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Polymorph characterization using the Nicolet iS50 Raman Module

Solid-dose drugs comprise a considerable segment of the pharmaceutical market. The solid-dose form can offer long shelf life, convenient drug delivery, and can be packaged into capsules, tablets, gel caps, and patches. *Polymorphism* is the ability of a molecule to exist in more than one stable or meta-stable crystalline state.

Distinct crystalline forms commonly exhibit differences in pharmacologically relevant properties of a single active pharmaceutical ingredient (API) in its solid dose form. These properties include bioavailability, solubility, absorption rate, duration, stability, manufacturability, and processability. Manufacturing factors also can influence polymorphism and can include solvents used in product formulation, the rate of product recrystallization, salt selection, and temperature. Since polymorphism can profoundly affect an API's pharmacodynamics and manufacturing process, it becomes critical to fully characterize and understand polymorphic behavior.

The information-rich content of Raman spectra and the availability of reliable commercial Raman instruments have given rise to the use of Raman spectroscopy in many aspects of drug development.[1-3] Raman spectroscopy has proven to be a valuable analytical method for differentiating between different polymorphs, salts, co-crystals, solvates, and hydrates of potential drug candidates. In many cases, the Raman spectra can distinguish between many of these existing forms and potentially identify new, unknown polymorphs. Raman

analysis offers several benefits, including the lack of special sample preparation for solid dosage forms and the ability to sample through packaging, like blister packs, without interference from the clear packaging material.

In this application note, we describe using the Thermo Scientific™ Nicolet™ iS50 Raman Module in the Thermo Scientific Nicolet iS50 FTIR Spectrometer sampling compartment to characterize different polymorphs of a model compound, 5-Methyl-2-[(2-nitrophenyl)amino]-3-thiophenecarbonitrile (ROY).[4-6] This compound was kindly provided by Professor Lian Yu of the University of Wisconsin, School of Pharmacy, Division of Pharmaceutical Sciences. This unique compound exhibits different colors for different polymorphs, and we describe here the analysis of three ROY polymorphs (red, orange, and yellow) seen in Figure 1.
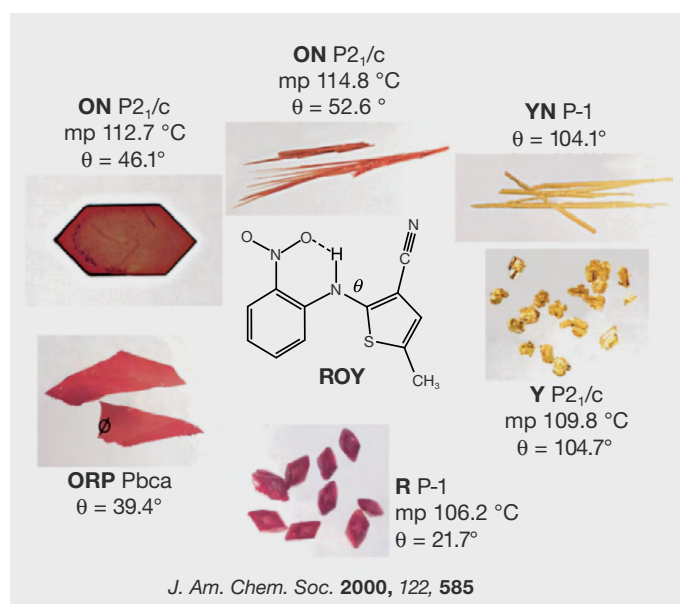

Figure 1: Pictures of the various polymorphs found for 5-Methyl-2-[(2-nitrophenyl)amino]-3-thiophenecarbonitrile (ROY).

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Experimental methods

The spectra described in this paper were acquired with the Nicolet iS50 Raman Module (Thermo Fisher Scientific, Madison, WI). The Raman module mounts in the sample compartment of the Nicolet iS50 Spectrometer and is equipped with a 1064 nm near-infrared laser which significantly reduces fluorescence interference relative to visible laser excitation. The samples were mounted in a mini-well plate that was placed on the fully automated X, Y, Z stage of the Raman module. Figure 2 shows a combined image of the wells containing the three samples that were acquired with the digital camera embedded in the module. This provided a video record of the precise position on the sample where the spectrum was acquired. The total data collection and analysis time for samples such as this depends upon the method employed. Using a single "aim-focus-collect" process (convenient for 3 samples) required about 3 minutes. The Array Automation software used here automates the analysis for a grid of samples, permitting unattended operation. Array Automation also links to spectral analysis tools, allowing the experiment to move from data collection to the final report automatically.
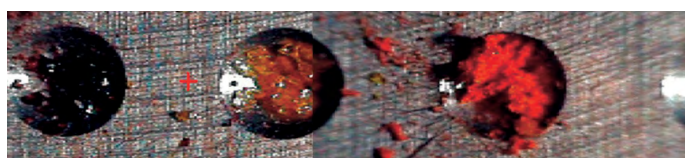

Figure 2: This composite image from the iS50 Raman Module shows the three ROY polymorphs in three 1 mm diameter wells in a mini-plate. Starting from the left; red, yellow, and orange polymorphs.
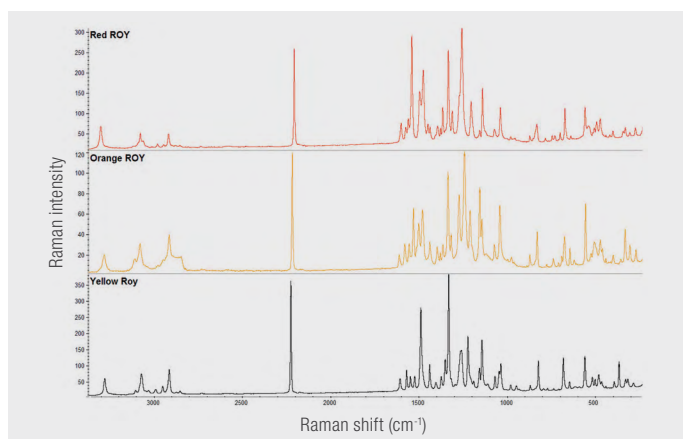

Figure 3: Full Raman spectra from three polymorphs of ROY.
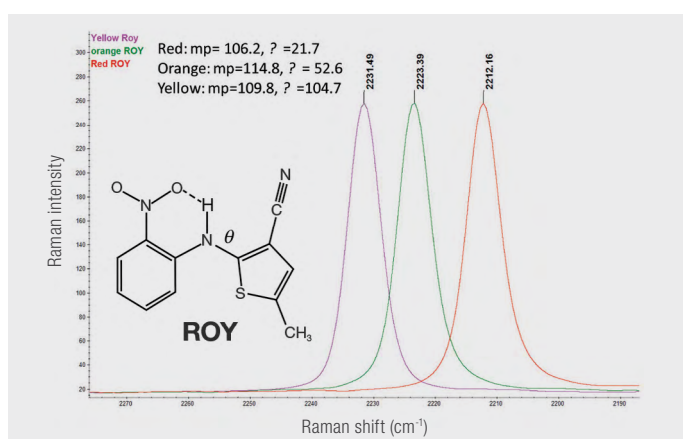

Figure 4: Expansion of the nitrile peak showing shift for three different ROY polymorphs.

The spectra from the three samples contain a number of significant spectral differences that are shown in Figure 3. One clear difference is the position of the nitrile peak near 2200 cm^{-1} that has been expanded in Figure 4. This peak shift appears to correlate with the different angle observed for the nitrile functional group in the different crystal forms.

Prolonged exposure of the orange polymorph to the excitation laser produced an interesting effect. Spectra collected over time at high laser power showed an increased presence of a second nitrile peak that closely matched the peak from the yellow polymorph. Figure 5 shows the resulting mixture spectra on the bottom with the three pure polymorphs displayed on top. We applied the Thermo Scientific OMNIC™ Curve Analysis Software to fit the mixture spectra. The results of one curve analysis are shown in Figure 6, demonstrating that Raman spectra can be used to determine the amount of different polymorphs present in a sample. The Thermo Scientific OMNIC TQ Analyst™ Multivariate Statistical Analysis Software would enable further analysis of this sample, including quantitative or qualitative analyses.

Raman spectroscopy provides pharmaceutical companies with a versatile tool for identifying the proper polymorph (or polymorph blend) during drug discovery. This allows optimization of the production process to yield the desired product and
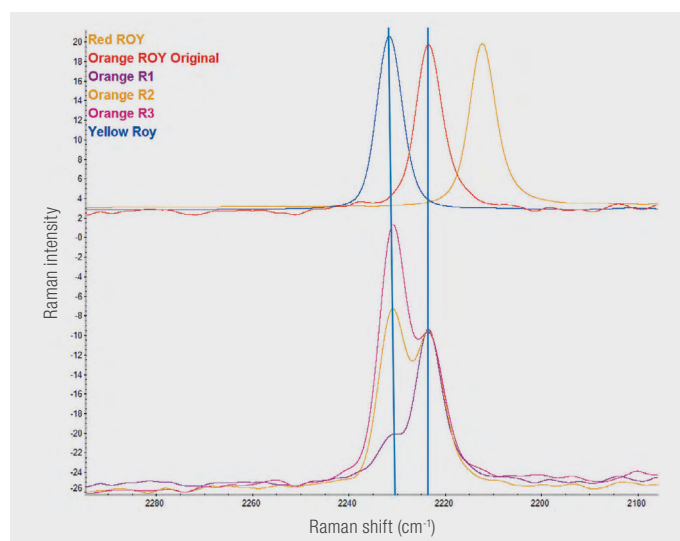

Figure 5: A comparison of the spectra from the pure materials (top) with spectra from samples containing a mixture of two polymorphs.
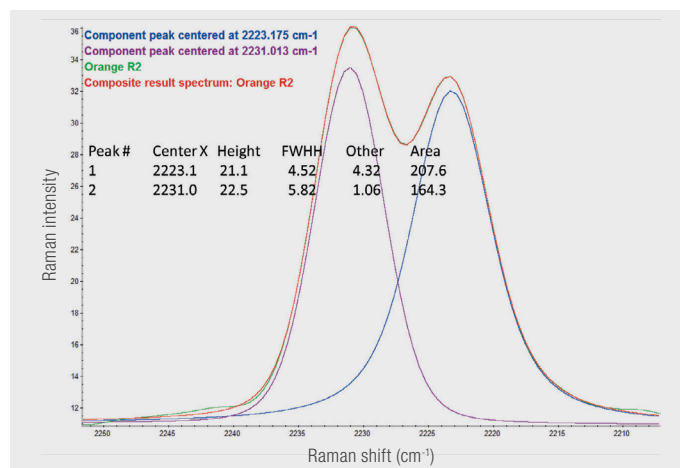

Figure 6: Results of curve fitting that provide a good estimate of relative concentrations from peak heights or areas for each polymorph.

speeds scale-up work into manufacturing. Post-launch, Raman assists in identifying counterfeit drugs or patent infringement issues to protect the intellectual property involved. Beyond pharmaceuticals, many laboratories—from research labs to forensic labs—can benefit from the additional information Raman spectroscopy offers in understanding the properties of solid-dose drugs.

Conclusion

Optimizing the choice of polymorphs of an API has become a significant step in the drug development process. The polymorphism and salt form of an API greatly affects a drug's performance and stability, drastically changing absorption and other characteristics in vivo. During the discovery process, identifying and comparing the properties of different polymorphs, salts, solvates, complexes, and co-crystals becomes vital for building intellectual property and creating a competitive advantage for pharmaceutical companies.

The Nicolet iS50 FTIR Spectrometer equipped with the iS50 Raman Module (Figure 7) can be extremely valuable for characterizing polymorphs and mixtures during drug development as well as providing a tool for identifying counterfeit drugs or patent infringement. The fully integrated system—with Raman and FTIR, plus other optional tools—offers an ideal workstation for drug development, including screening polymorphic forms. Raman analysis is fast, requires little to no sample preparation, and can be used to characterize bulk or single-crystal samples. Raman permits measurements through many packaging materials, such as blister packs. The iS50 Raman Module enables experiment automation and does not require consumables or extra safety precautions. Raman spectroscopy using the iS50 Raman Module represents an economical, reliable, and complementary technique to DSC (differential scanning calorimetry) or X-ray diffraction for characterizing APIs.

Figure 7: The Thermo Scientific Nicolet iS50 Spectrometer with the iS50 Raman module in the sample compartment with a well plate designed for analyzing solid dose tablets

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