Red-Excitation Dispersive Raman Spectroscopy is a Suitable Technique for Solid-State Analysis of Respirable Pharmaceutical Powders

REINHARD VEHRING
Nektar Therapeutics, 150 Industrial Rd., San Carlos, California 94070

Dispersive Raman spectroscopy with excitation by a red diode laser is suitable for quantitative crystallinity measurements in powders for pulmonary drug delivery. In spray-dried mixtures of salmon calcitonin and mannitol, all three crystalline polymorphs of mannitol and amorphous mannitol were unambiguously identified and their mass fractions were measured with a limit of quantification of about 5%. The instrument design offered high sensitivity and adequate background suppression, resulting in a low limit of detection in the range of 0.01% to 1%. This spectroscopy method has significant advantages over established techniques regarding specificity, sensitivity, and sample requirements.

Index Headings: Dispersive Raman spectroscopy; Crystallinity measurements; Polymorphism; Morphology effects; Quantification; Proteins; Saccharides.

INTRODUCTION

Pharmaceutical powders for drug delivery to the lungs must be carefully designed to achieve optimal performance.\(^1,2\) Among the attributes required for such powders are a particle size in the respirable range, good powder dispersibility, appropriate stability for long term storage, and robustness to short term environmental exposure. These requirements can be met by combining the active pharmaceutical ingredient with pharmaceutically acceptable carriers that provide stability and improved dispersibility. The resulting particles may be in an amorphous or crystalline form or may be a mixture of the two.\(^3\) It is important to accurately characterize the amorphous phase and, if present, any polymorphism in the crystalline phase, to enable rapid and successful product design and development.

Established analytical techniques used for the characterization of polymorphism and amorphous content, like X-ray diffraction (XRD) or differential scanning calorimetry (DSC), have some limitations regarding specificity and sensitivity. The crystallite size in respirable particles is typically less than 1 \(\mu m\), causing broadening of diffraction lines in XRD.\(^4\) It is also difficult to quantify a small amount of amorphous material in a mostly crystalline powder by XRD, because the amorphous signal is typically featureless and weaker than the crystalline signal.\(^5\) Interpreting DSC results in multi-component systems is complicated by the fact that the results are not component specific. It is thus desirable to use a supplementary technique to generate additional information on the nature of the observed thermal events.\(^6\)

Raman spectroscopy has been shown to have the necessary specificity and sensitivity for solid-state analysis of pharmaceutical products.\(^7-12\) Samples can be as small as individual particles with a mass of only a few nanograms,\(^13,14\) and usually no sample preparation is required. Glass and water are both weak Raman scatterers that do not interfere much with the Raman spectra of other components in the sample.

Fourier transform Raman spectroscopy has been applied to quantification of crystallinity and polymorphism in simple systems before,\(^5,15\) but dispersive Raman spectroscopy may be more sensitive, because lower excitation wavelengths can be used.\(^16\) Raman scattering cross-sections and charge-coupled device (CCD) detector sensitivity both increase with lower excitation wavelength.\(^17,18\) Dispersive Raman systems, using diode lasers with a wavelength longer than 780 nm,\(^19-21\) have been used for biological applications\(^22,23\) and are a popular choice for current commercial systems. The near-infrared excitation in these systems provides adequate suppression of the fluorescence background from a variety of samples.

Because active pharmaceutical ingredients and excipients must be of high purity, fluorescence caused by impurities is of less concern in pharmaceutical development compared to general biological applications. Therefore, excitation in the red spectral region becomes an attractive option. Many powders absorb less radiation in the visible than in the near-infrared spectral region, especially if residual moisture is present. Higher laser power at the sample then becomes feasible without negative effects of laser-sample heating.\(^24\) Consequently, the Raman signal level can be further increased.

In the work presented here, a red diode laser with a wavelength of 670 nm is implemented in a custom built Raman system, which was optimized for sensitivity. The laser provides 150 mW of power at a comparatively low cost with minimal maintenance requirements.

The performance of the Raman system was tested using spray-dried salmon calcitonin formulated with varying amounts of mannitol as a model system. Crystalline mannitol can exist in three different polymorphs, and it has been shown that the amorphous content and polymorphism of mannitol formulations is affected by processing conditions, protein content,\(^25\) or presence of electrolytes.\(^26\) Salmon calcitonin is a pharmaceutically active polypeptide containing 32 amino acids with a disulfide bridge.\(^27\)

This paper also provides insight into the theoretical background of Raman scattering on small particulate matter. Particle size and morphology effects are often ignored in powder analysis but become important when the particle size approaches the wavelength of the radiation,
as is the case for respirable particles. Based on the theoretical findings, the development of a straightforward quantification method appropriate for early stage product development is described.

THEORY

In homogeneous media, there exists a simple relationship between the radiant flux of the Raman scattering of a substance A, $\Phi_A$, and its molar concentration, $c_A$. The radiant flux is proportional to the number of molecules present in the scattering volume, $V$, and the radiant flux density of the incident laser beam, $\varphi_0$. The proportionality factor is the Raman scattering cross-section, $\sigma_A$, which depends on the fourth power of the frequency of the scattered radiation, $\nu$, and consequently, the frequency of the laser, $\nu_0$. The scattering cross-section further depends on the sample temperature, $T$. In mixed phases, the scattering cross-section of a component can be modified by interactions with other components, which are present in concentrations $c_i$. Thus, the radiant flux can be expressed as:

$$\Phi_A = c_A N_V \sigma_A (\nu_0, \nu, T, c_i) \varphi_0$$  \hspace{1cm} (1)

Many Raman studies are based on this simple relationship, assuming that the scattering cross-section and instrument response are constant, i.e., the Raman signal, measured by peak height or area, is proportional to the concentration. While this assumption has been shown to be valid in many cases, it is not obvious that it is generally applicable to the analysis of small particulate matter. Respirable particles typically have dimensions on the order of a micrometer, which approaches the wavelength of the radiation. Therefore, the particle morphology strongly affects the electromagnetic field of the excitation within an individual particle, the transmitted field, and also the electromagnetic field of the inelastically scattered radiation. To account for the effects of the particle morphology on the scattering process, a morphology factor, $P$, was introduced. Equation 1 can then be expressed as:

$$\Phi_A = c_A N_V \sigma_A P_A \varphi_0$$  \hspace{1cm} (2)

where $P_A$ is the morphology factor for component A. $P$ depends on the morphology of the particle and the internal distribution of the components. It further depends on the optical properties as described in the complex refractive indices of the components and the frequencies of the involved radiation fields. The morphology factor can only be calculated for simple geometries like spheres. Experimental studies show that Raman scattering from aerosols in the respirable size range is roughly proportional to the concentration of the measured component, if the signal is averaged over a sufficient particle size range. However, these studies were conducted on spheres or single component particles. So far, multicomponent particles of complex morphology have not been studied adequately. For particles that have an internal structure such as a core encapsulated by a shell, the morphology factor is generally component specific.

The Raman signal recorded at the detector, $S_A$, depends on the efficiency of the experimental apparatus, $E$, which is a function of the frequency of the scattered radiation, or the Raman shift, respectively. Using the total mass in the scattering volume, $m$, and the molecular weight of component A, $W_A$, Eq. 2 can be rewritten as:

$$S_A = E(\nu) \frac{m Y_A}{W_A} N_a \sigma_A P_A \varphi_0$$

where $Y_A$ is the mass fraction of component A. Because $P_A$ and $E$ cannot be easily determined, quantitative measurements can only be performed relative to a known component in the particle, which then serves as an internal standard. Once the calibration factor, $k_{A,Ref}$, is experimentally determined, the mass fraction of component A relative to the internal reference can be derived using Eq. 4:

$$Y_A = Y_{Ref} k_{A,Ref} \frac{S_A}{S_{Ref}} \frac{k_{A,Ref} W_A \sigma_{Ref} P_{Ref}}{k_{A,Ref} W_A \sigma_A P_A}$$

Because the calibration factor depends on the morphology factors, it should be derived from samples with a morphology that is representative of the samples to be analyzed. For example, in blends of particles with different size and shape the individual components will likely have different morphology factors than in homogeneously mixed multicomponent particles.

RAMAN SYSTEM

A dispersive Raman system with excitation in the red spectral region and detection by a cryogenically cooled CCD sensor was used in this study. A schematic of the instrument is shown in Fig. 1. The diode laser (#PI-ECL-670-150-FS, Process Instruments, Salt Lake City, UT) was operated at a wavelength of 669.85 nm, with a spectral bandwidth of 1 cm$^{-1}$, and a power of 150 mW. Spontaneous emissions from the laser cavity were suppressed by a holographic laser bandpass filter (#HLBF-668.0, Kaiser Optical Systems Inc., Ann Arbor, MI). Broadband dielectric laser mirrors were used to direct the laser beam and rotate the polarization to achieve vertical polarization in the scattering volume. An achromatic lens focused the laser beam onto the powder sample, which was placed in a conical aluminum cavity with a base diameter of 2 mm. The cavity reflected scattered laser radiation back into the powder sample, which slight-
ly improved the signal and lead to a more homogeneous radiation field in the sample, reducing morphology effects on the measurement. The volume of the cavity was 1.1 μL, holding a sample mass of typically 100 to 800 μg.

A camera lens (CTV20135/100, JML Optical Industries, Rochester, NY) with a focal length of 50 mm and a numerical aperture of 0.95 collected the scattered light from the sample under an angle of 90°. The spectrograph used in the setup was a modified single stage Czerny–Turner spectrograph (ARC 500i, Acton Research Corporation, Acton, MA) with an additional filter stage. It had a focal length of 500 mm and achieved an accuracy of ±0.2 nm and a repeatability of ±0.05 nm. The spectrograph was equipped with three different gratings. For this study, a ruled grating, blazed at 750 nm, with a groove density of 600 g/mm was used. One entrance port of the spectrograph was equipped with a modified Raman notch filter stage, to which a second entrance slit assembly (ARC # SP-716) was attached. This modification effectively changed the spectrograph to a two-stage design with a first non-dispersive filter stage and a second dispersive spectrograph stage. The slit assembly of the filter stage became the entrance slit, and the spectrograph slit became the middle slit of the system. A holographic band rejection filter (# HSPF-670.0-1.0, Kaiser Optical Systems Inc., Ann Arbor, MI) was mounted in the filter stage. It had an out-of-band transmission of greater than 80% in a wavelength range 700–1200 nm and achieved an optical density of greater than 6 in a band with a spectral width of 241 cm⁻¹ centered at 670 nm.

The sensor was a digital CCD spectroscopy system (# LN/CCD-400EHR-G1, Princeton Instruments, Trenton, NJ). It consisted of a cryogenically cooled camera head, a front-illuminated, deep depletion CCD array (EEV type 36, 1340 × 400 pixel format), and a large focal plane shutter. The sensor was controlled by a Princeton Instruments ST 133 camera controller (# 7355-0017) with a gain setting of 1.04 electrons/count.

The Raman system was calibrated using atomic emission lines from the cavity of a modified HeNe laser as absolute wavenumber standards. A hole was drilled into the laser housing such that the plasma emissions from the laser tube were scattered by the sample. The line position of the diode laser could be measured directly, with an accuracy of ±2 cm⁻¹, because the elastically scattered radiation from the sample was not completely blocked by the holographic notch filter. The Raman system was not affected by grating repositioning errors because it was designed to operate in a fixed grating position. It provided parallel spectral coverage of the fingerprint region from 200 to 1800 cm⁻¹ in one exposure.

**METHOD DEVELOPMENT**

**Reference Spectra.** The first method development step is the collection of high quality reference spectra from samples representative of all components present in the system. In this case, the components are salmon calcitonin, amorphous mannitol, and three known polymorphs of crystalline mannitol,

Because the Raman spectrum is sensitive to protein conformation and the secondary structure of proteins is affected by processing conditions, the reference sample was manufactured under the same conditions as the test samples. The salmon calcitonin reference sample was spray dried from a neat salmon calcitonin solution under similar conditions as the test samples. The raw spectrum was corrected for sensor inhomogeneities, a background subtraction was applied, and the spectrum was normalized to the strongest peak at 1450 cm⁻¹. Figure 2 shows the resulting reference spectrum. The spectrum is typical for peptides,

Attempts to produce pure amorphous mannitol were unsuccessful. To derive an amorphous mannitol reference spectrum, salmon calcitonin and mannitol were spray-dried in a ratio of 7:3. It was verified by XRD that this sample was amorphous. The salmon calcitonin contribution to the spectrum was then subtracted using the reference spectrum shown in Fig. 2. The remaining signal was corrected as described before and then normalized to the strongest peak. The resulting reference spectrum is the upper trace in Fig. 3. To verify the result, a spectrum was derived from a concentrated aqueous solution of mannitol by subtracting the broad-band Raman signal of water. The Raman spectra of concentrated solutions of saccharides are known to be similar to the spectra of the amorphous solid. This spectrum is the lower trace in Fig. 3. A comparison shows that the spectra are similar with some exceptions, most notably in the amide I region of the spectrum between 1550 and 1750 cm⁻¹. These differences are caused by conformational differences between neat salmon calcitonin and calcitonin formulated with mannitol. Saccharides have a stabilizing effect on protein conformation and have been used for this purpose for spray-dried products. The affected regions in the reference spectrum have been excluded during the deconvolution procedure.

The polymorphs of mannitol were produced in our laboratory and their identity was verified by XRD. The resulting reference spectra are shown in Fig. 4. The spectra are in good agreement with those published by Burger et al.

All components in the salmon calcitonin–mannitol sys-

![FIG. 2. Reference spectrum for salmon calcitonin.](image)
tem have unique spectra, which allow unambiguous deconvolution. The signal-to-noise ratios of all collected reference spectra are listed in Table I together with their exposure time and the Raman shift at which the signal quality was evaluated. At these positions the normalized signal strength is 0.5 for all spectra. The signal-to-noise ratio was excellent, exceeding 200:1 in all cases. The noise calculation assumes that the dominant sources of noise are shot noise from the background and the signal, while readout and dark noise are negligible. The signal-to-noise ratio is then the Raman signal after background subtraction divided by the square root of the signal including the background.

**Calibration.** Next the calibration factors described in Eq. 4 were determined. Salmon calcitonin served as internal standard because its mass fraction was accurately known from the formulation composition. The calibration factor for amorphous mannitol was derived from a binary formulation of salmon calcitonin and mannitol with a mannitol mass fraction of 0.2. The powder spray-dried from this formulation was verified to be amorphous by XRD. The calibration of the mannitol polymorphs made use of the fact that temperature or moisture stress above the glass transition temperature of amorphous mannitol leads to crystallization and subsequent polymorph transition into the α polymorph. The calibration factors for the α and δ polymorphs were derived from a spray-dried formulation of salmon calcitonin and mannitol with a mannitol mass fraction of 0.3. The mannitol fraction in this formulation was initially fully amorphous. Upon storage at 58% relative humidity (RH) the mannitol fraction in the spray-dried formulation crystallized, forming the δ polymorph. Storage at a higher RH of 84% caused the expected recrystallization into the α polymorph. Because no storage conditions were identified that lead to the formation of the β polymorph, the calibration factor for this polymorph was determined relative to the delta polymorph using physical mixtures of the ground, crystalline materials. Raman measurements on the mixture were repeated five times to reduce the effect of imperfect mixing.

Table I lists the calibration factors for the different mannitol forms relative to salmon calcitonin. Because the relative scattering cross-sections are dependent on the laser wavelength, these calibration factors cannot be applied to other Raman spectrometers with different excitation wavelength.

All mannitol forms have calibration factors less than 1, which means that they have higher Raman scattering cross-sections than salmon calcitonin on a mass basis. Compared to small molecules, proteins and peptides are weak Raman scatterers, because they usually consist of many different amino acid residues, which each contribute a unique spectrum. In amorphous mannitol the Raman lines are broadened due to the disorder in the solid state. Because the reference spectra are normalized to peak intensity, the resulting calibration factor is higher relative to the crystalline mannitol species, which concentrate the Raman scattering into narrow peaks.

**Laser Wavelength Correction.** Diode lasers have a broad gain bandwidth and, typically, a short cavity, which leads to narrow cavity mode spacing. Thus, the gain medium can support many different cavity modes. While diode lasers can be designed to reliably lase in a single mode, the frequency of the mode is dependent on several factors such as the temperature of the device, the power level, and the amount of back-reflection into the laser. The laser used in this study does not exactly reproduce the same laser wavelength in day-to-day operation. Therefore, Raman spectra measured on different days will have an apparent relative shift in the line positions. Because deconvolution of a sample spectrum into several components is dependent on the correct wavelengths, a laser wavelength correction is necessary to correct for the relative shifts.

---

**Table I. Signal-to-noise ratio and calibration factors of the reference spectra.**

<table>
<thead>
<tr>
<th>Component</th>
<th>S/N</th>
<th>Δν (cm⁻¹)</th>
<th>Exposure (min)</th>
<th>k_{A, ref}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmon Calcitonin</td>
<td>359</td>
<td>1250</td>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td>Mannitol amorphous</td>
<td>204</td>
<td>1140</td>
<td>20</td>
<td>0.69 ± 0.3</td>
</tr>
<tr>
<td>Mannitol alpha</td>
<td>212</td>
<td>879</td>
<td>1</td>
<td>0.20 ± 0.01</td>
</tr>
<tr>
<td>Mannitol beta</td>
<td>467</td>
<td>882</td>
<td>2</td>
<td>0.13 ± 0.02</td>
</tr>
<tr>
<td>Mannitol delta</td>
<td>289</td>
<td>883</td>
<td>5</td>
<td>0.19 ± 0.01</td>
</tr>
</tbody>
</table>
eral reference spectra requires that all spectra have a common Raman shift scale, variations in the laser wavelength have to be corrected. The approach taken in this work is to perform a coarse correction by measuring the laser position after each power-up of the system, and to incorporate fine adjustments into the data processing method.

Figure 5 shows how calibration of laser wavelength and dispersion is performed after system start-up. With the sample in place, the spectrograph is moved to a position where the laser line can be directly measured, because it is not completely blocked by the holographic band rejection filter. The effect of the filter can be seen between −100 and 100 cm⁻¹ where Raman signal and background are suppressed. The Raman shift axis is then corrected using the measured value. In Fig. 5, some atomic emission lines that were scattered off the sample are corrected using the measured value. In Fig. 5, some atomic emission lines that were scattered off the sample are indicated by arrows. The spacing of the lines is used to calibrate the spectrograph dispersion.

The top trace in Fig. 6 shows the signal difference of two independent measurements on a pure β polymorph sample. The signal difference is expressed as a percentage of the maximum signal level in the spectra. The background was removed, and the signal strength of the weaker spectrum was adjusted to allow complete subtraction. The trace shows artifacts at line positions corresponding to strong peaks in the mannitol spectrum shown in Fig. 4. These artifacts are caused by a small shift in the laser wavelength, which was not accurately accounted for in the coarse correction described above.

The following data processing method was applied to reduce these artifacts: linear interpolation between neighboring data points was used to transform the spectra into an equidistant dataset with significantly increased resolution. This allowed the spectra to be shifted relative to each other in smaller increments than the resolution of the CCD detector. Adjusting the Raman shift axis of the second spectrum by 0.133 pixels lead to the difference spectrum displayed in the lower trace of Fig. 5. This correction of the laser wavelength by as little as 0.2 cm⁻¹ leads to a twofold reduction in the amplitude of the subtraction artifacts.

These artifacts are a limiting factor in the deconvolution of multicomponent systems, because several reference spectra are subtracted from the measured spectrum, each adding artifacts. For quantitative measurements in these systems, the laser wavelength either should be measured with an accuracy of at least 10 times the resolution of the Raman system or corrected during data processing. Accurate experimental determination of the laser wavelength would require separate instrumentaton. The self-calibration offered by some commercial Raman systems is not desirable for this application.

**Deconvolution and Quantification.** The salmon calcitonin–mannitol system in this study was deconvoluted into five normalized reference spectra, $S_{i_{\text{norm}}}$, according to:

$$S(\Delta \nu) = B + \sum_i a_i S_{i_{\text{norm}}}(\Delta \nu + b_i)$$

(5)

Each reference spectrum was adjusted by a wavenumber shift correction, $b_i$. The last component of Eq. 5 is a slowly varying background function, $B$, a low-order polynomial. The results of the deconvolution are the intensity factors, $a_i$, that are used to calculate the mass fractions of the components in the sample relative to the mass fraction of salmon calcitonin, $a_{\text{Ref}}$, as described by:

$$Y_i = Y_{\text{Ref}} \cdot k_{i_{\text{Ref}}} \frac{a_i}{a_{\text{Ref}}}$$

(6)

In this study the deconvolution has been performed manually, by iteratively minimizing the residuals of selected marker peaks for the individual components. This procedure can be automated if desired, but this would require careful definition of marker positions, because the components show interactions in several regions of the spectrum, where the spectrum is no longer a simple linear superposition of the individual components.

**SAMPLE PREPARATION**

Solutions of salmon calcitonin (Peninsula Labs, San Carlos, CA) were spray-dried as binary mixtures of vary-
ing ratios with D-mannitol (USP grade, Mallinckrodt, St. Louis, MO) using a Büchi 190 spray dryer. The processing conditions are given elsewhere. Powders with an aerodynamic particle diameter in the respirable size range <5 μm were separated from the drying gas with a cyclone and collected in glass collectors. The dry powder was sampled and transferred into the cavity of the sample holder for the Raman measurements without further sample preparation. The powders had a residual moisture level of approximately 1% by weight.

RESULTS AND DISCUSSION

A typical spectrum, after background subtraction, from a sample with a salmon calcitonin mass fraction of 15% is shown in Fig. 7. The spectrum was measured with an exposure time of 20 min. The Raman shift range above 1500 cm\(^{-1}\) is magnified so that noise level, dynamic range, and sensitivity of the measurement can be assessed. The peak at 1615 cm\(^{-1}\) can be assigned to the single tyrosine residue in salmon calcitonin. The tyrosine residue contributes 4.8% of the mass of salmon calcitonin. For an estimate of the sensitivity of the system, the tyrosine can be treated as an individual component, present with a mass fraction of 0.72%. The narrow line at 1555 cm\(^{-1}\) is caused by atmospheric, gaseous oxygen in the void spaces of the powder. Using an estimated void fraction of 0.5, the mass of oxygen in the sample volume is on the order of 100 ng. Thus, relative to the other components the oxygen mass fraction is only about 200 ppm. This measurement demonstrates that the dispersive Raman system used here can detect components in a multicomponent mixture with mass fractions on the order of 0.1 to 1%. Under favorable conditions the limit of detection is on the order of 100 ppm.

The error in the deconvolution can be determined using the simple condition that all mass fractions must add up to 1. Because every component is measured relative to salmon calcitonin, the mass fraction of salmon calcitonin that fulfills this condition can be determined and compared to the nominal salmon calcitonin mass fraction.

Figure 8 shows the results of this test. The absolute error of the measured mass fraction was less than 3% throughout the whole composition range. For mass fractions between 5 and 10% the relative error reaches 10%, which is used here as a conservative estimate for the limit of quantification. The exposure times for the measurements shown in Fig. 8 were chosen such that the Raman signal level for salmon calcitonin stayed roughly constant regardless of the composition. The limit of quantification is significantly higher than the limit of detection, because all reference spectra and the sample spectrum contribute to the noise in the deconvolution, highlighting the need for reference spectra of very high quality.

Figure 9 shows the results of the deconvolution. The composition of the powder is displayed as a function of the mannitol mass fraction in the formulation. The data is plotted in a format that resembles a phase diagram.
with the mass fractions of the individual components adding up on the vertical axis. The open symbols denote repeated measurements that were performed to check the reproducibility of the results. The repeats were completely independent, including remanufacture of the samples.

Under the chosen processing conditions, no crystalline mannitol was detected up to a mannitol mass fraction of about 50%. For higher mannitol mass fractions the mannitol was found to be partially crystalline, initially forming only the delta polymorph in a mannitol mass fraction range between 50 and 65%, and as a mixture of polymorphs at higher mannitol mass fractions. Pure mannitol predominantly formed the β polymorph. In several powders all three polymorphs and amorphous mannitol were present, indicating a non-equilibrium state. Consequently, humidity stress on selected samples leads to transformation of the amorphous components and the polymorphs to the α polymorph. These results are presented elsewhere. 38

CONCLUSION

It has been demonstrated that red-excitation dispersive Raman spectroscopy can successfully be applied to quantification of amorphous content and polymorphism in a multicomponent dry powder formulation system. The use of visible excitation in combination with a sensitive Raman system designed for optimal sensitivity allows collection of spectra with outstanding signal-to-noise ratio in a reasonable measurement time. The fluorescence background at excitation with a laser wavelength of 670 nm is not a limiting factor for many proteins, peptides, and excipients at the high purity levels required for pharmaceutical applications. Dispersive Raman spectroscopy with red diode laser excitation provides higher sensitivity and typically less sample heating compared to dispersive or Fourier transform Raman systems with near-infrared excitation.

The interactive data processing method presented here delivers reproducible results and eliminates the need for accurate calibration of the laser wavelength. Once a suitable set of reference spectra is collected, the method can be adapted to different formulation systems in a matter of days, which meets the requirements for early stage product development.

ACKNOWLEDGMENTS

The author gratefully acknowledges the contributions of Willard R. Foss, John W.-Y. Lee, Nazli Egilmez, David Lechuga-Ballesteros, Trixie Tan, Jennifer Lobo, Lisa Williams, Hak-Kim Chan, and Jane C. Feeley.