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Utilization of Fourier transform-Raman spectroscopy for the study of pharmaceutical crystal forms[1]

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Abstract

It is well understood that the solid state physical characterization of a drug substance is necessary for successful development and approval of a pharmaceutical product AAPS [1]. Physical analytical techniques used include: XRD, IR, DSC, TG, and NMR. Recently, Fourier transform (FT) Raman spectroscopy has become a more common technique. Complimentary to IR, FT-Raman can be used to differentiate between different crystal forms of a drug substance. FT-Raman exhibits several advantages over IR and the other physical analytical techniques. Very little sample is required with no preparation (dilution), analysis time is quick, and since water is a weak scatter (Raman spectrum of water contains three low intensity peaks), crystallization studies of drug substances from aqueous solutions can be performed. Additionally, through the use of a variable-temperature accessory, phase diagrams can be determined for crystal systems, leading to further characterization of those systems. This paper introduces the use of FT-Raman spectroscopy for pharmaceutical development activities. Specific examples will be shown for investigations of crystal forms (qualitative and quantitative) and crystallization studies.

Keywords: Fourier transform-Raman spectroscopy; Pharmaceutical crystal forms; Crystallization; Quantitative analysis; Qualitative analysis

1. Introduction

Fourier transform (FT) Raman spectroscopy is becoming increasingly popular in the pharmaceutical industry for the analysis of a wide variety of materials [2–4]. Along with several other physical analytical techniques such as X-ray powder diffraction (XRD), infrared (IR) spectroscopy, differential scanning calorimetry (DSC), thermogravimetric analysis (TG), and nuclear magnetic resonance (NMR) spectroscopy, FT-Raman has been used for the characterization of drug substances [2]. Raman spectroscopy provides vibrational spectroscopic information complimentary

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to that obtained from IR analysis. It also demonstrates several advantages over traditional IR analysis of materials. Like IR, Raman spectroscopy can be used to differentiate solid-state pharmaceutical crystal forms. With the addition of specialized sampling accessories, the nature of crystal formation can be studied or a quantitative crystal form analysis performed. Raman spectroscopy can also be used for pharmaceutical development and clinical manufacturing support. This paper will describe how Raman spectroscopy can be used in the pharmaceutical analytical laboratory for drug substance characterization and quantitative crystal form method development.

2. Experimental

2.1. Instrumentation

All Raman spectra were acquired on a Nicolet model 950 Fourier transform Raman spectrometer. A water-cooled Nd:YAG laser (1064 nm excitation wavelength) set between 0.5–1.0 W of power was used to excite the sample. The specific laser power used for spectral acquisition of presented spectra is listed in each figure caption. The spectrometer utilizes a CaF_2 beamsplitter and a liquid nitrogen cooled Ge detector.

For variable temperature (VT) spectroscopic analysis, an AABSPEC model 95S-NIR-RAM Multi-mode cell was interfaced to the spectrometer. The temperature of the cell was regulated with the AABSPEC Digital Temperature Controller and Programmer (-120–200°C).

Qualitative and quantitative crystal form analysis utilized a Step-n-Repeat sampling accessory (Nicolet Instrument Corp., Madison, WI). This sampling accessory allows the operator to obtain spectra from various locations of the powdered sample, thus providing a more representative spectrum of the material (vida infra). The powdered sample is placed into a gold-coated sample cup (13 mm diameter, 2 mm depth), which is then placed in the accessory. The focus of the excitation laser is set by adjusting the height of the sample cup, and the circumference of rotation is adjusted by horizontal positioning of the sample cup. The number of steps per rotation is controlled through Nicolet OMNIC software.

2.2. Sample preparation

Variable-temperature crystallization studies were performed using a supersaturated menthol in ethanol solution. Menthol and ethanol were both obtained from Aldrich Chemical (Milwaukee, WI). The supersaturated menthol solution was prepared by adding excess menthol to warmed ethanol. The solution was then transferred to a 1 cm long glass NMR tube (Wilmad Glass, Buena, NJ). The inside surface of the glass tube was scratched lightly with aluminum oxide paper before insertion of the sample to help promote crystallization. The calibration samples for the quantitative Raman experiment were prepared using the previously described 'slurry technique' [5].

3. Results and discussion

3.1. Infrared and Raman spectroscopy

Infrared and Raman spectroscopies together provide complete vibrational information about a molecule. A vibration is infrared active when there is a change in the molecular dipole during the vibration. A vibrational mode is Raman active when there is a change in polarizability during the vibration.

Cyclohexane provides a good example for showing the complementary nature of IR and Raman active vibrations (Fig. 1). The CH_2 antisymmetrical and symmetrical vibrations, found at 2933–2915 and 2897–2852 cm^{-1}, are both IR and Raman active. The CH_2 scissoring motion at 1450 cm^{-1} has a strong IR absorption peak, and a medium Raman peak. The antisymmetric ring stretching at 903 cm^{-1} gives a strong IR peak, and the ring breathing motion of the chair form of cyclohexane gives a strong Raman peak at 802 cm^{-1} [6]. This is just a simple example of how IR and Raman spectroscopy can be used to provide complete vibrational spectral characterization of materials.

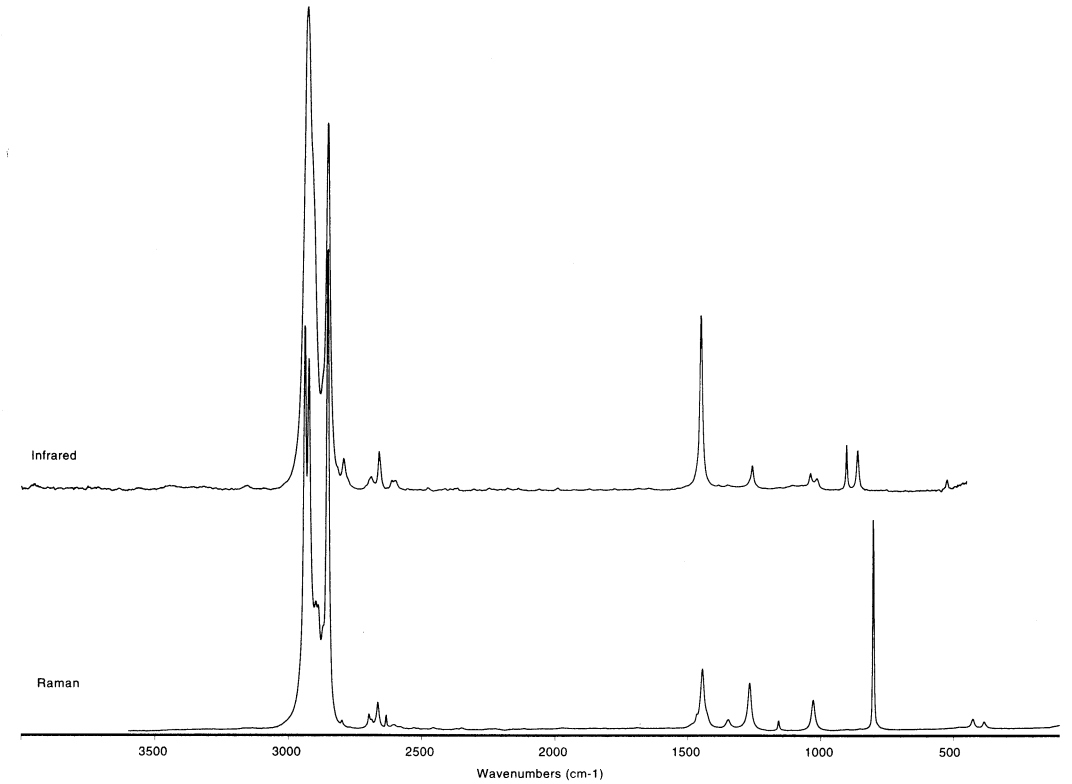

Fig. 1. Infrared and Raman spectra of cyclohexane. The Raman spectrum was acquired at a laser power of 1.0 W.

3.2. Advantages of Raman spectroscopy

Raman spectroscopy has several advantages over traditional infrared techniques. Like mid-infrared spectroscopy, Raman spectroscopy provides a direct probe of the molecular structure of the compound. Near-IR spectroscopy, often mistouted for identity testing and other applications because of its ease of use [7–10], displays only overtones and combinations of fundamental molecular vibrations [11]. Near-IR does not give a direct probe of the molecular structure.

Like near-IR, sample preparation for Raman analysis is minimal. Samples are often packed in a glass NMR tube, but they can also be analyzed directly in containers such as glass bottles, plastic bottles, and blister packs [4]. Sampling for Raman spectroscopy is further eased through the use of fiber optics. Although fiber optics have been developed and widely used for near-IR spectroscopy for years, optical limitations have prevented wide acceptance of mid-IR fiber optics [12]. Since most FT-Raman spectrometers use near-IR excitation and detection, fiber optics are widely available and useful. Fiber optic Raman spectroscopy can be used for process analysis in on-, at-, and in-line applications, and in a 'loading/receiving dock' environment for identity testing.

One of the major advantages that Raman spectroscopy has over IR spectroscopy is the ability to analyze samples in aqueous environments. Since water is a weak scatterer displaying three low intensity Raman peaks, in situ and other aqueous-based analyses are possible where water often overwhelms spectral features of interest in an IR spectrum. In pharmaceutical product development, Raman spectroscopy can be used in a variety of ways. Raman can be used for drug

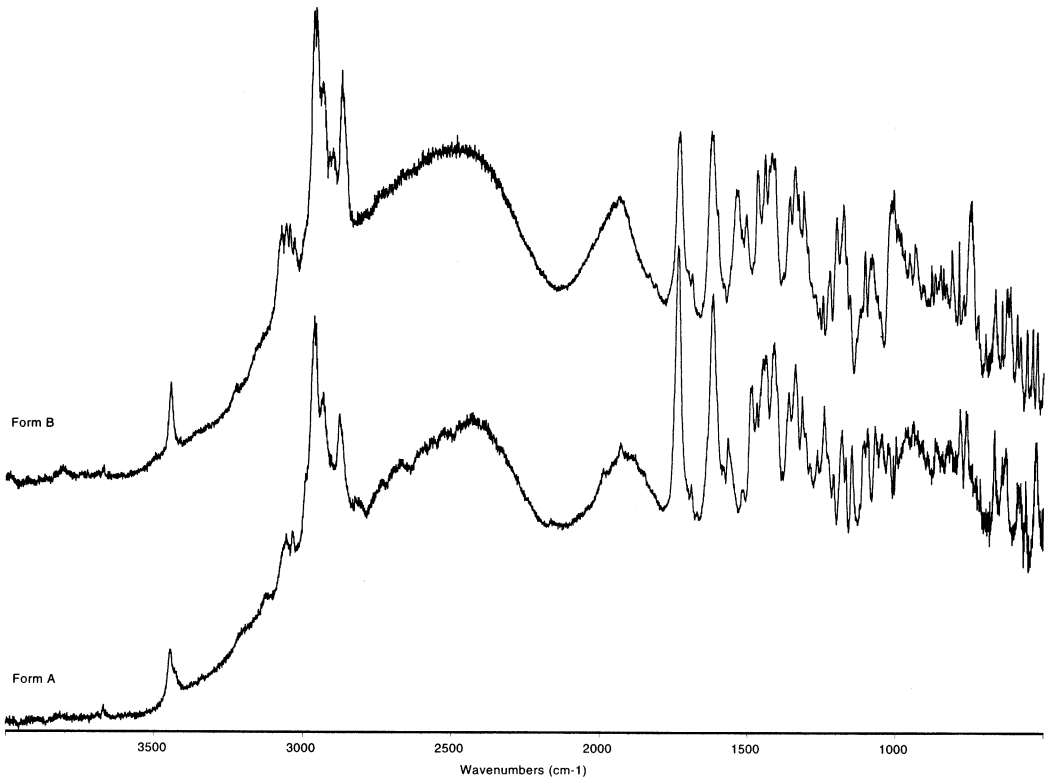

Fig. 2. Diffuse reflectance IR spectra of Forms A and B of a developmental compound.

candidate characterization, quantitative crystal form analysis, and other investigative studies.

Various crystal forms of a drug substance (including anhydrates, hydrates, and solvates) may display different physical properties such as melting point, solubility, density, morphology, stability, and bioavailability [5]. Physical analysis of drug substances has traditionally been performed using IR, XRD, DSC, TG, and NMR. FT-Raman spectroscopy provides another method for analyzing these types of materials. Together with mid-IR spectroscopy, Raman provides the complete fundamental vibrational analysis of a sample, leading to a more complete characterization.

Figs. 2 and 3 show an example of how IR and Raman can be used together for the characterization of drug substances. Fig. 2 shows the IR spectra (diffuse reflectance) of forms A and B of a developmental compound. Fig. 3 shows the Raman spectra of the same two samples. From the spectra, it is evident that the two different crystal forms can be differentiated by either technique, and that a quantitative method for detecting form B in bulk A material can be developed using either technique. Thus, Raman spectroscopy is another tool that the pharmaceutical analytical chemist can use for a variety of analyses. Although the advantages that Raman has over the more traditional IR spectroscopic techniques are numerous, it should not be seen as the one technique to solve all problems, but as one technique in a multidisciplinary approach to analysis.

3.3. Variable-temperature Raman spectroscopy

One of the advantages of Raman spectroscopy is the spectral range covered by a FT-Raman instrument. Traditional mid-IR spectrophotometers have a spectral range of 4000–400 cm^{-1}. Far-IR spectrophotometers have a spectral range

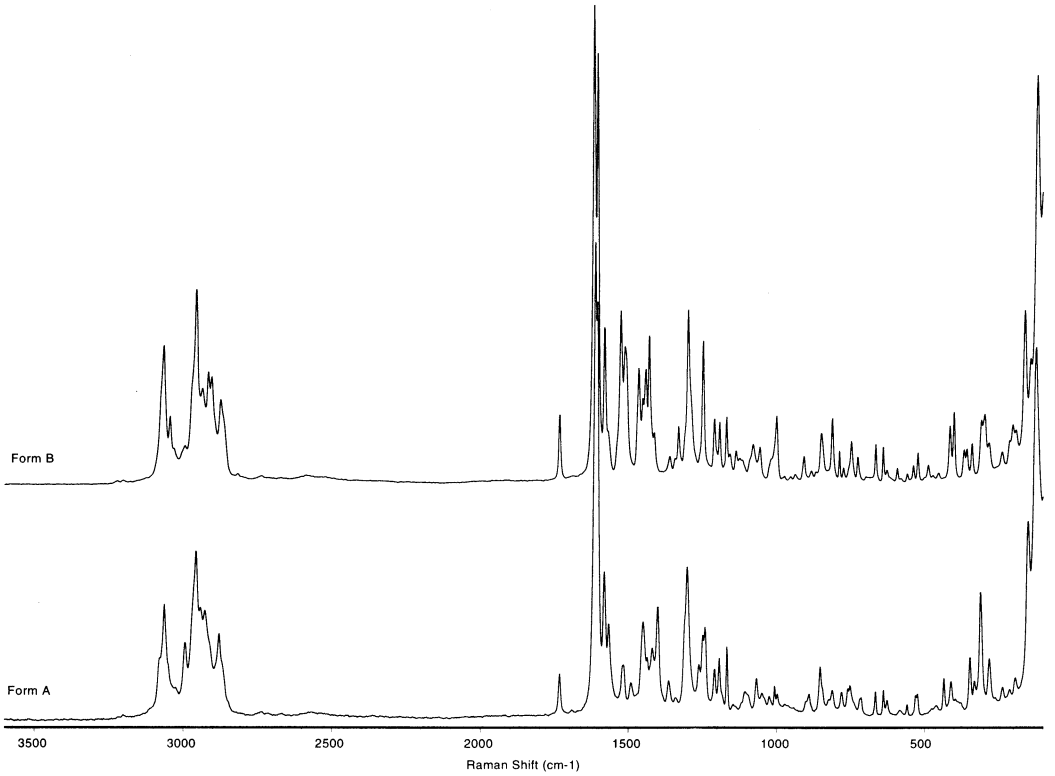

Fig. 3. Raman spectra of Forms A and B of a developmental compound. The Raman spectra were acquired at a laser power of 1.0 and 1.1 W, respectively.

of 500–50 cm^{-1}, but are not as easy to use due to less than optimum optics. Raman spectrometers cover the spectral range of 3600–25 cm^{-1}. It is this 500–25 cm^{-1} spectral range where Raman spectroscopy can be useful for the study of low frequency lattice vibrations.

Lattice vibrations of molecules help define the crystal structure of a molecule, including the space group and unit cell of the crystal. The vibrations of the crystal lattice can be either IR or Raman active, and are found below 300 cm^{-1} [13,14]. Within the low frequency region of the vibrational spectrum, other vibrational modes are also observed such as ring vibrations, heavy atom fundamental vibrations, and pure rotational spectra for gaseous samples [15]. One should not misinterpret lattice vibrations with these other molecular vibrations/rotations. Nonetheless, because of its spectral range, Raman spectroscopy is a good technique for studying these lattice vibrations.

The preferential formation of one crystal form of a compound over another is sometimes regulated by temperature [16]. With an accessory that controls the temperature of the sample, Raman spectroscopy can be used to study this dependence. This paper describes how variable temperature Raman spectroscopy was used to study the dynamics of crystallization of a pharmaceutical excipient from a solvent.

Menthol was selected as a model compound to study the effects of temperature on crystallization of a compound from a solvent. Menthol is a pharmaceutical excipient that is used as a flavor enhancer [17]. It displays well resolved IR and Raman spectra. Menthol is highly soluble in ethanol [17], and all experiments were performed using a supersaturated menthol in ethanol solu-

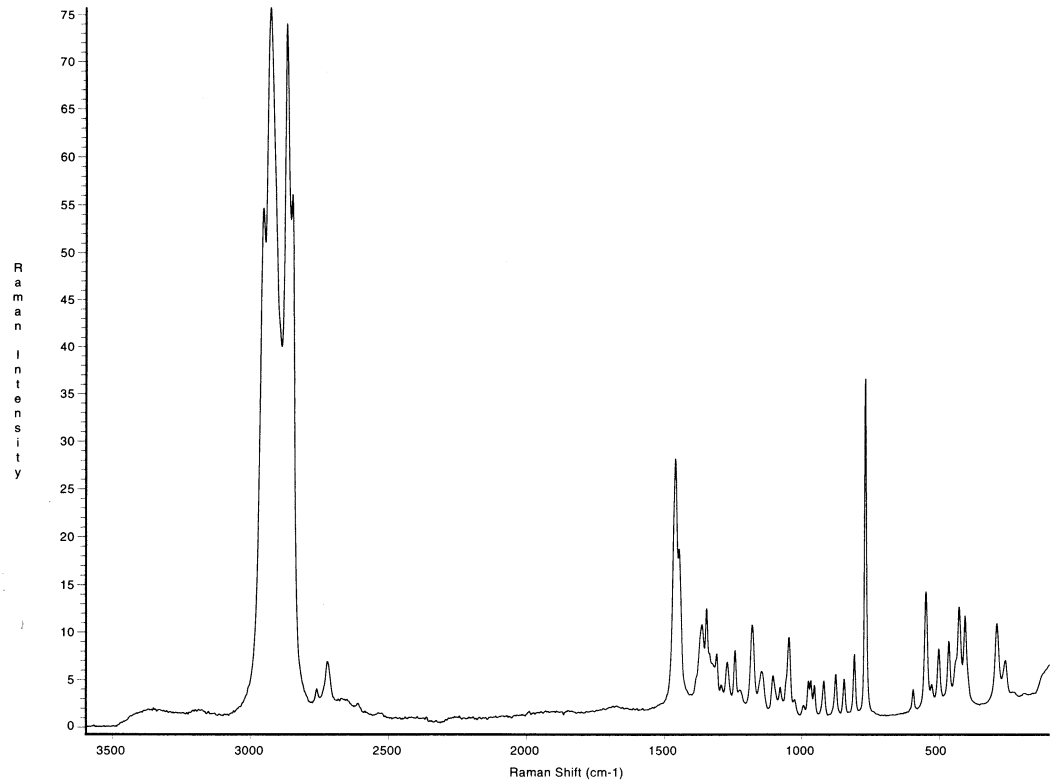

Fig. 4. Raman spectrum (acquired at a laser power of 1.0 W) of menthol supersaturated in ethanol.

tion. Fig. 4 shows the Raman spectrum of the supersaturated menthol solution.

As the solution was cooled, crystals of menthol formed. Fig. 5 displays the VT-Raman spectra of the supersaturated menthol solution at 30, 26, 25, 23, and 20°C, as well as bulk crystalline menthol. At 25°C, the first indication of crystallization is evident with a peak at 293 cm^{-1} increasing in intensity. At 20°C, peaks at 293 and 266 cm^{-1} are evident in the spectrum. Both of these peaks correlate with peaks found in crystalline menthol. These peaks are only observed as the crystalline menthol is formed, suggesting that they are lattice vibrations of the menthol crystal. Further theoretical calculations are in progress to determine the vibrational frequencies attributed to the lattice.

The potential future applications for VT-Raman spectroscopy are many. By changing the cooling rate of a supersaturated solution, it is possible to study the dynamics of crystallization of a polymorphic system, providing insight into why one polymorphic form is preferred over another under particular conditions. By increasing the temperature of a sample over ambient conditions, it is possible to study the temperature effects on actives and excipients in drug products. Temperature effects on drug substance synthesis procedures can also be studied.

3.4. Quantitative Raman spectroscopy

Raman spectroscopy can also be a quantitative technique. Quantitative solid-state Raman method development is similar to that of solid-state IR (usually diffuse reflectance) method development. Some of the criteria that must be critically considered when developing a quantitative method are homogeneous sample mixing, particle size, and instrument variability and reproducibility [5]. Each of these factors, if not con-

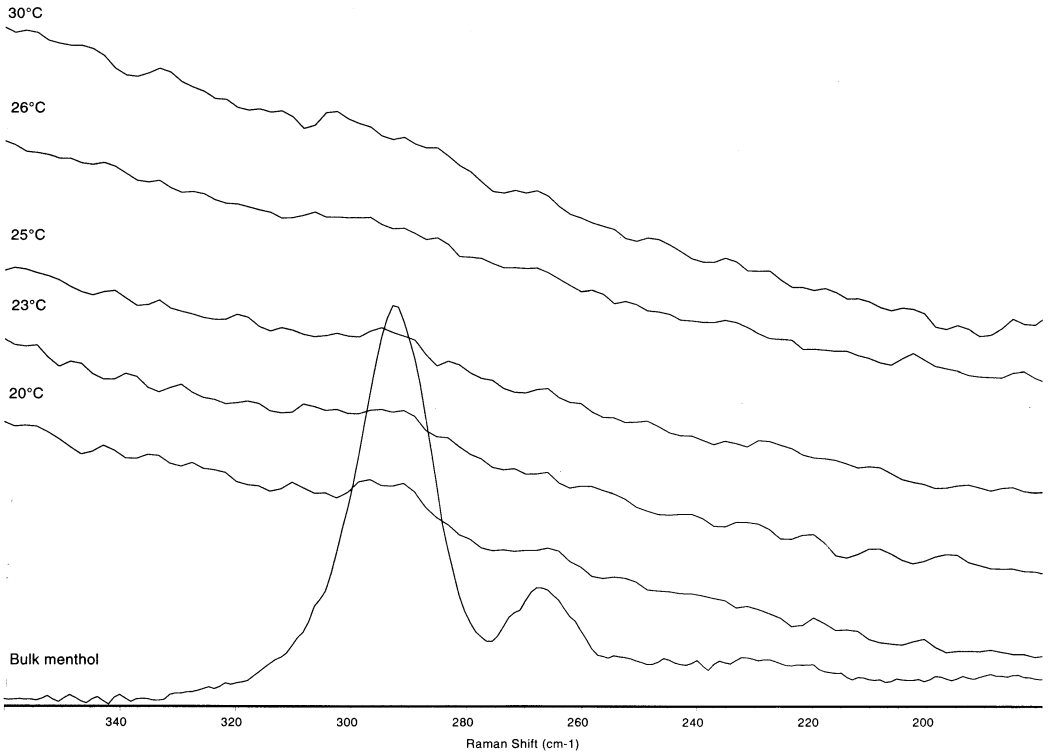

Fig. 5. Variable-temperature Raman spectra of a supersaturated menthol solution at 30, 26, 25, 23, 20°C, and bulk crystalline menthol. A laser power of 1.0 W was used for all spectral acquisition.

trolled, can add considerable error to a method. And like the diffuse reflectance technique, concurrent method development with another analytical technique, such as XRD or DSC, will assure a more rugged assay.

Sample homogeneity is always the primary concern when developing a quantitative solid-state spectroscopic method. Because a Raman laser excites only a very small portion of a sample (about 1 mm in diameter), the homogeneity issue is even more critical for Raman. One 1 mm spot of a sample in a 13 mm diameter sample cup or a 5 mm glass NMR tube is not representative of the entire sample if the sample is not homogeneous. For Raman spectroscopy, representative sampling can be achieved through a two part approach. Firstly, every effort is made to make the sample as close to homogeneous as possible. We have found that preparing samples using a slurry technique has been very successful [5]. Secondly, a technique that samples multiple spots of the material would further assure that a spectrum is truly representative of the material. The Step-n-Repeat sampling accessory is available to do this. With this accessory, any number of 'steps' around a circumference of the sample, packed in a 13 mm sample cup, can be obtained. Fig. 6 shows the Raman spectra of four 'steps' of a calibration sample of a developmental compound and the mean spectrum of the four steps. It can be seen from the four 'step' spectra, that there is inhomogeneity in the sample. This is most evident in the pair of peaks between 380–320 cm^{-1}, where there are changes in the relative peak heights of the pair between each of the 'steps'.

The Step-n-Repeat sampling accessory, or any accessory that allows multiple spectra of potentially inhomogeneous samples to be collected, is a key component in the development of a quantitative crystal form method. Although the time nec-

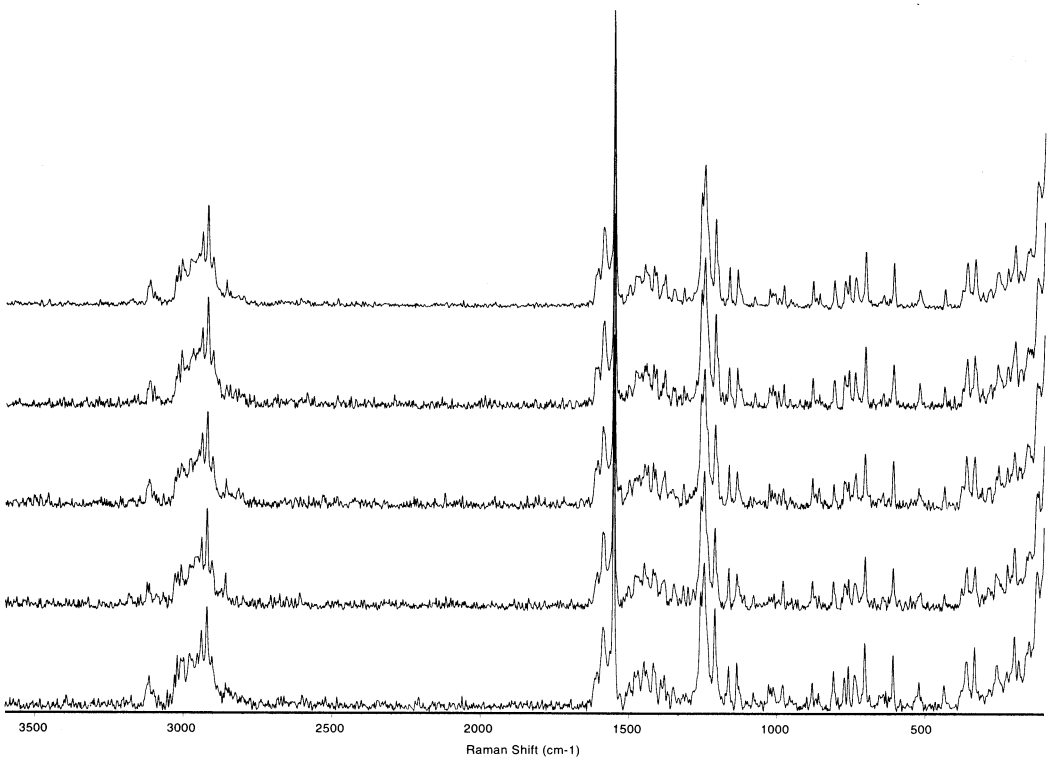

Fig. 6. Raman spectra of four 'steps' of a calibration sample of a developmental compound [top spectra] and the mean spectrum of the four steps [bottom].

essary to collect multiple spectra of a sample is greater than the time to collect just one spectrum, the evidence shown in Fig. 6 clearly indicates that potentially inhomogeneous samples should be analyzed in this manner.

3.5. Other Raman applications

There are a variety of qualitative and semi-quantitative applications where Raman spectroscopy is useful in the pharmaceutical industry. Because Raman probes the fundamental vibrational modes of a molecule (like mid-IR), it can be used for identity testing. FT-Raman spectral libraries can be created where only one spectrum per compound is representative of the material, as opposed to near-IR spectral libraries where a mean spectrum of perhaps 40–200 spectra per compound is required for compound representation. FT-Raman spectral libraries are also transferrable between instruments where the different instruments utilize the same laser excitation frequency (typically 1064 nm). Spectral wavelength and relative intensity reproducibility problems are minimized in these cases. With very little, or no sample preparation necessary for Raman spectral analysis, identity testing of materials can typically take less than 2 min. Sampling materials in their original containers, using a fiber optic probe or other sampling accessories, also eliminates issues such as human exposure to hazardous materials, contamination of the sample, and destruction of material during analysis.

Raman spectroscopy has been used in the drug substance salt selection process at Bristol-Myers Squibb. Together with XRD, DSC, IR, and TG/IR, Raman is used to characterize various salts of a drug candidate. Fig. 7 shows the Raman spectra of eight salts of a developmental drug. The characterization data, along with stability and solubil-

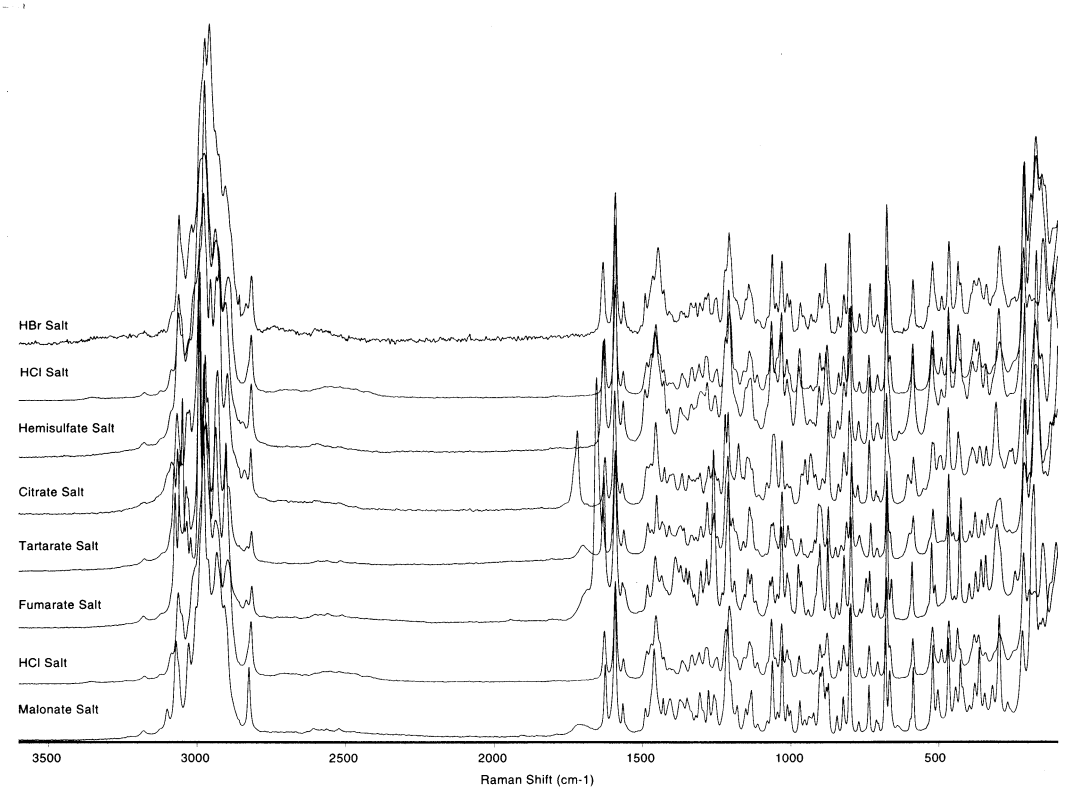

Fig. 7. Raman spectra of a variety of salts of a developmental compound. A laser power of 0.9 W was used for all spectral acquisition.

ity data, was used to select the optimum salt for drug product development.

Raman spectroscopy is also used for drug product evaluation. For products with similar tablet/capsule profiles but different potencies, Raman can be used as a non-destructive technique for differentiating samples of different potencies. Fig. 8 shows the Raman spectra of 150, 50, 25 mg potencies and the placebo of a product under development. For quantitation, it is very easy to differentiate the various potencies, and also select the samples that are placebos. This is very useful for clinical supplies support where samples can be non-destructively analyzed in their packages.

4. Conclusions

Raman spectroscopy can be used in the pharmaceutical analytical laboratory in a variety of ways. Traditional drug substance characterization is enhanced with the additional information provided by Raman spectroscopy and quantitative polymorph assays can be developed. Raman can also be used qualitatively and semi-quantita-

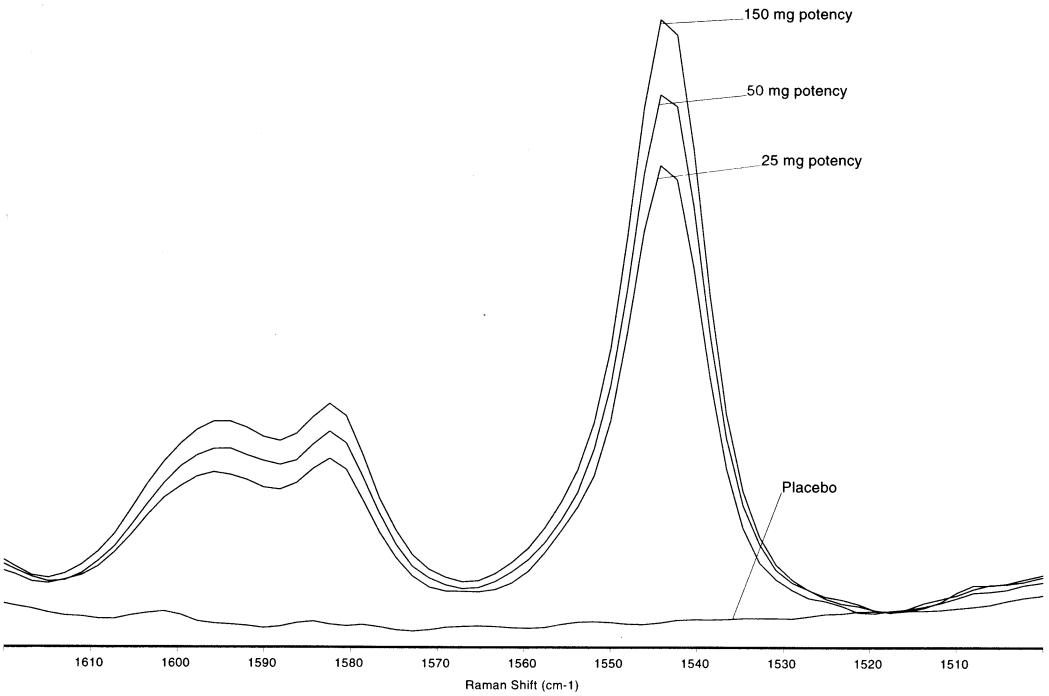

Fig. 8. Raman spectra of 150, 50 and 25 mg potencies, and the placebo of a product under development. All the spectra were acquired at a laser power of 1.0 W.

tively to support pharmaceutical development and clinical supplies operations by performing identity testing and potency differentiation.

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