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Review

Perspectives in the use of spectroscopy to characterise pharmaceutical solids

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ABSTRACT

Knowledge of the solid-state properties is one of the key issues in understanding the performance of drugs. Recent developments in spectroscopic techniques have made them popular tools for solid phase analysis; they are fast, accurate and suitable for real-time measurements during processing, and further, they can be used to obtain structural understanding of solid forms, for example, by the use of multivariate analysis and computational chemistry. In this article emerging topics related to spectroscopic analysis of pharmaceutical solids are reviewed. The following areas are highlighted: (1) the importance of multivariate methods in the analysis of solid forms when using spectroscopic techniques, (2) spectroscopic analysis of processing-induced solid phase transformations in the manufacturing setting, (3) novel spectroscopic techniques and pharmaceutical examples of their use, and (4) the advantages and the use of computational simulation of vibrational spectra. The topics listed are thought to be of the foremost importance in improving the understanding of pharmaceutical materials, processes and formulations.

Contents

1. Introduction

Solid formulations are the most important pharmaceutical dosage forms today. This is likely to remain true in the future, due to the convenience and acceptability of solid oral forms, including tablets and capsules, and the rising importance of chemically labile biopharmaceuticals, necessitating the formulation of freeze-dried products. With the advent of combinatorial chemistry in the drug discovery process, even classical small molecules will become increasingly larger, and will frequently show extensive polymorphism and poor aqueous solubility. As low solubility will often be due to crystallinity, rather than lipophilicity, advanced solid-state formulations, making use of metastable polymorphic forms and amorphous systems, will become more important in the future. These systems will contain drugs which are not in their thermodynamically stable state, and thus need to be stabilised against physical as well as chemical degradation. It is also clearly foreseeable that to increase the quality of the pharmaceutical manufacturing process, as well as the safety for the patient, process

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analytical technology (PAT) will become more important in the pharmaceutical field in general, and in the manufacturing of solid dosage forms in particular.

All this requires sophisticated and fast analytical techniques. In the solid state, we can differentiate analytical techniques with respect to the level at which they probe the solid. Raman and IR spectroscopy probe the solid state predominantly on the intramolecular level. X-ray diffraction and the various thermal analytical techniques predominantly probe the "lattice level", i.e. the intermolecular level. Terahertz spectroscopy is a spectroscopic technique that in the solid state also probes this level. Finally, we have a range of bulk techniques that analyse solids on the interparticulate level. Amongst all these techniques, the spectroscopic methods offer substantial advantages, in that they are usually fast, lend themselves to in-process monitoring, and provide chemical information. This is especially important in amorphous systems if one wants to investigate for example drug–excipient interactions. As spectroscopic techniques, such as Raman and IR, predominantly probe the molecular level, the identification and even more so the quantification of different solid-state forms of a drug in a dosage form requires the use of sophisticated, multivariate analytical techniques, as the vibrational spectra will remain quite similar for various solid-state forms of the same compound. Furthermore the spectra are complex and changes therein as a function of solid-state transformations need to be better understood to gain a deeper insight into the behaviour of solids. Computational techniques will therefore become more important in the pharmaceutical setting.

In this article we do not give a comprehensive review of the use of spectroscopic techniques in the solid state. Rather we want to highlight four areas that are emerging (or have already emerged to quite a sophisticated standard) and that in our view will be of the foremost importance in this field in the future. In the first part of this article we highlight the importance of multivariate methods in the analysis of solid forms when using spectroscopic techniques. We will then discuss the use of spectroscopy, to monitor and understand processing-induced solid phase transformations in the manufacturing setting. In the third part several novel spectroscopic techniques that are currently mainly used in the fields of physics, chemistry and physical chemistry will then be introduced and pharmaceutical examples of their use will be given. Finally we will investigate the advantages and the use of computational simulation of vibrational spectra.

2. Multivariate methods in the analysis of solid forms

During the last decade the use of multivariate methods of data analysis has become ever more popular in many areas of pharmaceutical research. This applies also to the analysis of spectroscopic data for characterisation of the solid state, and there are several good reasons for this. The most obvious one is the need for sophisticated data handling tools capable of analysing large data matrices. With modern equipment it is possible to record impressive amounts of high-resolution data very rapidly, and to make use of these improvements new approaches to data analysis have to be adopted. In multivariate spectral analysis regions of or whole spectra are being used to describe the same phenomenon whereas in univariate analysis only one value is used. It nowadays is – or at least should be – standard practice to use chemometrics to qualitatively and quantitatively analyse the data, because for qualitative purposes visual inspection of several hundreds or even thousands of spectra is certainly not reasonable, and for quantitative purposes univariate methods are not powerful or reliable enough. Multivariate data analysis can be used not only to streamline the analysis but also to help gaining essential knowledge of processes under investigation. It is recognised also in the regulatory guidelines that multivariate methods play an important part in increasing the quality of drugs via better understanding of the drug products and/or processes (FDA, 2004).

Bro (2003) has highlighted some advantages of chemometrics that support the opinion that proper analytical chemistry *must* incorporate a chemometric approach. The advantages include noise reduction and the ability to deal with interferents and detect outliers. When several data points, instead of only one, are used simultaneously to describe the given phenomenon, random noise is less likely to affect the results. The capability of coping with noisy data and still being able to obtain reliable results is particularly important when analysing data from measurements performed in rough conditions, such as *in situ* spectroscopic process analysis. Pharmaceutical processing and formulation often introduces various interferents (such as other chemicals than the drug under investigation) into the system. When performing quantification these interferents can hamper or disable univariate analysis, but with multivariate analysis the quantification can still be performed and, if the interferent is included in the calibration model, it can be quantified as well. There are several multivariate techniques of data analysis being used and developed in the chemometric community that have great potential and could be applied to pharmaceutical research. Of the various methods available for the researcher principal component analysis (PCA) and partial least squares (PLS) regression are probably the most often applied. For detailed description of the methods and how to apply them in practice see, for example, texts by Martens and Næs (1989), Wold et al. (1987, 2001), and Beebe et al. (1998).

PCA is an *unsupervised* technique, which means that no previous knowledge of the given system/process is needed for the analysis. Furthermore, it does not require calibration. PCA is an excellent tool for fast evaluation of data prior to quantification or in-depth structural analysis of solid-state events as it enables a comprehensive overview of the whole data set with one single analysis. Even large amounts of data can be visualised in a rather simple fashion by using the score plot. In the PCA score plot each score represents one spectrum. If the scores are located near each other in the score plot it means that the spectra they represent are similar whereas the scores of spectra with differences will be separated. Three-dimensional score plots can be used, and because PCA is a linear technique, the distances between the clusters are easy to analyse. This is not the case with all clustering techniques. Possible outliers, for example due to instrument malfunction or sampling error, can be detected in the score plot as they most probably are located away from the flawless scores. In addition to clustering and outlier detection, PCA can be used to obtain structural understanding from the recorded data by analysing the loadings. To explain how PCA can be used to obtain understanding of solid phase transformations an example is given. The PCA score plot of spectra recorded during a processing-induced solid phase transformation from solid form A to solid form B should comprise two distinct score clusters (of both solid forms A and B) and some scores in between the clusters representing the spectra measured while the transformation was underway. If the transformation is direct (so that no intermediate phenomena involved) then also the pathway of scores in between the clusters of forms A and B usually is direct. If only two forms are involved in the process a one-component model should be sufficient to explain the process. Such a simple transformation mechanism is merely the ideal case, and in practice some intermediate phenomena, such as metastable solid phases, are often involved and a more-than-one-component model is needed to explain the differences between the observed forms. If the transformation occurs via an intermediate solid phase it can be seen as a "detour" in the score path between the forms A and B. Such

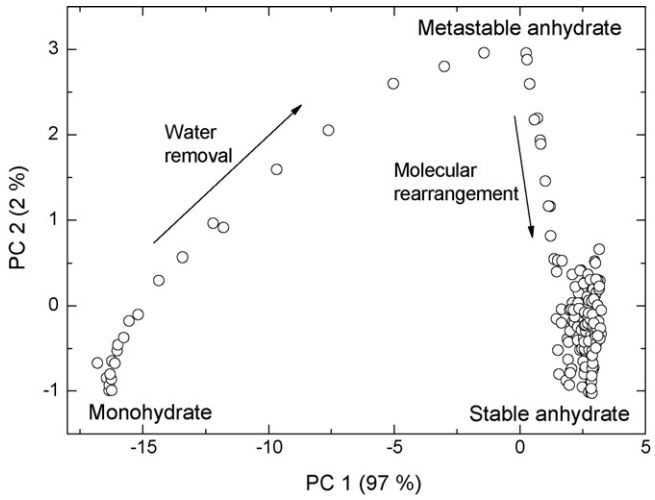

Fig. 1. PCA score plot of Raman spectra recorded in-line during fluidised bed drying of theophylline monohydrate granules at 60 °C. During drying theophylline monohydrate (scores in the lower left corner) undergoes a dehydration to the stable anhydrate form (lower right corner) via a metastable anhydrate (upper right corner).

a case, dehydration of theophylline monohydrate, is exemplified in Fig. 1. By analysing the loadings the underlying reasons for the observed score values can be determined. The variables (spectral regions) with the highest loading values correspond to the greatest differences between the spectra (with respect to a specific principle component), meaning that the vibrations these regions represent are those changing due to the solid phase transformation. The bands in these regions can then be assigned to specific vibrations which thus leads to structural and truly molecular level information of the solid phase transformation. The interpretation of the spectra and the band assignment can be further studied with quantum chemical modelling as discussed elsewhere in this paper. For detailed case studies of the above-described technique the reader is referred to a study by Jørgensen et al. (2006). In this extensive study Raman spectroscopy was used in combination with PCA to visualise and investigate dehydration of erythromycin dihydrate on a hot stage and crystallisation of amorphous lactose at high relative humidity. The PCA method was also applied to X-ray diffraction data recorded during dehydration of nitrofurantoin monohydrate and α-lactose monohydrate.

Quantitative solid phase analysis using spectroscopy is quite often problematic because the spectra of drugs themselves are very complicated and the characteristic peaks of various solid forms can (and usually do) overlap, fluorescence (which is frequently detected in Raman spectra) can occur, and various excipients further complicate the spectra. Often the spectra of various forms of a drug are very similar to each other, and the shifts of the peaks due to solid phase transformations can be in the magnitude of only a few wavenumbers making reliable identification, not to mention quantification by univariate analysis, practically often impossible. As discussed by Bro (2003), multivariate methods can extract the meaningful information out of noisy data recorded from samples with interferents. PLS regression is a *supervised* multivariate method with which quantitative analysis of multiple solid forms can be performed even if the differences between the spectra are minor. In a two-part study aiming at spectroscopic in-line quantification of four concurrently occurring solid forms of carbamazepine during processing, Kogermann et al. (2007, 2008) suggest a road map of how to develop a quantitative model and apply it in practice. In order to quantitatively analyse the solid phase transformation, the mechanism and the intermediate solid forms detected during the transformation need to be analysed first, because all the forms involved in the transformation have to be included in the calibration model. In the first part of the study (Kogermann et al., 2007), the mechanism of the solid phase transformation (dehydration of carbamazepine dihydrate) was investigated using partial least squares discriminant analysis (PLS-DA) of NIR and Raman spectra recorded during isothermal dehydration. Prior to multivariate modelling, a solid form screening was performed, and spectra of the four pure solid forms of carbamazepine prepared in the screening (anhydrate forms I and III, amorphous form and the dihydrate form) were recorded. In the PLS-DA, which is a qualitative classification method, spectra of the pure solid forms were used for calibration purposes. Thereafter the spectra recorded *in situ* during isothermal dehydration were introduced to the PLS-DA model and the solid-state transformation mechanisms were analysed by visual inspection of the PLS-DA scores. The dehydration process was found to involve all four forms with varying ratios depending on the dehydration temperature. After the elucidation of the transformation mechanism, quantitative analysis of the forms during dehydration could be performed. In the second part of the study, PLS regression was used: a calibration model including all the forms involved in the dehydration process was constructed and subsequently used to quantify the forms from the *in situ* spectra recorded in static conditions (on a hot stage) as well as dynamic conditions (in a fluidised bed dryer) (Kogermann et al., 2008). Overall, Raman spectroscopy was more accurate than NIR spectroscopy in the quantification of the solid forms of carbamazepine.

3. Spectroscopic analysis of processing-induced solid phase transformations

Early detection and quantification of solid phase transformations during processing can be of crucial importance for pharmaceutical companies. A phase transformation can occur during any stage of drug manufacturing or shelf-life and can lead to production problems in downstream processing or therapeutic failures due to decreased bioavailability. The detection of very small amounts of unwanted solid forms is important as even trace amounts can act as seeds and trigger the solid phase transformation. If a drug has to be withdrawn from the market the economic losses can be substantial. Ritonavir is a well-known example of extreme consequences originating from a sudden appearance of a new polymorph. It was originally formulated as a semisolid in a soft gelatine capsule using form I which was the only crystal form identified during development. Suddenly, after 240 lots of capsules had been successfully produced and no stability problems noticed, the capsules started to fail in dissolution tests because of the appearance of a new polymorph, form II, with lower bioavailability (Bauer et al., 2001). This started a long and expensive process during which the drug product was reformulated to suit form II, and the preparation of form I was revisited (Chemburkar et al., 2000). To avoid setbacks due to polymorphism two areas deserve special attention: first, a careful and comprehensive solid form screening needs to be performed to find out the possible solid forms and their thermodynamic relationship, and second, the monitoring of the solid form should be carried out during the whole drug production cycle, from the initial production of the raw materials through processing to stability studies, to detect the transformations as early as possible. Spectroscopic methods offer many solutions for solid phase analysis during processing, some of which are reviewed below and listed in Table 1.

In terms of spectroscopy, near-infrared (NIR) analysis has dominated the PAT field for a long time. There are certainly more PAT applications of NIR spectroscopy than any other spectroscopic

Table 1
Examples of in-line applications of spectroscopic solid-state analysis during different unit operations

Unit operation	Reference	Spectroscopic method	Solid-state phenomenon	Data analysis method
Crystallisation	Schöll et al. (2006)	Raman	Polymorphic transformation of L-glutamic acid	Bivariate
	Hu et al. (2005)	Raman	Polymorphic transformation of flufenamic acid	Bivariate
Freeze-drying	Romero-Torres et al. (2007)	Raman	Crystallisation and polymorphic transformations of mannitol	Univariate, PCA
	De Beer et al. (2007)	Raman, (at-line) NIR	Crystallisation and polymorphic transformations of mannitol	PCA
Wet granulation	Wikström et al. (2005)	Raman	Anhydrate to monohydrate conversion of theophylline	Trivariate
Drying	Aaltonen et al. (2007)	Raman, NIR	Dehydration of theophylline monohydrate	PLS
	Kogermann et al. (2008)	Raman	Dehydration of carbamazepine dihydrate	PLS

method (Räsänen and Sandler, 2007). However, for the analysis of solid forms NIR spectroscopy is not the most suitable method due to the fact that the bands in the NIR spectra consist of overtones and combinations of fundamental vibrations and thus cannot be specifically assigned. For the analysis of dehydration and hydrate formation NIR spectroscopy is a valuable tool as it can be used to differentiate the state of water molecules present in the solid sample (Zhou et al., 2003). Raman spectroscopy has evolved during the past two decades from a promising technique into a research tool used on a daily basis (Smith and Dent, 2005). Although there are plenty of recent publications dealing with PAT applications of Raman spectroscopy (Rantanen, 2007), the number of in-line quantitative studies is still rather low. The activity in this specific area is constantly growing, and as new approaches are developed to overcome the challenges related to spectroscopy/process interface (Rantanen, 2007), Raman spectroscopy will probably strengthen its position as a method of choice for in-line solid phase analysis. Another spectroscopic technique that has lately been increasingly used for solid-state analysis is terahertz pulsed spectroscopy. As it probes whole lattice vibrations it provides a complementary technique to infrared and Raman spectroscopy.

Crystallisation is one of the key unit operations in pharmaceutical manufacturing as raw material for dosage forms is produced. By monitoring and controlling the crystallisation process so that the material produced possesses optimal properties the number of subsequent processing steps can be minimised. As discussed earlier, polymorphism is one of the properties of utmost importance. The outcome of a crystallisation process is a result of the interplay between kinetic and thermodynamic factors, such as solvents, agitation, temperature and seeding to name but a few. Controlled crystallisation requires the monitoring of several properties simultaneously. In an article by Schöll et al. (2006) various properties were monitored *in situ* in a batch crystalliser during polymorphic transformation of L-glutamic acid including drug concentration (by ATR-FTIR spectroscopy), particle size (by focused beam reflectance measurement), morphology (particle vision measurement image analysis) and polymorphic form (Raman spectroscopy) (Fig. 2). The measured properties were subsequently used to mathematically model the process. It was concluded that the simulation allowed a deeper understanding of the influence of the process parameters. Hu et al. (2005) have used Raman spectroscopy in an intelligent way to determine the concentration of flufenamic acid in the solution as well as its polymorphic form during crystallisation process. The quantitative information on both the liquid and solid state was extracted from the same spectra by using characteristic peaks of different phases of flufenamic acid (forms I and III, and the solute). The authors also stated that Raman spectroscopy can be used to detect nucleation during crystallisation. In another Raman spectroscopic study Hu et al. (2007) investigated the solid phase transformation profiles of flufenamic acid at various temperatures to determine the polymorphic transition temperatures. The new method agreed well with results from the conventional van't Hoff approach and previously published literature. The authors concluded that the method could be used to assess the thermodynamic transition temperature, in particular for rapidly converting systems where it is not possible to measure equilibrium solubility of the metastable form.

Freeze-drying is another complex process where in-line monitoring of the solid form can provide valuable information, since the process involves long periods of vacuum as well as temperature treatment and thus cannot be stopped for sampling or invasive analysis. The polymorphism of mannitol during freeze-drying has been studied with in-line Raman spectroscopy combined with PCA by two research groups. In a study by Romero-Torres et al. (2007) two different fibre optic probes were used to analyse the samples in the freeze-drier through a quartz window in the freeze-drier door. A probe with larger sampling volume was found to produce more consistent results as sub-sampling effects could be minimised. In a study by De Beer et al. (2007) the fibre optic probe was located inside the freeze-drier such that the sample was irradiated and the scattered radiation signal collected from the sample directly to avoid disturbances of other materials such as the freeze-drier door or sample vial. In both studies the polymorphism of mannitol could be analysed and the mechanisms of the phase transformations during freeze-drying elucidated.

Wet granulation is a common pharmaceutical unit operation during which the drugs and excipients are subjected to mechani-

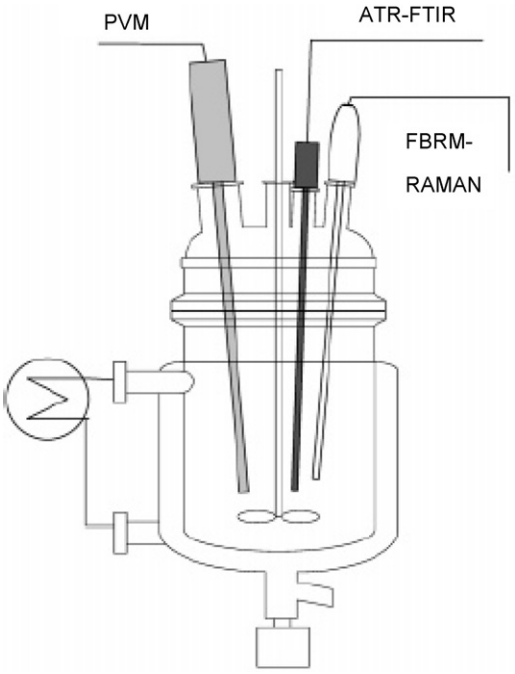

Fig. 2. Schematic of the 2-L batch crystalliser combining the different *in situ* process analytical technologies of FBRM, PVM, and ATR-FTIR and Raman spectroscopy. Reprinted with permission from Schöll et al. (2006). Copyright 2006 American Chemical Society.

cal and thermal stress and influence of solvents. Such conditions are a very probable cause for solid phase transformations. The feasibility of Raman spectroscopy for in-line monitoring of hydrate formation during high-shear wet granulation of theophylline has been evaluated (Wikström et al., 2005). The hydrate formation was quantitatively analysed using characteristic Raman peaks of the anhydrate and monohydrate forms. It was found that the fibre optic probe design and the placement of the probe in the bowl are critical to ensure representative sampling and prevent sticking of the material being granulated to the probe. NIR spectroscopy could not be used here because of the saturation of the signal due to bulk water. Also the drying process-induced dehydration of theophylline monohydrate has been studied with in-line Raman and NIR spectroscopy (Aaltonen et al., 2007). In this study a miniaturised fluidised bed dryer was used and the spectra were collected non-invasively through a quartz window in the drying chamber wall. The solid-state transformation was quantified using the multivariate method PLS regression. It was found that NIR and Raman spectroscopy are a particularly good combination for the analysis of dehydration during the fluidised bed drying process because, roughly said, NIR spectroscopy detects the water removal and Raman spectroscopy the change in the solid-state structure.

4. Selected new spectroscopic techniques to investigate pharmaceutical solids

A number of new techniques have emerged over the last few years that although not yet widely used show promise for the future. In the following section two of these techniques will be discussed in more detail including practical issues in their use. Raman spectroscopy has been successfully applied to many pharmaceutical problems and a number of excellent and recent reviews exist describing this area (Bugay, 2001; Wartewig and Neubert, 2005). Raman spectroscopy has a number of advantages over conventional infrared techniques as water does not show a strong Raman spectrum and thus does not obscure the spectral signatures of analytes of interest in wet matrices, which can be a problem in infrared spectroscopy. The main disadvantages of Raman spectroscopy, the expense and complexity of lasers and detector systems, have been ameliorated with technical advances. However a number of challenging problems still exist in using Raman measurements. One of these is sub-sampling; conventional Raman measurements are made using a focused laser with a beam diameter of 300 μm. Thus in an inhomogeneous sample such as a tablet the spot size may be too small to provide an average signal. This problem was recognised in a number of studies (Taylor and Zografi, 1998; Bugay, 2001; Stephenson et al., 2001; Roberts et al., 2002; Vergote et al., 2002) and is discussed in a study of illicit tablet compacts (Bell et al., 2004). In this study a conventional small spot size sampling arrangement was used (ranging from microscope size, 3 to 150 μm). A grid of up to 64 points over the tablets of interest was sampled. This provided a standard error of prediction of 1.1% for *N*-methyl-3,4-methylenedioxyamphetamine, the API. It was also found that lower density sampling grids gave poorer performance. This issue has been investigated by Johansson et al. (2005) in a study of tablets composed of microcrystalline cellulose and other excipients with a compound of interest. Each 157 mg tablet contained between 45 and 55 mg of API. In this study three distinct laser irradiation geometries were used, these were: (1) point source irradiation, in this geometry approximately 0.4% of the tablet surface area was sampled; (2) circle irradiation—this was achieved by using a small spot but spinning the sample. Using two spot positions it was possible to get a large diameter and smaller diameter circle. These gave area sampling coverages of 16 and 8%, respectively; (3) area sampling in which the spinning tablet was translated—this provided 39% area coverage. By comparing each side of the tablet the sub-sampling error could be evaluated and this was significantly greater for the point source than either the large circle or area illumination.

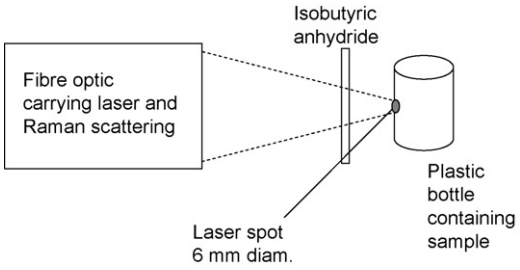

Fig. 3. Schematic of wide area illumination (WAI) experiment. See Kim et al. (2006).

One ingenious method to remove the sub-sampling problem is to use a wide area illumination (WAI) scheme (Fig. 3) (Kim et al., 2006). In these experiments the laser is focused from a fibre optic bundle and has a long focal length (248 mm) and a large spot size (beam diameter 6 mm, area about 28 mm^2). The WAI configuration is much less sensitive to sample position, an important consideration in PAT applications, and inhomogeneity (Kim et al., 2007a, 2007b; Nah et al., 2007). In a study of povidine in low-density polyethylene (LDPE) bottles (Kim et al., 2007c) it was found that the variation between samples when using a standard Raman configuration was almost an order of magnitude worse than using the WAI scheme (standard deviation for repeated measurements being 32.5 and 3.2%, respectively). They also found that they could further improve sample-to-sample variability by inserting a standard in the form of isobutyric anhydride in front of the sample area. This could be used to compensate for laser output/collection optics variability. The solution of these difficulties brings the use of Raman spectroscopy in a PAT environment closer to large-scale application.

A second challenge in using Raman spectroscopy is fluorescence from the sample; although this problem has been reduced with the advent of red excitation sources, such as 785, 830 and 1064 nm, it can still be difficult to measure samples in which the coating is emissive (Romero-Torres et al., 2006). A method that has been developed that completely circumvents the problem of emissive coating is spatially offset Raman spectroscopy. In this experiment the laser is focused on to a particular point on the analyte but the Raman scattering is collected from a different portion of the analyte (spatially offset) (Everall et al., 2004; Johansson et al., 2005; Matousek et al., 2005a,b; Matousek and Parker, 2006; Eliasson and Matousek, 2007a,b; Ricci et al., 2007). The Raman scattering emanating from the sampled portion is of much lower intensity than that from the surface where the laser strikes—but there is no emission from the sampled portion, thus the signal-to-noise with respect to the Raman signature may be better (Fig. 4).

Terahertz spectroscopy has attracted some attention as a tool for the study of pharmaceuticals. The terahertz region of the spectrum lies in the 3–100 cm^{-1} regime. In this regime the absorption phenomena are principally due to intermolecular motion – phonon modes – the transitions associated with intramolecular forces lie at higher energy in the mid-IR region (200–4000 cm^{-1}). The phonon modes are extremely sensitive to the type of forces between molecules and thus terahertz spectroscopy is well-suited to polymorph discrimination.

Although terahertz spectroscopy has been conducted for almost a century (Nichols, 1897; Kimmitt, 2003; Matousek, 2007) technical advances have made it a much more attractive technique in recent years. There are two types of experiments conducted, the first uses a modified FTIR spectrometer in which optics source and

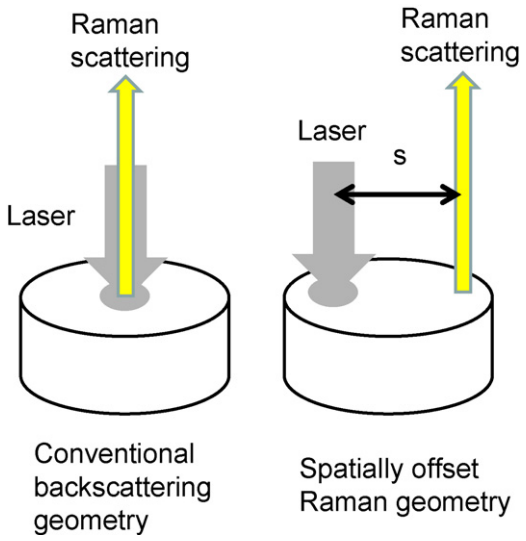

Fig. 4. Schematic of Raman illumination geometries. Adapted from Ricci et al. (2007).

detector are altered to improve terahertz generation, throughput and detection (Esenturk et al., 2007; Plusquellic et al., 2007). An assessment of this methodology has been recently reported with respect to its suitability for process analytical technology (Wu et al., 2007). This study examined tablets made up of theophylline with lactose, corn starch, magnesium stearate and microcrystalline cellulose. The study showed that a number of factors can alter the terahertz spectrum including desirable variations, such as composition, and less desirable variability associated with drying time. This is perhaps unsurprising as water shows very strong terahertz signals (Barnes et al., 1935) and indeed it is possible to quantify moisture content with terahertz spectroscopy (Hadjiloucas et al., 2002). In Wu's study (Wu et al., 2007) a careful analysis of the terahertz spectra revealed that the low wavenumber region (below 80 cm^{-1}) was not sensitive to drying time but above 80 cm^{-1} the spectral behaviour was complex (showing increases and decreases in signal heights with drying time). Using multivariate techniques quantification of API in such formulations was possible even with the complication of drying time. In these studies the spectral region 30–500 cm^{-1} was analysed.

The second method used pulsed laser excitation to create a pulse of terahertz radiation that interacts with the sample of interest. This so-called time-domain spectroscopy differs from that described above, and there are some advantages as the detection of the signal may be modulated with the terahertz pulse thus improving signal-to-noise. The spectral region observed generally lies between 20 and 100 cm^{-1}. This methodology also offers some advantages with respect to the imaging of samples.

Time-domain terahertz spectroscopy has been used to differentiate polymorphs of ranitidine hydrochloride (Taday et al., 2003). The differing crystal structures of each polymorph supported phonon modes at different energies and thus the terahertz spectra were distinct for each. However it has also been demonstrated that binary mixtures of pharmaceuticals may be quantified using terahertz methods (Strachan et al., 2004a, 2005). For example the crystalline forms of carbamazepine (form III in I) and enalapril maleate (form II in I) could be detected to 1.2% (w/w) and 0.7% (w/w), respectively. In these cases distinct spectral bands were observed for each component (Fig. 5). It is also possible to quantify crystalline forms in a predominantly amorphous mixture. This was demonstrated for indomethacin in which the limit of detection for the crystal form was 1% (w/w). In the case of the amorphous form

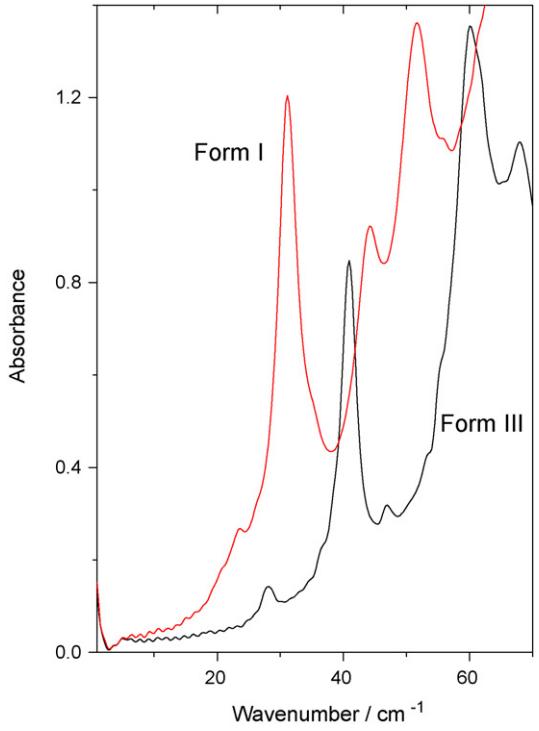

Fig. 5. Terahertz spectra of carbamazepine forms I and III.

there is no distinct terahertz absorption because there is no longer range order; instead the terahertz signal is featureless increasing with wavenumber in a smooth fashion. Nonetheless it was still possible to quantify such a system.

The speed of measurement for terahertz spectra is demonstrated in a kinetics study of the conversion of carbamazepine III to I (Zeitler et al., 2007). Using 30 s integration times kinetic traces were produced showing the conversion from form III to I; importantly it was found that the growth of form I was delayed with respect to the decay of III and this discrepancy was rationalised as involving compound in the gas phase thus providing some evidence for this earlier proposed mechanism (Behme and Brooke, 1991).

One of the exciting possibilities of terahertz time-domain spectroscopy is its potential in imaging. Because the terahertz radiation is produced as an ultra-short light pulse (80 fs) the reflections caused by its propagation through a material inform on the presence of each interface within the material. The thickness of each coating may be determined from the return time and intensity of each reflection if the refractive index of each layer is known. Using this approach a series of multiple layers present in Nurofen™ could be measured (Fitzgerald et al., 2005). The utility of the technique in measuring coating reproducibility, distribution and uniformity has also been demonstrated where coating defects could be observed and characterized (Ho et al., 2007).

5. Computational simulation of vibrational spectra in the pharmaceutical setting

Vibrational spectroscopy is invaluable for studying pharmaceutical systems on the molecular level, because the technique is sensitive to molecular structure, conformation and environment. However, to obtain molecular-level information spectral bands of interest must be assigned to the correct molecular vibrations. This is challenging even for small molecules in the pharmaceutical context. Interpretation can be significantly enhanced by the

computational simulation of vibrational spectra, and in recent years simulations have become increasingly used for this purpose (Meier, 2007). There are multitudinous computational and theoretical challenges associated with modelling of vibrational spectra, but recent advances in computational power and availability, suitable algorithms and user-friendly software have catalysed the use of computational chemistry as an aid to interpret vibrational spectra in the pharmaceutical setting.

The following discussion will briefly introduce approaches used for the simulation of vibrational spectra and then a few examples of predicted mid-infrared (IR), Raman, and terahertz spectra in the pharmaceutical setting. The focus is on applications, and not theory or method development to which the reader is referred to other texts, e.g. Foresman and Frisch (1995–1996), Young (2001).

5.1. Methods to predict vibrational spectra

The prediction of vibrational spectra is always a trade-off between accuracy and computational cost. The most accurate spectral prediction will come from the closest solution to the Schrödinger equation. Quantum mechanics-based calculations aim to do this, but approximations to the Schrödinger equation are always made. For very small single molecules (three or four atoms), comparatively few approximations are required for reasonable computational cost and vibrational spectra can be calculated to within a single wavenumber. However, for larger molecules, e.g. many active pharmaceutical ingredients, more approximations are typically made (including the harmonic approximation) as otherwise the computational cost is prohibitive. Nevertheless, sufficiently accurate vibrational spectra for many situations are still obtained (Young, 2001). Quantum mechanical-based methods may be subdivided into three classes: *ab initio* (e.g. Hartree-Fock), density functional theory (DFT) methods and semi-empirical methods. In the prediction of vibrational spectra of medium sized molecules, the DFT-based approaches have often exhibited the best accuracy at a reasonable computational cost and are thus currently the most popular (Young, 2001).

To obtain a predicted vibrational spectrum using quantum mechanics theory a suitable model chemistry (including basis set) is used to first optimise the molecular geometry (find a minimum energy conformation) and then predict the molecular vibrations. The band frequencies are determined using the gradient of the energy on the potential energy scan (PES) with respect to the vibrations of the molecule. With IR spectroscopy, the band intensities are determined by the change in dipole moment with respect to the vibrational mode. In Raman spectroscopy, the intensity is determined by the change in polarisability with the vibrational mode. For this to be accurately determined the energy of the system must be known with respect to the geometry change and the effect of the external electric field.

Quantum mechanics calculations are normally performed on single molecules (i.e. simulating the gaseous phase) or, perhaps, a small cluster of molecules. Obviously this environment is not representative for molecules in the pharmaceutical setting, where solids and liquids are the norm. If the intermolecular and long-range effects are paramount, much less accurate but less computationally demanding molecular mechanics-based methods using empirically derived force fields may be considered. Commonly, a molecular dynamics approach is used where the period of vibrations in an energy-minimised structure is monitored as a function of time using the empirically derived force fields. The result can then be Fourier-transformed to obtain a vibrational spectrum. Recently, hybrid quantum mechanics/molecular mechanics (QM/MM) methods have been applied to large systems with a core element of interest, e.g. solute molecules in solution. The core element (e.g. solute molecule) is modelled by accurate quantum mechanics-based methods, and the solution environment by less computationally expensive molecular mechanics and dynamics calculations.

All of the approaches mentioned above have been used in the prediction of vibrational spectra of pharmaceutical systems, and some examples will be discussed.

5.2. Computational analysis of mid-infrared and Raman spectra

Mid-IR radiation is resonant with fundamental intramolecular bond stretching and bending. Thus quantum mechanics calculations using the harmonic approximation on single molecules are typically used to predict the vibrational modes in this region. Such methods include Hartree-Fock (HF) theory and, now more commonly, DFT with the B3LYP functional (and a basis set 6-31G(d) or higher) (Foresman and Frisch, 1995–1996). There are many recent examples where IR and/or Raman spectra of drug molecules have been predicted using the isolated molecule and a comprehensive assignment of vibrational modes has been performed, for example, isoquinolone (Krishnakumar and Ramasamy, 2005), thalidomide (Cipriani and Smith, 2008) and non-steroidal anti-inflammatory drugs (Jubert et al., 2005). Despite experimental results being based on solid-state spectra and the calculations being based on the isolated gaseous molecule many band assignments can be made confidently, with the calculations representing a significant step forward over band assignments using group theory alone. Incorrect and controversial band assignments have been corrected using calculated spectra (Nolasco et al., 2006; Wysokinski et al., 2006). The ability of different computational methods and basis sets to predict experimentally observed spectra of pharmaceutical systems have been compared (Rastogi et al., 2000; Krishnakumar and Xavier, 2005; Sagdinc et al., 2007) and for specific kinds of molecules (e.g. those containing a transition metal), carefully chosen computational methods and basis sets may be particularly important (Wysokinski et al., 2006; Amado et al., 2007). In an effort to improve correlation between predicted and experimental spectra, some computationally demanding calculations incorporating anharmonicity have been performed on the small semi-rigid molecules, uracil and 2-thiouracil, using DFT with the B3LYP functional augmented with second-order perturbative theory (Barone et al., 2004). Some significant improvements in predicted frequencies were observed, however such calculations are very expensive and the calculation of anharmonic frequencies (e.g. those in near-infrared spectra) is still generally prohibitive for pharmaceutically relevant molecules (Gerber et al., 2002).

Strong intermolecular interactions, such as hydrogen bonding, can have an effect on intramolecular vibrations. Thus the band assignment of vibrational spectra of such systems may be significantly improved by incorporating such interactions. This was investigated computationally in a DFT study (Strachan et al., 2004b), where the carbamazepine monomer and hydrogen-bonded dimer were used to predict the IR and Raman spectra of the drug in the solid state. In all polymorphs, molecular dimers exist with cyclic hydrogen bonding between the $CONH_2$ moieties. While the experimental Raman spectra, mainly representing vibrations in the ring system, were relatively well predicted by the monomer calculation, the bands in the experimental IR spectra associated with the $CONH_2$ moiety were much better predicted when the hydrogen bonding interaction was considered. In another study, the influence of lattice bound water and intermolecular bonding on vibrational spectra of caffeine, theobromine and theophylline was considered by performing DFT calculations on different molecular dimers, considering each hydrogen bond in turn (Nolasco et al., 2006). This approach allowed a comprehensive assignment on

vibrational modes observed in the anhydrate and hydrate forms of each compound.

Computational prediction of vibrational spectra can be used to better understand intra- and intermolecular structure, including molecular conformation, and intermolecular bonding in solids and solutions. By comparing the vibrational spectra predicted from different conformers, molecular conformers present may be determined. The spectra of the two lowest energy conformations of ketoprofen were predicted, and by comparing the spectra with experimental spectra, it was found that only the lowest energy conformations are present in both the liquid and solid states (Vueba et al., 2006). In another study, prediction of the infrared spectra revealed that two tautomers of 6-thioguanine exist in roughly equal proportions in an isolated hydrophobic environment (Kasende, 2002).

Recently, there has been much effort to use computational chemistry to understand intermolecular interactions in solids and solutions. In solids, this is particularly useful where definitive X-ray structural analysis is not possible. To better understand the short-range order in amorphous indomethacin, the Raman and IR spectra of the indomethacin monomer and dimer, with cyclic hydrogen bonding between COOH moieties, were predicted using DFT and compared to the quench-cooled amorphous and γ-crystalline forms (Fig. 6) (Strachan et al., 2007). In general, the IR spectrum of the amorphous form was better predicted by the dimer calculation, with the main infrared-active mode involving the carboxylic acid moiety in the amorphous form (e.g. at 1716 cm^{-1} in the amorphous form) significantly better predicted by the dimer (1735 cm^{-1}) calculation than the monomer calculation (1791 cm^{-1}). This supports the theory that quench-cooled amorphous indomethacin is likely

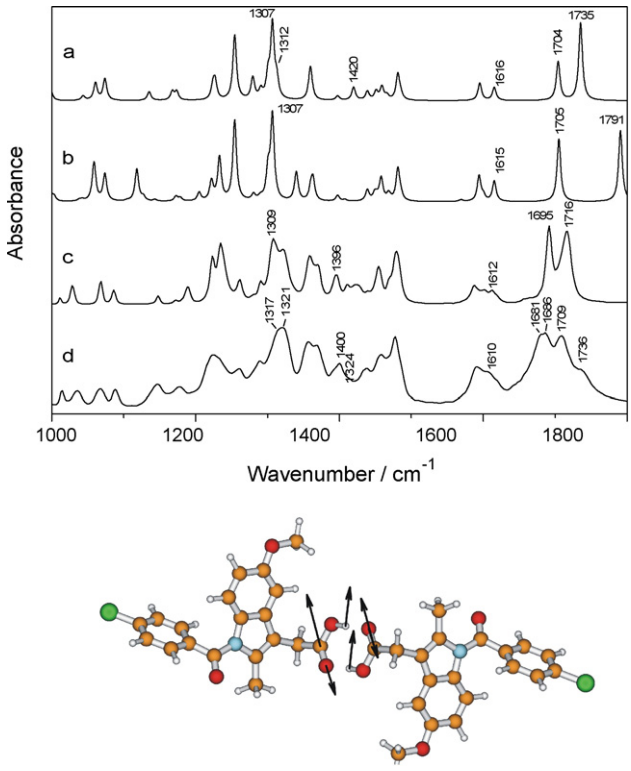

Fig. 6. Infrared spectra (top) of: (a) calculated IM dimer; (b) calculated IM single molecule; (c) experimental crystalline IM; (d) experimental amorphous IM between 1000 and 1800 cm^{-1}, and the predicted normal mode predicted at 1735 cm^{-1} (bottom) for the indomethacin dimer with the largest eigenvectors shown. Calculations were performed using *ab initio* density functional theory calculations. Reprinted with permission from Strachan et al. (2007). Copyright 2007 Pharmaceutical Press.

to exist predominantly as hydrogen bonded dimer motifs present in the γ-crystalline form. The prediction of vibrational spectra has also been used to propose the intermolecular bonding arrangement for caffeine anhydrate (Nolasco et al., 2006).

Drug–ligand interactions have also been studied. In a surface-enhanced Raman spectroscopy (SERS) experiment combined with DFT calculations, the binding of the anti-neoplastic agent cisplatin to the substrate guanine in dilute solution was modelled using DFT calculations (Giese and McNaughton, 2003). The findings supported the previously proposed drug–ligand binding mechanism. Despite some developments required to model the SERS substrate and drug complex interactions, the combined theoretical and experimental approach was suggested as a means to establish the relationship between new platinum complexes and their biological activity.

Simulation of vibrational spectra has also been used to study inclusion complexes. Rossi et al. (2006) have studied the structure of an indomethacin–cyclodextrin inclusion complex using Raman spectroscopy and a combined approach of quantum mechanics and force field molecular dynamics simulations. The HF method was used to simulate the vibrational spectrum of the indomethacin monomer and assign modes to the experimental vibrational spectrum of indomethacin in the γ-crystalline form and in the complex. The interaction between indomethacin and β-cyclodextrin (βCD) in water was then simulated using molecular dynamics calculations with the empirically derived AMBER force field and the density of vibrational states calculated for the resulting complex. From this combined approach, it was concluded that in solution the chlorobenzoyl unit of the indomethacin molecule inserts into the βCD cavity through the larger rim and the amide C=O stretch is affected by the inclusion process.

The solvent effects on the IR and Raman spectra of aspirin, caffeine and ibuprofen in aqueous solution were simulated by considering the long- and short-range solvent effects in DFT calculations (Bondesson et al., 2007). Long-range effects were considered using a polarisation continuum model, and short-range hydrogen bonding was considered by explicitly including water molecules hydrogen bonded to the drug molecules. Consideration of the solvent effects improved the agreement between calculated and experimental spectra, and the approach was proposed to characterise changes in intermolecular bonding during solvation as a means to better understand drug solubility.

5.3. Computational analysis of terahertz spectra

Terahertz spectroscopy has been used for both chemical and physical pharmaceutical analysis, most notably in solid-state analysis. However, the assignment of bands in terahertz spectra is still in its infancy for two main reasons. Firstly, historical neglect of far-IR spectroscopy has meant interpretation of terahertz spectra has been much less studied than that of mid- and even near-IR spectra. Secondly, since terahertz spectra of molecular materials are characterised largely by lower energy intermolecular interactions and, in crystals, phonon lattice modes, interpretation of terahertz spectra of liquids, and especially solids, must consider intermolecular interactions. However, the current interest in terahertz spectroscopy as an analytical technique and the need to understand the spectra has meant that computational modelling of terahertz spectra has recently become a very active area of research.

The first attempts to assign modes to terahertz spectra of molecules were based on the vibrational analysis of single isolated molecule calculations using the harmonic approximation. For example, Chen et al. (2004) used DFT calculations on the single molecule of 2,4-dinitrotoluene. Some inter- and intramolecular bands were suggested by comparing the calculated spectrum with

solid-state and solution spectra. Other examples of spectral interpretation using single molecule *ab initio* calculations exist, even from very recently (Li et al., 2005; Wang et al., 2007), but as discussed below, this approach can no longer be considered an appropriate approach for band assignment in terahertz spectra of solid or liquid systems, since intermolecular interactions are not considered.

Molecular clusters have been used in an attempt to consider some intermolecular interactions. Zeitler et al. (2005) used DFT calculations on the carbamazepine single molecule and hydrogen-bonded dimer structure. Some insight into possible intramolecular vibrations was gained. Fischer et al. (2002) calculated the low frequency modes of thymine in the crystalline state by including the four hydrogen-bonded molecules in the unit cell. By comparing the predicted and experimental spectra, the lowest frequency modes were assigned collectively to out-of-plane and in-plane vibrations of the hydrogen-bonded systems.

Despite some limited insight into vibrational modes with single molecule calculations and molecular clusters, for most crystalline solids periodic boundary conditions must be taken into account so that phonon modes can be considered. Furthermore, the stable molecular conformations due to extensive intermolecular bonding are more likely to be maintained during optimisation of the crystal structure. Periodic boundary conditions have been considered in a number of recently published papers, of which a significant proportion consider molecules of pharmaceutical relevance. In two recent studies, rigid molecule atomistic lattice dynamic calculations were performed on energy-minimised crystal structures (periodic boundary conditions were thus included in the calculations) (Day et al., 2006; Nguyen et al., 2007). In these cases the molecules were considered rigid bodies and thus only intermolecular vibrations were calculated. Day et al. (2006) calculated the terahertz spectra of the four known polymorphs of carbamazepine. Some agreement between the experimental and calculated spectra was obtained. Calculations involving rigid molecule structures may not, however, be appropriate for systems containing flexible molecular structures.

Recently, both intramolecular and intermolecular aspects of vibrations in the terahertz region have been considered (Allis and Korter, 2006; Allis et al., 2006, 2007; Korter et al., 2006; Saito et al., 2006a,b; Jepsen and Clark, 2007). These calculations are very computationally intensive; however, increased computing capabilities now make such calculations feasible. Allis et al. (2007) used DFT calculations on single molecules and crystal structures with periodic boundary conditions to predict the terahertz features of biotin and lactose monohydrate. The solid-state calculations, which incorporated anharmonic terms, suggested that the lowest frequency features were due to intermolecular hindered rotational modes, and not the intramolecular modes predicted by the single molecule calculations. In an impressive study by Jepsen and Clark (2007) density functional perturbative theory was used to predict the terahertz spectra of crystalline sucrose, benzoic acid and thymine (Fig. 7). Very good agreement between the experimental and theoretical results was observed, with the frequency and intensity of most experimental modes well predicted, especially with sucrose. The vibrational modes contained both intramolecular and intermolecular components.

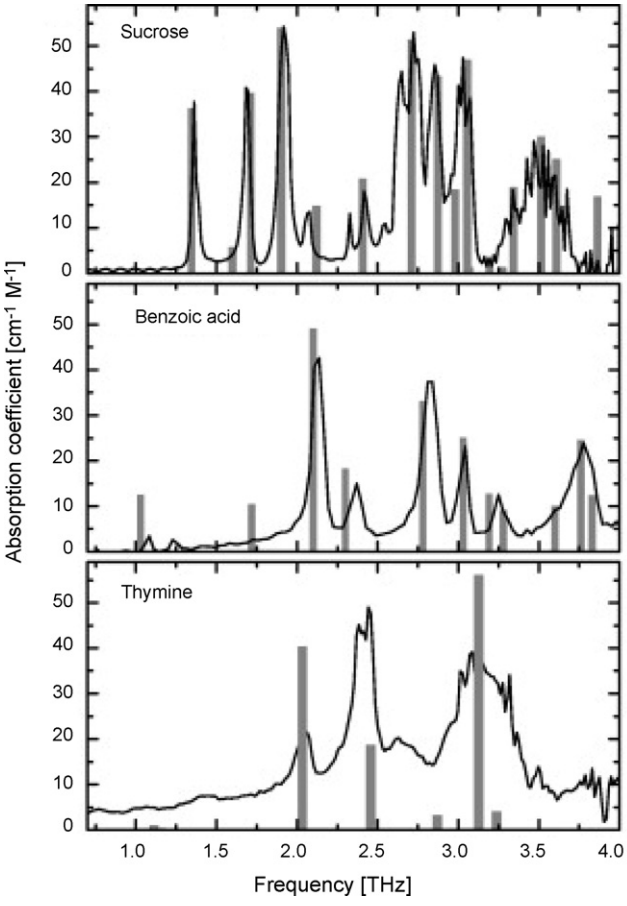

Fig. 7. Experimental terahertz spectra and predicted modes using *ab initio* density functional perturbative theory of the molecular crystals sucrose, benzoic acid, and thymine. Reprinted with permission from Jepsen and Clark (2007). Copyright 2007 Elsevier.

6. Conclusion and outlook

In our view the role of spectroscopy in the quantitative and structural analysis of pharmaceutical solids will further increase in the future. In combination with multivariate analytical tools, application in analysis of the end product and especially in the PAT setting will continue to rise. Novel techniques will give the pharmaceutical scientist new tools to better understand the nature of pharmaceutical solids, and to monitor and quantify changes in the solid state faster and with higher accuracy and precision. Finally, computational techniques will help us to gain a deeper understanding of solids, especially amorphous forms and solid dispersions.

References

Aaltonen, J., Kogermann, K., Strachan, C.J., Rantanen, J., 2007. In-line monitoring of solid-state transitions during fluidisation. Chem. Eng. Sci. 62, 408–415.
Allis, D.G., Korter, T.M., 2006. Theoretical analysis of the terahertz spectrum of the high explosive PETN. Chem. Phys. Chem. 7, 2398–2408.
Allis, D.G., Prokhovora, D.A., Korter, T.M., 2006. Solid-state modeling of the terahertz spectrum of the high explosive HMX. J. Phys. Chem. A 110, 1951–1959.
Allis, D.G., Fedor, A.M., Korter, T.M., Bjarnason, J.E., Brown, E.R., 2007. Assignment of the lowest-lying THz absorption signatures in biotin and lactose monohydrate by solid-state density functional theory. Chem. Phys. Lett., 440.
Amado, A.M., Fiuza, S.M., Marques, M.P.M., Batista de Carvalho, L.A.E., 2007. Conformational and vibrational study of platinum(II) anticancer drugs: *cis*-diamminedichloroplatinum (II) as a case study. J. Chem. Phys. 127, 185104.
Barnes, R.B., Benedict, W.S., Lewis, C.M., 1935. The far infrared spectrum of H_2O. Phys. Rev. 47, 918–921.
Barone, V., Festa, G., Grandi, A., Rega, N., Sanna, N., 2004. Accurate vibrational spectra of large molecules by density functional computations beyond the harmonic approximation: the case of uracil and 2-thiouracil. Chem. Phys. Lett. 388, 279–283.
Bauer, J., Spanton, S., Henry, R., Quick, J., Dziki, W., Porter, W., Morris, J., 2001. Ritonavir: an extraordinary example of conformational polymorphism. Pharm. Res. 18, 859–866.
Beebe, K.R., Pell, R.J., Seaholtz, M.B., 1998. Chemometrics: A Practical Guide. Wiley, New York.

Behme, R.J., Brooke, D., 1991. Heat of fusion measurement of a low melting polymorph of carbamazepine that undergoes multiple-phase changes during differential scanning calorimetry analysis. J. Pharm. Sci. 80, 986–990.
Bell, S.E.J., Beattie, J.R., McGarvey, J.J., Peters, K.L., Sirimuthu, N.M.S., Speers, S.J., 2004. Development of sampling methods for Raman analysis of solid dosage forms of therapeutic and illicit drugs. J. Raman Spectrosc. 35, 409–417.
Bondesson, L., Mikkelsen, K.V., Luo, Y., Garberg, P., Ågren, H., 2007. Hydrogen bonding effects on infrared and Raman spectra of drug molecules. Spectrochim. Acta Part A 66, 213–224.
Bro, R., 2003. Multivariate calibration: what is in chemometrics for the analytical chemist? Anal. Chim. Acta 500, 185–194.
Bugay, D.E., 2001. Characterization of the solid-state: spectroscopic techniques. Adv. Drug Dev. Rev. 48, 43–65.
Chemburkar, S.R., Bauer, J., Deming, K., Spiwek, H., Patel, K., Morris, J., Henry, R., Spanton, S., Dziki, W., Porter, W., Quick, J., Bauer, P., Donaubauer, J., Narayanan, B.A., Soldani, M., Riley, D., McFarland, K., 2000. Dealing with the impact of ritonavir polymorphs on the late stages of bulk drug process development. Org. Proc. Res. Dev. 4, 413–417.
Chen, Y., Liu, H., Deng, Y., Schauki, D., Fitch, M.J., Osiander, R., Dodson, C., Spicer, J.B., Shur, M., Zhang, X.-C., 2004. THz spectroscopic investigation of 2,4-dinitrotoluene. Chem. Phys. Lett. 400, 357–361.
Cipriani, P., Smith, C., 2008. Characterisation of thalidomide using Raman spectroscopy. Spectrochim. Acta Part A 69, 333–337.
Day, G.M., Zeitler, J.A., Jones, W., Rades, T., Taday, P.F., 2006. Understanding the influence of polymorphism on phonon spectra: lattice dynamics calculations and terahertz spectroscopy of carbamazepine. J. Phys. Chem. B 110, 447–456.
De Beer, T.R.M., Alleso, M., Goethals, F., Coppens, A., Van der Heyden, Y., Lopez De Diego, H., Rantanen, J., Verpoort, F., Vervaet, C., Remon, J.P., Baeyens, W.R.G., 2007. Implementation of a process analytical technology system in a freeze-drying process using Raman spectroscopy for in-line process monitoring. Anal. Chem. 79, 7992–8003.
Eliasson, C., Matousek, P., 2007a. Spatial offset broadens applications for Raman spectroscopy. Laser Focus World 43, 123–125.
Eliasson, C., Matousek, P., 2007b. Noninvasive authentication of pharmaceutical products through packaging using spatially offset Raman spectroscopy. Anal. Chem. 79, 1696–1701.
Esenturk, O., Evans, A., Heilweil, E.J., 2007. Terahertz spectroscopy of dicyanobenzenes: anomalous absorption intensities and spectral calculations. Chem. Phys. Lett. 442, 71–77.
Everall, N., Hahn, T., Matousek, P., Parker, A.W., Towrie, M., 2004. Photon migration in Raman spectroscopy. Appl. Spectrosc. 58, 591–597.
FDA, 2004. PAT—A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance.
Fischer, B.M., Walther, M., Uhd Jepsen, P., 2002. Far-infrared vibrational modes of DNA components studied by terahertz time-domain spectroscopy. Phys. Med. Biol. 47, 3807–3814.
Fitzgerald, A.J., Cole, B.E., Taday, P.F., 2005. Nondestructive analysis of tablet coating thicknesses using terahertz pulsed imaging. J. Pharm. Sci. 94, 177–183.
Foresman, J., Frisch, Æ., 1995–1996. Exploring Chemistry with Electronic Structure Methods, 2nd ed. Gaussian, Inc., Pittsburgh.
Gerber, R.B., Brauer, B., Gregurik, S.K., Chaban, G.M., 2002. Calculation of anharmonic vibrational spectroscopy of small biological molecules. PhysChemComm 5, 142–150.
Giese, B., McNaughton, D., 2003. Interaction of anticancer drug cisplatin with guanine: density functional theory and surface-enhanced Raman spectroscopy study. Biopolymers 72, 472–489.
Hadjiloucas, S., Galvao, R.K.H., Bowen, J.W., 2002. Analysis of spectroscopic measurements of leaf water content at terahertz frequencies using linear transforms. J. Opt. Soc. Am. A 19, 2495–2509.
Ho, L., Muller, R., Romer, M., Gordon, K.C., Heinämäki, J., Kleinebudde, P., Pepper, M., Rades, T., Shen, Y.C., Strachan, C.J., Taday, P.F., Zeitler, J.A., 2007. Analysis of sustained-release tablet film coats using terahertz pulsed imaging. J. Control. Release 119, 253–261.
Hu, Y., Liang, J.K., Myerson, A.S., Taylor, L.S., 2005. Crystallization monitoring by Raman spectroscopy: simultaneous measurement of desupersaturation profile and polymorphic form in flufenamic acid systems. Ind. Eng. Chem. Res. 44, 1233–1240.
Hu, Y., Wikström, H., Byrn, S.R., Taylor, L.S., 2007. Estimation of the transition temperature for an enantiotropic polymorphic system from the transformation kinetics monitored using Raman spectroscopy. J. Pharm. Biomed. Anal. 45, 546–551.
Jepsen, P.U., Clark, S.J., 2007. Precise *ab-initio* prediction of terahertz vibrational modes in crystalline systems. Chem. Phys. Lett. 442, 275–280.
Johansson, J., Pettersson, S., Folestad, S., 2005. Characterization of different laser irradiation methods for quantitative Raman tablet assessment. J. Pharm. Biomed. Anal. 39, 510–516.
Jørgensen, A.C., Miroshnyk, I., Karjalainen, M., Jouppila, K., Siiriä, S., Antikainen, O., Rantanen, J., 2006. Multivariate data analysis as a fast tool in evaluation of solid state phenomena. J. Pharm. Sci. 95, 906–916.
Jubert, A., Massa, N.E., Tévez, L.L., Okulik, N.B., 2005. Vibrational and theoretical studies of the non-steroidal anti-inflammatory drugs Niflumic [2-3((trifluoromethyl)phenylamino)-3-pyridinecarboxylic acid]; Diclofenac [[2-(2,6-dichlorpphenyl)amino]-benzeneacetic acid] and Indomethacon acids [1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indole-3-acetic acid]. Vib. spectrosc. 37, 161–178.
Kasende, O.E., 2002. Infrared spectra of 6-thioguanine tautomers. An experimental and theoretical approach. Spectrochim. Acta A 58, 1793–1808.
Kim, M., Chung, H., Woo, Y., Kemper, M., 2006. New reliable Raman collection system using the wide area illumination (WAI) scheme combined with the synchronous intensity correction standard for the analysis of pharmaceutical tablets. Anal. Chim. Acta 579, 209–216.
Kim, D., Chung, H., Kim, N., 2007a. Simple and reliable Raman measurement of an etchant solution directly through Teflon tubing. Appl. Spectrosc. 61, 447–450.
Kim, J., Noh, J., Chung, H., Woo, Y.A., Kemper, M.S., Lee, Y., 2007b. Direct, non-destructive quantitative measurement of an active pharmaceutical ingredient in an intact capsule formulation using Raman spectroscopy. Anal. Chim. Acta 598, 280–285.
Kim, M., Chung, H., Woo, Y., Kemper, M.S., 2007c. A new non-invasive, quantitative Raman technique for the determination of an active ingredient in pharmaceutical liquids by direct measurement through a plastic bottle. Anal. Chim. Acta 587, 200–207.
Kimmitt, M.F., 2003. Restrahlen to T-rays—100 years of terahertz radiation. J. Biol. Phys. 29, 77–85.
Kogermann, K., Aaltonen, J., Strachan, C.J., Pöllänen, K., Veski, P., Heinämäki, J., Yliruusi, J., Rantanen, J., 2007. Qualitative *in situ* analysis of multiple solid-state forms using spectroscopy and partial least squares discriminant modeling. J. Pharm. Sci. 96, 1802–1820.
Kogermann, K., Aaltonen, J., Strachan, C.J., Pöllänen, K., Heinämäki, J., Yliruusi, J. Rantanen, J., 2008. Establishing quantitative in-line analysis of multiple solid-state transformations during dehydration. J. Pharm. Sci., doi:10.1002/jps.21357.
Korter, T.M., Balu, R., Campbell, M.B., Beard, M.C., Gregurick, S.K., Heilweil, E.J., 2006. Terahertz spectroscopy of solid serine and cysteine. Chem. Phys. Lett. 418, 65–70.
Krishnakumar, V., Ramasamy, R., 2005. DFT studies and vibrational spectra of isoquinoline and 8-hydroxyquinoline. Spectrochim. Acta Part A 61, 673–683.
Krishnakumar, V., Xavier, R.J., 2005. Scaled quantum chemical studies of the structure and vibrational spectra of 2,4-diaminoquinazoline and 4-amino-2-methylquinoline. Chem. Phys. 312, 227–240.
Li, N., Shen, J., Sun, J., Liang, L., Xu, X., Lu, M., Jia, Y., 2005. Study on the THz spectrum of methamphetamine. Opt. Express 13, 6750–6755.
Martens, H., Næs, T., 1989. Multivariate Calibration. Wiley, Chichester.
Matousek, P., 2007. Deep non-invasive Raman spectroscopy of living tissue and powders. Chem. Soc. Rev. 36, 1292–1304.
Matousek, P., Parker, A.W., 2006. Bulk Raman analysis of pharmaceutical tablets. Appl. Spectrosc. 60, 1353–1357.
Matousek, P., Clark, I.P., Draper, E.R.C., Morris, M.D., Goodship, A.E., Everall, N., Towrie, M., Finney, W.F., Parker, A.W., 2005a. Subsurface probing in diffusely scattering media using spatially offset Raman spectroscopy. Appl. Spectrosc. 59, 393–400.
Matousek, P., Morris, M.D., Everall, N., Clark, I.P., Towrie, M., Draper, E., Goodship, A., Parker, A.W., 2005b. Numerical simulations of subsurface probing in diffusely scattering media using spatially offset Raman spectroscopy. Appl. Spectrosc. 59, 1485–1492.
Meier, R.J., 2007. Calculating the vibrational spectra of molecules: an introduction for experimentalists with contemporary examples. Vib. Spectrosc. 43, 26–37.
Nah, S., Kim, D., Chung, H., Han, S.H., Yoon, M.Y., 2007. A new quantitative Raman measurement scheme using Teflon as a novel intensity correction standard as well as the sample container. J. Raman Spectrosc. 38, 475–482.
Nguyen, K.L., Frišcic, T., Day, G.M., Gladden, L.F., Jones, W., 2007. Terahertz time-domain spectroscopy and the quantitative monitoring of mechanochemical cocrsytal formation. Nat. Mater. 6, 206–209.
Nichols, E.F., 1897. A method for energy measurements in the infrared spectrum and the properties of the ordinary ray in quartz for waves of great wavelength. Phys. Rev. 4, 297–313.
Nolasco, M.M., Amado, A.M., Ribeiro-Claro, P.J.A., 2006. Computationally-assisted approach to the vibrational spectra of molecular crystals: study of hydrogen-bonding and pseudo-polymorphism. Chem. Phys. Chem. 7, 2150–2161.
Plusquellic, D.F., Siegrist, K., Heilweil, E.J., Esenturk, M., 2007. Applications of terahertz spectroscopy in biosystems. Chem. Phys. Chem. 8, 2412–2431.
Rantanen, J., 2007. Process analytical applications of Raman spectroscopy. J. Pharm. Pharmacol. 59, 171–177.
Räsänen, E., Sandler, N., 2007. Near infrared spectroscopy in the development of solid dosage forms. J. Pharm. Pharmacol. 59, 147–159.
Rastogi, V.K., Jain, V., Yadav, R.A., Singh, C.D., Palafox, M.A., 2000. Fourier transform Raman spectrum and *ab initio* and density functional computations of the vibrational spectrum, molecular geometry, atomic charges and some molecular properties of the anticarcinogenic drug 5-fluorouracil. J. Raman Spectrosc. 31, 595–603.
Ricci, C., Eliasson, C., Macleod, N.A., Newton, P.N., Matousek, P., Kazarian, S.G., 2007. Characterization of genuine and fake artesunate anti-malarial tablets using Fourier transform infrared imaging and spatially offset Raman spectroscopy through blister packs. Anal. Bioanal. Chem. 389, 1525–1532.
Roberts, S.N.C., Williams, A.C., Grimsey, I.M., Booth, S.W., 2002. Quantitative analysis of mannitol polymorphs. FT-Raman spectroscopy. J. Pharm. Biomed. Anal. 28, 1135–1147.
Romero-Torres, S., Perez-Ramos, J.D., Morris, K.R., Grant, E.R., 2006. Raman spectroscopy for tablet coating thickness quantification and coating characterization in the presence of strong fluorescent interference. J. Pharm. Biomed. Anal. 41, 811–819.
Romero-Torres, S., Wikström, H., Grant, E.R., Taylor, L.S., 2007. Monitoring of mannitol phase behaviour during freeze-drying using non-invasive Raman spectroscopy. PDA J. Pharm. Sci. Technol. 61, 131–145.

Rossi, B., Verrocchio, P., Viliani, G., Scarduelli, G., Guella, G., Mancini, I., 2006. Vibrational properties of inclusion complexes: the case of indomethacin–cyclodextrin. J. Chem. Phys., 125.
Sagdinc, S., Kandemirli, F., Bayari, S.H., 2007. Ab initio and density functional computations of the vibrational spectrum, molecular geometry and some molecular properties of the antidepressant drug sertraline (Zoloft) hydrochloride. Spectrochim. Acta A 66, 405–412.
Saito, S., Inerbaev, T.M., Mizuseki, H., Igarashi, N., Kawazoe, Y., 2006a. Terahertz vibrational modes of crystalline salicylic acid by numerical model using periodic density functional theory. Jpn. J. Appl. Phys. 45, 4170–4175.
Saito, S., Inerbaev, T.M., Mizuseki, H., Igarashi, N., Ryunosuke, N., Yoshiyuki, K., 2006b. First principles calculation of terahertz vibrational modes of a disaccharaide monohydrate crystal of lactose. Jpn. J. Appl. Phys. 45, L1156–L1158.
Schöll, J., Bonalumi, D., Vicum, L., Mazzotti, M., Muller, M., 2006. In situ monitoring and modeling of the solvent-mediated polymorphic transformation of L-glutamic acid. Cryst. Growth Des. 6, 881–891.
Smith, E., Dent, G., 2005. Modern Raman Spectroscopy: A Practical Approach. Wiley, New York.
Stephenson, G.A., Forbes, R.A., Reutzel-Edens, S.M., 2001. Characterization of the solid state: quantitative issues. Adv. Drug Dev. Rev. 48, 67–90.
Strachan, C.J., Rades, T., Newnham, D.A., Gordon, K.C., Pepper, M., Taday, P.F., 2004a. Using terahertz pulsed spectroscopy to study crystallinity of pharmaceutical materials. Chem. Phys. Lett. 390, 20–24.
Strachan, C.J., Howell, S.L., Rades, T., Gordon, K.C., 2004b. A theoretical and spectroscopic study of carbamazepine polymorphs. J. Raman Spectrosc. 35, 401–408.
Strachan, C.J., Taday, P.F., Newnham, D.A., Gordon, K.C., Zeitler, J.A., Pepper, M., Rades, T., 2005. Using terahertz pulsed spectroscopy to quantify pharmaceutical polymorphism and crystallinity. J. Pharm. Sci. 94, 837–846.
Strachan, C.J., Rades, T., Gordon, K.C., 2007. A theoretical and spectroscopic study of γ-crystalline and amorphous indomethacin. J. Pharm. Pharmacol. 59, 261–269.
Taday, P.F., Bradley, I.V., Arnone, D.D., Pepper, M., 2003. Using terahertz pulse spectroscopy to study the crystalline structure of a drug: a case study of the polymorphs of ranitidine hydrochloride. J. Pharm. Sci. 92, 831–838.
Taylor, L.S., Zografi, G., 1998. The quantitative analysis of crystallinity using FT-Raman spectroscopy. Pharm. Res. 15, 755–761.
Vergote, G.J., Vervaet, C., Remon, J.P., Haemers, T., Verpoort, F., 2002. Near-infrared FT-Raman spectroscopy as a rapid analytical tool for the determination of diltiazem hydrochloride in tablets. Eur. J. Pharm. Sci. 16, 63–67.
Vueba, M.L., Pina, M.E., Veiga, F., Sousa, J.J., De Carvalho, L.A.E.B., 2006. Conformational study of ketoprofen by combined DFT calculations and Raman spectroscopy. Int. J. Pharm. 307, 56–65.
Wang, G., Shen, J., Jia, Y., 2007. Vibrational spectra of ketamine hydrochloride and 3,4-methylenedioxymethamphetamine in terahertz range. J. Appl. Phys. 102, 013106.
Wartewig, S., Neubert, R.H.H., 2005. Pharmaceutical applications of mid-IR and Raman spectroscopy. Adv. Drug Dev. Rev. 57, 1144–1170.
Wikström, H., Marsac, P.J., Taylor, L.S., 2005. In-line monitoring of hydrate formation during wet granulation using Raman spectroscopy. J. Pharm. Sci. 94, 209–219.
Wold, S., Esbensen, K., Geladi, P., 1987. Principal component analysis. Chem. Int. Lab. Syst. 2, 37–52.
Wold, S., Sjöström, M., Eriksson, L., 2001. PLS-regression: a basic tool of chemometrics. Chem. Int. Lab. Syst. 58, 109–130.
Wu, H., Heilweil, E.J., Hussain, A.S., Khan, M.A., 2007. Process analytical technology (PAT): effects of instrumental and compositional variables on terahertz spectral data quality to characterize pharmaceutical materials and tablets. Int. J. Pharm. 343, 148–158.
Wysokinski, R., Kuduk-Jaworska, J., Michalska, D., 2006. Electronic structure, Raman and infrared spectra, and vibrational assignment of carboplatin. Density functional theory studies. J. Mol. Struct. Theochem. 758, 169–179.
Young, D.C., 2001. Computational Chemistry: A Practical Guide for Applying Techniques to Real World Problems, 1st ed. John Wiley & Sons, Inc., New York.
Zeitler, J., Newnham, D., Taday, P., Strachan, C., Pepper, M., Gordon, K., Rades, T., 2005. Temperature dependent terahertz pulsed spectroscopy of carbamazepine. Thermochim. Acta 436, 71–77.
Zeitler, J.A., Taday, P.F., Gordon, K.C., Pepper, M., Rades, T., 2007. Solid-state transition mechanism in carbamazepine polymorphs by time-resolved terahertz spectroscopy. Chemphyschem 8, 1924–1927.
Zhou, G.X., Ge, Z., Dorwart, J., Izzo, B., Kukura, J., Bicker, G., Wyvratt, J., 2003. Determination and differentiation of surface and bound water in drug substances by near infrared spectroscopy. J. Pharm. Sci. 92, 1058–1065.