Microscope Mapping on Formulated Pharmaceutical Samples Using the Dispersive Raman Technique

Introduction

Product characterization, such as component identification, mixture consistency, and verification of formulation, is becoming increasingly important in the pharmaceutical industry. There are often stringent requirements for bulk measurements, such as screening of incoming raw materials, molecular level analyses of reaction products or properties such as intermolecular interactions, and solid compound crystallinity.

Dispersive Raman spectroscopy has many applications in the pharmaceutical industry because of the extensive amount of information that it provides and its advantages in sampling and data collection. Raman spectroscopy is very sensitive to aromatic compounds, molecules with double and triple bonds, and many organometallics. Raman can often provide information on the physical state of the sample such as compound backbone structure or crystalline form. Likewise, Raman is relatively insensitive to compounds with strong dipoles, so samples containing or dissolved in water can be directly analyzed. In addition, samples can often be analyzed without removing them from their sealed glass or plastic containers.

Another advantage is that the active ingredients in many pharmaceutical formulations provide very strong Raman spectra while the spectra of their excipients are fairly weak. This often allows the rapid, direct analysis of the active ingredients with no special sample preparation, not only saving time and effort, but also preserving the very physical state of the compound that is to be analyzed.

This technical note describes the analysis of a common pharmaceutical tablet using the dispersive Raman technique to identify the major components in the mixture. It illustrates that through the rapid collection of a single spectrum representing a large cross-section of the sample and simple manipulations of the data using spectral libraries, many of the major components will be quickly identified. This information is then used to automate data collection on a dispersive Raman microscope to probe a large area of the tablet with high spatial resolution and map out the distribution of the different components at the surface of the tablet.

Composition of a Tablet

Analysis of the bulk composition of a sample, whether a pharmaceutical mixture or any other chemical formulation, often helps identify the components or determine the relative breakdown of components. Whether this information is being applied to product testing and validation or for competitive analysis, it is important to analyze as many of the components as possible. In this investigation, the molecular composition of a common over-the-counter pain killer tablet was analyzed.

Figure 1 presents the spectrum of the sample collected on the 180° backscatter sampling configuration in the sample compartment of a Nicolet™ Almega™ dispersive Raman spectrometer. The spectrum exhibits a number of very strong, well-resolved peaks that can be used for compound identification. In this case, it is likely that the spectrum is actually a combination of spectra from the various components in the mixture. Therefore, it is useful to attempt to dissect the spectrum into its individual components. This can be done using OMNIC™ software and the Nicolet Aldrich® Raman library.
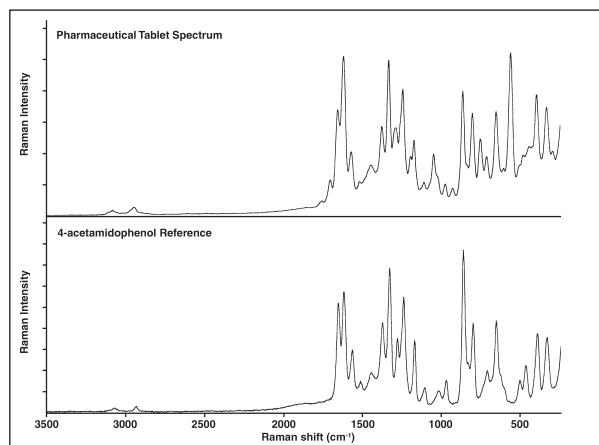

Figure 1: a) Spectrum of a pharmaceutical tablet collected using the 180° backscatter sampling configuration, and b) a reference spectrum of 4-acetamidophenol (acetaminophen)

A library search of the spectrum reveals that the main component is likely 4-acetamidophenol (acetaminophen), and this is confirmed by looking at the major peaks in the sample spectrum and the library spectrum. While these peaks are obviously present, there are a number of additional peaks that indicate the presence of other compounds.

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A spectral subtraction was performed in which the library spectrum of acetaminophen was subtracted from the original sample spectrum and the resultant spectrum is shown in Figure 2. This resultant spectrum was searched against the Nicolet Aldrich Raman library. A reference spectrum of the result is presented as spectrum b) in Figure 2. This suggests that acetylsalicylic acid (aspirin) is a second active component in the mixture.
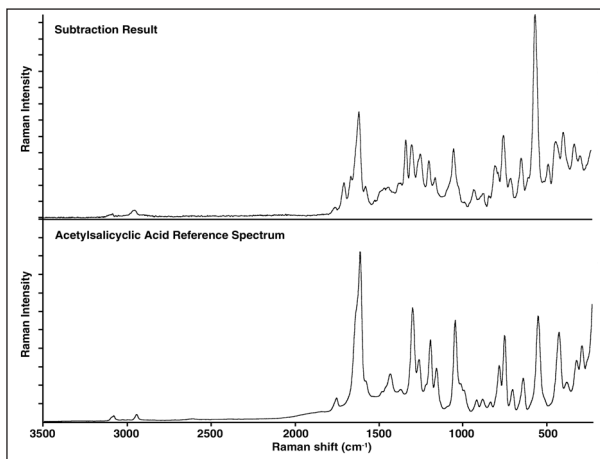

Figure 2: a) Subtraction result of acetaminophen from the original sample spectrum and b) a library spectrum of acetylsalicylic acid (aspirin) from a library search of the first subtracted spectrum

As with the first library search comparison, there are additional peaks in the subtracted sample spectrum, which can be attributed to compounds other than aspirin. While some of these peaks result from an incomplete acetaminophen subtraction, there are enough distinct peaks to identify at least one additional substance in the mixture.

Subtracting the aspirin spectrum from the spectrum of the first subtraction result produces a second subtraction result spectrum, shown in Figure 3. A library search of the second subtraction result suggests that caffeine is another main component in the mixture. It is also apparent that there are still peaks that are not accounted for by the caffeine spectrum. However, closer analysis reveals that the majority of these peaks arise due to acetaminophen. This suggests that acetaminophen is in a much larger quantity in the sample than the other components.
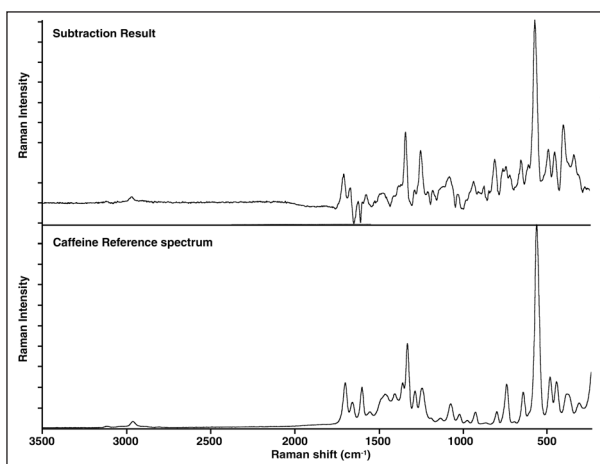

Figure 3: a) Spectrum resulting from the subtraction of the aspirin reference spectrum from spectrum of the first subtraction result and b) a reference spectrum of caffeine

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 tablet. This could assist in ascertaining the efficiency of mixing during manufacture, the likelihood of agglomerates, or component migration due to aging. Likewise, this information could be important in assessing likely tablet dissolution, active compound delivery rate, or shelf life. A very powerful technique for determining such spatial distribution is dispersive Raman microscope mapping.

Microscope mapping with the Nicolet Almega Raman spectrometer offers the ability to automatically collect spectra of many discreet points on the sample over a large pre-defined region. Since spectral mapping provides high sensitivity and full spectral information at every sampling point, it is possible to collect a single spectral map and quickly extract images that illustrate the distribution of many of the species in the sample.

Figure 4 shows the spectral information obtained by measuring a 150 x 150 micron region of the tablet and displaying the information as a function of overall spectral intensity. The map was obtained by taking 1-µm steps and collecting a single 5-second exposure at every point. This representation indicates that there is little differentiation over the mapped area in the relative spectral intensity, illustrating that, although the individual component spectra are changing, the overall intensity remains constant.
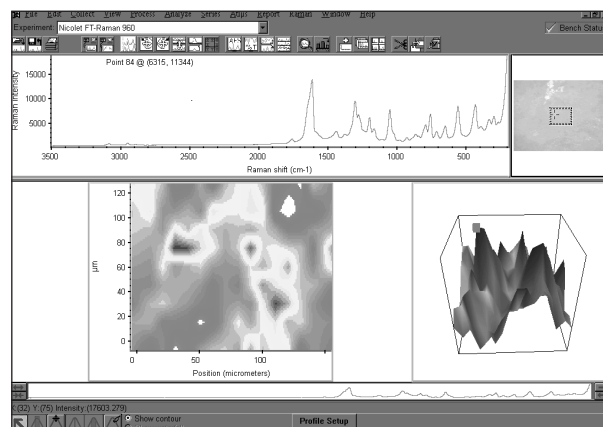

Figure 4: Atlµs™ software InterLink window provides several views of the sample data. (a) A video image of the sample area shows little detail, while the color contour image (b) and the three dimensional map (c) indicate a small amount of chemical variability across the tablet.

The power of spectral mapping can be realized by manipulating the data set in ways that present the spatial distribution of individual components. With Atlµs software mapping, all data collection and manipulation is automated through the software interface using simple on-screen commands. Once the map data is collected, chemical images are generated by pointing to peaks in the spectrum. This produces images based on peak, heights, peak areas, ratios of peaks, or correlations.

The unique Atlµs software InterLinking feature provides an efficient means of examining spectral features anywhere on the map by simply placing the cursor on the region of interest. By simply reprocessing the map data to look for the 857 cm^{-1} band of acetaminophen, a new image is extracted that is presented in Figure 5.

For peak height analysis, it is important to choose a peak that is free of interferences from the spectra of other components in the sample. In this case, the 857 cm^{-1} peak is unique to acetaminophen. The resulting chemical intensity contour map illustrates that the microscope is capable of resolving the different species in the area sampled.
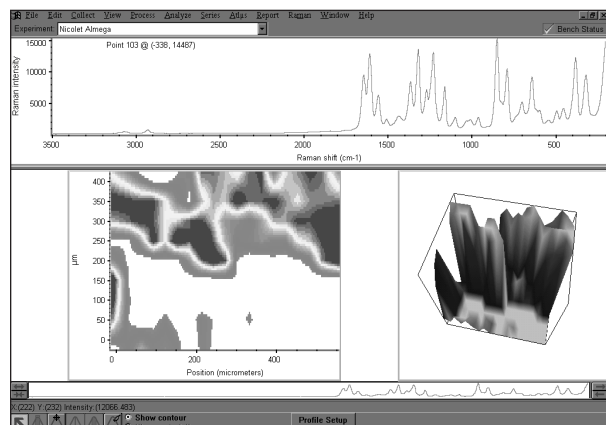
Figure 5: Contour display of the tablet sample mapping data, looking only at the peak intensity of the 857 cm^{-1} band of acetaminophen

Next, the data set is reprocessed to look for distributions of other components in the tablet. Figure 6 shows the chemical contour map extracted from the tablet data set, based on the intensity of the 1042 cm^{-1} band of aspirin. This feature was chosen to avoid interferences from strong peaks in other component spectra. The contour display shows a slightly different distribution of the aspirin component than we see for the acetaminophen component.
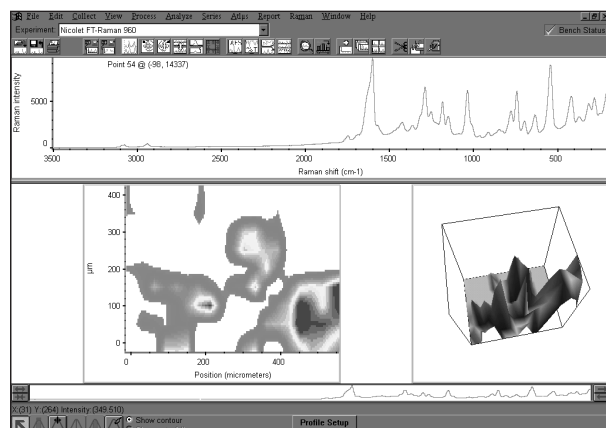

Figure 6: Contour display of the tablet sample mapping data, mapping the peak intensity at the 1042 cm^{-1} band of aspirin

Finally, an additional reprocessing of the mapped data, using the 1697 cm^{-1} band of caffeine, shows it is possible to obtain a chemical image of the distribution of this third component. This representation is shown in Figure 7.
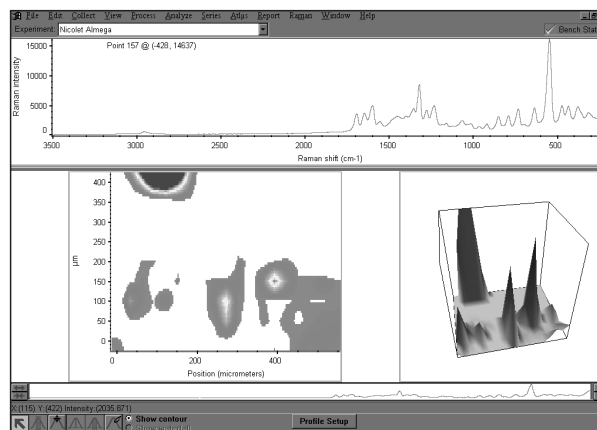

Figure 7: Contour display of the tablet sample mapping data, mapping the peak intensity at the 1697 cm^{-1} band of caffeine. This feature was chosen because it is in a region where none of the other components have noticeable peaks.

Figure 8 compares the three chemical images produced. The data show that these components are not uniformly distributed in the tablet, rather this view clearly shows that large crystal agglomerates of acetaminophen, aspirin, and caffeine account for a majority of the tablet surface. The minor regions not filled by these three compounds are likely occupied by various excipients, which typically have very weak Raman signals.
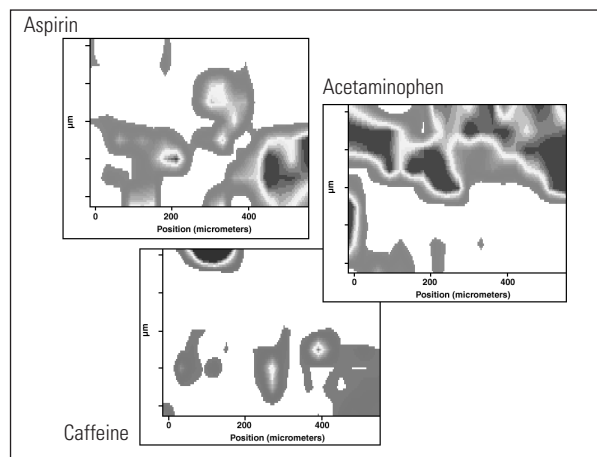

Figure 8: Comparison of the three chemical images produced from the single map of the tablet surface. This figure demonstrates that most of the surface mapped is composed of crystal agglomerates of acetaminophen, aspirin and caffeine.

Conclusion

Raman spectroscopy is a powerful analytical tool for the modern pharmaceutical laboratory. The ability to sample easily with no sample preparation and the abundant chemical information is increasing the demand for the technique in many aspects of research and quality control. Coupling the ability to look at bulk samples with the ability to automatically map microscopic component distribution makes dispersive Raman spectroscopy a necessity in the pharmaceutical laboratory.

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