CHAPTER 2

DRUG SUBSTANCES AND INSTRUMENTS EMPLOYED
2.1 Preparation of Pure polymorphic forms

Lamivudine polymorphic Form I and Form II drug substances were prepared in Aurobindo Pharma Limited Research Centre (Hyderabad, India).

Identification of the forms was carried out by polarized light microscopy, FT-IR spectrometer, FT-Raman spectrometer and Differential scanning calorimeter, as well as X-ray powder diffractometer. According to our own experiments, which agree with the published results 24.

2.2 Instrumentation
2.2.1 X-Ray Powder Diffractometer

![Fig. 2.1 X-Ray Powder Diffractometer](image)
Diffractograms of pure Lamivudine crystal forms were measured on a Seifert 3003 TT Diffractometer using Cu Kα radiation (λ = 1.5418 Å) with 40 Kv accelerating voltage and 30 mA anode current at a step width of 0.03° 2θ and step time 1 second. Diffractogram collected over the range of 3-40° 2θ and these were processed by Rayflex Analyze, Version 2.352 software.

**Principle:**

X-rays are electromagnetic radiation lying between ultraviolet and gamma rays in the electromagnetic spectrum. The wavelength of x-rays is expressed in angstrom units (Å); 1 Å is equal to 10⁻⁸ cm. Diffraction is a scattering phenomenon. When x-rays are incident on crystalline solids, they are scattered in all directions. In some of these directions, the scattered beams are completely in phase and reinforce one another to form the diffracted beams. Bragg’s law describes the conditions under which this would occur. It is assumed that a perfectly parallel and monochromatic x-ray beam, of wavelength λ, is incident on a crystalline sample at an angle θ. Diffraction will occur if

\[ nλ = 2d \sin θ \]
Where $d =$ distance between the planes in the crystal, expressed in angstrom units, and $n =$ order of reflection (an integer).

X-ray powder patterns can be obtained using either a camera or a powder diffractometer. Currently, diffractometers find widespread use in the analysis of pharmaceutical solids.
2.2.2 FT-Raman spectrometer

Raman spectra were collected using Thermo Nicolet 5700 NXR-FT Raman spectrometer equipped with Nd-YAG laser source at 1064 nm wavelength, InGaAs detector and data handling TQ Analyst 7.2.0.161 software; 150 scans were collected at 0.7 watt exciting power and 4 cm\(^{-1}\) spectral resolution. Samples of about 50 mg were placed in a Gold coated disk and were then mounted on the sample holder such a way that the flat surface of sample to be exposed for the laser radiation and scans were collected over the range of between 4000 and 200 cm\(^{-1}\).
Principle:

The Raman spectroscopy involves the study of vibrational-rotational energy changes in molecules by means of scattering of light. Raman spectra occurs as a result of oscillation of a dipole-moment induced in a molecule by the oscillating electric field of an incident wave. As the induced dipole-moment is directly proportional to the polarisability of the molecule, the molecule must possess anisotropic polarisability which should change during molecular rotation or vibration for vibrational or rotational-vibrational Raman spectra. When a substance is irradiated with a monochromatic light of definite frequency (ν), the light scattered at right angles to the incident light contains lines of (i) incident frequency.

Fig. 2.4 Energy level diagram depicting Raman scattering
and (ii) also of lower frequency. Sometimes lines of higher frequency are also obtained. Thus, certain discrete frequencies above and below that of the incident beam will be scattered. It is called Raman scattering. The lines with lower frequency are called stoke’s lines. Also the lines with higher frequency are called Antistoke’s lines. The line with the same frequency as that of the incident light is called Rayleigh line. It is observed that the difference between the frequency of the incident light and that of a particular scattered line is constant and depends only upon the nature of the substance which is irradiated. It is completely independent of the frequency of the incident light. If $v_o$ is the frequency of the incident light and $v_R$ is the frequency of a particular scattered line, then:

$$\Delta v \text{ (Difference)} = v_o - v_R$$

This difference is called Raman frequency or Raman shift. It may be noted that Raman frequencies for particular substance are characteristic of that substance.
2.2.3 FT-IR spectrometer

Fourier transform infrared spectra (FT-IR) were obtained using a Perkin Elmer FT-IR spectrometer (Spectrum One-model) at a spectral resolution of 4 cm\(^{-1}\). Samples were prepared by slightly grinding about 2 mg of sample with 200 mg of KBr and pressed to pellet using hydraulic pressure. IR spectra were recorded over the range between 400 and 3600 cm\(^{-1}\).
**Principle:**

The absorption of Infra-red radiations causes an excitation of molecule from a lower to the higher vibrational level. We know that each vibrational level is associated with a number of closely spaced rotational levels. Clearly, the Infra-red spectra are considered as vibrational-rotational spectra. All the bands in a molecule are not capable of absorbing infra-red energy but only those bonds which are accompanied by a change in dipole-moment of the molecule are called infra-red active transitions. Thus, these are responsible for absorption of energy in the infra-red region. On the other hand, the vibrational transitions which are not accompanied by a change in dipole-moment of the molecule are not directly observed and these are infra-red inactive. For example, vibrational transitions of C=O, N-H, O-H etc. bands are accompanied by a change in dipole-moment and thus, absorb strongly in the infra-red region. But transitions in Carbon-Carbon bonds in symmetrical alkenes and alkynes are not accompanied by the change in dipole-moment and hence do not absorb in the infra-red region. It is important to note that since the absorption in the infra-red region is quantized, a molecule of the organic compound will show a number of peaks in the infra-red region.

Infra-red (IR) spectroscopy, especially when measured by means of the Fourier transform method (FTIR), is another powerful technique for the physical characterization of pharmaceutical solids. In the IR method,
the vibrational modes of a molecule are used to deduce structural information. When studied in the solid, these same vibrations normally are affected by the nature of the structural details of the analyte, thus yielding information useful to the formulation scientist. The FTIR spectra are often used to evaluate the type of polymorphism existing in a drug substance. The FTIR method makes simultaneous use of all the frequencies produced by the source, thus providing a large enhancement of the signal-to-noise ratio when compared with that of a dispersive instrument.

2.2.4 **Differential scanning calorimeter (DSC)**

![Block diagram of DSC](image)

**Fig. 2.6 Block diagram of DSC**

Differential Scanning Calorimeter (DSC) thermograms were obtained using a Mettler DSC 821e with data handling STARE Software. Sample
weighed about 2 to 3 mg in an Aluminium crucible (40 μL) having perforated closure were scanned at 10°C/min from 30 to 200°C under nitrogen atmosphere.

**Principle:**

In the DSC method, the sample and reference are maintained at the same temperature and the heat flow required to keep the equality in temperature is measured. Hence DSC plots are obtained as the differential rate of heating (in units of watts/second, calories/second, or joules/second) against temperature. The area under a DSC peak is directly proportional to the heat absorbed or evolved by the thermal event. Two types of DSC measurement are possible, which are usually identified as power-compensation DSC and heat-flux DSC. In power-compensated DSC, the sample and reference materials are kept at same temperature by the use of individualized heating elements, and the observable parameter recorded is the difference in power inputs to the two heaters. In heat-flux DSC, one simply monitors the heat differential between the sample and reference materials.

DSC can be used to obtain useful and characteristic thermal and melting point data for crystal polymorphs or solvent species. This information is of great importance to the pharmaceutical industry since many compounds can crystallize in more than one structural modification.
2.2.5 Polarized light microscope

Microscope images were collected from Leica DMLP polarized microscope equipped with C PLAN 10x/0.22 pol objective lens, soft imaging system CCD colour camera, the light source of projection lamp (12v, 100w) and data handling system (Soft imaging systems analySIS FIVE Image analysis software and Microsoft. Excel or equivalent).

Principle:
The polarizing microscope is essentially a light microscope equipped with a linear polarizer located below the condenser, and an additional polarizer mounted on top of the eyepiece. A rotating stage is also found to be very useful, as is the ability to add other optical accessories. Polarization optical analysis is based on the action of the analyte crystal on the properties of the transmitted light. This method can yield several directly measured parameters, such as the sign and magnitude of any observed birefringence, knowledge of the refractive indices associated with each crystal direction, what the axis angles are, and what the relations among the optical axes are.

To conduct a polarizing microscope analysis, the light from the source is rendered linearly polarized by the initial polarizer. The second polarizer mounted above the sample (the analyzer) is oriented such that its axis of transmission is orthogonal to that of the initial polarizer. In this condition of “crossed polars,” no transmitted light can be perceived by the observer. The passage, or lack thereof, of light through the crystal as a function of the angle between the crystal axes and the direction of polarization is of key importance to the method.