Raman Instrumentation for the Pharmaceutical Industry

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Abstract

Over the past 5 years our Raman business has seen continually accelerating growth. The biggest component to this growth is in the pharmaceutical industry. Several factors contribute to this success. It is generally now known that it is easy to record a Raman spectrum, and that the information derived complements that of XRD (X-ray diffraction) and FTIR (Fourier Transform Infrared Spectroscopy). The spectral resolution at 1 μm is far greater than either of these techniques. And spectra can be recorded inside of glass and plastic containers. Of greatest interest is the differentiation between the various solid state forms, and distribution of the APIs (Active Pharmaceutical Ingredients) in final products. All of these issues are discussed in this article, with illustrative examples.

1 Introduction

With the revolution and evolution of Raman instrumentation over the past 10-12 years, the Raman market has been growing at an accelerated pace. Consequently we get more and more requests to provide systems specialized for particular applications, and some of our "special developments" evolve into becoming standardized products.

The largest area of growth that we are seeing is in the pharmaceutical industry. This is a result of several factors.

- It has become recognized that measuring a Raman spectrum is an "easy" experiment.
- There is information in the Raman spectrum that complements FTIR and XRD.
- There are several types of information of interest to the pharmaceutical industry that can be derived from Raman:

Differentiating Excipient vs. Active Ingredient, Differentiating polymorphic forms of the Active Ingredient, Monitoring Chemical changes of intermediate forms, and Measuring Composition of Solution.

Of particular interest to the pharmaceutical industry is information on polymorphy[*1] – for 2 reasons. Clearly the polymorph of the active ingredient will effect things like solubility and bioavailability. But, because of these properties, the pharmaceutical companies have been able to patent not only the chemical composition, but the polymorph as well.

What is important to recognize is that the polymorphy of small molecules is determined by chemical interactions. That means that intra- and/or inter-molecular interactions between specific molecular functional groups can determine the conformation of the molecules as well as the inter-molecular packing in crystals. While XRD can identify different polymorphs, extraction of detailed information requires a full fitting of the pattern. On the other hand, many molecular functional groups exhibit identifiable vibrational modes in the Raman and/or IR spectra, and these modes will shift due to chemical associations.

This article will show examples of results of measurements made on these types of materials that will demonstrate the power of the Raman instrumentation.

*1 By polymorphy, we include related phenomena of pseudomorphy in which waters of hydration are included in the crystal, and salt forms in which positive or negative functional groups are associated with anions or cations.

2 Excipients vs. Active Ingredients – Identification and Mapping

Pharmaceuticals are rarely sold as pure substances. APIs are mixed with excipients in order to provide bulk, extend shelf life, control solid state chemical reactions, and control the time of release (by wrapping APIs in polymer), etc.

An important characteristic of solid mixtures is the distribution of the species. In fact, the operation of mixing

itself becomes critical. It has been shown that vibration of a dry powder mixture of several species of particles of equal size but different densities will result in completely separating the species [1]! Thus, significant engineering and quality control has to be exerted in guaranteeing tablet composition.

Raman spectroscopy is an ideal tool for the differentiation of API from excipient. In fact, one could say it is custom tailored. Normally the API is a minor ingredient. But because it almost always has at least one aromatic group, its Raman signal is quite high compared to the other components.

The first example that we will present represents a study of a pharmaceutical tablet. Fig. 1 shows a microscopic image of a tablet. While it is possible to "see" particles, there is no chemical information in the picture.

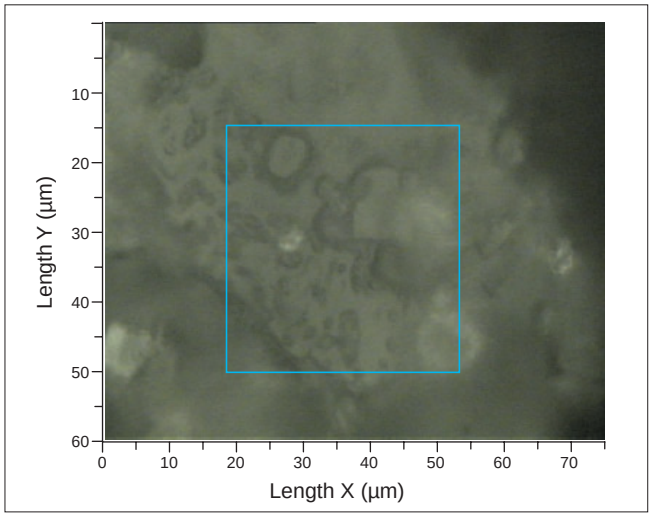

Fig. 1 Optical Microscopic Image of a Tablet

Spectral examination indicated the presence of at least three chemical species. It was decided to "map" the region indicated by the blue outline. In this operation, the sample is rastered in the X and Y directions. At each position, a full spectrum is acquired. The collection of spectra is used to map the position of each of the chemical species; in the map each color represents material whose spectrum is printed in the same color (Fig. 2). The two excipients have been identified as lactose (Fig. 2(a)) and starch (Fig. 2(b)) following a search of the spectra in a Raman spectral library. The third component, component C, has not been identified, but the presence of a strong line at ca. 1000 cm^{-1} indicates that it is an aromatic (Fig. 2(c)). Note also the intensity axis of component C is an order of magnitude higher than that of the other two components which confirms the expectation that aromatic API will produce more intense Raman spectra.

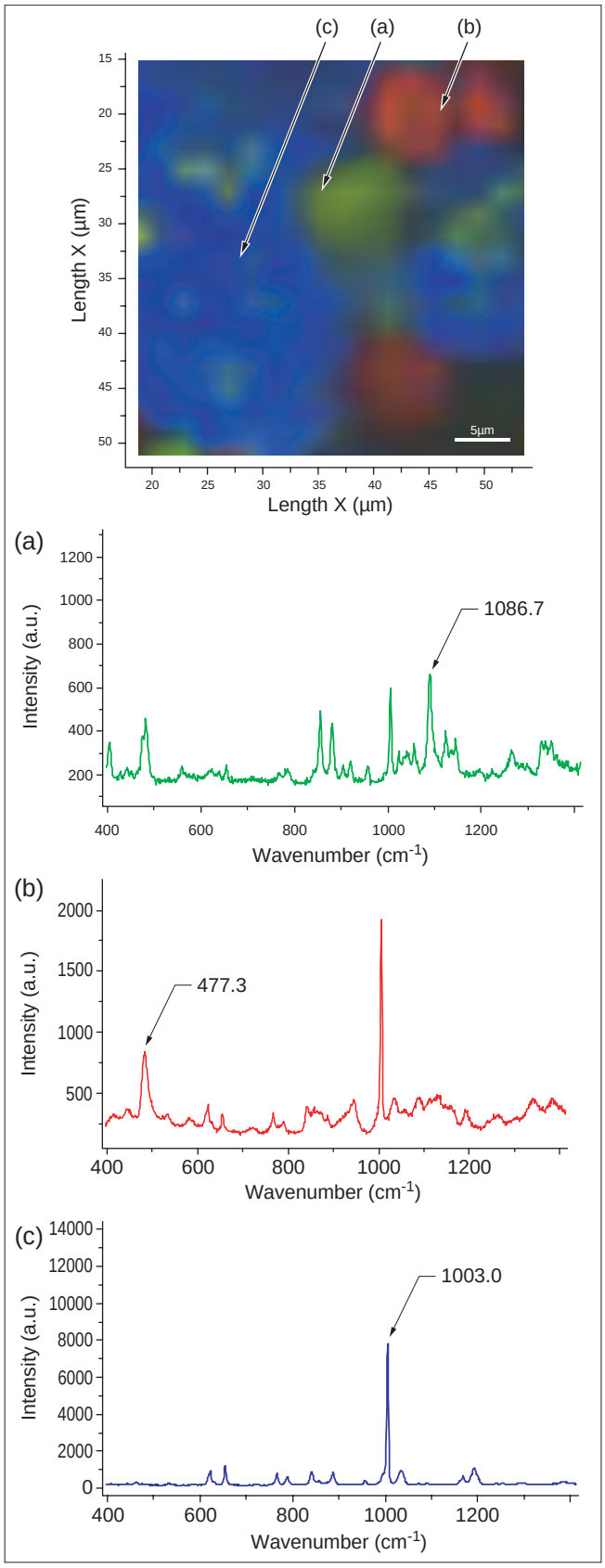

Fig. 2 Raman Map of a Tablet
(a) Raman Spectrum of Lactose
(b) Raman Spectrum of Wheat Starch + Component C
(c) Raman Spectrum of Component C
(assumed as aromatic series)

It was mentioned above that the identification of the excipients was done using Spectral ID, library-searching software. Fig. 3 shows the Raman spectra of lactose and starch, the two excipients mentioned above.

Fig. 4 is a reproduction of the Spectral ID screen showing the overlay of the top spectrum with that of a library spectrum of starch.

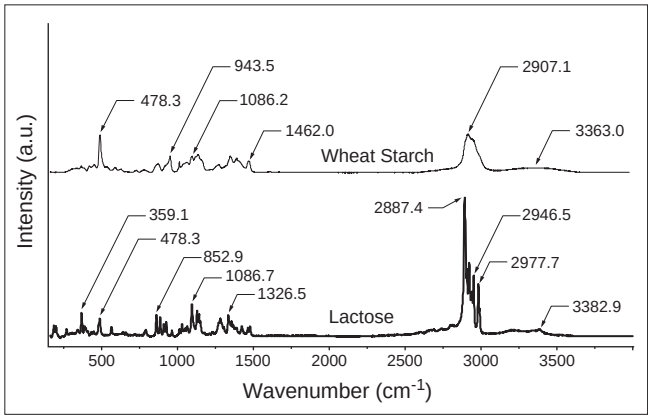

Fig. 3 Raman Spectra of Lactose and Wheat Starch

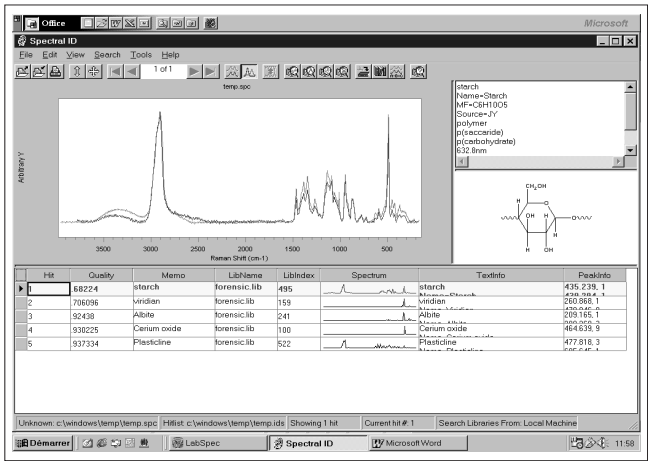

Fig. 4 Spectral ID Screen

3 Polymorphy

Control of polymorphy is a major issue in the pharmaceutical industry, as mentioned above. To re-iterate, solubility and bio-availability depend on polymorphy. Consequently, pharmaceutical companies want to control polymorphy, and patent-protect their products with polymorph spefications. Classically, polymorphy, and its variants as described above, were determined by XRD. However, XRD requires rather large samples, and does not provide chemical information directly. On the other hand, vibrational spectroscopy will indicate chemical interactions between identifiable functional groups. And the spatial resolution of vibrational techniques is determined by the physical diffraction limit and is quite good compared to XRD. For Raman, of course, the spatial resolution is about 1 μm. For infrared spectroscopy (FTIR) the spatial resolution is about 20 μm.

In the pharmaceutical industry, not only is the identification of the polymorph important, but its control during formulation of product is of great concern. Under certain circumstances it can be quite easy to convert from one polymorph to another. For instance, if relative humidity is not controlled, the degree of hydration of a crystal form can change, which changes its pseudopolymorphic phase. A different set of conditions that could require control might be the mixing of APIs with excipient and/or pressing into tablets. Under such operations one could also imagine the possibility of changing hydration or even salt forms.

The following spectra(Fig. 5) document the ability to identify polymorphy of Tylenol by Raman spectroscopy. The spectra represent the A and B forms.

FTIR spectra of the same species have been recorded on the LabRam IR and are shown in Fig. 6 – first the fingerprint part, and then the CH/NH part.

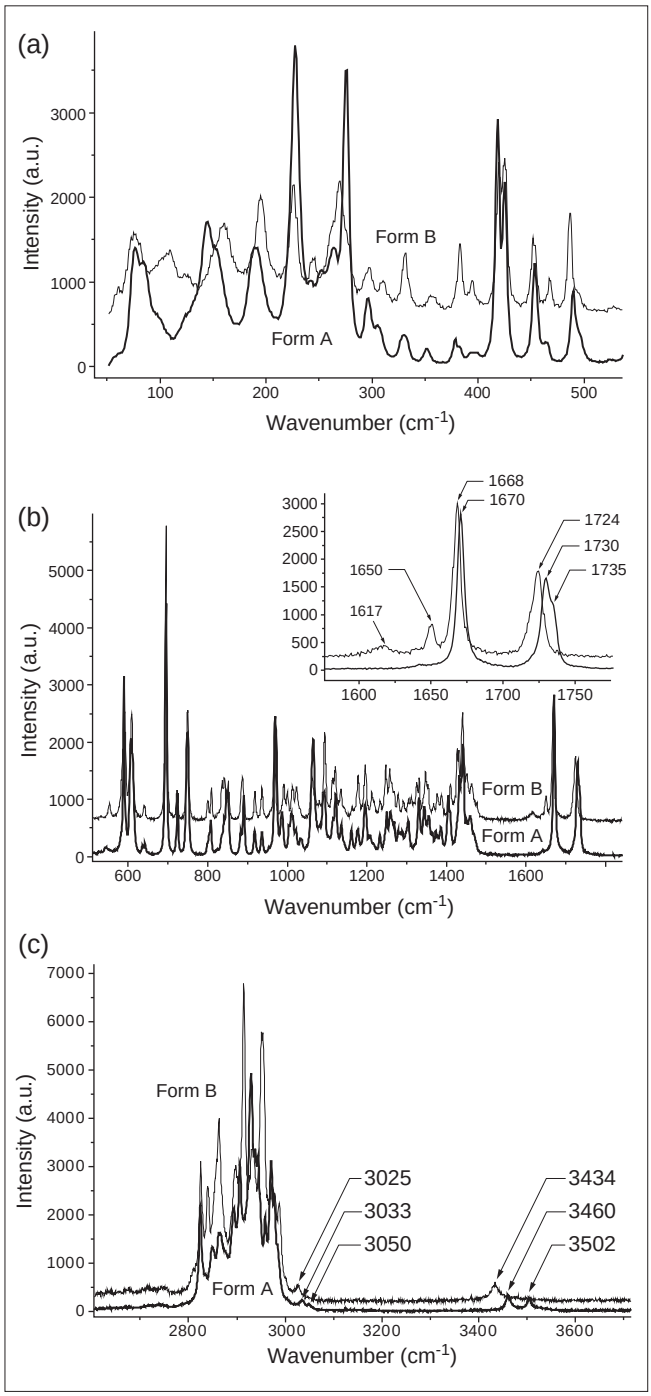

Fig. 5 Raman Spectrum of Tylenol Polymorphism
(a) Wavenumber Approx. 50 to 500 cm^{-1}
(b) Wavenumber Approx. 500 to 1800 cm^{-1}
(c) Wavenumber Approx. 2600 to 3700 cm^{-1}

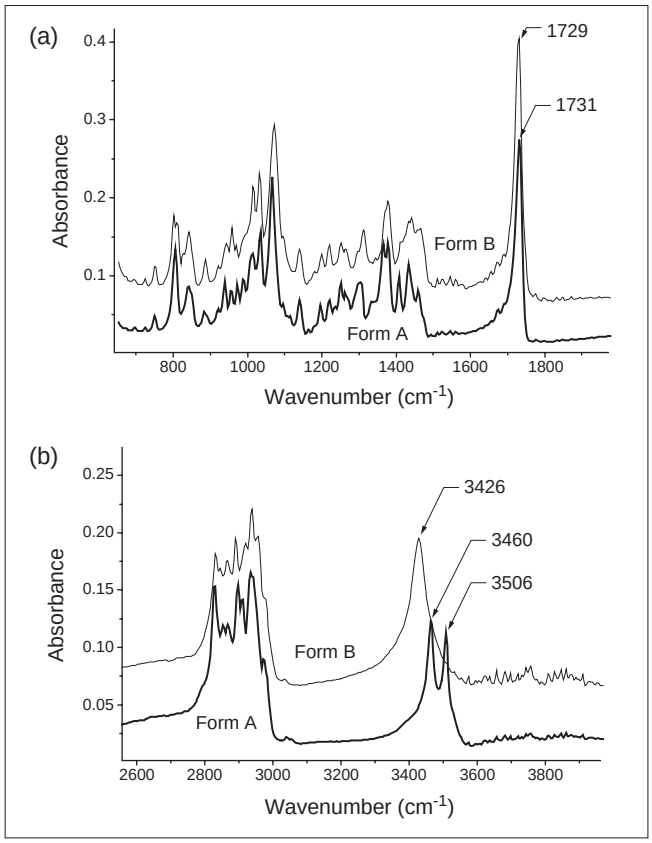

Fig. 6 Infrared Absorption Spectrum of Tylenol Polymorphism
(a) Fingerprint Region
(b) CH/NH Region

Recall that in the introductory section, we described how the interactions between functional groups can determine the way the molecule crystallizes. Now we can see how vibrational spectra can provide information on molecular associations. Table 1 tabulates the frequencies of >NH/-OH (3300-3600 cm^{-1}) and >C=O (1710-1740 cm^{-1}) bands.

Table 1 Raman and FTIR Peak Wavenumber for Tylenol Polymorphism

	Form A	Form B
Raman	3460/3502 cm^{-1} 1730 (sh 1735) cm^{-1}	3434 cm^{-1} 1724 cm^{-1}
FTIR	3460/3506 cm^{-1} 1731 cm^{-1}	3426 cm^{-1} 1729 cm^{-1}

There is overall agreement between the FTIR and Raman results. Form A has a doublet in the NH/OH region but a singlet in Form B. The carbonyl band has a frequency slightly higher in form A than form B. However, the difference in frequency is more pronounced in the Raman spectrum rather than the FTIR spectrum. This is somewhat ironic because the IR is believed to be more sensitive to carbonyl than Raman, which is, in fact, supported by the relatively low Raman intensities of this band. However, the insensitivity of the FTIR frequency could possibly be a result of the larger width of IR band.

These differences in frequencies can be assigned to phenomena such as hydrogen-bonding. A lower frequency means more H-bonding, which is also often reflected in a wider band. In this case form B is exhibiting stronger H-bonding.

4 High Throughput Screening and the Multiwell LabRam

High Throughput Screening (HTS) is the latest approach in analytical technology. Used particularly in the pharmaceutical industry and in life science laboratories, this method enables systematic screening of drug design and high throughput analysis of batch production.

The LabRam software has been adapted for automated study of Multiwell Plates. All of the phenomena discussed above are amenable to automated analysis for High Throughput Screening. Using a user-defined pre-programmed protocol for analysis, the software will output information in the form of an Excel spreadsheet. For example, the spreadsheet can report concentrations of API vs. excipient or different polymorphic forms as a function of well position. Fig. 7 shows the Raman Multiwell Analyzer.

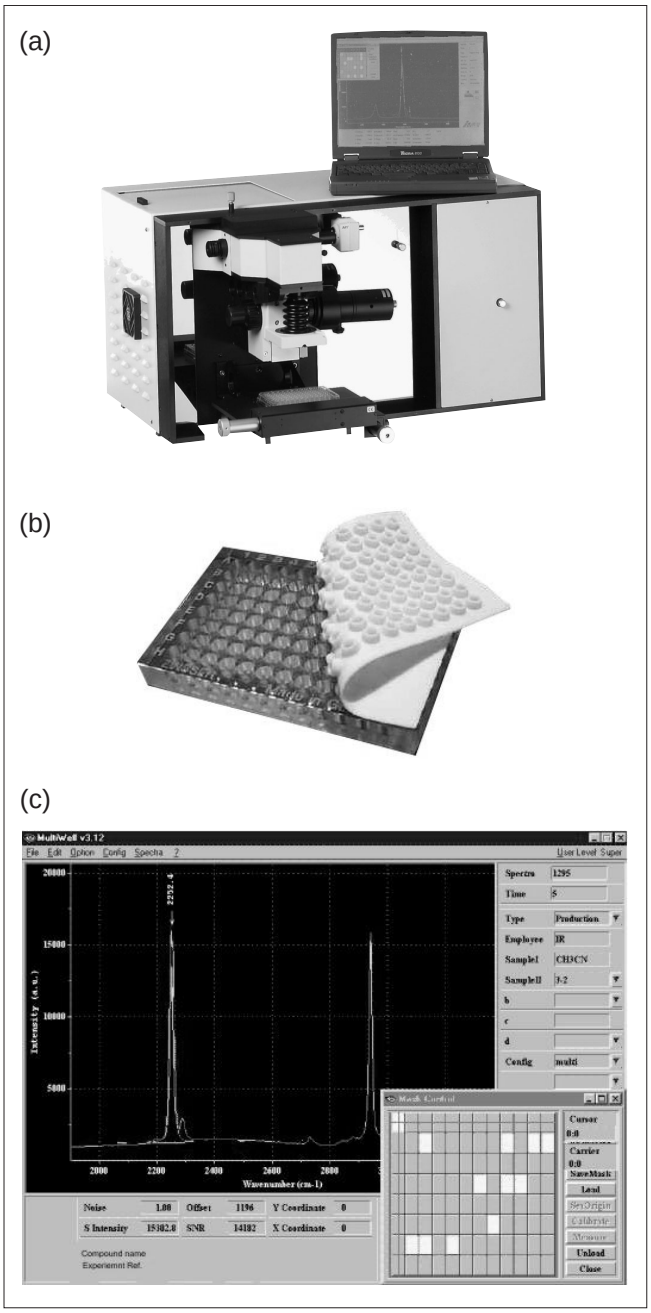

Fig. 7 Raman Multiwell Analyzer
(a) Multiwell LabRam
(b) Multiwell Plate
(c) Spectrum Screen

5 Conclusion

Developments of the last 75 years have paved the way for the implementation of sophisticated, automated Raman instruments in the analytical laboratory. These developments are in fact, two-pronged. On the one hand, the interpretation of spectra has to enable use of the phenomenon to extract useful information. On the other hand, the equipment has to produce the information with a minimum of effort. As this has converged, the pharmaceutical industry is now poised to take advantage of these developments to aid in the development of their products at all stages.

Reference

[1] Tom Mullin, "Mixing and De-mixing," Science 295, p.1851 (8 March, 2002)

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