

## Applications for Mid-IR Spectroscopy in the Pharmaceutical Process Environment

Mid-IR continues to prove its usefulness as a process analytical technology.

**Mark A. Druy, Ph.D.**

*Hello again, fellow analysts. I trust that you all had a jolly holiday season. I assume you all have had a chance to read the Process Analytical Technologies (PAT) Guidance and are eagerly awaiting the final draft. In the meantime, I will continue to highlight the tools of PAT. As promised, I am bringing you a taste of what can be done in PAT with that wonderful standby, midrange infrared spectroscopy. I have always agreed that no analytical lab can do its best work without an infrared spectrometer, but, now, Mark Druy (Director of Analytical Systems Development, Argose, Inc., Waltham, MA) has shown us that the old standby has a place in PAT, too.*



**Emil W. Ciurczak**

works as a consultant with Integrated Technical Solutions, 77 Park Road, Goldens Bridge, NY 10526. He can be reached via e-mail at: [emil@ciurczak.com](mailto:emil@ciurczak.com).

**M**id-infrared (mid-IR) spectroscopy is perhaps one of the most widely used vibrational spectroscopic techniques. However, when it comes to being used in a pharmaceutical process environment, it trails near-infrared (NIR) spectroscopy in popularity. This installment of Molecular Spectroscopy Workbench examines one of the key differences between NIR and mid-IR spectroscopy and highlights a couple of applications where mid-IR spectroscopy can be employed effectively in the pharmaceutical process environment.

The differences in the spectral properties of materials in the mid-IR and NIR are related to the fact that the absorption coefficients of the vibrational modes that are observed in the NIR are much weaker than the absorption coefficients associated with mid-IR vibrations. This is because the NIR vibrational modes are composed of overtones of fundamental molecular vibrations. The fundamental vibrations occur in the mid-IR spectral range. The absorption bands that are present in the NIR range ( $13,000\text{--}4000\text{ cm}^{-1}$ , or  $750\text{--}2500\text{ nm}$ ) are weaker in intensity. In addition, they consist of broad,

overlapping bands that arise from the combinations and overtones of the fundamental vibrations in the mid-IR ( $4000\text{--}400\text{ cm}^{-1}$ , or  $2500\text{--}25,000\text{ nm}$ ).

The Beer-Lambert law relates the absorbance of an observed band to the pathlength of the sample that the infrared energy passes through to its concentration and absorption coefficient. It can be expressed as:

$$A = \epsilon cl$$

where  $A$  is the absorbance and  $\epsilon$  is the absorption coefficient,  $c$  is the concentration, and  $l$  is the pathlength (or thickness of the sample).

One can look at this relationship in the following manner: as the absorption coefficient decreases, the pathlength of the sample must increase to measure the absorbance of a material. Conversely, if the absorption coefficient is strong, then the pathlength must decrease, otherwise the measured value of absorbance will saturate the detector of an infrared spectrometer.

**Table I. Vendors of Mid-IR Spectrometers and Probes for Process Applications**

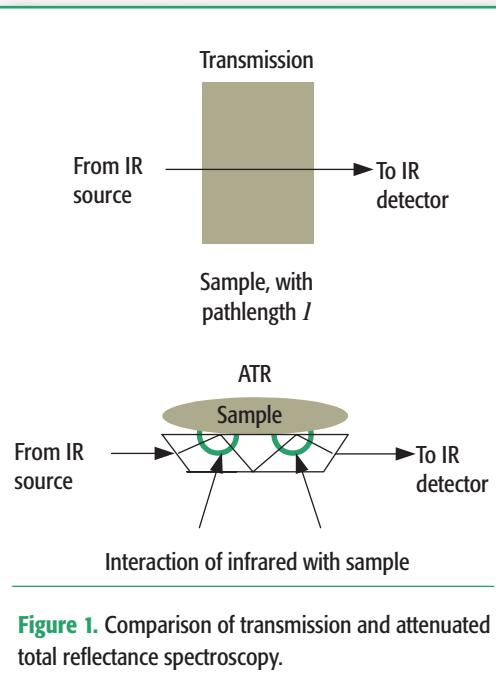
Vendor	Product	Web Info
Axiom Analytical	DPR-207, DMD-270 ATR probes	<a href="http://www.goaxiom.com">www.goaxiom.com</a>
Hamilton Sundstrand	Analect ChemEye	<a href="http://www.hs-ait.com">www.hs-ait.com</a>
Mettler Autochem	ReactIR 4000	<a href="http://www.asirxn.com">www.asirxn.com</a>
Midac	Illuminator	<a href="http://www.midac.com">www.midac.com</a>
Remspec	ReactionView Spotview probes and spectrometers	<a href="http://www.remspec.com">www.remspec.com</a>

Ultimately, the relationship expressed by the Beer-Lambert law is one of the key reasons for the use of NIR spectroscopy in the process environment in pharmaceutical development. The weak absorption coefficients mean that a long pathlength can be used for samples and the samples don't have to be diluted with infrared transparent materials for suitable analyses to be performed. In other words, in NIR spectroscopy sample preparation is virtually nonexistent and many processes can be monitored on-line. The use of cross-stream transmission cells for the analysis of liquids as well as diffuse reflectance fiber-optic probes for the analysis of powders are common techniques used in NIR.

Mid-IR spectroscopy, on the other hand, because of the strength of the absorption coefficients for the fundamental vibrations, is uniquely suited for certain aspects of the pharmaceutical process environment. Two examples that we will cover in this column are the ability to monitor the progress of chemical reactions *in situ* and the ability to determine the cleanliness of pharmaceutical equipment surfaces without the need for swabbing and off-line analysis of the swab.

### Monitoring of Chemical Reactions *In Situ*

The advent of the attenuated total reflectance (ATR) technique (1) has liberated users of mid-IR spectroscopy from laborious sample preparation techniques for obtaining high-quality spectra suitable for performing quantitative analysis. Before the development of ATR sampling accessories, because of the aforementioned strong absorption coefficients, one would have to dilute the material of interest in another infrared-transparent material, such as KBr or Nujol, before making a transmission measurement. With the



**Figure 1.** Comparison of transmission and attenuated total reflectance spectroscopy.



**Figure 2.** The ReactionView fiber-optic ATR probe.

development of the ATR technique and of chemically inert and hard diamond ATR optical elements, one now has the ability to obtain an infrared spectrum on almost any material imaginable. When one combines the ATR technique with fiber optics or optical conduits, the possibility of monitoring chemical reactions *in situ* is realized, as evidenced by the number vendors offering products for this application (Table I).

In particular, chemical reactions that are performed in slurries or other optically scattering media are quite easily monitored with ATR. This is because the optical pathlength through the

sample is quite short because the infrared beam doesn't pass through the entire sample (as in the transmission technique), but rather interacts with the surface and a few micrometers deep into the material (Figure 1).

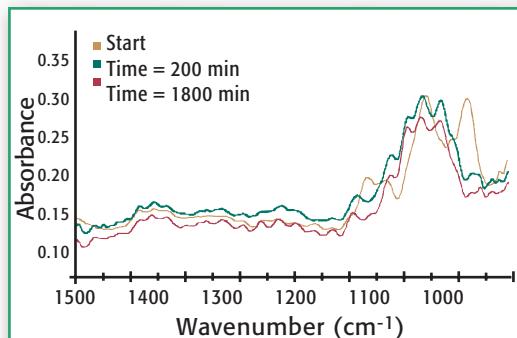
An example of the use of mid-IR spectroscopy for in-situ monitoring is following the fermentation of sucrose. This is an important application because of the development and commercialization of a wide range of biotechnology processes (2) and is meant as an example of how mid-IR will become increasingly important in the PAT initiative. The Web sites of the vendors cited in this column provide similar examples to the one discussed below.

In this investigation, a Remspec (Sturbridge, MA) ReactionView fiber-optic probe (Figure 2) and detector module were coupled to a Vector 22 FT-IR spectrometer (Bruker; Billerica, MA) to obtain spectra during the fermentation of *Saccharomyces cerevisiae* (baker's yeast) using a glucose substrate. Figure 3 shows a comparison of the spectra at the start of the experiment, at 200 min, and at 1800 min. The changes in the spectra are evident, but because of the overlapping nature of the spectra it is not possible to use a direct peak area analysis to develop a quantitative prediction for the changes in the reactants and the products as the reaction progresses.

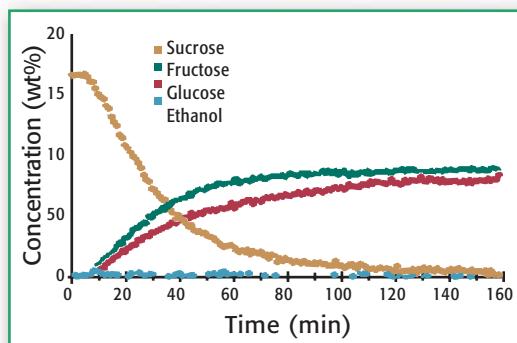
In this example, a partial least squares calibration was developed from more than 2000 spectra. A relationship between the concentration of the products (fructose, glucose, and ethanol) and the reactant (sucrose) was determined. Figure 4 shows these results for the first 160 minutes of the reaction.

### Cleaning Validation in a Pharmaceutical Reactor

The pharmaceutical industry has been discussing the problem of cleaning validation for the past decade (3). In the mid-1990's FDA published guidelines for validating cleaning processes that



**Figure 3.** Comparison of spectra obtained during sucrose fermentation at the start of the experiment, 200 min, and 1800 min.

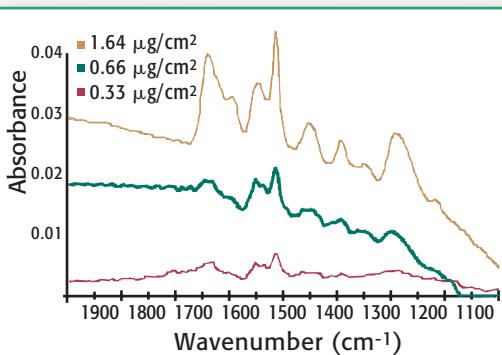


**Figure 4.** Time concentration profile (first 160 min) for the sucrose fermentation experiment.

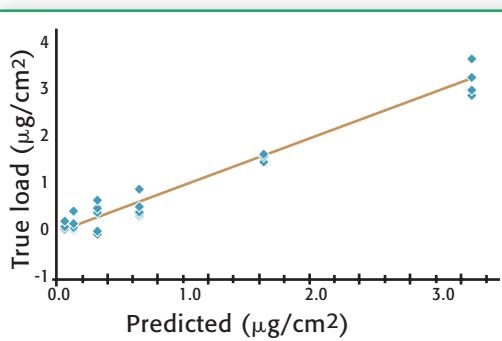
address the issue of residual acceptable limits (RALs) (4, 5).

To meet these guidelines, many industrial organizations adopted a technique known as swab testing (6). Swab testing is inherently an off-line process in which the swab that has been used to wipe the presumably clean surface must be taken to an analytical lab for analysis, typically with high performance liquid chromatography (HPLC). Many organizations have used the figure of merit of  $5 \text{ mg/cm}^2$  for RALs (7). Very few vibrational spectroscopic techniques have this level of sensitivity.

One such technique combines a mid-IR spectrometer with a reflection absorption accessory. Mid-IR spectrometers, when used with a grazing angle incidence reflection accessory, have the ability to detect low chemical concentrations on reflective surfaces such as metals (8). To bring this reflection technique and others into the process envi-



**Figure 5.** Spectra of acetaminophen on aluminum (0.33, 0.66, and 1.64  $\mu\text{g}/\text{cm}^2$ ).



**Figure 6.** First derivative calibration for predicting surface contamination.

ronment, several fiber-optic probes have been developed that provide the sensitivity to detect low chemical concentrations on reflective surfaces (9, 10). Figure 5 is an example of spectra obtained with the SpotView grazing angle fiber-optic probe (Remspec). The spectra are clear and recognizable even at concentrations less than 1  $\text{mg}/\text{cm}^2$ .

Figure 6 shows a calibration plot of predicted loading versus true loading that was obtained with this probe. These results show that the SpotView probe can quantitatively detect surface loadings below 1  $\text{mg}/\text{cm}^2$  and compare well with existing industry methods such as swabbing (11).

## Summary

The above examples illustrate how mid-IR spectroscopy can be employed *in situ* to monitor processes in pharmaceutical environments and in line with the PAT initiative. These examples take advantage of an inherent strength of the

mid-IR range — namely, the strong absorption coefficients of the fundamental vibrational modes and the availability of sampling interfaces that exploit these strengths.

In closing, before making the capital expense that results in the purchase of spectroscopic instrumentation for a process application, end users must have a firm understanding of what they are trying to measure and the limitations/advantages of the proposed measurement technique. The manpower cost resulting from a poor understanding inevitably will outweigh the cost of the capital equipment.

## References

1. B.C. Smith, *Fundamentals of Fourier Transform Infrared Spectroscopy*, (CRC Press, Boca Raton, 1996).
2. Z.A. Mosheky, P.J. Melling, and M.A. Thomson, *Spectroscopy* **16**(6), 15 (2001).
3. R.-C. Hwang, *American Pharmaceutical Review*, Fall 2002.
4. Food and Drug Administration, "Guide to Inspections of Validation of Cleaning Processes Division of Field Investigations," Office of Regional Operations, Office of Regulatory Affairs, pp. 1–7, July 1993.
5. Code of Federal Regulations, Food and Drugs, Title 21, Part 211.67 (U.S. Government Printing Office, Washington, DC).
6. "Cleaning Procedures and Protocols in Focus," Gold Sheet, 27 (1983).
7. M.J. Shifflet and M. Shapiro, *American Pharmaceutical Review*, Summer 2002.
8. P.R. Griffiths and J.A. DeHaseth, *Fourier Transform Infrared Spectrometry* (John Wiley, New York, 1986).
9. R.M. Carangelo, M.A. Druy, W.A. Stevenson, and P.J. Glatkowski, "Non-Contact Spectroscopy System and Process," U.S. Patent 5,841,546 (United States Patent and Trademark Office, Washington, DC).
10. P.J. Melling and P. Shelley, "Spectroscopic Accessory for Examining Films and Coatings on Solid Surfaces," U.S. Patent 6,310,348 (United States Patent and Trademark Office, Washington, DC).
11. M.A. Thomson and P. Melling, "Calibration of Fiber-Optic FTIR Spectroscopy for Cleaning Validation at Sub-Microgram Loadings", Eastern Analytical Symposium, November 2003. ■