the process of crystallization, some compounds have a tendency to trap the solvent molecules.

- Non-Stoichiometric inclusion compounds (or adducts):- In these crystals solvent molecules are entrapped within the crystal lattice and the number of solvent molecules are not included in stoichiometric number.

Depending on the shape they are of three types :-

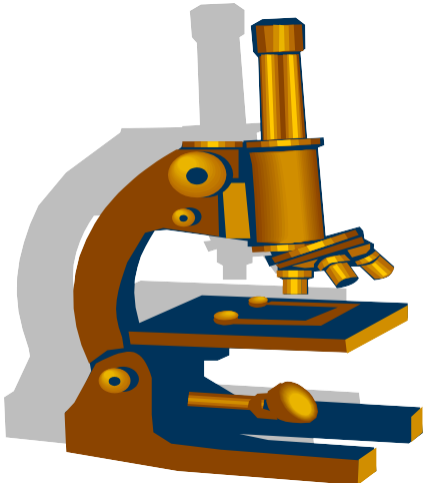
1. ***Channel:-*** When the crystal contains continuous channels in which the solvent molecule can be included. e.g . Urea forms channel.
2. ***Layers***:- Here solvent molecules are entrapped in between layers of crystals.
3. ***Clathrates*** (**Cage**):- Solvent molecules are entrapped within the cavity of the crystal from all sides.

Stoichiometric inclusion compounds (or stoichiometric adducts)

- Molecular complex has incorporated the crystallizing solvent molecules into specific sites within the crystal lattice and has stoichiometric number of solvent molecules complexed.
  - Incorporated solvent is water - Hydrates
  - Solvent is other than water - Solvates .
    - *Anhydrous* : 1 mole compound + 0 mole water
    - *Hemihydrate*: 1 mole compound + ½ mole water
    - *Monohydrate:* 1 mole compound + 1 mole water
    - *Dihydrate* : 1 mole compound + 2 moles water

ANALYTICAL METHODS FOR CHARACTERIZATION OF SOLID FORMS

- Microscopy
- Hot stage microscopy
- Thermal analysis
- X-ray diffraction



Microscopy

- Isotropic material have single refractive index and this substance do not transmit light with crossed polarizing filter and appears black.
- Material with more than one refractive index are anisotropic & appear bright with brilliant colors against black polarized background.
- The color intensity depends upon crystal thickness.

- Advantage :

  By this method, we can study crystal morphology & difference between polymorphic form.

- Disadvantage :

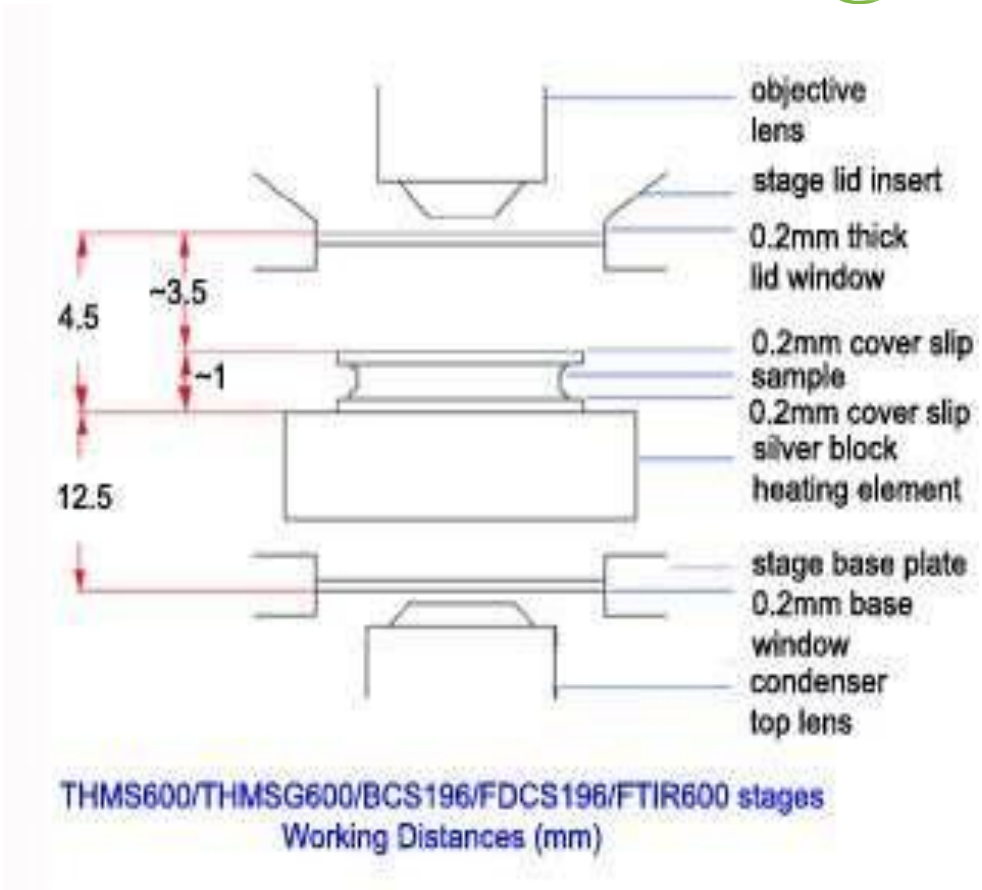
  This require a well trained optical crystallographer, as there are many possible crystal habit & their appearance at different orientation.

Hot stage microscopy

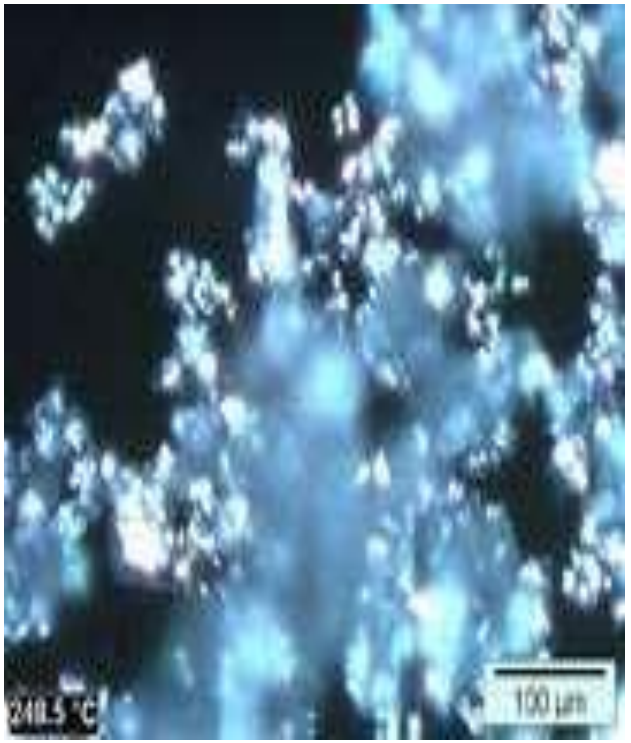
- The polarizing microscope fitted with hot stage is useful for investigating polymorphism, melting point & transition temp.

- Disadvantage :
  - In this technique, the molecules can degrade during the melting process.
  - Recrystalization of substance after melting.

Hot stage microscopy



Diagrammatic representation



Results of hot stage microscopy

THERMAL ANALYSIS

- In DTA instrument a record is produced where temperature difference (ΔT) (between the sample and reference material) is plotted against temperature (T) when two specimens are subjected to an identically controlled temperature regime.
- The reference material is alumina, keiselguhr.

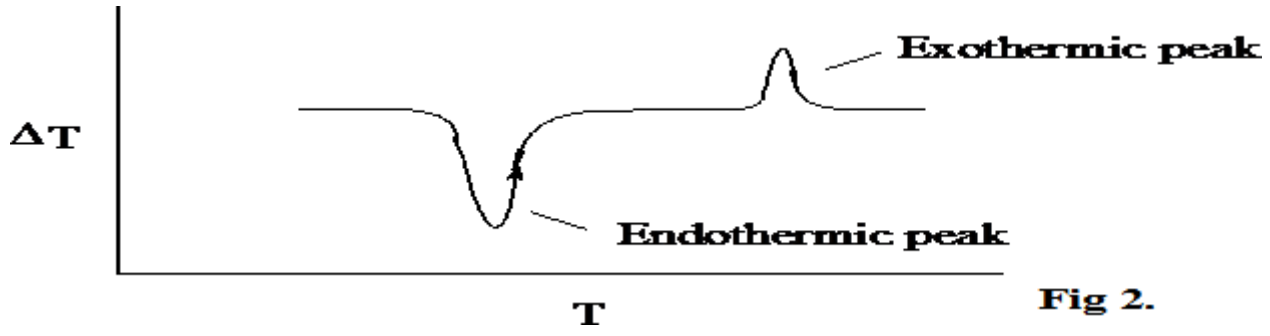


Fig 2.

DIFFERENTIAL SCANNING CALORIMETRY

- In DSC method the difference in energy inputs (ΔH) into a sample and reference material is measured as a function of temperature as the specimens are subjected to a identically controlled temperature programme.

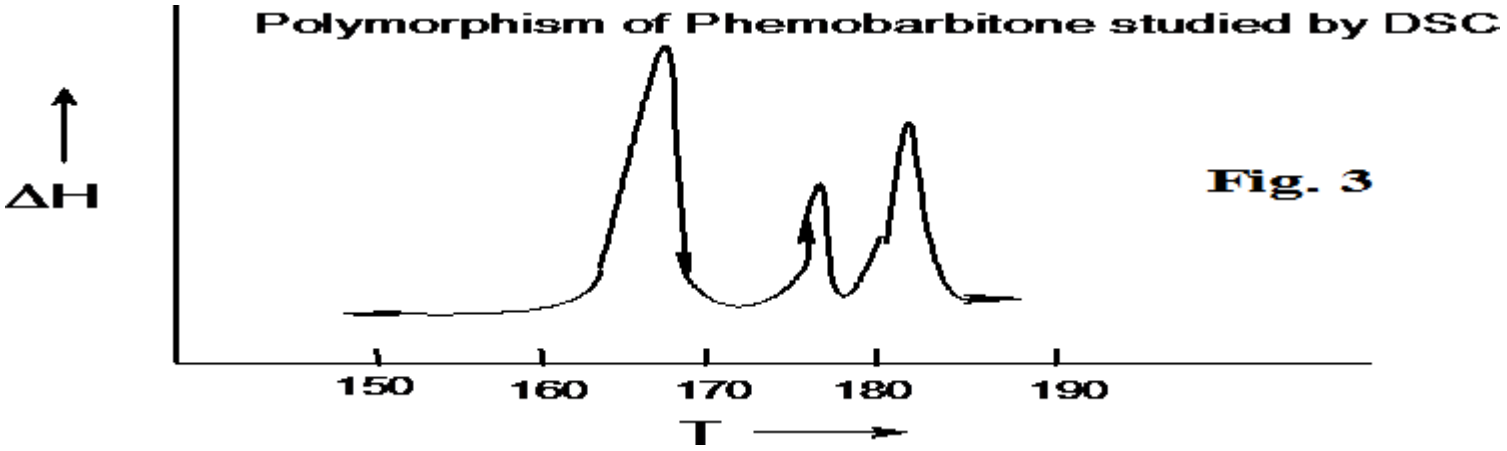


Fig. 3

Samples that may be studied by DSC or DTA

- Powders
- Fibers
- Single crystals
- Polymer films
- Semi-solids
- Liquids

Applications of DTA / DSC in Preformulation studies

- To determine the purity of a sample
- To determine the number of polymorphs and to determine the ratio of each polymorph.
- To determine the heat of solvation
- To determine the thermal degradation of a drug or excipients.
- To determine the glass-transition temperature (t_g) of a polymer.

X-RAY DIFFRACTION

- Working :

  When beam of non-homogenous X-ray is allow to pass through the crystal, X-ray beam is diffracted & it is recorded by means of photographic plate.

- Diffraction is due to crystal which acts as 3 dimensional diffraction grating toward X-ray.

- Random orientation of crystal lattice in the powder causes the X-ray to scatter in a reproducible pattern of peak intensities.

- The diffraction pattern is characteristic of a specific crystalline lattice for a given compound.

HYGROSCOPICITY

- Many pharmaceutical materials have a tendency to adsorb atmospheric moisture (especially water-soluble salt forms). They are called hygroscopic materials and this phenomenon is known as hygroscopicity.

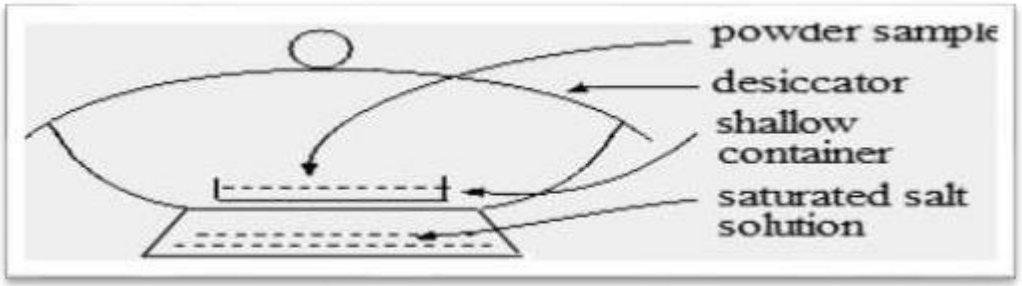
- *Deliquescent materials*: They absorb sufficient amount of moisture and dissolve completely in it. (e.g. anhydrous calcium chloride).

HYGROSCOPICITY

- Equilibrium moisture content depends upon:
  - the atmospheric humidity
  - temperature
  - surface area
  - exposure time
  - mechanism of moisture uptake.

TESTS OF HYGROSCOPICITY

- Bulk drug samples are placed in open containers with thin powder bed to assure maximum atmospheric exposure. These samples are then exposed to a range of controlled relative humidity (RH) environments prepared with saturated aqueous salt solutions.



- The amount of moisture adsorbed can be determined by the following methods:
- Gravimetry
- Thermogravimetric analysis (TGA)
- Karl-Fischer titration (KF-titration)
- Gas chromatography (GC)

- Time of monitoring depends on the purpose:
  - For the purpose of *'handling'* data points from 0 to 24 hours are taken
  - For the purpose of '*storage*' data points from 0 to 12 weeks are taken.
  - Moisture level in a powder sample may affect the flowability and compactibility which, are important factors during tableting and capsule filling.
  - After adsorption of moisture, if hydrates are formed then solubility of that powder may change affecting the dissolution characteristics of the material.
  - Moisture may degrade some materials. So humidity of a material must be controlled.

FINE PARTICLE CHARACTERIZATION

- Parameters those are measured:
  - Particle size and size-distribution
  - Shape of the particle
  - Surface morphology of the particles

PARTICLE SIZE

- Particle size is characterized using these terms :
  - Very coarse (#8)
  - Coarse (#20)
  - Moderately coarse (#40)
  - Fine (#60)
  - Very fine (#80)

- Particle size can influence variety of important factors :
  - Dissolution rate
  - Suspendability
  - Uniform distribution
  - Penetrability

METHODS TO DETERMINE PARTICLE SIZE

- Sieving
- Microscopy
- Sedimentation rate method
- Light energy diffraction
- Laser holography
- Cascade impaction

METHODS TO DETERMINE PARTICLE SIZE

- **Sieving method :** Range : 50 – 150 µm
  - Simple, inexpensive
  - If powder is not dry, the apertures get clogged.

- **Microscopy :** Range : 0.2 – 100 µm
  - Particle size can be determined by the use of calibrated grid background.
  - Most direct method.
  - Slow & tedious method.

- **Sedimentation method :**
- Range : 1 - 200 µm
- Andreasen pipette is used.
- Particle size is calculated by stoke's law :

$$d_{st} = \sqrt{18 n_o h / (\rho_s - \rho_o) g t}$$

Where,

h = distance of fall in time, t
n_o = viscosity of the medium
ρ_s = density of the particles
ρ_o = density of the dispersion medium
g = acceleration due to gravity

- **Light energy diffraction :** Range : 0.5 – 500 µm
  - Particle size is determined by the reduction in light reaching the sensor as the particle, dispersed in a liquid or gas, passes through the sensing zone.
  - Quick & fast.

- **Laser holography :** Range : 1.4 – 100 µm
  - A pulsed laser is fired through an aerosolized particle spray & photographed in three dimensional with holographic camera, allowing the particles to be individually imaged & sized.

- **Cascade impaction :**
  - The principle that a particle driven by an airstream will hit a surface in its path, provide that its inertia is sufficient to overcome the drug force that tends to keep in it in airstream.

PARTICLE SHAPE

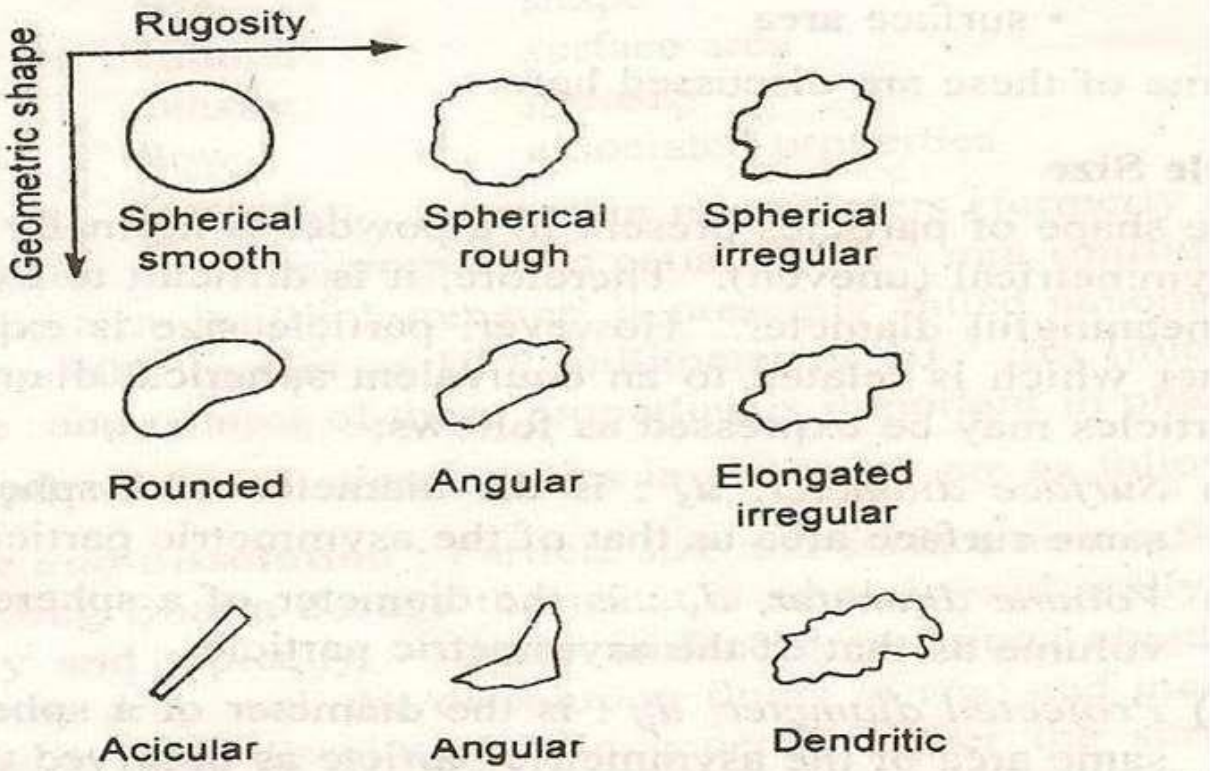


Figure 6-1. Characteristic particle shapes related to geometric shape and surface irregularity.

MEASUREMENT OF FLOW PROPERTY

- **Apparent Bulk Density (g/cm³)**
- Bulk drug powder is sieved through **40 mesh** screen. Weight is taken and poured into a graduated cylinder via a large funnel. The volume is called *bulk volume*.

- *Significance*

- Bulk density is required during the selection of capsule size for a high dose drug.
- In case of low dose drug mixing with excipients is a problem if the bulk densities of the drug and excipients have large difference.

Source of variation of bulk density

- Method of crystallization, milling, formulation.

Methods of correction

- By milling, slugging or formulation.

Tapped density (g/cm^3)

- Bulk powder is sieved through 40 mesh screen. Weight is taken and poured into a graduated cylinder.
- The cylinder is tapped 1000 times on a mechanical tapper apparatus. The volume reached a minimum – called *tapped volume*.
- Significance : Knowing the dose and tapped density of the formulation, the capsule size can be determined.

COMPRESSIBILITY

- Measurement of free flowing powder by compressibility.
- Also known as Carr's index.

- ✓ CARR'S INDEX(%) =(TAPPED DENSITY – POURED DENSITY) X 100 / TAPPED DENSITY

- It is simple, fast & popular method of predicting powder flow characteristics.

Carr's Index	Type of flow
5-15	Excellent
12-16	Good
18-21	Fair To Passable
23-35	Poor
33-38	Very Poor
>40	Extremely Poor

ANGLE OF REPOSE

- A greater angle of repose indicate poor flow.
- It should be less than 30°& can be determined by following equation.

$$\tan \theta = h/r.$$

where, θ = angle of repose.

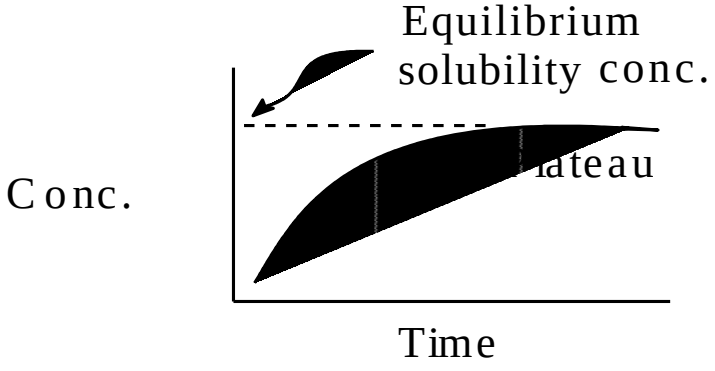
h=height of pile.

r= radius.

Angle Of Repose (In degree)	Type Of Flow
<25	Excellent
25-30	Good
30-40	Passable
>40	Very poor

SOLUBILITY ANALYSIS

- The drug is dispersed in a solvent. The suspension is agitated at a constant temperature. Samples of the suspension are withdrawn as a function of time, clarified by centrifugation, and assayed to establish a plateau concentration.
- *Solubility depends on*
  - pH
  - Temperature
  - Ionic strength
  - Buffer concentration



SOLUBILITY ANALYSIS

- *Solvents taken*
- 0.9% NaCl at room temperature
- 0.01 M HCl at RT
- 0.1 M HCl at RT
- 0.1 M NaOH at RT
- At pH 7.4 buffer at 37^{0}C

SOLUBILITY ANALYSIS

- *Drug concentration is determined by the following analytical methods*
  - HPLC
  - UV –Spectroscopy
  - Fluorescence Spectroscopy
  - Gas Chromatography

SIGNIFICANCE

- A drug for oral administrative should be examined for solubility in an isotonic saline solution and acidic pH. This solubility data may provide the dissolution profile invivo.
- Solubility in various mediums is useful in developing suspension or solution toxicologic and pharmacologic studies.
- Solubility studies identify those drugs with a potential for bioavailability problems.
  - E.g. Drug having limited solubility (7 %) in the fluids of GIT often exhibit poor or erratic absorption unless dosage forms are tailored for the drug.

IONIZATION CONSTANT (pKa)

- When a weakly acidic or basic drug partially ionizes in GI fluid, generally, the unionized molecules are absorbed quickly.
- Can be calculated by Henderson Hasselbach equation-

For acidic drugs….pH= pKa+ log $\frac{\text{[ionized drug]}}{\text{[unionized drug]}}$

For basic drugs….pH= pKa+ log $\frac{\text{[unionized drug]}}{\text{[ionized drug]}}$

METHOD OF DETERMINATION OF pKa OF A DRUG

(1) Detection of spectral shifts by UV or visible spectroscopy at various pH.

- *Advantage*: Dilute aqueous solutions can be analyzed by this method.

(2) Potentiometric titration

- *Advantage*: Maximum sensitivity for compounds with pKa in the range of 3 to 10.
- *Disadvantage*: This method is unsuccessful for candidates where precipitation of the unionized forms occurs during titration. To prevent precipitation a co-solvent e.g. methanol or dimethylsulfoxide (DMSO) can be incorporated.

(3) Variation of solubility at various pH.

EFFECT OF TEMPERATURE ON STABILITY

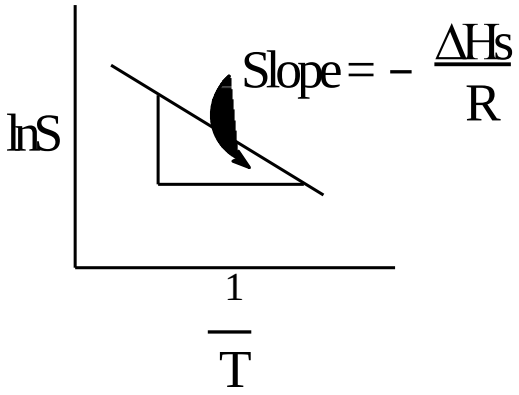
- Heat of solution, ΔH_S represents the heat released or absorbed when a mole of solute is dissolved in a large quantity of solvent.

- *Significance*
  - Most commonly, the solubility process is endothermic,
  - e.g. non-electrolytes, unionized forms of weak acids and bases $\Rightarrow$ ΔH is positive $\Rightarrow$ Solubility increases if temperature increases.
  - Solutes that are ionized when dissolved releases heat $\Rightarrow$ the process is exothermic $\Rightarrow$ ΔH_S is negative $\Rightarrow$ Solubility increases at lower temperature.

Determination of ΔH_S

The working equation where,

$$\ln S = -\frac{\Delta H_S}{R}\left(\frac{1}{T}\right) + C$$



- S = molar solubility of the drug at T°
- K and R = gas constant
- S is determined at 5°C, 25°C, 37°C and 50°C.

$$\Delta H_S = -\text{Slope} \times R$$

SOLUBILIZATION

- For drug candidates with poor water solubility, preformulation studies should include limited experiments to identify the possible mechanisms for solubilization.
- Means of increasing the solubility are:

(i) Addition of a cosolvent to the aqueous system e.g. ethanol, propylene glycol and glycerin.

- *MOA*: These co-solvents disrupt the hydrophobic interactions of water at the non-polar solute / water interfaces.

(ii) Solubilization in micellar solutions such as 0.01 M Tween 20 solution.

(iii)Solubilization by forming molecular complexes e.g. benzoic acid forms complex with caffeine.