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Polymorph Discrimination Using Low Wavenumber Direct Lattice Information

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Polymorphs play a crucial role in drug development and quality control in the pharmaceutical industry. Raman spectroscopy is an established technique for identifying and differentiating pharmaceutical polymorphs. To date, most work in this area has concentrated on the small Raman spectral changes observed in the "standard" Raman range (100 cm^{-1} to 3200 cm^{-1}) where lattice modes are probed indirectly through the study of molecular vibrations that occur in this region.

Renishaw's Near-Excitation-Tuneable (NExT) filter accessory for the inVia Raman microscope enables direct observation of the lattice vibrations. This creates some exciting new opportunities in the area of polymorphic differentiation and characterization:

- easier direct lattice differentiation of polymorphic active pharmaceutical ingredient (API) forms
- fast method to collect high quality low wavenumber data compared to traditional triple spectrometers
- permits testing of theoretical lattice vibrational predictive models against real data
- permits comparison of low wavenumber data with complementary techniques such as X-ray diffraction (XRD) and Terahertz spectroscopy.

The characterization can be aided by cooling the sample to −195 °C (using liquid nitrogen). This provides more distinct spectral features by reducing the natural line width of the Raman bands.

Arguably the most significant problem when using Raman analysis for the indirect differentiation of polymorphs is gaining a reliable and repeatable method to analyze the small spectral differences. Such studies have often been conducted but use complex chemometric methods.

This application note demonstrates how Renishaw's NExT filter accessory and inVia Raman microscope can be applied to directly observe polymorphic lattice information. The polymorphic discriminatory power is illustrated using different carbamazepine polymorphs as a real case example, where complex data interpretation is not required for unequivocal differentiation.

Introduction

What are polymorphs and why are they important?

Polymorphs are different stable or metastable crystalline states of the same molecule; often these exhibit different physiochemical and biological properties. There are many factors that can affect the polymorphic form of a species during crystallization. Such factors include recrystallization rate, solvent and salt used, pH, and temperature. The ability to isolate, differentiate, and characterize individual polymorphs is a major challenge to the pharmaceutical industry. One third of pharmaceutical drugs are estimated to be polymorphic, and this number is expected to increase (1).

Figure 1: Renishaw's inVia Raman microscope.

Why use Raman for low wavenumber analysis?

The advantages of Raman spectroscopy over other analytical techniques for the low wavenumber analysis of polymorphs are numerous:

- nondestructive nature of analysis
- no sample preparation necessary
- ability to analyze direct, assignable lattice vibrations (NExT filter)
- Wide tuneable spectral range (~ 800 cm^{-1})* compared to Terahertz spectroscopy
- fast collection of data (typically < 60 s)
- high spectral resolution (~ 1 cm^{-1})* necessary to maximizespectral differences

* configuration specific

What is carbamazepine and why is it important?

Carbamazepine (5H-dibenz[b,f]azepine-5-carboxamide) is an anticonvulsant and mood stabilizing drug. It is used extensively for the treatment of epilepsy and schizophrenic disorders. It works by controlling sodium channels within the brain and, therefore, reduces excessive neuron activity.

Three main polymorphic forms of carbamazepine (CBZ) exist. Forms I and II have very similar crystal structures where dimers form from the hydrogen bonding and the π–π interactions of the benzene ring offset the molecules. Form III has a different crystal structure as a result of a significant change in the ring spacing. The different forms exhibit varying dissolution rates and this relates directly to the effectiveness of the drug. CBZ species have been thoroughly investigated because of their model characteristics (2,3).

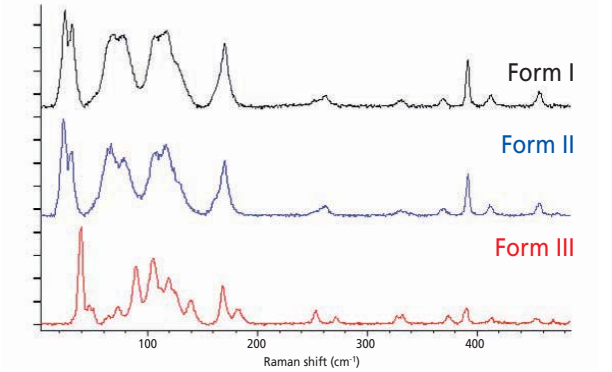

Figure 2: Typical low wavenumber polymorphic carbamazepine Raman spectra (room temperature, 25 °C).

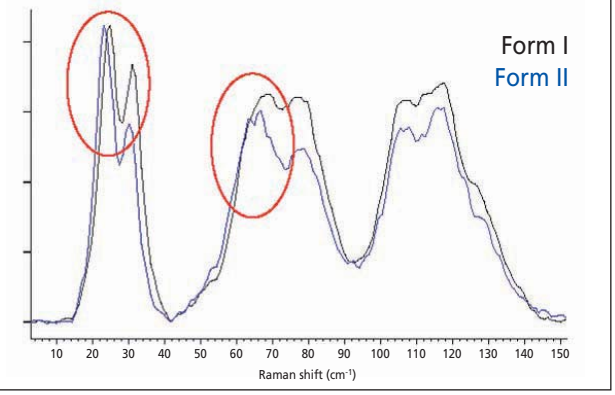

Figure 3: Form I and II low wavenumber polymorphic carbamazepine Raman spectra comparison.

Low wavenumber carbamazepine analysis using NExT filter

Raman spectroscopy is generally used to probe vibrations of discrete functional groups within molecules. As polymorphs contain the same functional groups with molecules arranged simply in a different crystalline order, the standard Raman spectrum is an indirect method for investigating these differences. Direct vibrational analysis of longer order structures, such as those present within polymorphs, occur at a significantly lower wavenumber (< 50 cm^{-1}).

Direct analysis of polymorphic lattice Raman bands can be achieved using Renishaw's NExT filter technology.[4] Such information allows far easier differentiation between polymorphs and, in some cases, is the only method that can be used. Renishaw's NExT filter is an accessory to the inVia Raman system and allows low wavenumber analysis (~ 10 cm^{-1})*.

The following sections describe how different carbamazepine polymorphs are used to demonstrate the power of the inVia Raman system combined with NExT filter technology for direct analysis of polymorphic information and how this information can be used for differentiation purposes.

* configuration specific

NExT analysis

The use of the NExT filter requires no additional considerations to the experimental collection parameters beyond those of a standard Raman measurement. The beam path is automatically selected through the WiRE 2 software.

Carbamazepine polymorphic forms I, II, and III were analysed using 514 nm Ar^+ laser excitation. The laser power was varied between 5 mW and 10 mW at the sample to ensure no thermal sample degradation occurred. A 60 s exposure time was used for each spectral collection and a quarter wave plate to scramble (circularly polarize) the incident and scattered light. This ensures Raman band variations resulting from polarization effects from the crystal orientation are minimized. Spectral differences are therefore attributable to the inherent crystal structure only. A spectral scan was collected between 2 cm^{-1} and 485 cm^{-1} using the NExT filter accessory. Further analysis was conducted at different sample temperatures using a Linkam TMS 600 hot/cold stage. Low temperature studies are used to reduce the entropic state of the system, sharpening the vibrational Raman bands and, therefore, aiding spectral interpretation and band assignment.

Each form was analyzed at 25 °C (room temperature), −50 °C, −100 °C, −150 °C, and −200 °C. On re-heating the room temperature, spectrum was re-collected to ensure the crystal phase had not been changed irreversibly. Typical low wavenumber Raman spectra of the three discussed carbamazepine forms are shown in Figure 2.

Each polymorph spectrum shows intense lattice vibrational information. In many cases this can be an order of magnitude greater then the corresponding vibrational information in the standard Raman range.

Form III has a vastly different spectrum compared to forms I and II. This is due to the different aromatic ring spacings within the crystal structure. This species is easily differentiated from forms I and II using the low wavenumber range.

The lattice similarities between forms I and II are consistent with their spectral similarity; only the low wavenumber bands at ~ 70 cm^{-1} and the shift in band position at 23 cm^{-1} permit unequivocal differentiation (Figure 3).

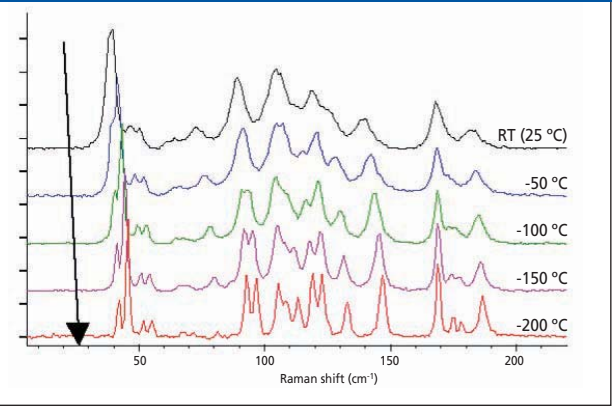

Figure 4: Change in Form III Raman spectrum with temperature.

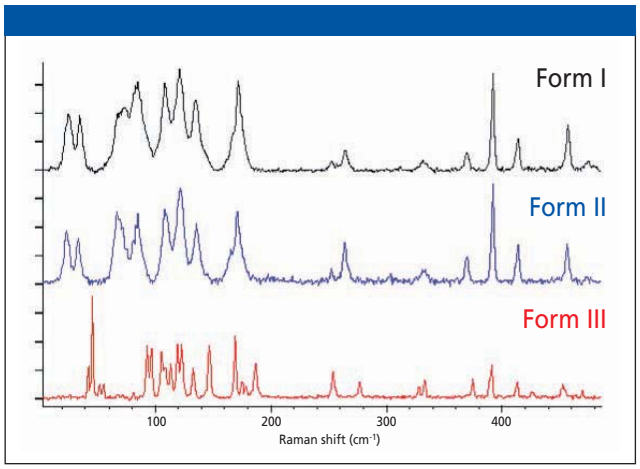

Figure 5: Typical low wavenumber polymorphic carbamazepine Raman spectra (−200 °C).

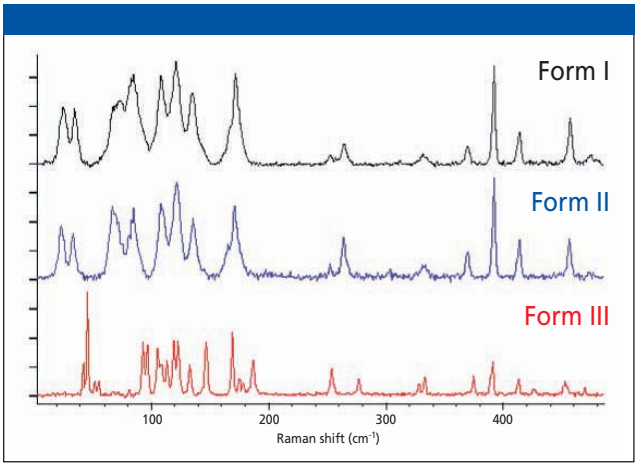

Figure 6: Form I and II low wavenumber polymorphic carbamazepine Raman spectra comparison (low temperature, −195 °C).

Variable temperature Raman analysis

At reduced temperatures, the lattice Raman bands shift to a higher wavenumber position. This is due to the shrinking of the crystal lattice afforded by the weak van der Waals forces. This coincides with an expected sharpening of the band system as the molecules are less energetic and vibrations are more specifically defined. Figure 4 shows these changes for form III carbamazepine.

The 38 cm^{-1} band, collected at room temperature, shifts by about 6 cm^{-1} when the sample is cooled to −195 °C. It also clearly splits into a major and minor band, ultimately revealing the true makeup of that band system. This is typical of such analysis and provides a means to gain accurate single band positional information. Characterization and predictive modeling benefit from this, as deconvolution errors are removed.

Collecting the Raman spectral data at a variety of temperatures can provide additional information. In this case it is also observed that no intermediate species are created. The polymorphic structures were preserved after reheating back to room temperature and identical spectra were collected from each form.

The low temperature Raman spectrum for each carbamazepine polymorph is shown in Figure 5.

A comparison of forms I and II collected at low temperature clearly allows the spectral differences to be more easily observed (Figure 6). In this case, the splitting of the 75 cm^{-1} band system confirms the change between the different forms originally observed at room temperature.

Conclusion

Low wavenumber Raman analysis of polymorphic species has an important role to play in the pharmaceutical industry. Renishaw's unique NExT filter technology allows such information to be rapidly and easily collected — a major advancement over traditional Raman instruments. Such data can be used in conjunction with Terahertz and XRD spectroscopy to allow direct lattice information to be gained for characterization and differentiation purposes. This technology ascribes the user data collection speed and quality of low wavenumber information unrivaled in the industry, both of which are beneficial for polymorphic discrimination in new drug development.

Acknowledgment

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References

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