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Application of mid-IR spectroscopy for the characterization of pharmaceutical systems

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ABSTRACT

In this article, the use of mid-IR in pharmaceutical settings is reviewed. An overview of mid-IR instrumentation and sampling techniques is provided. Subsequently, different pharmaceutical applications of the technique are described, including structure elucidation and identification, characterization of crystalline (polymorphs, hydrates, salts and co-crystals) and amorphous forms, as well as quantitative and remote sensing applications. Finally, an overview of current trends in FTIR-based imaging applications is provided, with special attention paid to currently emerging FTIR–AFM coupled techniques which have high spatial resolution.

1. Introduction

Mid-infrared (mid-IR) radiation covers light in the spectral region of 4000–400 cm^{-1}, corresponding to wavelengths between 2500 and 25,000 nm. The radiation covers energies between 4.8 (400 cm^{-1}) and 48 kJ/mol photons (4000 cm^{-1}), which are too low to cause electron transitions in molecules. Rather, vibrational transitions result from the absorption of mid-IR radiation, hence mid-IR spectroscopy belongs to the broader field of *vibrational spectroscopy*. These transitions occur at distinct wavelengths that depend on the molecular structure of the compound as well as on the local environment surrounding the molecules. As such, mid-IR spectral analysis can be used to provide information on molecular structure as well as to gain insight into a molecule's local environment, which will be affected by factors such as polymorphic form. Furthermore, as mid-IR absorption obeys a law similar to the Beer–Lambert law well known from UV–vis spectroscopy, quantitative information can be derived using mid-IR spectroscopy.

Mid-infrared (mid-IR) spectroscopy has been and continues to be intensively applied in a pharmaceutical setting. Several excellent reviews have been published, typically describing the use of mid-IR as one of several characterization techniques for pharmaceutical systems (e.g. Aaltonen et al., 2008; Brittain, 1997; Bugay, 2001; Gendrin et al., 2008; Heinz et al., 2009; Jørgensen et al., 2009; Stephenson et al., 2001; Wartewig and Neubert, 2005). This review is devoted solely to the application of mid-IR spectroscopy. First, a brief overview is provided of the most popular instrumentation, sampling accessories, and sample methodologies used for mid-IR spectroscopy. Subsequently, qualitative pharmaceutical applications of the technique are discussed, with special attention paid to the use of the technique for the characterization of different solid state forms. Next, the use of mid-IR in pharmaceutical settings for quantitative analysis and remote sensing applications are discussed. Finally, recent developments in the field of mid-IR imaging are discussed.

2. Instrumentation and sampling methodologies

Dispersive spectrometers were introduced in the mid-1940s and consist of three basic components: a radiation source, a monochromator, and a detector. The radiation source is typically an inert solid, heated electrically to 1000–1800 °C, producing a specific continuous radiation profile. The broad spectrum of radiation is dispersed by the monochromator by either prisms or gratings. Detectors either measure the heating effects produced by IR radiation (thermal detectors) or the current or voltage resulting from the interaction of IR radiation with a semiconducting material (photon detectors).

The introduction of Fourier transform infrared (FTIR) instrumentation has truly revolutionized the field of mid-IR spectroscopy, and has currently almost entirely replaced the used of dispersive instrumentation. While the IR sources used in these instruments are similar to those of dispersive instruments, the approach taken to differentiate and measure the absorption at the differ-

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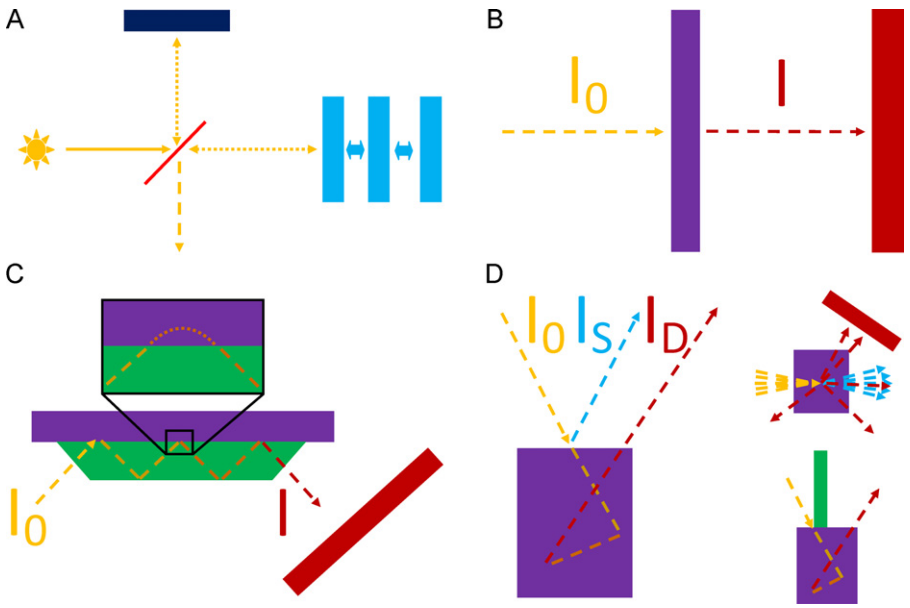

Fig. 1. A Schematic of a Michelson interferometer: radiation from the source (sun, orange) impinges on the beam splitter (red), which splits the radiation (dotted lines). Half of the radiation is directed to and reflected by the fixed mirror (dark blue), the other half to the moving mirror (light blue). Upon recombination of both beams at the beam splitter, the radiation directed towards the sample (dashed line). Due to changes in the relative position of the moving mirror, an interference pattern is generated. B Schematic of a transmission setup. The incident radiation with intensity I_0 (dashed line, orange) travels through the sample (purple) and absorption occurs. The intensity I of the outgoing radiation (dashed line, red) is recorded by the detector (red). C Schematic of an attenuated total reflectance (ATR) setup (multiple reflections). The incident radiation with intensity I_0 (dashed line, orange) travels through the ATR crystal (green). Absorption of the evanescent wave (inset, dotted line, orange) occurs in the sample (purple). The intensity I of the outgoing radiation (dashed line, red) is recorded by the detector (red). D Simplified schematic of the diffuse reflectance infrared Fourier transform spectroscopy technique (DRIFTS). Left: specular reflectance, directly reflected off the surface with an angle of reflectance equal to that of incidence (I_S, dashed line, blue), and diffuse reflectance, which penetrates into the sample and then scatters in all directions (I_D, dashed line, red). Right: minimization of the specular reflectance component by geometrical discrimination against specularly reflected radiation (upper right) or physically blocking of the specular component with an opaque shield (green), touching the sample (lower right).(For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

ent component frequencies varies considerably. For instance, the monochromator is replaced by an interferometer, with the Michelson interferometer being the most frequently used for this purpose.

A classic two-beam Michelson interferometer is depicted in Fig. 1A. The device consists of three main components: a moving mirror and a fixed mirror, placed perpendicular to each other, and a beam splitter. The beam splitter is a semi-reflecting device, often made by depositing a thin film of germanium onto a flat KBr substrate. As the radiation emitted by the IR source impinges on the beam splitter, half of the radiation is directed to the fixed mirror, while the other half travels to the moving mirror. After reflection by the mirrors, both spatially coherent beams are recombined at the beam splitter and, due to changes in the relative position of the moving mirror, an interference pattern is generated. The resulting beam then interacts with the sample resulting in absorption, and is eventually focused on the detector. The resulting interferogram is a complex summation of the superimposed sinusoidal waves to which the detector is responsive, with each wave corresponding to a single frequency. The mathematical operation of converting the interferogram (an intensity profile expressed in a positional domain) into a spectrum (an intensity profile expressed in its inverse domain, i.e. frequency) is termed Fourier transformation, hence the name Fourier transform infrared spectroscopy. Deuterated trigycine sulfate (DTGS) or mercury cadmium telluride (MCT) detectors are typically used in FTIR instruments, as detectors used in dispersive instruments are generally too slow for the rapid scan times applied (1 s or less). The DTGS detector is a pyroelectric detector that delivers rapid responses because it measures the change in temperature rather than the value of temperature. The MCT detector is a photon detector that depends on the quantum nature of the radiation and like the DTGS detector, also exhibits very fast responses. Although the MCT detector is faster and more sensitive compared to the DTGS detector, the fact that it must be maintained at liquid nitrogen temperature to be effective makes the room temperature operating DTGS detector a more convenient option. A number of distinct advantages of FTIR instrumentation over dispersive spectrometers can be noted, the most important being (i) higher speed of measurements (1 s versus 10–15 min for a similar S/N ratio), (ii) increased optical throughput (no energy wasting slits are needed in the interferometer as no dispersion or filtering is needed), (iii) the use of an internal laser reference providing automatic calibration with high accuracy (eliminating the need for external calibration), (iv) a simpler mechanical design (moving mirror) resulting in less wear and better reliability, and (v) elimination of stray light and emission contributions (as the interferometer modulates all frequencies).

Some major advantages of mid-IR spectroscopy are that samples can be measured in different states (liquid, solid, gas), and measurements can be conducted either in transmission or reflectance mode. A simplified scheme illustrating a transmission experiment can be found in Fig. 1B. As many materials strongly absorb IR radiation, dilution and dispersion of the sample is often needed prior to measurement in transmission mode. Alternatively, one can prepare samples as thin films, e.g. by spin coating on an IR transparent substrate, which is an under-utilized preparation technique for pharmaceutical samples. For example, this method has been found to be extremely useful to study amorphous solid dispersions (Konno and Taylor, 2006). For dilute solutions of solids and liquids in IR transparent solvents, liquid cells can be used for transmission measurements. Solvent selection, however, should be based on the spectral area of interest, as no single solvent is transparent over the entire mid-IR region. In addition, it is important to note that solute–solvent interactions can have an effect on the resulting spectrum since they can, for example, hydrogen bond with the solute. In addition to gathering information about a “simple” solution spectrum of a given compound or mixture, solution IR spectroscopy

has been used to probe the association of molecules as a function of solvent and concentration e.g. to better understand crystallization behavior (Boyd et al., 2010; Towler and Taylor, 2007). Solution IR studies of solutes have also been used to provide fundamental information about the hydrogen bonding of various functional groups (Laurence et al., 2009). Polar solvents such as water and alcohols are seldom used because their absorption in the mid-IR region is strong. For situations where an aqueous environment is a necessity [e.g. analysis of proteins (Dong and Caughey, 1994)], thin cells of water-insoluble materials such as BaF_2, AgCl or KRS-5 (a thallium bromide–thallium iodide mixture) can be used for transmission measurements. Typical solution concentrations for measurements in liquid cells are 0.5–10%, with a sample chamber thickness of 0.1–1 mm. For more concentrated solutions, attenuated total reflectance accessories (discussed below), can be used.

For the dilution of solids, alkali halides are often used (typically potassium bromide). Here, the finely ground sample is intimately mixed with the ground alkali halide at a concentration of 0.5–1 wt% (0.5–1 mg in 100 mg) e.g. using a mortar and pestle. The powder mixture is then pressed into a transparent disk or pellet using an appropriate press. It is essential that particles are ground to sizes smaller than the shortest wavelength used (2.5 μm) to minimize band distortion due to particulate scattering effects. In addition, great care should be taken to ensure dryness of the alkali halide, as moisture present will result in the appearance of additional absorption bands. Furthermore, water can result in hydrate formation and/or hydrolysis of the sample (Baker, 1957). It should also be noted that the grinding and the application of stress during sample compression can induce solid state transformations such as polymorphic transitions and crystallization of amorphous phases (Cleverley and Williams, 1959; Mutha and Ludemann, 1976). In rare cases, reaction with the halide material can also occur, e.g. ion exchange when an ionic material is blended with the diluting halide. For these reasons, sampling accessories where less material manipulation is required have become popular for pharmaceutical samples, which are discussed in more detail below. One less common alternative to alkali halide pellets is the use of mulls (e.g. mineral oil, Nujol, Fluorolube or hexachlorobutadiene). Here, the sample (1–5 mg) is ground with 1–2 drops of a mulling agent to form a viscous two-phase mixture, and is subsequently pressed between two IR-transparent plates to form a thin film. Again, care should be taken that the particle size of the analyte are smaller than the shortest wavelength used, to minimize band distortions due to scattering.

For gases and low-boiling point liquids, gas cells can be used. As the concentration of these samples is typically much lower compared to condensed samples, longer path lengths are needed, which can be accomplished by repeatedly reflecting the IR beam through the use of internal mirrors. Generally, a good spectrum can be obtained at a partial pressure of 50 torr in a 10 cm cell. The most common application of gas analysis in pharmaceutical development is where FTIR analysis of evolved gases has been used in conjunction with thermogravimetric analysis to determine the identity of solvents evolved from heating powdered samples (Rodriguez and Bugay, 1997). In addition to the aforementioned sampling setups, a range of microsampling accessories have been designed for the evaluation of microquantities of samples.

As an alternative to measurements in transmission mode, reflection measurements can be performed. One important reflection technique which is very useful for the analysis of solid pharmaceutical samples (although it is widely used for liquids and semisolids as well) is attenuated total reflection (ATR, see Fig. 1C). ATR occurs when a beam of radiation enters from a medium with a high index of refraction (the ATR crystal, made of e.g. zinc selenide, diamond, silicon, germanium) into a medium with lower index of refraction (in practice, the sample). When the angle of incidence increases, the fraction of the incident beam reflected increases, and above a critical angle that is a function of refractive index, all incident radiation is completely reflected at the interface. However, although complete reflection occurs at the interface, the beam actually penetrates a very short distance (typically a depth of a few μm) beyond the interface into the low refractive index medium prior to complete refraction. This penetrating wave is termed the evanescent wave. In regions of the spectrum where the low refracting index medium absorbs, the intensity of the beam will be reduced or attenuated. When the low refractive index medium consists of the sample of interest, the attenuation observed will be characteristic of the absorptive properties of the sample, resulting in a spectrum similar (although not identical as band intensities can differ) to that measured in transmission mode. As the beam can be reflected in the ATR crystal with either a single reflection or multiple reflections, different designs of ATR accessories will have different sensitivities. A distinct advantage of ATR compared to transmission measurements is that minimal or no sample preparation is needed; however, an intimate contact between sample and the ATR crystal is crucial. While easily obtained in the liquid state, this is typically accomplished for solids by applying pressure on the sample. ATR is a very valuable alternative for samples that cannot be readily examined by transmission measurements in normal transmission mode, such as highly absorbing and/or thick solids and liquids. As such, it is an excellent method for the rapid analysis of pharmaceutical powders (Silva et al., 2009), tablets (Matero et al., 2007; Msimanga and Ollis, 2010) as well as the rapid discrimination of polymorphs (McArdle et al., 2005). Heated ATR units are also available, and hence variable temperature measurements can be easily performed (Peresypkin et al., 2008; Qi et al., 2010; Tang et al., 2002a). Since the main requirement for obtaining a spectrum using an ATR accessory is a good extent of contact between the sample and the ATR crystal, the technique can be used for powders, tablets, as well as liquids. For example, Matero et al. (2007) used ATR-FTIR for the quantitation and detection of drug and excipient distribution during dissolution in starch acetate matrix tablets containing riboflavin sodium phosphate. Furthermore, since the depth of penetration into a sample is dependent on the refractive index of the ATR crystal used as well as the angle of the incident radiation, ATR can be also used for depth profiling (Harrick, 1987). In other words, information about surface versus sub surface composition can be obtained. Some important factors to bear in mind when using ATR spectroscopy are that the technique is surface sensitive (as mentioned above) thus the spectrum obtained may not be representative of the bulk composition, as well as the fact that the depth of penetration (and hence absorption) of the radiation is wavelength dependent. Software programs are available to correct the ATR spectrum for wavelength dependent effects. More information regarding specific applications of ATR spectroscopy is also provided below from the perspective of imaging applications.

A second although more complex type of measurement conducted in reflection mode is diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS, see Fig. 1D), which can be used to analyze rough surface solids and powdered samples. IR radiation, focused onto the surface of a solid sample in a cup results in two types of reflection: (i) specular reflectance, directly reflected off the surface with an angle of reflectance equal to that of incidence, and, (ii) diffuse reflectance, which penetrates into the sample and then scatters in all directions. To minimize the specular reflectance component, one can geometrically discriminate against the specularly reflected radiation or physically block the specular component. Typically, the obtained data needs to be further transformed, often using the Kubelka–Munk theory, prior to further interpretation. A serious drawback of this technique is the strong particle size dependence (Fuller and Griffiths, 1978). An example of the use of DRIFTS can be found in Morris et al. (1994). Here, variable temperature

and humidity DRIFTS accessories were used to study solid state properties as a function of environment.

3. Pharmaceutical applications

3.1. Structural elucidation and compound identification

Many functional groups result in characteristic mid-IR absorption at specific narrow frequency ranges. Numerous tabulated overviews of these characteristic absorption bands have been compiled [e.g. Lin-Vien et al. (1991) and Silverstein et al. (1981)], which can be used to aid in the structural elucidation of compounds. For example, an IR spectrum of a new chemical entity is commonly included in the Chemistry, Manufacturing and Control (CMC) information to provide support for the identity of the compound when submitting an Investigational New Drug application (IND) to the Food and Drug Administration. For systemic evaluation, the mid-IR spectrum is typically split into three regions. Although an in-depth discussion of these regions is beyond the scope of this review, a brief overview follows. The first region is the functional group region, 4000–1300 cm^{-1}. Strong absorption bands in the region 4000–2500 cm^{-1} typically result from stretching vibrations between hydrogen atoms and atoms with a mass of 19 or less. O–H and N–H frequencies can be found in the 3700–2500 cm^{-1} region. Hydrogen bonding significantly influences peak shapes and intensities, generally resulting in peak broadening and peak shifts to lower frequencies (higher wavelengths). C–H stretching vibrations can be found between 3300 and 2800 cm^{-1}, while stretching vibrations of triple bonds and allenes (C=C=C) are located between 2700 and 1850 cm^{-1}. Finally, stretching bands of double-bonded functional groups can be found between 1950 and 1450 cm^{-1}. The second region, termed the 'fingerprint' region, spans 1300–910 cm^{-1}. Absorption in this region includes contributions from complex interacting vibrations, resulting in a unique 'fingerprint' of the molecule. Apart from these unique contributions, characteristic absorptions found in this region can also help to elucidate molecular structure. The aromatic region (910–650 cm^{-1}) is the third and last region in the mid-IR spectrum, whereby no strong bands in this region usually points to the absence of aromatic character. One interesting application of IR spectroscopy in the area of structure determination is establishing the predominant tautomeric form in a given phase of the compound. An example thereof can be found in İde and Topaçli (1997). Based on the observation of the absorption band due to the stretching vibration of the C=O group, the authors concluded that chlorzoxazone has the amino tautomeric form in the crystalline state. Another application is to determine if salt formation has occurred, an example of which can be found in Romañuk et al. (2010). Mid-IR spectroscopy is a powerful technique for structural elucidation, but is typically complemented by other techniques providing additional insight in the molecular structure, including nuclear magnetic resonance (NMR), mass spectrometry and elemental analysis.

A second important application is compound identification, which stems from the unique character of the infrared spectrum of a given substance. For unknown or ambiguous samples, a mid-IR spectrum can be obtained and then compared to either a spectral library or a particular reference spectrum. Automated spectral matching software is available which provides a percentage match to a given reference spectrum. An excellent review on the use of IR microspectroscoy for forensic analysis of pharmaceuticals has been written by Aldrich and Smith (Aldrich and Smith, 1999). A specific example of the use of DRIFTS for identification purposes can be found in Ryan et al. (1991). Here, the technique was used to identify and verify the content of solid dosage forms using spectroscopic matching software. A study where ATR-FTIR in conjunction with multivariate analysis was used to differentiate between different Tylenol brands can be found in Msimanga and Ollis (2010). Again, complementary techniques should be ideally performed for a complete verification of the identity of an unknown substance.

3.2. Solid state characterization and quantification

Apart from information purely relating to the molecular structure of the compound, as discussed above, the mid-IR spectrum of a compound is also influenced by its local environment, in particular by the formation of intermolecular interactions or molecular conformation. This is of major importance for solid state characterization, as different solid state forms will often result in differences in the local environment and/or conformation of the constituent molecules. However, it is important to note that for some polymorphic systems, spectroscopic differences may not be present between the different forms. For example, Burger and Koller (1999) observed no difference in the IR spectra of three polymorphs of tiaprofenic acid. In what follows, the utility of IR spectroscopy for solid state characterization will be discussed, using relevant published examples.

3.2.1. Characterization of crystalline forms

Mid-IR spectroscopy has been widely used for the characterization of and discrimination between crystal forms including polymorphs, and solvates. When analyzing spectra obtained from crystals, several potential effects must be considered when interpreting the data. For instance, if the unit cell contains more than one molecule which are conformationally different, splitting of the fundamental vibrations can occur. Hence, if a unit cell contains two crystallographically independent molecules with different geometry, each fundamental peak can theoretically split into two peaks. In practice, however, not all of the peaks may be IR active and some may be degenerate (energetically equivalent). For a given functional group, more than one peak can be also seen if for example, a portion of the groups is hydrogen bonded, whereas another fraction is free or hydrogen bonded to a different extent. In general, it is important to recall that the molecules in a crystal lattice are influenced by the molecules around them, both in terms of the intermolecular interactions formed as well as more complex coupling effects. This means that the IR spectrum of a crystalline material contains a wealth of information, only some of which is readily interpretable. Indomethacin provides a good example of the complex IR spectrum of a crystalline material. In Fig. 2, the molecular structure of indomethacin, a crystallographic packing diagram showing the hydrogen bonding interactions that can be found in the α form, and the IR spectrum of the carbonyl region of the same polymorph are shown. Based on the structure of indomethacin, it would be expected that the gas phase spectrum would have two peaks in the carbonyl region, one arising from the asymmetric stretch of carbonyl group of the carboxylic acid, the other from the benzoyl carbonyl. The IR spectrum of the crystalline α polymorph has at least four discernible peaks. By referring to the hydrogen bond patterns of the crystal, as well as the position of the peaks in other forms, these peaks have been assigned as follows (Towler and Taylor, 2007): (a) the highest wavenumber carbonyl peak at 1734 cm^{-1} arises from the carboxylic acid carbonyl that does not form a hydrogen bond. (b) The next peak at 1692 cm^{-1} is most likely due to the asymmetric stretching mode of the carboxylic acid carbonyl that forms a dimer with another carboxylic acid function. (c) The peak at 1680 cm^{-1} is assigned to the non-hydrogen bonded benzoyl carbonyl. Finally, the peak at 1648 cm^{-1} is due to the benzoyl carbonyl that is hydrogen bonded to the carboxylic acid of an adjacent molecule. Thus the complex carbonyl region of the mid-IR spec-

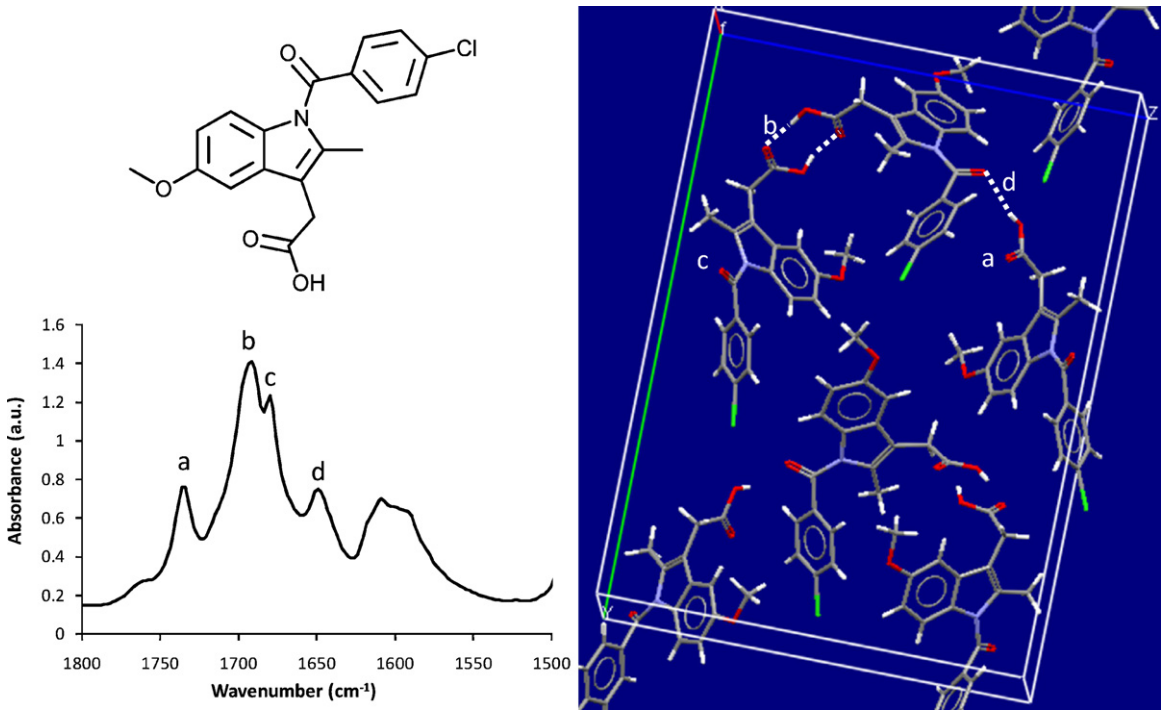

Fig. 2. Molecular structure of indomethacin, infrared spectrum of the carbonyl region of the α form of indomethacin and crystallographic packing diagram of the α polymorph. See text for further detail on the annotations in the crystallographic packing diagram.

trum for α indomethacin can be interpreted based on its crystal structure.

The relationship between the peak position of a hydrogen bonding group, such as an NH or OH function, and the strength of the hydrogen bonding interaction has been extensively explored in crystals. Typically this has been done by comparing the peak position of the O–H or N–H group with the relevant interatomic distances as measured from single crystal diffraction data. Although such correlations are not linear over large ranges in hydrogen bonding strength for a variety of reasons [for a detailed discussion, see Jeffrey (1997) and references therein], good correlations are often observed for a related series of compounds or acceptor-donor combinations over modest wavenumber ranges. Thus comparison of the peak position of a particular hydrogen bonding functional group between polymorphs or structurally similar compounds can be used to make inferences about the relative strength of hydrogen bond donor–acceptor interactions. One pharmaceutically relevant example, taken from the work of Tang et al. (2002b) is shown in Fig. 3. Here, the N–H peak positions of a series dihydropyridine calcium channel blockers are plotted against the corresponding N–O interatomic distances as extracted from single crystal structures. It can be seen that shorter interatomic distances which are characteristic of stronger hydrogen bonds, correspond to NH stretching vibrations that occur at a lower wavenumber. Hence by comparing the IR spectra, it is possible to evaluate the relative strength of the hydrogen bonding within different compounds.

3.2.1.1. Polymorphs. FTIR spectroscopy is very valuable for distinguishing different polymorphs and numerous publications can be found where FTIR was applied for this purpose. Since it is not possible to review all of these studies, the following discussion will be limited to a few relevant examples. An extensive list of studies where mid-IR has been used to characterize different polymorphic forms has been published (Bugay, 2001). One of the earliest studies demonstrating the utility of IR spectroscopy to differentiate between polymorphs was described by Higuchi et al. (1969). In this study, ATR spectroscopy was used to better understand the dissolution behavior of methylprednisolone. The authors found that the slower than expected dissolution rate of the form II polymorph was caused by conversion to the form I polymorph on exposure to the aqueous medium, nicely demonstrating the correlation between solid state properties and dissolution rate. The use of IR spectroscopy to differentiate and characterize polymorphs is now routinely conducted. However, as previously mentioned, in some instances spectroscopic differences between polymorphs are minimal. For example, FTIR spectroscopy was applied for the characterization of ciprofloxacin saccharinate polymorphs (Romañuk et al., 2010) where it was found that the spectra of both forms were extremely similar. This outcome was in agreement with solid state NMR (SSNMR) results, which suggested that the intermolecular interactions were qualitatively similar between the two polymorphs. From the IR spectra, the authors were able to also confirm proton transfer between the drug and acidic counterion to form

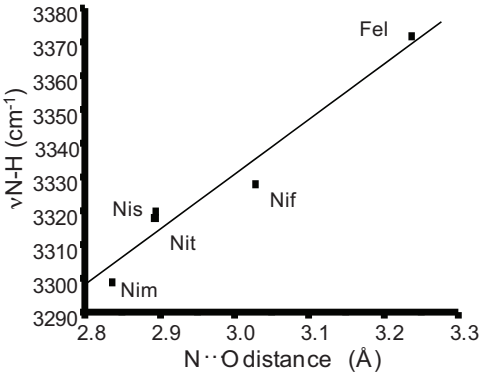

Fig. 3. Relationship between the N–O interatomic distance in the crystal and the NH stretching frequency for dihydropyridine calcium channel blockers. The R^2 correlation coefficient obtained upon linear regression analysis was 0.974. Key: Fel – felodipine, Nif – nifedipine, Nis – nisoldipine, Nit – nitrendipine, Nim – nimoldipine. Figure adapted from Tang et al. (2002b).

a salt. Finally, they elucidated from the IR spectra that neither of the polymorphs contained a zwitterion, in contrast to the non-salt form of ciprofloxacin. In an attempt to further understand the origin of spectroscopic differences between polymorphs, Strachan et al. (2004), used FTIR spectroscopy in combination with both FT-Raman spectroscopy and *ab initio* density functional theory calculations to study polymorphic forms I and III of carbamazepine. The conformation of molecules in the two polymorphs is very similar due to the rigid structure of carbamazepine, hence many of the IR peaks were found to occur at the same wavenumber for both polymorphs and the positions were well predicted by theoretical calculations. However, clear differences could be observed between the polymorphs for peaks arising from IR stretching vibrations of the carboxamide group, which forms a hydrogen bonded dimer motif in both polymorphs. Interestingly, differences seen in the Raman spectra were much more subtle in nature, presumably because the carboxamide group does not give rise to strong Raman peaks. The authors concluded that polymorphic systems with greater molecular conformational differences will result in IR spectra with greater spectral contrast. Piroxicam pivalate is exhibits conformational polymorphism (Caira et al., 1998), whereby the infrared spectra for the two polymorphs are quite different, further illustrating the utility of this method for differentiating conformational polymorphs.

3.2.1.2. Hydrates. Hydrates are another type of crystalline form extensively investigated using FTIR spectroscopy. For differentiation between hydrates and the corresponding anhydrate, the presence of a peak arising from O–H stretching can be used to indicate the presence of water. In principle, it should also be possible to extract information about the environment of the water molecules in the crystal lattice. However, it should be noted that interpretation of IR spectra of crystal hydrates can be extremely complex, in particular when several water molecules are present (Falk and Knop, 1973). In general, O–H stretching vibrations occurring at lower wavenumbers and having a higher intensity indicate that hydrogen bonding is stronger. However, vibrational coupling may lead to several bands for a given vibration, which in turn can lead to increased bandwidths due to unresolved components as well as frequency shifts, complicating interpretation. In order to deduce information about the number of structurally different water molecules from IR spectra, it is usually necessary to use the isotopic dilution technique, where vibrational coupling is reduced by obtaining spectra of partially deuterated species (Falk and Knop, 1973).

A useful example showing the potential of FTIR in the differentiation between the anhydrous forms and hydrates is provided in Brittain et al. (1988). Here it was observed that the anhydrous form of ampicillin only showed broad features in the 3000–4000 cm^{-1} region, whereas a sharp and strong peak could be observed in the trihydrate at 3334 cm^{-1}. The position and shape of the peak were consistent with the single crystal structure, which indicates that the water molecules occupy specific sites in the crystal lattice and that hydrogen bonding with the host molecule occurs. It should be stressed, however, that water in a hydrate does not always give rise to an extremely sharp peak in the O–H stretching region. An example thereof is provided by Chen et al. (2005). In this study, the monohydrate of mometasone furoate, a channel hydrate, clearly shows a peak that can be associated with the O–H stretching vibration. However, the observed peak is rather broad and occurs at a relatively high wavenumber (3500–3600 cm^{-1}), suggesting that the water is less strongly hydrogen bonded in mometasone furoate monohydrate relative to water in ampicilin trihydrate, as described above [note the O–H stretching peak of water in the gaseous state occurs at 3655 cm^{-1} (Brittain et al., 1988)]. FTIR in combination with SSNMR spectroscopy was used to investigate water in different hydrates of nedocromil sodium (Chen et al., 2000). The authors observed that in general, the intensity of the O–H stretching peak increased as the number of water molecules in the crystal increased, however no direct quantitative relationship between intensity and number or water molecules could be found. In addition, it was observed that the resolution of the O–H peaks varied depending on the hydrate formed. It was found that hydrate structures which contained isolated water molecules in the crystal structure gave rise to better defined peaks.

3.2.1.3. Salts and co-crystals. FTIR spectroscopy provides a powerful method to evaluate molecular complexes and to determine whether proton transfer has occurred between two components present in a crystalline material, and hence is ideal to evaluate if salt formation (ionic species are present) has taken place or if a co-crystal (both molecules in the crystal lattice are neutral) has been formed (Childs et al., 2007). Romañuk et al. (2009) studied the solid state properties of a series of saccharinate salts of fluoroquinolone antibiotics and used IR spectroscopy to provide evidence of an ionic interaction between the antibiotic and saccharin. In another study, Umeda et al. (2009) used IR spectroscopy to evaluate the nature of the complex formed between the acid indomethacin and the base lidocaine. Their results clearly showed the loss of the indomethacin carboxylic acid carbonyl peak, which is consistent with the formation of an ionic complex between the two components, as confirmed by single crystal studies. Likewise, Brittain used IR peaks associated with the carboxylic acid group to ascertain if salt formation had occurred between benzoic acid and benzylamine (Brittain, 2009a). A large decrease in the frequency of the carboxylic acid carbonyl band was observed, which was interpreted as the formation of a carboxylate anion band by deprotonation of the carboxylic acid functional group following salt formation. Co-crystal formation between benzoic acid and benzamide was studied using a variety of solid state characterization techniques including IR spectroscopy (Brittain, 2009b). The author found that in the co-crystal the IR peaks arising from carbon–carbon and carbon–hydrogen modes were relatively unperturbed relative to the pure component spectrum. In contrast, bands associated with the carboxylic functional group of benzoic acid and the amide functional group of benzamide were significantly altered in the co-crystal spectrum, with new bands being observed. Childs et al. (2007) studied 20 complexes of theophylline with a variety of guest molecules using a combination of IR spectroscopy and single crystal data to probe the state of ionization in the various complexes. The authors observed a total of 13 co-crystals, five salts and two complexes with mixed ionization states.

Infrared spectroscopy has also proven to be useful in assessing excipient mediated changes in API ionization state during formulation and processing. Rohrs et al. (1999) used IR spectroscopy to show that delaviridine mesylate was partially converted to the free base in tablets in the presence of moisture and croscarmallose sodium, whereby the latter was added as a disintegrant. In a study by Mallick et al. (2008), a solid state reaction between ibuprofen and aluminum hydroxide upon comilling was elucidated based on changes in the carbonyl region of the infrared spectrum. Guerrieri and Taylor (2009) studied disproportionation, the phenomenon whereby a salt converts back to the free form, for various pharmaceutical salt–excipient combinations using IR and Raman spectroscopy.

3.2.2. Characterization of amorphous phases

FTIR spectroscopy is a very valuable tool to probe amorphous materials, which are increasing in relevance due to the intensified use of solid dispersion technology to improve the delivery of

poorly water soluble drugs. Amorphous forms of a given drug give rise to IR spectra that differ from their crystalline counterparts. The origin of these differences relate to both the wider range of conformations typically present in an amorphous solid, which normally leads to the presence of broader peaks relative to those found in the crystalline spectrum, as well as differences in intermolecular interactions. Thus in some cases, FTIR can be used to better understand the local intermolecular interactions in an amorphous solid relative to the hydrogen bonding patterns present in crystal forms. For example, Taylor and Zografi (1997) used a combination of IR and Raman spectroscopy to determine that amorphous indomethacin predominantly contained hydrogen bonded carboxylic acid dimers, with a small amount of non-hydrogen bonded groups, and had a similar hydrogen bonding motif to that found in the γ polymorph.

A study by Tang et al. (2002b) used FTIR spectroscopy to characterize differences between crystalline and amorphous phases of dihydropyridine calcium channel blockers. For all compounds, the amorphous and crystalline samples gave rise to different spectra. In this study, special attention was given to the strength and extent of hydrogen bonding in the different forms, by studying the N–H and C=O stretching peaks. It was found that for the various compounds the average hydrogen bonding strength in the amorphous phases was very uniform, whereby the variation in the N–H stretching peak of the amorphous compounds was only 5 cm^{-1}. In sharp contrast, differences seen in the N–H stretching peak of the crystalline materials amounted up to 70 cm^{-1}. It was thought that these differences originated from the constraints that crystal packing imposes on the crystalline materials. Somewhat surprisingly, a number of compounds (felodipine, nicardipine, isradipine) had stronger average hydrogen bonding in the amorphous state compared to the crystalline state.

In a study by Tong and Zografi (1999), FTIR was used to characterize amorphous indomethacin and amorphous sodium indomethacin. The authors observed that while a peak characteristic for carboxylic acid dimers could be observed in amorphous indomethacin, this peak was eliminated in amorphous sodium indomethacin. It was suggested that ionization of the carboxyl group and its electrostatic interaction with sodium ions was responsible for the peak loss. In another study, Tong et al. (2002) used IR spectroscopy to study interactions between the counterion and the carboxylate group for a series of indomethacin salts formed with group I metal counterions. The spectroscopic observations made supported the assumption that a difference in ionic interactions affects the observed variation in T_g for the different salts

FTIR is a very powerful technique to study complex amorphous systems consisting of more than one component. These include amorphous solid dispersions, which typically consist of an amorphous drug formulated with a polymer added to inhibit crystallization and improve the dissolution rate. For these systems, it is important to characterize the state of mixing between the drug and the polymer. In other words, in addition to knowing whether the state of the drug in the dispersion is crystalline or amorphous, it is of interest to obtain further insight into the actual composition of the amorphous phase(s), if formed. In solid dispersions, an amorphous drug phase can exist as a system where the drug is molecularly mixed with the polymer, or alternatively, separate drug- and polymer-rich domains can be formed. Because of its ability to detect intermolecular interactions, FTIR is ideally suited to address these questions, as long as the appropriate control spectra are obtained. An example where FTIR was used to characterize interactions in amorphous solid dispersions can be found in Taylor and Zografi (1997). From the FTIR spectra obtained from solid dispersions of indomethacin and polyvinylpyrrolidone (PVP) of varying composition, it was observed that hydrogen bonds were formed between the carboxylic acid functional group of the drug and the carbonyl acceptor functional group of the polymer. The authors surmised that these drug–polymer hydrogen bonds occurred at the expense of the carboxylic acid dimers formed in the pure amorphous drug. This study demonstrated that it was necessary to compare the solid dispersion spectra with the spectra of both the pure amorphous drug (rather than the crystalline forms) and the (dry) polymer in order to elucidate the presence or absence of drug–polymer hydrogen bonds. Comparing spectra of solid dispersions with those of physical mixtures of the same composition proved to be the easiest way to identify the presence of specific interactions. Drug–polymer interactions in felodipine dispersions have been extensively probed using IR spectroscopy (Konno and Taylor, 2006). In a comparison of dispersions made with three polymers [PVP, hydroxypropyl methyl cellulose (HPMC) and hydroxypropyl methyl cellulose acetate succinate (HPMCAS)], it was found that PVP formed the strongest hydrogen bonds with the drug, as inferred from the shift in the position of the drug NH group in the solid dispersions. The presence of concentration-dependent specific interactions between the drug and polymer indicate that the two components are mixed at the molecular level. It is important to establish these insights, since, for a polymer to be an effective crystallization inhibitor, it needs to be in intimate contact with the drug. For an amorphous dispersion of felodipine and PVP, it was observed that the system remained miscible even when heated to the melting temperature of the drug, whereby drug–polymer hydrogen bonding interactions were still present (albeit weakened) at higher temperatures (Marsac et al., 2010). Rumondor et al. (2009a) used principal components analysis on IR spectra of solid dispersions and physical mixtures to evaluate miscibility of the solid dispersions. They found that the score values for the physical mixtures were similar to those of the dispersions of equivalent concentrations for poorly miscible systems. For miscible systems, on the other hand, larger differences were seen between the scores of the solid dispersions and those of the physical mixtures.

When trying to infer the presence of drug–polymer hydrogen bonds using IR spectroscopy, it is essential to eliminate the effects of sorbed water through rigorous drying of the sample prior to IR analysis. Marsac et al. (2010) showed that water absorbed into an amorphous solid dispersion partially obscured absorption bands arising from drug–polymer interactions, making them much harder to characterize. The same study showed that the presence of moisture actually disrupted the drug–polymer hydrogen bonding, resulting in amorphous–amorphous phase separation. Additional studies have confirmed the ability of moisture to disrupt drug–polymer hydrogen bonding interactions in a number, but not all of the drug–polymer systems studied (Rumondor et al., 2009b; Rumondor and Taylor, 2010).

3.2.3. Quantitative studies

In addition to qualitative information, mid-IR studies can also be used to extract quantitative information on the phase composition of pharmaceutical systems. Both univariate and multivariate analysis approaches can be used for this purpose, although it is worth mentioning that multivariate analysis [e.g. partial least-squares analysis (PLS)] is growing in popularity since it provides a potentially more powerful means to analyze differences in the spectral data. As an extensive discussion of these approaches is beyond the scope of this review, the interested reader is referred to more detailed literature on the subject (e.g. Schoonover et al., 2003).

One of the first demonstrations of the utility of IR spectroscopy for quantification of mixtures of solid state forms is highlighted by the study of Black and Lovering (1977). Here, the authors estimated the crystallinity of milled digoxin samples using both IR and X-ray powder diffraction, with good agreement being observed between the two techniques. In an early application of multivariate data analysis for quantification of solid state forms, Deeley et al. (1991)

compared Raman and diffuse reflectance IR spectroscopy for the quantification of different polymorphs of cortisone acetate, using a principal components regression approach to analyze the spectra. The authors found that both methods gave similar precision for predictions of the amount of each polymorph with a standard error of prediction of less than 3.5%. Because of its ease of use and minimal sample preparation, ATR spectroscopy is gaining in popularity as a method to characterize solid state mixtures. In a study by Helmy et al. (2003), ATR was employed to quantitate the relative amounts of two polymorphs of aprepitant in powder blends. The authors applied a second derivative to their spectra and normalization to a reference band prior to quantification. Upon comparison of the IR results with those obtained with X-ray powder diffraction, good agreement was observed between the two methods. Additionally, both methods were able to detect small amounts of the polymorphic impurity of interest. These studies, in combination with many others, have laid the foundations for the routine use of FTIR spectroscopy for the characterization of solid state forms in powdered blends.

3.3. Remote sensing

Important advancements in mid-IR instrumentation have been realized by the development of fiber optics, enabling remote sensing (Mizaikoff, 2003). An important application is the use of fiber optics in combination with ATR spectroscopy, i.e. fiber-optic evanescent wave spectroscopy (FEWS; Saito and Kikuchi, 1997). The main application of these probes in a pharmaceutical setting is process control of crystallization (Braatz et al., 2002; Yu et al., 2004). As the measurements are based on absorption of the evanescent waves generated at the boundary between the ATR crystal and the sample, only material in close contact with the probe is sampled. Hence, when applied to slurries, the liquid solution is measured and crystals do not significantly affect the infrared spectra collected. As such, this technology has been extensively used for the measurement of solubility and supersaturation in crystallization processes. While the initial publications on the subject typically focused on the evaluation of the feasibility of the technique for measurement of solubility and/or supersaturation in slurries of relatively simple systems [e.g. citric acid/water (Dunuwila et al., 1994), maleic acid/water (Dunuwila and Berglund, 1997a,b), potassium dihydrogen phosphate/water (Togkalidou et al., 2001), bifenox/methanol (Lewiner et al., 2001), isoproturon/ethanol (Lewiner et al., 2001)], there has been an evolution to more complex systems [e.g. multicomponent pharmaceutical systems (Togkalidou et al., 2002)] and/or applications [e.g. determination of optimal cooling curves for crystallization (Feng and Berglund, 2002), the separation of mandelic acid enantiomers by direct crystallization (Profir et al., 2002), pharmaceutical antisolvent crystallization (Yu et al., 2006; Zhou et al., 2006), concentration controlled batch crystallization (Nagy et al., 2008) and selective crystallization of a specific polymorph (Kee et al., 2009a,b)].

Another application where fiber optics is used is the detection and quantification of trace amounts of compounds on surfaces, using mid-IR grazing angle fiber optics probes. These studies are relevant for cleaning validation purposes, as the downtime of equipment during current cleaning validation, typically performed by high performance liquid chromatography analysis of rinsewater or swabs taken from one or several regions of the equipment, can be significantly reduced using *in situ* infrared reflection-absorption spectroscopy (IRRAS). In a number of publications (Mehta et al., 2003; Teelucksingh and Reddy, 2005; Hamilton et al., 2005, 2006; Perston et al., 2007, 2008), the feasibility of detecting and quantifying small amounts of pharmaceutically relevant substances has been evaluated.

3.4. Imaging applications

To obtain spatially resolved infrared spectra, one can either restrict radiation at the sample plane or segment the transmitted radiation beam at the detection plane (Levin and Bhargava, 2005). With the former technique, which is the simplest approach, an opaque mask with an aperture of controlled size physically restricts radiation absorption to the sample area of interest. By moving the sample, spectra can be obtained from neighboring points. This technique is known as 'mapping' and suffers from several drawbacks, the most important ones being the extremely low sample throughput and relatively low spatial resolution (Levin and Bhargava, 2005). Samples are typically analyzed in either transmission or reflectance mode. Transmission analysis, however, requires that samples are inherently thin or can be sliced into sections a few microns thick, thus transmission measurements are difficult for compacted solids. Reflectance measurements can be difficult for pharmaceutical solids due to their relatively rough surface as the resultant combination of diffuse and specular reflectance renders the obtained spectra hard to interpret. Hence, although mapping has evolved into a well established technique over the years, due to the some of the issues described above, the pharmaceutical application of this methodology has been somewhat limited and is therefore not further discussed.

Segmenting the radiation beam at the detector plane has become possible by the advent of focal plane array (FPA) detectors (Lewis et al., 1995), which consist of many small, individual detectors laid out in a grid pattern. No apertures are necessary in this setup as the microscope images the sample plane directly on the detector array (Levin and Bhargava, 2005). The latter technique is termed 'imaging', as, depending on the detection array and the collection parameters, thousands of moderate resolution spectra can be acquired at near diffraction-limited spatial resolution in minutes (Levin and Bhargava, 2005). Further details on the technique will not be provided here, and more information is available in the literature: Bhargava and Levin (2001), Chan and Kazarian (2003), Levin and Bhargava (2005), Kazarian and Chan (2006, 2010), Koenig and Bobiak (2007), Steiner and Koch (2009), Davis et al. (2010a,b). In the following section, a number of pharmaceutical applications of imaging will be discussed, with the experiments typically conducted either in transmission mode or using ATR accessories.

3.4.1. Characterization of heterogeneous solids

The first group of applications relates to the characterization of solids, in particular the identity and distribution of various components in complex mixtures. Both micro- (high spatial resolution, smaller sampling area) and macro-ATR (lower spatial resolution, larger sampling area) FTIR imaging has been applied to investigate homogeneity in lightly compacted pharmaceutical powder blends (Chan et al., 2003). The sample area measured by the macro-ATR approach was approximately 1 mm^2 and the spatial resolution was around 15 μm. Using micro-ATR, a much better spatial resolution was obtained (around 4 μm) at the expense of a smaller measured area (approximately 250 μm^2). Micro-ATR was found to be better for more clearly visualizing the domains of the different components, and components with concentrations below 0.5% and small particle sizes (less than 15 μm) could be detected using this method.

As previously mentioned, for ATR spectroscopy, good contact between the sample and the ATR crystal is a prerequisite. For powders, this is normally achieved by using an ATR crystal made from a hard material, such as diamond, and an anvil which can apply a constant and predefined pressure to ensure adequate contact. Unfortunately, preformed tablets may be difficult to analyze using conventional ATR approaches due to fragmentation of the tablet under the applied pressure. To overcome this limitation, Chan et al.

(2005), used ATR-FTIR to study different tablet components by compacting the powder *in situ* on the ATR crystal by means of a specially constructed cell. The distribution of the various components in a compact thus formed can then be analyzed using ATR imaging as described above. The authors successfully imaged the distribution of tablets composed of three components of similar chemical composition, namely lactose, microcrystalline cellulose, and HPMC. Using this experimental setup, it was also possible to investigate the density of the tablets from the absorption intensity, whereby more dense tablets would be expected to result in a higher absorption. It was observed that addition of 5 wt% of magnesium stearate to HPMC led to an increase of the density of the tablet by lubricating the HPMC particles, causing an increase in absorbance.

Elkhider et al. (2007) studied the effect of pressure and moisture on tablet compaction of ibuprofen–HPMC mixtures, using an ATR setup combined with a controlled humidity cell. Using a compaction pressure of 103 MPa, the distribution of the drug was complementary to the image of water up to 40% relative humidity. In other words, regions of high drug concentrations corresponded to relatively low water contents, consistent with the hydrophobic nature of ibuprofen. Histograms depicting the number of detector pixels at different integrated values of HPMC absorbance bands were used to assess the density distribution of compacted tablets in a semi-quantitative manner.

FTIR imaging is often combined with other imaging techniques in order to enhance the information content extracted from the samples. For example, FTIR imaging was used in combination with X-ray fluorescence imaging to study model inorganic pellets and a model pharmaceutical pellet containing acetaminophen (Patterson and Havrilla, 2006). The combination of both techniques was found to be useful to build two dimensional maps showing the distribution of acetaminophen, calcium phosphate and HPMC, although the magnesium stearate that was present could not be detected with the imaging methods. Wray et al. (2008) studied the distribution in caffeine compacts prepared using different polymer matrixes (microcrystalline cellulose, HPMC and lactose) with FTIR imaging, using X-ray microtomography as a complementary technique.

The application of FTIR imaging for the analysis of counterfeit pharmaceuticals has been the subject of a number of publications. Ricci et al. (2007a) used FTIR imaging and desorption electrospray-ionization linear ion-trap mass spectrometry as complementary techniques for the analysis of counterfeit anti-malarial tablets. Based on results from ATR-FTIR spectra, it was concluded that while the genuine tablets contained artesunate in combination with talc and avicel, no artesunate could be detected and calcium carbonate was the main component in some of the counterfeits analyzed. In addition, the fake tablets were observed to contain a variety of different drug substances. In another study (Ricci et al., 2007b), spatially offset Raman spectroscopy was used as a complementary technique to IR spectroscopy to study potentially counterfeit artesunate anti-malarial tablets. ATR-FTIR imaging enabled the identification of paracetamol, dipyridone, and talc in a number of fake tablets, and suggested that artemisinin, an artesunate precursor, was present in others. Lanzarotta et al. (2009) used imaging to compare and contrast the spectra of a genuine and suspected fake tablet. In this instance, the suspect tablet was found to contain the API, but was missing an absorption band arising from an excipient that was present in the authentic tablet core. This discrepancy in the excipient composition confirmed that the suspected tablet was indeed a counterfeit product. A forensic application is provided by Ricci et al. (2006), where FTIR imaging was used to detect the model drugs paracetamol and ibuprofen on different types of tape as well as directly on a contaminated newspaper surface. Diacetylmorphine (heroin) was used as the model compound for the forensic case study.

Chan and Kazarian (2004a) applied FTIR imaging to study water sorption in compacted physical mixtures of griseofulvin and polyethylene glycol (PEG) as a function of relative humidity. It was observed that, not unexpectedly, the PEG domains absorb a significantly greater amount of water compared to the griseofulvin-rich domains. Changes in relative humidity did not have a significant effect on the spatial distribution of PEG and griseofulvin. Hence, the technique enabled the differentiation of water sorption in different domains of the sample.

Transmission macro-FTIR imaging has been used for the study of crystallization of a nifedipine glass under a controlled environment (Chan and Kazarian, 2006a). In this study, it was observed that the absorbance of a thin film of amorphous nifedipine was not uniform due to a variation in the thickness of the film, highlighting an inherent problem of transmission measurements. On exposure to moderate temperature and high relative humidity conditions (38 °C, 80% RH), it was observed that the β polymorph crystallized and appeared to nucleate from a few sites in the film. Based upon IR measurements, crystallization was denoted as complete after 4 h. After longer exposure times, conversion to the stable α polymorph was observed, commencing at the center of the film.

3.4.2. Drug dissolution and release

Another comprehensive set of studies relates to the investigation of drug dissolution and release. For example, Coutts-Lendon et al. (2003) used FTIR imaging for the characterization of drug release from systems consisting of testosterone dispersed in PEG. Here a 5 μm film of the dispersion was prepared between two IR windows to enable the sample to be imaged in transmission mode. The authors found that if the drug dissolved at the same rate as the dissolution front of the polymer, the polymer dissolution rate controlled the rate of drug release, and this trend was seen for 10% and 20% testosterone loadings. Representative images of drug and polymer distribution from the beginning, middle and end of the release process of a 20% testosterone sample are provided in Fig. 4. Conversely, the dissolution rate of the drug was the controlling factor for drug release at 30% and 40% testosterone loadings, with large portions of the drug remaining intact (undissolved) after the polymer dissolves. The authors were able to monitor the kinetics of both the polymer and drug dissolution and apply mathematical models to describe the release profiles, with the Peppas model best able to characterize both the polymer dissolution and drug release.

In a study by van der Weerd et al. (2004), a compaction cell was designed that enabled the compression of the tablet formulation directly onto an ATR device, whereby the resultant tablet covered half of the ATR crystal. This facilitated the study of the interaction of the tablet with water, whereby water could be circulated around the tablet and the dissolution process monitored. This novel design was tested by studying the distribution of HPMC, caffeine, and water in the tablet prior to and after exposure of the tablet to water. In a follow-up study (van der Weerd and Kazarian, 2004a), the technique was further expanded by integrating a UV detector in the effluent water stream to enable the determination of the dissolved drug concentration (niacinamide). Partial least squares (PLS) calibration was used for accurate quantitative analysis of the concentrations of the different compounds (niacinamide, HPMC and water) based upon the measured spectra. Good similarity was seen between the dissolution profiles of niacinamide determined by both FTIR imaging and those resulting from UV spectroscopy of the effluent.

Dissolution and swelling of HPMC tablets was investigated using a macroscopic ATR-FTIR imaging setup (van der Weerd and Kazarian, 2004b). The rate of water intake into the HPMC tablets was found to be essentially unaffected by the compaction pressure over the range of pressures studied, or by the type of ATR crystal used. The authors were able to measure quantitatively the rate of

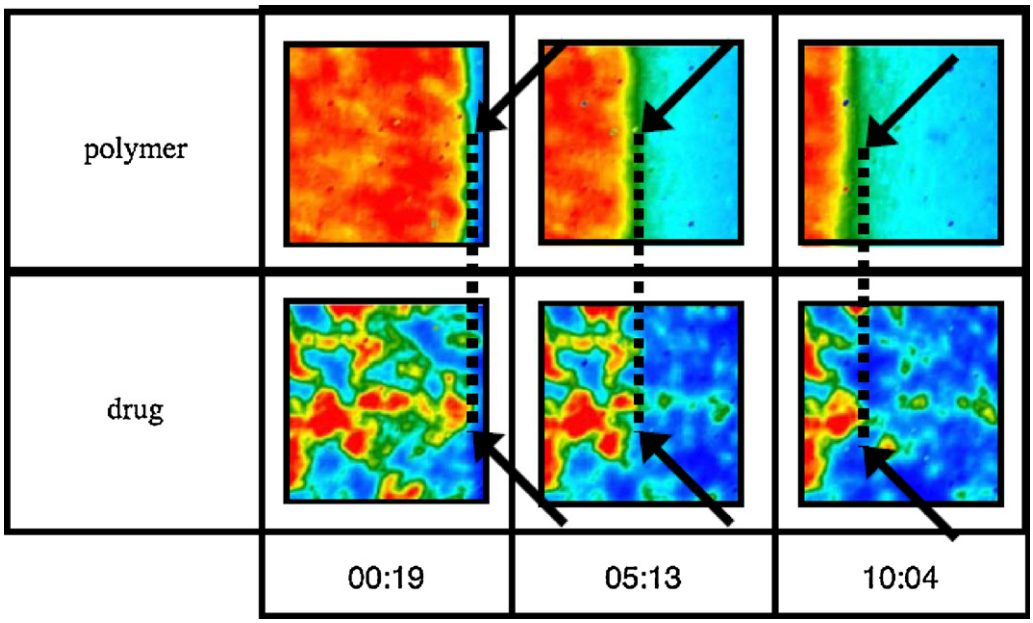

Fig. 4. Images of the polymer and drug extracted from the beginning, middle and end of the release process of a formulation containing 20% testosterone dispersed in PEG. The sustained alignment of the polymer and drug dissolution fronts relative to one another indicates the polymer is dictating the release mechanism. From Coutts-Lendon et al. (2003), with permission.

water ingress into the tablets, and swelling was found to effectively double the radius of the tablet.

The combined setup of FTIR microscopy of tablets and UV spectroscopy of the effluent has been further optimized for sodium diclofenac/HPMC tablets by mixing the effluent with a NaOH solution prior to UV analysis (van der Weerd and Kazarian, 2005). As such, precipitates of the free acid form of diclofenac, formed due to the use of a dissolution medium with a pH of 4, could be redissolved prior to analysis. It was concluded that the release rate from the tablet was independent of the pH (4 or 11) of the dissolution medium. It should be mentioned, however, that the authors defined the release rate as the rate at which drug was removed, regardless of its physical state. Dissolution differences seen between different media (as detected by the UV detector) in a setup where the (re)dissolution step was omitted were interpreted as being caused by drug precipitation into the acidic medium subsequent to drug release. In order to obtain insight into 'fronts' moving into tablets, a colored substance, buflomedyl pyridoxal phosphate (BPP) was incorporated into HPMC tablets. Images during dissolution were obtained with both visible photography and with FTIR imaging (Kazarian and van der Weerd, 2008). Based on the results from FTIR imaging, the authors concluded that the three fronts observed with visible photograhy were due to (1) true water penetration, possibly combined with (partial) dissolution of BPP, (2) total gellification of HPMC, and (3) the erosion front.

Imaging has also been used to probe dissolution in more complex formulations, in particular various solid dispersions formed using PEG as the carrier substance. In such dispersions, the drug can be present in either as a crystalline, amorphous, or mixed form. IR imaging can be used not only to evaluate the release of the drug from the matrix, but also to determine if any changes in solid state form occur on contact with the dissolution medium. Dissolution of solid dispersions of nifedipine in PEG was studied using ATR-FTIR imaging (Chan and Kazarian, 2004b). Nifedipine was observed to be initially amorphous in the solid dispersions with a low drug loading, however upon addition of water, both the PEG and the drug were observed to dissolve in a uniform fashion. Solid dispersions containing a higher loading of nifedipine (>10%) contained a mixture of crystalline and amorphous drug. Following exposure to moisture, the amount of crystalline drug increased in certain locations of the dispersion. In a subsequent study (Kazarian et al., 2005), tabletted nicotinamide/PEG dispersions were evaluated. Samples prepared either by mechanical mixing or by solvent evaporation showed significant differences in their dissolution profiles, and the profiles compared well with the data obtained from the hyphenated UV dissolution test. Profiles obtained from solvent evaporation were smoother compared to those obtained from mechanically mixed samples. Dissolution of ibuprofen/PEG formulations has also been studied using FTIR imaging (Kazarian and Chan, 2003). Initially, ibuprofen was found to be molecularly dispersed in the PEG matrix, however upon addition of water, either as a static or a flowing system, a layer of crystalline ibuprofen was observed to form at the front of the tablet. It was shown that ibuprofen crystallization could be prevented by inclusion of cyclodextrins into the formulations.

3.4.3. *FTIR imaging for high throughput applications*

The potential of FTIR imaging for high throughput analysis has been the focus of a number of publications. In a study by Chan and Kazarian (2005), the authors showed that it was possible to obtain "chemical snapshots" from a spatially defined array of many different polymer/drug formulations, deposited using a microdroplet-on-demand device under identical conditions. Using a heated nozzle, drops of a molten PEG/ibuprofen or PEG/nifedipine mixture were dispensed directly onto the ATR crystal, such that the resultant droplets had a diameter of approximately 200 μm. A total of 117 samples were deposited onto the ATR crystal, and these were analyzed simultaneously.

In a follow-up study (Chan and Kazarian, 2006b), different ibuprofen/PEG formulations were evaluated simultaneously at a controlled humidity and temperature. It was observed that above a certain weight fraction of ibuprofen in PEG (about 30 wt%), dimerization of ibuprofen occurred. Below this concentration, it was concluded that ibuprofen was molecularly dispersed in PEG. At elevated temperatures, formulations with a higher ibuprofen load lost

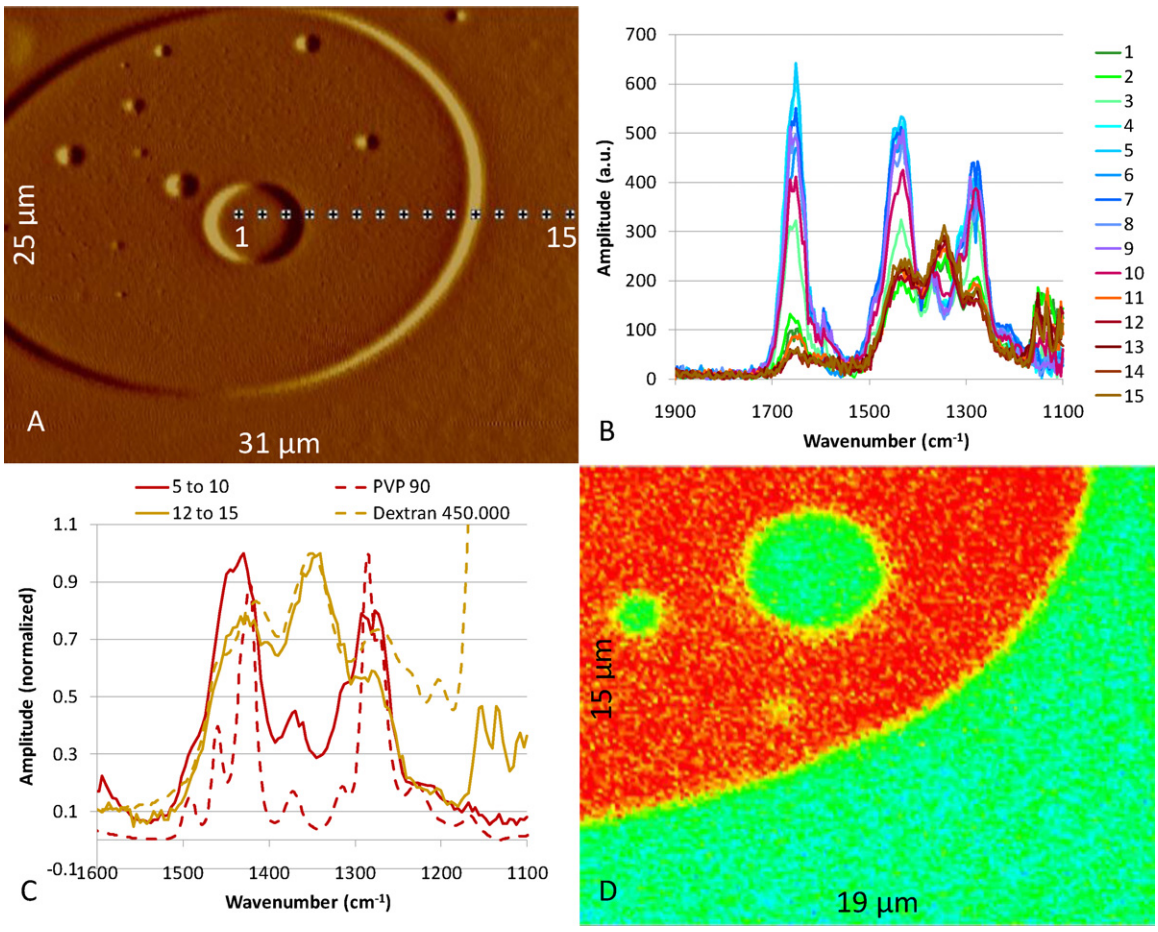

Fig. 5. PTIR data obtained on a spin coated 50/50 (w/w) dextran (M_w 450.000)/PVP K90 film. (A) Deflection image, numbers indicate the positions of local spectral measurements, (B) local spectra obtained at the different positions, covering a spectral region of 1100–1900 cm^{-1}, (C) averages of different sets of local spectra (solid lines) and comparison with standard transmission spectra of the pure components, (D) IR absorbance image at 1440 cm^{-1}. Data taken from Van Eerdenbrugh et al. (2010).

a significant amount of drug due to sublimation, while the amount of ibuprofen in molecularly dispersed formulations changed by a much lower amount.

Various other high thoughput applications have also been described. A multi-channel dissolution grid constructed of polydimethyl siloxane placed in contact with an ATR crystal, combined with a FPA detector, enabled parallel evaluation of the dissolution behavior of multiple samples (five) of various drug formulations (Chan and Kazarian, 2006c). For the high-throughput evaluation of drug polymorphism, FTIR imaging in transmission mode was evaluated (Chan et al., 2007), using melt quenched glasses of nitrendipine without/with different ratios of nifedipine. The authors observed that the crystallization tendency of both compounds was much reduced in binary mixtures subjected to elevated temperatures and relative humidities. Finally, the feasibility of ATR-FTIR imaging for protein crystallization has been recently demonstrated (Chan et al., 2009) by studying crystallization of thaumatin and lysozyme in a high-throughput manner, simultaneously from six solutions.

3.4.4. Current trends – towards higher temporal and spatial resolution

From the above discussion, it can be concluded that FTIR imaging can be successfully applied for the characterization of complex solids and dissolution processes, either performed on single samples or in a high throughput mode. However, there are a number of current efforts ongoing to enhance the temporal and spatial limitations, whereby reducing the temporal resolution between images would enable faster processes to be captured. An example thereof is step scan FTIR imaging, which enables the monitoring of reversible dynamic processes with millisecond order time resolution (Bhargava and Levin, 2003, 2004). To the best of our knowledge, no pharmaceutical applications using these higher temporal resolutions have been described to date, although it can be expected that these will emerge in the near future. In terms of spatial resolution, an interesting, rapidly evolving approach is the application of photothermal induced resonance spectroscopy (PTIR), whereby an AFM probe is used to measure local thermal expansion from IR laser light pulses incident upon a sample (Dazzi et al., 2005, 2006, 2007, 2008; Mayet et al., 2008; Dazzi, 2009; Houel et al., 2009; Hill et al., 2009; Felts et al., 2010; Kjoller et al., 2010; Rice, 2010). As an AFM probe is used for signal detection using this approach, it is possible to achieve spatial resolutions well below the diffraction limit of the incident radiation. As such, it is possible to obtain images, corresponding to absorption at a certain wavelength, with a sub-100 nm spatial resolution. Alternatively, local full spectral information can be obtained by varying the wavelength of the incident radiation with the probe held stationary at a certain location. The capability of the technique thereby clearly exceeds that of standard AFM measurements, as an unambiguous identification of different phases using the latter technique can be hard to achieve. An example of a pharmaceutically relevant sample is provided in Fig. 5, showing data obtained on an immiscible dextran/PVP system (Van Eerdenbrugh et al., 2010). While standard contact frequency (Fig. 5A) images reveal differ-

ent domains in the sample, suggesting immiscibility, assignment of the different phases is not straightforward based solely on the information contained in this image. Local spectra obtained using PTIR at the positions provided in Fig. 5A clearly demonstrate that the different domains seen correspond to different chemical compositions (Fig. 5B). Upon averaging sets of similar local spectra and further comparison with spectra obtained on the pure components (Fig. 5C), it is clear that the contact frequency image corresponds to small dextran-rich domains embedded in a PVP-rich domain that is surrounded by a dextran-rich phase. This is further confirmed in the absorbance image, collected using an excitation wavelength of 1440 cm^{-1} (Fig. 5D). A related technique currently under development, where the resultant temperature fluctuations rather than the thermal expansion is probed as a function of frequency, is photothermal (FT-IR) microspectroscopy (Hammiche et al., 1999; Harding et al., 2008). Published applications of the technique include the study of drug–excipient compatibility of acetylsalicylic acid and magnesium stearate (Harding et al., 2008) and the chemical identification of materials in multicomponent systems (Dai et al., 2009).

4. Conclusions

From the numerous examples discussed in this review, it is clear that mid-IR is and continues to be an indispensable characterization and quantification technique for pharmaceutical systems and processes. A wealth of information can be obtained with mid-IR spectroscopy and, as illustrated by recent developments discussed herein, it can be expected that future development of mid-IR based applications will advance our understanding of pharmaceutical materials, systems and processes.

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