Solid state properties: Preparation and characterization

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ABSTRACT

The field of solid state characterization is central to the pharmaceutical industry, as drug products are manufactured as solid materials. Selection of the optimum solid form is a critical aspect in the development of pharmaceuticals, due to their ability to exist in more than one form or crystal structure (polymorphism). These polymorphs show different physical properties which can affect their biopharmaceutical properties. The possible result of changing crystal forms during late-stage of drug development had gave a good reason for the well thought out and early characterization of polymorphism. A detail understanding of polymorph characteristics allows selection of the best form to market. The focus is on recent developments and combined description strategy for different types of polymorphs using spectroscopic, crystallographic, microscopic and thermal methods of analysis.

Keywords: Characterization of drug; Polymorphism; Solvatomorphs; Spectroscopy

INTRODUCTION

Most drug based active medically helpful agents are given as solid dosage forms. Over the past ten years more importance and focus was on parts of chemical purity of drug substance but the situation has changed extremely over the period. Now the focus is on the safety and efficacy of drug as per needed things by the legal bodies. This has led to the increased attention being given to the physical properties of solids that contain a dosage form. [1]

The Physical properties and Solid state properties including polymorphism, pseudo polymorphism, can affect a lot the important properties that are essential to the successful development of drug candidate viz. solubility, intrinsic dissolution rates (IDRs), bioavailability and stability with respect to accelerated conditions and formulation excipients. [2]

During preformulation stage solid state properties of Active pharmaceutical ingredient (API) and the conditions under which the candidate drug should be formulated are examined. Key issues include investigation of polymorphism, the ability of a compound to exist in more than one crystalline form, and careful selection of the solid form for further development.

Preformulation studies strengthen the scientific foundation of the guidance, provide regulatory relief and conserve resources in the drug development and evaluation process, improve public safety standards, enhance product quality, facilitate the implementation of new technologies, facilitate policy development and regulatory decision making. Preformulation studies give directions for development of formulation in choice of drug form, excipients, composition, physical structure, helps in adjustment of pharmacokinetic and biopharmaceutical properties, support for process development of drug substance support for PAT (Process Analytical Technology) (critical process parameters), produce necessary and useful data for development of analytical methods. According to ICH, all technical requirements for the application of drug approval were harmonized in CTD format which are scientifically more elaborate by USFDA in QOS - QbR format. QbR is based on the principle of Quality by Design (QbD) which increased efficiency in the Food and Drug Administration (FDA) review process. [3]
1.1 Solid state properties [4]
Polymorphism is defined as the ability of a compound to exist in more than one form which is similar in their composition. (Fig.1)

Usually there are two types of Polymorphs, they are:

- Enantiotropic polymorph is the one which can be reversibly changed into another form by altering the temperature or pressure e.g.; sulfur
- Monotropic polymorph is the one that is unstable at all temperatures and pressures e.g.; glyceryl stearates.

Polymorphs may include solvates or hydrates (also known as pseudo polymorphs) and amorphous forms. (Fig.3)

- Solvatomorphism is defined as the situation where the various crystal forms of a compound differ in their solvation or hydrated state. It includes solvates and hydrates. (Fig.2).
- These type of molecular adduct in which the solvent molecules are incorporated in the crystal lattice of the solid are called as the solvates and the trapped solvent as solvent of crystallization.
- When the solvent in the association with the drug is water, the solvate is known as a hydrate.

There are three types of hydrates:
- Isolated lattice site H$_2$O: In this situation, the water molecule is not in contact with each other i.e. they are separated by drug molecules.
- Lattice channel H$_2$O: In this situation water molecule lie in channels and are hydrogen bonded, perform a space-filling role.
- Metal-ion co-ordinated H$_2$O: This situation arises in salts of weak acids.
There are certain rules for assigning the nature of phase relationship for polymorphic system given in Table 1

1.2 Impact of Solid state properties on solubility, bioavailability and stability [6]
The difference in the lattice energies (and entropies) associated with physical forms (amorphous, different polymorphs or solvates) leads to measurable differences in physical properties. Some physicochemical properties that may be affected include melting point, hygroscopicity, solubility, dissolution rate, stability (both physical and chemical), refractive index, thermal conductivity, surface activity, density, habit, electrostatic, mechanical and optical properties. Biopharmaceutical properties may also be affected.
Table 1: Rules for Assigning the Nature of Phase Relationships in Polymorphic Systems [5]

<table>
<thead>
<tr>
<th>Rules</th>
<th>Enantiotropic System</th>
<th>Monotropic System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fundamental Definition</td>
<td>Form 1 is the most stable polymorphic form at temperature below the transition point, while Form 2 is the most stable polymorphic form at temperature above the transition point</td>
<td>Form 1 is the stable polymorph at all temperatures below that of the melting point.</td>
</tr>
<tr>
<td>Heat of fusion</td>
<td>The enthalpy of fusion of Form-1 is less than the enthalpy of fusion of Form-2</td>
<td>The enthalpy of fusion of Form-1 is greater than the enthalpy of fusion of Form-2</td>
</tr>
<tr>
<td>Heat of Transition</td>
<td>The phase transition of Form-2 to Form-1 is endothermic</td>
<td>The phase transition of Form-2 to Form-1 is exothermic</td>
</tr>
<tr>
<td>Entropy of Fusion</td>
<td>The melting points of both Form-1 and Form-2 are less than the temperature of the transition point</td>
<td>The melting point of the most stable polymorph is higher than the temperature of the transition point</td>
</tr>
<tr>
<td>Phase Transformation</td>
<td>The phase transformation at the transition point is reversible.</td>
<td>The phase transformation at the transition point is irreversible.</td>
</tr>
<tr>
<td>Solubility</td>
<td>Form-1 is the most soluble polymorphic form at temperatures below the transition point, while Form-2 is the most soluble polymorphic form at temperatures above the transition point</td>
<td>Form-1 is the most soluble polymorph at all temperatures below that of the melting point.</td>
</tr>
<tr>
<td>Density</td>
<td>The density of Form-1 is less than the density of Form-2</td>
<td>The density of Form-1 is greater than the density of Form-2.</td>
</tr>
</tbody>
</table>

a) Solid state properties and solubility
The term solubility hints that the process of solution (or dissolution) has reached a balanced state such that the solution has become saturated. The intrinsic or built-in solubility of a substance depends on the particular solid phase (solvate or anhydrate) that is present. Since lattice energies of physical forms (amorphous, polymorphs or solvates) are responsible for the difference in solubilities and dissolution rates, the largest difference in solubility is observed between amorphous and crystalline materials. Since the entropy of the amorphous powder is high, the solubility of the amorphous form is the highest among all crystalline forms.

The solubility difference between different polymorphs is usually less as compared to solubility between amorphous and crystalline material.

According to the thermodynamic theory of solubility of solvates, the solvates are always less soluble in the solvent forming the solvate than the original solid. Thus, hydrates are less soluble in water than the corresponding anhydrous solid but solvates formed from other solvents, are more soluble in water, if the solvent is water-miscible than the corresponding non-solvated form. For example, caffeine hydrate is much less soluble in water than anhydrous caffeine, but the hydrate is much more soluble in ethanol than anhydrous Caffeine.

b) Solid state properties and bioavailability
The process of drug absorption requires initial transport through GI membrane for the delivery of drug molecule into systemic circulation. The mechanism is governed by the dissolution and initial permeability of drug molecule. The driving force of dissolution is aqueous solubility of the molecule. Hence, greater the aqueous solubility greater is the rate of dissolution.

Due to the effect of solid state forms on solubility and dissolution rate, the use of amorphous, different polymorphs or solvates it is expected to show its effect on the bioavailability. As the entropy of the amorphous powder is high, the solubility of the amorphous form is the highest among all crystalline forms. Hence, it can be concluded that the high solubility characteristic is an advantage in the design of a product with high bioavailability.

The effect of polymorphs on the bioavailability of chloramphenicol palmitate suspension is a classic example. Chloramphenicol palmitate exists in four crystal forms. Form B gave much higher blood levels than Form A after oral dosing of the suspension formulations. A particular suspension formulation even exhibited an unsatisfactory therapeutic effect. This was attributed to the fact that this particular formulation contained too much Form A rather than Form B.

c) Solid state properties and stability
For any test to provide reliable results, compounds need to have adequate chemical strength and stability. For compounds to be bio available, they need to have adequate stability in the gastric and intestinal fluid. For compounds to be successfully formulated into products, they need to be stable for the shelf-life of the products. These stability requirements have different meanings and different scales, thus require different studies and judging requirements. If a compound is chemically unstable as the crystalline material, the challenge to develop an oral dosage form will be very significant. If the compound is stable as crystalline material but not stable as amorphous material, the risk may
not be as high but controlling the amorphous content in drug substance manufacturing and formulation process may become a significant challenge.

For most pharmaceutical degradation reactions, because of the importance of molecular mobility, reaction rates are typically the greatest in the liquid or solution states and least in crystalline state, with intermediate rates occurring in the amorphous state. For example, NCEX is a PPAR-dual agonist for the treatment of type II diabetes. It exists in two monotonically related crystal forms, Forms I and II, with melting points of 160 and 140°C, respectively. After 4 weeks storage at 40°C/75% RH, Form I shows 0.3% total degradation compared to 6% for Form II and 20% for the amorphous material.

Considering the impact of Solid state properties on stability, solubility and bioavailability, it is not surprising that instances arise where crisis situation develop due to variability in physical properties of API, which could have been avoided if these had been better characterised. Ignoring the physical aspects of a formulation can be disastrous, because a variety of solid state reactions can compromise the stability of a drug entity.

Proper physical characterisation must be systematic in its approach and should follow a protocol which is rationally designed to obtain all needed information.[5]

One of the areas where physical characterisation of solids has become important is study of polymorphs and solvatomorphs. The polymorph or the crystal form used to produce the dosage form must be known, as should how crystal form changes might affect the performance or stability of the drug product. The nature of the crystal structure adopted by the given compound upon crystallisation exerts a profound effect on solid-state properties of that system, and these variations can translate into significant differences in properties of pharmaceutical importance. Hence, when designing formulations, it is imperative to know which crystal form of a drug is present at the various stages of a process and at the end of the process. [1]

1.3 Polymorphic Screening
The selection of the solid form of the active pharmaceutical ingredient (API) is made after polymorph screening, during which several solid forms of the candidate drug are generated and analyzed. Even though it is called polymorphic screening, it is understood that other solid forms cannot be neglected, since they may possess properties which make them the most suitable for development. (Fig.4)

There are important reasons for performing a polymorphic screening of API
i) Polymorphs have different physicochemical properties such as solubility, dissolution rate and melting point which influence their biological activity, pharmacodynamic properties and stability. Therefore polymorphic forms of API are considered by drug authorities (FDA) to be different entities.
ii) Drug product may not be thermodynamically stable in its current polymorphic form and during formulation of the drug product or during storage it may transform to another polymorph, which could lead to rejection of the API.
iii) The desired forms can be consistently manufactured
iv) The effect of storage condition on the dosage form can be evaluated and predicted.
• Polymorphic screening includes two steps:
i) Preparation of polymorphs
ii) Characterization of polymorphs
a) Physical characterization
b) Chemical characterization
Drug substance

1. Conduct polymorphism screen on drug substance
   Can different polymorphs be formed?
   YES
   Characterize the forms: e.g.,
   - X-ray powder diffraction
   - DSC/thermoanalysis
   - Microscopy
   - Spectroscopy
   GO TO 2.
   NO
   No further action

2. Do the forms have different properties? (solubility, stability, melting point)
   YES
   Is drug product safety performance or efficacy affected?
   NO
   No further test or acceptance criterion for drug substance
   GO TO 3.
   YES
   Set acceptance criterion for polymorph content in drug substance

3. Does drug product performance testing provide adequate control if polymorph ratio changes (e.g., dissolution)?
   YES
   Establish acceptance criteria for the relevant performance test(s).
   NO
   Monitor polymorph form during stability of drug product.
   Does a change occur which could affect safety or efficacy?
   NO
   No need to set acceptance criteria for polymorph change in drug product.
   YES
   Establish acceptance criteria which are consistent with safety and/or efficacy.

Fig.4: Tree diagram of polymorph screening
2.1 Preparation of polymorphs and solvatomorphs [2]

The preparation and identification of polymorphs and solvatomorphs have become extremely important, since the nature of the crystal structure adopted by a given compound upon crystallisation exerts a profound effect on the solid state properties of that system. It is well established that different crystal forms have different solubility characteristics, as well as different intrinsic dissolution rates (IDRs) and possibly even different bioavailabilities. In addition, different forms may have different degrees of stability with respect to humidity, temperature, light, and formulation excipients.

Practically, every protocol for the study of polymorphs and solvatomorphs, the first sequence of study entails crystallisation of the substance out of a variety of solvents and under a variety of conditions. Since it has been found that the crystal form of a substance often depends on the nature and identity of the crystallisation solvent, it is necessary to precipitate the drug substance from a wide range of solvent types and under a variety of crystallisation rates.

Owing to their differing chemical and physical properties, crystallizing solvents can often exert a strong influence on the nature of polymorphism associated with a given compound.

Solvents have been classified on the basis of their proton-donating, proton-accepting and dipole-interaction abilities.

<table>
<thead>
<tr>
<th>Solvent System Type</th>
<th>Preferred Solvents</th>
<th>Alternative Solvents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dipolar aprotic</td>
<td>Acetonitrile</td>
<td>Dimethyl formamide, dimethyl sulfoxide</td>
</tr>
<tr>
<td>Protic</td>
<td>Water (pH 3, 7, 10), methanol</td>
<td>Acetic acid, ethanol, 2-propanol, n-butanol</td>
</tr>
<tr>
<td>Lewis Acidic</td>
<td>Dichloromethane</td>
<td>Chloroform</td>
</tr>
<tr>
<td>Lewis Basic</td>
<td>Acetone, ethyl acetate</td>
<td>Tetrahydrofuran, methyl ethyl ketone, methyl butyl ether,</td>
</tr>
<tr>
<td>Aromatic</td>
<td>Toluene</td>
<td>Xylene, pyridine, anisole, ethylbenzene</td>
</tr>
<tr>
<td>Nonpolar</td>
<td>Hexane</td>
<td>Heptanes, cyclohexane</td>
</tr>
</tbody>
</table>

Solvents are also classified as:

1. **Class I solvents**: These solvents are to be avoided and these includes human carcinogens.
   E.g. Benzene, carbon tetrachloride, 1,2 dichloroethane.

2. **Class II solvents**: Solvents to be limited. These are non-genotoxic, animal carcinogens or possible causative agents. eg. acetonitrile, cyclohexane, toluene, methanol, chloroform etc

3. **Class III solvents**: Solvents with low toxic potential. eg. acetic acid, acetone, Ethanol, ether.

It is hoped that following information will prove useful in devising a screening protocol for the preparation of the various solid state forms of pharmaceuticals.

2.3 Methods for preparation of Polymorphic forms:

A) **Sublimation** [7]

On heating approximately two thirds of all organic compounds are converted partially from the solid to gaseous state and back to solid i.e. they sublime.

The sublimation temperature and the distance of the collecting surface from the material undergoing sublimation have a great influence on the form and size of crystal produced.

B) **Crystallization from a single solvent** [8]

Slow solvent evaporation is a valuable method for producing crystals. solutions of the material being crystallized, preferably saturated or nearly so are filtered to remove most nuclei and then left undisturbed for a reasonable period of time.

C) **Evaporation from a binary mixture of solvent** [9]

If single solvent solution do not yield the desired phase, mixtures of solvents can be tried. Multicomponent solvent evaporation methods depend on the difference in the solubility of the solute in different solvents.
D) Vapour diffusion
In this a solution of the solute in a good solvent is placed in a small, open container that is then stored in a larger vessel containing a small amount of a miscible, volatile nonsolvent. The larger vessel is then tightly closed. As solvent equilibrium is approached the nonsolvent diffuses through the vapour phase into the solution and saturation or super saturation is achieved.

E) Thermal Treatment [10]
Sometimes there is an exothermic peak between the two endotherms, representing a crystallization step. In these cases it is possible to prepare higher melting polymorph by thermal treatment.

F) Crystallization from the melt [11]
The cooling of melts of polymorphic substances often yields the least stable modification which rearrange to the stable modification in stages.

G) Rapidly changing solution pH to precipitate acidic or basic substances [12]
Many drug substances fall in the category of slightly soluble weak acids or bases, whose salt forms are much more soluble in water. Upon addition of acid to an aqueous solution of a soluble salt of weak acid or alkali crystals often results.

H) Thermal desolavation of crystalline solvates [13]
Desolvated solvates has been applied to compounds that were originally crystallized as solvents but from which the solvent has been removed by heat and vacuum. There is only small change in the crystal lattice parameters hence it is called as pseudopolymorphic solvates.

I) Growth in the presence of additives [14]
The presence of impurities can have profound effect on the growth of crystals. Some impurities can inhibit growth completely and some may enhance the growth.

J) Grinding [15]
Polymorphic transformation have been observed to occur on grinding of certain materials. This transformation require the three steps a) molecular loosening (nucleation by separation of the lattice b) solid solution formation c) separation of the product (crystallization of the new phase).

2.4 Method for preparation of Hydrate forms [16]
Pharmaceutical solids may come into contact with water during processing steps. When water is incorporated into the crystal lattice of the compound in stoichiometric proportions, the molecular adduct formed are referred as hydrates.

2.5 Method for preparation of solvate forms [17]
When solvents are used in purification of new drug substances by recrystallization, it is observed that the isolated crystals include solvent molecules either entrapped within empty spaces in the lattice or interacting via hydrogen bonding or vander waals force with molecule constituting the crystal lattice.

2.6 Method for preparation of Amorphous materials
A) Solidification of the melt
Amorphous solids are often created by rapidly cooling a liquid so that crystallization nuclei can neither be created nor grow sufficiently, whereas the liquid then remains in the fluid state well below the normal freezing point.

B) Reduction of particle size [18]
Reduction of the particle size of crystalline materials to the microcrystalline level can yield a material incapable of exhibiting on x-ray powder diffraction pattern.

C) Spray drying [19]
Spray drying is used to dry heat sensitive pharmaceuticals, to change the physical form of materials. In the spray drying process, a liquid feed stream is first atomized for maximal air spray contact. The particles then dried in airstream. It can produce spherical particles that have good flow properties.

D) Lyophilisation [20]
This technique is useful for compounds susceptible to decomposition in the presence of moisture but that are more stable as dry solids.
E) Removal of solvent from solvate or hydrate [21]
Solids can sometimes be converted to amorphous forms by simply allowing solvent molecules of crystallization to evaporate at modest temperature.

F) Precipitation of acids or bases by change in pH [22]
If the level of supersaturation is carefully controlled, it is often possible to avoid crystallization when a water soluble salt of weak acid is precipitated with a base or water soluble salt of weak base is precipitated with an acid.

G) Miscellaneous methods [23]
In this doping of crystals is done.

RESULTS AND DISCUSSION

3.1 Characterisation of solids (polymorphs and solvatomorphs)
It is important to characterize polymorphism for reasons such as: [6]

✓ Crystal forms may be patentable
✓ The types of solid forms that a molecule produces may be variable. Thus, some molecules yield true polymorphs and others solvates; some crystallize easily, others form glasses; and some polymorphs crystallize predictably, whereas others are elusive (‘disappearing polymorphs’)
✓ Regulatory expectations for the characterization of new drug products have expanded to include the polymorph types and their purity levels
✓ Polymorphism provides a unique opportunity to study structure–property relationships in organic solids and the thermodynamic and kinetic control of crystallization
✓ Solid forms may be ‘engineered’ to optimize certain physical properties (e.g. dissolution rate and bioavailability)

3.1.1 PHYSICAL CHARACTERIZATION [1,5]
A systematic approach to the physical characterisation of pharmaceutical solids is outlined. Physical properties are classified as being associated with

The molecular level – properties associated with individual molecules.
The particulate level - properties pertaining to individual solid particles.
The bulk level - properties associated with an assembly of particulate species.

A) Properties Associated With The Molecular Level [29,30];
Molecular properties may be defined as those material characteristics which theoretically could be measured for a small ensemble of individual molecules. Due to minimal sample requirements, molecular properties can be determined at the earliest stages of drug development.

I) VIBRATIONAL SPECTROSCOPY
1) Infrared spectroscopy
An extremely powerful method for study of pharmaceutical solids is Fourier transform infrared (FTIR) spectroscopy, with the vibrational modes of a compound being used for a variety of investigational purposes. (Fig.5)

IR spectroscopy is based on the conversion of IR radiation into molecular vibrations. For a vibration to be IR active, it must involve a changing molecular dipole (asymmetric mode). For example, vibration of a dipolar carbonyl group is detectable by IR spectroscopy. IR has been traditionally used as an aid in structure elucidation; vibrational changes also serve as probes of intermolecular interactions in solid materials. Sampling techniques for IR include pellets, mulls and diffuse reflectance. Diffuse reflectance is the best choice for crystal form determination because minimal sample manipulation is required. Mulls can also be used for form identification, but peaks that result from the suspension medium could interfere with the peaks of interest.

The acquisition of high quality infrared spectra on solid materials is possible only with FTIR method. Since transmission and beam attenuation problems are minimized. All FTIR spectrometers use a Michelson interferometer. Radiation entering the interferometer is split into two beams by means of a beam splitter. Beam A follows a path of fixed distance before being reflected back into the beam splitter, while Beam B travels a variable distance before being recombined with beam A. The recombination of these two beams yields an interference pattern. The detector is placed so that radiation in the central image of the interference pattern will be incident upon it, and therefore intensity variations in the recombined beam are manifest as phase difference. The frequency domain spectrum is obtained from the interferogram by performing the mathematical operation called Fourier transformation (FT). The overall detector output as a function of time is termed as interferogram and is sum of all the waves for each frequency component.
Importance

- Identification and characterization of polymorph in drug substance when the structural characteristics of a solid perturb the pattern of vibrational motion for a given molecule, these alteration can be used as a means to study the solid-state chemistry of the system.
- Identification of polymorphic forms in drug substance and can be very useful to study the water combined within hydrate species.
- Monitoring the stability of polymorph in drug substance and drug product
- Identification of raw materials and excipients

Kendall et al., [31] while studying spectra and structure of pigments found that IR technique can be conveniently applied to distinguish between different crystalline forms of substance of same chemical structure.

Blanco et al., [32] has studied potential of near infrared (NIR) spectroscopy for the characterization of polymorphs in the active principle of a commercial formulation prior to and after the manufacturing process. Polymorphism in active principles is extremely significant to the pharmaceutical industry. Polymorphic changes during the production of commercial pharmaceutical formulations can alter some properties of the resulting end-products. Multivariate curve resolution–alternating least squares (MCR–ALS) methodology was used to obtain the “pure” NIR spectrum for the active principle without the need to pretreat samples. This methodology exposed the polymorphic transformation of Dexketoprofen Trometamol (DKP) in both laboratory and production samples obtained by wet granulation. No polymorphic transformation, however, was observed in samples obtained by direct compaction. These results were confirmed using by X-ray powder diffractometry (XRD) and differential scanning calorimetry (DSC) measurements. Pure crystalline polymorphs of DKP were available in the laboratory but amorphous form was not, nevertheless the developed methodology allows the identification of amorphous and crystal forms in spite of the lack of pure DKP.

2) Raman spectroscopy

Another technique of vibrational spectroscopy that is ideally suited for characterization of solids is Raman spectroscopy. In this methodology, the sample is irradiated with monochromatic laser beam and the inelastic scattering of the source energy is used to obtain a vibrational spectrum of the analyte. Although most of the transitions observed by Raman spectroscopy are associated with internal vibrations and are thus insensitive to changes in the three-dimensional arrangement of the API molecules in the crystal, vibrations associated with functional groups capable of forming hydrogen bonds are affected by changes in the crystalline structure and often give easily detectable shifts. The Raman Effect originates from the interaction of the oscillating induced polarization or dipole moment of the medium with the electric field vector of the incident radiation. Raman spectra are measured by passing a laser beam through the sample and observing the scattered light either perpendicular to the incident beam. The scattered light is analyzed at high resolution by a monochromator and ultimately detected by suitable device.
Importance
✓ It has found widespread use for the qualitative identification and study of different solid state forms of compounds having pharmaceutical interest.
✓ Identification and spatial distribution of chemical components using Raman Microscope.
✓ Distribution of Active Pharmaceutical Ingredient and Excipients in solid dosage forms using Raman microscope and mapping.

Simone et al., [33] has done Significant work for the calibration of Raman spectroscopy to monitor the presence and amount of solid polymorphs in suspensions during crystallization, as well as the liquid concentration. Nevertheless, a clear and systematic approach to Raman calibration is missing in the literature. The present work has the aim of developing a methodical strategy for Raman calibration, taking into account the principal factors that can affect the Raman spectra of a specific compound in solution, such as solid type, solute concentration, temperature, crystal size and suspension density.

Dracinsky et al., [34] given that depending on crystallization conditions, many organic compounds can form crystals of different structure. In the present study, Raman spectroscopy combined with the density functional calculations is suggested as a complementary method to the X-ray and other higher resolution techniques. The potential to discriminate structural differences in polymorphic crystalline forms is documented on three model compounds of industrial importance. Methacrylamide, piracetam, and 2-thiobarbituric acid were crystallized under various conditions, and their Raman spectra were recorded using 532 and 1064 nm laser excitations. X-ray diffractometry and nuclear magnetic resonance spectroscopy were used as complementary techniques to verify sample composition and structure. To interpret the observed differences in Raman frequencies and intensities, three computational strategies were explored based on single molecule, a cluster model, and a plane-wave periodic boundary conditions calculation. The single-molecule modeling was found inadequate, whereas the plane-wave approach provides the most realistic spectra. For all compounds, the differences in the Raman spectra of polymorphic forms could be unambiguously assigned to the simulations. The modeling revealed that the spectral differences were caused by the molecular structure itself as well as by crystal packing. The relative importance of these factors significantly varied across the investigated samples. Owing to its simplicity, Raman spectroscopy appears to be a promising technique capable of reliable discriminating between organic crystal polymorphic states.

II) NUCLEAR MAGNETIC SPECTROSCOPY
The ultimate Characterization of pharmaceutical solids concerns the chemical environment of each atom in the compound and this information is best obtained through the use of NMR spectroscopy. NMR spectroscopy probes atomic environments based on the different resonance frequencies exhibited by nuclei in a strong magnetic field, hydrogen and carbon atoms are the most studied. Different crystal structures of a compound can result in perturbation of the chemical environment of each nucleus, resulting in a unique spectrum for each form. Once resonances have been assigned to specific atoms of the molecule, information on the nature of the polymorphic variations can be obtained. This can be useful early in drug development when the single crystal structure might not be available. Long data acquisition times are common with solid-state NMR, so it is often not considered for routine analysis of samples. However, it is usually a sensitive technique and sample preparation is minimal. This technique offers little interference from many excipients but the data acquisition can be complicated and lengthy.

Importance
✓ NMR spectroscopy can be used either qualitatively or quantitatively, and can provide structural data, such as the identity of solvents bound in a crystal.
✓ It is normally not difficult to assign functional groups to observed resonances, solid- state NMR can be used to deduce the nature of polymorphic variations.

Park et al., [35] gives that Donepezil hydrochloride is a reversible acetylcholinesterase inhibitor that is used in the treatment of Alzheimer’s disease to improve the cognitive performance. It shows different crystalline forms including hydrates. Therefore, it is very important to confirm the polymorphic forms in the formulations of pharmaceutical materials because polymorphs of the same drug often exhibit significant differences in solubility, bioavailability, processability and physical/chemical stability. In this paper, four different forms of donepezil hydrochloride were prepared and characterized using X-ray powder diffraction, Fourier transform infrared, and solid-state nuclear magnetic resonance (NMR) spectroscopy. This study showed that solid-state NMR spectroscopy is a powerful technique for obtaining structural information and the polymorphology of pharmaceutical solids.

Pacilio et al., [36] studied that High-resolution solid-state NMR (SSNMR) spectroscopy has many advantages as a tool to characterize solid-phase material that finds applications in polymer chemistry, nanotechnology, materials science, biomolecular structure determination, and others, including the pharmaceutical industry. The technology
associated with achieving high resolution has evolved to where SSNMR spectroscopy has become routine. To highlight SSNMR spectroscopy capability, an experiment exploring polymorphism in a pharmaceutical compound is described. Polymorphism can be studied by one-dimensional 13C NMR spectroscopy, presenting a straightforward experiment to highlight the techniques of cross-polarization, magic-angle spinning, and decoupling. To aid those unfamiliar with solid-state NMR methods, a detailed tutorial on the associated techniques is provided. The polymorphs of cimetidine, the active pharmaceutical agent of Tagamet, were selected to study.

B) Properties Associated With The Particulate Level:
Particulate properties are defined as material characteristics which theoretically can be determined by the analysis of one or a few particles. These properties can be investigated as soon as a drug candidate is available in milligram quantities.

I) X-RAY DIFFRACTION
X-ray diffraction techniques used for characterizing pharmaceutical solids include the analysis of single crystals and powders. (Fig.6) This technique is based on Bragg equation which describes the diffraction of a monochromatic X-ray beam impinging on a plane of atoms. Parallel incident rays strike the crystal planes at an angle \( \theta \) and are then diffracted at the same angle \( \theta \) and this knowledge, together with knowledge of the X-ray wavelength, can be used to calculate the spacing between the planes.

\[
n\lambda = 2d \sin \theta
\]

where,
\( n \) = order of the diffraction pattern
\( \lambda \) = wavelength of the incident beam
\( d \) = distance between the planes in the crystal
\( \theta \) = angle of beam diffraction

Bragg established that the diffraction angles were governed by the spacing’s between atomic planes within a crystal. He also reported that the intensities of diffracted rays were determined by the types of atoms present in solid and their rearrangement within a crystalline material. To measure a powder pattern a randomly oriented powdered sample is prepared so as to expose all the planes of a sample. The angle \( \theta \) is determined by slowly rotating the sample and measuring the angle of the incident beam. Knowing the wavelength of incident beam, the spacing between the planes \( (d) \) is calculated using Bragg’s law.

X-ray powder diffraction (XRPD) is the analysis of a powder sample with typical output being a plot of intensity versus the diffraction angle \( (2\theta) \). The value \( 2\theta \) is used based on the configuration of the instrument. Such a plot can be considered a fingerprint of the crystal structure and is useful for determining the crystallographic similarity of samples by pattern comparison. A crystalline material will exhibit peaks indicative of reflections from specific atomic planes; these patterns are representative of the structure but do not give positional information about the atoms in the molecule. One peak will be exhibited for all repeating planes with the same spacing. By contrast, an amorphous sample will exhibit a broad hump in the pattern called an amorphous halo.
Importance

✓ XRPD is a common choice for pharmaceutical dosage form analysis because it is a direct measure of the crystal form of a material.

✓ Powder X-Ray Diffraction Analysis (PXRD) is the primary tool for characterizing the crystalline and amorphous materials.

✓ Identification and characterization of polymorph in drug substance and drug product

✓ Monitoring the stability of polymorph in drug substance and drug product.

Davidovich et al., [37] has studied that Polymorphism has the potential to affect many aspects of drug development in the pharmaceutical industry. Multiple crystal forms with different solid-state properties can exhibit differences in bioavailability of the active drug substance, shelf life of the drug and behave differently during processing. Powder X-ray Diffraction is a powerful tool in identifying different crystal phases by their unique diffraction patterns. i. The most recent USP has included the criteria in the general chapter on using x-ray powder diffraction for phase identification and for the equivalence of two powder patterns ii. High-resolution PXRD patterns generally provide ample data to assess phase purity of crystalline samples. Small changes in the X-ray powder patterns in the form of new peaks, additional shoulders, or shifts in the peak position often imply the presence of a second polymorph.

Blanton et al., [38] has given that X-ray powder diffraction (XRD) is utilized for the determination of polymorphism in crystalline organic materials. Though convenient to use in a laboratory setting, XRD is not easily adapted to in situ monitoring of synthetic chemical production applications or thin film depositions. Near-infrared spectroscopy (NIR) can be adapted to in situ manufacturing schemes by use of a source/detector probe. Conversely, NIR is unable to conclusively define the existence of polymorphism in crystalline materials. By combining the two techniques, a novel simultaneous NIR/XRD instrument has been developed. XRD can detect crystalline phase changes and NIR can monitor solvent loss and/or water uptake.

II) THERMAL METHODS OF ANALYSIS:

Thermal methods of analysis are defined as those techniques in which property of the analyte is determined as a function of an externally applied temperature. Thermal methods can be extremely useful in preformulations studies, since the carefully planned studies can be used to indicate the existence of possible drug-excipient interactions in a prototype formulation. Thermal analysis is normally used to monitor endothermic reactions (melting, boiling, sublimation, vaporisation, desolvation, solid-solid phase transition and chemical degradation) as well as exothermic reactions (crystallization and oxidative decomposition).

Thermal methods include Differential Scanning colorimetry (DSC), Differential thermal analysis (DTA) and Thermogravimetry analysis (TGA).

1) Differential Thermal Analysis (DTA) involves monitoring of the difference in temperature between a sample and a reference as a function of temperature. Differences in temperature between the sample and the reference are observed when changes occur which require a finite heat of reaction. If $\Delta H$ is positive (endothermic reaction), the temperature of the sample will lag behind that of reference and yield positive going peak. If $\Delta H$ is negative (exothermic reaction) temperature of the sample will exceed that of reference and yield negative going peak.

2) Differential Scanning Calorimetry (DSC) measures the rate of heat flow and is used to measure the heat of transition. As all the transitions in materials involve flow of heat (into the sample in endothermic events and out of the sample for exothermic events) DSC is the universal detector for measuring the wide variety of transitions in pharmaceutical materials.

In this method, the sample and the reference are kept at same temperature and the heat flow required maintaining the equality in temperature is measured. This is achieved by placing separate heating elements in the sample and reference cells, with the rate of heating by these elements being controlled and measured. DSC plots are obtained as the differential rate of heating (W/sec, J/sec) against temperature. The area under the peak of DSC is directly proportional to the heat absorbed or evolved by the thermal event, and integration of these peak areas yield the heat of reaction.

3) Thermogravimetric analysis (TGA) measures physical changes in materials. Thermogravimetry is a measure of the thermally induced weight loss of a material as a function of the applied temperature. TGA provides quantitative measurement of mass change in materials associated with transition and thermal degradation. Thermogravimetric analysis (TGA) uses heat to force reactions and physical changes in materials. TGA records change in mass from dehydration, decomposition, and oxidation of a sample with time and temperature. The thermo gram data is a characteristic for specific materials and / chemical compounds due to unique physicochemical reactions occurring
over specific temperature ranges and heating rates. These unique characteristics are related to the molecular structure of the sample. TGA is restricted to studies which involve either a mass gain or a mass loss and is most commonly used to study desolvation processes and compound decomposition.

**Importance**
- **DSC** is used for analysis and characterization of Amorphous compound
- Detection of amorphous material in semi-crystalline compound
- Analysis and characterization of crystalline compounds crystallinity, melting and crystallization, Purity.
- Thermal stability
- The major use of TG analysis is in the quantitative determination of the total volatile content of a solid.
- TG analysis of compound decomposition can also be used to compare the stability of similar compounds.
- TGA is used for characterization of hydrates, solvates and the thermal behavior of compounds.

Atef et al., [39] has studied Raman spectroscopy as a quick and reliable method to quantify the alpha (α) and gamma (γ) polymorphic forms of indomethacin compared to differential scanning calorimetry (DSC). Binary mixtures with different ratios of α and γ indomethacin were prepared and analyzed by Raman and DSC. The Raman method was found to be more reliable and superior compared to DSC. The partial conversion of the alpha to gamma polymorphic form during the DSC measurement was the major limitation for the use of full DSC as a quantitative method and resulted in difference between the calculated and measured enthalpy of both polymorphic forms.

Chadha et al., [40] focused on estimation of transition temperature and stability of various forms of lamivudine. The forms were recrystallized from variety of solvents and preliminarily identification on the basis of SEM revealed existence of three forms (Forms I, II, III). DSC scans of Forms I and III show that these are metastable and undergo heat mediated transformation to Form II and Form III, respectively. Form II is phase pure with single sharp melting endotherm at 178.6°C. The thermal events are visually observed by hot stage microscopy. Enthalpy of solution of the forms is endothermic and magnitude varies in the order Form II > Form I > Form III suggesting Form III to be least crystalline which is well correlated with XRPD data. The transition temperature of the polymorphic pairs II/I and III/I/III derived from enthalpy of solution and solubility data revealed monotropy whereas enantiotropy exists in III/I. The slurry experiments showed Form II to be thermodynamically most stable. Forms I and III though stable in water are converted to Form II in ethanol, acetonitrile, and propanol after 1 day. Form III is converted to Form I in water after 7 days and the observation is of importance as this instability can effect the pharmaceutical preparations whereas Form I shows a balance between stability and solubility.

**III) PARTICLE MORPHOLOGY:**
Most pure pharmaceutical materials consist of small micro crystals aggregated into much larger composite structures. The nature of aggregate species can be quickly determined, and preliminary estimates regarding average particle sizes can be obtained. Microscopy is the best choice for the study of such aggregated species and both optical and electron microscopies.

1) **Optical microscopy** can be easily used to determine whether compound polymorphs are either enantiotropic or monotropic in their relation to each other. These experiments are carried out using the combination of a variable-temperature hot stage and polarizing optics. 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, the refractive indices associated with each crystal direction, the axis angles, and the relationships among the optical axes.

2) **Scanning electron microscopy (SEM)** is the technique of choice to obtain information at high magnification levels or when a three-dimensional view of particle surface is required. A conventional SEM is similar to an inverted light microscopy in that source lies above the specimen, the interrogating electron beam is focussed by a series of lenses, and the image is constructed on the basis of scattered electromagnetic radiation. In SEM the surface is scanned by a focused electron beam, and the intensity of secondary electrons is monitored. The output from the secondary electron detector modulates the raster of a cathode-ray tube, which is scanned in synchronization with the focused electron beam. Each point on the cathode ray tube raster corresponds to a point on the surface of the sample, and the strength of the image at each point varies according to the production of secondary electrons on the surface. As a result, SEM is able to give investigator an excellent picture of the details of the sample surface.

Carver et al., [41] has studied the particle sizes, morphologies, and structures are presented for succinic acid particles formed from the evaporation of uniform droplets created with a vibrating orifice aerosol generator. Particle sizes are monodisperse, and solvent choice is found to be the dominant factor in determining the final morphology and structure. The external particle morphologies range from round to cap shaped, while the surface roughness ranges
from fairly smooth to extremely rough and pitted. Internally, the particles have significant void space and noticeable crystals. X-ray diffraction confirms that the particles are crystalline. Thus, the morphologies of the particles take on a crystal filled structure that is unique in comparison to previous particles formed through droplet evaporation. The structure of the particles contains β succinic acid; however, the particles formed from water also contain α succinic acid. α Succinic acid has not previously been able to be formed from solution at near atmospheric conditions. The unique morphologies and ability to identify unexpected polymorphs provide for a potential tool to not only enhance particle engineering but also to identify metastable polymorphs.

Dalvi et al., [42] has carried out Precipitation of curcumin, a poorly water-soluble drug, by the liquid antisolvent technique in the presence of ultrasound and stabilizers. Curcumin particles with varied morphology were observed to have formed during precipitation in the presence of ultrasound and additives such as sodium dodecyl sulfate (SDS), Tween 80, hydroxyl propyl methylcellulose (HPMC), polyvinyl pyrrolidone (PVP), and bovine serum albumin (BSA). Characterization of the precipitated particles reveals that curcumin particles precipitated with ultrasound and large polymeric stabilizers such as HPMC, PVP, and BSA are superstructures formed by aggregation of several primary curcumin nanoparticles. The particles in these cases appeared to be loose aggregates (of ~1–5 µm in size) composed of several curcumin nanoparticles (~50–200 nm in size). The results obtained in this work suggest that curcumin particles with the desired physical form and morphology can be engineered through a careful manipulation/choice of ultrasound and additives during precipitation.

IV) PARTICLE SIZE DISTRIBUTION:
The particle size distribution of drugs and excipients will exert profound effects on mixing phenomenon and on possible segregation in mixed materials. The particle size distribution in a powdered material can affect the bioavailability of certain active drugs and exert a major effect on powder flowability. All pharmaceutical dosage forms must be produced in uniform units, and good content uniformity will be possible only when the particle size of active component is carefully controlled.

A variety of methods is available for the determination of the particle size distribution of powdered solids. These are optical microscopy (usually combined with image analysis), laser light scattering of particles suspended in inert solvents, electrical zone sensing or sieve analysis. Microscopy and sieving are normally carried out on dry powders and are therefore, useful as true indicators of the actual particle size of a powdered solid.

In principle, Sieve analysis represents the simplest method for the determination of particle sizes. Particles are allowed to pass through a series of screens and the amount retained on each screen is determined. The smaller particles pass through the screen and are termed as fines, while larger particles remaining on the screen are termed as coarse particles. Coulter principle can also be used for particle size distribution in which the measurement of electrical pulses caused by the passage of particles through a sensing zone is used to deduce size distribution.

Table 1b summarizes the information provided by each technique for different types of polymorphs. From this summary, the inter-disciplinary nature of polymorph characterization is indicated clearly.

Hu et al., [43] Raman spectroscopy has been widely used to monitor various aspects of the crystallization process. Although it has long been known that particle size can influence Raman signal, relatively little research has been conducted in this area, in particular for mixtures of organic materials. The aim of this study was to investigate the effect of particle size on quantification of polymorphic mixtures. Several sets of calibration samples containing different particle size fractions were prepared and Raman spectra were collected with different probes. Calibration models were built using both univariate and multivariate analysis. It was found that, for a single component system, Raman intensity decreased with increasing particle size. For mixtures, calibration models generated from the same particle size distribution as the sample yielded relatively good predictions of the actual sample composition. However, if the particle sizes of the calibration and unknown samples were different, prediction errors resulted. For extreme differences in particle sizes, prediction errors of up to 20% were observed. Prediction errors could be minimized by changing the sampling optics employed.

Kale et al., [44] Particle size enlargement is the process of transforming fine particles into larger particles by the introduction of external forces, and is a value-added step in many processes involving powdered materials. There are many different reasons to enlarge particle size, including increased flowability and improved product shape and appearance. With the multitude of options available to achieve enlarged particle product, it can be difficult to narrow down the best method for the desired application. The main factor in selecting the right kind of method is to specify the type of end product required. In the pharmaceutical industry in particular, uniform flow of solid mixtures is one of the most important considerations in solid dosage manufacture. The particle size distribution, shape, hardness, and
moisture content of the powder particles govern its flow and compactibility property. This article outlines making of enlarged particles and understanding their behavior.

C) Properties Associated With The Bulk Level:
Bulk material properties may be conveniently defined as those characteristics of a solid which require a large assembly of particles for measurement. Once a solid formulation has reached the bulk manufacturing stage, these bulk physical properties are certainly of the highest importance.

1) POWDER FLOW CHARACTERISTICS
One of the more important parameters of interest to formulators is the flowability of the powdered solid material. Carr described method which is extremely useful in the evaluation of the flowability of powdered solids. Powder flowability is evaluated using the angle of repose (defined as the angle formed when a cone of powder is poured onto a flat surface), compressibility (obtained from measurement of the bulk and tapped material densities) and cohesion (relating to the attractive forces which exist on particle surface). The overall summation of these permits deductions regarding the degree of powder flow ability.

2) SURFACE AREA
The surface area of a solid material is important in that provides information on the available void spaces on the surfaces of individual particles or aggregates of particles. The surface area of a solid is obtained by first adsorbing a monolayer of inert gas onto the solid surface at reduced temperature and then desorbing this gas at room temperature. Any inert gas can be used for BET measurements, but the preferred gases are nitrogen and krypton. Nitrogen is used for most samples exhibiting surface areas of 2$m^2$/g or greater, but materials with smaller surface areas should be measured using krypton.

Surface areas are calculated using the following equation:

$$\frac{P}{V(P_0 - P)} = \frac{1}{VmC} + \frac{1}{VmCP_0}$$

Where, $V$ = volume of gas adsorbed at pressure, $P$ = partial pressure of adsorbate, $Vm$ = volume of gas adsorbed in monolayer, $P_0$ = saturation pressure of adsorbate at experimental temperature, $C$ = A constant exponentially relating the heats of adsorption and condensation of the adsorbate.

Using various concentration of adsorbate, a graph of $P/V(P_0-P)$ against $P/P_0$ yields a straight line. The value of $Vm$ is obtained as the reciprocal of the sum of the slope and intercept.

The total surface area of the sample is calculated using,

$$St = \frac{\{Vm N_0 Acs\}}{M}$$

Where, $St$ = total surface area, $N_0$ = Avagadro’s number, $Acs$ = cross- sectional area of the adsorbate

The specific surface area is finally obtained $St$ by the sample mass taken.

D) CHANGES DURING PROCESSING [7]
The presence of different physical forms in various dosage formulations could be a result of the initial API that was used to manufacture the drug product or to changes that occurred during processing. Form changes can occur at various stages of the formulation process, including wet granulation, drying (including lyophilization), compression, handling and storage (including stability testing). Examples of possible changes that could occur during the formulation process are shown schematically in Fig. 7.
Common steps in the formulation process where form changes can occur will be discussed here; however, it should be noted that form changes can occur at almost any point in the process.

**i) Wet granulation and drying: the influence of moisture and heat**

Wet granulation is a frequent way of creating new forms, especially if hydrated forms are already known for the drug substance. Testing materials in the presence of water or aqueous solvents can help determine the feasibility of using wet granulation. In the case of cimetidine, anhydrous Form A was found to convert to hydrated Form B in water over a period of up to 12 months.

It was postulated that in a high shear mixer other mechanisms would likely increase the solid form transformations. In the case of an HMG-CoA reductase inhibitor (SQ33600), dry blending the formulation resulted in no change in crystal form; however, wet granulation transformed the crystalline material into amorphous.

**ii) Compression: the influence of mechanical force**

Tablets are one of the most common dosage forms used in pharmaceuticals and form changes upon compression is an active area of study. Several investigations have been performed on chlorpropamide. It is postulated that chlorpropamide Form A and C will partially convert to a highly unstable amorphous material upon compression and a form change occurs from the amorphous material. It was observed that the stable Form A will convert to the metastable Form C upon compression. Compressing the material at elevated temperatures will produce more of Form C. Compression of Form C at ambient temperatures results in the formation of Form A; however, at elevated temperatures, Form C is maintained. Compression pressures will also affect the conversion to another form.

Bauer has given awareness, knowledge, and understanding of polymorphism are important throughout the product lifecycle. Polymorphism may impact product development, clinical studies, product manufacturing, product quality, and product stability. Change management and validation in API manufacturing and product manufacturing should address the potential impact of formulation and process changes on API polymorphism.

**iii) Storage**

Storage conditions can have a crucial role in form stability. The effects of moisture and temperature can readily cause transformation between hydrated and anhydrous species but temperature alone can also induce solid transformations between unsolvated polymorphic forms. Recent studies at Purdue University (http://www.purdue.edu) have shown that seeding also accelerates transformations. Suspensions are also at risk for form transformations due to possible dissolution and recrystallization phenomenon, especially at elevated temperature conditions.

**iv) Chemical stability**

Chemical stability in dosage forms is also a concern and initial excipient compatibility studies are usually performed during development of the dosage form. Formulation can accelerate chemical degradation as a result of: (1) interaction with excipients, (2) processing effects and (3) induction by excipients (but not involving chemical
reactions with the excipients). Various reactions, such as oxidation, cyclization and hydrolysis, are common degradation pathways and excipient interactions, such as acid and Maillard reactions, also need to be considered during formulation development.

Stability information on both drug substance and product is required as a part of the registration dossier and serves to assign the shelf life, determine appropriate storage conditions and assure that the quality of the product is unchanged from the time of manufacture to the time of administration to the patient.

Stability of a pharmaceutical preparation can be defined as “the capability of particular formulation in a specific container/closure system to remain within its physical, chemical, microbiological, therapeutic and toxicological specifications throughout its shelf life.

3.1.2 Chemical Characterization [8]
i) Analytical Methodology and Validation
In Chemical characterization of API the methods are developed for identification and assay of drug substances and methods for deducing its profile of impurities. At the earliest state of development, it is most convenient to develop methods of chemical analysis that are either titrimetric or chromatographic in nature. However, if the methods are to be sufficiently reliable, they must be validated to an appropriate degree. The degree of validation will generally be less than that expected for later-stage work, but still must prove the quality of the method.

Various types of assay methods included in chemical characterization are:

1) Category I assays are those developed for the quantitation of major components of bulk drug substances or active ingredients in finished pharmaceutical products

2) Category II assays are those that are used for the determination of impurities in bulk drug substances or degradation products in finished pharmaceutical products and can include either quantitative assays or limit tests.

3) Category III methods are used to determine performance characteristics (such as dissolution profile, disintegration time, rate of drug release etc.) of a drug substance or dosage form.

For analytical methodology the procedure should be validated as to its precision, accuracy, specificity, limits of detection and quantitation, linearity and range.

Validation of analytical methods [27, 28, 45] in general, has been extensively covered in the ICH guidelines Q2A and Q2B in the FDA guidance and by USP. There are several other reports in literature, which have reviewed the concept, either in general or specifically the validation of spectroscopic non-chromatographic and chromatographic methods.

Overall, there are two stages in the validation of a Stability Indicating Assay Method. First stage is early in the development cycle when drug substance is subjected to forced decomposition studies and the SIAM is established based on the knowledge of drug degradation behaviour.

The main focus of validation at this stage is on establishment of specificity/selectivity, followed by other parameters like accuracy, precision, linearity, range, robustness, etc. The limits of detection and quantitation are also determined finds application in the analysis; of stability samples of bulk drug for determination of its retest or expiry period.

In the second stage, when the SIAM so developed is extended to formulations or other matrices, the emphasis gets limited to just prove the pertinence of the established validation parameters in the presence of excipients or other formulation constituents. Here only parameters critical importance like specificity/selectivity, accuracy and precision are revalidated.
Table 3: Recommended Validation Characteristics of the Various Types of Tests.

<table>
<thead>
<tr>
<th>Type of Tests / Characteristics</th>
<th>Identification</th>
<th>Testing for Impurities</th>
<th>Assay Dissolution (Measurement Only), Content/Potency</th>
<th>Specific Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Quantitative Limit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accuracy</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+4</td>
</tr>
<tr>
<td>Precision-Repeatability</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+4</td>
</tr>
<tr>
<td>Precision Intermediate –Precision</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+4</td>
</tr>
<tr>
<td>Specificity</td>
<td>+2</td>
<td>+</td>
<td>+5</td>
<td>+5</td>
</tr>
<tr>
<td>Detection Limit</td>
<td>-</td>
<td>-3</td>
<td>+</td>
<td>+4</td>
</tr>
<tr>
<td>Quantitation Limit</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Linearity</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Range</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Robustness</td>
<td>-</td>
<td>+</td>
<td>-3</td>
<td>+4</td>
</tr>
</tbody>
</table>

* Signifies that this characteristic is not normally evaluated.
+ Signifies that this characteristic is normally evaluated.
1. In cases where reproducibility has been performed, intermediate precision is not needed.
2. Lack of specificity for an analytical procedure may be compensated for by the addition of a second analytical procedure.
3. May be needed in some cases.
4. May not be needed in some cases.
5. Lack of specificity for an assay for release may be compensated for by impurities testing.

CONCLUSION

Solid-state analysis of API in drug products covers a wide variety of topics, ranging from the form present in the final dosage form to possible changes that can occur upon processing. It is important to note that form changes can occur at almost any stage during formulation depending on your solid-state system. Several analytical techniques are available for determining the crystal forms present in the drug product. These techniques can be used for qualitative or quantitative testing. It is important from a regulatory, as well as an intellectual property, perspective to know the crystal form(s) present in the marketed drug product and stability of drug product.

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