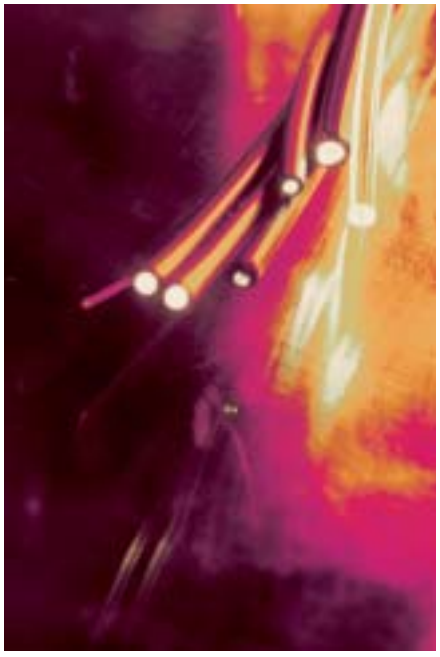

Development of an In Situ Spectroscopic Method for Cleaning Validation Using Mid-IR Fiber-Optics

Narinder K. Mehta, Javier Goenaga-Polo, Samuel P. Hernández-Rivera, David Hernández, Mary A. Thomson, and Peter J. Melling

A fiber-optic, mid-IR spectroscopy probe combined with a grazing-angle reflectance sampling head can be used as a solvent-free, in situ method for validating cleanliness with substantial improvement in accuracy.

Narinder K. Mehta *is a professor in the department of chemical engineering,* ***Javier Goenaga-Polo*** *is president, and* ***Samuel P. Hernández-Rivera*** *is a professor in the department of chemistry at the University of Puerto Rico;* ***David Hernández****, is a scientisti at Pfizer Pharmaceuticals in Puerto Rico; and corresponding author* ***Mary A. Thomson*** *is director of marketing and applications development, and* ***Peter J. Melling*** *is president and chief engineer at Remspec Corporation, 512 Leadmine Road, Sturbridge, MA, 01566, 508.248.1462 ext. 203, fax 508.248.1463, mat@remspec.com, www.remspec.com.*

Good manufacturing practices (GMPs) require the pharmaceutical and biopharmaceutical industries to strive for the highest manufacturing standards (1). All phases of the manufacturing process must be controlled for predictability and for final production of a finished product that consistently meets predetermined quality standards and specifications. Cleaning processes for manufacturing equipment are closely inspected because inadequate cleaning procedures can result in adulterated or contaminated products. An important factor in that control is validating reactor cleanliness; FDA has published guidelines for validating cleaning processes (2,3).

Current Practices

Various approaches are used for cleaning validation, including a widely used technique called *swab testing* (1,4). Clean, usually wet, swabs are applied to areas that have been cleaned, and the swab or an extract of that swab is analyzed to show the effectiveness of that cleaning. Swab testing, however, is expensive, time consuming, and subject to errors caused by factors such as incomplete removal (and underestimation) of contaminants (5). Cross-contamination can result from handling and treatment of samples between swab collection and subsequent analytical work.

Unfortunately, no infallible algorithm has been identified for either cleanliness validation or contaminant detection in final products, and validation practices normally depend on previous experience. The most effective way to prevent the presence of a contaminant in a finished product is to develop increasingly reliable and foolproof cleaning validation methods.

Fourier transform–infrared (FT-IR) spectroscopy in the middle-infrared (mid-IR) range is a more sensitive technique than most for detecting low concentrations of organic compounds such as pharmaceuticals. Reflectance spectroscopy is a well-known technique for obtaining FT-IR spectra from powdered samples and from surfaces (6). Mid-IR grazing-angle spectroscopy is the most sensitive optical absorption technique available for measuring low chemical concentrations on reflective surfaces such as metals (6).

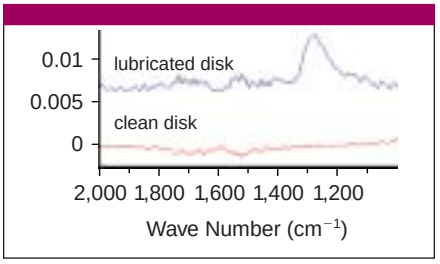

Figure 1. Grazing-angle FT-IR fiber-optic spectrum of a 19 angstrom (0.2µg/cm^2) lubricating layer on a hard disk drive compared with a spectrum from a clean disk drive

The disadvantage of conventional spectroscopic techniques for applications such as cleaning validation is that the test materials must be placed physically within the spectrometer's sample compartment for measurement. That is impractical when examining reactor surfaces, for example.

FT-IR spectroscopy can now be used outside the confines of the sample compartment. Fiber-optic cables (FOCs) that transmit in the mid-IR range have made it possible to develop a range of spectroscopic probes for in situ analysis (7).

Grazing angle sampling probe. In our study, we used a specially designed sampling head that operates at the grazing-angle (8) to detect and quantify small amounts (a few µg/cm^2) of organic material on metal surfaces.

We have previously used this grazing-angle probe to measure very thin coatings of fluorocarbon lubricant on computer hard drives. Figure 1 is an example of the spectra that were obtained from that work (9). The

thickness of the coating (19 angstroms) was confirmed by ellipsometry. That loading is equivalent to 0.2 µg/cm^2, well within the range of values that are of interest in cleaning validation.

Materials and Methods

Solution preparation. A pure active pharmaceutical ingredient (API) from Pfizer Inc. (Barceloneta, Puerto Rico) was selected as a test compound. A stock solution in HPLC-grade methanol was prepared, then diluted to the µg/L range.

Cleaning procedures. After preliminary cleaning and degreasing, the substrate metal coupon was cleaned ultrasonically in spectroscopic-grade toluene. The coupon was rinsed with fresh toluene, then cleaned with HPLC-grade methanol, air-dried at room temperature, and stored in a humidity-controlled cabinet until needed.

Standard preparation using an airbrush aerosol spray. Precleaned, precision-made, 3 in.× 8 in.× 0.03125 in. aluminum metal plates were subjected to the standard cleaning procedure with the following change: One side of the coupon was roughened using abrasive paper (#50 grit) to promote adhesion of the analyte before ultrasonic cleaning. Before aerosol spraying, the cleaned plates were weighed to a precision of five decimal places. We used a Paasche (www.paascheairbrush.com), double-action, internal-mix airbrush set for aerosol spraying with a high-purity, gas chromatography (GC) grade helium.

Extensive practice test runs used methanol to control the velocity of the helium-generated aerosol. Once conditions were optimized, we used a stock (0.1838 g API per 100 mL methanol) solution to deposit the analyte on each plate. Resulting analyte loading ranged about 4–21 µg/cm^2 (determined by the weight difference of the plates before and after coating and drying). A loading of 4 µg/cm^2 proved to be the lowest practical limit using the weight measuring technique. A loading of 1 µg/cm^2 would require determining the weight difference of approximately one part in 5×10^{-8}, which available equipment could not provide.

Mid-IR spectroscopy. Grazing-angle spectra were obtained using a Remspec mid-IR grazing-angle probe attached to a Bruker (www.optics.bruker.com) Vector-22 spectrometer with a Remspec external mercury–cadmium–telluride (MCT) detector

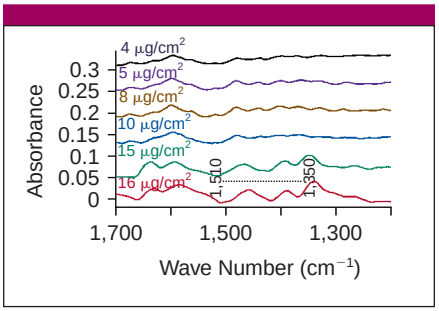

Figure 2. Selected fiber-optic, grazing-angle spectra (shown at the same scale) for the active pharmaceutical ingredient at different loading levels; dotted line indicates the region used for calibration

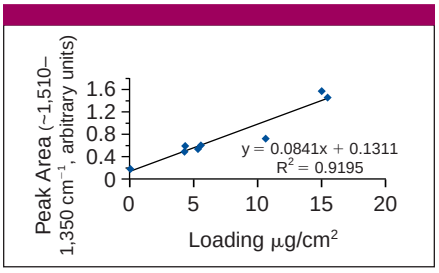

Figure 3. Calibration curve for the 1,510–1,350 cm^{-1} peak area compared with surface loading of aerosol-sprayed active pharmaceutical ingredient using a graze-angle probe

and Bruker Opus software. Spectra were collected across the range of 900–5,000 wave numbers (cm^{-1}), and we used a clean coupon to collect background spectrum. Sample spectra were recorded at 4-cm^{-1} resolution using 256 scans (approximate collection time was 2.25 min/spectrum). We used GRAMS-32 software (Thermo Galactic, www.galactic. com) for peak area calculations.

Results and Discussion

Grazing angle reflectance. In the FT-IR experiment, the signal amount acquired from a sample depends on both the area illuminated by the IR beam and the path length traveled by the mid-IR radiation through the sample. The grazing-angle head uses carefully aligned mirrors to deliver the mid-IR beam to the sample surface at the *grazing angle* (approximately 80° from normal) (6), to collect the reflected beam, and to return it to a mid-IR detector (in this case, an MCT detector). The signal is delivered from the spectrometer to the head and returned to the MCT detector by IR-transmitting fiber optic cables (cables are approximately two meters long). The specially configured head illuminates a large spot on the sample surface (about 4.5 cm^2) and maximizes the distance traveled through the surface layer by the IR beam before it returns to the detector. That combination of factors increases the sensitivity and signal-to-noise performance of the probe by several fold when compared with conventional reflectance methods using a mid-IR beam normal to the sample surface.

Collecting FT-IR spectra from grazing-angle probe. We prepared standard samples as described. FT-IR spectra were collected from each sample using the grazing-angle probe (selected samples in Figure 2). The spectra are shown at the same scale, and the intensity of the spectra decreases with lower API loading, as would be expected.

Initial calibration of the spectra against the known API surface used well-known spectroscopic quantitation methods of estimating the area under a selected peak (dotted line in Figure 2). Figure 3 is the resulting calibration curve. A linear relationship clearly exists between the peak area and the surface API concentration, although the data are insufficient for a robust calibration.

Comparing grazing-angle and swab methods. A second study was performed jointly with an industrial partner (Pfizer, Inc. of Barceloneta, Puerto Rico). Metal coupons were prepared by the aerosol-spray technique, and we compared the data obtained with the grazing-angle mid-IR probe with results obtained using Pfizer's routine cleaning evaluation procedure, an HPLC and swab technique

A predetermined area of each coupon was cleaned with a solvent-soaked swab. Material collected from the surface was removed from the swab by solvent extraction and quantitated by HPLC. The results of the grazing-angle spectroscopy were quantified by comparing relevant peak areas with the calibration curve in Figure 3. The grazing-angle and HPLC–swab methods were compared with the API loading in Table 1. Clearly, low (µg/cm^2) levels of API can be detected and measured on a metal surface with quantitative results that compare favorably with those from the HPLC–swab method.

Chemometrics. Measuring surface concentration using the peak area method is simple conceptually and easy to use, but it

Table 4. Percentage error for three methods of determining surface contamination loading

Sample Number	HPLC Swab	Grazing Angle (Peak Area)	Grazing Angle (PLS)[a]
1	42.76	10.02	0.77
2	8.76	0.87	1.11
3	6.54	8.03	1.10
4	28.08	34.50	1.58
5	23.61	30.86	1.03
6	22.39	0	0.80
7	13.75	15.00	0.06
Avg.[b]	20.84	14.18	0.92

[a]Partial least squares
[b]Average

has limitations. The method is univariate (the concentration is determined with a single spectral peak), and it depends on a linear correlation between the concentration and the spectral response. The results can, therefore, be undermined by perturbations such as fluctuations caused by detector noise, temperature variations, or molecular interactions.

The training set. Statistically based multivariate calibrations use spectral features over a wider range. Information from a calibration spectral set (a training set) was compared to independently determined concentration data using partial least squares regression (PLS1). The method is based on the assumption that systematic variations in the spectra are a consequence of concentration changes. A detailed explanation of chemometric methods is published elsewhere (10).

We assembled a training set of grazing-angle FT-IR spectra and used the API surface concentration determined by weighing the test coupons before and after treatment as the independent variable. We combined two sets of grazing-angle spectra from the study to obtain a training set of reasonable size (21 spectra).

The calibration model. We built the calibration model using the Quant 2 (Bruker Optics) package, an add-on to the Opus spectroscopy software. Quant 2 uses PLS1 based on principal components analysis (PCA) of the training set. In our study, the model parameters were automatically optimized and the spectral region 1,280–1,842 cm^{-1} was used, and the first derivative of each spectrum was taken. We cross-validated the resulting model using the "leave one out" method in which each spectrum is omitted in turn from the training set and then tested against the model built with the remaining spectra. Table 2 gives the results, which are illustrated in Figure 4. The root mean square error of the cross validation was 0.114, and R^2 was 99.92.

Table 3 shows the results of the chemometric calibration for selected cases compared with the results of the HPLC–swab tests; Figure 5 illustrates the same data. The percent relative error reduced drastically versus the data shown in Table 1 by using the PLS1–PCR method.

Table 1. Quantitation of active pharmaceutical ingredient on metal coupons by gazing-angle fiber-optic FT-IR and HPLC–swabbing compared with actual loading

	Surface Loading (µg/cm^2)		
Sample Number	Deposited	Grazing Angle	HPLC–Swab
1	4.26	4.69	2.44
2	5.30	5.25	4.83
3	5.55	6.00	5.19
4	4.33	5.82	5.54
5	10.70	7.37	8.14
6	15.52	15.50	12.01
7	15.13	16.71	13.02

Table 2. Active pharmaceutical ingredient data for known versus predicted values (in µg/cm^2)

	Quant 2		
Sample Number	Known	Prediction	Difference
1	4.263	4.296	−0.033
2	4.273	4.209	0.064
3	4.290	4.383	−0.093
4	5.296	5.238	0.058
5	5.301	5.374	−0.073
6	5.282	5.243	0.039
7	5.554	5.642	−0.088
8	5.652	5.475	0.177
9	5.479	5.636	−0.157
10	4.327	4.375	−0.048
11	4.313	4.295	0.018
12	4.326	4.322	0.004
13	10.656	10.550	0.11
14	10.557	10.780	−0.22
15	10.755	10.610	0.14
16	15.514	15.490	0.01
17	15.456	15.470	−0.01
18	15.645	15.650	0
19	15.048	15.170	−0.12
20	15.125	15.260	−0.13
21	15.501	15.230	0.27

An In Situ Method

This case study suggests that mid-IR reflectance spectroscopy using a fiber-optic probe with a grazing-angle head is a viable method for detecting and measuring low (µg/cm^2) quantities of organic contaminants on metal surfaces. Adding standard chemometric methods, which are developed and automated easily, leads to a powerful technique for surface contamination detection and measurement. Table 4 compares the percentage error in measuring surface contamination loading using industry-standard HPLC–swab methods with that associated with grazing-angle spectroscopy with and without using chemometrics.

Our results suggest that the grazing-angle mid-IR spectroscopy method may provide a performance advantage over the HPLC–swab method even when simple peak-fitting techniques are used to analyze the spectra.

Future Work

Further work is needed on the grazing-angle FT-IR fiber-optic method to successfully implement it for cleaning validation. Our earlier results on computer hard drives

Table 3. Data from Table 1 recalculated using Quant 2 software

Sample Number	Known Value (µg/cm^2)	Quant 2 Prediction (µg/cm^2)	Absolute Error (µg/cm^2)	Percent Relative Error	HPLC Swab (µg/cm^2)	Absolute Error (µg/cm^2)	Percent Relative Error
1	4.263	4.296	0.033	0.77	2.440	1.823	42.8
2	5.296	4.375	0.048	1.11	4.832	0.464	8.76
3	5.554	5.238	−0.058	1.10	5.191	0.363	6.54
4	4.327	5.642	0.088	1.58	5.542	1.215	28.1
5	10.66	10.55	−0.11	1.03	8.143	2.513	23.6
6	15.50	15.17	0.12	0.80	12.03	3.470	22.4
7	15.05	15.49	−0.01	0.06	12.98	2.068	13.7

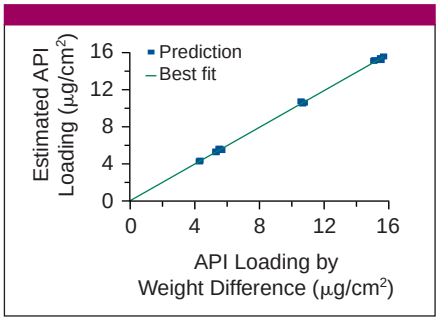

Figure 4. Prediction of the active pharmaceutical loading using Quant 2 software compared with actual loading

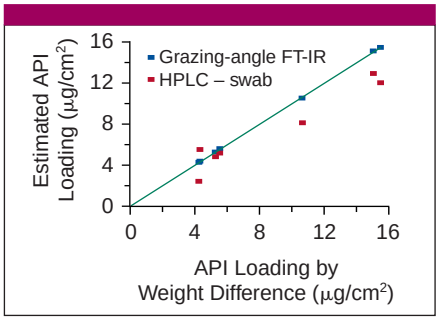

Figure 5. Prediction of the active pharmaceutical ingredient surface loading (blue) and the HPLC–swab results (red) compared with actual loading

indicates that detection of organics smaller than 1μg/cm^2 is possible, but that needs to be confirmed using independent calibrations effective for that range.

The method should be tested with a range of possible reactor contaminants including APIs, intermediates, and cleaning materials such as detergents. The effect of different reactor materials and geometries also needs to be investigated

Alternative definitions for surface cleanliness using the absence of spectral activity in certain defined ranges should be investigated. Because of the potential gains in decreased downtime and the possibility of improved accuracy in contaminant quantitation are considerable, we are aggressively continuing to develop the technique.

Acknowledgments

Funding support from Puerto Rico's Industry-University (INDUNIV) Consortium is gratefully acknowledged. Thanks are due to Vilmary Negrón and Luis Sanchez of Pfizer Pharmaceuticals for the laboratory support and especially for carrying out the HPLC–swab analytical work.

References

(1) "Current Good Manufacturing Practices for Finished Pharmaceuticals," *Code of Federal Regulations, Food and Drugs*, Title 21 Part 211.67 (U.S. Government Printing Office, Washington, DC), revised April 1990.

(2) Office of Regulatory Affairs, *Guide to Inspections of Validation of Cleaning Processes* (FDA, Rockville, MD), 1993. Available at www.fda.gov/ora/inspect_ref/igs/valid.html

(3) Office of Regulatory Affairs, *Guide to Inspections of Bulk Pharmaceutical Chemicals* (FDA, Rockville, MD), revised May 1994. Available at www.fda.gov/ora/inspect_ref/igs/bulk.html.

Table 4. Percentage error for three methods of determining surface contamination loading

Sample Number	HPLC Swab	Grazing Angle (Peak Area)	Grazing Angle (PLS)[a]
1	42.76	10.02	0.77
2	8.76	0.87	1.11
3	6.54	8.03	1.10
4	28.08	34.50	1.58
5	23.61	30.86	1.03
6	22.39	0	0.80
7	13.75	15.00	0.06
Avg.[b]	20.84	14.18	0.92

[a]Partial least squares [b]Average

(4) "Cleaning Procedures and Protocols in Focus," *Gold Sheet* 27 (1983).

(5) G. Amer and P. Deshmane, "Ensuring Successful Validation: The Logical Steps to Efficient Cleaning Procedures," *BioPharm* 14(3), 26–32 (2001).

(6) P.R. Griffiths and J.A. De Haseth, *Fourier-Transform Infrared Spectrometry* (John Wiley, New York, 1986), p.194.

(7) J.R. Berard et al., "Optical Fiber Coupled Devices for Remote Spectroscopy in the Infrared," U.S. Patent 5,170,056 (United States Patent and Trademark Office, Washington, DC).

(8) 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).

(9) P.J. Melling, unpublished work.

(10) R. Kramer, *Chemometric Techniques for Quantitative Analysis* (Marcel Dekker, New York, 1998). **BP**