CHAPTER 1

GENERAL INTRODUCTION
1.1 Polymorphism of drug substances - Introduction and significance of polymorphism in pharma industry

It has been known since the middle of 18\textsuperscript{th} century that many substances could be obtained in more than one crystalline form\textsuperscript{1} but the subject of drug polymorphism has received extensive academic and industrial attention since the early pioneering reports of Aguiar and colleagues at Parke-Davis in which effect of polymorphism on dissolution and bioavailability were highlighted for Chloramphenicol palmitate\textsuperscript{2-3}. The existence of different crystal structures of the various polymorphs of a substance often causes these solids to exhibit a variety of different physical and chemical properties. Because of differences in the dimensions, shape, symmetry, capacity (number of molecules), and void volumes of their unit cells, the different polymorphs of a given substance have different physical and chemical properties arising from differences in molecular packing. Such properties include molecular volume, molar volume (i.e., molecular volume multiplied by Avogadro's number), density, refractive index along a given crystal axis, thermal conductivity, electrical conductivity, hygroscopicity, hardness, solubility, rate of dissolution in different solvents, chemical stability and interactions with biological systems. Differences in melting points of the various polymorphs arise from differences of the cooperative interactions of the molecules in the solid state compared with the liquid state. Also observed
are differences in spectroscopic properties, kinetic properties, and some surface properties. Differences in packing properties and in the energetics of the intermolecular interactions (i.e., thermodynamic properties) among polymorphs give rise to differences in mechanical properties. These differences in physical properties among the crystal forms of a polymorphic system have become extremely interesting to pharmaceutical scientists because their manifestation can sometimes lead to observable differences that have implications for processing, formulation, and drug availability. At the present time regulatory authorities require pharmaceutical companies to investigate and control polymorphism of drug substances to ensure product quality, safety and performance. Manufacturers have to declare that their API and product does not suffer solid phase transformation within the shelf life, which could affect bioavailability as well as to prove the non-infringement with respect to the of claimed polymorph form. Stability relationships between different solid forms of the substance and the storage conditions avoiding phase transitions have to be established. Gaining such information needs suitable solid state analytical methods to be able to differentiate polymorphic forms of the substance and often, methods of quantifying these solid forms.

In principle, a variety of different methods such as thermo analytical methods (e.g. DSC), X-ray powder diffractometry (XRPD), Solid-state
NMR, NIR, IR and Raman spectroscopy can be used for identification and quantification of specific crystal forms in drug substances. Above techniques for differentiating polymorphic forms of drug substances have been reviewed by several authors 8-11. Each method has its limits and a single analytical procedure is thus not likely to be applicable to all systems and arising problems. X-ray powder diffractometry (XRPD) is a widely used technique for the identification and quantification of polymorphic forms of drug substances since it is non-destructive in nature and requires relatively small amounts of sample. However, when the different polymorphic forms of the drug substance do not exhibit a distinct X-ray powder pattern and not having more intense characteristic peaks of unwanted polymorphic form of drug substance, then it is difficult to quantify the unwanted form with low limit of detection by XRPD. Raman spectrometry is not very sensitive to the physical apparition of the sample, which means that many solid samples can be studied directly without sample preparation, but it is sensitive to conditions at the molecular level. Thus, differences are often seen between the Raman spectra from different crystal forms of a compound, or between crystalline and amorphous forms. The advantage of Raman spectroscopy has been emphasized by several authors 10, 12-14. The technique requires no sample preparation and shows minimum interference from water. Chemometrics is equally applicable for the
analysis of Raman spectral data when univariate analysis is inappropriate 15-21.

1.2 Scope and Objective of the research work

In our current study the combination of chemometrics with FT-Raman spectroscopy method has been developed for the quantification of Lamivudine Form II content in Lamivudine Form I drug substance. Lamivudine, [cis-4-Amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one], which is a 1,3-oxathiolane nucleoside analogue, has proven anti-viral activity and exists in at least two polymorphic modifications.

Fig. 1.1 Molecular structure of Lamivudine
This reverse transcriptase inhibitor is in clinical use for HIV-positive and hepatitis B-positive patients\textsuperscript{22}. The existence of two polymorphic forms of Lamivudine viz., needle-shaped crystals (Form I) and bipyramidal crystals (Form II) are known \textsuperscript{23}. It has also been established that Form I is a hydrate (having one molecule of water to every five molecules of Lamivudine). It is stated that when Lamivudine is crystallized from aqueous solution or methanol, needle-shaped crystals (Form I) are obtained and when it is crystallized from non-aqueous solvents substantially bipyramidal crystals (Form II) are obtained \textsuperscript{24}. Further, another patent state that Form II is a more stable polymorphic form and used for the preparation of pharmaceutical drug products\textsuperscript{23}. It also discloses that Form I crystals are less stable and in certain pharmaceutical unit operations such as milling / granulation may cause conversion of Form I to Form II, which is an undesirable characteristic to manufacture a solid dosage form and thus is not favoured for the pharmaceutical formulation. It also suggested that Form II crystals can be obtained by grinding or milling of Form I, also Form II has been prepared by slurring Form I in solvents such as Methylated spirit. All these indicate the instability of Form I known in prior art. However, we have prepared the Lamivudine Form I crystals, which do not convert into Form II, during the preparation of solid pharmaceutical dosage forms as well as during storage at 80°C for 72 hours\textsuperscript{25}. The comparative studies were done with FT-Raman spectroscopy by using different chemometric
models with combination of different pathlength types. The results as well as the advantages of the FT-Raman spectroscopy method relative to other analytical techniques were discussed for Lamivudine drug substance. The present study demonstrates that FT-Raman spectroscopy can be used as a fast and efficient technique that is very suitable for the identification and quantification of polymorphic forms of Lamivudine drug substance according to regulatory requirements.