Application Note: 50766

Polymorph Analysis by Dispersive Raman Spectroscopy

Key Words

- Dispersive
- Identification
- Imaging
- Microscopy
- Pharmaceutical
- Polymorphs
- Raman
- Spectral Mapping

Introduction

Product analysis, such as chemical identification and composition verification, is becoming increasingly important in pharmaceutical manufacturing and research. In addition to identifying the chemical composition of active ingredients in formulations, it is often necessary to indicate the crystalline form of the substances of interest. This has ramifications not only in the desired chemical interactions and drug effectiveness but also in the ability of a company to protect its exclusive rights to exploit a formulation that it develops.

For these reasons, there is an increased interest in an important physical property: the polymorphic form of active ingredients. Polymorphism refers to the different crystalline forms of a substance, which exhibit the exact same empirical and chemical formula. This property is of importance for several reasons. First, the crystalline form can affect the way in which the active ingredient bonds to other molecules, thus affecting its ability to produce the desired biological effect. Differences in polymorphs can also influence drug dissolution, thereby altering or even eliminating desired drug delivery.

In addition to affecting the activity of the formulation, full chemical and physical characterization of polymorphic mixtures is important from a legal point of view. Substantial research resources are typically invested in the discovery and development of pharmaceutical formulations. Manufacturers want to protect this investment by retaining exclusive rights to manufacture and dispense the drug – a step typically accomplished through patents and licenses. Recent rulings have indicated that both chemical formulation and polymorphic form can be considered in patent protection or infringement. Therefore, it is increasingly necessary to fully characterize this aspect.

Dispersive Raman spectroscopy offers the ability to analyze both the chemical and physical form of such compounds. The unique Raman selection rules and spectral information provide a vibrational spectrum that is a chemical fingerprint, which can be used for unambiguous chemical identification. In addition, Raman spectroscopy is highly sensitive to molecular backbone and branching structures. Thus the Raman technique can be used to analyze chemical identity and confirm polymorphic form as well as quantitatively predicting the relative amounts of the various forms in a single formulation.

The Nicolet Almega dispersive Raman spectrometer

In this note, the Nicolet™ Almega™ dispersive Raman spectrometer will be used to determine the polymorphs of a common pharmaceutical compound and to identify the different forms. Additional studies will follow the formation of the different polymorphs as a function of time. Finally, a formulation will be analyzed to determine the spatial distribution of the two forms in a single tablet.

Physical Composition of a Mixture

To fully characterize pharmaceutical formulations by dispersive Raman spectroscopy, it is necessary to use both microscopic and macroscopic methods. By first analyzing a large area of the sample in the full-sized sampling compartment, it is possible to confirm the identity of the active ingredient in the formulation and to confirm the form because the measurement effectively produces a spatial "average" of the components present. Subsequent microscopy can then be used to probe the individual component distribution.

Figure 1 presents such a measurement of a confidential pharmaceutical compound. Product formulation was designed to produce a specific compound and form, but it was important to confirm this identity. The spectra in Figure 1 show not only the new formulation

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under analysis, but also a reference of the pure polymorphic form (Form A) of the expected product.

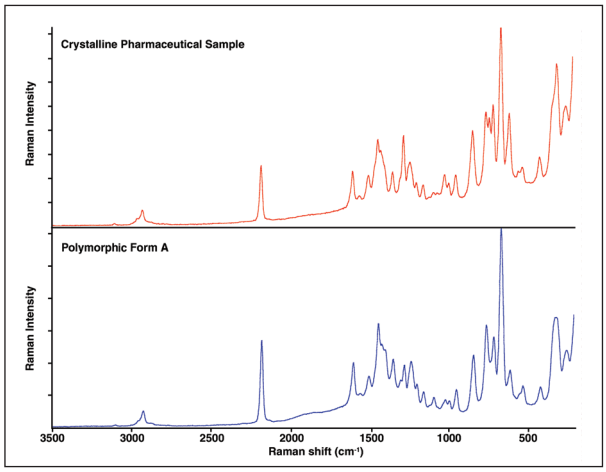

Figure 1: (top) A sample spectrum of the pharmaceutical formulation to be analyzed and (bottom) a spectrum of the pure polymorphic form of the desired compound. Both spectra were collected in the full-sized sample compartment of the Nicolet Almega dispersive Raman spectrometer. Spectra were collected with 5 second exposures using a 785 nm laser in the sample compartment in the vials without sample preparation.

This spectral information indicates that the compound in question is very similar to the known reference sample. However, differences are seen. These differences might be the result of a mixture of different polymorphs during manufacture. Another possibility is that, with time, the desired polymorph transforms into another.

To investigate this, it was necessary to confirm the second form of the compound. This is done by comparing to another known polymorph (Form B) of this compound. Figure 2 shows the comparison of the two pure forms of the polymorph to the formulated compound analyzed. The data in this figure for the new formulation show bands that are different from the spectrum of pure Form A. The additional bands appear to originate from a mixture of Form A and Form B.

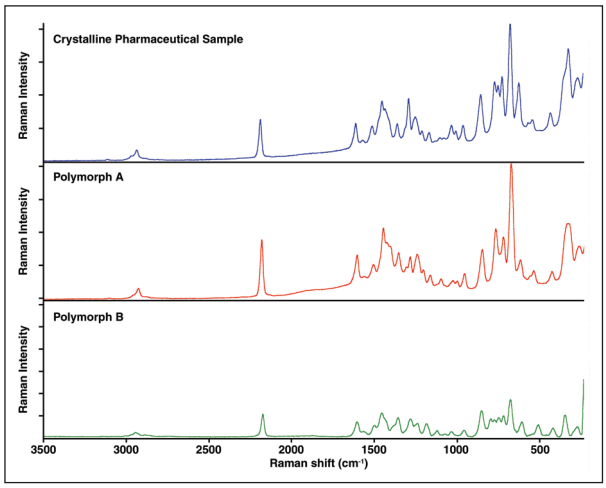

Figure 2: (top) A spectrum of the sample formulation, (middle) a spectrum of the desired polymorph (Form A), and (bottom) a spectrum of an alternate polymorph (Form B).

Having established the existence of the second polymorph in the compound, it was desirable to determine its origin. In this case, it was assumed that the initial formulation only contained Form A, so it was necessary to determine whether Form B impurity arose from incorrect formulation or whether it resulted from transformation over time. To test this, a pure formulation was analyzed over the course of time to establish the possible transformation with shelf life. The sample was stored under ambient conditions and periodically analyzed for the two polymorphs.

Figure 3 shows the spectra at times t = 0 through t = 4 (2 week intervals) for the measurements conducted. By paying special attention to the spectral region between 1000 and 300 cm^{-1}, it is apparent, that although the change is small, there is likely an increase in polymorphic Form B over time. This suggests that the original sample mixture could have indeed been composed of pure Form A, but with time could have converted to Form B.

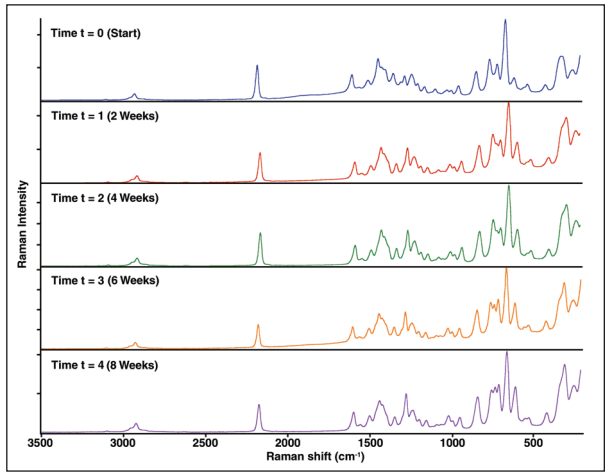

Figure 3: Time dependence study of polymorph conversion showing the transition from initial pure Form A composition to a mixture containing Form A and Form B.

Compositional Mapping of the Sample

Quite often it is not sufficient to simply identify the individual components in a formulation, but it is equally important to determine the distribution of these components in the final tablets. This could also assist in determining whether the observed aging is a thermal effect or an atmospheric one, since the predominance of Form B at the surface of the sample material could indicate that the transformation was influenced by atmospheric effects such as humidity, oxygen, or other atmospheric conditions. A more uniform distribution would suggest that the formation of Form B is a kinetic process that occurs uniformly and spontaneously.

To investigate this, a cross-section of the formulation was taken and a dispersive Raman microscope map was collected from the outer surface inward. Figure 4 presents the line map of a tablet formulation.

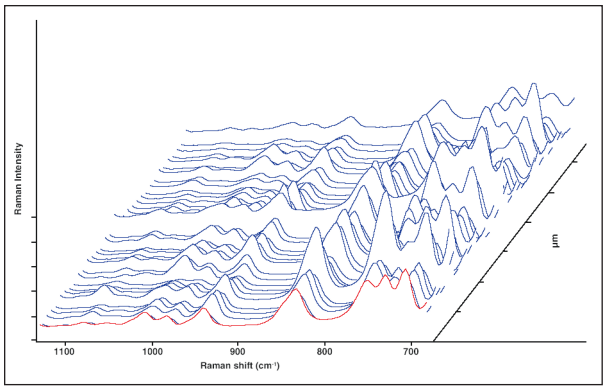

Figure 4: Line map of a pharmaceutical formulation of pure Form A that has been stored for a period of time.

The line map data indicate that the distribution of polymorphic Form A and Form B are relatively random over the region of the sample mapped. The spectral map is truncated to highlight both the relative and absolute peak intensities across the area of investigation. The absolute peak intensities of all of the bands appear to change across the map, corresponding to the topology of the tablet surface. Since the tablet is composed of individual crystals, the surface is not completely flat on the size scale of the microscope spatial resolution, so some crystals are in better focus than others.

The relative peak heights within a single measurement and the appearance or disappearance of individual peaks are good indicators of the distribution of the different forms. This initial investigation indicates that the transformation from Form A to Form B is a spontaneous process that is likely only influenced by temperature and time, being little affected by the ambient environment.

A final note of interest highlights the power of dispersive Raman microscopy. While the macroscopic view of the sample is useful in determining the relative amounts of the various components, often the identity of the components is not known. By focusing the microscope on isolated single crystals or crystal agglomerates it is possible to collect a spectrum of the pure component and to thereby identify the individual components in the mixture.

Figure 5 shows the spectrum of a single crystal of Form A, taken at high magnification. By isolating on a small crystalline region, it is possible to get a pure spectrum of one form of the active ingredient without interferences from other compounds in the tablet. If the components were unknowns, it would be possible to isolate the individual compounds by locating pure crystals or crystalline agglomerates and collecting pure spectra for identification.

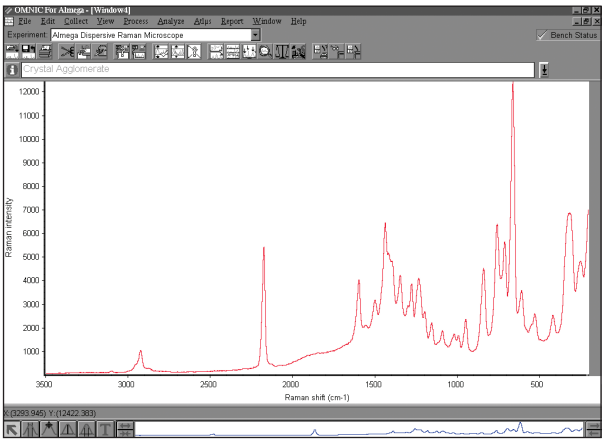

Figure 5: Spectrum of a crystal agglomerate region that is composed of a single component measured by dispersive Raman microscopy. The spectrum indicates that the agglomerate contains only Form A.

Conclusion

Raman spectroscopy is a powerful analytical tool for the modern pharmaceutical laboratory. The ability to unambiguously identify not only chemical composition but also physical form of the chemical entities makes it possible to both confirm composition and to study polymorphic transitions. Dispersive Raman spectroscopy allows the qualitative and quantitative identification of the polymorphic forms of compounds and also allows spatial mapping to determine the spatial distribution in final formulation.

The combination of ease of sampling, no sample preparation, and abundant chemical information that Raman offers is increasing the demand for the technique in many aspects of research and quality control. Coupling the ability to look at bulk samples in their original form with the ability to automatically map microscopic component distribution makes dispersive Raman spectroscopy a necessity in the pharmaceutical laboratory.

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