CHAPTER 1

Preformulation I (Physical Form: Crystal and Amorphous)

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Learning Outcome

After completing this chapter, you will be able to understand the

- Basic concept of preformulation
- Need of preformulation in drug formulation development
- Various components of preformulation studies
- Effect of crystal and amorphous form on drug formulation
- Characterization of crystal form

Lesson Plan

- Concept of Preformulation
- Need of Preformulation
- Introduction to Preformulation process
- Amorphous form
- Crystal form & habit
- Effect of crystallinity on drug delivery or formulation
- Characterization of crystal form

Dear students let us begin our discussion on preformulation with an example from the kitchen. Can you answer these questions?

Why do not you add turmeric in green vegetable?

Why don't you cook pumpkin in pressure cooker?

Why do you use oil for preparing dough for puris and not for chapatis?

What do we need to know before preparing a recipe in kitchen?

Of course, we should be familiar with the main ingredients, their nature, properties, quality, and effectiveness and about the desired characteristics of the product to be prepared. Then only we can go ahead. Or if we are preparing something for first time or developing a recipe, we follow some standard ways of establishing the method.

Drug Formulation Development

In pharmaceutical sciences same holds true. We must have the knowledge of "Preformulation" before formulating a drug or active pharmaceutical in gradient (API) in to a dosage form.

API or drug is a chemical entity with affinity to the receptors and with positive or negative or nil intrinsic activity.

Timing of Preformulation: After the drug discovery cycle when preclinical and clinical trial confirm a medicinal effect of a drug, we plan to formulate it in a form that is called dosage form.

Or in case of developing a new dosage form of an existing API.

API is never administered in a raw chemical form. With the help of some excipients and additives a formulation is prepared to deliver the drug and it is called a dosage form. Like tablet, capsule, emulsion suspension etc.

The efforts are focused to have a dosage form with

- High degree of uniformity: in physical characteristics (weight. Content, hardness etc.) and drug release.
- Physiological availability, Bioavailability
- Therapeutic quality.

Preformulation

All the activities of characterization of physicochemical properties of the drug under study which are important to develop a stable effective and safe dosage form.

So, please note these three words- **Stable, Effective and Safe**

All the Preformulation activities revolve around these three words. Anyone point is skipped, the formulation fails.

Challenges

1. **Very small amount of API is available for the studies:** In initial stage of drug discovery, we have only a very tiny amount of API available sometimes in few mg. In natural products when you are synthesizing a drug or active ingredient from the natural product you have the yield in 0.1 mg, 0.2 or 0.5 mg sometimes. So, we have only a little amount (that to impure), this is the first challenge.
2. **Only preliminary data like melting point, spectral data and structure is available:** Now next question comes in mind. What kinds of properties we need to focus?

Let's move to these properties one by one.

(a) ***Physical properties:*** Physical form (crystal & amorphous), polymorphism, particle size, shape, flow properties, solubility profile (pKa, pH, partition coefficient),

(b) ***Chemical Properties:*** Hydrolysis, oxidation, reduction, racemization, polymerization

(c) ***Biopharmaceutical Classification System (BCS):*** dissolution & permeability

Planning Preformulation

1. First identify the dosage form (solid/ semi solid/ liquid) to be selected for development and then focus the efforts accordingly. Got my point? It would not be suitable to expect a complete solubility in aqueous solution, if you are just planning for tablet preparation. But on the other hand, it is the utmost requirement in injectable.
2. Pick and study the relevant physicochemical properties (as per the desired dosage form) and take it in priority.

A poor Preformulation study may lead to the disasters.

- Unstable/ ineffective or less effective and unsafe dosage form
- Loss of development time (You have put your energy, time, money, all things will go in vain if preformulation fails)
- Increased expenditure on development

- Triggering repeated need for in vivo bioavailability/bioequivalence studies (if the preformulation fails)

The data and protocols of preformulation studies are passed on to the F&D department for further formulation work.

Physical Properties

- Solid state properties
- Physical form
- Crystal & amorphous

Solid state is the most preferred state of API for developing any dosage form. Why?

- API can easily be crystallized
- Can easily be purified (by crystalizing)
- easy to handle than liquids
- better chemical stability than that of liquids

Solids may be of three types as per the internal structure (physical)

- Amorphous
- Liquid Crystal
- Crystal

Then the crystals can be further classified as per this Figure 1.1.

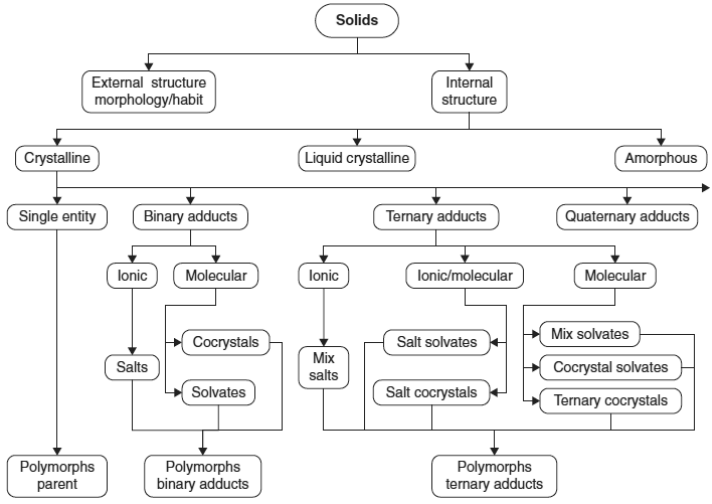


Figure 1.1 Classification of solids.

Amorphous

"These are the solids which do not exhibit long-range order in any of the three physical dimensions."

There may be the existence of short-range order for amorphous solids.

If you compare an amorphous phase with crystalline phase of a same drug, the amorphous phase always shows higher free energy, enthalpy, and entropy than the crystalline one. Let me clear you.

To be simple and straight, the amorphous form has the small particle size so more surface area and hence it is easily attacked by the solvent or the moisture. So, it is more hygroscopic. And more energy means less stability.

Use of Amorphous Form

These phases are used in improving solubility and hence the oral bioavailability of the poor water-soluble drugs. But these are less stable as compared to their crystal phase. To be precise, there exists some amount of crystal form in amorphous forms also. And this two-state model is well documented in USP. ***"According to the USP, the degree of crystallinity depends on the fraction of crystalline material in the mixture"***

So, the challenge lies in overcoming its stability issues. Being thermodynamically unstable, they might change into crystalline form (specially in suspension or in some solid dosage forms) with the passage of time or are very hygroscopic or prone to hydrolytic degradation. In general, the complete amorphous drug is typical to be formulated. The matter of fact is that only few amorphous drugs containing dosage form are marketed (**Table 1.1**).

Table 1.1 Drugs approved by FDA as amorphous drugs.

• Itraconazole, • Nelfinavir mesylate • Paroxetine • Celecoxib • Cefuroxime axetil • Cefepodoxime proxetil • Novobiocin

So, instead of complete amorphous phase many a times partial amorphization of a drug can be done using some techniques like solid dispersion, cyclodextrin-complexation etc. But the amorphous form should be

avoided until the difference in solubility make a significant impact on bioavailability.

Liquid Crystals

If the internal structure is having long-range order but only one or two dimensions, they are liquid crystalline materials. On the basis of number of components, these can be further classified as single, binary, and ternary LCs. But these are not of much use in pharmaceuticals.

Crystal

A majority of APIs are crystalline in nature. These are the solids with the internal structure having long-range order in all three dimensions. The logical method of classification of crystal is based on the angle between the faces.

If three dimensions are given by a, b, and c, then these crystals may be of several types on the basis of length of the faces and angle between these faces.

If a = b = c and angle between all the faces is 90 degree it is called simple cubic crystal (three equal axes each at right angle). And in the same way tetragonal, orthorhombic etc. Several common crystal forms are shown in **Figure 1.2**.

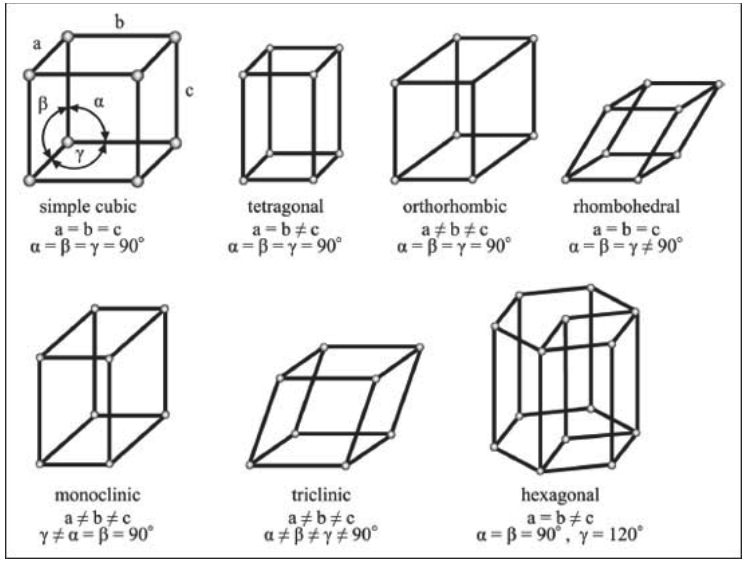


Figure 1.2 Common crystal forms.

But in practical, the cubic crystal may be perfect symmetrical cube, plate, needle or an aggregate of the imperfect crystals. The angle between the faces is 90 degree to each other is the sole criteria for a cubic system and not the relative length of the faces. These are crystal form.

Crystal Habit

We always talk about that "Habits die hard", actually it is our environment which makes our habit. By the form, we are unique. But our habits change, because we adapt with the environment or sometimes the environment forces us to adapt ourselves.

It is the relative development of different types of faces. Let's take the example of

NaCl in aqueous solution → Crystallizes into → Cubic face

NaCl in aqueous solution → Crystallizes in to → Octahedral (with small amount of Urea)

The crystals may have different habit depending on the process, impurities or conditions. (It refers to the types of faces developed and not the shape of the faces)

On the basis of number of components, they can be further classified as single entity, binary, and ternary adducts.

Single Entity

"The ability of a substance to exist as two or more crystalline phases that have different arrangements and/or conformations of the molecules in a crystalline lattice is called polymorphism."

We will discuss this property and pseudo polymorphism exclusively in coming chapters because it is very important in pharmaceutical sciences and preformulation.

Further, if the drug forms a binary composite of crystalline lattice with another chemical it is called binary adduct. And so on like ternary.

On the basis of the ionization states of these species, the adducts may be ionic, molecular or ionic/molecular.

Cocrystals

"Two or more molecules are hydrogen bonded to each other."

"Structurally homogeneous crystalline molecular adducts, made from components that are apparently neutral, that are by themselves solids at ambient conditions. The components are held together by interactions other than covalent or ionic bonds (hydrogen bonding, $\pi - \pi$, van der Waals, charge-transfer, halogen–halogen, etc.). "

The choice of cocrystal formation, depend on the need.

To control the hygroscopy of caffeine, its cocrystals with oxalic acid were prepared and showed the better stability even at high humidity also (Tarsk et al. 2005).

Carbamazepine-sachharin cocrystals showed better bioavailability, suspension stability and same stability as compared to its immediate release tablet (Hickey 2007)

Importance of Crystallinity in Preformulation

1. Solubility of drug candidates can be altered by modifying the crystal form:
2. Solubility can be improved by partial amorphization through developing adducts or binary composites of drug.
3. Onset of action can be controlled by using the crystalline form. Using a crystalline form cane delay the onset of action and prolong the drug release. Amorphous acts quite early but duration of action is not longer one. But the crystalline form acts slowly but the duration of action is longer. So, if you mix both of the form you can have both the advantages with quicker onset of action (from amorphous form) and prolonged duration of action (from crystalline form). The classical example is: lente insulin, a physical mixture of 70% crystalline ultralente and 30% amorphous semilente insulin give quick action and prolonged release.

   So you can blend the both the forms.
4. The purity standards are laid down by the properties of a pure crystal.

   US FDA states

   "It is mandatory to establish whether or not the API being studied exist in more than one crystalline form. If yes, what are the properties of all different crystal forms. Like melting point, solubility, stability, safety and efficacy."

How Crystals affect Solubility

In general, when a crystalline molecule is to be dissolved, firstly it is to come out from the crystal lattice. The amorphous solute molecule are free to move in

a solvent so easily dissolved. The enthalpy consideration delays the entry of drug molecule from crystalline lattice to solvent **Figure 1.3**.

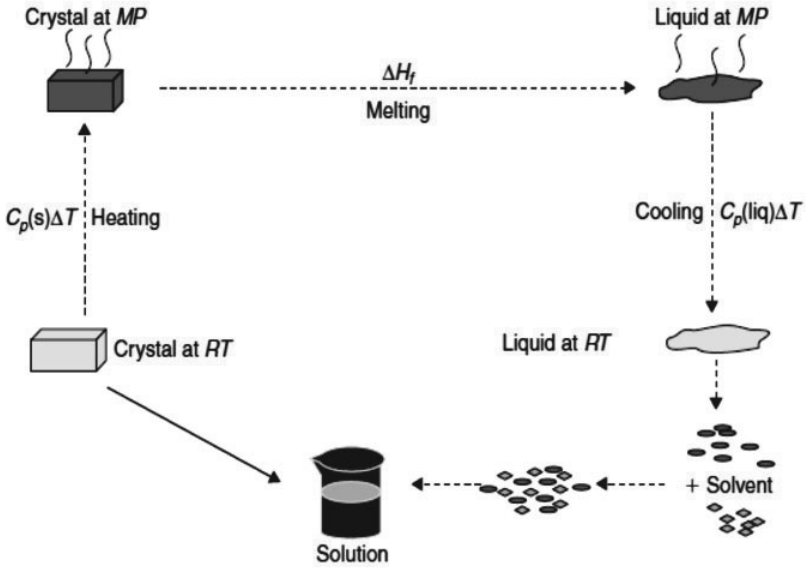


Figure 1.3 Role of crystallinity in solubility.

Characterization

Melting point: Melting point can indicate the type of crystal form. Melting point can be determined by

- **Capillary melting:** We observe the melting in acapillary tube in a heated metal block and note the melting range. (it is difficult to pinpoint the m.p. by this method)
- **Hot stage microscopy:** visual observation of melting under a microscope. The heating rate of sample is controlled and upto three transitions may be observed and recorded (onset of melting, half melt, completion)

Thermal Analysis or Differential Scanning Calorimetry (DSC)

The standard thermal analysis technique is either done by DSC or DTA. DSC stands for Differential Scanning Calorimetry while the DTA stands for Differential Thermal Analysis. In DTA the difference of the temperature (between the sample and a reference) is measured as a function of temperature or time (**Figure 1.4)**.

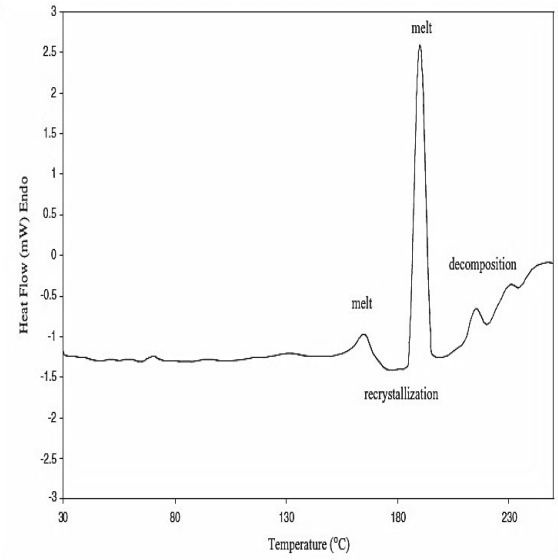


Figure 1.4 Heat flow in DTA.

While in DSC, all things are same like DTA except additional measurement of enthalpy or energy required to keep the sample at same temperature as that of reference.

This is the most suited characterization method for preformulation because it requires only 2-5 mg of sample.

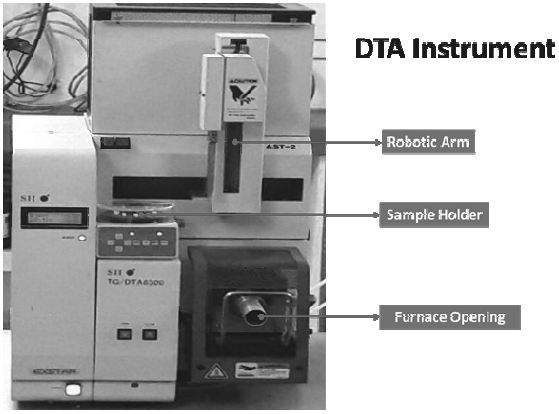
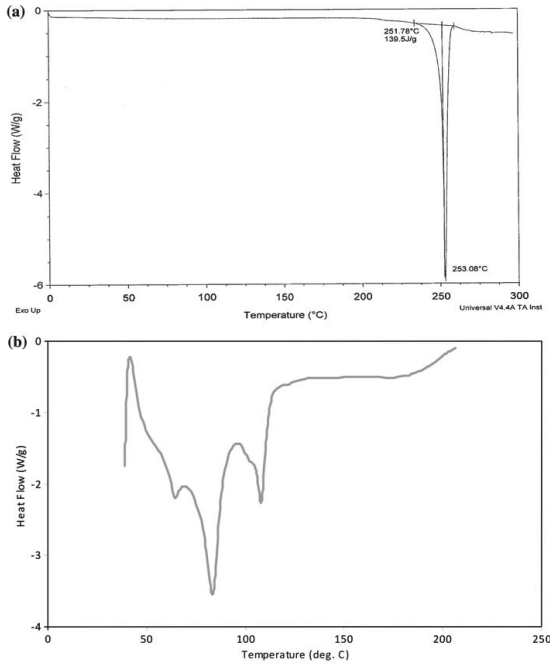


Figure 1.5 Standard DTA instrument; Courtesy: Institute Instrumentation Centre, IIT Roorkee.

A standard thermal analysis instrument DTA is shown in Figure 1.5.

In the first step the sample is prepared for that sample is taken in the crucible or the sample holder. Now the robotic arm will do the rest of the work. It picks up the sample holder and places it in the furnace. In the furnace another reference sample holder is present. The comparison of heat flow between the sample and reference is the observable parameter for DTA. The sample and the reference, both are heated at a constant rate and difference of the temperature is measured as a function of temperature or time. In DSC, all things are same like DTA except additional measurement of enthalpy or energy required to keep the sample at same temperature as that of reference. As there is loss of heat or gain of heat by the sample when it changes the phase upon heating, exothermic or endothermic signals are registered and recorded. The crystalline fusion, transition, evaporation and sublimation stages can easily be visualized through these thermograms. Thermal analysis gives a lot more information than the m.p. In this particular example (Figure 1.6) the first thermogram is the DSC of drug which shows clear endothermic peak which was found shifted in case of its complex (the bottom one) with other carrier. It is visible that the complex was showing the unique peaks unlike the drug and the carrier. Now after completion of the analysis, the sample is removed by the robotic arm. Please be cautious while removing the sample holder and never use bare hands to touch the crucible just after analysis. During the analysis it gets very high temperature, and it might burn your skin.



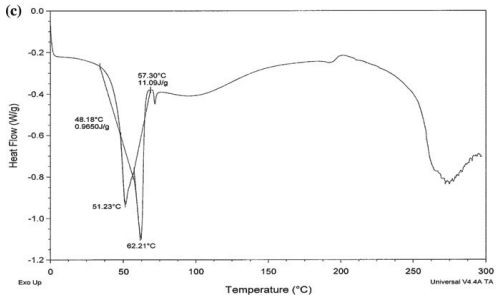


Figure 1.6 DSC thermograms of (a) drug; (b) carrier and (c) its complex (Semalty et. al., Journal of Inclusion Phenomena and Macrocyclic Chemistry, 2010, 67, 253-60).

X Ray Powder Diffraction (XRPD)

X rays are Electro Magnetic Radiations between UV and gamma rays. These are expressed in angstrom units.

When X rays are incident on crystalline solids, scattering of x rays takes place (Figure 1.7). This scattering is called diffraction. This diffraction is unique for a single pure crystal. And it is available in the repositories XRD data bank.

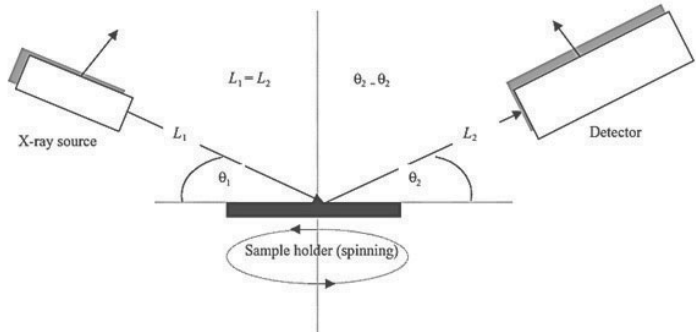


Figure 1.7 Principle of X Ray diffraction.

Bragg's law define the diffraction

$$n\lambda = 2d \sin \theta$$

Where λ is wavelength of a perfectly and monochromatic X-ray beam

θ is the angle of incident beam on crystalline sample

n is the order of reflection (an integer, usually 1)

d is the distance between planes in crystal

As shown in (Figure 1.8), it is a huge instrument and generally housed in a cabinet with glass window. Sample preparation is a very crucial step in case of X-RPD analysis. The sample should be properly dried. First step of sample preparation is to clean the sampling wells properly. Then the sample is placed in the sample holder. The sample should fill the sample well properly which can be achieved be using small pressure over the sample holder. Now the prepared samples are placed on the sampling area of the instrument and rest of the work is automated. Using the software based controlling we can vary the parameter of the analysis as per our need and start the process.

Figure 1.8 Standard XR powder diffractometer;
Courtesy: Institute Instrumentation Centre, IIT Roorkee.

Xray diffraction pattern gives the clear information about the crystallinity of the sample. Even the percent crystallinity can also be calculated using the X ray diffraction pattern. This is a non-destructive method. Sample can be recovered. But the sample size required is quite high, about 500 mg-1 g or more, depending on the instrument. The powder pattern consists of a series of peaks that have been collected at various scattering angles, which are related to d-spacings such that unit cell dimensions can be determined. In most cases, measurement of the d-spacings will suffice to positively identifying a crystalline material. The removal, shift and change in intensity of diffraction peaks give qualitative and quantitative information about the crystallinity of the sample in the crystal composite Figure 1.9. In the "Crystallographic Open Data Base" each drug molecule's unique XRD pattern is given for the reference. In this example, you can see that the sharp crystalline peak of drug is changed in XRD of its complex.

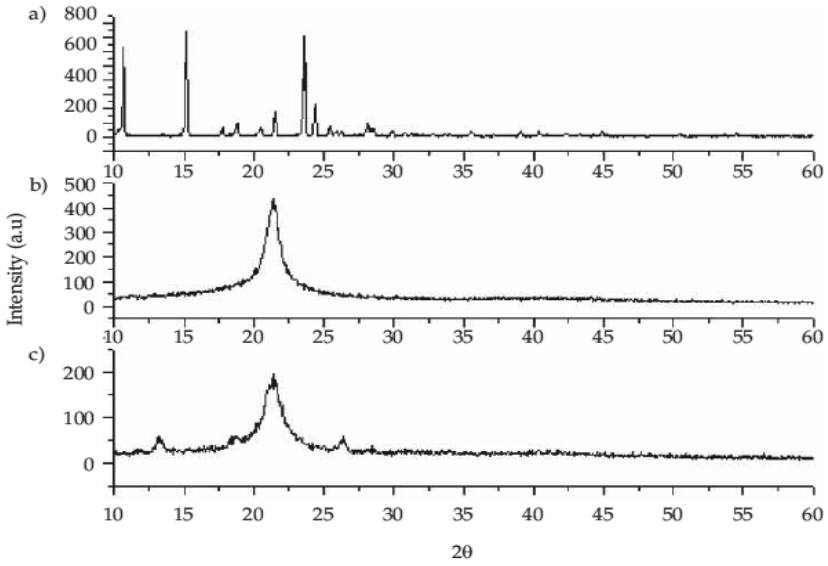


Figure 1.9 XRD of (a) drug; (b) carrier and (c) its complex
(Semalty et. al., Acta Pharmaceutica, 2009, 59, 335–344).

After completion of analysis, the samples are removed from sampling area and the sample holders are cleaned properly, again.

IR Spectroscopy

Infrared spectrum provides qualitative information about the solid under consideration. Different arrangement of atoms in solid state leads to a different molecular environment and subsequently leads to different stretching frequencies. This change can be used to distinguish a polymorphic form. Functional groups, change, and interactions may be detected with the help of IR.

IP and USP like official books possess a data bank of IR of standard drugs. This technique aids to XRD in confirming the purity of molecule.

Scanning Electron Microscopy (SEM)

It is useful technique for surface morphology and particle size measurement. The crystal habit can be visualized with SEM (Figure 1.10).

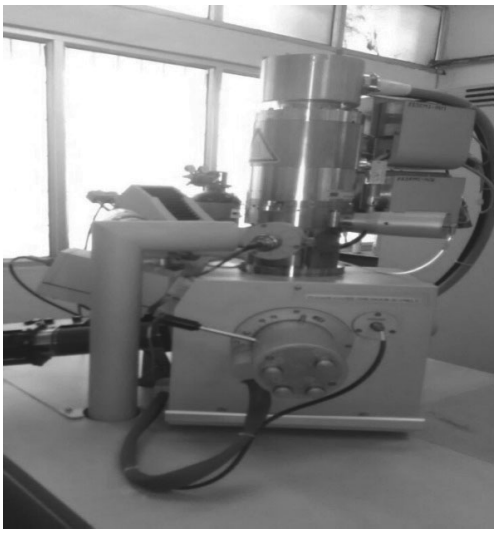


Figure 1.10 Scanning Electron Microscope (SEM);
Courtesy: Institute Instrumentation Centre, IIT Roorkee

First step in SEM is sampling preparation. The sample should be clean and free from moisture as the SEM instrument uses Vacuum in the process and presence of any moisture or dust can compromise the results as well as the analytical process. The sample preparation is done by coating the sample using gold thin film. The sample is stick to sampling disk and the disk is than placed in the sample preparation instrument. Vacuum is applied and flushing of instrument is done several times using argon gas. The sputtering is applied for required time while the vacuum of the sample preparation chamber is kept constant approximately 10-1 mbar. After sputtering stops, the sample disk is removed from the sample preparation chamber and placed in the SEM instrument. Now by using the software based controls, the parameters are set to the desired limits and imaging is started. Now the sample images are being shown in the computer screen. Freeze it, save and remove the sample. Precaution: Sampling area should not be kept open for a long time.

Misc. Methods of Characterization: Synchrotron radiation, solid state Raman spectroscopy, solid state NMR etc.

Summary

- Preformulation is the heart of formulation development.

- Safety, stability and efficacy are the three desired key elements of preformulation.
- Amorphous form- unordered form, more soluble, high free energy, less stable
- Crystal form- defined shape, less soluble, more stable, less free energy
- Crystals can be characterized by XRPD, IR, DSC and SEM.

Further Readings

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