CHAPTER 2

Crystal growth and characterization techniques

2.1 Crystal growth

2.2 Methods of crystal growth
   2.2.1 Concentration gradient methods
   2.2.2 Thermal gradient method
   2.2.3 Crystal growth by gel technique

2.3 Characterization techniques
   2.3.1 Single crystal X-Ray Studies (SXRD)
   2.3.2 Powder X Ray studies (PXRD)
   2.3.3 Fourier transform infrared spectroscopy (FTIR)
   2.3.4 Elemental analysis
   2.3.5 UV-VIS-NIR spectroscopy
   2.3.6 Dielectric studies
   2.3.7 Thermal analysis
   2.3.8 Scanning electron microscopy
CHAPTER 2

Crystal growth and characterization techniques

Crystallization is the process of arranging atoms or molecules that are in a fluid or solution state into an ordered solid state. Growth of new crystals are essential and important in various research fields like surface physics, crystallography, condensed matter physics, pharmaceutical materials and materials science. Though research in this field is going on for more than 100 years, still it is regarded as an important field when one considers its theoretical and practical applications.

2.1 Crystal growth

The main objective of crystal growth is to produce good quality crystals of suitable size. The shape of a crystal depends on the internal symmetry of matter and the relative growth rate of the faces. The crystallizing particles that bind most securely contribute to rapidly growing crystal faces and these faces are usually small and less well developed. Larger faces are usually formed in a direction where there are only weak intermolecular interactions. The factors that affect crystal growth and size of the crystal are solvent, nucleation, mechanics and time.

2.2 Methods of crystal growth

All crystallization methods change the physical state of a system from some non-equilibrium to an equilibrium state. Depending on the performance of this transformation, the crystallization methods can be classified into two categories namely concentration gradient and thermal gradient. In the concentration gradient method the concentration of the sample is increased either by removing the solvent or transferring the sample to another solvent system in which the material is less soluble. The thermal gradient method adopts the cooling of the sample to produce crystals.

2.2.1 Concentration gradient methods

1. Evaporation

It is one of the easiest methods to crystallize small molecular compounds. The choice of the solvent is so important that it can either influence the mechanism of crystal
growth or can incorporate in the crystal lattice. The rate of crystallization can be controlled by either reducing the rate of evaporation or by controlling the temperature. The number of nucleation sites may be increased either by seeding the solution or by scratching the surface of the vessel exposed to the solution.

2. Liquid and vapor diffusion

This method is applicable when evaporation method does not have fruitful results. The foremost step is to choose two solvents or solvent mixtures in which the sample is soluble in one solvent and insoluble in the other. These solvent systems should be immiscible or nearly immiscible for liquid diffusion and should be miscible for vapor diffusion.

In liquid diffusion method the less dense solvent is carefully layered on top of the more dense system in a narrow tube. The sample can be dissolved in either of the solvent. To avoid immediate precipitation or to grow crystals of large size, another solvent immiscible to both the solvents and the sample can be layered in between the solutions.

Vapour diffusion is carried out by dissolving a small amount of the sample in a solution taken in a small vial and carefully placing this vial in a large sealed vessel containing an insoluble solvent. During the crystallization process the vapor of the solvent in the outer vial will diffuse in to the inner vial causing precipitation of the sample. Protein crystals are often grown by a modified form of this technique called hanging drop method.

2.2.2 Thermal gradient method

Thermal gradient methods are useful in the production of high quality crystals. This method includes slow cooling of sealed saturated solutions, refluxing of saturated solutions, sublimation and zonal heating. Zonal heating is usually used to crystallise solid solutions or mixtures. Zonal refluxing of a saturated solution can be used to grow larger crystals. If sublimation is carried out in a sealed vessel it can take an advantage of evacuating or filling the free space with inert gases.

2.2.3 Crystal growth by gel technique

Gel technique is one of the easiest and inexpensive method used to grow crystals at ambient temperature (Henisch 1970, Dalal and Saraf 2006). The method involves
diffusion of two reagents in gel at a reasonably slow and controlled rate to yield a sparingly soluble reaction product. Being chemically inert, by preventing convection currents, the gel medium itself provides a soft three dimensional network in which the crystal nuclei are delicately held in the position of their formation (Desai 1987, 1988). Since the crystal grows at ambient temperature, one can ensure the presence of minimum equilibrium defects (Chauhan, 2009).

Gels can be classified as chemical and physical gels according to their formation. Chemical gels are formed by chemical reactions like hydrolysis or polymerization. Example: silica, polyacrylamide, tetramethyoxysilane etc. The physical gels are prepared from physical processes such as cooling. Example: gelatin, agar and clay.

The various methods used to grow crystals in gel include:
1. The chemical reaction method

In this method one can adopt two different types of growth procedure. In the former one, the two soluble reactants are allowed to diffuse through the gel where they react and forms an insoluble or relatively less soluble (in the solvent used) crystalline product. In this case crystals are formed well inside the gel. In the latter one, one of the reactants is allowed to diffuse through the gel impregnated with the other reactant and here crystals are formed both inside and on the surface of the gel.

2. The complex dilution method

There are materials whose solubility increase in the presence of another soluble material in a non linear way. This method of crystallization is suitable for such materials. During the diffusion process the concentration of the combined solutions decreases and hence the material reappears as crystals.

3. The reduction of solubility method

In this method an aqueous solution of the material is incorporated with the gel. On the surface of the set gel, a solution which reduces the solubility of the substance is added to induce crystallization. In some cases the pH of the solution determines the solubility of the material. Adding suitable alkaline or acidic solutions on the surface of the set gel impregnated with the material, crystal growth can be ensured by tuning the pH values.
4. The gel diffusion method

Some extremely insoluble compounds will precipitate immediately after synthesis. Crystals of desired size of such compounds can be grown by decreasing the rate of diffusion. This can be achieved by allowing the two reactants to diffuse through the two ends of a u-tube containing some gel medium. The only drawback of this method is the comparatively long time for crystallization depending on the rate of diffusion.

If one of the reactants is easily soluble in an alkaline solution, sodium metasilicate solution itself can be used as the solvent and the other reactant can be poured slowly over the surface of the set gel impregnated with the former one. The gel diffusion technique can also be applied to this method by adding a small volume of neutral gel on the surface of the previously set gel containing one of the reactants. The second reactant can then be poured over the surface of this neutral gel to enhance the quality of the crystals.

2.3 Characterization techniques

Different kinds of techniques are used to identify and study the physical and chemical properties of grown crystals. Identification is usually carried out by a combination of spectroscopic methods and chemical analysis. Each crystalline solid has its own characteristic powder X-ray diffraction pattern which may be used as a fingerprint for its identification. Once the identification is completed the next step is to characterize it. No single technique is capable of providing a complete characterization of a crystal, so a combination of diffraction, spectroscopic and microscopic methods is usually applied. In addition to this, other techniques like thermal, magnetic, and electrical measurements can be used to study the physical properties of the crystal which may give valuable information in certain application fields. So a brief description of the characterization techniques used in this study is presented here.

2.3.1 Single crystal X-Ray Studies (SXRD)

Single crystal X-ray diffraction studies can provide information about the molecular structure, internal lattice, unit cell dimensions, bond length and angles and details of site ordering. The data generated from the X-ray analysis is used to interpret and refine the crystal structure. A Brucker Kappa Apex 2 CCD diffractometer is used to collect data at
room temperature for the present investigation. The structures were solved by direct methods using the program package SIR-92 (Altomare et al. 1994) and refined using a full-matrix least square procedure on F² using SHELXL 97 (Sheldrick 1997). Anisotropic displacement parameters were applied to non hydrogen atoms in full matrix least square refinement based on F². Hydrogen atoms were refined as riders excepting those belonging to aqua ligands, which were refined isotropically in positions previously determined in the corresponding fourier map. Distance (DFIX) and angle (DANG) restraints were used for the aqua ligands in order to achieve reasonable geometric parameters. The IUCR software MERCURY (ver.2.3) is used for molecular graphics.

2.3.2 Powder X Ray studies (PXRD)

Powder X-ray diffraction is a non-destructive analytical tool used to characterize crystalline solid state materials. The development of fast X-ray detectors and new software has significantly improved the data collection and analysis and added new fields of application to this technique from materials science and engineering to medicine. From the PXRD line profile, one can observe the parameters such as peak position, intensity and width/breadth and shape, and each have specific applications.

Each crystalline material has its own unique powder XRD pattern and it can be used as an identity (finger print) of that material. As most of the pharmaceutical materials are polymorphic, this analytical tool provides information about structural changes associated with phase transitions and chemical transformations.

In the present work the powder X-ray diffraction measurements were carried out using a Bruker AXS D8 Advance machine with maximum usable angular range from 3° to 135° using a wave length of 1.5406 Å.

2.3.3. Fourier transform infrared spectroscopy (FT-IR)

This is the most widely used technique for the detection of functional groups and identification of organic compounds. Usually an infrared spectrometer records the absorption spectrum of pairs or group of atoms in the region 4000 to 400 cm⁻¹ region, that is in the middle frequency region of IR spectrum. The complexity of infrared spectra in the 1450 to 600 cm⁻¹ region makes it difficult to assign all the absorption bands, but the patterns found there are unique and therefore called the fingerprint region. Absorption
bands in the 4000 to 1450 cm\(^{-1}\) are usually due to stretching vibrations of diatomic units and is called the group frequency region.

In the present study the FT-IR of the crystal was taken in a Thermo Nicolet Avtar 370 DTGS spectrometer by KBr pellet method.

2.3.4 Elemental analysis

Elemental analysis is carried out to determine the stoichiometric ratio of the elements in the complex and hence to derive an empirical molecular formula. The Elementar Vario EL III machine, with precision > 0.1% abs, auto sampler and ultra microbalance, is used to determine the percent composition of carbon, hydrogen, nitrogen and sulphur in the present work.

2.3.5 UV-Vis-NIR spectroscopy

Visible and UV spectroscopy have several applications associated with the structure of materials, because the positions of the absorption bands are sensitive to coordination environment and bond character. UV absorption spectroscopy is one of the best methods for determination of impurities in organic molecules. Additional peaks can be observed due to impurities in the sample and it can be compared with that of standard raw material. The absorption spectrum can be used to determine the band gap. The relation between absorption coefficient \(\alpha\) and the incident photon energy \((h\nu)\) is given by Tauc relation (Tauc 1968),

\[
(\alpha h\nu)^{1/n} = A (h\nu - E_g)
\]

where \(A\) is a constant, \(E_g\) the band gap of the material, \(\alpha\) the absorption coefficient, \(h\) the Planck's constant, \(\nu\) the frequency and the exponent \(n\) depends on the type of transition (Joshi et al. 2003). An approximate value of band gap regardless of band structure can be determined by plotting the absorbance against energy, and extrapolating the curve to zero absorbance (Manoj and Beena 2011). The polarisability is highly sensitive to band gap and hence the empirical relationship,

\[
\alpha = \left[1 - \frac{\sqrt{E_g}}{4.06}\right] \frac{M}{\rho} \times 0.396 \times 10^{-24} \text{cm}^3
\]

Where \(M\) is the molecular mass and \(\rho\) the density, can be used to calculate the electronic polarisability (Chauhan and Arora 2009).
The drug–DNA interaction is mainly based on the electron transferability and electronic polarisability (Murthy et al. 2007a, Murthy et al. 2007b). The electronic polarisability has been correlated with nerve toxicity of a wide variety of chemicals acting on nerves of frogs, rabbits and human (Hansch and Kurup 2003).

Here in this work, a Varian Carry 5000 UV-Vis-NIR Spectrometer capable of recording data in the range 190-3200 nm with resolution 0.05 nm in UV and 0.2 nm in NIR is used to record UV-Vis-NIR data.

### 2.3.6 Dielectric studies

Dielectric properties relate to the ability of a material to polarise under the influence of an external electromagnetic field. The technique for measuring dielectric properties is known as dielectric spectroscopy. Most of the pharmaceutical materials are dielectric in nature. The information obtained from dielectric studies can be used for two purposes. First of all, the dielectric data can be used as a fingerprint with which samples prepared at different conditions can be compared to assure quality control. Secondly, each spectrum can be used to analyze the structure and behavior of the sample so that more specific information of the sample may be obtained (Duncan 2005). Dielectric spectroscopy can be used to monitor charge mobility of drugs since drugs act by ionizing into cations which then interact with anionic groups of DNA or phospholipid head groups. Also, the dielectric response provides information on structural characteristics of polymer, the interfacial properties of molecular films and water content and states of water and the effect of water as a plasticizer. The knowledge of water content of pharmaceutical materials is essential for microwave drying (Heng et al. 2010). Dielectric studies may be of use to distinguish between different crystal forms of the same drug (Shablakh et al. 1983). Simons and Williams (1992) by measuring the capacitance of a solid-liquid suspension showed that a change in the solid concentration will result in a change in the effective permittivity. The dielectric response may therefore be used to give an idea of the concentration of the solid phase present in the drug suspension.

Penn proposed a simple model for an isotropic semiconductor with electrons in a sphere of momentum space and are characterized by an isotropic energy gap \((E_g)\) which is the minimum energy required to excite an electron (Penn 1962, Ravindra et al. 1979,
Ravindra et al. 2007). To an extent, this theory can also be applied to dielectric materials (Chauhan and Arora 2009, Verma 2009). Plasmon—the quantum of plasma energy—depends on the number of free valance electrons. Considering this factor, the average valance electron plasma energy, $\hbar \omega_p$, can be calculated as,

$$\hbar \omega_p = 28.8 (Z \rho / M)^{1/2}$$ (3)

Where $Z$ is the total number of valence electrons, $\rho$ is the density, and $M$ is the molecular weight of the crystal. The Penn gap $E_p$ (average energy gap) and the Fermi energy $E_F$ can be calculated using the relations (3) and (4) (Suresh et al. 2011).

$$E_p = \hbar \omega_p / (C_{\infty})^{1/2}$$ where $C_{\infty}$ is the dielectric constant at high frequency (4)

And the Fermi Energy,

$$E_F = 0.2948 \left( \hbar \omega_p \right)^{4/3}$$ (5)

The polarisibility $\alpha$ can be calculated using the formula,

$$\alpha = \left[ \frac{\left( \hbar \omega_p \right)^2 S_0}{\left( \hbar \omega_p \right)^2 S_0 + 3 E_F^2} \right] \times \frac{M}{\rho} \times 0.396 \times 10^{-24} \text{ cm}^3$$ (6)

Where $S_0$ is a constant given by,

$$S_0 = 1 - \left[ \frac{E_p}{4 E_F} \right] + \frac{1}{3} \left[ \frac{E_p}{4 E_F} \right]^2$$ (7)

The value of $\alpha$ can also be calculated using Clausius-Mossotti relation,

$$\alpha = \frac{3}{4 \pi N_p} \left( \frac{\varepsilon - 1}{\varepsilon + 2} \right) \text{ where } N \text{ is the Avogadro number.}$$ (8)

The variation of dielectric constant with frequency of the grown crystals were studied at room temperature using a HIOKI 3532 LCR HITESTER meter, in the frequency range 350Hz to 6MHz.

### 2.3.7 Thermal analysis

Thermo Gravimetric/Differential Thermal Analysis (TGA/DTA) and Differential Scanning Calorimetry (DSC) techniques are applied for the evaluation and comparison of the thermal stabilities of drugs and pharmaceutical materials. The kinetic parameters (activation energy, frequency factor and order of reaction) are extremely useful in pharmaceutical quality control, for pre-formulation studies and lead optimization. (Venkataram et al. 1995, Cides et al. 2006). The most important objective of a pharmaceutical lead optimization is the improvement of ligand binding. The logarithm of binding affinity is proportional to the gibb’s free energy $\Delta G$. Since $\Delta G$ is a function of
the binding enthalpy $\Delta H$ and entropy $\Delta S$, its accurate determination is essential for drug design (Ferenczy and Keseru 2010, Alves et al. 2010). In addition to this, $\Delta H$ also reflects the interactions between the ligand and the target (Luque and Freire 2002). The knowledge of these parameters are useful in development, quality control, lead discovery and optimization in pharmaceutical industry (Ferenczy and Keseru 2010, Alves et al. 2010).

The thermo gravimetric analysis of a sample measures the amount and rate of change in mass as a function of temperature or time in a controlled manner in a selected atmosphere. The curve plotted with temperature versus mass is used to determine thermal and/or oxidative stabilities of materials as well as their compositional properties. The derivative thermogravimetric curve (DTG) is the first derivative of TG curve and shows peaks at maximum loss points. DTA measures the difference in energy provided to the substance and a reference material as a function of temperature or time. When the sample undergoes an endothermic or exothermic reaction, the differential heat flux necessary to keep the sample and the reference at the same temperature can be measured. Kinetic and thermodynamic parameters at different decomposition stages can be calculated from these graphs. The kinetic parameters of a sample can be calculated by Coats and Redfern methods (Coats and Redfern 1964, Ashok and Achar 2008),

$$\ln \frac{1-a^{1-n}}{T^2(1-n)} = \ln \left[ \frac{A}{\beta E_a} \left( 1 - \frac{2RT}{E} \right) \right] - \frac{E}{R \beta T} \text{ for } n \neq 1$$

(9)

$$\ln \frac{-\ln a}{T^2} = \ln \left[ \frac{A}{\beta E} \left( 1 - \frac{2RT}{E} \right) \right] - \frac{E}{R \beta T} \text{ for } n = 1$$

(10)

Where $E$ is the activation energy (J/mol), $R$ the universal gas constant (8.314 J/mol-k), $T$ the absolute temperature ($^\circ$K), $T_{max}$ the maximum decomposition temperature, $A$ the Arrhenius pre-exponential factor (S$^{-1}$), $n$ the reaction order, $\beta$ the heating rate ($^\circ$C/min), $a$ is the fractional weight loss and is calculated as $a = (w_t - w_\infty)/(w_0 - w_\infty)$, where $w_0$, $w_t$, and $w_\infty$ are the weights of the sample before degradation, at time $t$ and after total decomposition respectively.

A graph can be plotted with $\ln [g(a)/T^2]$ versus 1000/T

where $g(a) = \frac{[1-(1-a)]^{1-n}}{(1-n)}$ and $E$ and $A$ can be calculated from the slope and intercept of the plot. A sample plot of Coats and Redfern equation is shown in fig 2.1.
The thermodynamic parameters of the samples were evaluated by the following equations (Laidler 1987, Parikh et al. 2007).

change in entropy ($\Delta S$) = $2.303 \times R \times \log[\frac{A_h}{kT_m}]$

\hspace{1cm} \text{(11)}

where $k$ is the Boltzmann constant, $h$ the Planck’s constant, $T_m$ the peak temperature and $A$ the frequency factor.

change in enthalpy ($\Delta H$) = $E_nRT_m$

\hspace{1cm} \text{(12)}

the standard Gibbs energy $\Delta G = \Delta H - T_m \Delta S$

\hspace{1cm} \text{(13)}

The TGA/DTA studies for the present thesis work were performed using a Perkin Elmer Diamond TGA/DTA instrument with TG sensitivity: 200mg; DTA sensitivity: $\pm 1000\mu$V and temperature range of ambient to 1200°C.

2.3.8 Scanning electron microscopy

Scanning electron microscope (SEM) generates high energy electrons and focus them on the surface of the solid state sample and collects signals from electron-sample interactions. This collected signal contains the information about the external
morphology, crystalline structure, particle size and the orientation of the materials making up the sample. It also permits the observation of the material in the macro and submicron ranges. A JEOL Model JSM - 6390LV microscope is used to scan samples in this work.