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Vibrational spectroscopy used in polymorphic analysis

Andrei A. Bunaciu [a], Hassan Y. Aboul-Enein [b], Vu Dang Hoang [c,*]

[a] SCIENT – Research Center for Instrumental Analysis, (Cromatec Plus S.R.L.), Bucharest, Romania
[b] Pharmaceutical and Medicinal Chemistry Department, Pharmaceutical and Drug Industries Research Division, National Research Centre, Dokki, Giza 12622, Egypt
[c] Department of Analytical Chemistry and Toxicology, Hanoi University of Pharmacy, Hanoi, Vietnam

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ABSTRACT

Vibrational spectroscopy includes several techniques, the most important being mid-infrared (MIR), near-IR (NIR) and Raman spectroscopies. Vibrational spectrometry covers a series of well-established analytical methodologies suitable for both qualitative and quantitative purposes. Raman and MIR spectroscopies are complementary techniques and usually both are required to measure the vibrational modes of a molecule completely. In the first part of this review, we focus on theoretical aspects related to vibrational techniques, while, in the second part, we discuss important articles published during the period 2000–14 relating to polymorphic analysis.

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1. Introduction

"Polymorphism" comes from the Greek word, *polus* = many and *morph* = shape, so it is defined as the ability of a substance to exist as two or more crystalline phases that have different arrangements or conformations of the molecules in the crystal lattice. It essentially means that, in different polymorphs, the same molecule exists in different ways (i.e., a material that exhibits different crystal structures within the solid state but has identical characteristics in solution). Polymorphism is a technical term used in biology and crystallography. It is estimated that 80–90% of organic compounds are capable of existing in polymorphic forms.

As a result of the polymorphism phenomena, the molecules can present different arrangements in the unit crystal cell and can thus have different physical properties [1], including packing, thermodynamic (e.g., solubility, free energy, and melting point), spectroscopic, kinetic properties (e.g., dissolution rate and stability), and mechanical (e.g., hardness, compatibility, tableting, and tensile strength). Polymorphism is very important in those areas of chemical research where full characterization of a material has a pivotal role in determining its ultimate use (e.g., pharmaceutical, pigment, agrochemical, explosive and fine-chemical industries) [2].

Taking into account the pharmaceutical literature, it is very clear that the physical characterization of the active principle ingredient (API) is crucial for the development of each drug [3,4]. It has been known for a long time that a pharmaceutical can exist in more than one solid form (i.e., crystal or amorphous). The different solid forms of a drug can display significantly different physical and chemical properties, including color, morphology, stability, dissolution, and bioavailability [5]. These properties can have direct effects on the ability to process and/or manufacture the drug substance and the drug product, and drug-product stability, dissolution and bioavailability. Polymorphism can affect quality, safety and efficacy of the drug product [2,6]. The most thermodynamic stable form is chosen for development of the final dosage product, but, more recently, metastable forms were used to enhance dissolution or bioavailability profiles. For example, carbamazepine is a poorly soluble drug that exists in four well-characterized anhydrous crystal

* Corresponding author. Tel: 84-4-38254539; Fax: 84-4-38264464.
E-mail address: hoangvd@hup.edu.vn (V.D. Hoang).

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forms [i.e., P-monoclinic (III), triclinic (I), trigonal (II) and C-monoclinic (IV)]. The P-monoclinic polymorph is marketed in commercial preparations because it is relatively stable at ambient temperature and has higher solubility and bioavailability, while the C-monoclinic polymorph can be formed under various conditions possible during drug formulation.

Piracetam is a nootropic drug that has five reported polymorphs, and at least two hydrated forms (a monohydrate and a dihydrate). Two forms, FIV and FV are generated under high pressure while FI is generated when FII or FIII is heated to 127°C (below the melting points of both polymorphs) and then quenched to room temperature. The FII and FIII polymorphs are both kinetically stable at room temperature and FIII is the thermodynamically more stable polymorph with FII being metastable.

In the past, physical characterization of the API was especially performed with optical microscopy, X-ray powder diffraction (XRPD) and thermal analysis, including differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). After 1940, infrared (IR) spectroscopy became one of the most used techniques in the multi-disciplinary approach to physical characterization of pharmaceutical solids, while recently Raman and solid-state nuclear magnetic resonance (ssNMR) spectroscopy also became used for solid-state characterization. In addition, time-of-flight secondary ion mass spectrometry (TOF-SIMS) was added to these techniques to achieve better characterization of solids [7].

Chemical imaging can also be achieved using IR and Raman spectroscopy with the additional advantage that a specific component can be located within a drug product based upon spectral response, and the spectra can also potentially differentiate different solid forms of the API [8].

The polymorphic behavior of drugs is a major concern of the pharmaceutical industry as it may have considerable formulation, therapeutic, legal and commercial implications [9], so it is crucial to be able to identify and to quantify different polymorphic forms of drugs adequately.

Since polymorphic forms are generally extremely sensitive to physical treatments, such as milling, grinding or heating, many physico-chemical techniques are not useful in the study of substances that can undergo polymorphic transitions. The main advantage of Raman spectroscopy is that no sample manipulation is required, so, in the case of polymorphs that are susceptible to transformation, the spectra can be obtained with complete certainty of the identity of the sample under investigation. The absence of sample preparation makes Raman spectroscopy a more robust testing procedure [10].

The appropriate vibrational spectroscopy measuring mode (IR or NIR) will be dictated by the optical properties of the samples (Fig. 1). Transparent materials are usually measured in transmittance mode (Fig. 1A). Turbid liquids or semi-solids and solids may be measured in diffuse transmittance mode (Fig. 1B), diffuse reflectance mode (Fig. 1C) or transflectance mode (Fig. 1D and E), depending on their absorption and scattering characteristics.

Every molecule has a unique fingerprint of vibrational frequencies, which makes Raman and Fourier-transform IR (FTIR) spectroscopy highly specific techniques for molecular identification. Both techniques can be employed non-invasively, making them ideal for any applications. Raman and FTIR spectroscopy are sometimes referred to as “sister” techniques and provide complementary information about molecules, but they differ in fundamental ways.

Polymorphism is a widespread phenomenon in polymers; under the right conditions, almost all semi-crystalline polymers can form polymorphs. Biodegradable polymers have attracted increasing interest in fundamental research and technology, due to their potential to address environmental concerns and biomedical applications. Biodegradable polymers break down in physiological environments by macromolecular chain scission into smaller fragments, and ultimately into simple, stable end-products. The degradation may be due to aerobic or anaerobic microorganisms, biologically active processes (e.g., enzyme reactions) or passive hydrolytic cleavage [11].

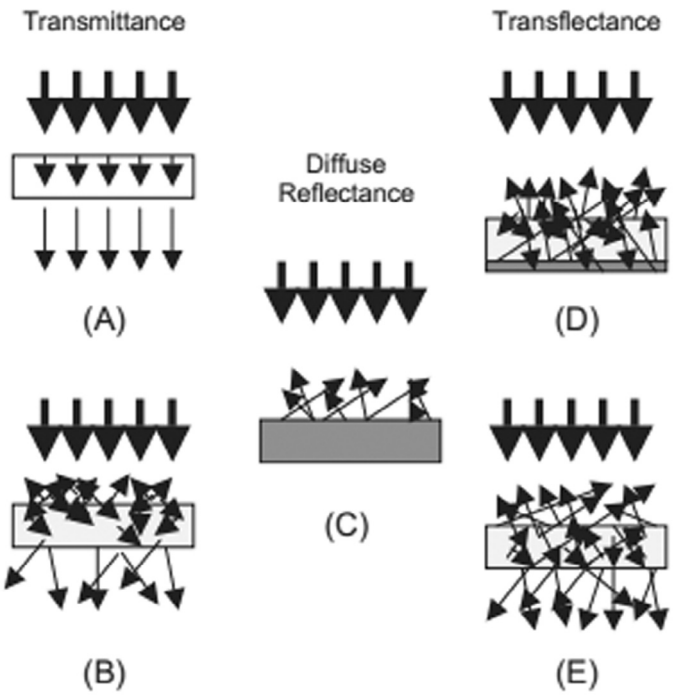

Fig. 1. Infrared spectroscopy measuring modes: (A/B) transmittance, (C) diffuse reflectance and (D/E) transflectance.

The objective of this article is to review new developments in applications of vibrational spectroscopy (Raman and FTIR) in polymorphic analysis, covering the period 2000–14. Prior to the review, it is useful to give a short introduction to the concept of the vibrational spectroscopy followed by discussion of the quantitative and qualitative applications of these techniques.

2. Theoretical aspects

Vibrational spectroscopy includes several different techniques, but the most important techniques are mid-IR (MIR), near-IR (NIR), and Raman spectroscopy. Both MIR and Raman spectroscopies provide characteristic fundamental vibrations that are employed for the elucidation of molecular structure and are the topic of this review. NIR spectroscopy measures the broad overtone and combination bands of some of the fundamental vibrations (only the higher frequency modes) and is an excellent technique for rapid, accurate quantitative determination.

Vibrational spectroscopy offers complete information on the chemical composition of samples regarding both major and minor compounds, which present many characteristic bands in the range studied. Additionally, the presence of trace compounds can be modeled in some cases through the multivariate treatment of the whole IR or Raman spectra of well-characterized samples based on the influence of molecules at low-concentration levels on the size and the shape of the bands of major compounds.

Molecular vibrations can be excited via two physical mechanisms (i.e., the absorption of light quanta and the inelastic scattering of photons), as can be seen in Fig. 2.

Direct absorption of photons is achieved by irradiation of molecules with polychromatic light that includes photons of energy matching the energy difference between two vibrational energy levels [i.e., the initial (ground state) and the final (first excited state)

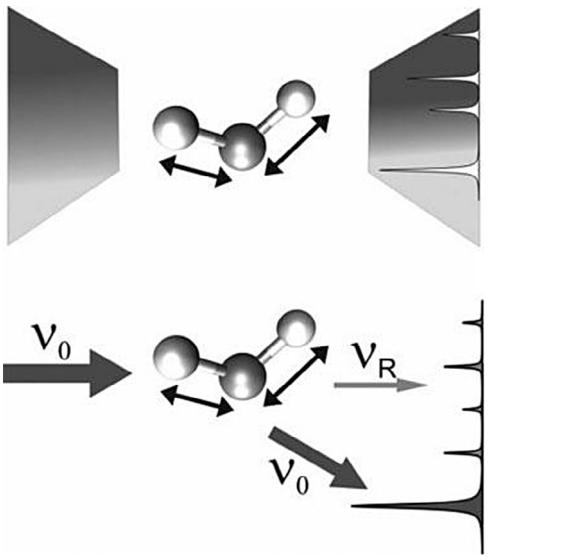

Fig. 2. The excitation of molecular vibrations in IR (top) and Raman (bottom) spectroscopies.

vibrational state]. In IR spectroscopy, the vibrational transitions are induced by absorption of light quanta from a continuous light source in the IR spectral region. Vibrational Raman transitions correspond to inelastic scattering (ν_R; thin arrow) of the incident monochromatic light (ν_0), whereas the elastic scattering (ν_0) is represented by the thick arrow.

There are many reasons why scientists want to measure the Raman spectra of compounds. First, many bands that are weak in the IR spectrum are among the strongest bands in the Raman spectrum. Second, some Raman bands are found at very characteristic frequencies. Samples for Raman spectrometry can be mounted in standard glass tubes, making sample handling for Raman far easier than for IR. Finally, low-frequency bands are far more easily measured by Raman spectrometry than by IR.

We further present the main aspects related to vibrational spectroscopy methods involved in polymorphic analysis.

IR spectroscopy has a long history in the characterization of pharmaceutical solids. It can be used to probe the nature of polymorphism on the molecular level, being useful in many cases where full crystallographic characterization is impossible [12]. The advantage of IR transmission spectroscopy on halide pellets is that only a small amount of sample is required (usually 1 mg) and a good spectrum can be obtained very quickly. But the great disadvantages are due to the pressure requirement to form the glassy disc and possible halide exchange between KBr (or KCl) and the sample of interest [13].

The diffuse-reflectance IR Fourier-transform spectroscopy (DRIFTS) method is the most used solid-state technique for pharmaceutical polymorphism analysis [14]. DRIFTS is used extensively in the MIR and NIR spectral regions. Samples may be investigated neat or diluted with a non-absorbing matrix, such as KBr or KCl (usually at a 1–5% w/w concentration). The great advantage of this method is that the polymorphic character remains intact due to the limited sample handling [15]. The technique involves irradiation of the sample by an IR beam, where the incident radiation undergoes absorption, reflection and diffraction by the particles of the sample. One disadvantage is that this technique depends on particle size.

Attenuated total reflectance (ATR) spectroscopy is a simple technique that can be used easily for solid-state characterization [16]. The IR beam is directed through the crystal, penetrating the surface of the sample a few micrometers or tenths of micrometers, so obtaining spectral information. An advantage of this technique is that it requires only a very small sample. The disadvantage is that cannot be used for polymorphic analysis, taking into account the penetration of the IR radiation through the sample.

NIR spectroscopy is another technique used for solid-state characterization, but the absorption bands arise from overtones or combinations of fundamental vibrations, in addition to combinations of overtones. Overtone absorption bands are the result of forbidden transitions arising from the ground vibrational energy level and are less intense than their corresponding fundamental absorption bands. Typically, NIR spectroscopy is used for the detection of water [17].

Raman spectroscopy needs very small samples (nanogram level) and sample preparation is simple, and powders must be interfaced with the laser beam. Sometimes, the information is influenced by fluorescent materials [18]. The Raman effect is relatively weak, so the material needs to be present to a level of ~1% for accurate measurements, whereas IR can be used to detect materials <1.0%.

The use of optical microspectroscopy is a natural course of action for any solid-state investigations to provide more information about a sample. Depending on the sample morphology, thickness and transmittance properties, a reflectance or transmittance spectrum can be acquired. The advantages of the technique are sampling is non-destructive, measurements are reflectance and/or transmittance, sample requirements are minimal, and solid-state transformations can be monitored with a hot-stage interface – all important for polymorphic analysis [16]. Analogous to IR microspectra, Raman spectra can be obtained from small amounts of sample using a Raman microprobe [18].

Recently, the most exciting new application of IR and Raman spectroscopy was chemical imaging of a two-dimensional area of a sample [8]. This technique relies on the interface of an optical microscope, equipped with a motorized stage, to an IR or Raman spectrometer, and a contour plot can be obtained showing the spatial position and concentration of a chemical entity.

For pharmaceutical polymorphism, the structure–property relationships are mainly governed by differences in the spatial arrangement of the constituent molecules in the crystal, and, in some cases, by variations in molecular conformation.

We can conclude that each technique presented above (IR, NIR and Raman) has its limitations that must be clearly understood before starting a solid-state characterization.

Table 1 summarizes the vibrational spectroscopy techniques used in polymorphic analysis, including their advantages and disadvantages [19].

3. Analytical applications

Several reviews were published in relation to characterization of pharmaceuticals using vibrational spectroscopy [10,11,19]. Different factors affect the process of crystallization and make it difficult to select a polymorph in a straightforward way. As a general rule, the crystallization of the most stable or the easiest form to crystallize is achieved and a polymorphic transition follows. To achieve the transition and to obtain the desired crystal form, water activity needed to be controlled [20]. Excipients from the pharmaceutical formulations play an important role in bioavailability, so they were studied too [21].

Calibration models were developed for dry solid mixtures and slurries of polymorphs. A good calibration practice (GCP) procedure was also proposed [22]. Polymorphic forms of the same chemical compound can have different physical and chemical properties that can strongly affect the manufacturing process. For this reason, determining and monitoring polymorphic transformations have become very important, especially in the pharmaceutical industry. Univariate and multivariate calibration techniques were

Table 1
Vibrational spectroscopy techniques used for solid-state characterization

Technique	Information	Advantages	Disadvantages
Mid- IR	- Intramolecular vibrations, H-bonding - Polymorphic forms: unique bands, peak shifting - Amorphous form: broadening of peaks - Complementary to Raman spectroscopy - Spatial chemical information with imaging set-up	- Small sample size - Relatively fast data acquisition - Availability of spectral libraries - Instruments readily available - No sample preparation required for ATR	- Sample preparation in FT-IR and DRIFTS can induce solid-state transformation - Interference from environmental humidity
Raman	- Intramolecular vibrations - Polymorphic forms: unique bands, peak shifting - Amorphous form: broadening of peaks - Complementary to IR spectroscopy - Spatial chemical information with imaging set-up	- Small sample size - No sample preparation required - Non-destructive method - Ability to penetrate through glass containers - Insensitive to water – experiments in aqueous environment possible - Relatively fast data acquisition - Fiber-optic probes available - At low frequencies Raman spectroscopy can be used to analyze particulate level properties (lattice vibrations)	- Local heating of sample - Sample fluorescence - Photodegradation
NIR	- Overtones and combinations of vibrations in the MIR region - Sensitive to different water states - Spatial chemical information with imaging set-up	- Fast data acquisition - Non-destructive method - No sample preparation required - Ability to penetrate glass containers - Fiber-optic probes available - Can also be used for particulate level (e.g., particle size) and bulk level (e.g., water content) measurements with proper calibration	- Low sensitivity and selectivity (weak intensity) - Significant baseline slope - May require chemometrics to analyze NIR spectra

investigated using pre-processing techniques to optimize the signal. The results are combined in a systematic GCP procedure, proposed for the first time in this work. The approach has been applied for the quantitative monitoring of the polymorphic transformation of ortho-aminobenzoic acid (OABA).

Next, we present some recent articles related to the use of vibrational spectroscopy for studying different drugs that contain pharmaceutical active principles known to have different polymorphic forms.

Acetaminophen [N-acetyl para-amino phenol (APAP)] is known to have three crystalline polymorphs [23]. Monoclinic type I APAP is the thermodynamically-stable polymorph found in commercial products. Orthorhombic type II APAP is a metastable polymorph that is stable on a timescale of months in powder form at room temperature [24]. Types I and II of APAP can be distinguished from one another by their melting temperatures, X-ray structures and spectroscopic characteristics. Type III APAP is highly unstable, and its single-crystal X-ray structure has not been determined to date, but it has been observed spectroscopically in thin-film samples, and powder X-ray diffraction patterns have been published [25]. Kauffman et al. [26] reported recently the simultaneous application of DSC and Raman microscopy for the observation of thermally-driven transitions between polymorphs of pharmaceutical materials. Raman-detected DSC (RD-DSC) was used to monitor the DSC thermograms of super-cooled liquid acetaminophen and confirmed the assignment of two exothermic transitions to specific polymorphic transformations. Principal component analysis (PCA) of the Raman spectra were used to determine the number of independent components that participated in the phase transformations, and multivariate regression was used to determine transition temperatures from the spectral data. The results demonstrated the advantage of simultaneous Raman spectroscopy and DSC for the unambiguous assignment of thermally-driven polymorphic transformations.

A spectroscopy method based on FT-NIR spectroscopy and chemometric techniques was introduced to classify paracetamol preparations according to polymorphic changes [27]. X-ray diffraction (XRD) and FT-NIR studies were carried out on standard samples, paracetamol preparations (acetaminophen tablet), and also the additives. A direct comparison was performed between the spectroscopic data and those obtained by XRD. The NIR and XRD analyses of paracetamol preparations showed some distinct differences, particularly in the Iranian tablet. These differences were related to polymorphism and paracetamol purity.

Ampicillin or α-aminobenzyl penicillin belongs to the antibiotic penicillin class, which possesses a β-lactam ring in its structure. Ampicillin may exist in two anhydrous polymorphic forms (γ form also named B or I; δ form also named II) and hydrate forms (trihydrate; monohydrate or form A) [28]. The trihydrate and anhydrous form I have been the most studied forms, while the monohydrate and anhydrous form II have not received similar attention. Amorphous monohydrate and three crystalline forms, the trihydrate, the crystal form I and the crystal form II, were investigated by FT-IR and micro-Raman [29]. Also, data obtained by DSC, TGA, XRPD and hot-stage Raman spectroscopy were reported. Finally, quantum mechanical calculations were performed by density functional theory to assist the assignment of spectroscopic experimental bands. Experimental and theoretical results allowed identification of specific features for polymorph characterization. Bands typical of the different polymorphs were identified in IR and Raman spectra.

Bicalutamide is a pharmaceutically-active compound that possesses anti-androgenic activity and is very useful in treating prostate cancer. In a recent paper, Vega et al. focused their interest on the stability of two crystalline forms and an amorphous phase of bicalutamide, providing some physical information that supported the temperature-stability-diagram interpretation [30]. Fig. 3 shows the Raman spectra for forms I and II, where a characteristic maximum was observed for form I at very low wavenumber (around 150 cm^{-1}) and three characteristic maxima appear for form II (around 600 cm^{-1}, 1450 cm^{-1} and 1700 cm^{-1}). Moreover, as expected in this case because of the conformational polymorphism, some other differences were observed in a medium region (500–1700 cm^{-1}) as a fingerprint that provides several features to distinguish between both polymorphs.

A detailed overview on vibrational spectroscopy studies of polymorphic forms of carbamazepine based on literature data followed by new experimental and theoretical results was presented [31]. Raman, FT-MIR and, for the first time, FT-far-IR (FT-FIR) spectra of carbamazepine were measured and interpreted by means of the

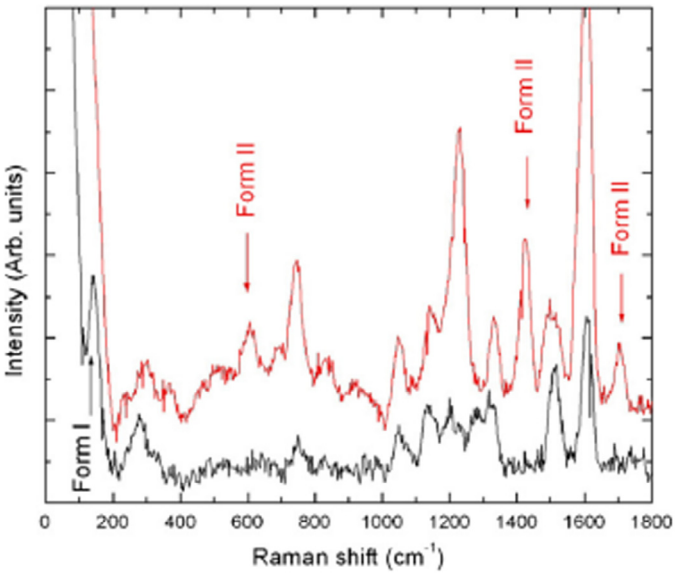

Fig. 3. Room-temperature Raman spectra of forms I and II of bicalutamide.

B3LYP/6–311++G** theoretical calculations combined with the potential energy-distribution analysis. Relatively good agreement was found between experimental and theoretical spectra.

The application of Raman spectroscopy and PCA was investigated for the quantitative analysis of polymorphic mixtures of carbamazepine forms III and I, based on individual peak areas and heights in the Raman spectra and whole wavenumber ranges [32]. Various linear regressions indicated that generally using every fourth wavenumber value of a larger region of the Raman spectrum gave better quantitative results than choosing defined peak areas and peak heights, as the spectral differences between the polymorphs were only subtle. This study demonstrated that PCA of Raman spectroscopic data provided a sensitive method for the quantitative analysis of polymorphic forms, with a limit of quantitation (LOQ) < 2%, if the spectral input data were selected carefully.

Cimetidine, one of the first blockbuster drugs, is (Z)-N"-cyano-N-methyl-N'[2-[[(5-methyl-1H-imidazol-4-yl)methyl]thio]ethyl] guanidine, and is a specific competitive antagonist of the histamine H2-receptor at the parietal cells, which inhibits the histamine-stimulated secretion of gastric acid and reduces pepsin output. Cimetidine has several relevant solid-state forms, including four polymorphs (A, B, C and D), an amorphous form (AM) and a monohydrate (M1). Dehydration of M1 has been reported to yield mixtures of polymorphs A, B and C or just a single form [33]. It was demonstrated that, under the experimental conditions, dehydration of M1 initially resulted in the formation of polymorph B; after melting and upon cooling, polymorph B yielded an AM. It was concluded that the ATR-FTIR in association with a multivariate curve resolution (MCR) is a promising, useful technique for monitoring solid-state phase transformations.

The feasibility of applying ultraviolet-visible and shortwave NIR diffuse-reflectance spectroscopy (UV-Vis-SWNIR-DRS) coupled with chemometrics for qualitative and simultaneous quantitative analysis of drug polymorphs, using cimetidine as a model drug, was also investigated [34]. This research indicated that UV-Vis-SWNIR-DRS can be used as a simple, rapid, non-destructive qualitative and quantitative method for the analysis of drug polymorphs.

ATR-FTIR was coupled to PCA and used for the classification of physical mixtures of Cimetidine and excipients, as well as laboratory-made and commercial tablets, according to their polymorphic composition [35]. It was demonstrated that two principal components (PCs) suffice to classify the samples of the four forms of cimetidine into distinct groups, and that method performance is optimum when the second and third PCs are used for the classification process. The application of the method for commercial tablets containing cimetidine also gave good results, confirming that they employed the correct polymorph (form A).

Raman spectroscopy with far-red excitation has been used to study seized, tableted samples of MDMA (*N*-methyl-3,4-methylenedioxyamphetamine) and related compounds [MDA (3,4-methylenedioxyamphetamine), MDEA (N-ethyl-3,4-methylenedioxyamphetamine, MBDB (N-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine), 2C-B (4-bromo-2,5-dimethoxyphenethylamine)] and amphetamine sulfate), as well as pure standards of these drugs [36]. It was noticed that by using far-red (785 nm) excitation the level of fluorescence background even in untreated seized samples is sufficiently low that there is little difficulty in obtaining good quality data with moderate 2 min data accumulation times. The spectra can be processed to give unambiguous identification of both drug and excipients (even when more than one compound has been used as the bulking agent) and the relative intensities of drug and excipient bands can be used for quantitative or at least semi-quantitative analysis.

Indomethacin can exist in at least two non-solvated monotropic polymorphic crystalline forms: the γ-form, which is stable at room temperature, and the metastable α-form [37]. A study assessed the ability of vibrational spectroscopy combined with multivariate analysis to quantify ternary mixtures of different solid-state forms, including the amorphous form [38]. Raman and NIR spectroscopy were used to quantify mixtures of α-, γ-, and amorphous indomethacin. Partial least squares (PLS) regression was employed to create quantitative models. The results show that both spectroscopic techniques in combination with multivariate modeling are well suited to rapidly quantify ternary mixtures of crystalline and amorphous indomethacin. Furthermore, this study shows that quantitative analysis of powder mixtures using Raman spectroscopy can be performed in the presence of limited fluorescence.

Furosemide, a loop diuretic drug, indicated for the treatment for hypertension, edema, and nephrotic syndrome, was studied to evaluate the ability of NIR chemical imaging (NIR-CI), NIR (NIR), Raman and ATR IR (ATR-IR) spectroscopy to quantify three polymorphic forms (I, II, III) in ternary powder mixtures [39]. For this purpose, PLS regression models were developed, and different data preprocessing algorithms such as normalization, standard normal variate (SNV), multiplicative scatter correction (MSC) and 1st to 3rd derivatives were applied to reduce the influence of systematic disturbances. This study proves that NIR-CI, NIR, and Raman are well suited to quantify forms I–III of furosemide in ternary mixtures as shown in Fig. 4. Because of the pressure-dependent conversion of form II to form I, ATR-IR was found to be less appropriate for an accurate quantification of the mixtures.

Mebendazole, {methyl-(5-benzoyl-1H-benzimidazol-2yl) carbamate} is a synthetic broad-anthelmintic drug, active against nematodes and cetodes, being indicated in the treatment of single or mixed infestation by *Enterobius vermicularis*, *Trichuris trichiura*, *Ascaris lumbricoides*, *Ancylostoma duodenale*, *Necator americanus*, *Strogyloides stercoralis*, *Taenia app.* in human and veterinary therapeutics. An FT-IR spectrometric method, diffuse reflectance IR Fourier transform spectroscopy (DRIFTS), was developed for the rapid, direct measurement of mebendazole in drugs [40]. Conventional KBr spectra and DRIFTS spectra were compared for best determination of active substance in drug formulations. DRIFTS-spectra were chosen because better differences of the spectra were observed in this case, as can be seen in Fig. 5. Two chemometric approaches, partial least-squares (PLS2) and principal component regression (PCR+) methods were used in data processing. The best results were obtained with the PLS2 method.

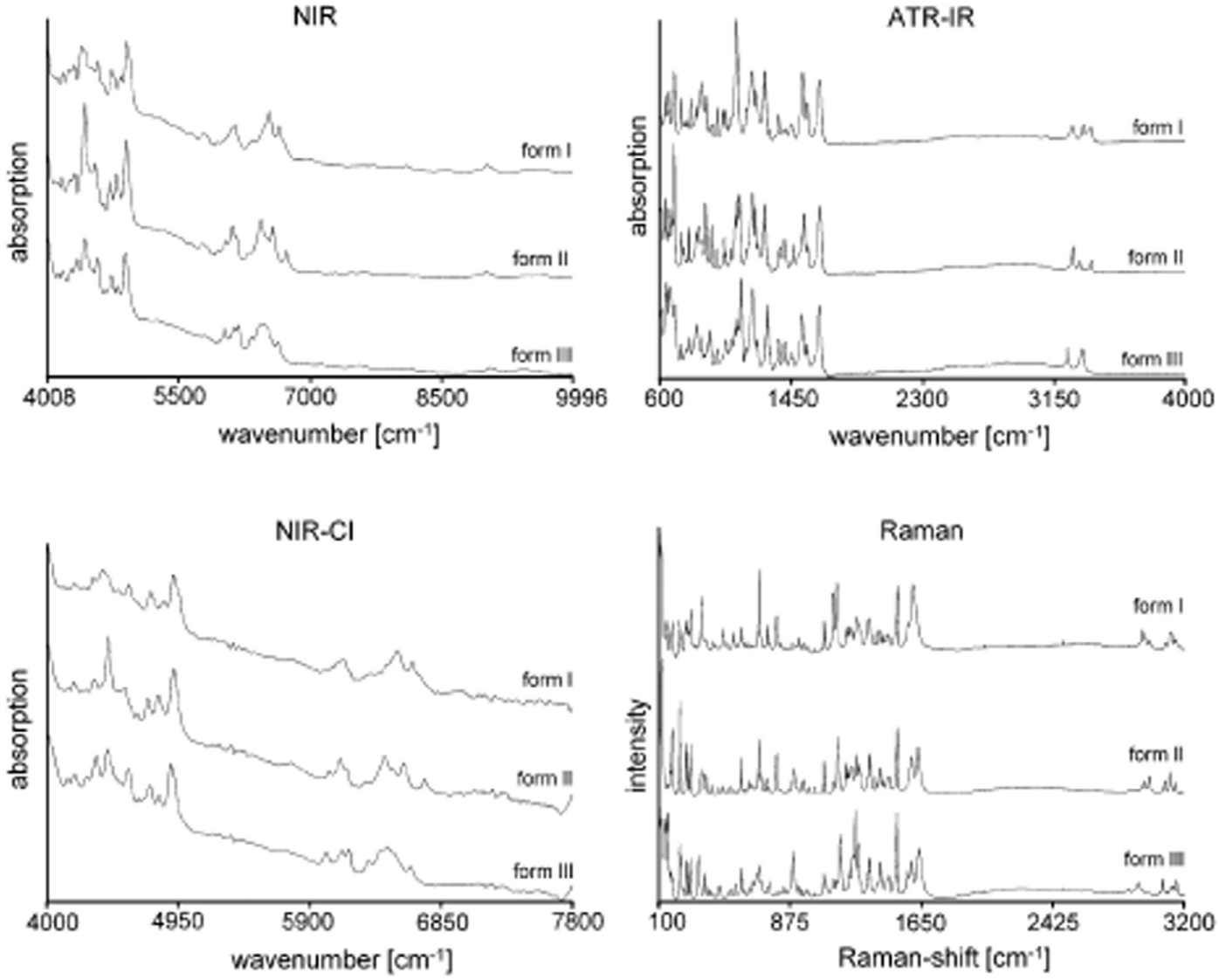

Fig. 4. Spectra of pure polymorphic forms I, II, and III of furosemide using different spectroscopy techniques.

In another study, a simple method based on DRIFTS and artificial neural network (ANN) modeling was developed for the simultaneous quantitative analysis of mebendazole polymorphs A–C in powder mixtures [41]. Spectral differences between the polymorphs elucidated by computationally assisted band assignments on the basis of quantum chemical calculations, and subsequently, the spectra were preprocessed by calculation of 1st and 2nd derivatives. Application of the ANN to commercial samples of raw material and formulation (suspension) proved its suitability for the prediction of polymorphic content.

Mebendazole mesylate monohydrate (MEB), a new stable salt of mebendazole, has been synthesized and fully characterized [42]. It was obtained from recrystallization of MEB forms A, B, or C in diverse solvents with the addition of methyl sulfonic acid solution. The vibrational behavior is consistent with the crystal structure, the most important functional groups showing shifts to lower or higher frequencies in relation to the MEB polymorphs. Thermal analysis indicates that the compound is stable up to 50°C.

Piroxicam (4-hydroxy-2-methyl-N-(2-pyridyl) 2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide) is a nonsteroidal anti-inflammatory and analgesic drug that presents an interesting case of polymorphism. Three polymorphic forms (I, II and III) and one monohydrate form can be obtained by crystallization from saturated solutions in various solvents [43]. Since polymorphism can change the pharmacokinetic and pharmacodynamic characteristics of piroxicam [44], it is important to develop quality control methodologies for the polymorphic determination of this drug [45]. NIR spectroscopic and multivariate statistical control charts based on the net analyte signal (NAS) were applied to the polymorphic characterization of piroxicam samples. Three different polymorphic forms (I, II and III) were studied, using XRPD and scanning electron microscopy as reference techniques. The use of multivariate control charts provides a rapid evaluation of purity and the polymorphic composition of pharmaceutical formulations based on piroxicam.

In another work, piroxicam polymorphic forms 1 and 2 were studied using NIR chemical imaging (NIR-CI) technology to map the distribution of both species in pharmaceutical formulations [46]. In this direction, the PLS method was used to construct calibration models of concentrations per pixel of the sample. The RMSEP (Root-Mean-Square Error of Prediction) results for both models of the polymorphic forms remained below 4% (w/w). It was also possible to distinguish local and global information of the constituents through this method, as can be seen in Fig. 6. These results seem

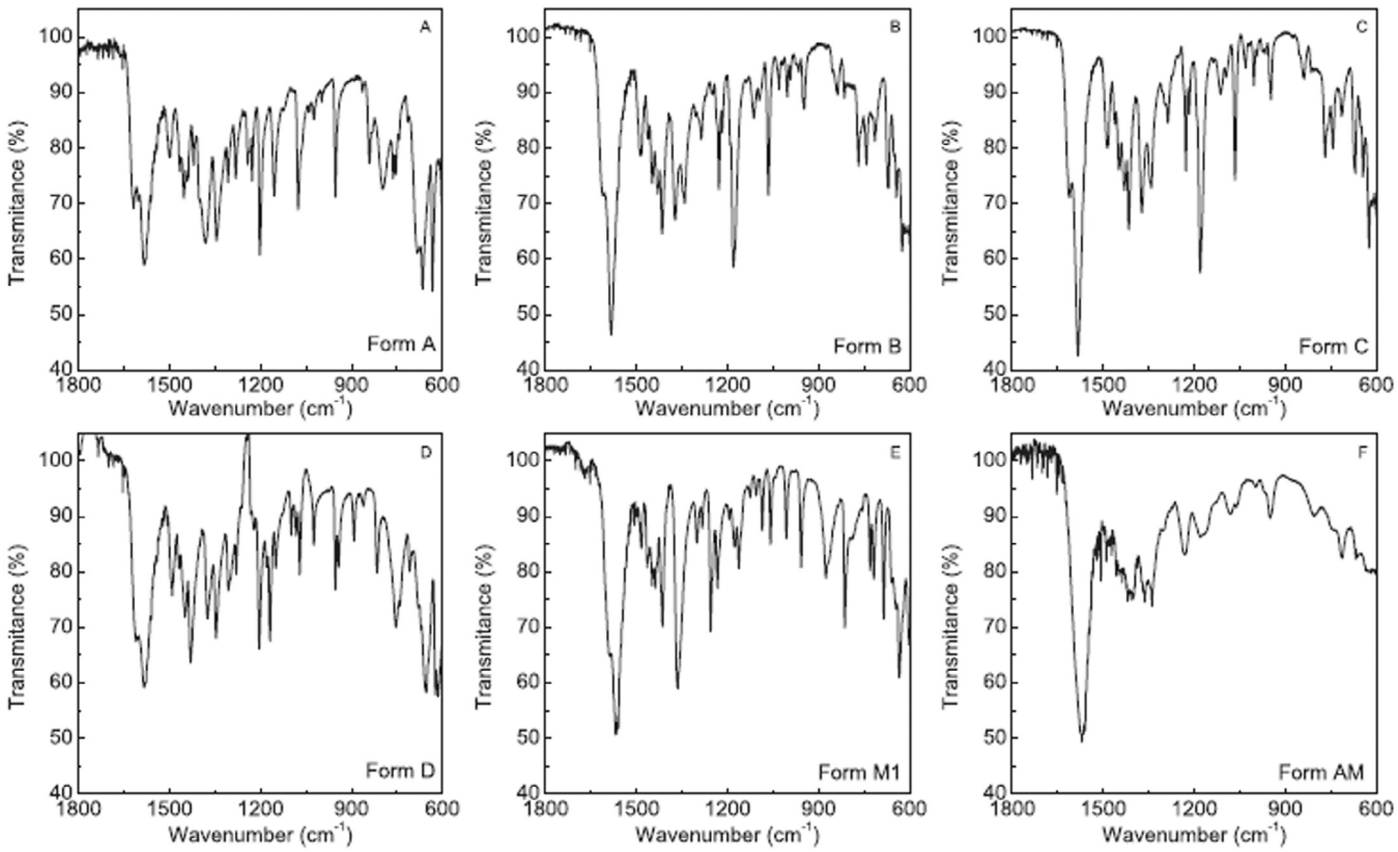

Fig. 5. Attenuated total reflectance (ATR)-Fourier-transform infrared (FTIR) spectra of different forms of cimetidine. *Note*: Spectra correspond to the polymorphs: A (A), B (B), C (C) and D (D); monohydrate M1 (E) and amorphous AM (F).

to be a suitable tool for quality process control and final product quality assurance.

Ranitidine hydrochloride exists as two polymorphs, forms I and II, both of which are used to manufacture commercial tablets. Raman spectroscopy can be used to differentiate the two forms but univariate methods of quantitative analysis of one polymorph as an impurity in the other lack sensitivity. PCA was applied to Raman spectra of binary mixtures of the two polymorphs and binary mixtures prepared by adding one polymorph to powdered tablets of the other [47]. This study demonstrated that PCA of Raman spectroscopic data provided a sensitive method for the quantitative analysis of polymorphic impurities of drugs in commercial tablets with an LOQ of less than 2%.

Chieng et al. [48] developed a reliable quantification procedure for mixtures of three solid forms of ranitidine hydrochloride using XRPD and Raman spectroscopy combined with multivariate analysis. The effect of mixing methods of the calibration samples on the calibration model quality was also investigated. PCA was used to study the effect of the mixing method, while PLS regression was used to build the quantification models. MSC without scaling gave the

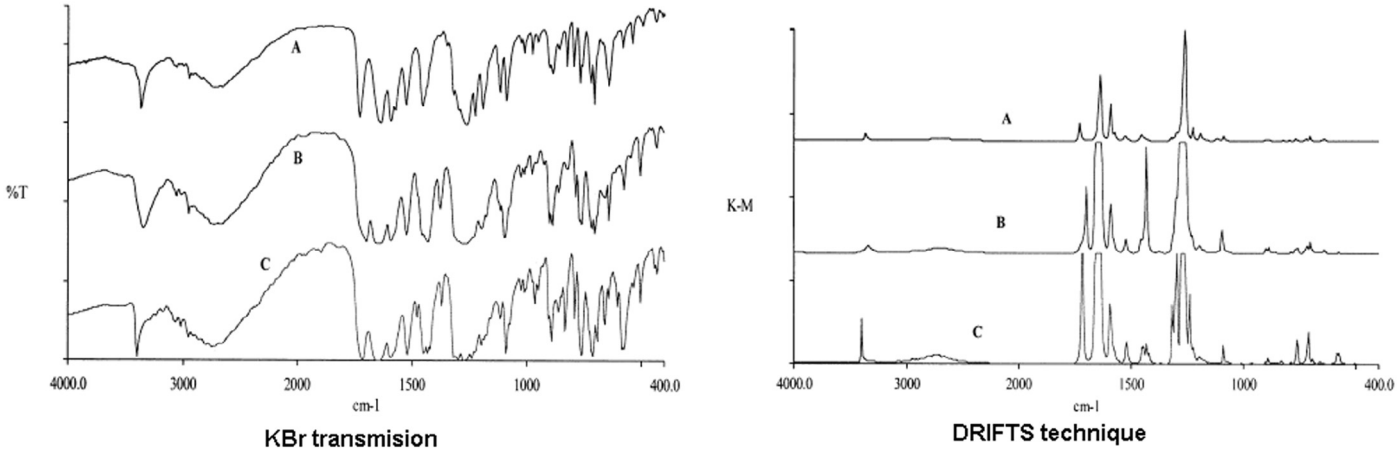

Fig. 6. Infrared spectra of mebendazole A, B, C polymorphs in blend with potassium bromide (KBr) using different spectroscopy techniques.

best PLS regression model for XPRD, and standard normal variate (SNV) transformation with centering gave the best model for Raman spectroscopy. Raman spectroscopy gave better PLS regression models than XRPD, allowing more accurate quantification.

McGoverin et al. [49] investigated the accuracy of NIR spectroscopy when quantifying polymorphic binary mixtures of ranitidine hydrochloride. Ranitidine-hydrochloride polymorphs may be identified and quantified from NIR spectra, but not to the level of accuracy displayed by Raman spectroscopy.

The simultaneous quantitative analysis of sulfathiazole polymorphs (forms I, III and V) in ternary mixtures by ATR-IR, NIR and Raman spectroscopies combined with multivariate analysis was reported [50]. To reduce the effect of systematic variations, four different data pre-processing methods (MSC, SNV, and first and second derivatives) were applied and their performance evaluated using their prediction errors. NIR spectroscopy exhibited the smallest analytical error, highest accuracy and greatest robustness. When these advantages are combined with the greater convenience of the "in glass bottle" sampling method of NIR, both ATR-IR and Raman methods appear less attractive.

A combination of DSC and hot-stage microscopy with image analysis was used to investigate the polymorphism of sulfathiazole [51]. The results of the experiments proved that sulfathiazole tended to crystallize as mixtures of polymorphs, even though the literature methods for producing pure polymorph were followed.

The quality assurance of a product during the whole development and manufacturing cycle of pharmaceuticals through increased level of understanding the process is the main aspect to be considered within process analytical technology (PAT). Development of new tools using, e.g., multivariate methods for quality control at different steps of the manufacturing process are very important [52]. In this context, DRIFT-IR spectroscopy together with multivariate statistical process control (MSPC) analysis, soft independent modeling of class analogy (SIMCA), orthogonal signal correction (OSC), preprocessing and partial least squares (PLS) regression methods were applied to the polymorphic characterization of crystalline bulk product. XRPD was used as a reference technique. The use of several multivariate techniques in rapid evaluation of purity and polymorphic composition of bulk samples was demonstrated.

4. Conclusions

In recent decades, the importance of polymorphism became evident in the pharmaceutical industry. Well-established analytical techniques available for quantification purposes in solid-state analysis were extensively utilized and described. In particular, vibrational spectroscopic techniques, such as Raman, NIR and MIR spectroscopies are important because they allow differences to be probed on the molecular level while being fast and non-destructive. Raman and NIR spectroscopies offer advantages over other spectroscopic techniques for PAT applications, since they can be readily coupled to probes and require minimal sample preparation. The role of spectroscopy in the quantitative and structural analysis of pharmaceutical solids will increase in the future.

In the past decade, vibrational spectroscopic techniques, including IR, NIR and Raman, became popular for detecting, characterizing and monitoring solid-state transformations. Studies focused on solid-state conversions that occur during steps of pharmaceutical manufacturing, such as crystallization, wet granulation, milling, freeze-drying, spray-drying and tableting, and tried to shed light on the mechanism and the kinetics of transformations and factors that influence the conversions. In this context, vibrational spectroscopy has several advantages over other commonly used analytical methods, including thermal techniques and XRPD.

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