Application Note: 51184

Calibrationless Semi-Quantitative Analysis of a Heterogeneous Sample Using Raman Microscope Mapping

Koichi Nishikida, Steve Lowry, Thermo Fisher Scientific, Madison, WI, USA

Key Words

- Calibrationless Semi-quantitative Analysis
- Heterogeneous Sample
- Imaging Analysis
- Multivariate Curve Resolution
- Raman Microscopy

Introduction

Microscopy is frequently used to assess the microheterogeneity and identify components in solid samples. One common application in the pharmaceutical industry is the mapping and quantification of excipients and active pharmaceutical ingredients in tablets. Both FT-IR and Raman microscopy have proven useful for this application. We reported on results obtained using the Thermo Scientific Nicolet Continuμm XL FT-IR imaging microscope from Thermo Fisher Scientific.[1] Raman microscopy is particularly valuable for this application, since Raman spectroscopy can identify not only the molecular species present, but can also distinguish between crystal polymorphs and amorphous forms. In addition, unlike FT-IR imaging microscopy, Raman spectra are not subject to artifacts generated by differences in the sample surface morphology. Dispersive Raman microscopes, such as the Thermo Scientific Nicolet Almega XR, use excitation lasers in the visible wavelength range. This results in excellent spatial resolution down to 1 μm.

Thermo Scientific OMNIC Atlμs imaging software incorporates sophisticated chemometric and image analysis tools. When combined with library search capabilities, dispersive Raman tablet mapping using Atlμs™ software is now able to identify the tablet components and perform a calibrationless semi-quantitative analysis of their distribution and relative concentration. This application note presents the results from mapping a tablet of painkiller using the Nicolet™ Almega™ XR Raman microscope.

Experimental

The workflow for calibrationless quantitative analysis of a heterogeneous tablet sample using Raman microscopy consists of the following steps:

1. Map the area of interest by collecting Raman spectra using the Almega XR Raman dispersive microscope.
2. Perform multivariate curve resolution (MCR) to determine the number of components, calculate component concentrations, and generate result vectors that are used to identify components through library searches.
3. Apply the OMNIC™ Atlμs image analysis feature to digitize the concentration maps into binary maps of the constituents.
4. Calculate the area occupied by each sample constituent from the binary maps.
5. Convert the percent surface area of each component to percent weight.

A commercially available, over-the-counter painkiller tablet with surface coating was sliced in half. The Nicolet Almega XR dispersive Raman microscope equipped with a 10X objective was used to map the cut surface of the tablet using the 780 nm excitation laser and the OMNIC software suite with Atlμs imaging software. Raman spectra over the fingerprint region (200–1800 cm^{-1}) were collected from a 6 x 5.5 mm area at 50 μm intervals. A total of 13,542 spectra were collected. Spectral preprocessing consisted of a baseline correction, followed by normalizing the maximum peak intensity in each spectrum to unity.

Results

Figure 1 is a screen shot showing the results obtained using the Atlμs mapping software. The Figure shows a video image of the tablet surface and a chemical image based on the Raman intensity at 551 cm^{-1}. It also shows the Raman spectrum acquired at the position in the video and chemical images that is marked with the red cross hairs. The chemical map shows three distinct areas. A spectral library search on representative spectra from each area suggested that the three areas represented the distribution of caffeine, aspirin and acetaminophen. However, since the step size in the map is 50 microns and the laser spot size with the 10X objective is 10–20 microns, spectra from many pixels in the map are likely to show spectral features corresponding to more than one component. This precludes using simple Raman intensity maps such as this for quantitative analysis.

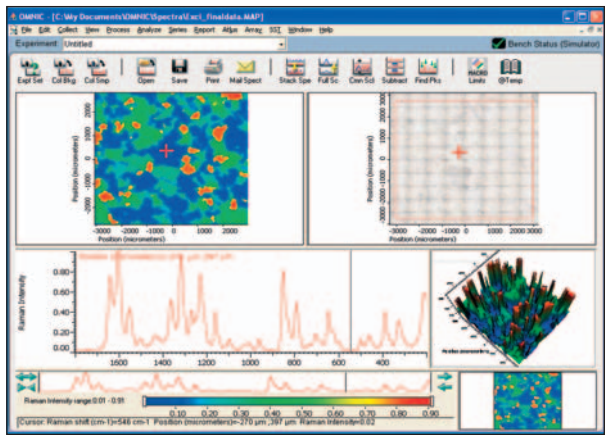

Figure 1: Map of a painkiller tablet. This screen shot of results using OMNIC Atlμs software shows the video image of the mapped tablet (upper right) and a chemical image (upper left) generated using the intensity of the Raman signal at 551 cm^{-1}.

It is possible to extract the pure component spectra by using Multivariate Curve Resolution (MCR), a feature of the Atlμs mapping software package. MCR is a statistical analysis procedure that deconvolves the spectral data in the sample space into the product of a pure spectral matrix and a concentration matrix. The resulting spectral components are shown in Figure 2. Three of the resulting vectors generated by MCR were very similar to the spectra of the three components already identified by the library search. In addition, a fourth component was identified. The unknown component (Figure 2(a)) showed

three characteristic peaks in the low frequency region. A library search identified this component as titanium dioxide, which is frequently used for whitening and opacity in formulation coating materials. The remaining components were confirmed to be acetaminophen, caffeine, and aspirin. One advantage of using MCR is that the resulting components are sufficiently free from cross contamination by other components that they can usually be identified by performing a library search.

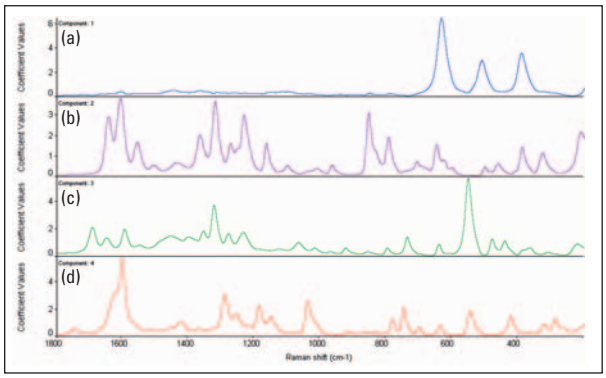

Figure 2: The four painkiller tablet components generated using multivariate curve resolution of the hyperspectral mapping data collected using OMNIC Atlμs mapping software.

The distribution of the four components can be displayed as concentration maps. Using image analysis features in Atlμs, the maps of the four components were digitized to generate binary images (Figure 3). Assuming the densities of the four components are approximately the same, the relative concentrations of each can be calculated from the relative areas in the binary maps. The results are shown in Table 1 and are comparable with the reported percent by weight for the three main components.

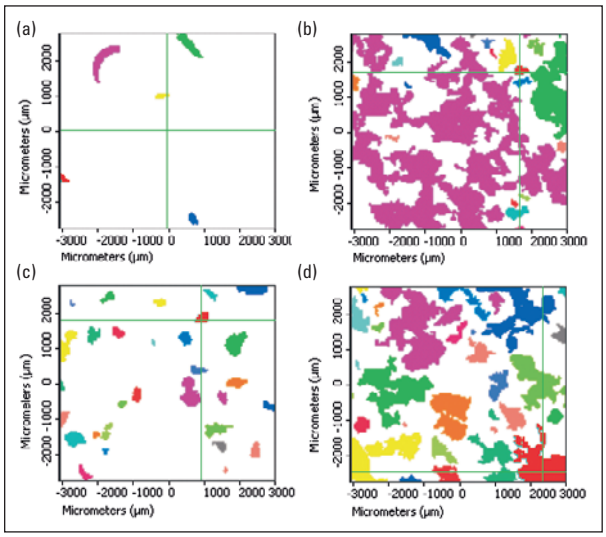

Figure 3: Digitized area maps showing the areas occupied by (a) titanium dioxide; (b) acetaminophen; (c) caffeine; (d) aspirin.

Component	Compound	Area Percent	Formulation Weight Percent
1	TiO_2	1.4	-
2	Acetaminophen	45.9	44.3
3	Caffeine	8.1	11.4
4	Aspirin	44.6	44.3

Table 1: Percent by weight composition of painkiller tablet calculated from the binary image maps and compared with the reported formulation weight percent.

The advantage of using MCR rather than using trial-and-error inspection of spectra to identify the sample components is that MCR makes it much easier to identify minor contaminants. This is illustrated in Figure 4. The spectrum in Figure 4(a) is of pure aspirin, whereas that in Figure 4(b) is from aspirin contaminated with 5% TiO_2. Without prior knowledge, it would be difficult to detect and identify the contaminant.

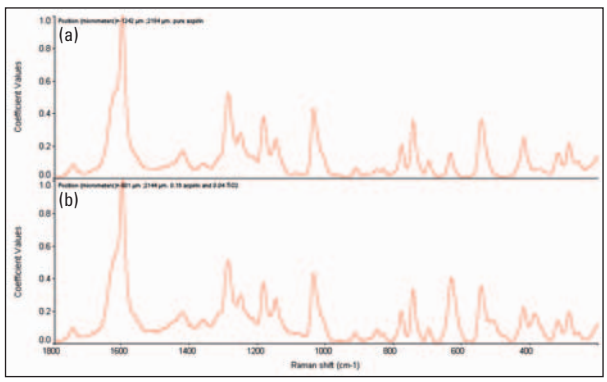

Figure 4: The benefits of using MCR. The Raman spectrum of pure aspirin (a) is barely distinguishable from the spectrum of aspirin contaminated with approximately 5% TiO_2 (b).

Summary

We have established a method to achieve calibrationless semi-quantitative analysis of a heterogeneous sample by using the Nicolet Almega XR dispersive Raman microscope to map the surface of a painkiller tablet. The MCR analysis and library searching capabilities of OMNIC plus Atlμs mapping software were used to identify the tablet components. From the digitized concentration maps generated using Atlμs image analysis, the relative areas and thus the relative concentrations of the tablet components were calculated. The calculated results were in good agreement with those provided by the tablet manufacturer. By revealing the presence and obtaining the spectrum of a minor impurity, the results clearly demonstrate the benefit of using multivariate curve resolution in this application.

References

1. Koichi Nishikida, Jonathan A. Tarr, Federico Izzia, N. Simon Nunn, *"Standardless Semi-Quantitative Image Analysis of Heterogeneous Microscopic Materials,"* Poster, Pittsburgh Conference, March 2006.

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

Africa-Other
+27 11 570 1840
Australia
+61 2 8844 9500
Austria
+43 1 333 50 34 0
Belgium
+32 2 482 30 30
Canada
+1 800 530 8447
China
+86 10 8419 3588
Denmark
+45 70 23 62 60
Europe-Other
+43 1 333 50 34 0
Finland/Norway/Sweden
+46 8 556 468 00
France
+33 1 60 92 48 00
Germany
+49 6103 408 1014
India
+91 22 6742 9434
Italy
+39 02 950 591
Japan
+81 45 453 9100
Latin America
+1 608 276 5659
Middle East
+43 1 333 50 34 0
Netherlands
+31 76 579 55 55
South Africa
+27 11 570 1840
Spain
+34 914 845 965
Switzerland
+41 61 716 77 00
UK
+44 1442 233555
USA
+1 800 532 4752

www.thermo.com

Thermo Electron Scientific Instruments LLC, Madison, WI USA is ISO Certified.

AN51184_E 04/09M