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EMERGING TECHNIQUES FOR POLYMORPH DETECTION

Deepali Manish Gangrade[1*], Priyanka Yashwant Kulkarni[2], Swati Devendra Bakshi[3]

[1] Assistant Professor, Department of Pharmaceutical Chemistry, Vivekanand Education Society's College of Pharmacy, Chembur, Mumbai, Maharashtra, India.

[2,3] Quality Assurance, Vivekanand Education Society's College of Pharmacy, Chembur, Mumbai, Maharashtra, India.

***Corresponding author:** deepali.gangrade@ves.ac.in

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ABSTRACT

Polymorphism presents a challenge to pharmaceutical industry to produce drugs of consistent quality. Polymorphic conversion occurs due to various sources like change in the temperature, presence of air, mechanical stress, humidity and lead to formation of new polymorph. Their presence can adversely affect the bioavailability, stability, safety and efficacy of the pharmaceutical products. To avoid these adverse effects, detection of polymorphic impurity is needed. Identification of polymorphic impurity can be done by various techniques either alone or in combination. The PXRD technique identifies different crystal phases by their unique diffraction patterns by capturing the three dimensional diffraction patterns in a two dimensional plot. DSC is a thermal method of analysis which is used to study thermal transitions. Infrared and Raman spectra identify a pure crystal form from different crystalline forms of the same organic compound. NMR spectroscopy indicates the presence of configurational or conformational multiplicity present in the solid state. Optical microscopy determines the optical properties and morphological properties of particles. Scanning Electron Microscopy determines surface topography and type of crystals (Polymorphism and crystal habit). HSM is an analytical technique enables the solid state characterization of materials by combining the properties of microscopy and thermal analysis. When single technique fails to identify the polymorphs, then combination techniques are used. DSC-FTIR technique gives spectroscopic and thermodynamic information about a solid or liquid sample undergoing thermal modification. DSC-PXRD allows simultaneous measurements of thermochemical and thermophysical events, while following changes in crystalline structure(polymorphism) during these events. This article briefly reviews, the importance of detection of polymorphism in pharmaceuticals, types of polymorphism and its characterization.

Keywords – *Polymorph ; detection of polymorph ; PXRD ; DSC ; FTIR*

1.INTRODUCTION

In any formulation study the investigation of drug polymorphism is important because polymorphism have a considerable influence on solid-state properties that may modify biopharmaceutical and technological behavior of drug. 'Polymorphism' comes from the Greek word, *Polus* = many and *morph* = shape. Polymorphism is ability of a product to exist in more than one crystalline forms with different space lattice arrangements.[1] Over 85% of active pharmaceutical ingredients (APIs) have been reported to possess more than one polymorphic form in the solid state .[2,3] As a result of polymorphism, molecules have different arrangements in the unit cell of its crystal

and thus display different physical and chemical properties. This difference in physical and chemical properties lead to changes in its solubility, stability, dissolution, bioavailability and efficacy of the drugs. [4,5]

2.POLYMORPHISM

Polymorphism is defined as the ability of a substance to exist as two or more crystalline phases that have different arrangements or conformations of the molecules in the crystal lattice.[1] The molecules present in the crystal lattice of a polymorph exhibit different types of non-covalent interactions like hydrogen bonds, van der waals forces and electrostatic interactions etc. Changes in such non-covalent interactions lead to different crystalline packing in polymorphs. These are two ways in which different crystal structures can arise:

2.1 Arrangement polymorphism

When rigid molecules of the same conformation are packed in different ways, it is called Arrangememnt Polymorphism e.g. Acetaminophen orthorhombic and monoclinic forms.[6]

2.2 Conformational polymorphism

When flexible molecules with different conformations are packed in different ways, it is called Conformational Polymorphism e.g. Imatinib Mesylate forms α and β.[7]

2.3 Types of polymorphism

In 1888, Lehmann coined the term monotropy and enantiotropy to distinguish between two different polymorphic behaviour.

Transition Temperature:

It is a temperature at which a material changes from one crystal (allotrope) to another. When one polymorphic form can change into another at a definite temperature and the two forms have a common vapour pressure, then it is known as 'Transition temperature'.

2.3.1 Enantiotropy

When the change of one form to the other at the transition temperature is revesible, the phenomenon is called Enantiotropy and the polymorphic forms are called enantiotropes e.g. : Rhombic sulphur (a-sulphur) on heating changes to monoclinic sulphur (ß sulphur) at 95.6ºc (transition temperature) and on cooling, again changes to rhombic sulphur at 95.6°c.

2.3.2 Monotropy

When one form is stable and the other is metastable. The metastable changes to the stable form at all temperature and the change is not reversible. Thus there is no transition temperature as the vapor pressures are never equal. This phenomenon is known as monotropy and the polymorphic forms are called monotropes e.g. : Graphite and diamond, graphite is stable and diamond is meta-stable, the change is infinitely slow.

3.NEED OF DETERMINING POLYMORPHISM

When and how polymorphic forms should be monitored and controlled is outlined by The International Conference on Harmonization's Q6A guideline, Specification: Test Procedure and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances.[8]The most stable form is normally used in the formulation for stability concern. The metastable polymorphic form may be inadvertently generated due to temperature, moisture and mechanical treatment during processing or storage of the drug product.[19]

Sometimes presence of different polymorphic forms in various batches of drug substances does not affect the performance of drug product, for example if the drug product is solution and all polymorphs are highly soluble whereas, in other case polymorphic form of drug is most important for the performance of drug product, for example, the original formulation of protease inhibitor ritonavir (Norvir) was the capsule containing hydroalcoholic solution of ritonavir. Appearance of new & dramatically less souble polymorph of ritonavir made it impossible to manufacture this product and necessitated the change in formulation.[10,11,12] Presence of polymorph affects the solubility,bioavailability,stability of drug products; to avoid these problems, detection of polymorphism is necessary.

4.INFLUENCE OF POLYMORPHISM ON DRUG PRODUCT

4.1 Influence on Solubility, Dissolution, and Bioavailability (BA) and Bioequivalence (BE)

Apparent solubility of the drug substance is significantly influenced by the solid-state properties of a drug substance. Because of differences in the internal solid-state structure, a drug substance that exists in various polymorphic forms can have different aqueous solubilities and dissolution rates.[13] The Biopharmaceutics Classification System (BCS)[14,15] provides a useful scientific framework for regulatory decisions regarding drug substance polymorphism. For a drug whose absorption is only limited by its dissolution, large differences in the apparent solubilities of the various polymorphic forms are likely to affect BA/BE. On the other hand, for a drug whose absorption is only limited by its intestinal permeability, differences in the apparent solubilities of the various polymorphic forms are less likely to affect BA/BE.

Examples where polymorphism affects drug product bioavailability include chloramphenicol palmitate [16] and carbamazepine. [17]

4.2 Influence on Manufacturing of the Drug Product

Drug substance polymorphic forms exhibit different physical and mechanical properties which include hygroscopicity, density, flowability, particle shape and compactibility,which affect processing of the drug substance and/or manufacturing of the drug product. Pharmaceutical processes such as crystallization, milling, freeze drying, spray drying, wet granulation and solid dispersion give rise to new polymorphic forms.[18]

The solid-state properties of the active ingredient affects the manufacture of the drug product, when drug product is manufactured by direct compression. On the other hand, the solid-state properties of the active ingredient are often masked by the resultant granulation, and the solid-state properties of the active ingredient are less likely to affect the manufacture of the drug product when drug product is manufactured by wet granulation.

4.3 Influence on Stability

Drug product stability is affected by the factors, like formulation, manufacturing process, and packaging, it is the stability of the drug product and not stability of the drug substance polymorphic form that should be the most relevant measure of drug quality.

Eg : Aspirin : During grinding process, aspirin form I gets converted to polymorphic form II; form II increases the degradation rate of drug in suspension. This ultimately affects the stability of suspension.

5.FACTORS RESPONSIBLE FOR GENERATION OF POLYMORPHS

5.1 **Temperature**

Change in temperature condition cause polymorphic transition.This gives rise to new polymorphic form of drug e.g. Sulphur crystal has critical temperature of 96^0C.Above this temperature monoclinic crystal of sulphur gets converted to rhombic sulphur.

5.2 **Wet/dry granulation**

Wet granulation causes change in the crystal structure of the drug, which leads to formation of new polymorphic form. e.g. Theophylline monohydrate form gets converted to anhydrous form during wet granulation method.

5.3 **Effect of Solvent**

The packing in the crystal lattice may differ with the polarity of the solvent ,hence results in new polymorphic form.

e.g. Lamivudine : with H_2O & CH_3OH it gives acicular crystals; whereas with Non-aq solvent it gives bipyramidal crystals.

5.4 **Effect Of Compression Pressure**

Force of compression cam cause polymorphic transition,which leads to formation of new polymorphic form. e.g.Phenylbutazone form III gets converted to form II at compression pressure > 2000 kg/cm^2 .

Tablet compression has been reported to cause a polymorphic transition for many other drugs, such as acetaminophen, carbamazepine, piroxicam and chlorpropamide and to alter the physico-chemical properties of these drugs and, finally, to influence the dissolution rate and bioavailability of the final products [19-23]

5.5 **Equipment**

A change in the equipment used for drying the final drug substance can also result in formation of new polymorphs.

6.DETECTION OF POLYMORPHS

6.1 DSC (Differential Scanning Calorimetry)

In the DSC method, the sample and the reference are kept at the same temperature and the heat flow required to maintain the equality in temperature between the two is measured. This equality can be achieved by placing separate heating elements in the sample and the reference cells, where the rate of heating by these elements is controlled and measured. This method of measurement is termed power-compensation DSC, and it yields positive-going peaks for endothermic transitions and negative-going peaks for exothermic transitions. DSC plots are obtained as the differential rate of heating (in units of watts per second, calories per second or Joules per second) against temperature and thus they represent direct measures of the heat capacity of the sample.

e.g. : Four polymorphic phases of Terfenadine were identified by using DSC melting curves.[24]

The DSC curves for the two polymorphic forms of the fluconazole were recorded at the heating and cooling rates of 5^0C/min in flowing nitrogen, employing differential scanning calorimeter. As a result, a single endothermal peak was observed at 139.3 °C for sample A , indicating polymorph III ,the melting point of sample B was observed at 138.6 °C indicating the presence of polymorph II .[25-29]

6.2 PXRD : (Powder X-Ray Diffraction)

The molecules in a crystalline compound are ordered in a three-dimensional array called as lattice. When a collimated beam of X-rays is incident up this lattice,X-rays are diffracted. Every crystal form of a compound produces its own characteristic X-ray diffraction pattern. This technique is useful for distinguishing between solid-state forms of a bulk drug substance and for

characterizing changes in the solid state (e.g.,distinguishing between polymorphs, hydrates and solvates and characterizing phase transitions between them).

The analysis of x-ray diffraction data is divided into three parts.[30,31] The first is the geometrical analysis,where the exact spatial distribution of x-ray reflections is measured and used to compute the size and shape of a unit cell. The second phase entails a study of the intensities of the various reflections,using this information to determine the atomic distribution within the unit cell. Finally, the x-ray diagram is examined to deduce qualitative information about the quality of the crystal within the solid.

To measure a powder pattern,a randomly oriented powdered sample is prepared so as to expose all the planes of a sample.The scattering angle is determined by slowly rotating the sample and measuring the angle of diffracted x-rays (typically using a scintillation detector) with respect to the angle of the incident beam. Alternatively, the angle between the sample and the source can be kept fixed,while moving the detector to determine the angles of the scattered radiation. Because the wavelength of the incident beam is known, the spacing between the planes is calculated using Bragg's law.

$$\sin\vartheta = n\lambda / 2d$$

where,

ϑ is the angle of incidence of the X-ray,

n is an integer,

λ is the wavelength, and

d is the spacing between atom layers.[32]

The X-ray diffractograms of the powder of fluconazole were recorded at room temperature. The mixtures were prepared by grinding together known quantities of pure polymorphs III and II using pestle and mortar. The diffraction lines of sample A were identical to those of crystalline polymorph III while sample B matched polymorph II. Whereas sample C indicated the coexistence of polymorph III and polymorph II.[25-29] Powder X-ray diffraction was applied to characterize the new solid form C of Norfloxacin.[33]

6.3 FT-IR : (Fourier Transform Infa-Red)

Infrared (IR) absorption spectroscopy, specially measured by FTIR, is a powerful technique for the physical characterization of pharmaceutical solids. The principle of FTIR is based on the fact that bonds and groups of bonds vibrate at characteristic frequencies. A molecule that is exposed to infrared rays absorbs infrared energy at frequencies which are characteristic to that molecule.

In a molecule, the differences of charges in the electric fields of its atoms produce the dipole moment of the molecule. Molecules with a dipole moment allow infrared photons to interact with the molecule and causes excitation to higher vibrational states.

During FTIR analysis,a spot on the specimen is subjected to a modulated IR beam. The specimen's transmittance and reflectance of the infrared rays at different frequencies is translated into an IR absorption plot consisting of reverse peaks. The resulting FTIR spectral pattern is then analyzed and matched with known signatures of identified materials which helps to analyse the presence of polymorph.

Infrared spectroscopy applied to characterize the new solid form C of Norfloxacin. Only slight differences can be detected between polymorphs B and C, whereas significant differences between the polymorphic forms A and C can be seen in the whole spectral region.[34] Two forms of Linezolid have been characterized by means of FT-IR spectroscopy.[35]

6.4 Raman Spectroscopy

Raman spectroscopy is an ideal technique for polymorphic detection. Raman spectroscopy offers several particularly important advantages over other analytical techniques:

(1) As a vibrational spectroscopy technique, Raman is very sensitive to molecular geometry,

(2) Raman analysis requires little or no sample preparation,

(3) Collecting Raman spectra is much more rapid than previous techniques, and

(4) Raman spectrum can easily be acquired from very small samples

It is well established that vibrational spectroscopy is an effective technique for characterizing polymorphs and solvates. Raman spectroscopy, in particular, yields important information that can ultimately be related to the geometric structure of a molecule and its environment. Changes in crystal geometric structure or salt electrostatic environment will cause band shifts in the spectra. Gross changes in symmetry with different crystal packing geometries will cause overall band splitting, coalescence, or relative intensity changes, as a result polymrph is detected.

A model tablet system with two excipients and a 10% API concentration, where the API is a mixture of the F-II and F-III polymorphs of piracetam was prepared. Using Transmission Raman spectroscopy (TRS) and NIR spectroscopy it was possible to detect F-II polymorph contamination in these model tablets.[36]

6.5 Microscopy :

6.5.1 Scanning Electron Microscopy

A scanning electron microscope (SEM) is a type of electron microscope with a focused beam of electrons that produces images of a sample by scanning it. The electrons interact with atoms in the sample, and produces various signals that can be detected and that contain information about the sample's surface topography and composition. The electron beam is generally scanned in a raster scan pattern, and the beam's position is combined with the detected signal to produce an image. Resolution achieved by SEM is better than 1 nanometer.

Morphology of Atorovastatin Calcium (ATC) drug samples by SEM showed crystalline samples (ATC-2 to ATC-6) to consist primarily of rod shaped crystals, whereas ATC-1 showed irregular particles. All amorphous samples (ATC-7 to ATC-12) comprised of irregular shaped particles.[37]

6.5.2 Optical Microscopy

The Difference of molecular structures of polymorph usually leads to their different macroscopic crystal properties such as morphology, size, colour which can be easily distinguished by optical microscopy.

For example, optical microscopy enabled visualization of the transformation of F-V sulphathiazole.[38]

6.5.3 Polarised Light Microscopy

Difference in the crystal structures of different polymorphic forms lead to differences in their optical properties, such as colour, refractive indices, extinction angle, and optical dispersion. These unique properties can be observed using plane polarised light and between crossed polarisers to distinguish between different solid forms either as a pure form or as a mixture of forms. Crystal twinning, particle size distribution, agglomeration, variation in crystal habit and mesomorphism can also be observed by polarised light microscopy.

In 1992, Ritonavir was discovered and in 1996, the FDA approved the sales of drug. About 2 years later the drug product failed dissolution tests, and investigation to control crystallisation of API was necessary. Examination by polarised light microscopy revealed a new crystal form which was thoroughly characterised as form II of ritonavir.[39]

6.5.6 HSM : (Hot Stage Microscopy)

Thermo microscope or hot stage microscope is a microscope coupled with a hot-stage accessory (either open or closed) which provides an information of physical characterization of pharmaceutical materials. The stage consists of a large area temperature control element with excellent heating and cooling systems with the temperature varying from -200 °C to 500°C. The material under investigation is placed onto the microscope stage. A color camera is attached to the microscope for observation of the visual changes. A high resolution color video camera is especially used when the pharmaceutical substances exhibit multiple transitions in small temperature ranges. The hot stage controller which is attached to the system monitors the temperature program as well as transmits the thermal analysis data to a computer for processing and analysis .

Hot-stage microscopy (HSM) combines the best properties of microscopy and thermal analysis to enable the solid state characterization of materials as a function of temperature.[40] Hot-stage microscopy is used to obtain visual and semi-quantitative information with regard to the transition of pharmaceutical polymorphs. This approach provides a unique insight into the polymorphic transitions and thermal behaviour exhibited by different crystal forms of a compound [41,42.]

Hot-stage microscopy is the most widely used technique for identifying different polymorphs of a given API through visualization of their diverse crystal habits and determining their unique solid state transformations.

Investigation the polymorphic transformations and thermal events occurring in sulfathiazole was done by using combination of HSM and differential scanning calorimetry with image analysis and light intensity profiles. Approved methods in literature were used for obtaining pure polymorphs. However, the experiments conducted showed that sulfathiazole tends to crystallize as mixtures of polymorphs.[43]

The solvates prepared from toluene and n-tridecane were observed by HSM technique. The HSM technique detected the gas evolved during the desolvation process of the solvates. It was observed that polymorphic transformations in crystals prepared from toluene occurred rapidly than those prepared from n-tridecane[44].

6.5.7 Solid State NMR : (Nuclear Magnetic Resonance)

Solid-state NMR spectroscopy is a powerful technique for analyzing the structural, chemical and physical properties of pharmaceutical solid.[45] This technique is usually non-destructive and non-invasive. In addition, the samples to be analyzed require no special preparation. Compared to other solid-state characterization techniques, solid-state NMR spectroscopy provides more vital information on the local environment in crystal packing and mobility in pharmaceutical solids.[46] In practice, solid-state NMR spectroscopy is used widely to characterize and identify polymorphs, pseudopolymorphs with different hydrate and amorphous compounds.

Solid-state 13 C NMR was used to determine the amounts of carbamazepine anhydrate and dihydrate [47] and, recently, to quantify relative amounts of delavirdine mesylate polymorphs and pseudopolymorphs. [48]

Different polymorphic forms of solid benoxaprofen, nabilione and pseudopolymorphic crystal forms of cefazolin have been reported by using solid state NMR.[49]

Indomethacin structural characterization was done using NMR spectroscopy. [50] The obtained spectra elucidated the structural differences between the polymorphs.

Coupling, or hyphenating, two instruments can uncover results and insights not possible with individual techniques.When single techniques fails to detect the polymorphs, then combinnation techniques like DSC-FTIR, DSC-PXRD are used.

6.5.8 DSC-FTIR

DSC–FTIR technique gives simultaneous thermodynamic and spectroscopic information about a solid or liquid sample undergoing thermal modification. DSC measures the endothermic and exothermic responses of the samples, at the same time the FTIR analysis observes their changes in chemical and physical composition. FTIR can provide

qualitative information that complements the heat flow changes measured by DSC.[51]

A sample of polyethylene terephthalate (PET) was also analyzed by DSC/FTIR. DSC was used to detect the glass transition, multiple melting endotherms and recrystallization of the polymer as it was heated and cooled. FTIR was used to monitor structural changes in the sample as it went through these thermal transitions.[52]

6.5.9 DSC-PXRD

XRD-DSC executes X-ray diffractometry (XRD) and Differential Scanning Calorimetry (DSC) at the same time as it heats or cools a sample at a particular heating or cooling rate or keeps the sample at a particular temperature. Simultaneous XRD and DSC measurements can give the outline of physico-chemical changes of the sample under heating or cooling processes within a short time without problems arising from separate measurements of XRD and DSC such as specimen inhomogeneity and difference in the temperature distribution.

XRD-DSC can easily reveal phase transitions, their temperatures and energy during heating and cooling using only 5–10 mg sample.

Special features :

(1) Simultaneously XRD and thermal data can be measured under the same environmental conditions of temperature and humidity;

(2) XRD and DSC data can complement each other for understanding reaction processes;

(3) Observation of reaction processes under controlled humidity conditions;

(4) Small amount of sample is required (<10mg) for analysis.

Dehydration process of trehalose dihydrate was first observed by DSC-PXRD. Structural changes of trehalose dihydrate with increasing temperature were divided into five stages. No appreciable change was first observed in trehalose dihydrate up to the temperature of 63°C (stage I). At the temperature of ~89°C, the XRD peaks of dihydrate disappeared which identifies dehydration of trehalose dihydrate and formation of anhydrate (stage II). After a small endothermic DSC peak at 120-133°C, this intermediate anhydrate phase changes into an amorphous phase as revealed by the XRD pattern (stage III). Around an exothermic DSC peak region of 175-200°C this amorphous phase were further changed into a crystalline anhydrate identified by the XRD patterns (stage IV). Finally, it melts at 205-218°C, indicated by the endothermic DSC peak and loss of crystalline peaks in the XRD pattern (stage V). [53]

CONCLUSION

Identification and characterization of polymorphic drug substances and products is significant due to widespread existence of polymorphs. The polymorphic forms of drug molecules have similar chemical structure, molecular formula and molecular configuration, but differ in physico-chemical properties like stability and solubility; this enables the identification of different polymorphs. XRD, DSC, HSM techniques are used in the initial characterization of the compounds. They provide physical analysis of the compound. The results are obtained within a short time utilizing a small amount of the sample. NMR technique provides more precise information about the crystalline packing of molecules in a unit cell. NMR also helps to resolve the conflicting results sometimes obtained by XRD and DSC techniques. All these techniques may be used separately or together.. Knowledge of the behavior of polymorphs allows the utilization of the proper form of the drug substance to achieve the best performance. An efficient characterization strategy helps selection of the best form of the compound for the formulations in the industry.

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