Application note

Rapid infrared microscopy in pharmaceutical product development, quality control and biologics formulation

FTIR infrared microscopy in the pharmaceutical industry

Fourier transform infrared (FTIR) microscopy provides critical data that pharmaceutical companies use to improve time-to-market and reduce product failures. Formulation scientists may want to identify components in a solid dosage form for reverse engineering or ascertain the distribution of API, excipients or polymorphic forms of API in their product. Pharmaceutical QC laboratories need to verify composition prior to release of drug batches, and they must understand batch-to-batch variation of solid dosage forms. Pharmaceutical labs may need to identify foreign particulates in a biologic/parenteral drug or assess the make-up of a tablet or transdermal patch. Many organizations use IR microscopy for the detection and analysis of counterfeit pharmaceuticals.

As new challenges emerge, FTIR microscopy is being called upon to deliver flexible solutions that combine crisp, clear visual tools with precise infrared analysis and powerful software analytics. The Thermo Scientific™ Nicolet™ RaptIR™ FTIR Microscope provides the required capabilities and outstanding performance in both visual and IR operations, while enabling novice and expert users to obtain useful results. It is ideally suited to address the diverse emerging challenges in the pharmaceutical industry.

Example applications in pharmaceuticals

The Nicolet RaptIR FTIR Microscope combines speed, agility and accuracy to provide analytical results in minutes to a formulation scientist, QC analyst or a complaint investigator, regardless of skill level. Here are some examples of how pharmaceutical labs can take advantage of the system's enhanced analytical capabilities.

Example 1: Product development of metered dose inhalers

During product development, R&D scientists aim to control and optimize dissolution or distribution of materials through refinement and adjustment of formulations and processes. Inhaled drug products are often delivered with metered dose inhalers (MDIs). During the development of MDIs, the evaluation of the drug distribution, agglomerates and crystallinity of the APIs are of utmost importance.

For this work, the sample was prepared by actuating the MDI at a set distance from a gold-plated slide. The slide was then evaluated using Reflectance mode. The Nicolet RaptIR FTIR Microscope provides fast chemical mapping of large areas allowing an R&D scientist to study the sprayed profile (distribution) of the drug, and to simultaneously obtain information about the crystallinity of the API and to evaluate/characterize agglomeration of the particles. Both crystallinity and agglomeration can affect the efficacy of the drug in patient use.

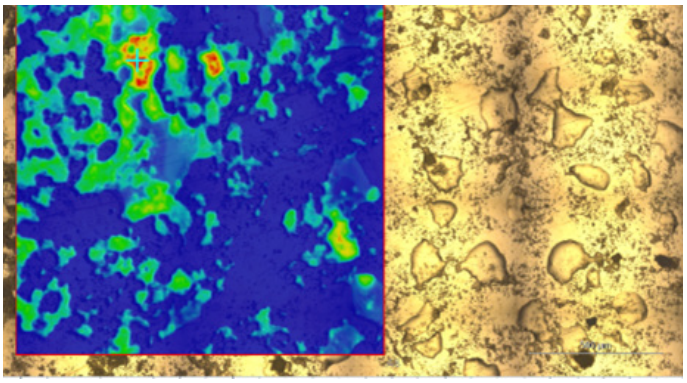

Figure 1. Wide view image of a sprayed drug and area of interest (blue region outlined in red) selected for infrared analysis.

In Figure 1, a wide field of view image of the sprayed drug is shown, and the areas of interest selected for infrared analysis are highlighted (the colored region). The visible backdrop appears to be relatively evenly distributed, but the profile, or chemigram, shows the highest density of inhalable drugs in the areas colored green and red. Drug delivery from the MDI is not uniform in this case.

A multivariate curve resolution (MCR) processing of this data in Figure 2 reveals the red and green spectra are indeed salbutamol sulfate, which is the API of the inhaled drug product. The apparent Interaction between the propellants and the API can be established by profiling the infrared spectra across the image, generating a color-coded image of the product distribution.

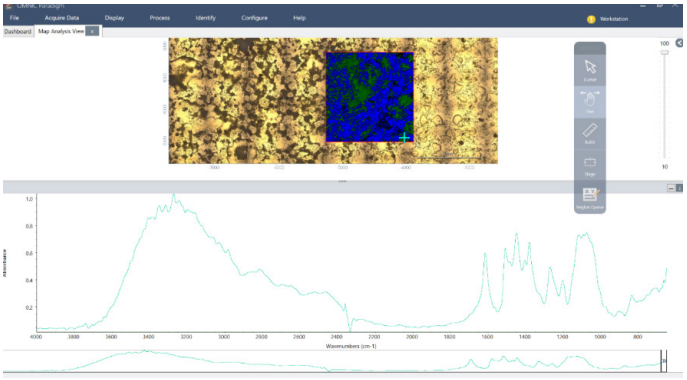

Figure 2. MCR analysis of MDI spray to examine API distribution.

While the preferred test for the particle size distribution of MDI's is the cascade impactor, the US Pharmacopeia (USP) states that microscopy can be used to evaluate the number of large particles, agglomerates, and foreign particulates in the emissions of MDIs. The key advantages of FTIR microscopy inspection lie in the additional information gained, including the detection of large particles, observation of any changes in morphology of the drug substance particle, the extent of agglomeration and crystal growth, and the detection and identification of foreign particulate matter. All these factors can affect the bioavailability, performance, stability, and other properties of the drug product.

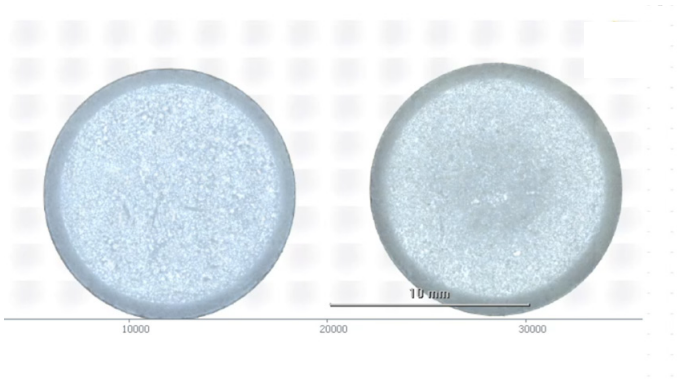

Figure 3. High quality visual images of two tablets with identical ingredients.

Example 2: Analyzing tablets in the quality control laboratory

In this example, two tablets with identical ingredients in the same concentrations were analyzed to map the distribution of API and excipients. First, high quality visual images of the tablets were acquired (figure 3). The Thermo Scientific™ OMNIC™ Paradigm Software enables multiple areas of interest to be acquired in serial fashion automatically, even across the separate tablets.

In this case, the IR data was collected using a Ge ATR (a contact method with very high signal-to-noise) using a 25 µm step size to map 1200×950 µm areas on each tablet.

Once the chemical map was acquired, MCR was utilized to decompose the spectra into pure components. MCR is a very powerful analytical tool for identifying unknown components for exploratory analysis such as in reverse engineering.

MCR images in Figures 3 and 4 of the two tablets reveal the distribution of three components; component 1 is shown in blue, component 2 in green and component 3 in red. Areas in black are unresolved by the three components. The MCR image shows a significant difference in that tablet 1 has more homogenous distribution of components compared to tablet 2.

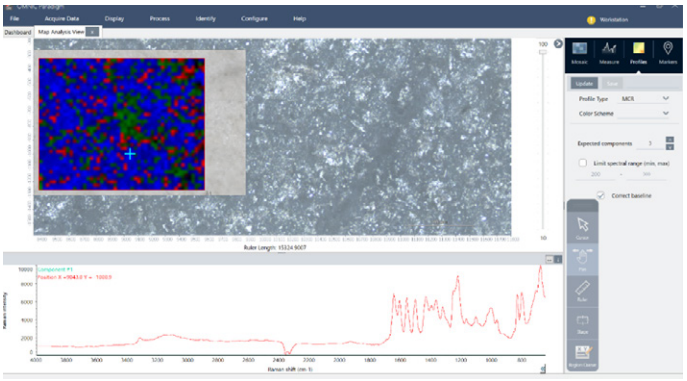

Figure 4a: MCR of tablet 1.

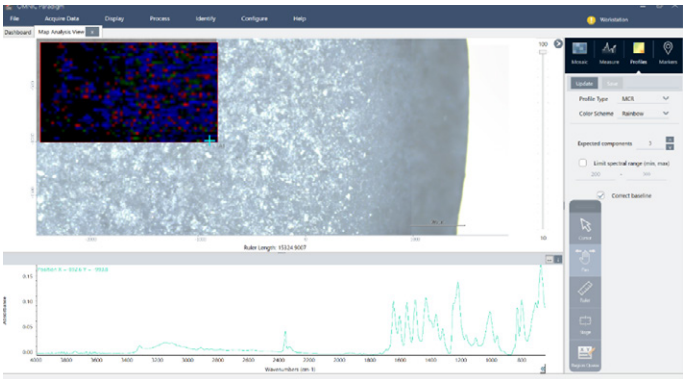

Figure 4b. MCR of tablet 2.

Analysis of the three modeled components revealed acetaminophen, sodium bicarbonate and starch as ingredients 1, 2, and 3, respectively. So, in one pass, the identity, distribution and differences in the two tablets were determined.

Figures 5-8 show the specific distributions of acetaminophen (5,6) and starch (7,8) in the two tablets.

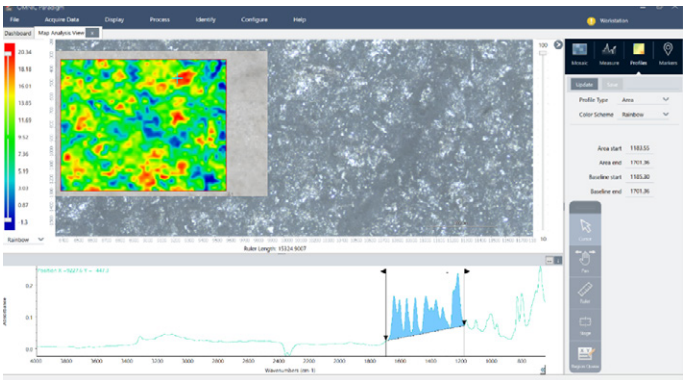

Figure 5. Acetaminophen profile for tablet 1.

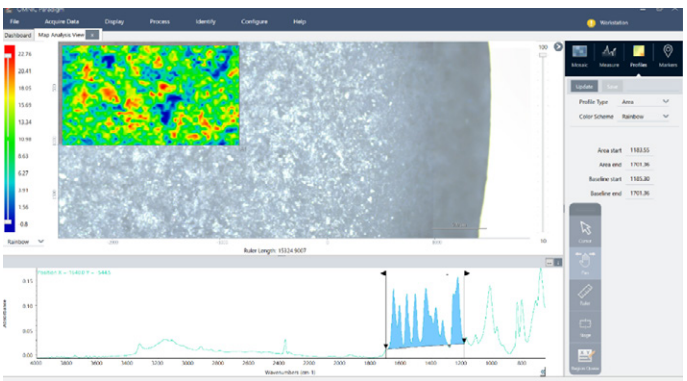

Figure 6. Acetaminophen profile for tablet 2.

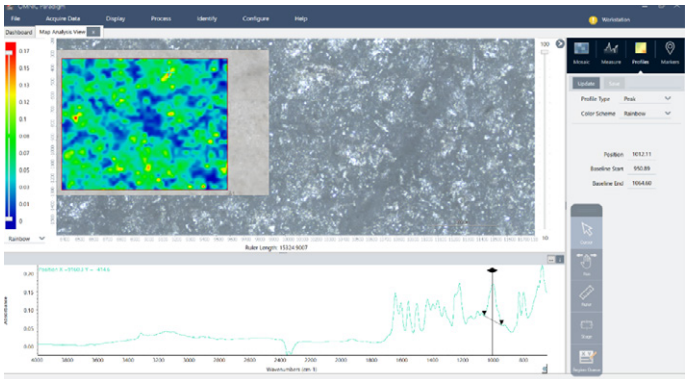

Figure 7. Starch profile for tablet 1.

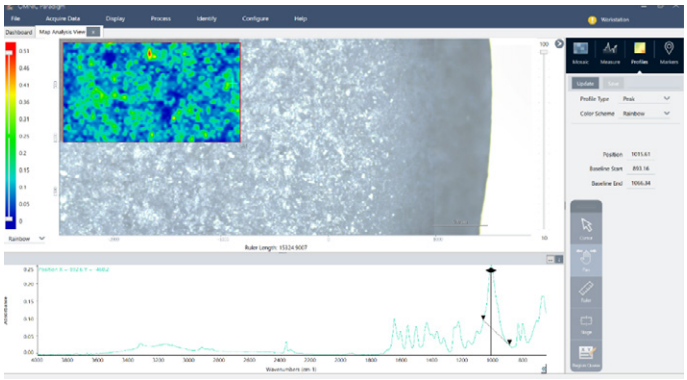

Figure 8. Starch profile for tablet 2.

In both cases, tablet 2 seems to show a smaller domain size indicating a difference in the tablet production (perhaps in the compression used or the preparation of the two formulations). This seems apparent, even from the visual images.

Example 3: Biologics formulation evaluation

Biotherapeutics containing bioproteins are an important class of biopharmaceuticals. During formulation of such biotherapeutics, it is essential to evaluate their stability. Chemical and physical factors such as variable temperatures or shear rate exposure under storage conditions can lead to misfolding, nucleation, and subsequent fibril formation of the proteins. These will strongly affect the bioactivity of the therapeutic. Infrared microspectroscopy can be utilized for secondary structure analysis to investigate protein misfolding and fibril formation.

For this work, 1 µL of a commercially available insulin product was dried onto a barium fluoride window. Additional depositions of the sample on the window enhanced the signal and hence the detection limit in reflection mode. The infrared spectrum from one point (Figure 9) shows a composite spectrum. This is easily seen comparing the red (sample) and blue (library spectrum of insulin). The red spectrum was run through OMNIC Paradigm Software's multi-component search algorithm. The result showed the presence of insulin and the excipients glycerol, phenol and meta cresol in the formulation.

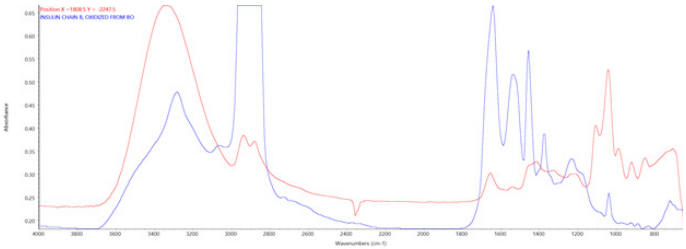

Figure 9. Infrared spectrum of a prepared insulin sample.

A 10 mm×10 mm square area was mapped in reflection mode. Figures 10 and 11 show the visual image of the sample, the infrared map, and a spectrum associated with one location on the map.

As before, various profiles were selected to view the distribution of insulin, glycerol and phenol and were applied to create the distribution images. Figure 10 was profiled to show the protein material of the insulin. This covers the majority of the window, with a speckling due to the drying.

Figure 11 emphasizes the glycerol distribution in the dried material. Note by comparison, the glycerol and insulin have largely separated as they dry.

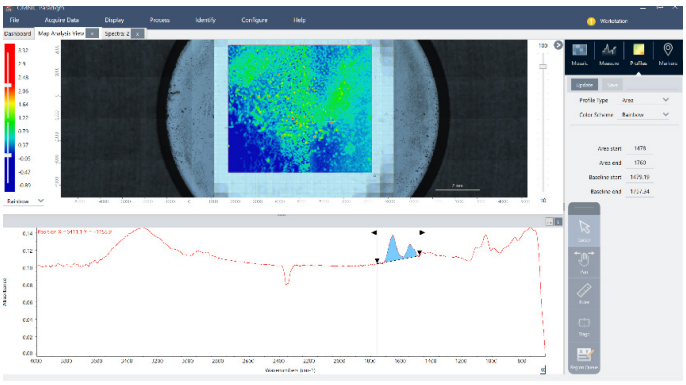

Figure 10. Visual image of an insulin sample, infrared map, and spectrum associated with one location on the map profiled to show protein material.

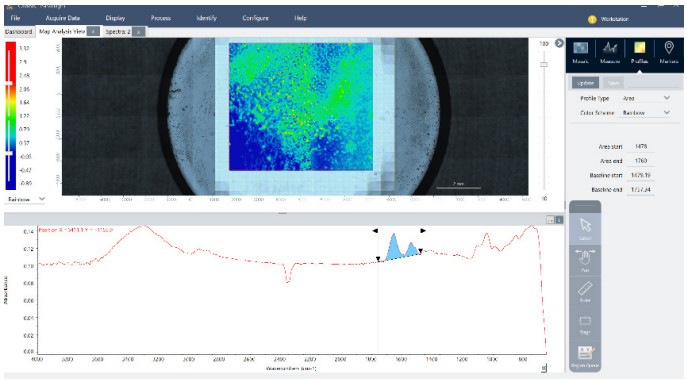

Figure 11. Visual image of an insulin sample, infrared map, and spectrum associated with one location on the map emphasizing glycerol distribution.

Conclusion

The Nicolet RaptIR FTIR Microscope provides outstanding visual images (<1 micron) and high spatial resolution infrared data (<5 microns without ATR) and a suite of powerful software tools to understand the results. The simplicity of operation enables even novice users to begin to extract information from microscopic samples. The breadth of applications makes this an ideal tool for use in pharmaceutical settings from R&D to QA/QC laboratories, even as new analytical challenges arise.

References

Hannah Tiernan, et al. ATR-FTIR spectroscopy and spectroscopic imaging for the analysis of biopharmaceuticals, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, vol. 241, 2020, 118636.

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