Characterization of the solid state: quantitative issues

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Abstract

Quantitative analysis of solid state composition is often used to ensure the safety and efficacy of drug substances or to establish and validate the control of the pharmaceutical production process. There are a number of common techniques that can be applied to quantify the phase composition and numerous different methods for each technique. Each quantitative option presents its own issues in ensuring accuracy and precision of the solid state method. The following article describes many of the common techniques that are used for quantitative phase analysis and many of the considerations that are necessary for the development of such methods.

Keywords: Crystallinity; X-ray powder diffraction; Solid state; NMR; IR; Near IR; Raman; Spectroscopy

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1. Introduction

It is well established that different solid forms (crystal, amorphous) of drugs exist, and can influence pharmaceutical drug products differently with respect to stability, dissolution, bioavailability, etc. [1]. As a result, significant effort is placed in identifying suitable solid forms of drug substances for use in pharmaceutical drug products. While instances may arise where metastable forms are desirable based upon a physical attribute, such as rapid dissolution rate or increased bioavailability, in most cases the
thermodynamically most stable solid form is selected. For polymorphic solids where the stability difference between two crystal forms is comparatively small, even in cases where the desired form of a drug can be manufactured in pure form, the extreme conditions used in processing a formulation into a dosage form can alter this form. For instance, wet granulation can result in crystallization of an amorphous form or the formation of a hydrated form. A reaction may occur between acidic and basic components of the formulation. Dehydration can result in a loss of crystallinity or formation of an anhydrate due to drying of a wet granulation. In suppositories, transdermal patches and pulmonary delivery systems, uncontrolled crystal transformations can result in differences in bioavailability.

Because the physical form of a drug can impact pharmaceutical drug product performance, there are occasions when it is not sufficient to merely qualitatively identify the forms present in the API or final product. In such cases, one may need to develop a quantitative method to monitor the production process and ensure that the active pharmaceutical ingredient (API) remains within manufacturing control limits and the drug product performance is not compromised. To meet regulatory requirements for drug product registration, flow charts were constructed for investigators to use as guidelines for characterizing compounds under development [2]. According to Byrn et al., quantitative methods are called for only in cases where mixtures of polymorphs or hydrates, that are known to have different physical properties that are relevant to dosage form performance (bioavailability or chemical stability) or manufacturing reproducibility, cannot be avoided. In such cases, validated methods would be needed to ensure that the ratio of forms is reproducible and the production process is controlled.

A variety of physical techniques (crystallography, spectroscopy, thermal analysis, and microscopy) are useful for characterizing the solid forms of pharmaceuticals and have recently been reviewed [3]. These techniques rely on differences in periodicities of atoms in crystals (X-ray diffraction), energies of bond stretching/bending vibrations and lattice vibrations (IR, Raman), electronic environments of nuclei (NMR), heat flow or weight change (thermal analysis), and morphology (optical microscopy), for the qualitative differentiation of solid forms of drugs. In this review, we focus on quantitative aspects of the techniques most commonly used in our labs for quantitative phase analysis. Specifically, we examine the use of X-ray powder diffraction, FT-Raman, mid IR, near IR, and solid-state NMR spectroscopy for quantitative analysis of solid-state forms of pharmaceuticals.

2. Quantitative methods of analysis

2.1. X-ray powder diffraction

X-ray powder diffraction has been used extensively for quantitative analysis of mixtures of crystal forms and to a lesser extent the determination of the degree of crystallinity. There are two primary methods for quantification; using either individual peaks or the whole patterns to establish the relationship between phase composition and the intensity of individual peaks or of patterns of the phases being quantified. The basic elements to quantitative analysis of powder mixtures and the mathematical relationships between pattern intensity and composition were first outlined by Klug and Alexander in 1948 [4]. The primary assumptions of the diffraction method rely on the particle size to be sufficiently small that extinction and micro-absorption effects are negligible. Furthermore, accurate quantification relies heavily on our ability to minimize the effects of preferred orientation. With inorganic samples, this is typically accomplished by grinding of the sample. With organic compounds, this may not be so readily accomplished. The potential of phase inter-conversion while reducing particle size is of major concern. Crystalline samples can be made amorphous, solvates can desolvate, and metastable phases can convert to more thermodynamically stable forms. This problem is particularly troublesome during the earliest stages of development when only limited amounts of unmilled material are available and lot sizes are smaller. Obtaining a non-preferentially oriented powder diffraction pattern representative of the pure metastable phase can be particularly problematic. There are numerous methods used to generate standards of smaller particle sizes, the most common being grinding the sample using an analytical mill or
mortar and pestle. In our laboratory we have found
that sonication is a good method for maintaining less
stable phases in its native state during particle size
reduction. By this approach, a solvate can be stabil-
ized by using its solvent of crystallization. If the
form is metastable and non-solvated, it is best to use
a non-solvent such as octane. The non-solvent re-
duces the potential for phase inter-conversion to the
more stable form by minimizing the rate of solvent-
mediated transformations. An additional method of
overcoming preferred orientation is to mix the
sample with an inert amorphous component, as was
proposed in the first quantitative pharmaceutical X-
ray powder diffraction method published [5]. This is
highly convenient in the pharmaceutical industry
since many of the commonly employed excipients
are amorphous. Furthermore, if the crystal structures
of the components being analyzed are available, the
success of eliminating preferred orientation can be
determined by comparison of the relative intensities
of the phases being quantified in the experimental
powder patterns to their respective calculated powder
patterns.

The general expression underlying quantitative
diffraction analysis was presented by Alexander and
Klug [4].

\[
I_1 = \frac{K_i x_1}{\rho_1 (\mu_1^* - \mu_M^*) + \mu_M^*}
\]  

(1)

The intensity of a reflection, \(I_1\), is related to the
phase composition using a proportionality constant,
\(K_i\), that is a function of a number of physical
constants; the dimensions of the diffractometer (slit
size, goniometer radius), Lorentz-polarization fac-
tors, and reflection multiplicity of the phase of
interest. The intensity of the reflection is also a
function of the phase density, \(\rho_1\), its weight fraction,
\(x_1\), and the mass absorption coefficients of the
analyte and the sample matrix, and \(\mu_1^*\) and \(\mu_M^*\),
respectively. In the special case of a mixture of
polymorphic forms of a substance, the mass absorp-
tion coefficient of the polymorph being quantified is
the same as the sample matrix, that is \(\mu_1^* = \mu_M^*\). The
intensity of a given peak is directly proportional to
its concentration such that linear analysis can be
made directly by a plot of intensity versus con-
centration. When the absorption coefficient of the
phase of unknown composition differs from that of
the matrix, that is \(\mu_1^* \neq \mu_M^*\), the more general, Eq.
(1), needs to be considered to accurately quantify
crystal forms in the formulation or in mixtures of
solvates and non-solvated forms. The concentration
versus weight fraction of the analyte will deviate
from linearity. In purely organic systems, the devia-
tion from linearity is not as severe as with inorganic
systems [6], since their mass absorption coefficients
are relatively low and such samples generally do not
have as large of a range of mass absorption co-
efficients.

In the pharmaceutical industry, typically one will
examine the previously defined (API) – either as a
mixture of polymorphic forms or as a mixture of
crystalline and amorphous phase (both having a
simple linear intensity proportionality to concen-
tration). Alternatively one may encounter a mixture
of hydration/solvation states, in which cases the
intensity would not be necessarily directly propor-
tional to concentration. Fig. 1 demonstrates the
deviation from linearity resulting from differences
in mass absorption coefficients between the analyte
and the sample matrix. In the example, the mass absorp-
tion coefficient of the pentahydrate form of cromolyn
sodium was calculated and a theoretical intensity
versus concentration curve was generated for it as a
mixture with an anhydrous form. Since this repre-
sents a highly hydrated system, this indicates that in
general, hydrates will not result in a significant
deviation from linearity due to a difference in mass
absorption coefficients (despite its not being a truly
polymorphic system). Neglecting to account for the
difference in mass absorption coefficient will intro-
duce a relatively small error compared to other errors
influencing the accuracy of quantitative measure-
ment. In contrast, curves calculated based on a
hypothetical methylene chloride solvate mixed with
the anhydrous crystals would result in significant
inaccuracy if the different mass absorption coeffi-
cient of the analyte is not accounted for. Similar
considerations apply if one were to quantify the
amount of a pharmaceutical salt versus its unionized
form, it is always best to account for the difference
in mass absorption coefficient.

When quantifying polymorphic composition of a
drug substance in a formulation, one makes standard
mixtures of two polymorphic forms added to a
constant amount of excipient. The sample’s mass absorption coefficient is constant and a linear relationship results from a plot of peak intensity and its polymorphic composition (this again is approximately true for a mixture of drug hydration states). The overall intensity of the peaks associated with the API will be reduced, since it is diluted relative to pure API. Consequently, the range of quantification is reduced and detection limit is higher than for quantitative methods of formulated products relative to pure API methods. On the other hand, powder diffractionists are often relied upon for forensics-type work and inevitably encounter mixtures of inorganic phases. In such instances, differences in mass absorption coefficients between the analyte and the sample’s matrix may result in severe deviation from linearity during quantification and must be accounted for accurate analysis.

There are numerous variants to quantitative analysis by X-ray powder diffraction. Some methods use direct analysis of an individual phase concentration based upon the intensity of single peak relative to its pure phase intensity. Other methods reference the analyte’s intensity to an internal standard, while still others rely on a change in diffraction response as a result of spiking or dilution of the sample. The best approach to use is dependent upon many factors. These approaches may be developed using single peaks representative of the individual phases comprising the sample or they may use the entire diffraction pattern. Single line methods generally require less knowledge about the phases to be quantified than whole pattern methods, see Table 1. They will often be the most sensitive approach, since the method may be developed to quantify based on the intensity of only the most intense peak of the diffraction pattern. Such methods suffer from greater variability due to the influence of factors such as

Fig. 1. Theoretical intensity-concentration curves for several mixtures of the pentahydrate form of cromolyn sodium versus anhydrate form I (and vice versa) demonstrate the minor impact of hydration state on quantification. In contrast, the theoretical intensity of a ‘hypothetical’ methylene chloride solvate of a cromolyn sodium versus anhydrate composition (and vice versa) shows significant deviation from linearity as a result of differences in mass absorption coefficient.
Table 1
Tabulation of the degree of phase information required performing quantitative analysis by the various X-ray powder diffraction approaches

<table>
<thead>
<tr>
<th>Method</th>
<th>Standards</th>
<th>Indexation</th>
<th>Structural model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single peak methods</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole powder pattern methods</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>PLS principle component analysis</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Smith’s method</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Toraya’s method</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Rietveld method</td>
<td>X</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Such approaches are considered ‘standardless,’ however at some point a pure standard was obtained (i.e. its crystal structure was determined).

preferred orientation, but are the least sophisticated methods and are often ideally suited for long-term quality control applications.

Most whole pattern methods require a greater level of knowledge about the phases to be analyzed than the single peak methods, see Table 1. Two exceptions would be the whole pattern approaches such as factor-based Partial Least Squares (PLS) or the whole pattern method described by Smith et al. [7]. Factor-based PLS is a multivariate method that has found widespread analytical application [8]. Such methods involve establishing a calibration set that is used to derive a predictive model for analysis of future data sets. The calibration set should contain as many sources of sample variation as possible. In doing so, one might expect to be able to empirically correct for (or at least partially compensate for) the influence of preferred orientation [9]. Such methods require no more information than single peak methods because they rely on empirically-derived correlation of intensity/composition through training sets measuring the response of a single region or multiple regions of the powder pattern as a function of concentration.

The program GMQUANT, developed by Smith et al. [7], uses a whole powder pattern approach that does not require indexation of the individual components of the mixture. Indexation of pharmaceuticals can be highly problematic due to their low symmetry and commonly large unit cell axes. This requirement is perhaps the greatest limitation to the utility of the whole powder pattern approaches described later. GMQUANT uses least squares minimization of the difference between the digitized experimental pattern of a mixture and that of a convolution of the digitized pattern of the individual phases related by weighting factors. This approach represents perhaps the most easily applied whole pattern method and is suitable for quality control applications, since it requires minimal interaction of the analyst.

In whole powder-pattern decomposition methods (WPPD), the integrated intensity parameters, unit-cell parameters, and the peak profile parameters are refined by least squares fitting procedures along with an overall scale-factor relating the individual phases (or even amorphous background). The intensity of the diffraction pattern profile intensity, $Y$, at individual steps, $i$, of $2\theta$ is decomposed

$$Y(2\theta_i) = B(2\theta_i) + \sum_{k=1}^{N} S_k \sum_j f_{jk} P(2\theta_j)$$

where $B(2\theta_i)$ is the background function, $P(2\theta_j)$ is the profile function and $S_k$ is the scale factor. There are a number of different background functions and profile functions that describe the diffraction profile [10].

The Whole Powder Pattern Decomposition (WPPD) approach implemented by Toraya et al. [11] decomposes the pattern into peak profiles and background functions to obtain the best fit to the experimental powder pattern of the individual pure phase data by least-squares refinement. The integrated intensities of the pure phases are then stored with the other refined parameters, such as the profile parameters and the unit-cell parameters of the phases to be quantified. During quantification step, the integrated intensity of the phase being quantified is scaled, as defined in Eq. (3), such that the total of the...
scale factors for the individual component phases sum to unity. The scale factors of the individual components are then refined by least-squares methods until a best fit is observed with respect to the pattern of unknown composition.

\[ S_i = \sum w_i (Y(2\theta_i) - Y(2\theta_{ci}))^2 \]  

(3)

where the weighting function is \( w_i = 1/Y(2\theta_i) \). If an amorphous phase is present, its composition can be determined by addition of a known quantity of a crystalline standard

\[ S_{\text{amorphous}} = 1 - \sum_{k=1}^{n-1} S^k_{\text{crystalline}} \]  

(4)

In the Rietveld method, essentially the same approach is used as in Toraya’s method except a structural model (typically from a crystallographic determination) is used to calculate the intensities of the individual phases, as shown in Eq. (5).

\[ Y_{ei} = S \sum_K L_K |F_K|P_{\text{Bragg}}(2\theta - 2\theta_K)PO_KA + Y_{\text{background}} \]  

(5)

where \( Y_{ei} \) is the intensity calculated at an individual point, \( i \), in the diffraction pattern, \( S \) is a scale factor, \( L_K \) contains the Lorentz, polarization, and multiplicity factors, \( K \) represents the individual Miller indices, \( h, k, l \) for the reflection, \( F_K \) is the structure factor for the \( K \)th Bragg reflection, \( P_{\text{Bragg}} \) is the peak profile function, \( PO_K \) is a function describing the sample’s preferred orientation, \( A \) is an absorption factor, and \( Y_{\text{background}} \) is the intensity of the background. The function minimized is based on the difference (residual) in intensity between the experimental pattern of the mixture and the scaled ratio of the calculated powder patterns of the phases being quantified.

Toraya’s WPPD approach is quite similar to the Rietveld method; it requires knowledge of the chemical composition of the individual phases (mass absorption coefficients of phases of the sample), and their unit cell parameters from indexing. The benefit of this method is that it does not require the structural model required by the Rietveld method. Furthermore, if the quality of the crystallographic structure is poor, contains disordered pharmaceutical or poorly refined solvent molecules, quantification by the WPPD approach will be unbiased by an inadequate structural model, in contrast to the Rietveld method. If an appropriate internal standard of known quantity is introduced to the sample, the method can be applied to determine the amorphous phase composition as well as the crystalline components [11]. The Rietveld method uses structural-based parameters such as atomic coordinates and atomic site occupancies are required for the calculation of the structure factor, in addition to the parameters refined by the WPPD method of Toraya. The additional complexity of the Rietveld method affords a greater amount of information to be extracted from the data set, due to the increased number of refinable parameters. Furthermore, the method is commonly referred to as a standardless method, since the structural model serves the role of a standard crystalline phase. It is generally best to minimize the effect of preferred orientation through sample preparation. In certain instances models of its influence on the powder pattern can be used to improve the refinement [12].

There seems to be an endless number of approaches to quantification by X-ray powder diffraction, some of which have been briefly discussed herein. When deciding what approach to use, there are many considerations one must take into account; a method suitable for the development of a process may not be appropriate for a quality control application. Many factors influence the lab-to-lab or instrument-to-instrument transferability of a diffraction method. For instance, it might not be advisable for one to transfer a Rietveld method to a quality control laboratory since the success of quantification relies heavily on obtaining a global minimum from a nonlinear least squares refinement process. The robustness of such a methodology is highly dependent upon the skill level of the analyst and may not be readily automated. Since organic compounds may decompose with time, consideration must be made about the long-term availability of standards if standard curves are to be used. When instruments are changed/upgraded over time, many of the geometrical factors that influence \( K \) in Eq. (1) may be affected. Standard curves will need to be regenerated even when changing X-ray tubes for most methods other than the ‘standardless’ Rietveld method whose standardization is based upon an existing structural model. Development of a good quantitative method
requires careful consideration of many factors and oftentimes depends on trade-off between ease and sophistication of approach.

2.2. Spectroscopy

Spectroscopic methods are non-destructive and can be used in conjunction with other solid-state techniques (TGA, microscopy, DSC, XRD) for the quantitative analysis of pharmaceutical solids. Once suitable spectral features, which arise from observed crystallographic differences, are identified, they can be used to develop methods for the quantitative analysis of one polymorph (or solvate) in the presence of the other [13]. One advantage of spectroscopic methods over diffraction methods for quantitative analysis is that these methods are often superior for the quantitative analysis of crystallinity, since Raman, IR, or SSNMR spectra of amorphous phases give specific, albeit broadened signals. The non-destructive nature of spectroscopic methods renders these techniques superior to thermal methods of analysis in most cases.

2.2.1. Vibrational spectroscopy (infrared (mid IR, NIR), Raman)

In contrast to X-ray powder diffraction, which probes the orderly arrangement of molecules in the crystal lattice, vibrational spectroscopy probes differences in the influence of the solid state on the molecular spectroscopy. As a result, there is often a severe overlap of the majority of the spectral features for different forms of the pharmaceutical. Sometimes complete resolution of the vibrational modes of a particular functional group suffices to differentiate the solid-state form and allows direct quantification. In other instances, particularly with NIR spectroscopy, the overlap of spectral features results in the need to rely on more sophisticated approaches for quantification. Of the spectroscopic methods which have been shown to be useful for quantitative analysis, vibrational (mid IR absorption, Raman scattering, and NIR) spectroscopy is perhaps the most amenable to routine, on-line, off-line, and quality-control quantification.

Diffuse reflectance infrared Fourier transform spectroscopy, DRIFTS, has become an attractive alternative to mulls with the introduction of DRIFTS cell by Griffiths [14], later modified by Yang [15]. Since materials are dispersed in a non-absorbing medium and are not subjected to thermal or mechanical energy during sample preparation, DRIFT spectroscopy is especially suitable for the qualitative/quantitative analysis for polymorphs, which are prone to solid-state transformations. The Kubelka–Munk equation [16], which is analogous to Beer’s law for transmission measurements, is used to quantitatively describe diffusely-reflected radiation:

$$F(R_s) = \frac{(1 - R_s)^2}{2R_s} + \frac{2.303ac}{s}$$  \hspace{1cm} (6)

where $F(R_s)$ is the K–M function, $R_s$ the absolute reflectance of an ‘infinitely-thick’ sample ratioed to that of a non-absorbing reference, $a$ is the molar absorption coefficient, $c$ is the molar concentration of the analyte, and $s$ is the scattering coefficient. Clearly for a linear relationship between intensity and concentration to exist, the scattering coefficient must be constant. At low analyte concentrations, the scattering coefficient will depend on the non-absorbing dispersant, while at higher concentrations, the linearity of the DRIFTS analysis may be limited by the analyte. To minimize the effects of the analyte on the scattering coefficient, i.e. to maximize the linear calibration range, care must be taken to control sample homogeneity, bulk density, particle size and shape. Specular reflectance must also be negligible.

In developing a quantitative method based on vibrational spectroscopy, measures must be taken to ensure homogeneous sample mixing, particle size, and instrument variability and reproducibility [17]. Roston et al. recommend grinding a spectroscopic-grade dispersant, e.g. KCl, for 2 min in a laboratory mixer to ensure uniform, consistent particle size, a prerequisite for run-to-run and day-to-day consistency [18]. Unique spectral features must be identified and calibration studies must be performed on samples of known composition using peak areas determined by integration. Multivariate calibration methods (e.g. PLS) or regression analysis are typically required.

Near infrared reflectance analysis (NIRS) shares many of the same advantages as DRIFT spectroscopy; however, the need to dilute the sample is eliminated. This is due to the relatively weak molar
absorptivities of the transitions responsible for the peaks observed in the near infrared region, which are typically two or more orders of magnitude less intense than those found in the mid infrared region. Absorbance peaks in the NIR region arise from combination bands (1900–2500 nm), harmonic overtones (1500–2000 nm, first overtone; 1100–1600, second overtone; and 700–1100, third overtone) and are weak due to the fact that, in a quantum mechanical sense, they are forbidden transitions of the simple harmonic oscillator. They arise only because of the deviation of the actual system from the harmonic oscillator (anharmonicity), causing a slight overlap of the energies (wave functions) of the initial and final states. As one might expect, the absorptivities of the second overtones are much weaker than the first overtones, and the third weaker than the second. As a consequence, this weak interaction of light in the 700 to 1500 nm region affords a greater depth of penetration into the sample. Thus, instruments are now commercially available which can record a transmission spectrum through a tablet several millimeters thick, as demonstrated by Jee et al., for 300 mg paracetamol tablets, 4 mm thick [19]. With powder samples, scattering, which is also wavelength dependent, competes with absorption. The combination of the effects of scattering and absorption determine the depth of penetration of the light into the sample, and thereby the effective sample size. For microcrystalline cellulose powder samples, depth of penetration was found to vary from tenths of millimeters (1500–2500 nm range) to a few millimeters (700–1100 nm range), as reported in a paper by Berntsson et al., which also included a rigorous discussion of reflectance theory using the radiative transfer equation [20].

One limitation of NIRS is that the absorbance peaks in the NIR region arise primarily from hydrogen to heteroatom bonds. This is simply due to the fact that the frequencies of these vibrations, because of the large mass difference of the atoms, are high enough for their overtones and combinations to fall outside the mid IR region. The frequencies of the combinations and first overtones of other IR active bonds for atoms closer in mass (e.g. C–O, C–N, etc.) fall within the IR region, and are obscured by the stronger, primary absorbance peaks. Therefore, NIR is ill suited to compounds that do not contain hydrogen. At the same time, water absorbs strongly in the NIR region, and its peaks are resolved from alcohols and amines, making NIR ideal for analysis of hydrates.

Compared to spectra obtained in the mid infrared region, NIR spectra contain fewer, less resolved, peaks. Due to scattering and other effects, a set of NIR spectra on similar samples often exhibits constant baseline offsets from one to the next. To eliminate these baseline offset differences, reduce (but not eliminate) scattering effects, and increase the resolution of neighboring peaks, first- or second-derivatization is often applied to NIR spectra prior to their use in calculations. Other preprocessing techniques, such as standard normal variate (SNV) or multiplicative scatter correction (MSC), may be applied to more effectively reduce scattering effects that arise from particle size differences among samples [21].

When developing quantitative calibrations, it is important to understand that since a single compound typically absorbs at many different wavelengths, the absorbance values in a given spectrum are highly collinear. Thus, multivariate calibration is often utilized to relate spectral information to some analytical property (chemometrics). Some of the most popular approaches include multiple linear regression (MLR), principle components analysis (PCA) and partial least-squares (PLS); for an excellent description of the basis for these approaches, see the textbook by Martens and Naes [22]. Given the large number of data-points in a spectrum, the chances of finding random spectral information in a set of spectra, which correlate to any analytical property, are quite good. Thus, one must carefully validate calibration models by testing them with spectra that are completely independent from the spectra used to develop the calibrations.

Once a valid calibration has been established, the calibration curve is stored in computer memory for ongoing usage. Modern NIR instruments are highly stable with respect to sensitivity drift, and extensive calibration and diagnostic test procedures have been developed which may be routinely applied to ensure this stability [23]. Thus, a compelling advantage of NIRS analysis is the possibility of quantitative analysis without daily standardization or sample manipulation. When using an instrument equipped with a fiber-optic probe, samples may be analyzed without any preparation steps. This can afford both a
rapid, and a precise measurement, since preparation steps (such as dilution and mixing with KBr for DRIFTS) that introduce variation have been eliminated. The ability to record spectra through a glass sample container is another major advantage of NIRS, especially for hygroscopic substances, where handling and manipulation could cause a form conversion and/or change water content [24]. Spectra may even be recorded through polymeric films such as bags or packaging materials. A multivariate calibration can be developed that is not affected by the contribution of polymer absorbance, or a transparent spectral region may be found. Also, the sensitivity of reflectance analysis to changes in sample particle size may be exploited for additional selectivity in identification testing. Using an instrument equipped with a fiber-optic probe, O’Neil et al., have successfully developed NIRS calibrations for the median particle diameter of a number of powdered drugs and excipients [25].

Raman spectroscopy is similar to DRIFT spectroscopy in the sense that it is a surface technique, where particle size is an important parameter to control. The smaller bandwidths in Raman (relative to IR) make this method more powerful for differentiating polymorphs than IR, and therefore potentially more powerful for quantitative analysis. No sample preparation is required (sampling can be done through containers), however, because of the small excitation range used in Raman spectroscopy, sample homogeneity must be considered. Bugay recommends either of two approaches, (1) a slurry technique utilizing a standard sample accessory [17] or (2) using a Step-n-Repeat sampling accessory to acquire spectra of several regions of a sample [26]. Langkilde et al. reported a sample rotation method (∼1 mm diameter of sample is typically excited by a Raman laser) to address sample inhomogeneity concerns of quantitative Raman spectroscopy and was able to achieve linear concentration curves (1–15%) [27]. To ensure a rugged assay, Bugay recommends concurrent method development using another analytical technique [27].

2.2.2. NMR spectroscopy

Solid-state NMR spectroscopy may also be used for the quantitative analysis of pharmaceutical solids. The advantage of this technique over other spectroscopic techniques lies in its high degree of spectral resolution. Several solid-state NMR experiments are available for quantitative analysis, depending on the nature of the sample. Multiple pulse sequences have made it possible to collect solid-state \(^1\text{H}\) NMR spectra, however these measurements are hardware intensive and difficult [28,29]. Furthermore, because the isotropic chemical shift range of \(^1\text{H}\) is only 12 ppm, solid-state \(^1\text{H}\) NMR spectra are overwhelmed by peak broadening effects of proton–proton dipolar interactions that span several ppm, effectively limiting even qualitative applications of \(^1\text{H}\) NMR spectroscopy. The significantly larger isotropic chemical shift range of \(^{13}\text{C}\) is better suited for quantitative analysis, since highly resolved solid-state \(^{13}\text{C}\) NMR spectra are possible. Hays [30], and later Harris [31], have provided excellent descriptions of the quantitative aspects of high resolution solid-state NMR spectroscopy. We review here the quantitative aspects of \(^{13}\text{C}\) NMR spectroscopy as they apply to problems of pharmaceutical interest. This discussion can be extended to other nuclei, such as \(^{31}\text{P}\) or \(^{15}\text{N}\), which also give high-resolution spectra.

To obtain high resolution solid-state \(^{13}\text{C}\) spectra, strong \(^1\text{H}–^{13}\text{C}\) dipolar interactions and the chemical shift anisotropy (CSA) of the \(^{13}\text{C}\) nuclei must be overcome. The CSA pattern arises from the simultaneous observation of chemical shifts for every molecular orientation with respect to the applied magnetic field. A combination of magic angle spinning (MAS) [32], which averages the CSA to zero, and high power proton decoupling, which reduces strong \(^1\text{H}–^{13}\text{C}\) dipolar interactions (MAS also helps to reduce \(^1\text{H}–^{15}\text{C}\) dipolar interactions), is used to obtain high resolution, solid-state \(^{13}\text{C}\) spectra. Because high spinning speeds are oftentimes required to collect high resolution SSNMR spectra, metastable forms that may undergo phase transitions may not be amenable to characterization by this technique. In cases where MAS can be used for quantitative analysis, the magic angle must be precisely set to 54°44’ [33], since deviations will cause line broadening and intensity deviations proportional to the CSA of each nucleus.

One complication of magic angle spinning is the appearance of spinning sidebands, which arise from insufficient sample spinning rates relative to the shielding anisotropy of the nucleus. Spinning sidebands, which are separated from the centerbands, i.e. isotropic peaks, by the spinning rate (in Hz) and
therefore may be readily identified as the peaks which shift in spectra acquired at different spinning speeds, are particularly significant at high magnetic fields or using low spinning rates. MAS not only affects the distribution of sidebands, but also their relative intensities. Therefore, in cases where spinning sidebands are significant and the relative peak intensities do not appear to be sensible, the entire spinning sideband manifold must be considered. For quantitative analysis, peak areas should be used as opposed to peak heights in adding back the signal intensity of the sidebands into the centerband intensity. Eliminating spinning sidebands by TOSS [34] (total suppression of spinning sidebands) or improving baselines of MAS spectra by spin echo sequences is generally not recommended for quantitative work [31].

The key to developing a quantitative NMR method (solution or solid state) is to ensure that experimental conditions are selected, such that the integrated NMR signal is proportional to the number of nuclear spins producing it. For accurate integrations, the recycle time between successive pulses must be properly set to allow for the net magnetization ($M_z$) to return to its equilibrium state ($M_{eq}$) after the radio frequency-induced spin transition. The choice of recycle delay will depend on the pulse sequence used for quantification, which in turn is determined by the nature of the sample. Single pulse magic angle spinning (SP/MAS) experiments, using normal 90° pulses (or lower pulse angles) and high power proton decoupling, are recommended for mobile systems. If SP/MAS is to be used for quantitative analysis, $T_1$ values should be precisely determined for the carbons of interest using inversion recovery pulse sequences [35] and recycle delays of $5T_{1C}$ then selected to allow for complete relaxation. If cross polarization magic angle spinning (CP/MAS) is used for quantitative analysis, as is generally recommended for rigid, proton-containing samples (with long $T_{1C}$), the recycle time will depend on the magnetization transfer process and relaxation behavior of the protons, not the carbons. The net result is that the recycle delay will be shorter, on the order of the proton $T_1$’s, i.e. 1’s to 10’s instead of 100’s of seconds.

Cross polarization was introduced by Pines, Gibby, and Waugh to address the low sensitivity associated with collecting NMR spectra of dilute spin-1/2 nuclei, such as $^{13}$C [36]. The basic CP pulse sequence is shown for $^{13}$C–($^1$H) in Fig. 2. Polarization transfer between $^1$H and $^{13}$C will occur by transferring the proton magnetization to the $x'$ axis with a 90° pulse, phase shifting the magnetization to the $y'$ axis by an on-resonance spin locking pulse, and then applying an on-resonance pulse to the $^{13}$C spins of precise magnitude so as to achieve the Hartmann–Hahn matching condition ($\gamma_cB_{1C} = \gamma_HB_{1H}$). Because the rare spin ($^{13}$C) takes on the magnetization and relaxation behavior of the abundant spin ($^1$H), not only is the sensitivity of the $^{13}$C experiment enhanced by use of the CP pulse sequence, but as mentioned earlier, the recycle delay (which depends on the magnetization transfer process and the $^1$H spin-lattice relaxation time) is also significantly reduced.

To understand how the CP/MAS experiment can be applied to quantitative analysis, the magnetization transfer and relaxation processes, which directly affect the signal intensity, must be considered. Cross polarization is mediated by $^1$H–$^{13}$C dipolar interactions, so the magnetization build-up will occur at different rates for different types of carbons. In general, the cross polarization rate ($T_{CP}^{-1}$) increases with the degree of protonation [37,38]. That is, $T_{CP}$ is generally shorter for methylene and methine carbons than for quaternary carbons, Fig. 3. Motional modulation of the $^{13}$C–$^1$H dipolar interaction, however, can significantly attenuate the magnetization build-up. Thus, for mobile methyl groups, the static dipolar interactions that allow for cross polarization
start to average out, and longer contact times are required for full polarization [30].

At the same time the proton magnetization is being partially transferred to the carbon spins ($T_{\text{CP}}$), it relaxes to the lattice ($T_{1\text{pH}}$, proton spin lattice relaxation in the rotating frame). The net result of the competing relaxation and polarization transfer processes is that $^{13}$C magnetization reaches a maximum. Once the $^1$H and $^{13}$C spins reach a common spin temperature, both spins relax to the lattice at a rate of $T_{1\text{pH}}^{-1}$. The exponential rise and decay of $^{13}$C magnetization of a single species as a function of contact time is given by [39].

$$I(\tau) = \frac{M_0 \left( \frac{\gamma_H}{\gamma_C} \right) \left[ \exp\left(-\frac{\tau}{T_{1\text{pH}}}\right) - \exp\left(-\frac{\tau}{T_{CH}}\right) \right]}{1 - \frac{T_{CH}}{T_{1\text{pH}}}}$$

(7)

where $I(\tau)$ is the peak intensity at variable contact time, $\tau$. The main requirement for efficient cross polarization is that the contact time ($\tau$) is larger than $T_{\text{CP}}$, but smaller than $T_{1\text{pH}}$.

For homogeneous solids, all protons have identical relaxation times due to proton spin diffusion, which is mediated by homonuclear dipolar coupling. Spin diffusion is not fast enough, however, to average the proton relaxation times of materials with spatially distinct regions greater than 1 nm across [40]. As a result, heterogeneous materials may have multiple proton relaxation times associated with the different regions. Different relaxation behavior is evident from the signal decay of the CP curves of different crystal forms of a developmental drug substance, Fig. 4. For these samples, both the cross polarization rates and proton relaxation times in the rotating frame must be considered for each component to ensure that the signal is truly proportional to the amount of species present. It is also important to recognize that $T_{1\text{pH}}$, which is sensitive to mobility in molecular solids, may change drastically with variations in temperature. Therefore, sample temperature must be carefully selected and controlled to ensure quantitative results.

Clearly, in order to correctly apply CP pulse sequences for quantitative analysis (or even qualitative analysis), many relaxation processes ($T_{1C}$, $T_{1\text{pH}}$, $T_{1\text{H}}$, $T_{\text{CP}}$) must be considered and spectral acquisition parameters appropriately set. While a CP
Fig. 4. Signal intensity of a diagnostic $^{13}$C resonance in the SSNMR spectra of several crystal forms of a developmental drug substance as a function of the contact time during the CP experiment.

Spectrum may be obtained when $T_{1c} \gg T_{1H} \geq T_{1pH} \gg T_{CP}$, a quantitative CP spectrum requires that the recycle delay is sufficient (on the order of $T_{1H}$) for the protons to be uniformly relaxed at the beginning of the contact time [41] all proton magnetization spin locked in the rotating frame decays at the same rate ($T_{1pH}^{-1}$), and the contact time is sufficient to allow complete cross polarization (at least five times the longest $T_{CP}$) [42]. Except when relative peak intensities are constant and appear to be correct, single contact time measurements should be avoided. Instead, $^{13}$C spectra and $^1$H relaxation times should be measured and complete magnetization curves analyzed.

To correct the signal intensities in CP/MAS spectra for $T_{CP}$ and $T_{1pH}$, complete magnetization curves should be constructed by varying the contact time and plotting the log of the signal intensity as a function of contact time. For homogeneous solids (with full spin diffusion for $T_{1H}$ and $T_{1pH}$), the correct signal intensity may be obtained by simply extrapolating the signal to zero contact time [31]. Rethwisch et al. recommends collecting 10–15 spectra with ~75% of the data at contact times greater than $5T_{CP}$ for determining correct signal intensities by this method [43]. An approximate $T_{CP}$ can be obtained by analysis of the initial rise in intensity, i.e. at short contact times, while an approximate $T_{1pH}$ value can be obtained from the exponential decrease in intensity at long contact times [37]. For heterogeneous samples with complex spin diffusion (multi-exponential $T_{1pH}$) and regions of different $T_{CP}$, line fitting of the CP curves may be required to obtain the correct signal intensity, as well as $T_{1pH}$ and $T_{CP}$ values [43]. To improve the quantitative reliability of CP/MAS NMR spectra in cases where overlapping peaks (with different polarization and relaxation time constants) prevent the signal intensities from being corrected directly, a mathematical correction to $T_{1pH}$ may also be applied [44].
3. Applications of quantitative analysis to pharmaceuticals in the solid-state

3.1. Quantification of mixtures of crystalline forms

Christ et al. reported the first application of quantitative X-ray powder diffraction to a pharmaceutical system in 1948 [5]. In this work, X-ray powder diffraction was used to quantify the amount of crystalline sodium penicillin G in samples containing a mixture of five other related substances from the fermentation process. Sodium penicillin G content was determined by measuring the intensity of the reflection versus an external standard and plotting its ratio versus concentration. A straight-line working curve was obtained. They found that addition of carbon black to the sample reduced the effect of preferred orientation. We have found this approach effective for minimizing preferred orientation of a number of other pharmaceuticals. The advantage of carbon black is that its color provides a visible indication of the homogeneity of mixing. Being relatively inert and amorphous, it is non-reactive and disfavors successive layering of platy crystals, as was the case with penicillin G samples. In 1963, Shell studied the application of quantitative XRPD to pamoic acid, sulfonamide, tetracycline, and novobiocin. He further surveyed the use of direct simple calibration curves and the use of internal standards. In this work, Shell demonstrated the use of quantitative XRPD for the completion of a salt forming reaction. Duddu and coworkers studied the reaction between two enantiomers to form a racemic crystal [45]. In the method, physical mixtures were made of the racemic crystalline form and one of the enantiomers. A linear relationship was observed in a plot of the peak area versus the weight fraction. The standard curve was used to determine the amount of crystalline racemate formed during the solid-state reaction.

More recently, the development of quantitative methods by the group of Suryarananan to investigate carbamazepine has occurred. A single-peak powder diffraction method was developed to quantify the relative amounts of anhydrous carbamazepine and carbamezepine dihydrate to study the kinetics of transformation upon suspending the anhydrous form in water [46]. Although the study used a single-peak method, which may be most affected by preferred orientation, its influence was minimized by selection of lines least influenced by preferred orientation. This was accomplished by systematic evaluation of the standard deviation of the individual peak areas as the result of repeat sample preparation. Another important aspect of the study was that it demonstrated the minimal influence of a change of hydration state on the mass absorption coefficient of the compound, changing from 5.21 to 5.87 cm² g⁻¹ in the anhydrous versus hydrated phase. In most of the pharmaceutical literature, this difference is not considered. The lack of correction for differences in mass absorption of different states of hydration of pharmaceuticals being quantified can be expected to introduce a relatively small, albeit unnecessary error in such analyses.

In another study by Suryanarayan, a powder diffraction method was developed to quantify the active ingredient in intact tablets [47]. Two model drugs were examined, lithium carbonate and carbamazepine. The drugs of various weight fractions were mixed with microcrystalline cellulose, as well as starch in the case of carbamazepine, and then compressed into tablets. Though use was not made of the entire diffraction pattern, the intensities of several different reflections were used for quantification, thereby resulting in a more robust method. The objective was to monitor the drug content in individual tablets during accelerated stability studies. The method was carried out on intact tablets and resulted in a simple procedure that could readily be automated.

In a recent study, Bugay et al. used mid IR spectroscopy and X-ray powder diffraction to quantify the relative ratio of cefepime monohydrate in cefepime dihydrate [17]. The article presents many of the considerations that one must make in the development of a method to support the new drug application (NDA) for a pharmaceutical substance. The objective was to be able to establish the detection limits for the dihydrate and to ensure that no crystal form conversion occurred during sample preparation or analysis. The powder patterns are distinctly different and a small region of the pattern, 12 to 15° 2θ, was chosen that contained significant
peaks of both forms for quantification, Fig. 5. The X-ray powder diffraction method used a linear calibration curve of intensity versus concentration of the dihydrate phase, see Fig. 6. Correction for the small difference in the mass absorption coefficient between the dihydrate phase and the sample matrix was neglected over the narrow range of quantification. The limit of detection of the dihydrate was 0.75% and a minimum quantifiable level of 2.5% was determined with a relative standard deviation of 7.8% over the range of 2.5 to 15% dihydrate was established. These results are fairly typical of what one can expect of a well-developed quantitative XRD method for crystalline pharmaceutical systems. In the mid IR region, two distinct bands appeared at 3574 and 3432 cm\(^{-1}\) representing the dihydrate form, whereas only a single band at 3529 cm\(^{-1}\) was characteristic of the monohydrate form. The amount

![Graph showing the X-ray powder diffraction patterns of the dihydrate and monohydrate phases of cefepime 2HCl](image)

Fig. 5. The X-ray powder diffraction patterns of the dihydrate and monohydrate phases of cefepime 2HCl enables a quantification over the angular range of 12 to 15° 2θ (reproduced, with permission, from Ref. [17]).

![Graph showing the linear correlation curves for the determination of dihydrate content in cefepime 2HCl monohydrate by IR spectroscopy and XRD analysis](image)

Fig. 6. The linear correlation curves for the determination of dihydrate content in cefepime 2HCl monohydrate by IR spectroscopy and XRD analysis (reproduced, with permission, from Ref. [17]).
of the dihydrate present in the sample was determined using standard curves and the background-subtracted spectral integration response of the absorption band at 3574 cm\(^{-1}\) versus concentration. In the study, a detection limit of 5% was found in the mid IR assay, a limit of detection of 0.3% (w/w) with a minimum quantifiable limit of 1.0% and an RSD of 12.7% over a range of 1.0–8.0% (w/w) was determined. A technique for obtaining well-mixed standards for quantification involving slurrying the mixture in a volatile non-solvent, filtering it and then removal of residual volatile solvent prior to analysis was discussed.

No general rule can be made regarding relative sensitivity of FT-IR as opposed to FT-Raman spectroscopy, however if the polymorphic change involves differences in hydrogen bonding, FT-IR will be highly sensitive [48]. On the other hand, FT-Raman spectroscopy is extremely useful when the analyte is diluted within a polar matrix. For example, relatively non-polar components will be highly Raman active and most excipients are poor Raman scatterers, making Raman an excellent method for dosage form analysis. Similarly, Raman is uniquely suited for studying hydration kinetics of a relatively non-polar pharmaceutical when dispersed in water, providing a high degree of sensitivity. A comparison made between the diffuse reflectance FT-IR, near IR, and FT-Raman methods found that similar precision was observed. Excellent accuracy, precision, and convenience of measurement make near IR and FT-Raman valuable tools for quantification of polymorphic composition. In the article, similar problems were observed for each method; packing, sample positioning, particle size, surface reflection (Fresnel) and diffuse reflection affected signal intensity [48].

Miyazaki developed a quantitative IR method (nujol mulls) for following the rate of transformation of the \(a\) and \(\beta\) crystal forms of chlortetracycline hydrochloride at different relative humidity and temperature [49]. ATR and DRIFT spectroscopy are considered to be attractive alternatives to mulls and pellets for the analysis of powdered samples. Higuchi suggested the use of ATR–FTIR for analysis of polymorphs [50] Kang reported use of ATR for qualitative identification of pharmaceutical solids [51]. Hartauer reported the quantitative analysis of sulfamethoxazole and trimethoprim in pharmaceutical formulations by circle-cell ATR [52]. Salari and Young were the first to report an application of ATR–FTIR to the quantification of polymorphs [53]. In this work, three polymorphs of ganciclovir were quantified using PLS. Quantitative analysis of sulfamethoxazole polymorphs by IR was reported by Hartauer et al. using diffuse reflectance [54]. Roston et al. reported the use of DRIFT spectrometry for the detection of a low melting polymorph in high melting samples over a range of 0–25% w/w [18]. Tudor demonstrated that Raman spectroscopy can be used to quantify the solid phases in cortisone acetate mixtures and also for a hypolipidaemic drug [55]. Tudor also applied Raman spectroscopy and factor analysis to quantify mixtures of chlorpropamide polymorphs [56].

One of the more thorough investigations of quantitative analysis of polymorphs using FT-Raman spectroscopy was conducted by Jalovsky et al. [57]. Six polymorphs of cimetidine were analyzed by FT-Raman to examine quantification of the mixtures of crystalline forms. Characteristic bands were found and a variety of mathematical models were examined of which PLS was found to be best. FT-Raman was convenient, linear regression and classic least squares methods gave satisfactory results particularly when curve fitting procedures were used for band areas. Significantly better results were achieved by the PLS technique, though with a more limited accuracy for ternary polymorphic form mixtures.

Forbes et al. demonstrated that loracarbef dihydrate, monohydrate, and anhydrate could be easily distinguished by NIRS [58]. They found that NIRS was capable of quantifying the isopropyl alcohol solvate of loracarbef, in a matrix of desolvated loracarbef (anhydrate), down to 0.3% alcohol (~2% solvate), see Figs. 7 and 8. Demonstrating the ability of NIRS for discriminating crystal forms that were not merely different states of hydration, Corti et al. examined polymorphs of several pharmaceuticals utilizing the Mahalanobis distance method [59]. Two forms of paracetamol, three apparent forms of gemfibrozil, and four polymorphs of chenodeoxycholic acid were distinguished. The \(l\)-form, levamisole, was able to be distinguished from the racemic form of tetramisole. While many of the example spectra show differences in relative intensities of absorbance peaks for differ-
ent polymorphs, some spectra appeared to be very similar, except for apparent differences due to scattering effects. The authors did not describe whether attempts were made to control differences in particle size among the forms. Given the sensitivity of the technique to sample particle size, this should be examined carefully, before concluding that the NIR spectra for polymorphs are indeed different. Exploiting the effect of particle size differences, the authors demonstrated that micro- versus macro-crystalline nitrofurantoin, and high- versus low-density ibuprofen could be distinguished.

Norris followed a slurry conversion of trovafloxacin mesylate in butanol, demonstrated the utility of NIRS for on-line process monitoring involving polymorphs [60]. Two anhydrous forms of trovafloxacin mesylate, with distinctly different X-ray powder patterns, were identified. While the authors concluded that the differences in mid IR spectra obtained by DRIFTS were too small to be useful in discriminating polymorphs, the reflectance NIR spectra, also showing subtle differences, were found to be more useful when analyzed with principle components and hierarchical clustering. When water was present, the two forms readily converted to a monohydrate, exhibiting distinctive water peaks at 1906 and 1952 nm. Utilization of NIRS as an on-line monitoring tool could be quite useful in the event of difficulties in obtaining reproducible nucleation and crystal growth of a second form. Such a system would enable routine production to proceed without probability of continued processing of a un- or partially converted batch before being detected off line.

Solid-state NMR spectroscopy has been used as a quantitative tool in the field of polymer science [61]. To date, most applications of SSNMR spectroscopy for characterizing pharmaceutical solids, i.e. polymorphism in bulk drug substances [62] and in formulations [63], however, have utilized this tech-
nique as a qualitative tool [64]. SSNMR spectroscopy has been suggested for the quantitative analysis of polymorphism/pseudopolymorphism by many researchers [65], but in fact has only been reported in a few instances [66]. Jakobsen et al., utilized $^{31}$P SP/MAS NMR spectroscopy to assess the enantiomeric purity of organophosphorus compounds [67]. $^{13}$C CP/MAS NMR spectroscopy has been used primarily as a semi-quantitative tool, utilizing ratios of integrated peak intensities, with no corrections to $T_{1\rho H}$ and $T_{CP}$, to estimate the levels of phase impurities. For example, Harris used relative intensities (isotropic peaks + spinning sidebands) as a basis for the semi-quantitative analysis of cortisone acetate polymorphs by $^{13}$C CP/MAS NMR spectroscopy [68]. More recently, Stockton reported that $^{13}$C CP/MAS NMR spectroscopy could be used to detect less than 2% of a polymorphic impurity pendimethalin, however, details of the quantitative analysis were not provided [69].

A prerequisite to developing any quantitative method is that spectral features be identified which can be used to differentiate species in multi-component samples. Gao used diagnostic resonances of delavirdine mesylate to quantify polymorphic and pseudopolymorphic mixtures. Fig. 9 [70]. By showing that the $T_{1\rho H}$ and $T_{CP}$ values of the polymorphic forms VIII and XI were virtually identical (Fig. 10), the mole ratio in form VIII/XI mixtures could be obtained directly from the ratio of selected peak intensities over a dynamic concentration range of 2–50% (w/w) and with a limit of detection of 2–3% of the minor component (Fig. 11). In the case of the pseudopolymorphic forms XI and XII, however, the $T_{1\rho H}$ and $T_{CP}$ values were significantly different (Fig. 10), resulting in different relative intensities of the diagnostic peaks on a per mole basis. In addition to the different cross polarization kinetics, quantitative analysis was complicated by the diagnostic carbon resonances being not fully resolved. Therefore, to quantitative mixtures of forms XI and XII, peak intensities of XII were extracted from the difference spectra of pure form XI and various XI/XII mixtures, and compared to those of the original spectra of the mixtures. The relative intensities of XI and XII were then related to the mole ratio of the forms,

Fig. 9. $^{13}$C CP/MAS NMR spectra of delavirdine mesylate forms (A) VIII, (B) XI, and (C) XII. The methyl resonances (19–24 ppm) were used to quantify VIII/XI and XI/XII mixtures. (Reproduced, with permission, from Ref. [70]).
Fig. 10. The evolution of $^{13}$C peak intensities for delavirdine mesylate forms VIII, XI, and XII. (Reproduced, with permission, from Ref. [70]).

Fig. 11. The composition of delavirdine mesylate form VIII/XI mixtures determined by CP/MAS NMR, $w_{\text{VIII}}^{\text{NMR}}$, plotted against the known mass fraction of the form VIII, $w_{\text{VIII}}^{\text{mass}}$, in VIII/XI mixtures. (Reproduced, with permission, from Ref. [70]).
factoring in the different $T_{1\text{ph}}$ and $T_{CP}$ values. Using this approach, as low as 3% form XII could be detected and up to 25% quantified in form XI/XII mixtures.

Typically, polymorphs/pseudopolymorphs are differentiated by their unique chemical shifts. For example, Harris used the different chemical shifts of cortisone acetate polymorphs as a basis for the semi-quantitative analysis by $^{13}$C CP/MAS NMR spectroscopy [68]. In this application, integrated peak intensities of isotropic peaks and spinning sidebands were combined to yield signal intensities.

In the case of the anhydrate and dihydrate forms of carbamazepine, which are not differentiated by their chemical shifts, quantitative methods could still be developed by taking advantage of the significantly different proton spin lattice relaxation rates of the pseudopolymorphs [71]. As described earlier, the efficiency of cross polarization at a given contact time depends on $T_{1\text{ph}}$. Thus, if the $T_{1\text{ph}}$ values are different, as in the case of carbamazepine dihydrate and anhydrate, cross polarization may be used to discriminate between different parts of the sample. By selecting a contact time in which only the dihydrate form was observed, Suryanarayanan was able to reference the spectra to glycine, which was used as an internal reference standard, and to construct a calibration curve for the dihydrate form, see Fig. 12.

3.2. Quantification of degree of crystallinity

Clearly, a variety of techniques are available to differentiate crystalline and amorphous solids. The assessment of crystallinity by any technique depends on just how the technique detects the organization of molecules in the solid pharmaceutical. For example, X-ray diffraction measurement of crystallinity requires ‘long range’ ordering of molecules whereas solid-state NMR is more sensitive to local or ‘short-range’ order, approximately 100 versus 5 Å, respectively. Thus, even if quantitative solid-state NMR measurements are made, the estimate of percent crystallinity may differ from values obtained from X-ray diffraction methods [72]. Furthermore, the amorphous component of the pharmaceutical may actually be due to disorder of the crystal lattice itself, i.e. consistent with the single state model, rather than a distinct separate phase, as in the dual state model of crystallinity [73]. If the disorder is a part of the unit cell of the crystal lattice itself, one would expect significant differences in the quantitative results of X-ray diffraction and solid state NMR, since the range over which the disorder occurs is intermediate between ‘long’ and ‘short’ range.

Takahashi and coworkers investigated the effect of grinding and drying on the solid-state stability of ampicillin trihydrate [74]. The effects of removal of water from the ampicillin trihydrate structure resulted in the chemical instability of the drug. They found that the water that was freed from hydrogen bonding during grinding was then able to participate in a hydrolytic solid-state reaction in the impaired crystal lattice. They quantified the degree of crystallinity by ratioing the peak area under the diffraction peaks to the total area of the diffraction pattern. A linear correlation curve was observed between concentration and peak area divided by total area of diffraction. Kaneniwa et al. examined the effect of grinding in an agate centrifugal ball mill on the transformations of polymorphs of chloramphenicol palmitate using XRD [75]. They used the ratio of two peaks representing the individual crystalline phases.

In one recent study of the degree of crystallinity, the intensity of X-ray diffraction at a specific angle in the ‘amorphous halo’ region of the diffraction pattern of imipenem, a β-lactam antibiotic, was correlated with degree of crystallinity [76]. A linear relationship of concentration versus diffraction intensity was observed for standard mixtures of crystalline and amorphous components. One distinct advantage of this approach over methods that measure crystalline content based upon the intensity of a single diffraction peak is that the influence of preferred orientation is eliminated. The method requires a calibration curve whenever the study is conducted, since intensity of diffraction is highly dependent upon tube intensity, which inevitably deteriorates with age. The method was found to be quick, convenient, and suitable for the optimization of the lyophilization process of imipenem.

In a study of the kinetics of crystallization of indomethacin, the ratio of the area of a single crystalline peak representing each phase was ratioed against the peak area of an external standard, LiF.
over time to monitor the relative rate of crystalliza-
tion to the two polymorphic forms. The use of the
internal standard compensates for any changes in
photon output from the X-ray tube during the period
of the study [77]. FT-Raman spectroscopy has also
been used to study the degree of crystallinity of
indomethacin [78]. In the study, a ratio of peak
intensities of the crystalline and amorphous phases
resulted in a linear correlation curve over a range of
0–100%. The method was capable of measuring
down to 1% amorphous or crystalline component of
indomethacin α and γ phases. The greatest source of
eroerror was attributed to inhomogeneous mixing of
calibration and validation samples. Inhomogeneity of
mixing is perhaps the greatest sources of error for all
quantitative methods, particularly with amorphous
and crystalline components where the bulk density
difference is more extreme than with crystalline
d polymorphs. Of course this is not only true for
calibration and validation samples, we have found
that there can be considerable segregation of crys-
talline and amorphous components of the API as it is
stored in fiber drums.

Both FT-IR spectroscopy and X-ray powder dif-
fraction methods were developed to quantify the
degree of crystallinity of cefazolin sodium [79]. The
FT-IR method used the intensity ratio of a peak
representative of the crystalline and amorphous
phases, respectively, versus a reference peak whose
intensity was independent of crystalline state of the

over time to monitor the relative rate of crystalliza-
tion to the two polymorphic forms. The use of the
internal standard compensates for any changes in
photon output from the X-ray tube during the period
of the study [77]. FT-Raman spectroscopy has also
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down to 1% amorphous or crystalline component of
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eroerror was attributed to inhomogeneous mixing of
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mixing is perhaps the greatest sources of error for all
quantitative methods, particularly with amorphous
and crystalline components where the bulk density
difference is more extreme than with crystalline
d polymorphs. Of course this is not only true for
calibration and validation samples, we have found
that there can be considerable segregation of crys-
talline and amorphous components of the API as it is
stored in fiber drums.

Both FT-IR spectroscopy and X-ray powder dif-
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degree of crystallinity of cefazolin sodium [79]. The
FT-IR method used the intensity ratio of a peak
representative of the crystalline and amorphous
phases, respectively, versus a reference peak whose
intensity was independent of crystalline state of the

Fig. 12. Left: $^{13}$C spectra of mixtures of carbemazepine anhydrate (I), dihydrate (II), and glycine (internal reference standard). Form II is
observed exclusively with a recycle delay of 10 s (from bottom to top, mole fraction of II (in I) is 0, 0.25, 0.5, 0.75, and 1.0). Right: the
peak area ratio of carbemazepine II to glycine as a function of mole fraction of II (in I) (reproduced, with permission, from Ref. [71]).
substance. Specifically, the β-carbonyl band intensity was constant and independent of the crystalline state whereas the position of the amide II band differed for the crystalline and amorphous pharmaceutical, see Fig. 13. The X-ray powder diffraction method used standard mixtures of amorphous and crystalline forms to generate a calibration curve. Crystallinity was measured by integrating the intensity of X-ray scattering of the crystalline region of the sample, the area of the sharp peaks above the ‘amorphous halo,’ versus the integrated intensity of the entire diffraction pattern. Different results were found by the FT-IR and X-ray powder diffraction methods. The difference was attributed to the high degree of disorder associated with crystalline pentahydrate phase (α form) of the pharmaceutical.

Qualitatively, crystalline and amorphous phases can be readily identified by solid-state NMR spectroscopy. Whereas discrete, sharp signals are typically observed in solid-state NMR spectra for molecules in crystalline environments, amorphous components will give rise to broad resonances due to the distribution of all possible molecular conformations and/or orientations in the non-crystalline solid. Because amorphous materials are also generally characterized by high molecular mobility relative to their crystalline counterparts, a qualitative or quantitative evaluation of crystallinity by solid-state NMR spectroscopy may require both CP and SPE techniques. LeBotlan reported an example of the quantitative analysis of crystallinity by SSNMR spectroscopy [80]. Relaxation delays shorter than 5T₁ for the analysis of crystalline samples were used to avoid lengthy experiments along with a continuous method (CONTIN program) for the analysis of relaxation curves for quantification of crystallinity in mixtures of sugars.

4. Conclusions

There are numerous techniques and methods that allow quantification of solid-state forms of pharmaceuticals. It is difficult to predict which method is best suited for a particular type of analysis a priori. There are numerous considerations that go into the development of a good analytical method, sensitivity, specificity, method ruggedness, and skill level of the person conducting the analysis. One will not typically find the precision or accuracy that is commonly found with chromatographic methods. The solid-state methods suffer from an inherent difficulty of achieving sample uniformity. A well-developed method will often be capable of quantifying down to 5% and approximately 10% relative standard deviations.

There are numerous opportunities for further development of quantitative methods for pharmaceuticals. One can expect to see more frequent application of ‘whole pattern’ approaches to quantitative X-ray powder diffraction analysis of pharmac...

Fig. 13. Regression lines for cefazolin sodium as a function of percentage crystallinity using IR spectroscopy (left) and X-ray diffractometry (right): α form (a) and disordered/amorphous dehydrated α form (b). (Reproduced, with permission, from Ref. [79]).
ceuticals. Due to the enhanced sensitivity of FT-Raman spectroscopy and the advantages of minimal sample preparation and greater specificity relative to near IR, one may anticipate its increased application in quantitative analysis. Because of the ever-increasing need to reduce particle size to enhance bioavailability of highly permeable-water insoluble compounds, there continues to be a largely unmet need for the detection of low levels of an amorphous component present in crystalline pharmaceuticals.

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