CHAPTER 2
CHARACTERIZATION TECHNIQUES

2.1 SINGLE CRYSTAL X-RAY DIFFRACTION

Single crystal X-ray diffraction is an analytical technique in which X-rays are employed to determine the actual arrangement of atoms within a crystalline specimen. Single crystal X-ray diffraction is a non-destructive tool to analyze crystal structure of compounds, which can be grown as single crystals. The molecular structure, atomic coordinates, bond lengths, bond angles, molecular orientation and packing of molecules in single crystals can be determined by X-ray crystallography. Single crystal X-ray diffractometer collects intensity data required for structure determination. The monochromatic X-rays incident on a plane of single crystal at an angle theta are diffracted according to Bragg's relation \( 2d \sin \theta = n \lambda \) where \( d \) is the interplanar spacing of the incident plane, \( \lambda \) is the wavelength of X-rays and \( n \) is a positive integer. The intensity of the diffracted rays depends on the arrangement and nature of atoms in the crystal. Collection of intensities of a full set of planes in the crystal contains the complete structural information about the molecule. Fourier transformation techniques are used to determine the exact coordinates of atoms in the unit cell from this data.

In the present study, the single crystal X-ray diffraction analysis was performed using an ENRAF (BRUKER) NONIUS CAD 4 single crystal X-ray diffractometer. ENRAF NONIUS CAD4-MV 31 single crystal X-ray diffractometer is a fully automated four circle instrument controlled by a
computer. It consists of an FR 590 generator, a goniometer, CAD4F interface and a microVAX3100 equipped with a printer and plotter. The detector is a scintillation counter. A single crystal is mounted on a thin glass fiber fixed on the goniometer head. The unit cell dimensions and orientation matrix are determined using 25 reflections and then the intensity data of a given set of reflections are collected automatically by the computer. An IBM compatible PC/AT 486 is attached to microVAX facilitating the data transfer on to a DOS floppy of 5.25" or 3.5". Mo and Cu targets are available. Maximum X-ray power is 40 mA x 50 KV. Polaroid camera is available. UPS backed power supply takes care of the instrument during power failure for short periods. The ideal dimensions of the single crystal required are approximately 0.3 x 0.3 x 0.3 mm³. However, these dimensions may vary based on the habit of crystal growth and the diffracted beam intensity.

2.2 POWDER X-RAY DIFFRACTION

Single crystal X-ray diffraction is a powerful technique that is commonly used to determine the structures of new materials. However, the technique is limited by the ability to grow nearly perfect crystals that are suitable for diffraction. Due to this limitation and the time and cost-intensive nature of the technique, single crystal diffraction is not used for routine structural characterization of known materials. For routine structural characterization of materials, X-ray powder diffraction is far more common. The samples for powder diffraction may be large crystals, or they may be in the form of a powder composed of micro crystals that are too small to be seen by the human eye. The underlying principles of the experiment are the same in both powder diffraction and single crystal diffraction, although the data analysis is much simpler in powder diffraction.

Powder X-Ray Diffraction Analysis (PXRD) is the primary tool for characterizing the crystalline and amorphous materials. Characterization and
monitoring of solid-state properties of the active ingredients and excipients are fundamental elements of the pharmaceutical development since batch-to-batch inconsistency can cause crucial problems in the manufacturing of the pharmaceutical dosage form, the quality of the formulation, the bioavailability and drug stability.

In the present study, the powder X-ray diffraction analysis was performed using a Bruker AXS D8 Advance X-ray diffractometer. The Bruker AXS D8 Advance X-ray diffractometer consists of a copper X-ray source, a solid state detector, and a computer to control the diffractometer and collect and analyze the diffraction data. In this experiment, we will be observing Bragg diffraction of the X-rays from repeating planes of atoms in the structure of the sample. During data collection, the sample remains in a fixed position and the X-ray source and detector are programmed to scan over a range of 2θ values (2θ is the sum of the angles between the X-ray source and the sample and the detector). Routinely, a 2θ range of 5° to 70° is sufficient to cover the most useful part of the powder pattern. Choosing an appropriate scanning speed (measured in ° 2θ/min) depends on balancing the desire to collect a powder pattern quickly with obtaining a reasonable signal-to-noise ratio for the diffraction peaks. Usually how fast we can scan depends on the crystallinity of the sample. In most cases, we will begin with a scanning speed of 2°/min and recollect the diffraction pattern at a slower speed if the background is too noisy.

X-ray powder diffraction is a powerful tool for characterizing the products of a solid state synthesis reaction. At the simplest level, diffraction patterns can be analyzed for phase identification, which is, determining what crystalline substances are present in a given sample. More quantitatively, the peak positions can be used to refine the lattice parameters for a given unit cell. Unit cells in three-dimensional repeating structures have different shapes
based upon the symmetry of the structure. In all cases, the unit cells are parallelepipeds, but the different shapes arise depending on restrictions placed on the lengths of the three edges (a, b, and c) and the values of the three angles (α, β, and γ). The seven different unit cell shapes or the so-called seven crystal systems that result from these restrictions are listed below in Table 2.1.

Table 2.1 Seven crystal systems and lattice parameters of the unit cell

<table>
<thead>
<tr>
<th>Crystal System</th>
<th>Lattice Parameter Restrictions</th>
</tr>
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<tbody>
<tr>
<td>Cubic</td>
<td>a = b = c</td>
</tr>
<tr>
<td></td>
<td>α = β = γ = 90°</td>
</tr>
<tr>
<td>Tetragonal</td>
<td>a = b ≠ c</td>
</tr>
<tr>
<td></td>
<td>α = β = γ = 90°</td>
</tr>
<tr>
<td>Orthorhombic</td>
<td>a ≠ b ≠ c</td>
</tr>
<tr>
<td></td>
<td>α = β = γ = 90°</td>
</tr>
<tr>
<td>Monoclinic</td>
<td>a ≠ b ≠ c</td>
</tr>
<tr>
<td></td>
<td>α = γ = 90°, β ≠ 90°</td>
</tr>
<tr>
<td>Triclinic</td>
<td>a ≠ b ≠ c</td>
</tr>
<tr>
<td></td>
<td>α ≠ β ≠ γ ≠ 90°</td>
</tr>
<tr>
<td>Hexagonal</td>
<td>a = b ≠ c</td>
</tr>
<tr>
<td></td>
<td>α = β = 90°, γ = 120°</td>
</tr>
<tr>
<td>Trigonal</td>
<td>a = b ≠ c</td>
</tr>
<tr>
<td></td>
<td>α = β = 90°, γ = 120°</td>
</tr>
</tbody>
</table>

The difference between trigonal and hexagonal systems is the symmetry. A hexagonal unit cell has C₆ symmetry, whereas a trigonal unit cell only has C₃ symmetry. Each crystalline substance has a unique X-ray
diffraction pattern. The number of observed peaks is related to the symmetry of the unit cell (higher symmetry generally means fewer peaks). The $d$-spacings of the observed peaks are related to the repeating distances between planes of atoms in the structure. And finally, the intensities of the peaks are related to what kinds of atoms are in the repeating planes. The scattering intensities for X-rays are directly related to the number of electrons in the atom. Hence, light atoms scatter X-rays weakly, while heavy atoms scatter X-rays more effectively. These three features of a diffraction pattern: the number of peaks, the positions of the peaks, and the intensities of the peaks, define a unique, fingerprint X-ray powder pattern for every crystalline material.

Each peak in a diffraction pattern arises from a unique set of repeating planes in the structure. These sets of planes are oriented in all different directions in three-dimensional space. However, in order to see diffraction from a specific set, the planes must be oriented relative to the incident X-ray beam. Therefore, X-ray powder diffraction relies on a large number of crystallites in random orientations in order to observe the most diffraction peaks. Of course, the proper orientation is only one factor. Diffraction from a particular set of planes may not be observed or the peak intensity may be low due to symmetry (patterns of systematic absences) or other factors that contribute to low intensity. The powder diffraction of a substance is characteristic of the substance and forms a sort of fingerprint of the substance to be identified. The peaks of the X-ray diffraction pattern can be compared with the standard available data (Joint Committee on Powder Diffraction Standards (JCPDS) PCDFWIN) for the confirmation of the structure.
2.3 CHN ANALYZER

CHN Analysis is a form of Elemental Analysis concerned with determination of only Carbon (C), Hydrogen (H) and Nitrogen (N) in a sample. The most popular technology behind the CHN analysis is combustion analysis where the sample is first fully combusted and then the products of its combustion are analyzed. The full combustion is usually achieved by providing abundant oxygen supply during the combustion process. In this setup the analyzed products: Carbon, Hydrogen and Nitrogen oxidize and form carbon dioxide - CO$_2$, water - H$_2$O, and nitric oxide - NO, respectively. These product compounds are carefully collected and weighted. The weights are used to determine the elemental composition, or empirical formula, of the analyzed sample.

In the present study, the elemental analysis was performed using an Elemental Vario EL III C-H-N Analyzer.

2.4 INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETER (ICP-OES)

Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES) is an emission spectrophotometric technique, exploiting the fact that excited electrons emit energy at a given wavelength as they return to ground state after excitation by high temperature Argon Plasma. The fundamental characteristic of this process is that each element emits energy at specific wavelengths peculiar to its atomic character. The energy transfer for electrons when they fall back to ground state is unique to each element as it depends upon the electronic configuration of the orbital. The energy transfer is inversely proportional to the wavelength of electromagnetic radiation, $E = \frac{hc}{\lambda}$ (where $h$ is Planck's constant, $c$ the velocity of light and $\lambda$ is wavelength), and hence the wavelength of light emitted is also
unique. Although each element emits energy at multiple wavelengths, in the ICP-OES technique it is most common to select a single wavelength (or a very few) for a given element. The intensity of the energy emitted at the chosen wavelength is proportional to the amount (concentration) of that element in the sample being analyzed. Thus, by determining which wavelengths are emitted by a sample and by determining their intensities, the analyst can qualitatively and quantitatively find the elements from the given sample relative to a reference standard.

In the present study, the ICP – OES analysis was performed using a Perkin Elmer Optima 5300 DV analyser. Emission spectrometry is based on the principle that atoms or ions in an excited state tend, to revert back to the ground state and in so doing emit characteristic wavelength and intensity of that light is proportional to the concentration of that particular element in the sample solution

2.5 FOURIER TRANSFORM INFRA RED SPECTROMETER (FTIR)

For each spectroscopic method, it is helpful to understand how much energy corresponds to each wavelength and how this relates to the physical process after absorption of radiation. Organic molecules can absorb IR radiation between 4000 cm$^{-1}$ and 400 cm$^{-1}$ which corresponds to absorption of energy between 11 kcal/mole and 1 kcal/mole. This amount of energy initiates transitions between vibrational states of bonds contained within the molecule. In a conventional IR spectrophotometer, a sample IR beam is directed through the sample chamber and measured against a reference beam at each wavelength of the spectrum. The entire spectral region must be scanned slowly to produce good quality spectrum. The heart of an FTIR Spectrophotometer is a Michelson Interferometer built around the sample chamber. Radiation from an IR source is directed through the sample cell to a
beam splitter. Half of the radiation is reflected from a fixed mirror while the other half is reflected from a mirror which moved continuously over a distance of about 2.5 micrometers. When the two beams are recombined at the detector, an interference pattern is produced. A single scan of the entire distance takes about 2 seconds and is stored in the computer. In order that several scans may be added, they must coincide exactly. Obviously, this would be impossible considering the thermal fluctuations and vibrations in the laboratory. In order to solve this problem, a helium-neon laser is simultaneously directed through the Michelson Interferometer and the interference pattern of the laser is used as a frequency reference. Due to the sensitivity of the FTIR instrument, the most convenient and satisfactory method involves simple evaporation of a solution of the sample (chloroform, ether, dichloromethane; or even a CDC13 NMR sample may be used) onto a KBr salt plate and acquisition of the spectrum from the thin film remaining. This method provides excellent spectra with flat baseline unless the thin film is too powdery in which case excessive scattering of the light leads to an irregular baseline.

The PERKIN ELMER SPECTRUM RX I grating Infrared spectrophotometer was used for the present study.

2.6 UV-VIS-NIR SPECTROPHOTOMETER

A beam of light from a visible and/or UV light source (colored red) is separated into its component wavelengths by a prism or diffraction grating. Each monochromatic (single wavelength) beam in turn is split into two equal intensity beams by a half-mirrored device. One beam, the sample beam (colored magenta), passes through a small transparent container (cuvette) containing a solution of the compound being studied in a transparent solvent. The other beam, the reference (colored blue), passes through an identical cuvette containing only the solvent. The intensities of these light beams are
then measured by electronic detectors and compared. The intensity of the reference beam, which should have suffered little or no light absorption, is defined as I₀. The intensity of the sample beam is defined as I. Over a short period of time, the spectrometer automatically scans all the component wavelengths in the manner described. The ultraviolet (UV) region scanned is normally from 200 to 400 nm, and the visible portion is from 400 to 800 nm. Near-infrared spectroscopy (NIRS) is a spectroscopic method that uses the near-infrared region of the electromagnetic spectrum from about 800 nm to 2500 nm. If the sample compound does not absorb light of a given wavelength, I = I₀. However, if the sample compound absorbs light then I is less than I₀, and this difference may be plotted on a graph versus wavelength, as shown on the right. Absorption may be presented as transmittance (T = I/I₀) or absorbance (A = log I₀/I). If no absorption has occurred, T = 1.0 and A= 0. Most spectrometers display absorbance on the vertical axis, and the commonly observed range is from 0 (100% transmittance) to 2 (1% transmittance).

The PERKIN ELMER LAMBDA 35 UV-Visible spectrophotometer was used for the present study.

2.7 DIFFERENTIAL SCANNING CALORIMETRY

The basic principle underlying this technique is that when the sample undergoes a physical transformation such as phase transitions, more or less heat will need to flow to it than the reference to maintain both at the same temperature. Whether less or more heat must flow to the sample depends on whether the process is exothermic or endothermic. For example, as a solid sample melts to a liquid it will require more heat flowing to the sample to increase its temperature at the same rate as the reference. This is due to the absorption of heat by the sample as it undergoes the endothermic phase transition from solid to liquid. Likewise, as the sample undergoes exothermic
processes (such as crystallization) less heat is required to raise the sample temperature. By observing the difference in heat flow between the sample and reference, differential scanning calorimeters are able to measure the amount of heat absorbed or released during such transitions. DSC may also be used to observe more subtle physical changes, such as glass transitions. It is widely used in industrial settings as a quality control instrument due to its applicability in evaluating sample purity and for studying polymer curing. The result of a DSC experiment is a curve of heat flux versus temperature or versus time. There are two different conventions: exothermic reactions in the sample shown with a positive or negative peak, depending on the kind of technology used in the experiment. Differential scanning calorimetry or DSC is a thermoanalytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature. Both the sample and reference are maintained at nearly the same temperature throughout the experiment. Generally, the temperature program for a DSC analysis is designed such that the sample holder temperature increases linearly as a function of time. The reference sample should have a well-defined heat capacity over the range of temperatures to be scanned.

The NETZSCH DSC 200F3 instrument was used for the present study.

2.8 NLO TEST-KURTZ POWDER SHG METHOD

Second harmonic generation (also called frequency doubling or abbreviated SHG) is a nonlinear optical process, in which photons interacting with a nonlinear material are effectively "combined" to form new photons with twice the energy, and therefore twice the frequency and half the wavelength of the initial photons. It is a special case of sum frequency generation. Growth of large single crystal is a slow and difficult process.
Hence, it is highly desirable to have some technique of screening crystal structures to determine whether they are noncentrosymmetric and it is also equally important to know whether they are better than those currently known. Such a preliminary test should enable us to carry out the activity without requiring oriented samples. Kurtz and Perry proposed a powder SHG method for comprehensive analysis of the second order nonlinearity. The starting point is the Q-switched Nd: YAG laser with a fundamental laser beam of 1064 nm wavelength which serves as source. The power of the incident beam was measured using a power meter. The laser beam then passes through the energy and polarization control which controls the energy flow. The beam is then focused onto the sample through the focusing lens. The crystal was ground as powder and was packed densely between two transparent glass slides or capillary tube usually made of quartz and exposed to laser radiations. A sample of potassium dihydrogen phosphate (KDP), also powered to the same particle size as the experimental sample, was used as reference material.

The transmitted fundamental wave was passed over a monochromator which separates 532 nm (second harmonic signal) from 1064 nm. This is then collimated onto the spectral filters. The SHG detector usually employed is a photomultiplier tube. The green light was detected by a photo multiplier tube and displayed on a storage oscilloscope. The emission of green radiation confirms the NLO property of the material under study.