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Title	Application of vibrational spectroscopy to study solid-state transformations of pharmaceuticals
Author(s)	Erxleben, Andrea
Publication Date	2016-09-01
Publication Information	Andrea, Erxleben. (2016). Application of Vibrational Spectroscopy to Study Solid-state Transformations of Pharmaceuticals. <i>Current Pharmaceutical Design</i> , 22(32), 4883-4911. doi: <a href="http://dx.doi.org/10.2174/1381612822666160726110103">http://dx.doi.org/10.2174/1381612822666160726110103</a>
Link to publisher's version	<a href="http://dx.doi.org/10.2174/1381612822666160726110103">http://dx.doi.org/10.2174/1381612822666160726110103</a>
Item record	<a href="http://hdl.handle.net/10379/6533">http://hdl.handle.net/10379/6533</a>
DOI	<a href="http://dx.doi.org/10.2174/1381612822666160726110103">http://dx.doi.org/10.2174/1381612822666160726110103</a>

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# Application of Vibrational Spectroscopy to Study Solid-state Transformations of Pharmaceuticals

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**Abstract:** Understanding the properties, stability and transformations of the solid-state forms of an active pharmaceutical ingredient (API) in the development pipeline is of crucial importance for process-development, formulation development and FDA approval. Investigation of the polymorphism and polymorphic stability is a routine part of the pre-formulation studies. Vibrational spectroscopy allows the real-time *in situ* monitoring of phase transformations and probes intermolecular interactions between API molecules, between API and polymer in amorphous solid dispersions or between API and coformer in cocrystals or coamorphous systems and thus plays a major role in efforts to gain a predictive understanding of the relative stability of solid-state forms and formulations. Infrared (IR), near-infrared (NIR) and Raman spectroscopies, alone or in combination with other analytical methods, are important tools for studying transformations between different crystalline forms, between the crystalline and amorphous form, between hydrate and anhydrous form and for investigating solid-state cocrystal formation. The development of simple-to-use and cost-effective instruments on the one hand and recent technological advances such as access to the low-frequency Raman range down to 5 cm<sup>-1</sup>, on the other, have led to an exponential growth of the literature in the field. This review discusses the application of IR, NIR and Raman spectroscopies in the study of solid-state transformations with a focus on the literature published over the last eight years.

**Keywords:** IR spectroscopy, NIR spectroscopy, Raman spectroscopy, polymorphism, amorphous phase, hydrates, cocrystals, amorphous solid dispersions

## 1. INTRODUCTION

Vibrational spectroscopy has become an indispensable tool in pharmaceutical solid-state chemistry. Its applications range from routine stability studies, quality assurance and process-monitoring where inexpensive, non-intrusive and fast analytical techniques are needed to sophisticated mechanistic studies of solid-state phase transformations and solid-state reactions such as the mechanically-induced formation of pharmaceutical cocrystals and salts.

Polymorphism, the ability of a compound to exist as more than one crystalline phase, is a widespread phenomenon in pharmaceuticals (see [1,2] and references therein). Recent estimates suggest that about 80 - 90 % of organic compounds are capable of crystallizing in two or more solid-state forms with different molecular arrangements and / or conformations in the crystal lattice [3]. It is well established that polymorphs can exhibit significantly different physicochemical properties, such as density, melting point, physical stability, reactivity, and dissolution rate [4]. As these can directly affect absorption, bioavailability, and shelf-life of the final drug product, characterization and experimental evidence for the solid-state form is required in the final specifications for FDA approval [5,6]. In extreme cases, an undesired polymorph can even be toxic [7]. The identity of the polymorphic form is also relevant to patent protection [8].

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Usually, the most thermodynamically stable form of an active pharmaceutical ingredient (API) is selected for development of the final drug product formulation. However, there are instances, where a metastable polymorph is utilized on account of more favorable physicochemical properties. In this case spontaneous transformation to a more stable polymorph may occur during processing, formulation or storage. Very often, the stability differences between polymorphs are relatively small and even if the most stable form is used, mechanical or thermal stress during milling or compression, or unexpected severe storage conditions (temperature, humidity) can induce a polymorphic transformation [9]. Low aqueous solubility hindering oral delivery is one of the main hurdles in drug development today and many poorly soluble drug development candidates fail to reach the market. One approach to overcome poor water solubility is to convert the crystalline API into its more soluble amorphous form [10-12]. However, the solubility advantage comes at the cost of thermodynamic instability and recrystallization during storage is a major problem. On the other hand unwanted loss of crystallinity of a crystalline phase can occur during drying, milling or compression. Solvates – in the pharmaceutical context usually hydrates –

are often referred to as pseudo-polymorphs and like true polymorphs undergo mechanically, thermally or moisture-induced solid-state transformations.

Vibrational spectroscopies including mid-infrared (IR), near-infrared (NIR) and Raman spectroscopy require minimal sample preparation and handling, are non-destructive and allow the *in situ* monitoring of phase changes in real time. They are also quantitative techniques and the absorbance at single characteristic wavenumbers can be used to obtain composition-time profiles. However, often the spectral differences between polymorphs are small and more sophisticated approaches to data analysis are needed. Nowadays, multivariate methods such as partial least-squares (PLS) regression that utilize spectral information from the whole spectrum or selected spectral regions are generally considered more effective and superior to the traditional univariate approaches. The availability of accurate quantification methods is essential for understanding the transformation kinetics of an API and thus for assessing the stability range of a given solid-state form. Vibrational spectroscopies probe intermolecular interactions and are therefore studied as potential tools for predicting the stability of solid-state formulations [13]. Moreover, NIR and Raman spectroscopy which can be easily coupled to fiber-optic probes, are also ideally suited as process analytical technology (PAT) tools.

Vibrational spectroscopy is increasingly used to address important fundamental questions in pharmaceutical solid-state chemistry, such as whether a polymorphic transformation proceeds via the transient amorphous state or as a direct crystal-crystal transformation. The pathway of the mechanochemical formation of pharmaceutical cocrystals which are of significant current interest can be investigated by spectroscopic methods. Spectral data can give insight into the much debated question of 'polyamorphism'. Imaging techniques, such as Raman mapping and NIR spectroscopic imaging provide 2D spatial information on phase changes in solid-dosage forms and have been widely applied to investigate dissolution and drug release mechanisms of amorphous APIs [14-17].

Several excellent review articles on the application of vibrational spectroscopy for the solid-state analysis of pharmaceuticals and for the detection and monitoring of solid-state transformations of crystalline polymorphs, hydrates and amorphous forms have been published [18-21]. However, the literature is rapidly growing and the aim of this article is to present an update on the use of mid-IR, NIR and Raman spectroscopies in pharmaceutical research covering the literature of the last eight years (>2009). First a brief description of the spectroscopic methods and of spectral data analysis will be given. Then recent applications will be discussed with a focus on mechanistic studies of phase transformations in the solid state. Spectroscopic techniques are often the method(s) of choice for monitoring the polymorphic form during cooling crystallization or in slurries [22-28]. However, solution crystallization processes are beyond the scope of this review. Neither will terahertz spectroscopy which has recently been reviewed by Sibik and Zeitler be covered [29].

NIR, mid-IR and Raman spectroscopies cover the wavenumber ranges 12,500 – 4000, 4000 – 400 and 4000 – 10  $\text{cm}^{-1}$ , respectively. Recent advances in filter technology have led to the growing use of low-frequency Raman spectroscopy (400 – 10  $\text{cm}^{-1}$ ) which gives access to the lattice vibrations of organic compounds [30]. As described in the following sections, each technique has its advantages and disadvantages. Very often they provide complementary information and in most studies aimed at detecting and investigating phase transformations in the solid state a combination of two or more spectroscopic methods is applied.

### 2.1. Mid-IR spectroscopy:

Mid-IR is routinely used for the characterization and identification of APIs including different solid-state forms. It provides information about H bonding in organic or pharmaceutical compounds and is widely applied to study API-excipient interactions. However, for the quantitative analysis of a polymorphic API, sample preparation can be a major problem and source of error. The classical IR sampling technique of alkali halide pellet preparation for transmission mode measurements may lead to solid-state transformations due to the pressure applied during tablet pressing. KBr, in particular, can lead to salt formation. Even in the absence of pressure-induced solid-state conversions during sampling, the time requirement for sample preparation often makes the method unsuitable for kinetic measurements. Attenuated total reflectance (ATR) IR spectroscopy allows the neat sampling of powder samples, but again the effect of pressure has to be controlled. Additionally, the small sampling size is a major source of error in ATR-IR spectroscopy and the low penetration depth of only a few micrometers is a significant shortcoming with regard to bulk material analysis of polymorphic mixtures. Moreover, ATR-IR spectra are particularly affected by packing and crystal orientation problems. The mineral oil mull preparation technique is less likely to cause phase transformations, probably because the oil carries away most of the heat generated during grinding, but the intense IR bands of the oil can overlap with specific absorption bands and this may be a particular problem for quantitative analysis. Furthermore, in some cases nujol itself has been found to mediate polymorphic conversion [31]. The preparation of thin films by spin coating on an IR transparent substrate is rarely used for pharmaceutical samples [32]. Diffuse reflectance IR-FT spectroscopy (DRIFTS) is probably the most extensively used method for polymorph analysis, as compounds can be sampled neat or diluted in KBr or KCl powders without exposing the sample to any compression energy. Besides for routine characterization and analysis purposes, the method can be employed to study temperature- and humidity-mediated solid-state transformations using an environmental chamber attachment [33]. A disadvantage of the diffuse reflectance technique is the particle-size dependency [34]. For accurate and reliable analysis the particle size of all calibration, validation and test samples must fall in a specified range. Likewise, sample homogeneity, bulk density and particle shape must be carefully controlled. A method that has proven to be an effective neat sampling technique for quantitative polymorph

## 2. SPECTROSCOPIC METHODS

analysis is photoacoustic spectroscopy (PAS), since the particle size has a minimal effect and depth profiling is possible [35-37]. In FT-IR PAS the sample is placed into a closed cell that is fitted with a coupling gas. Absorbed modulated IR radiation is converted into heat. The increase in temperature produces pressure changes in the surrounding gas (sound waves) that occur at the frequency of the modulated light. Detection of the acoustic wave by a very sensitive microphone and Fourier transformation of the subsequent electrical signal generates the spectrum. IR microspectroscopy is a valuable technique in polymorphism research, as single crystals or individual particles can be analyzed, either in transmission or in reflectance mode [38,39]. Other useful methods for the solid-state investigation of polymorphic APIs include techniques that are amenable to *in situ* or real-time studies. Hot stages for IR spectrometers, for example, offer the possibility to monitor temperature-induced polymorphic transformations.

## 2.2. NIR spectroscopy:

The absorption bands in the NIR spectrum arise from overtones and combinations of O-H, N-H, C-H, and S-H stretching modes. Therefore NIR spectra are particularly useful for the quantification of polymorphs with their different H bonding patterns. However, the NIR bands are broad and spectra are generally less resolved than spectra in the mid-IR region so that usually multivariate methods are required to extract quantitative information from NIR spectra. NIR spectra contain chemical and physical information. To reduce the influence of physical properties such as particle size, particle shape and surface texture spectra are routinely pre-processed prior to multivariate analysis using for example scatter correction methods or derivation. The weak interaction of NIR radiation in the 14,000 to 6000  $\text{cm}^{-1}$  region due to the low absorptivities of the second (9000 - 6000  $\text{cm}^{-1}$ ) and third (14,000 - 9000  $\text{cm}^{-1}$ ) overtones results in a greater depth of penetration into the sample and transmission spectra can be measured of opaque samples or through tablets of several millimeter thickness. NIR spectroscopy (NIRS) is very sensitive to O-H vibrations and is therefore often used to measure the water content or to investigate the hydration behavior of pharmaceuticals. It is also well suited to *in situ* monitoring and is in fact often utilized in industry for process monitoring [40]. However, when unit operations such as freeze-drying or wet granulation involve large amounts of water, NIR spectroscopy can fail as an analytical method, as the water signal can become saturated. As NIR spectra are much more complicated for interpretation than IR spectra due to the overlapping of overtones and combination bands, not many reports have been published on the characterization of pharmaceuticals by NIR spectroscopy, although NIR spectra have been used for the chemical identification of APIs and polymorphs in the past [41,42]. For difficult and non-routine analysis, two-dimensional correlation NIR spectroscopy can be useful, for example for the detection of specific bands which are not clearly observed [43].

## 2.3. Raman spectroscopy:

Raman spectroscopy has become increasingly popular as a vibrational spectroscopic method for the characterization and study of solid-state forms and transformations, mainly

because its adaptability to *in situ* monitoring, high sensitivity and better selectivity compared to NIRS. Several comprehensive reviews on the application of Raman spectroscopy to the solid-state characterization of pharmaceuticals are available in the literature [19,44-46]. Raman spectroscopy is based on the inelastic scattering of monochromatic laser light. Nowadays, both FT-Raman instruments and single-stage dispersive Raman systems are widely used in pharmaceutical applications. Generally, Raman spectra feature sharp and well-resolved bands that can be easily assigned to functional groups. The speed of measurement – data acquisition usually takes no more than a few seconds - makes it an ideal method for high-throughput screening studies. Raman spectroscopy probes changes in molecular conformation, H bonding interactions and long- and short-range order and is often used in mechanistic studies of phase transitions. Due to its low sensitivity to water, Raman spectroscopy is especially effective for the analysis of solvent-mediated transformations. The spectral intensities are determined by polarizability changes. Aliphatic excipients are therefore often poor Raman scatterers compared to aromatic, heterocyclic APIs with extended  $\pi$ -conjugated systems. Consequently, APIs are usually easily detected, even when present at low concentration in a formulation. Major disadvantages of conventional backscattering Raman spectroscopy are subsampling and limited penetration depth which is a particular problem for heterogeneous samples or formulations. These issues were addressed by expanding the laser deposition and Raman collection areas [47] and Raman spectroscopy in transmission mode [48].

### 2.3.1. Transmission Raman spectroscopy:

Transmission Raman spectroscopy (TRS) which relies on the diffuse rather than the ballistic scattered light allows for a deeper penetration range of several millimeters into opaque samples and thus combines the specificity of Raman spectroscopy and the depth probing of NIR spectroscopy [49-52]. TRS has been known since the early days of Raman spectroscopy, but has only recently been recognized as a powerful method for the analysis of pharmaceutical formulations [53-56]. It can be applied to intact tablets and capsules as it effectively suppresses fluorescence and Raman signals originating from coating or capsule layers [55]. Burley's group were the first to show the superior performance of TRS compared to conventional backscattering Raman spectroscopy for the chemometric quantification of polymorphic mixtures using flufenamic acid as a model API [57]. Griffin *et al.* have recently significantly reduced the acquisition time of TRS by using an enhancing "photon diode" [58].

### 2.3.2. Low-frequency Raman spectroscopy:

There are an increasing number of reports in the literature on the use of low-frequency (LF) or phonon-mode Raman spectroscopy in pharmaceutical solid-state chemistry. The LF region (below 200  $\text{cm}^{-1}$ ) is composed of molecular skeleton deformations, librations and translations. It provides a second fingerprint region that reflects the long-range order in crystals and is very sensitive to polymorphic changes and structural disorder. LF Raman spectroscopy (LFRS) is particularly useful to study polymorphic systems that do not

exhibit conformational polymorphism, as the intramolecular fingerprint regions of the vibrational spectra of non-conformational polymorphs are typically very similar. The use of LFRS as a screening method has led to the discovery of new polymorphs [59]. The lattice vibrations in the LF Raman spectrum are also sometimes referred to as external vibrations, intermolecular vibrations or phonon modes. Like the intramolecular vibrations, lattice vibrations depend on molecular-site symmetry and the number of Raman active phonons is determined by the degree of crystal symmetry [60].

Since amorphous solids lack long-range order, a broad band, also called the boson peak, is observed in the LF spectrum of an amorphous phase instead of the relatively sharp peaks of a crystalline solid. Therefore, LFRS not only easily discriminates between different crystalline forms, but also presents a potential probe for crystallinity and disorder and has been applied to study polymorphic transformations, amorphization and crystallization processes [61-64].

Double or triple monochromators are typically used to record the LF Raman range [30,65,66]. Recently, ultra-narrow band laser rejection filters have become available that allow measurements at  $< 10 \text{ cm}^{-1}$  [67]. Like conventional Raman spectra, LF Raman spectra can be recorded within seconds and only milligram quantities or less are required. Bands arising from phonon-mode vibrations are typically several times more intense than those arising from intramolecular vibrations. As in the high-frequency range aromatic APIs have characteristic and intense Raman bands below  $200 \text{ cm}^{-1}$ , while the typically aliphatic excipients exhibit weak bands. Carbamazepine, theophylline and caffeine, for example, exhibit LF bands of ten- to twenty-fold higher intensity than those of the common excipients sucrose, lactose and microcrystalline cellulose [68]. Furthermore, LF Raman spectra are less affected by fluorescence interference compared to conventional Raman spectra.

Matzger's group compared the utility of LFRS and conventional Raman spectroscopy for the discrimination of the different phases of ten polymorphic pharmaceuticals including the widely studied model polymorphic API paracetamol [69]. The discriminating power of the LF range was found to be clearly superior to that of the conventional Raman range and to present a fingerprint region for polymorphs. In an independent study, Burley's group confirmed the higher sensitivity of the LF region to phase transitions of paracetamol by using rigorous statistic methods [70]. Different crystalline phases of the same compound give rise to specific peaks in the LF range, while in the conventional Raman range different polymorphs give peak shifts of a few wavenumbers, peak broadening or sharpening or a change in peak shape. On the other hand, in the case of carbamazepine two of the five known polymorphs, form I and II, have very similar lattice structures and this is reflected in the similarity of their LF Raman spectra. Matzger and coworkers suggested that the LF range could be used to assess packing similarities in the absence of crystallographic data [69]. Very often the combined analysis of the phonon and intramolecular vibrations prove to provide useful complementary information on phase transformations.

### **2.3.4. Coherent anti-Stokes Raman scattering:**

Coherent anti-Stokes Raman scattering (CARS) uses two pulsed lasers to generate a coherent anti-Stokes signal. CARS microscopy has recently been applied by Strachan and coworkers to monitor solid-state transformations in oral dosage forms of theophylline anhydrate during dissolution [71]. However, the requirement for rather specialized equipment makes CARS a not commonly used method for studying pharmaceutical compounds and materials.

### **2.3.5. Imaging techniques:**

Vibrational microscopy and imaging techniques provide information on the 2D spatial distribution of solid-state forms in solid-dosage forms. Imaging techniques include point-by-point scanning, line scanning and focal plane scanning [72-76]. In theory, IR light allows a spatial resolution of  $0.5 \mu\text{m}$  compared to  $1\text{-}2 \mu\text{m}$  in the case of NIR light. In practice, however, typical resolutions are  $4$  and  $6 \mu\text{m}$ , respectively [77]. By contrast, submicron level resolution can be achieved with Raman spectroscopy [78,79]. In mid-IR spectroscopic imaging that uses transmission, reflectance or ATR mode, the intensity is measured with a focal plane array (FPA) detector which enables the recording of thousands of spectra from the sampled area simultaneously. The acquisition speed of FPA detectors allows the investigation of dynamic systems. FT-IR imaging has been significantly developed during the last decade. In particular ATR-FTIR imaging has been recognized as an effective method for dissolution studies of pharmaceutical formulations, as accurate quantitative distribution profiles can be obtained [80]. Reference [81] provides an excellent overview on the theory and applications of ATR-FTIR spectroscopic imaging.

As in conventional NIR spectroscopy, chemometric methods are usually required to extract information on the spatial distribution of different solid-state forms from NIR mapping data.

Raman mapping is widely used to study the distribution of different components in solid dosage forms [80,82]. In various works Raman mapping has been employed to study polymorphic transformations, the formation of hydrates or the recrystallization of an amorphous component in solid-dosage forms in a temporally and spatially-resolved manner. Šašić and Mehrens recently proposed a statistically optimized sampling grid to detect unwanted solid-state forms of a development API by Raman mapping of the tablet surface (API concentration  $1 \text{ \% w/w}$ ) [83]. The method was able to identify  $0.025 \text{ \% (w/w)}$  of the amorphous form and  $0.05 \text{ \% (w/w)}$  of two other relevant polymorphs without the requirement of multivariate data analysis, as the different forms showed clearly distinct Raman peaks.

## **3. SPECTRAL DATA ANALYSIS**

As already mentioned, spectral differences between different solid-state forms are often small and chemometric approaches are required to extract information from the spectral data. A spectrum with  $n$  spectral data can be represented as a point in  $n$ -dimensional space and the basic concept of chemometric methods is to reduce the dimensionality of the data set [84].

Principal component analysis (PCA) re-organizes information in a data set of samples and identifies a set of underlying variables, called principal components (PCs), which are orthogonal in nature and encode the spectral variance. PCA decomposes the spectral data matrix  $X$  into a score matrix  $T$  multiplied by a loading matrix  $P^T$  and a residual matrix  $E$  [85]:

$$X = TP^T + E$$

The scores are the values of individual samples when the PCs are used as new variables. Each PC is a linear combination of the original measurement variables multiplied by a coefficient (loading).

$$PC_a = \sum_{k=1}^K c_{a,k} \text{variable}_k$$

The decomposition of the spectral data matrix allows the visualization of the data in a low dimensional space as scores plots and loadings plots with the latter showing the relationships between the PCs and the original measurement variables. Thus, PCA allows the classification of samples according to their vibrational spectra. Other chemometric methods include soft independent modelling of class analogy (SIMCA), hierarchical clustering, k-nearest neighbor, linear discriminant analysis (LDA), and partial least squares discriminant analysis (PLS-DA). However, a discussion of these techniques is beyond the scope of this article and the interested reader is referred to recent review articles [86,87].

Although studies solely aimed at the development of new analytical methods for the quantification of different solid-state forms are not the focus of this review, it should be mentioned here that imaging and non-imaging vibrational spectroscopy, in particular NIR and Raman spectroscopy, in conjunction with chemometric tools receives significant attention in analytical chemistry and for quality assurance applications [88-100]. While the traditional method of monitoring the area or height of a selected band or the area or height ratio of two bands gives satisfactory results in some cases, nowadays multivariate methods that use the entire spectrum, a spectral region or a combination of spectral regions are routinely applied, when accurate quantitative data are needed. This is particularly true, when NIR spectroscopy is used as the spectroscopic technique. The potential of chemometrics in modern pharmaceutical analysis has been discussed in a recent review by El-Gindy and Hadad [101]. The most frequently used methods for building multivariate calibration models for the quantitative analysis of polymorphic mixtures include multiple linear regression (MLR), principal component regression (PCR) and partial least-squares (PLS) regression. In particular, PCR and PLS are well recognized as powerful tools for the quantitative analysis of spectroscopic data and the availability of various software packages has led to their widespread application in pharmaceutical analysis. All three methods are based on a least-squares regression. While MLR models use a discrete number of data points, PCR and PLS include all spectral data (or a spectral range) and are therefore often more accurate than MLR. In contrast to MLR that searches for a single factor that correlates data and concentration, PCR tries to find the multidimensional directions through the data that explain most of the variability before regressing the response variable  $Y$  on selected principal components. Like PCA, PCR and PLS

perform data decomposition into loadings and scores before regressing the concentration information onto the PC scores. PLS uses both spectral and concentration data for data decomposition, whereas in PCR only spectral information is utilized. The squared covariance of the score vectors of the predictor variable matrix  $X$  (spectral data) and the score vectors of the response variable matrix  $Y$  (concentration) is maximized during PLS model building, while unrelated data are neglected. A major limitation of PLS calibration models for spectroscopic data is the possibility of chance correlation, *i.e.* spectral variations that exist within the calibration spectra but do not originate from the analyte concentration [102-105].

While PCR and PLS are the most popular methods, several other chemometric techniques are used in combination with quantitative IR and NIR spectroscopy, such as artificial neural networks (ANN) [106] or non-linear calibration techniques. Multivariate curve resolution (MCR) is aimed at extracting pure-component spectra from the overlapped spectra of mixtures, without the need for building a calibration model, *i.e.* when the spectra of the pure components are not available [107].

## 4. STUDIES OF SOLID-STATE TRANSFORMATIONS USING VIBRATIONAL SPECTROSCOPY

### 4.1. Polymorphic transformations:

Table 1 gives an overview of examples from the recent literature on the application of vibrational spectroscopy in polymorphism research. Paracetamol is without doubt among the most studied polymorphic APIs. Vibrational spectroscopy as a means to analyze mixtures of paracetamol polymorphs is very well established [108-110], while the application of Raman, NIR and IR spectroscopy for the investigation of solid-state transitions of paracetamol continues to be of significant interest. Paracetamol form I is the most thermodynamically stable form at ambient temperature and pressure, forms II and III are metastable. As form II has better tableting properties than commercially used form I, understanding the solid-state properties and transformations of this polymorph is particularly important. In order to gain more insight into the crystallization and transition behavior of paracetamol forms I and II the dynamics of the intermolecular  $\text{OH}\cdots\text{O}$  and  $\text{NH}\cdots\text{O}$  H bonds were studied by variable-temperature (5 – 300 K) polarized Raman spectroscopy of oriented single-crystals of these two polymorphs [111]. It was shown that the higher stability of form I is a cumulative effect rather than the result of the greater strength of individual  $\text{OH}\cdots\text{O}$  and  $\text{NH}\cdots\text{O}$  H bonds. That is all H bonds together stabilize the structure of form I to a larger extent than that of form II. A comprehensive analysis of the temperature dependence of the wavenumbers, widths and intensities of the Raman bands indicated that in both forms the disorder increases on cooling, albeit with different preferential disorders in the two types of H bond ( $\text{OH}\cdots\text{O}$  and  $\text{NH}\cdots\text{O}$ ). The  $\nu(\text{C}=\text{O})$ ,  $\nu(\text{O}-\text{H})$ , and  $\nu(\text{N}-\text{H})$  vibrations of form I appear at higher wavenumbers than those of form II suggesting that form II has the stronger  $\text{O}-\text{H}\cdots\text{O}$  hydrogen bonds. This contradicts

conclusions drawn from X-ray data which showed that the H bonds in form II are longer and more compressible compared to form I and shows that single-crystal data do not unambiguously reflect the strength of H bonding interactions. It was also pointed out that along with the difficulty of obtaining accurate hydrogen atom positions by X-ray analysis, X-ray analysis gives the averaged structure while the typical time-scale of Raman spectroscopy is suited to detect dynamic processes. In another study the phonon range of the Raman spectrum, *i.e.* the “polymorphic fingerprint” region, was used to monitor the solid-state transformations undergone by paracetamol upon heating [59]. Zimmerman *et al.* used paracetamol as model API to develop a screening method for polymorphic transformations based on temperature-dependent reflectance and transmittance IR measurements, 2D-IR data presentation and baseline analysis [112]. This method detected previously unobserved phase transitions, *e.g.* the crystallization of form III during cooling. The pressure-induced form I → form II transition could be observed by spectral changes in the Raman spectrum [113]. On increasing the pressure to 5.9 GPa two new bands appeared and the  $\nu(\text{C}=\text{O})$  band shifted to lower wavenumbers suggesting a strengthening of the H bonding interactions upon conversion to form II in line with the earlier Raman study described above. Changes to the  $\rho(\text{CH}_3)$  and  $\text{CH}_3$  umbrella modes were attributed to an

increase in intramolecular H bonding and/or an increase in steric constraints between the  $\text{C}=\text{O}$  and  $\text{CH}_3$  groups. In addition spectral changes affecting the phenyl modes occurred consistent with the expected reorganization of the molecular layering. More importantly, pronounced shifts in relative intensities and wavenumbers and the appearance of new bands at 8.1 GPa gave evidence for the existence of a new polymorph (form IV). A further increase in pressure resulted in additional change in the Raman spectrum indicating the formation of a second previously unknown polymorph (form V). Raman mapping in conjunction with MCR analysis was performed to monitor the solid-state transformation of unstable paracetamol trihydrate to stable, anhydrous form I [114]. Earlier work [115] suggested that the trihydrate converts directly to the most thermodynamically stable polymorph in violation of Ostwald’s rule of stages which states that the least stable state lying nearest to the original state in free energy is initially formed. It was proposed that the loss of water gives rise to a density difference resulting in the collapse of the structure to the more favorable anhydrate. By contrast, the recent Raman mapping results presented evidence of a transient phase (form X) with clear spectral differences both in the phonon and in the molecular regions. Based on the presence of a unique O-H band form X was suggested to be a hydrated form.

Table 1. Recent (after 2009) studies on solid-state transformations of pharmaceuticals using vibrational spectroscopy

API/Excipient	Method	Study	Reference
amlodipine besylate	NIR, Raman, PCA, PLS, interval PLS	quantitative monitoring of solid-state transformations during stability measurements and wet granulation	[160]
atorvastatin, Ca salt	hot-stage Raman, PCA	monitoring of heating-induced solid-state transformations	[269]
berberine chloride	IR	qualitative monitoring of the heating-induced transformation of the di- and tetrahydrate; qualitative monitoring of phase changes of pharmacopoeial grade berberine chloride dihydrate during wet granulation	[270]
BN83495	Raman mapping	polymorph analysis of opacified tablets exposed to high temperature	[119]
caffeine	LFRS	phase transformations in caffeine tablets	[128]
caffeine	LFRS	solid-state transformations induced by hydrostatic compression of forms I and II	[62]
caffeine	LFRS	orientational and dynamic disorder in forms I and II	[182]
caffeine	Raman	quantitative analysis of the kinetics of hydration and dehydration	[271]
caffeine hydrate	Raman, LFRS	mechanistic studies of the dehydration process	[148]
carbamazepine	NIR imaging, MCR	monitoring of the thermal-induced form I → III conversion on the surface of tablets	[126]
carbamazepine	Raman, PLS, PLS-DA	solid-state transformations during dissolution	[174]
carbamazepine	Raman hyperspectral imaging, MCR-ALS	monitoring of the thermal-induced polymorphic transformations in tablets containing two heterogeneously distributed polymorphs	[127]
carbamazepine	Raman	<i>in situ</i> monitoring of the transformation of the anhydrate to the dihydrate during dissolution in simulated intestinal fluid and fasted state simulated intestinal fluid	[272]
carbamazepine dihydrate	2D-FT-IR correlation spectroscopy	crystal water dynamics	[161]
carbamazepine dihydrate	NIR, Raman, PLS	quantitative monitoring of solid-state transformations during isothermal dehydration; application of Raman spectroscopy to quantify solid-state transformations during dehydration in a fluidized bed dryer	[159]
cefuroxime	Raman	<i>in situ</i> monitoring of the conversion to the acetonitrile solvate	[273]
chlorpropamide	Raman	detailed analysis of the Raman spectra of four polymorphs and study of the low-temperature phase transition of form I	[274]
etravirine	ATR-IR	crystallization kinetics	[275]
fingolimod hydrochloride	Raman	monitoring of polymorphic transformations during heating to complement XRPD study	[276]
fluconazole	DRIFTS, PLS	monitoring of the solid-state transformation of metastable form I to stable form II upon grinding, compression, heating, seeding and	[116]

fluconazole	Raman	exposure to solvent vapors	[118]
flufenamic acid	LFRS	discovery of two new polymorphs at high pressure	[59]
gabapentin	hot-stage FT-IR	<i>in situ</i> monitoring of heating-induced polymorphic transformations	[277]
gabapentin	microspectroscopy	<i>in situ</i> monitoring of heating-induced dehydration and polymorphic transformations	[278]
gabapentin	FT-IR microspectroscopy	monitoring of milling-induced transformations of four polymorphs in the presence and absence of additives	[278]
glibenclamide	Raman, PCA	milling-induced disorder and recrystallization kinetics	[172]
glycopyrronium bromide	Raman	polymorph distinction by using a new Raman setup	[178]
griseofulvin	LFRS	detection and quantification of low levels of crystallinity in tablets; comparison of low- and high-frequency Raman spectroscopy	[177]
griseofulvin	Raman	detection of disorder by the superimposition of diffuse scattering on the Raman lines	[184]
griseofulvin	CARS	stabilization of the amorphous form by loading into mesoporous silica	[243]
ibipinabant	Raman, multivariate analysis	stability monitoring of ASD tablets	[201]
ibuprofen	ATR-FTIR spectroscopic imaging	qualitative monitoring of the crystallization of the amorphous form in pH resistant tablet formulations during dissolution	[279]
ibuprofen	Raman, NIR, MCR	real-time monitoring of recrystallization during acoustic levitation	[173]
ibuprofen	Raman	identification of metastable form II as a transient phase during heating-induced recrystallization of the amorphous phase	[64]
imidafenacin	NIR, PLS	quantification of form III (monohydrate) during form II → form III transformation using a humidity-controlled 96-well plate; comparison with glass bottle method	[152, 153]
imipramine hydrochloride	LFRS	screening study; new polymorphs	[59]
indomethacin	NIR, PCA	monitoring of the recrystallization of the amorphous form under different storage conditions using a humidity controlled 96-well plate	[280]
indomethacin	Raman, LFRS	sensitivity of the low- and high-frequency range to low levels of crystallinity	[61]
indomethacin	NIRS, Raman, PCA	comparison of NIR, Raman and DSC combined with univariate and multivariate data analysis for monitoring the recrystallization of the amorphous form prepared by different methods	[281]
indomethacin	Raman, PLS, PLS-DA	solid-state transformations during dissolution	[174]
indomethacin	IR, PLS	comparison of the suitability of IR and XRPD to predict performance of stored amorphous samples in dissolution testing	[176]
indomethacin	Raman	pressure-induced solid-state transformations	[183]
indomethacin	Raman mapping	crystallization of the amorphous phase; particle-size dependency of the transformation kinetics	[185]
indomethacin	Raman, PLS	recrystallization of the amorphous form confined into microcontainers	[244]
indomethacin	Raman mapping, chemometrics analysis	detection of crystallinity of amorphous indomethacin loaded into mesoporous silica	[242]
itraconazole	CARS	imaging of loaded mesoporous silica	[243]
lamivudine	IR, Raman	quantitative monitoring of the heating-induced dehydration of the dihydrate	[282]
mannitol	Raman	SMPT of the $\alpha$ - to the $\beta$ -form	[130]
mannitol	NIR, PCA	effect of drying method and conditions on the solid-state form in protein formulations	[133]
mannitol	Raman mapping, PLS-DA	spatial distribution of solid-state forms on the surfaces of spray-dried mannitol/lysozyme powders and single levitation droplet particles	[134]
mannitol	NIR, correlation coefficient (CorrCoef)	identification of polymorphs in freeze-dried end products	[283]
mannitol	Raman	polymorph distinction by using a new Raman setup	[178]
metoclopramide hydrochloride	DSC-FTIR, Raman microspectroscopy	heating-induced dehydration, recrystallization and phase transformation of the hydrate	[156-158]
mitemincinal fumarate	NIR	quantification of the hydrate : anhydrate ratio in tablets; monitoring of the hydrate during the tableting and film coating process	[284]
paracetamol	Raman	dynamics of the intermolecular OH...O and NH...O H bonds	[111]
paracetamol	LFRS	monitoring of heating-induced solid-state transformations	[59]
paracetamol	IR	monitoring of heating-induced solid-state transformations	[112]
paracetamol	Raman	monitoring of pressure-induced transformations of form I; discovery of two new polymorphs	[113]
paracetamol	DSC-Raman, PCA	crystallization of the supercooled phase	[110]
paracetamol	Raman, Raman mapping	crystallization of the amorphous phase	[189]
paracetamol	Raman	polyamorphism	[191]
paracetamol trihydrate	Raman mapping, MCR	solid-state transformation to form I	[114]
paroxetine hydrochloride	IR	corroborating XRPD, DSC, TGA and DVS results that indicate that form I of the API has been incorrectly classified as an anhydrate	[150]
pazopanib hydrochloride	diffuse reflectance NIR, PLS	real-time monitoring of solid-state transformations on a manufacturing scale	[168]
piroxicam	IR	identification of a solid-state form transition during the formation of poloxamer 188 stabilized nanocrystals; complementary to XRPD analysis	[285]
piroxicam	Raman	recrystallization of the amorphous form during layer-coating	[166]
piroxicam	Raman, PCA	in-line monitoring of solvent-mediated recrystallization of the amorphous phase; dependency on starting polymorph	[171]

piroxicam	Raman, PLS	<i>in situ</i> monitoring of SMPT of the anhydrate under different pH conditions	[167]
piroxicam monohydrate	NIR, Raman, PLS	quantitative monitoring of solid-state transformations during isothermal dehydration	[159]
piroxicam monohydrate	NIR hyperspectral imaging, MCR-ALS	mapping of the simultaneous dehydration of piroxicam dihydrate and lactose dihydrate on the surface of tablets	[162]
rivastigmine tartrate	hydrogen IR	qualitative monitoring of solid-state transitions during ball-milling	[286]
simvastatin	DRIFTS	recrystallization of the amorphous phase; comparison with XRPD	[287]
sulfamerazine	NIR, PLS	quantitative monitoring of transformations of sulfamerazine form I and II during milling	[121]
sulfamerazine	Raman	correlation of slip planes and mechanical instability of form III with very soft phonon modes	[192]
sulfathiazole	NIR, PLS	quantitative monitoring of transformations of sulfathiazole polymorphs during milling	[124]
sulfathiazole	NIR, PLS	recrystallization pathways of cryomilled sulfathiazole	[175]
sulfathiazole	Raman mapping	characterization of form II crystals having a form IV middle layer	[125]
tauroursodeoxycholic acid	NIR, chemometrics	amorphization during milling and recrystallization during storage	[169] [170]
theophylline	Raman	<i>in situ</i> monitoring of the solid-state form in lipid matrices during intrinsic dissolution testing	[288]
theophylline	NIR, PLS	quantification of theophylline monohydrate and water content during agitated granulation	[289]
theophylline	Raman, NIR	in-line monitoring of the solid-state form in theophylline/lactose/PVP blends during continuous drying	[165]
theophylline	Raman, NIR, PCA	in-line, real-time monitoring of the solid-state form of theophylline during the granulation of a theophylline/lactose blend; influence of process parameters	[290]
theophylline	DSC-NIR	monitoring of the dehydration kinetics	[154] [155]
theophylline	Raman	determination of the hydrate/anhydrate transition temperature from temperature-dependent transformation kinetics	[163]
theophylline monohydrate	NIR, PLS	in-line quantification of different solid-state forms during dehydration in a vacuum contact dryer	[164]
trehalose	simultaneous gravimetric vapor sorption/NIR	detection of crystallinity; distinction of free and bound water during sorption	[291]
warfarin	Raman, PLS	quantitative analysis of crystallinity	[292]

Obaidat *et al.* developed a calibration model based on DRIFTS and PLS to quantify binary mixtures of fluconazole forms I and II [116]. The model was applied to investigate the kinetics of the isothermal form II  $\rightarrow$  form I transformation and to study the effects of relative humidity (RH), seeding, solvent vapors, grinding and compression on the transformation rate. The Prout-Tompkins model for solid-state reactions which accounts for sigmoidal time profiles by assuming that the transformation rate is controlled by linearly growing, “branching” nuclei that are terminated more rapidly as the number of nuclei increases [117] provided the best fit of the kinetic data and was used to derive the activation energy for the transformation (329 kJ mol<sup>-1</sup>). In a later study Gorkovenko *et al.* used Raman spectroscopy to complement X-ray powder diffraction (XRPD) data on pressure-induced polymorphic transformations on fluconazole [118]. Changes to the C-H and CH<sub>2</sub> stretching modes and the appearance of new Raman bands were taken as evidence for the formation of two new polymorphs at high pressure.

Martins *et al.* investigated the polymorphism of an development API, BN83495 which has three known polymorphs [119]. Crystals of the low-temperature form I were found to progressively turn opaque on heating above

*ca.* 138 °C. The opacified parts were identified as form III by Raman mapping. Interestingly, more form III was observed at 20  $\mu$ m depth than on the surface. It was suggested that nucleation of form III is promoted by pre-coding defects in line with Mnyukh’s theory [120].

MacFhionnghaile *et al.* studied the solid-state conversions of sulfamerazine forms I and II during milling [121]. Compared to an earlier XRPD study [122], the NIR spectroscopic monitoring of the milling process gave further insight into the grinding-induced form I  $\rightarrow$  form II conversion: the composition-time profile showed that the milling-induced form I  $\rightarrow$  form II conversion is not direct, but takes place *via* the transient amorphous state. Milling at low temperature resulted in the complete amorphization of both sulfamerazine polymorphs which will be discussed in a later section. The conclusion that the amorphous phase is an intermediate rather than a by-product of room-temperature milling was drawn from a detailed quantitative kinetic analysis showing that the percentage of amorphous sulfamerazine in the mixture went through a maximum after 20 min. Thus, the construction of accurate multivariate calibration models was crucial. Form I, form II and amorphous sulfamerazine give distinct peaks in the 5700 – 7000 cm<sup>-1</sup> and 4000 – 5200 cm<sup>-1</sup> regions and binary PLS models were built using the combination of the 6475 – 6965

$\text{cm}^{-1}$  and the 4000 – 5115  $\text{cm}^{-1}$  ranges to exclude the broad water bands at 5200 and 7070  $\text{cm}^{-1}$ . Root mean square error of prediction (RMSEP) values for the detection of low levels of one form in another one were  $\leq 0.8\%$  and limits of detection (LOD) and limits of quantification (LOQ) of  $< 1$  and  $< 3.2\%$  were obtained. RMSEP values of  $\leq 5.1\%$  were obtained for mixtures containing all three forms. NIRS as the most accurate method outperformed XRPD and Raman microscopy applied in an earlier report [123]. NIRS was also distinctly superior to IR spectroscopy, although there were clear differences between the IR spectra of the different forms [121]. For example, as a consequence of the lower symmetry of the form I structure that contains pseudo-centrosymmetric  $\text{NH}\cdots\text{N}$  dimers in contrast to the centrosymmetric  $\text{NH}\cdots\text{N}$  dimers in form,  $\nu_{\text{as}}(\text{NH}_2)$  and the  $\text{NH}_2$  scissoring vibration give rise to split bands in the case of form I. NIRS was also used to investigate the mechanically activated polymorph conversions of the related sulfa-drug sulfathiazole [124]. Sulfathiazole has five known polymorphs, forms I to V. The conversion of forms II – V to metastable form I upon milling at room temperature was monitored. PCA of the NIR spectra again indicated that the transformation involves an intermediate amorphous stage. The transient amorphization or the transient formation of an unstable crystalline phase followed by a rapid recrystallization process is one of the possible pathways by which milling-induced polymorphic transformations can proceed. Alternatively, the formation of another polymorph can take place *via* a direct crystal-crystal transformation. The latter was found for humidity-induced solid-state transformations of (nonmechanically activated) sulfathiazole [124]. Forms I and V convert to mixtures of form II and form IV at RH  $> 70\%$  and the rates of transformation were monitored using the NIR bands at 6872 and 6318  $\text{cm}^{-1}$ , respectively. The scanning electron microscopy images showed no change in the external form of the crystals before and after exposure to the humid atmosphere. The NIR spectra did not detect any uptake of water confirming a solid-state process. Munroe *et al.* analyzed sulfathiazole form II crystals that had a distinctive middle layer of 1 to 10  $\mu\text{m}$  thickness by specific point analysis with Raman spectroscopy [125]. Spectra collected from the middle layer featured a peak at 950  $\text{cm}^{-1}$  which is specific to form IV. This peak was absent in spectra collected from any other area that showed the characteristic form II peak at 850  $\text{cm}^{-1}$  instead.

NIR spectroscopic imaging combined with matrix-augmented MCR analysis was shown to be a suitable method for quantifying the concentrations of two carbamazepine polymorphs, forms I and III, on tablet surfaces and for monitoring the thermally induced form III  $\rightarrow$  I conversion by recording concentration distribution maps at different time intervals [126]. Using the 7800 – 5400  $\text{cm}^{-1}$  spectral range that encompasses the first overtones of C-H stretching vibrations and the double-first overtones of the symmetric and asymmetric  $\text{NH}_2$  stretching vibrations, a calibration model with errors of  $< 5\%$  was obtained. Raman hyperspectral imaging in conjunction with MCR-alternating least squares (MCR-ALS) analysis was shown to provide deeper insight into the thermal-induced transformation in carbamazepine tablets containing two heterogeneously distributed polymorphs [127]. The spatial information

obtained allowed to conclude on parallel transformations of the two polymorphs to the stable form. LF Raman microspectroscopy was used to map phase transitions in caffeine tablets [128].

In the presence of solvent, polymorphic transformations can occur *via* two mechanisms: solid-state polymorphic transformation (SSPT) and solution-mediated polymorphic transformation (SMPT) [129]. The former involves a positional rearrangement in the solid state, while in the latter the stable polymorph nucleates following dissolution of the metastable form. Su *et al.* reported that the metastable  $\alpha$ -form of mannitol converts to the stable  $\beta$ -form *via* the SMPT route based on a combined Raman spectroscopy, focused beam reflectance measurement (FBRM) and particle vision measurement (PVM) study [130]. The transformation was monitored *in situ* by the disappearance of the characteristic band of the  $\alpha$ -form at 1355  $\text{cm}^{-1}$  and the appearance of the characteristic  $\beta$ -form band at 1365  $\text{cm}^{-1}$ . The dependence of the transformation rate on the temperature, substrate mass and solvent composition was studied and the interpretation of the Raman data was confirmed by monitoring the change in particle size and morphology by FBRM and PVM. Solid-state transformations of mannitol are widely studied, as mannitol is often added to freeze-dried protein formulations as a stabilizer. Variations in the solid-state form are known to affect the performance and stability of the lyophilized biopharmaceutical formulations [131,132]. Grohganz *et al.* published a NIR spectroscopic study on the effect of the drying method (freeze-drying vs. spray-drying), drying temperature and protein concentration on the solid-state form of mannitol in the end-product [133]. PCA of the 4200 - 4500  $\text{cm}^{-1}$  region where the combination bands of the C-H vibrations appear showed a clear clustering of the samples depending on the drying technique and protein concentration. The drying process was found to be more important in determining the polymorphic composition than the temperature. For freeze-dried samples, a change from  $\beta$ -mannitol as the predominant polymorph to  $\delta$ -mannitol was observed with increasing protein content, while in spray-dried samples the formation of  $\alpha$ -mannitol was enhanced with increasing protein content. The NIR data were in agreement with XRPD analysis. The spatial distribution of mannitol solid-state forms on the surfaces of spray-dried mannitol / lysozyme powders and of single levitation droplet particles was investigated by Raman mapping [134]. PLS-discriminant analysis (PLS-DA) of the Raman data allowed for a quantitative analysis and showed a more even distribution of the different solid-state forms in the case of spray-dried samples. The observed co-localization of  $\alpha$ -mannitol and lysozyme corroborated that the presence of protein affects the polymorphism of mannitol as also found in an earlier study [135]. NIR and Raman spectroscopies are extensively used to monitor and investigate solid-state transformations of mannitol and other carbohydrate-based stabilizers during freeze-drying of protein formulations or of the pure sugar [136,137] which, however, is beyond the scope of this review.

#### 4.2. Hydrate formation and dehydration:

The study of transformations between the hydrate and the anhydrous form of an API is essential for predicting the hydrate's stability range and selecting appropriate drying and

dehydration protocols. During and after dehydration the original crystal lattice can be retained (as a high-energy and usually highly hygroscopic structure) or the removal of the water molecules of crystallization can destabilize the lattice leading to the collapse of the original crystal lattice and structural reorganization. In the latter case, a new crystal lattice different from the original one can form or the amorphous phase may be obtained. Hydrates may also undergo several consecutive solid-state transformations during dehydration.

Vibrational spectroscopy is a valuable and widely applied tool to probe hydrate water. Water gives strong absorption bands at *ca.* 5150 and 6900  $\text{cm}^{-1}$  and NIR spectroscopy is generally considered the best suited spectroscopic method for the qualitative and quantitative analysis of hydrates. In fact, one of the first pharmaceutical applications of NIR spectroscopy was the determination of water [138]. NIR spectroscopy not only easily distinguishes hydrates from anhydrites, it is also very sensitive to different water states and distinguishes hydrate from bulk water, different hydrates of the same API as well as water molecules bound at different sites in the crystal lattice. Furthermore, it allows probing the degree of hydrogen bonding of the water molecules [139-142]. Because the energetic distribution of the OH vibrations of the water molecules bound in a crystal lattice is rather uniform, crystal water gives sharper bands than bulk water and hydrogen bonding gives rise to shifts to longer wavelengths [143-146].

In principle, hydrates and anhydrites can also be distinguished by the diagnostic OH stretching bands between 3200 and 3700  $\text{cm}^{-1}$  in the mid-IR region. In particular isolated site hydrates (as opposed to channel hydrates) show sharp OH bands. However, misinterpretation is easily possible as for example the NH stretching vibration of secondary amines (3205 – 3380  $\text{cm}^{-1}$ ) also appears in this range. In contrast to NIR and IR spectra, conventional Raman spectra do not feature strong water bands, as the polar water molecule is a rather poor Raman scatterer. Moisture, however, can lead to shifts and changes in the shape of Raman bands due to water-API interactions and this may be used as an indirect detection method [147]. On the other hand, water of crystallization gives a characteristic water band in the LF range of the Raman spectrum (around 80  $\text{cm}^{-1}$ ) and LFRS is well-suited for studying dehydration mechanisms by simultaneously monitoring the disappearance of the water of crystallization and the reorganization of the crystal lattice using the phonon modes [148]. When the high water sensitivity of NIR spectroscopy becomes a disadvantage because of overlap of the strong OH absorptions with other diagnostic bands, the combination of NIR and conventional Raman spectroscopy which is more sensitive to conformational changes can provide useful complementary information.

Besides dehydration, the study of the reverse reaction, the conversion of the anhydrate into the hydrate form of an API in bulk state or in pharmaceutical formulations, is equally important. As the hydrate form is often the more thermodynamically stable form, it usually has a lower solubility and dissolution rate than the anhydrous form. There are well-known cases, where the uncontrolled hydrate formation in tablets during storage can result in therapeutic

failure [149]. In this regard the distinction between hydrate formation and hygroscopic behavior of the anhydrous form of an API is relevant with a view to solid-state properties, processing and handling of the API, yet is often not straightforward. For example, a recent study using a combination of XRPD, differential scanning calorimetry (DSC), thermal gravimetric analysis (TGA), dynamic vapor sorption (DVS) and ATR-FTIR spectroscopy suggested that paroxetine hydrochloride, originally characterized as an anhydrate should in fact be classified as a non-stoichiometric hydrate [150].

The application of vibrational spectroscopy for probing water of crystallization during pharmaceutical processing has been reviewed by Jørgensen *et al.* in 2009 [151]. Since then, a number of recent reports on the use of IR, NIR and Raman spectroscopy to monitor hydrate formation, dehydration and crystallization processes of hydrates have been published (Table 1). Rapid screening methods are of particular interest with a view to process development. Uchida *et al.* developed a microanalytical method based on a combination of chemometric NIR spectroscopy and a humidity-controlled 96-well plate that allowed to qualitatively and quantitatively evaluate the stability and pseudopolymorphic transformation of metastable imidafenacin form II to the monohydrate (form III) upon moisture uptake [152,153]. They compared PLS models for the quantification of imidafenacin form III during form II  $\rightarrow$  form III conversion using the 96-well plate and the conventional glass-bottle method and showed that the 96-well plate method is an efficient technique to study solid-state transformations with small amounts of drug samples. The standard error of cross validation (SECV) value (0.318 %) was found to be only slightly higher than that of the conventional glass bottle method (0.213 %). Beard *et al.* proposed a combined DSC and NIR method that uses a fiber-optic probe located over the sample within the DSC lid. This technique was used to monitor the dehydration kinetics of theophylline monohydrate by simultaneous DSC and NIR measurements [154,155]. A combination of DSC and vibrational spectroscopy was also utilized to monitor the temperature-induced dehydration, recrystallization and phase transformation of metoclopramide hydrochloride hydrate [156,157]. In this case DSC data were collected together with FT-IR spectra at high spatial resolution. A Raman microspectroscopic study of the system was also reported [158]. Kogermann *et al.* compared NIR and Raman spectroscopy for the quantitative monitoring of the solid-state transformations of carbamazepine dihydrate and piroxicam monohydrate during isothermal dehydration [159]. They constructed multivariate calibration models using PLS to quantify binary mixtures of piroxicam monohydrate and anhydrate and quaternary mixtures of the four carbamazepine forms that occur during drying, carbamazepine dihydrate, form III, form I and amorphous carbamazepine. In both cases, NIR spectroscopy gave the better performing model (root mean square error (RMSE) values 3.6 – 5.9 % for carbamazepine and 1.96 – 2.16 % for piroxicam) compared to Raman spectroscopy (RMSE values 4.0 – 7.1 % for carbamazepine and 2.78 – 2.85 % for piroxicam). In particular, the carbamazepine data were found not to be randomly distributed about the calibration line. The poorer performance of the Raman model was attributed to problems arising from fluorescence, smaller sampling

volume and different sensitivities. However, while the Raman model allowed to differentiate all four carbamazepine solid-state forms during isothermal dehydration, NIR spectroscopy failed to distinguish the different anhydrous forms of carbamazepine. Both techniques revealed similar dehydration profiles. As NIR spectroscopy is more sensitive to crystal changes related to water content while Raman spectroscopy is more sensitive to crystal structure changes, the authors concluded that changes in the molecular conformation and arrangement take place concurrently with the loss of water during isothermal drying and that the transformation from piroxicam monohydrate to the anhydrate is direct. Raman spectroscopy also showed that the amount of amorphous carbamazepine formed strongly depended on the dehydration temperature. At lower temperature the crystal structure collapses leading to the amorphous form, while at higher temperature, the higher mobility results in rapid nucleation and crystal growth. The Raman model was further applied to analyze solid-state transformations of carbamazepine during dehydration in a fluidized bed dryer. Koradia *et al.* monitored the transformation of amlodipine besylate anhydrate, amlodipine besylate monohydrate and amorphous amlodipine besylate to the dihydrate during solubility testing, wet granulation and drying using at-line Raman and NIR measurements [160]. Multivariate calibration models were developed for the quantification of ternary mixtures of the anhydrate, mono- and dihydrate. Replicate samples grouped much closer in the NIR scores plot than in the Raman scores plot. While both spectroscopic methods gave satisfactory RMSE values, Raman spectroscopy was found to be superior for monitoring the solid-state composition during wet granulation and drying which was attributed to the higher sensitivity of NIR spectroscopy to particle size and shape. In particular, Raman spectroscopy outperformed NIR spectroscopy in the case of wet granulation due to the influence of water on the NIR spectrum. Kachrimanis and Griesser used 2D-FT-IR correlation spectroscopy to investigate the crystal water dynamics of carbamazepine dihydrate by monitoring N-H and C=O vibrations associated with H bonding interactions with water of crystallization [161]. They demonstrated that the two water molecules that are bound at different sites dehydrate at different rates. Alexandrino *et al.* showed that the solid-state transformations of two different hydrates - piroxicam monohydrate and lactose monohydrate - that dehydrate simultaneously can be mapped separately on the surface of tablets using multi-series NIR hyperspectral imaging with multivariate data analysis (MCR-ALS) [162]. Hedoux *et al.* investigated the dehydration of caffeine hydrate by *in situ* low- and high-frequency Raman spectroscopy [148]. Analysis of the phonon region showed that during the dehydration process at room temperature an anhydrate is formed that is metastable and transient between the two anhydrous polymorphs of caffeine. The dehydration kinetics monitored by LFRS indicated a two-step mechanism with the first step involving a molecular rearrangement before water removal and the second step involving a disordering process of the caffeine molecules accompanying the water removal. Further insight into the molecular-level mechanism was obtained from the high-frequency regions of the Raman spectra which probed the breakdown of the caffeine-water H bonds and the H bonds forming the caffeine network. The combined spectral data led to the following

conclusions: The rapid breakdown of the caffeine-water H bonds at the early stages of the room temperature dehydration kinetics enables the long-range reorganization of the caffeine molecules. Upon breakdown of the H-bonded caffeine network, the structure collapses gradually enabling the escape of water.

Wilkström *et al.* have shown for three model APIs that the transition temperature of an anhydrate/hydrate system can be obtained by measuring temperature-dependent transformation kinetics in an aqueous slurry with Raman spectroscopy [163]. The transition temperature corresponds to the temperature at which the transformation rate is zero. The method works for transition temperatures up to 85 °C. At higher temperatures the kinetics become too fast. For highly soluble APIs interference from the solution spectrum can be problematic.

Vibrational spectroscopy is very often the method of choice for monitoring the water removal or uptake in real time during processing with a view to PAT applications. NIR spectroscopy in conjunction with PLS regression was utilized for the in-line quantification of different solid-state forms of theophylline during dehydration in a vacuum contact dryer [164]. Touil *et al.* constructed a calibration model for the determination of theophylline monohydrate, the stable and metastable anhydrous form and water with standard error of calibration (SEC) and prediction (SEP) values for the theophylline forms of < 2.5 and < 3.1 %. This model was applied to study the influence of the process parameters on the kinetics of the transformation of theophylline monohydrate to theophylline anhydrate *via* the metastable anhydrous form. Fonteyne *et al.* showed that the solid-state form of theophylline in theophylline/lactose/polyvinylpyrrolidone blends during continuous drying in a six-segmented continuous fluid bed drying unit can be monitored by in-line Raman and NIR spectroscopy [165]. Raman spectroscopy could differentiate between theophylline monohydrate, anhydrous theophylline and a metastable form of theophylline anhydrate based on specific bands in the 1650 – 1750 cm<sup>-1</sup> region, while NIR spectroscopy could only distinguish the monohydrate and the anhydrate, but not the two anhydrous forms. The rapid transformation of amorphous piroxicam to crystalline piroxicam monohydrate during layer-coating was identified by Raman spectroscopy. The solid-state transformation affected not only the dissolution rate but also the coating adhesion, porosity, uniformity and thickness [166]. *In situ* Raman spectroscopy together with PLS was also applied to slurries of piroxicam anhydrate to quantify the solvent-mediated solid-state transformations of the crystalline anhydrate to the monohydrate under different pH conditions in order to gain insight into the dissolution behavior of the anhydrate and the effect of surfactants on the dissolution behavior [167]. Three forms of pazopanib hydrochloride, the monohydrate (as an intermediate), an undesired acetonitrile solvate and the desired anhydrate were observed during process development. NIR spectroscopy could clearly distinguish the three forms and identify them in slurries, as in this case free water does not overlap with the band of the hydrate water. An *in situ* diffuse reflectance NIR spectroscopy method coupled with PLS was successfully developed for real-time monitoring of the transformation of

the monohydrate to the anhydrate on a manufacturing scale [168].

### 4.3. Amorphization and recrystallization:

Mechanical stress during grinding or compression can lead to unwanted amorphization. On the other hand, amorphization is a possible strategy to improve the bioavailability of poorly soluble APIs, as the amorphous phase has a higher apparent solubility and dissolution rate than the crystalline solid. However, the amorphous state is a high energy state and thus thermodynamically unstable. Amorphous materials often tend to recrystallize during processing or storage. The transformation to a crystalline form can be triggered by moisture or traces of remaining crystalline particles that act as crystallization seeds. Therefore, there is a need for accurate and reliable analytical tools to detect traces of crystallinity in amorphous samples, to quantitatively monitor the amorphization process and to predict the storage stability of an amorphous API. Very often, the crystalline and amorphous form show clear spectroscopic differences with one or more characteristic bands of a crystalline polymorph disappearing or new bands appearing on amorphization. Furthermore, amorphous solids generally have broader IR and NIR bands than their crystalline counterparts because of the greater range of molecular environments available in the amorphous state. In many cases the combination of vibrational spectroscopy with multivariate analysis allows the construction of accurate calibration models with low LODs for traces of crystallinity in an amorphous solid or low levels of amorphous content in crystalline materials. Examples from the recent literature for the study of amorphization and recrystallization processes by vibrational spectroscopy are summarized in Table 1.

Xu *et al.* utilized NIR spectroscopy combined with multivariate analysis to quantitatively monitor the amorphization of tauroursodeoxycholic acid during milling and to determine the storage stability of the hydrated amorphous form (form III) [169]. Later, the study was extended to tauroursodeoxycholic acid anhydrate (form II) and the amorphous anhydrate (form IV) [170]. Form I undergoes a direct crystal-crystal transformation to form II on heating to 100 °C, while form IV converts to form III. Both solid-state transformations are rapid at room temperature and their monitoring requires a fast analytical method. The form II → form I and form IV → form III transformations could be quantitatively assessed by in-line NIR spectroscopy. The data were analyzed by using characteristic peaks of forms II and III at 5130 and 5129 cm<sup>-1</sup>, respectively, and complementary PCA.

In-line Raman spectroscopy was used to monitor the solvent-mediated recrystallization of amorphous piroxicam [171]. The recrystallization kinetics were found to depend on the starting form with samples prepared from anhydrous piroxicam form I recrystallizing faster than those prepared from anhydrous piroxicam form II. This was explained by a higher residual order in X-ray amorphous samples obtained from form I. The recrystallization to form I and III during storage was also monitored by Raman spectroscopy along with complementary XRPD measurements and PCA. Raman and XRPD analysis gave the same general results, although the PCA model of the Raman data was more sensitive to

sample differences. While the XRPD model identified a sample stored for 48 h at room temperature as almost pure crystalline form I, the Raman scores plot showed a movement to, but still a difference from form I. Again, the recrystallization kinetics and polymorphic form depended on the starting form. Mah *et al.* compared Raman spectroscopy with XRPD and DSC as analytical techniques to monitor the degree of disorder in glibenclamide during milling and to predict the recrystallization of milled samples during dissolution [172]. The fingerprint regions in the spectra of crystalline and amorphous glibenclamide feature various spectral differences. Raman spectroscopy combined with PCA suggested that samples were fully amorphous after a milling time of 60 min in marked contrast to the DSC and XRPD analyses that indicated complete amorphization after 120 and 30 min, respectively. Raman spectroscopy is more sensitive to the presence of small nanocrystals than XRPD which is also not able to distinguish between crystal defects and amorphous regions. However, Raman spectroscopy is less sensitive to remaining crystalline seeds than DSC so that samples analyzed spectroscopically appeared amorphous after a shorter milling than samples monitored by DSC. When the solid-state changes of milled samples during intrinsic dissolution testing were monitored by *in situ* Raman spectroscopy in conjunction with PCA, negligible crystallization was found for DSC amorphous glibenclamide samples, while samples that were XRPD- or Raman-amorphous recrystallized which resulted in a decrease in dissolution rate. Thus, in this case Raman spectroscopy is inferior to DSC in predicting the dissolution behavior of the amorphous API.

Rehder *et al.* compared real-time Raman and NIR spectroscopy for the determination of the recrystallization kinetics of amorphous ibuprofen during acoustic levitation [173]. When MCR analysis was applied, the transformation profiles obtained by both methods could be fitted satisfactorily (average R<sup>2</sup> = 0.992 and 0.991 for the NIR and Raman data, respectively) and comparable empirical recrystallization rate constants were calculated. However, Raman spectroscopy gave a significantly shorter crystallization onset time compared to NIR spectroscopy (1.42 ± 0.15 min vs. 2.01 ± 0.08 min). The authors proposed that the nucleation was influenced by local heating effects originating from the high energy density of the Raman laser beam compared to the less focused W light source used to collect the NIR data.

Savolainen *et al.* reported an example where *in situ* Raman spectroscopy combined with chemometric tools revealed information about solid-state transitions during dissolution that was not accessible by XRPD measurements [174]. They used Raman and multivariate analysis (PLS, PLS-DA) to study the crystallization of amorphous indomethacin and carbamazepine. In the case of carbamazepine, the scores of the PLS-DA travelled instantly from the amorphous form towards the anhydrate (form I) and later to the dihydrate demonstrating that the recrystallization pathway involves the transient formation of anhydrous form I *via* solid-state transformation followed by solution-mediated transformation of form I to the dihydrate. PLS-DA of the Raman data of amorphous indomethacin indicated a direct transformation to the  $\alpha$ -form. In both cases, the solid-

state transformation observed by *in situ* Raman spectroscopy explained the dissolution profiles of the amorphous APIs.

Sulfathiazole has five different polymorphs and is a widely used model drug in polymorphism research. Sulfathiazole form I and form III were shown to undergo complete amorphization on cryomilling [175]. The NIR spectra of sulfathiazole form I, form III and amorphous sulfathiazole show clear differences and the recrystallization of cryomilled sulfathiazole was investigated by NIR spectroscopy. Application of PCA to the NIR data demonstrated the dependence of the recrystallization pathway on the starting form and the storage conditions. Amorphous sulfathiazole prepared by cryomilling form I and kept under vacuum crystallized back to the original polymorph, while the recrystallization of cryomilled form III was more complicated. NIR analysis also revealed that moisture not only increased the recrystallization rates but also affected the recrystallization pathway. In contrast to samples stored in humid atmosphere, samples stored under vacuum did not follow Ostwald's rule of stages. It was suggested that the catalytic effect of water reduced the kinetic barrier to the crystallization of the different polymorphs [175]. The kinetics and pathway of the recrystallization of the cryomilled, amorphous form of the related sulfa-drug sulfamerazine were also investigated by NIR spectroscopy [121]. The multivariate analysis of the NIR data gave composition-time profiles that revealed that when stored under vacuum, amorphous sulfamerazine crystallizes directly to the most thermodynamically stable polymorph as observed for sulfathiazole. The speed of spectral acquisition and minimal sampling handling required for NIR spectroscopy was pivotal to obtaining reliable composition-time profiles, as amorphous sulfamerazine recrystallizes within hours.

ATR-FTIR spectroscopy is a surface-biased technique and because of this can be more suitable for predicting the dissolution behavior of amorphous APIs than XRPD as shown in an interesting study by Priemel *et al.* [176]. PLS models for the determination of crystallinity in binary mixtures were developed for both ATR-FTIR and XRPD analysis. When the calibration models were applied to stored samples of amorphous indomethacin, markedly different recrystallization rates and profiles were obtained. The IR data indicated a rapid and linear decrease in amorphous content with recrystallization being complete after 5 days, while from the XRPD data, amorphous content was still present after 100 days. This was explained by the effect of surface crystallization on the penetration depth of IR light which leads to a disproportionately high crystalline content being measured for the whole sample. By contrast, XRPD can underestimate the amount of the amorphous form, as nanocrystalline particles appear as "X-ray amorphous". On the other hand, dissolution is a surface-mediated process so that the surface crystallinity detected by the IR method was found to be a better indicator of the performance of the stored samples in dissolution testing than the total crystallinity measured by XRPD.

Amorphous solids give rise to a broad peak in the LF-frequency Raman spectrum which corresponds to the envelope of the phonon peaks of the crystalline phase. The high sensitivity of the LF Raman spectrum to detect small

traces of crystallization in a matrix of amorphous material makes LFRS a well-adapted technique to analyze recrystallization kinetics with high accuracy. The LF range was shown to be considerably more sensitive to small amounts of crystallinity (significantly lower than 20 %) in an amorphous sample of indomethacin than the C=O stretching region [61]. Amorphous griseofulvin tablets are another example that shows the capability of LFRS to detect and quantify low levels of crystallinity. A recent study compared conventional FT-Raman spectroscopy using a 785 nm laser and a CCD detector for the simultaneous collection of low- and high-frequency spectra [177]. A much higher intensity was observed for the low-frequency region compared to the high-frequency region. The Raman data were subjected to multivariate data analysis. The best-performing PLS model and lowest LOD was obtained for the FT-Raman data (RMSEP value of 0.65 %, LOD of 0.58 % vs. RMSEP values of 1.2 and 1.4 % and LODs of 1.11 and 1.51 % for the low- and high-frequency regions collected with the 785 nm system), but the good RMSEP values and LODs for the LF region (1.2 and 1.11 %) confirmed that LFRS is able to detect trace crystallinity with high accuracy and sensitivity. The PLS models were applied to monitor the recrystallization of the amorphous griseofulvin tablets during storage. FT-Raman and low-frequency Raman analysis detected crystallinity earlier than high-frequency Raman spectroscopy using the 785 nm setup. The 785 nm setup also gave larger error bars in the recrystallization profiles which was attributed to more subsampling due to the eight times smaller sampling volume compared to the FT-Raman system. Vehring's group recently described a Raman setup that combines ultra-narrow band notch filters with traditional dispersive Raman setup and reported high sensitivity, high resolution and access to the LF range. They demonstrated the capability of their system to distinguish the crystalline and amorphous forms of glycopyrronium bromide and formoterol fumarate and to identify three mannitol polymorphs by their lattice modes [178].

Caffeine is another popular model pharmaceutical and LFRS was also employed to investigate solid-state transformations induced by hydrostatic compression of the two anhydrous polymorphs of caffeine, forms I and II. Band shape analysis of the LF region of both forms suggested a pressure-induced amorphization of form I *via* a transient state close to form II. It was pointed out that the detection of this metastable intermediate state was only possible due to the short acquisition time of LF Raman spectra [62].

LFRS is also a very suitable technique to study orientational disorder in rotator phases [179-181]. Hedoux *et al.*, for example, monitored the isothermal transformation of orientationally disordered caffeine form I into the stable form II. Band analysis of the LF range of the Raman spectra showed the existence of a similar orientational and dynamic disorder in form II as in form I, albeit with a weaker rotation amplitude [182].

Various Raman spectroscopic studies were reported that used a combination of the analysis of the low- and high frequency range to gain insight into solid-state transformations. The 1550 – 1750  $\text{cm}^{-1}$  region where C=O stretching vibrations are usually observed can be very sensitive to changes in H bonding. Hédoux *et al.* carried out

a detailed study on pressure-induced solid-state transformations of  $\gamma$ -indomethacin using LFRS [63,183]. Upon increasing the pressure from ambient pressure to 4 GPa, two additional phonon peaks appeared in the low-frequency range of the Raman spectrum of solid indomethacin indicating an ordering process. This corresponds to a change from the relatively poor packing of dimers in the  $\gamma$ -form to a more efficient packing of trimers in the  $\alpha$ -form. Furthermore, changes in the range encompassing the C=O stretching vibrations were consistent with an extensive rearrangement in the H bonding network and long-range order. In particular, the width of the benzoyl C=O stretching band of  $\gamma$ -indomethacin at 1697  $\text{cm}^{-1}$  could be used to probe and monitor the long-range order in the  $\gamma$ -polymorph. The 1550 – 1750  $\text{cm}^{-1}$  region in the spectrum of the  $\alpha$ -form is more complex due to the presence of three different molecular conformations and additional H bonding. The pressure-induced transformation to  $\alpha$ -indomethacin could be detected by the emergence of the 1648  $\text{cm}^{-1}$  band (C=O stretch of H bonded carboxyl group) and the 1678/1688  $\text{cm}^{-1}$  doublet (H bonded and non-H bonded benzoyl C=O stretch). Interestingly, the changes in the LF region appeared before changes in the 1550 – 1750  $\text{cm}^{-1}$  region were observed (*i.e.* at lower pressure). Thus, the authors concluded that the H bonding network remains stable, while lattice modifications and probably conformational changes take place prior to the breakdown of the dimer chains of the  $\gamma$ -form. A further increase in pressure resulted in the disappearance and merging of the bands in the C=O region and in the disappearance of the lattice modes in the LF region which is indicative of a disordering process. However, the Raman line shapes of the pressure-induced amorphous phase clearly differed from those of the thermal glass obtained by quench-cooling and this was attributed to the existence of a high-density amorphous state. The two-step formation of the high-density amorphous state – ordering process to a higher density  $\alpha$  form-like structure (according to Le Chatelier's principle) followed by disordering is different from the direct loss of long-range order upon grinding or undercooling the liquid state. On release of the pressure the high-density amorphous form transforms first into the thermal glass and finally to the  $\gamma$ -phase.

With the help of LF and conventional Raman spectroscopy the metastable phase II of ibuprofen was identified as a transient metastable state in the heating-induced recrystallization of the glassy state [64]. The LF Raman susceptibility showed the typical band shape of disordered molecular systems and the 600 – 1800  $\text{cm}^{-1}$  region of the spectrum indicated a local order in phase II similar to that of the glassy state. Upon cooling to 100 K, structuring of the spectrum was observed with the spectrum resembling more and more the envelope of the phonon peak of phase I in line with phase II representing an intermediate state between the under-cooled liquid and phase I and becoming less disordered at lower temperature. The time-dependence of the quasi-elastic component was used for a kinetic analysis of the isothermal transformation of the under-cooled liquid to phase II. A sigmoidal curve typical of a nucleation and growth process was obtained. It should be noted that the presence of positional disorder in phase II was not detectable in the XRPD pattern which gave distinct

Bragg peaks, as XRPD measures the average molecular organization.

In the works described so far the boson peak and/or line broadening in the Raman spectrum were taken as an indicator of disorder. Zarow *et al.* have shown that the superimposition of diffuse scattering on the Raman lines can also be used to detect disorder [184]. Localized defects give rise to new Raman peaks in the molecular vibration frequency region. Fluorescence produces background intensity that depends on the excitation wavelength. Milling-induced particle size reduction to dimensions in the order of the excitation wavelength can additionally cause excitation-wavelength independent Mie scattering. Cryomilled and thermally generated amorphous griseofulvin were used as examples. The Raman spectra of both, the cryomilled and the amorphous samples showed a broad, excitation-wavelength independent, inelastic scattering background. Solid-state fluorescence spectra confirmed the interpretation of the Raman spectra of cryomilled griseofulvin as being the result of disorder and Mie scattering rather than localized defects. Exposure of the cryomilled griseofulvin to high humidity led to a decrease in the background intensity reflecting crystal growth.

Imaging techniques, *i.e.* Raman mapping or NIR spectroscopic imaging, have also been effectively applied to amorphous pharmaceuticals. The crystallization of amorphous indomethacin, for example, was studied by Raman mapping which allowed the quantification of crystallinity with a LOD of 4.7 % and an RMSEP value of 1.47 % [185]. In contrast to XRPD for which a LOD of 6.2 % was found, Raman mapping detected traces of crystalline material in freshly prepared samples. Kinetic analysis revealed particle-size dependent, non-uniform crystallization rates with larger particles crystallizing more slowly due to their smaller relative surface area compared to smaller particles. The Raman data were fitted to the Kolmogorov-Johnson-Mehl-Avrami equation

$$x = 1 - \exp(-k(t - t_0)^n)$$

where  $x$  is the crystallinity at time  $t$ ,  $t_0$  is the induction time,  $k$  is the crystallization constant depending on nucleation and growth and  $n$  is the nucleation and crystal growth exponent. The crystal growth exponent is an indicator for the growth mechanism. Generally, a crystal growth exponent of 1, 2 and 3 is associated with 1D (rod-like), 2D (disk-like) and 3D (spherical) growth [186]. The determination of  $k$  and  $n$  using data obtained from larger particles and from the whole sample area showed a decrease of  $k$  for larger particles compared to the whole area. The crystal growth exponent increased from 1.1 for the whole area to 1.38 for larger particles suggesting a change in crystal growth mechanism.

As already pointed out, the solid-state chemistry of paracetamol has been and is attracting considerable attention. The recrystallization behavior of amorphous paracetamol has been widely studied and the recrystallization pathway and rate seemed to be highly sensitive to small changes in the experimental conditions and setup [110,187,188]. Kauffman *et al.* studied the crystallization of super-cooled liquid paracetamol by simultaneous DSC and Raman microscopy in conjunction with PCA and multivariate regression and found, for example, that the formation of intermediate form

III depends on whether the super-cooled liquid form was prepared in an open or covered crucible [110]. A recent Raman study in conjunction with multivariate data analysis gave detailed insight into the crystallization pathway of amorphous paracetamol. Nanubola and Burley used *in situ* variable temperature Raman spectroscopy and Raman mapping to gain molecular-level information on the crystallization behavior of amorphous paracetamol [189]. PCA of the phonon and molecular regions were carried out. The scores plot clearly reflected the occurrence of intermolecular and intramolecular changes during the phase transformations with the lower wavenumber region showing larger spectral differences between the different phases. From the spectroscopic data it was concluded that in uncovered samples surface crystallization predominates and forms I and II crystallize while in samples covered with a quartz coverslip surface crystallization was inhibited and metastable form III selectively nucleated leading to bulk crystallization of this form. It should be noted that this crystallization behavior of paracetamol under covered conditions was also seen in a recent study using terahertz spectroscopy [190]. In an interesting study, Thi *et al.* used Raman spectroscopy to support evidence from synchrotron X-ray diffraction for polyamorphism in paracetamol [191]. Amorphous paracetamol prepared by solution evaporation from different solvents was found to recrystallize to different polymorphs. The Raman spectra of the different amorphous forms showed differences in the 2800 – 3100  $\text{cm}^{-1}$  range. It was suggested that distinct local order of the amorphous forms already contains polymorph-specific information which is controlled by the solvent used for the preparation.

The relationship between the presence of slip planes, very low-energy lattice vibrations, millability and mechanical and thermodynamic instability is discussed in the literature [192]. Quantitative monitoring of the cryomilling-induced amorphization of sulfamerazine form I and form II showed that form I was fully amorphous after 30 min, while milling for 2 h was required to convert form II to the amorphous phase [121]. The better millability of form I was attributed to the presence of slip planes in the crystal structure of form I [121,193]. The slip planes of form I also lead to higher plasticity, compressibility and tableability compared to form II [194]. A recent combined theoretical and experimental study using *ab initio* lattice dynamics calculations, Raman spectroscopy and temperature-dependent X-ray analysis gave further insight into the origin of the different mechanical properties of the polymorphs of sulfamerazine [192]. Raman spectroscopy was used to identify the low-frequency collective motions that are particularly low in energy and thermally accessible at low to moderate temperatures in order to understand how the structure may preferentially deform under mechanical shear during milling. A set of harmonic-phonon calculations were performed and phonon DOS curves were compared to the experimental mid-IR and Raman spectra. The analysis of the phonon eigenvectors obtained from the lattice dynamics calculations indicated a large number of low-frequency modes related to the rocking of the phenyl ring of sulfamerazine about the S-C bond. It was argued that these motions should facilitate the separations of the layers in form I and hence the slip action. Furthermore, modes were observed that correspond to the layers sliding past one

another as well as partial slip modes in which one layer slips while the other gives ring bending, additionally aiding the layer separation. While form II also has a layer structure, the layers in form II are zigzag-shaped and interlock [195]. Due to the dense herringbone structure modes similar to those in form I had higher frequencies indicating that motions that contribute to layer separation are energetically more difficult than the soft lattice vibrations in form I. The third polymorph of sulfamerazine, form III, can be prepared in milligram quantities only [196]. The vibrational modes contributing to slipping were found at significantly lower frequencies compared to form I. It was suggested that the presence of very soft phonons leads to mechanical and thermodynamic instability of this rare polymorph.

The extremely rapid recrystallization of amorphous nifedipine prepared by evaporating a small amount of an acetone solution pipetted onto a glass slide was studied by Raman spectroscopy. The short data acquisition time allowed the identification of the  $\beta$ -polymorph as an intermediate and the determination of the amorphous  $\rightarrow \beta$ -form and  $\beta$ -form  $\rightarrow \alpha$ -form transformation rates which are in the order of  $10^{-6} \text{ m}\cdot\text{s}^{-1}$  [197]. Such rates are incompatible with a classical diffusion mechanism and suggest the presence of an arrangement of pre-ordered nifedipine entities in the amorphous phase.

Strategies to prevent recrystallization of an amorphous API during storage include coamorphization and the dispersion of the API into a polymer. A coamorphous system is a stoichiometric mixture of an API and a small-molecule excipient or of two compatible APIs [198-200] as opposed to a non-stoichiometric API/polymer amorphous solid dispersion (ASD). Vibrational spectroscopy coupled with chemometrics has proven to be a valuable tool for the quantitative analysis of ASDs, to detect trace crystallinity and to measure the recrystallization kinetics of the API in the polymer matrix in order to predict the stability range of the formulation (Table 2). FT-Raman spectroscopy, for example, gave accurate multivariate models that allowed the detection of less than 5 % (w/w) of the crystalline form of ibipinabant in ASD tablets (less than 0.05 % of the total mass of the tablet) [201]. Stability monitoring and fitting the data to the Johnson-Mehl-Avrami model along with optical microscopy revealed a sporadic nucleation mechanism with an induction period followed by 1D (rod-like) crystal growth based on the Avrami exponent and observed crystal habit.

IR and Raman spectroscopy are also important tools to study changes in intermolecular interactions upon amorphization and the presence of drug-polymer or drug-coformer interactions that stabilize the system [202-208,287]. H bonding between the drug and the polymer or coformer can affect the molecular mobility of the API, inhibit H bonding between two API molecules and can impact on the crystallization driving force due to the relative strength of API-polymer/coformer and API-API H bonds. On the downside strong drug-polymer interactions can have an impact on dispersibility and dissolution rate, *i.e.* actually hinder drug release [209]. While for many systems a correlation between intermolecular interactions and crystal growth inhibition has been found as discussed below, other stabilization mechanisms are also debated and various examples have been reported where the IR spectrum did not

differ significantly from that of the physical mixture [210,211,288] suggesting a lack of interaction at the molecular level. Resveratrol is a fast crystallizer and was selected by Wegiel *et al.* for this reason as a model API to study the potential correlation between the strength of H bonding interactions between API and polymer in ASDs and crystallization inhibition [212]. In the mid-IR spectrum a significant shift of the  $\nu(\text{C}=\text{O})$  band of polyvinylpyrrolidone (PVP) K29/32 of  $26\text{ cm}^{-1}$  was observed (30 % resveratrol), while the OH band of resveratrol shifted from  $3238\text{ cm}^{-1}$  for the crystalline API (pure amorphous resveratrol could not be prepared) to  $3193\text{ cm}^{-1}$ , suggesting that out of a range of different polymers tested PVP forms the strongest H bonds with resveratrol. The resveratrol/PVP ASD was found to be extremely stable towards crystallization under various storage conditions. The dimethylamino group of Eudragit E100 gave two bands at  $2820$  and  $2770\text{ cm}^{-1}$ . These bands disappeared in the spectrum of the ASD indicating that in addition to  $\text{C}=\text{O}\cdots\text{HO}$  H bonding ionic interactions occur in resveratrol/E100 ASDs, which again were found to be highly stable. Curcumin has phenol groups that form intramolecular H bonds. It was suggested that the intramolecular H bonds of the keto-enol group and between neighboring phenol and methoxy substituents reduce the tendency of curcumin to

interact with the polymer at a molecular level resulting in most polymers being poor crystallization inhibitors for this natural product [213]. Small band shifts in the mid-IR spectrum indicated that any H bonding interactions between curcumin and cellulosic polymers are weak. Again, the two bands at  $2820$  and  $2770\text{ cm}^{-1}$  of the dimethylamino group of Eudragit disappeared in E100 ASDs confirming the presence of ionic interactions that stabilize the amorphous form as in the case of resveratrol, while H bonding is limited or weak. Using quercetin and naringenin as model compounds, Taylor's group confirmed that the analysis of the relative strength of ionic and H bonding interactions by mid-IR spectroscopy presents an efficient tool for predicting the ability of a polymer to prevent recrystallization of a given API [13]. Similar results were obtained in a study on felopidine ASDs in which an inverse correlation between the strength and extent of H bonding between API and polymer (as judged from the IR data) and crystal growth was established [214]. Drug-polymer interactions in ASDs of ketoconazole were also evaluated by IR and solid-state NMR spectroscopy and correlated to the molecular mobility and crystallization onset [215].

Table 2. Recent (after 2009) studies reporting the utilization of vibrational spectroscopy for the characterization, stability assessment, and drug release mechanism of ASDs and coamorphous systems

API	Polymer/Cofomer	Method	Study	Reference
acyclovir	citric acid	IR	intermolecular interactions	[293]
aprepitant	various	ATR-FTIR imaging	drug release mechanism	[14]
artemether / lumefantrine	Soluplus	IR, Raman	intermolecular interactions and mapping of the API distribution	[294]
atorvastatin	carvedilol	IR	intermolecular interactions	[295]
atorvastatin	glibenclamide	IR	intermolecular interactions	[295]
$\beta$ -azelnidipine	maleic acid	IR	intermolecular interactions	[296]
bicalutamide	Copovidone VA64	Raman mapping, MCR	dissolution of compacts	[16]
bifonazole	various	IR	effect of intermolecular interactions on crystallization	[208]
BMS-A	PVP	confocal Raman microscopy	mixing homogeneity	[219]
carbamazepine	arginine, tryptophan, phenylalanine	IR	intermolecular interactions in binary and ternary mixtures	[234]
celecoxib	Eudragit	Raman, chemometrics	detection of crystallinity and in-line monitoring of extrudates	[218]
curcumin	artemisin	IR	intermolecular interactions	[297]
curcumin	various	IR	effect of intermolecular interactions on crystallization	[213]
curcumin	various	IR, Raman	drug-polymer interactions	[298]
felopidine	various	IR	effect of intermolecular interactions on crystallization	[214]
felopidine	Copovidone VA64	Raman mapping; MCR	tablet dissolution	[15]
fenofibrate	Kollidon VA64	Raman microscopy, chemometrics	detection of crystallinity; comparison with XRPD	[217]
glibenclamide	simvastatin, various amino acids	IR	intermolecular interactions in binary and ternary mixtures	[236]
glimepiride	Soluplus®-PEG 4000	Raman	drug-polymer interactions	[299]
ibuprofen	Copovidone, Soluplus	ATR-FTIR imaging	drug release mechanism	[17]
indomethacin	acetylated saccharides	IR	drug-polymer interactions	[207]
indomethacin	nicotinamide	ATR-FTIR imaging	drug release mechanism	[220]
indomethacin	Soluplus	IR	drug-polymer interactions	[225]
indomethacin	polyethyleneglycol, HPMC	ATR-FTIR imaging	crystallization pathways; API-polymer distribution in tablets; release mechanism	[226]
indomethacin	PVP	IR, Raman	intermolecular interactions	[227]
indomethacin	ranitidine hydrochloride	IR	intermolecular interactions	[198]

indomethacin	naproxen	IR	intermolecular interactions	[199, 231]
indomethacin	arginine, tryptophan, phenylalanine	IR	intermolecular interactions in binary and ternary mixtures	[234]
itraconazole	PVP	Raman mapping, MCR-classical least-squares	long-term stability	[216]
itraconazole	various	Raman mapping, MCR-ALS	monitoring of the long-term stability of ASDs prepared by electrospinning	[216]
ketoconazole	various	IR	effect of intermolecular interactions on crystallization	[215]
lurasidone hydrochloride	saccharin	IR	intermolecular interactions	[300]
metoprolol tartrate	(MPT) – Eudragit® RL	Raman	quantitative in-line monitoring of hot-melt extrusion	[301]
naproxen	Eudragit	IR	ionic interactions	[302]
naproxen	various	IR	drug-polymer interactions	[203]
naproxen	cimetidine	Raman	intermolecular interactions	[232]
naproxen	various APIs	IR	heating-induced recrystallization; intermolecular interactions	[233]
naproxen	tryptophan, proline, arginine	IR	intermolecular interactions in binary and ternary mixtures	[235]
naringenin	various	IR	effect of intermolecular interactions on crystallization	[13]
nateglinide	metformin hydrochloride	IR	intermolecular interactions	[303]
nifedipine	various	Raman	crystallization pathway and kinetics	[223]
nimesulide	various	IR	effect of intermolecular interactions on crystal growth inhibition	[208]
(R)-3((1R)-3-oxocyclopentyl)-2-[3-chloro-4-methylsulfonyl]phenyl-N-pyrazin-2ylpropanamide	Eudragit, PVP	IR	drug-polymer interactions	[304]
paracetamol	various	IR	intermolecular interactions	[305]
piroxicam	Soluplus	IR, Raman, PCA, MCR-ALS	recrystallization kinetics	[223] [224]
piroxicam	HPMC	Raman	characterization and stability monitoring of nanofibers of ASDs prepared by electrospinning	[306]
quercetin	various	IR	effect of intermolecular interactions on crystallization	[13]
repaglinide	saccharin	IR	intermolecular interactions	[307]
resveratrol	various	IR	effect of intermolecular interactions on crystallization	[212]
ritonavir	indomethacin	IR	intermolecular interactions	[308]
ritonavir	quercetin	IR	intermolecular interactions	[309]
simvastatin	glipizide	IR	intermolecular interactions	[200]
sulfathiazole	various multifunctional carboxylic acids	NIR, PCA	recrystallization pathway and intermolecular interactions	[240]
tacrolimus	HPMC	IR, Raman, NIR, NIR imaging, PLS	recrystallization and spatial distribution of crystallinity	[230]
tanshinone 2A	hydroxyapatite	IR	intermolecular interactions	[310]

Démuth *et al.* used Raman mapping to evaluate the long-term stability of itraconazole ASDs prepared by electrospinning [216]. Due to the small differences in the spectra and overlap with polymer bands, classical least squares analysis proved unsuitable to quantify crystalline material. Instead, MCR analysis was used to obtain the pure component spectra and to decompose the multicomponent spectra in order to map the ratio of amorphous and crystalline itraconazole. Widjaja *et al.* showed that Raman mapping combined with band-target entropy minimization (BTEM) or target transformation factor analysis (TTFA) can present a powerful method to identify trace crystallinity in ASDs and to determine the spatial distribution of the amorphous API, trace crystallinity and polymer [217]. BTEM is a self-modelling curve resolution technique that does not require calibration samples, while TTFA uses pure

reference spectra. For the example of fenofibrate-Kollidon VA64 ASDs Raman microscopy was found to be more sensitive for the detection of crystalline fenofibrate than XRPD. Likewise, in-line Raman spectroscopy combined with chemometric analysis was found to be more sensitive to low levels of crystallinity in celecoxib/Eudragit than XRPD and DSC [218]. The solid-state properties of celecoxib/Eudragit extrudates during hot-melt extrusion were monitored with a fiber-optic Raman probe built into the die head of a twin-screw extruder. PCA analysis of the Raman data allowed to distinguish between glassy solid solutions and crystalline dispersions.

A critical attribute of ASDs that affects the stability of the API towards crystallization is drug-polymer mixing homogeneity. The presence of a single glass transition in the DSC traces intermediate between the glass transition temperatures ( $T_g$ ) of the two pure components is generally

considered proof for homogeneous mixing. Qian *et al.* reported that different batches of an ASD of BMS-A and PVP-VA exhibited different stabilities, although a single  $T_g$  was observed and no trace crystallinity was detected by DSC or XRPD, *i.e.* DSC and XRPD suggested identical properties [219]. Confocal Raman microscopy and analysis of the Raman images by peak height ratio analysis of a BMS-A characteristic peak at *ca.*  $1630\text{ cm}^{-1}$  and a PVP-VA band at  $1735\text{ cm}^{-1}$  demonstrated that the API was distributed over a much wider concentration range in the less stable batch. Thus, Raman mapping provided critical solid-state characterization not detectable by bulk characterization methods.

Exposure to moisture during storage can lead to amorphous-amorphous phase separation. The formation of API-rich and polymer-rich domains in turn can result in accelerated API recrystallization. Rumondor *et al.* have shown for a series of systems that resistance to moisture-induced amorphous-amorphous phase separation is linked to strong drug-polymer interactions [220]. It was discussed that the redshift of the  $\nu(\text{C}=\text{O})$  band of PVP in ASDs represents a quantitative measure of API-API H bonding interactions and thus may represent an indicator for the tendency of an API/PVP ASD to undergo amorphous-amorphous phase separation.

The dissolution advantage of the amorphous form of an API in an ASD can only be maintained, as long as contact with the dissolution medium does not trigger recrystallization. Therefore it is clear that suitable analytical methods that give insight into the release mechanism including potential solid-state transformations during dissolution are essential for optimizing ASD formulations. Towards this end, imaging techniques that allow to spatially resolve drug-polymer interactions and to monitor the release of the individual components over time are particularly attractive. Puncochova *et al.* studied the dissolution mechanism of ASDs of aprepitant in different polymers [14]. Water, polymer, amorphous aprepitant and the precipitation of crystalline aprepitant were qualitatively observed by ATR-FTIR spectroscopic imaging. Complementary magnetic resonance imaging and classical dissolution testing allowed a quantitative analysis. Studies of this kind that give insight into the effect of different polymers on drug release and release mechanism provide valuable information towards rationally selecting compatible API/polymer combinations.

Raman mapping has been applied by several research groups to investigate the dissolution mechanism of ASDs. Burley and co-workers studied the dissolution of compacts of a solid dispersion of felodipine in copovidone VA64 as a model formulation [15]. Real-time Raman mapping and MRC analysis of the dosage form during dissolution showed that at high drug loading (50 %) the rapid loss of highly soluble copovidone leads to a build-up of felodipine-rich amorphous regions on the surface of the compact which is followed by crystallization proceeding from the surface to the core. The MRC Raman maps further revealed different crystallization rates for different regions of the compact surface suggesting heterogeneous nucleation. Phase transitions in compacts of solid dispersions of bicalutamide in copovidone VA64 during dissolution were also studied by

real-time Raman mapping combined with MCR [16]. The metastable polymorph form II, stable form I and the bicalutamide solid dispersion are distinguishable in the  $1055 - 1724\text{ cm}^{-1}$  range and analysis of the conversion rates amorphous  $\rightarrow$  form II, amorphous  $\rightarrow$  form I and form II  $\rightarrow$  form I as well as spatially correlating the data revealed that the stable form I crystallized mainly directly from the amorphous solid dispersion, *i.e.* in a parallel (“non-Ostwald”) crystallization mechanism. Again, recrystallization was found to take place from the outside of the compact following dissolution of the polymer and formation of an amorphous shell prone to crystallization.

*In situ* ATR-FTIR spectroscopic imaging provided insight into the origin of the different drug release rates of ibuprofen-copovidone, ibuprofen salt-copovidone, ibuprofen-Soluplus and ibuprofen salt-Soluplus ASDs [17]. Changes in the spectra of ibuprofen-copovidone and ibuprofen-Soluplus indicated the presence of H bonding interactions between drug and polymer. For example, a reduction in intensity and a shift from  $1627$  to  $1596\text{ cm}^{-1}$  was observed for the C=O band in the spectrum of ibuprofen-Soluplus compared to pure Soluplus. This shift is greater than the shift experienced by the C=O band on water interaction suggesting that the H bond to ibuprofen is stronger than to water. As a consequence water cannot displace the drug and the lack of water ingress into the tablets of the ibuprofen-Soluplus formulation was confirmed by the FTIR imaging spectra. By contrast, ibuprofen salt is released rapidly as the salt form is not a H bond donor.

ATR-FTIR imaging was also applied to study the effect of drug-drug interactions in multiple-drug tablet dosage forms on drug release. By monitoring absorption bands indicative of interactions information can be obtained on whether drug-drug intermolecular interactions are maintained during drug release. This was recently shown by Ewing *et al.* for homogeneous mixtures of amorphous indomethacin and nicotinamide [221]. Indomethacin and nicotinamide are known to hydrogen-bond with each other giving characteristic shifts of the  $1611$  and  $1124\text{ cm}^{-1}$  bands to  $1624$  and  $1114\text{ cm}^{-1}$ , respectively. At low indomethacin loading spectroscopic imaging revealed that the H bonding interactions are maintained, *i.e.* that nicotinamide acts as a carrier transporting indomethacin into solution, while at high indomethacin loading, the H bonding is not maintained, but highly soluble nicotinamide serves as disintegrant.

There are two potential routes of crystallization of an amorphous API during dissolution: matrix and solution crystallization. In the former, the solid crystallizes directly, while in the latter the solid first dissolves to give a supersaturated solution from which crystallization occurs [222]. Taylor and co-workers studied the crystallization behavior of amorphous nifedipine in the absence and presence of different polymers using Raman spectroscopy [223]. Polarized light microscopy had revealed that upon contact with buffer containing pre-dissolved polymer nifedipine rapidly crystallized, mainly through the matrix route. By contrast, nifedipine in ASD crystallized *via* the solution route. Raman spectroscopy and MCR allowed a detailed quantitative analysis of the crystallization behavior showing that irrespective of the crystallization pathway, the metastable  $\gamma$ -polymorph formed first and then converted to

the stable  $\alpha$ -polymorph. From the concentration *vs.* time profile it was evident that the crystallization and conversion through the solution route was faster compared to the matrix route. In case of neat amorphous nifedipine, the crystallization to the  $\gamma$ -form was complete within 10 min at 25 °C, while the conversion to the  $\alpha$ -form took about 40 min. It should be noted that the  $\gamma$ -polymorph could be detected as an intermediate phase only because of the speed of Raman analysis. The presence of pre-dissolved polymer did not affect the crystallization of amorphous nifedipine but slowed down the  $\gamma \rightarrow \alpha$  transformation. From the lag-time for the formation of the  $\alpha$ -form seen in the concentration *vs.* time profile and the similarity of the slope of the curves in the presence and absence of pre-dissolved polymer it could be concluded that the polymer interferes with the nucleation of the  $\alpha$ -polymorph.

Kogermann *et al.* studied solid-transformations of amorphous piroxicam-polymer solid dispersions prepared by solvent evaporation [224] and ball-milling [225]. The stability of amorphous piroxicam-Soluplus SDs under different storage conditions was examined by ATR-FTIR spectroscopy and Raman spectroscopy with PCA and MCR-ALS. The disappearance of the piroxicam N-H stretching vibration in the IR spectrum at 3337  $\text{cm}^{-1}$  was attributed to strong H bonding between piroxicam and the C=O group of Soluplus. The Raman spectrum also showed clear changes such as the broadening and intensity loss of the band at 1334  $\text{cm}^{-1}$  specific to piroxicam anhydrate. However, to monitor solid-state transitions during storage, Raman spectroscopy was combined with PCA. At medium and high RH, the PCA scores travelled towards the physical mixture of piroxicam anhydrate and Soluplus. Applying MCR-ALS to the Raman data allowed to quantitatively analyze the recrystallization. At 40 % RH recrystallization could be detected after one day and samples were found to transform to the anhydrate and subsequently to the monohydrate within 2 to 3 months. At low RH, clear clustering of the samples around the freshly prepared sample was observed indicating that the samples are stable for at least 6 months. By contrast, ball-milled SDs crystallized within 2 months even at low RH. The authors concluded that the different preparation methods led to different sizes of nuclei, degrees of mixing, molecular arrangements and drug-polymer interactions, although the latter was not discussed in terms of the spectroscopic data.

Lin *et al.* determined the relative amounts of amorphous indomethacin in indomethacin-Soluplus ASDs prepared by two different methods, air-drying and heat-drying, using IR spectroscopy with curve-fitting of the 1800 - 1530  $\text{cm}^{-1}$  region [226]. In both cases the SDs were stable towards recrystallization under accelerated conditions. However, interestingly, only heat-dried samples showed shifts of the IR bands, *e.g.* a shift of the amide carbonyl band of Soluplus from 1636 to 1633  $\text{cm}^{-1}$  and of the ester carbonyl stretching band of Soluplus from 1735 to 1731  $\text{cm}^{-1}$  (1 : 1 indomethacin : Soluplus, w/w) suggesting that H bonding interactions occur in heat-dried indomethacin-Soluplus SDs, but not in air-dried SDs. Ewing *et al.* studied the stability and dissolution behavior of indomethacin in polyethylene glycol (PEG) and HPMC [227]. In contrast to the indomethacin/HPMC ASD, the ATR-FTIR spectra of the ASD of indomethacin in PEG showed a band specific to the  $\alpha$ -form after 24 h and a characteristic band of the  $\gamma$ -

polymorph after 48 h indicating recrystallization to  $\gamma$ -indomethacin *via* the metastable form. Spectroscopic imaging of tablets of the ASDs showed that amorphous indomethacin was evenly distributed in HPMC but not in PEG. Spectroscopic imaging also revealed the accumulation of indomethacin as PEG dissolves and again recrystallization of  $\gamma$ -indomethacin was detected by the appearance of characteristic IR bands in the  $\nu(\text{C}=\text{O})$  region which hindered drug release into solution. The 1500 – 1800  $\text{cm}^{-1}$  ranges of the IR and Raman spectra of solid dispersions of indomethacin and PVP prepared by solvent evaporation or melt extrusion gave evidence of H bonding interactions between the hydroxyl group of indomethacin and the carbonyl group of PVP inhibiting the crystallization of  $\gamma$ -indomethacin [228,229]. By contrast, no significant changes in the Raman band-shapes of solid dispersions prepared by grinding were observed suggesting that crystallization inhibition was not due to H bonding interactions, but due to a partitioning effect of PVP which prevents the growth of indomethacin nuclei [183].

Zidan *et al.* compared vibrational spectroscopies with XRPD and DSC as methods to detect and quantify recrystallized tacrolimus in SDs with HPMC [230]. Multivariate calibration models were constructed that gave RMSEC values of 2.91, 5.36, 7.07 and 11.58 % for the NIR, XRPD, Raman and DSC method, respectively, showing that NIR spectroscopy outperforms Raman spectroscopy, as well as DSC and XRPD. Furthermore, NIR imaging combined with PLS analysis confirmed a homogenous distribution of the components and allowed a spatial analysis of crystallinity. Characterization of the SDs by IR spectroscopy indicated H bonding interactions between the functional groups of tacrolimus and HPMC, as identified by the pronounced upfield shift of the C=O and C=C vibration at 1698 and 1637  $\text{cm}^{-1}$  to 1715 and 1651  $\text{cm}^{-1}$  and the disappearance of the C=O band at 1728  $\text{cm}^{-1}$ .

Several recent publications discuss the vibrational spectra of coamorphous systems of two APIs or an API and a small-molecule excipient in order to rationalize the stability of the formulations towards recrystallization (Table 2). When a stoichiometric mixture of indomethacin and ranitidine hydrochloride, a drug-drug combination suitable for multidrug therapy, was coamorphized, the DRIFTS spectra showed three bands in the CO stretching region that were not observed in the spectra of the pure amorphous components [198]. The bands that were assigned to the benzoyl and carboxyl  $\nu(\text{C}=\text{O})$  vibrations of indomethacin and the  $\nu(\text{C}=\text{N})$  vibration of ranitidine hydrochloride gave evidence of the disruption of the homodimers present in amorphous indomethacin and the presence of indomethacin-ranitidine hydrochloride interactions, although the exact nature of these interactions remained elusive. A detailed analysis of the IR spectrum of coamorphous indomethacin-naproxen explained why the 1:1 mixture is more stable than the 2:1 and 1:2 mixtures despite not having the highest  $T_g$  [199]. The most diagnostic IR band was the band at 1702  $\text{cm}^{-1}$ . As this band is positioned between the  $\nu(\text{C}=\text{O})$  bands of the homodimers in amorphous indomethacin (1708  $\text{cm}^{-1}$ ) and amorphous naproxen (1697  $\text{cm}^{-1}$ ), it was attributed to the formation of indomethacin-naproxen heterodimers. Furthermore, a  $\nu(\text{C}=\text{O})$  stretching vibration at 1733  $\text{cm}^{-1}$  indicated that a fraction of the carboxyl groups in the coamorphous mixture

is not involved in hydrogen bonding. This description of the molecular near range order in coamorphous indomethacin-naproxen was supported by a later theoretical study involving the DFT prediction of the IR spectra of the optimized monomers, homodimers and heterodimer of the two drugs [231]. As in the case of drug-polymer ASDs, understanding the interactions between the components of a coamorphous system at the molecular level can shed light on the dissolution behavior: The synchronized release of indomethacin and naproxen and the faster dissolution of the former compared to pure amorphous indomethacin was attributed to the formation of the indomethacin-naproxen heterodimer [199].

Allesø *et al.* studied the molecular interactions in coamorphous naproxen-cimetidine by Raman spectroscopy [232]. The disruption of  $\pi$ -stacking in naproxen on coamorphization was concluded from the shift of the C=C vibration from 1630 to 1634  $\text{cm}^{-1}$ . Ueda *et al.* used IR spectroscopy to monitor the heating-induced recrystallization of various naproxen-drug combinations [233]. Coamorphization of simvastatin and glipizide was shown to lead to a significant stability enhancement [200]. However, only minor bathochromic shifts of the C=O and S=O vibrations occurred. In contrast to the binary mixtures of indomethacin-ranitidine hydrochloride, naproxen-cimetidine and naproxen-indomethacin, no evidence for specific intermolecular interactions between simvastatin and glipizide were obtained from the FTIR spectra. The absence of significant interactions was confirmed by PCA of the spectral data. In the same paper, the differences in the IR spectra of the amorphous and crystalline forms of simvastatin were used to complement XRPD stability studies of coamorphous simvastatin-glipizide samples prepared by different methods and stored under different conditions. In some cases FTIR spectroscopy detected the onset of recrystallization earlier than XRPD. As already discussed, IR spectroscopy as a surface technique is more sensitive to traces of crystallization which usually starts on the surface.

FTIR spectroscopy was also applied to analyze molecular interactions in ternary coamorphous mixtures [234-236]. Löbmann *et al.* [234] and Jensen *et al.* [235] coamorphized indomethacin, carbamazepine and naproxen with two different amino acids. As evident from the examples described above, shifts diagnostic of intermolecular interactions are often small and in many cases the interpretation of the data is not straightforward. The spectral analysis of ternary mixtures is even more challenging because of the additional complexity of the spectra and more band overlap. Furthermore, when the amino acids cannot be amorphized on their own, a comparison with the IR data of the pure amorphous amino acids is not possible. This latter problem was circumvented by preparing coamorphous amino acid blends [234]. A comparison of the spectra of the indomethacin-arginine-phenylalanine and arginine-phenylalanine systems, for example, allowed to conclude that indomethacin and arginine interact *via* salt formation, while the involvement of phenylalanine in intermolecular interactions remained unclear. Coamorphization of carbamazepine and tryptophan resulted in the merging of the carbamazepine bands at 1589 and 1670  $\text{cm}^{-1}$  with the tryptophan bands at 1659 and 1581  $\text{cm}^{-1}$  to a broad double band suggesting the disruption of the homodimers present in

amorphous carbamazepine. Further shifts and changes in the shape of the  $\nu(\text{C}=\text{C})$  and  $\nu_{\text{ring}}$  vibration bands were consistent with  $\pi$ - $\pi$  interactions between carbamazepine and tryptophan. Likewise, the spectral data of the ternary system carbamazepine-tryptophan-phenylalanine gave evidence of H bonding and  $\pi$ - $\pi$  interactions. In contrast to the indomethacin-arginine-phenylalanine system, differences between the spectra of carbamazepine-tryptophan and carbamazepine-tryptophan-phenylalanine revealed the participation of phenylalanine in specific interactions with the drug molecule and/or tryptophan. Changes in the aromatic region indicating  $\pi$ - $\pi$  interactions were also seen in the spectrum of ternary mixtures containing naproxen, tryptophan and proline, while on coamorphizing naproxen with arginine and proline naproxen and arginine converted to the salt [235]. In both cases, the spectra confirmed that proline interacts with naproxen and/or arginine. Laitinen *et al.* studied binary and ternary mixtures of simvastatin, glibenclamide and various amino acids [236]. In the IR spectrum of amorphous glibenclamide the  $\nu(\text{C}=\text{O})$  band of the carbamino group appeared at 1626  $\text{cm}^{-1}$  (shifted from 1616  $\text{cm}^{-1}$  in the crystalline form) and a shoulder was observed at 1655  $\text{cm}^{-1}$  that was assigned to the rare imidic acid tautomer. Interestingly, this shoulder was not visible in the spectra of coamorphous glibenclamide-serine and glibenclamide-threonine. It was proposed that intermolecular interactions of glibenclamide and the amino acid prevent the tautomeric conversion seen in the amorphous API.

Coamorphous systems of the polymorphic sulfa-drug sulfathiazole and a range of multifunctional carboxylic acids as cofomers were studied by NIRS [237]. L-tartaric acid and citric acid were shown to stabilize amorphous sulfathiazole for up to one month at low RH. At  $>10\%$  RH, recrystallization occurred and PCA was applied to the NIR data to elucidate the recrystallization pathway for different storage conditions. The PCA scores scatter plots showed clustering of the samples indicating that the ratio of the different polymorphs formed depended both on RH and on the starting sulfathiazole polymorph used to produce the amorphous form. To gain insight into intermolecular interactions between sulfathiazole and the acid that may lead to the enhanced stability of the coamorphous systems, samples were further analyzed by IR spectroscopy. It was concluded that the coamorphous solids contain small particles of the components which are stabilized by particle-to-particle hydrogen bonding.

Another strategy to stabilize the amorphous form is by confinement into the pores of porous silica [238,239]. Silica surfaces may influence the polymorphism and the crystallization behavior of the API [240,241]. Furthermore, the pores should be filled evenly and to maximum capacity. Non-imaging and imaging vibrational methods can provide useful information on the properties and stabilities of API/silica systems. Hellstén *et al.* assessed Raman mapping combined with multivariate data analysis (PLS, PCA) as a tool to study the occurrence and distribution of different solid-state forms of an API loaded in porous silica [242]. Using PLS regression a calibration model was developed for the determination of the  $\alpha$ -,  $\gamma$ - and amorphous form of the model API indomethacin using the C=O stretching range (1500 – 1700  $\text{cm}^{-1}$ ). Despite problems arising from fluorescence and sample burning, the model detected the

presence of crystalline and amorphous forms and the even distribution of the crystalline form on the silica surface with larger crystalline clusters forming upon storage at higher temperature and RH. As a limitation of the method the authors pointed out that it was not possible to estimate the exact penetration depth of the measurement. Fussel *et al.* investigated the loading of itraconazole and griseofulvin into mesoporous silica using CARS microscopy [243]. The hyperspectral images and extracted CARS spectra of itraconazole- and griseofulvin-loaded MCM-41 particles confirmed that both APIs were in their amorphous state. Nielsen *et al.* used FT-Raman spectroscopy in conjunction with PLS regression to examine the recrystallization of indomethacin confined into microcontainers of different sizes [244]. In 174 and 223  $\mu\text{m}$  microcontainers the recrystallization rate to stable  $\gamma$ -indomethacin was significantly reduced ( $29.0 \pm 2.6\%$  and  $38.3 \pm 1.5\%$  after 30 d at 30 °C and 23 % RH compared to 51.0 % for bulk indomethacin). The PLS model also revealed that amorphous indomethacin confined in 73  $\mu\text{m}$  microcontainers converted to the  $\gamma$ -form and no enhanced stability was observed. Possible reasons for the different behavior of the 73  $\mu\text{m}$  system were discussed: capillary condensation, slightly different cooling rates experienced by the material in the differently sized microcontainer during melt-quench preparation and the larger relative amount of indomethacin in contact with the moisture-absorbing microcontainer walls.

#### 4.4. Cocrystallization:

Cocrystals are defined as stoichiometric multi-component systems connected by non-covalent interactions where all the components present are solid under ambient conditions. Pharmaceutical cocrystals are prepared by cocrystallizing two compatible APIs or an API and a pharmaceutically acceptable cofomer in order to improve the stability, dissolution behavior or processability of the API. Traditionally, cocrystals are grown from solution and characterized by X-ray analysis, but nowadays high-throughput screening methods are becoming increasingly important. In particular, solid-state milling, either neat or in the presence of catalytic amounts of solvent, is a more effective approach to cocrystal synthesis. XRPD as the classical method to characterize and identify cocrystals has several drawbacks, such as long measurement times, preferred orientation effects, inability to distinguish isostructural crystals and therefore does not lend itself to high-throughput screening applications, in contrast to vibrational spectroscopy which allows the analysis of a large number of samples within a short time. The importance of spectroscopic methods for the characterization, purity assessment, stability studies and monitoring of the formation of cocrystals is well-documented in the recent literature (Table 3). Raman spectroscopy, in particular, is gaining popularity for the characterization of cocrystals. If a cocrystal is to be patented, it has to be demonstrated that it is present in the formulation rather than the raw API. For quality control of cocrystal formulations it is essential to confirm that the cocrystal does not convert into a physical mixture of its components over time. Like APIs, cocrystals can exhibit polymorphism and polymorphic cocrystals may undergo solid-state transformations during storage or during the manufacturing process.

Various studies on the use of vibrational spectroscopy for the quantitative analysis of pharmaceutical cocrystals are reported in the literature (Table 3). Soares and Carneiro showed that Raman spectroscopy in combination with MCR-ALS is a suitable method for monitoring the conversion of ibuprofen and nicotinamide to the cocrystal in aqueous slurry [245]. In a later study the same researchers developed multivariate calibration models for the quantification of ternary mixtures containing the ibuprofen - nicotinamide cocrystal, ibuprofen and nicotinamide to evaluate the purity of the cocrystallization product and showed that Raman spectroscopy outperforms mid-IR spectroscopy, XRPD and DSC [246]. Raman spectroscopy combined with PLS regression gave root mean square error of cross validation (RMSECV) and RMSEP values of  $< 5\%$  for all three components, whereas the mid-IR models for the determination of the cocrystal and nicotinamide had RMSECV and RMSEP values of  $> 15\%$  due to the similarity of the spectra. Maheshwari *et al.* used ATR-FTIR spectroscopy in conjunction with PLS regression to quantitatively analyze the spontaneous cocrystal formation between carbamazepine and nicotinamide during the storage of physical mixtures under different conditions and to study the effect on the formation rate, when the individual components are milled prior to mixing [247]. The usefulness of Raman spectroscopy for the characterization of intermolecular interactions in cocrystals is also well documented in the literature. Elbagerma *et al.*, for example, have recently reported a detailed analysis of the Raman spectrum of the paracetamol - citric acid cocrystal [248]. Du *et al.* used Raman spectroscopy to show that upon grinding a physical mixture of piracetam and 3-hydroxybenzoic acid formation of the piracetam - 3-hydroxybenzoic acid cocrystal is complete after 35 min [249]. Kelly *et al.* evaluated NIR spectroscopy as a PAT tool to monitor the twin-screw extrusion-based, solvent free continuous cocrystallization of ibuprofen and nicotinamide [250]. They used a high temperature NIR probe in the extruder die and PLS analysis of the 4959 – 5080  $\text{cm}^{-1}$  region of the spectra where the N-H and N-H/C=O absorptions of nicotinamide appear. Comparison with off-line XRPD data confirmed the suitability of in-line NIR spectroscopy to follow cocrystal formation during twin extrusion. Sarraguca *et al.* used the formation of furosemide – adenine cocrystals as a case study for the applicability of NIR spectroscopy in conjunction with chemometrics as a PAT tool for the on-line monitoring of cocrystallization processes [251]. Zhang *et al.* investigated the formation of the indomethacin - saccharin cocrystal upon grinding and heating by FTIR microspectroscopy combined with quantitative curve-fitting analysis [252].

The anhydrous, solvent-free mechanochemical cocrystallization of different sulfathiazole polymorphs with L-glutaric acid was monitored by XRPD, NIR and IR spectroscopy [237]. PCA of the NIR data gave some insight into the solid-state transformation. The rates at which the PCA scores travelled toward the cocrystal region varied significantly with the starting polymorph with sulfathiazole form III requiring the longest milling time. Furthermore, the PCA score plots of the NIR spectra clearly showed that sulfathiazole forms I and V form the cocrystal directly while form II transforms to form IV before the cocrystal is formed. Interestingly, when oxalic acid was used as a cofomer,

proton transfer was observed. Monitoring of this solvent-free salt formation between sulfathiazole and oxalic acid upon grinding by NIR and IR spectroscopy along with XRPD showed that complete amorphization took place prior to salt formation. While amorphous sulfathiazole was identified by two characteristic IR bands at 3464 and 3364  $\text{cm}^{-1}$  that disappeared when the salt was formed, NIR spectroscopy proved useful to monitor the time-dependence of the reaction for different starting polymorphs. In this study, the milling process was interrupted and samples were analyzed off-line. By contrast, Lin *et al.* inserted the fiber-optic probe of a portable Raman analyzer directly into the milling assembly to monitor the mechanochemical cocrystal formation between  $\gamma$ -indomethacin and saccharin and between theophylline and citric acid in real time [253]. No peaks specific to  $\alpha$ -indomethacin or amorphous indomethacin ( $\nu(\text{C}=\text{O})$  at 1692 and 1680  $\text{cm}^{-1}$ , respectively) were observed, indicating that the cocrystal formation was direct without polymorph transformation or prior amorphization. A different approach to the real-time, *in situ* monitoring of cocrystal formation during milling was taken by Gracin *et al.* [254]. Using translucent Plexiglas milling jars time-resolved Raman scattering signals were obtained from the milled sample without interrupting the milling process. A similar setup was used by Batzdorf *et al.* who investigated the

milling-induced formation of the theophylline - benzoic acid cocrystal and found that the conversion was direct without the formation of the amorphous form or other intermediate [255]. In this case the Raman data were complemented with simultaneous XRPD measurements. On the basis of the small shift of the C=O bands of theophylline the milling product was identified as a cocrystal rather than a salt. Lin's group introduced simultaneous DSC-FTIR microspectroscopy as a rapid, real-time, one-step screening method for cocrystal formation [256]. This method was applied for example to screen for the formation of indomethacin [257] and metaxalone [258] cocrystals with dicarboxylic acids and to follow the thermally induced cocrystal formation in ground physical mixtures of indomethacin and nicotinamide [259] as well as H bond and cocrystal formation between piroxicam and saccharin on heating or liquid-assisted grinding [260]. Hsu *et al.* showed by DSC-FTIR microspectroscopy and MCR that upon grinding a physical mixture of theophylline and citric acid gradually converts to the cocrystal obtained by solution crystallization [261]. They also applied DSC-FTIR spectroscopy to confirm that the cocrystal forms upon heating and during storage of the physical mixture at 55 °C and 75 % RH and is stable under these conditions [261,262].

Table 3. Recent (after 2009) studies reporting the utilization of vibrational spectroscopy for the characterization, formation, purity and stability assessment of pharmaceutical cocrystals

API	Cofomer	Method	Study	Reference
apixaban	various dicarboxylic acids	LFRS	distinction of isostructural cocrystals	[68]
caffeine	4-hydroxybenzoic acid	LFRS	analysis of tableted formulation	[267]
caffeine	glutaric acid	IR	purity controls	[314]
carbamazepine	nicotinamide	IR, PLS	quantitative analysis of the spontaneous cocrystal formation during storage	[247]
carbamazepine	succinic acid	IR	characterization and stability	[311]
carbamazepine	malonic acid	IR	intermolecular interactions	[312]
flurbiprofen	nicotinamide	LF Raman mapping, chemometrics	Kofler experiment	[263]
flurbiprofen	nicotinamide	TRS, statistical data analysis	analysis of tableted formulation	[53]
furosemide	adenine	NIR, chemometrics	online monitoring of the cocrystallization process	[251]
ibuprofen	nicotinamide	Raman, MCR-ALS	in-line monitoring of cocrystal formation in aqueous slurry	[245]
ibuprofen	nicotinamide	Raman, IR	purity control	[246]
ibuprofen	nicotinamide	NIRS, PLS	in-line monitoring of the twin-screw extrusion-based continuous cocrystallization process	[250]
indomethacin	nicotinamide	IR, Raman	characterization and stability	[313]
indomethacin	nicotinamide	DSC-FTIR	monitoring of the thermally induced cocrystal formation	[259]
indomethacin	saccharin	IR, quantitative curve-fitting analysis	grinding- and heating-induced cocrystal formation	[252]
indomethacin	saccharin	Raman	<i>in situ</i> monitoring of cocrystal formation during milling	[253]
indomethacin	trans-cinnamic acid	IR, Raman	intermolecular interactions	[313]
indomethacin	various dicarboxylic acids	DSC-FTIR	cocrystal screening	[256]
metaxalone	various dicarboxylic acids	DSC-FTIR	cocrystal screening	[258]
nicotinamide	suberic acid	Raman	<i>in situ</i> monitoring of the cocrystal formation during milling	[254]

paracetamol	citric acid	Raman	characterization	[248]
piracetam	3-hydroxybenzoic acid	Raman	monitoring of the cocrystal formation during milling	[249]
piroxicam	saccharin	IR, DSC-FTIR	monitoring of the cocrystal formation during milling	[260]
rhein	L-arginine	NIR, PLS	purity assessment	[315]
sulfathiazole	glutaric acid	NIR, PCA	transformation pathway of different sulfathiazole polymorphs	[237]
theophylline	nicotinamide	Raman	spontaneous cocrystal formation in physical mixtures	[316]
theophylline	citric acid	Raman	<i>in situ</i> monitoring of cocrystal formation during milling	[252]
theophylline	citric acid	DSC-FTIR, MCR	cocrystal formation and stability	[261]
				[262]

In an interesting study the group of Burley applied confocal Raman microscope mapping to a Kofler melt of the model flurbiprofen - nicotinamide cocrystal system using both the low- and high-frequency range [263]. Analysis of the spatial distribution of flurbiprofen and nicotinamide by chemometric methods not only gave new insight into the fundamentals of Kofler melts but also revealed implications for the use of Kofler melts as a screening method for cocrystals. In a Kofler experiment, the two components are allowed to come into contact between a glass microscope slide and a cover slip and to form a composition gradient (by melting). Cocrystal formation is usually examined by optical microscopy. Although not widely used to screen for cocrystals, it has been occasionally applied in pharmaceutical cocrystal studies [264-266]. Chemometric analysis of the Raman data of the flurbiprofen - nicotinamide system showed that the composition gradient in the Kofler melt is not a linear function of position as had been previously believed. Instead, there appears to be a sharp interface of the component with the higher melting point with the rest of the Kofler melt preparation, *i.e.* a step change in the composition. As a consequence, potential cocrystals with compositions rich in the higher melting point component may be experimentally inaccessible. Burley *et al.* also investigated the ability of TRS to analyze tableted formulations of cocrystals using the flurbiprofen - nicotinamide cocrystal as model cocrystal and magnesium stearate, avicell and lactose as examples for common excipients [53]. Cocrystal formation was clearly evidenced by the disappearance of a specific flurbiprofen peak in the phonon region. However, while the visible inspection suggestion that the chemical composition (cocrystal vs. physical mixture) mainly accounted for the variance and overall spectral data structure, statistical data analysis (hierarchical agglomerative clustering and PCA) showed that the main data structuring is due to the API content. The authors concluded that TRS can be a valuable tool to study intermolecular interactions in formulations but that there are also potential pitfalls in the visual inspection of datasets without detailed statistical data analysis.

Hisada *et al.* showed that LFRS can clearly distinguish between the caffeine - 4-hydroxybenzoic acid cocrystal and a physical mixture of the two components in tablets containing 90 % (w/w) microcrystalline cellulose [267]. While the conventional regions of the Raman spectra of the cocrystal and the physical mixture were rather similar and suffered

from fluorescence interference, the low frequency range proved suitable to visualize the distribution of the dispersed cocrystal and the physical mixture in the tablets under ambient atmosphere. It was pointed out that a spatial resolution of ca 16  $\mu\text{m}$  could be obtained which is significantly lower than the typical spatial resolution of terahertz spectroscopy (ca 600  $\mu\text{m}$ ) in addition to vacuum conditions not being necessary.

XRPD does not distinguish isostructural cocrystals with identical molecular packing, aromatic ring stacking and H bonding interactions which may be formed by related cofomers, such as a series of dicarboxylic acids with slightly different spacers. Isostructural cocrystals should also give very similar LF Raman spectra. However, they are anticipated to differ in the conventional Raman region because of the different molecular structure of one or both component(s). This was confirmed by Larkin *et al.* who compared the Raman spectra of a range of isostructural apixaban - dicarboxylic acid cocrystals having the same space group, stacking and H bonding interactions [68].

Loratadine forms an inclusion complex with hydroxypropyl- $\beta$ -cyclodextrin as evidenced by a shift of the C=O stretching band of loratadine from 1703 to 1676  $\text{cm}^{-1}$  and of the C-O stretching band from 1227 to 1235  $\text{cm}^{-1}$ , while the O-H stretching vibration of the cyclodextrin shifts from 1646 to 1640  $\text{cm}^{-1}$ . FTIR spectroscopy with curve-fitting analysis can discriminate loratadine, the cyclodextrin and the inclusion complex in mixtures and can be used to monitor the formation of the inclusion complex by co-grinding [268].

## CONCLUSION

Mid-IR, Raman and NIR spectroscopies continue to be important analytical techniques for understanding solid-state transformations of polymorphs, pseudopolymorphs and amorphous forms of pharmaceuticals and for relating the stability of solid-state forms to structural properties. Raman and NIR spectroscopy – usually in conjunction with chemometric tools – are often the method(s) of choice for measuring transformation rates. Several examples discussed in this review have shown that in various cases transient phases can be detected by vibrational spectroscopy that are missed by other methods. The low-frequency region of the

Raman spectrum has several unique advantages over the conventional range and the last decade has seen a significant increase in the use of LFRS in the study of pharmaceutical phase transitions. LFRS has led to the discovery of new polymorphs, has contributed to a deeper understanding of phase properties and has proved to be suitable for quantitative analysis. In contrast to Raman and NIR spectroscopy, IR spectroscopy is still mainly used as an off-line method. It is a valuable tool in studies on drug-polymer/coformer interactions in ASDs and coamorphous systems with a view to understanding drug release mechanisms and crystallization inhibition and developing rational design strategies for amorphous formulations.

## CONFLICT OF INTEREST

The author declares no conflict of interest.

## ACKNOWLEDGEMENTS

This work was supported by Science Foundation Ireland under Grant No. [07/SRC/B1158] as part of the Synthesis and Solid State Pharmaceutical Centre (SSPC).

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