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Is This Surface Clean? Cleaning Validation by Direct Spectroscopic Surface Analysis

by Peter Melling and Mary Thomson

Pharmaceutical manufacturers and formulators must be able to confirm that the equipment they use is clean before it is utilized.[1,2] In particular, they must ensure that active pharmaceutical ingredients (APIs) are not carried over from one batch to the next. Cleaning validation is often done by swabbing selected areas of the contact surfaces of pharmaceutical equipment, then analyzing the swabs by, for example, solvent extraction and HPLC. This approach is laborious and slow, requires access to laboratory equipment or services, and often entails considerable delay before equipment can be released for use. In addition, the swabbing method is subject to considerable errors in overall recovery of the target compound, and in its reproducibility.[3]

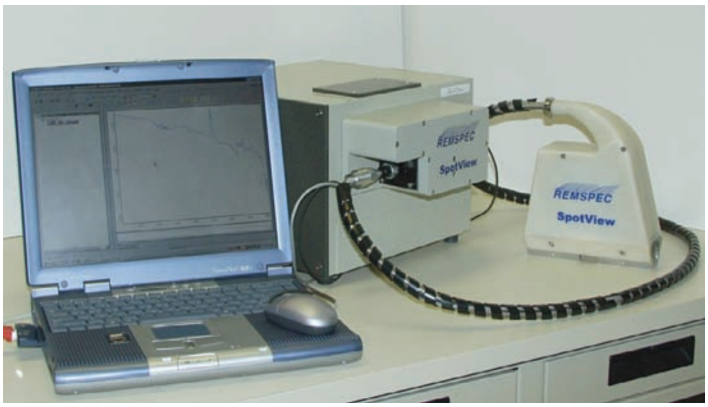

Figure 1 SpotView system, including the mid-IR spectrometer with fiber optic cable attached to the sampling head (at right) and the laptop computer used for operation.

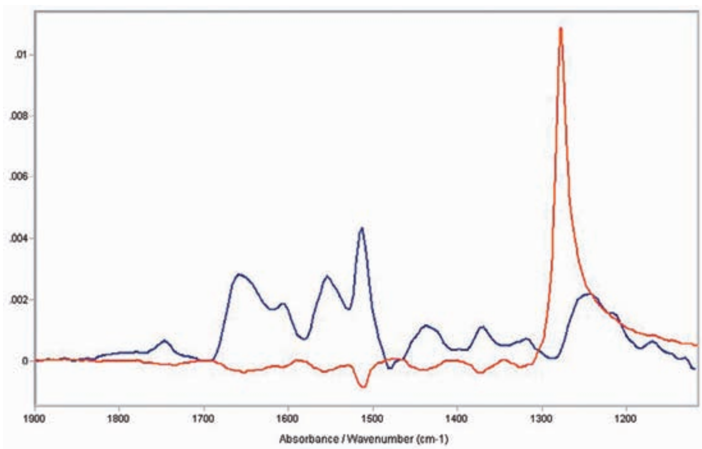

Figure 2 SpotView spectra of approx. 0.35 µg/cm^2 acetaminophen on aluminum (blue trace) and glass (red trace).

A more direct method, which can be used in situ and is readily calibrated at microgram levels for one or more compounds on a variety of different surfaces, is grazing-angle midinfrared spectroscopy. The portable SpotView® system (**Remspec Corp.,** Sturbridge, MA) (see *Figure 1*) comprises a compact spectrometer; mid-IR fiber optic cable; and a patented sampling head design for rapid spectroscopic examination of potentially contaminated surfaces, simply by placing the head against the surface and collecting the resulting spectrum. Mid-IR spectroscopy is a very sensitive technique, and it is highly specific for individual compounds, especially in the "fingerprint" region of the spectrum between about 1000 and 2000 cm^{-1}. For example, the upper (blue) trace in *Figure 2* shows the fingerprint region of the SpotView spectrum of acetaminophen on an aluminum surface. The spectrum is well resolved and distinctive, even at a surface concentration considerably below 1 µg/cm^2. This is in the range of surface concentration that is required for most APIs. The amount of residual API that is permitted on a cleaned surface varies depending on the potency and toxicity of the particular material. A typical

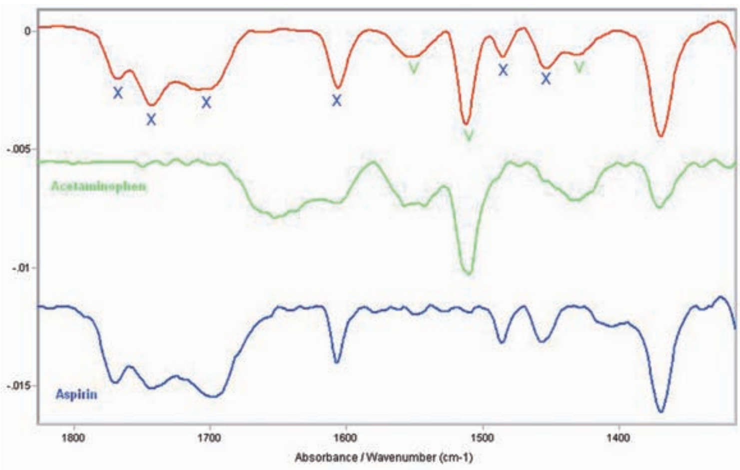

Figure 3 SpotView spectra of aspirin (blue line), acetaminophen (green line), and a mixture of the two (red line) on a glass surface. Prominent features in the mixture spectrum are tagged to indicate the matching pure compound spectrum (X = aspirin, V = acetaminophen).

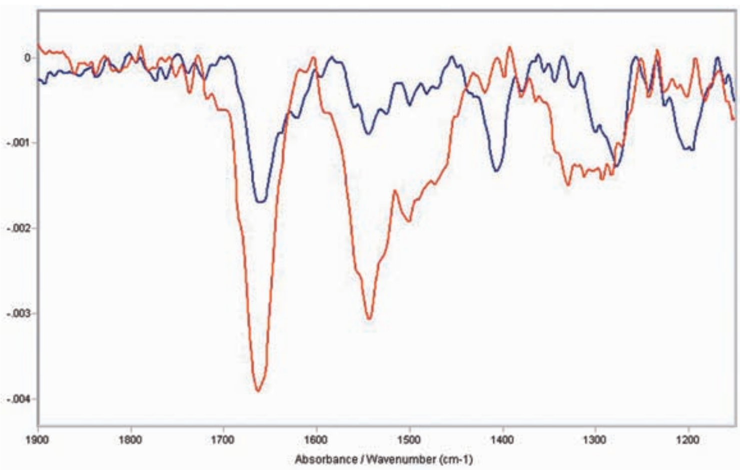

Figure 4 SpotView spectra of bovine serum albumin deposited on an EPDM surface from 50-ppm (dark blue line) and 100-ppm (red line) aqueous solutions.

value is 0.45 µg/cm^2 (2.9 µg/in.^{2}) for a conventional drug; values for modern high-potency drugs can be even lower.

Metal, glass, and plastic surfaces

Mid-IR reflectance methods work on a variety of different surfaces. Reflectance from metals is the easiest to interpret, since the spectrum contains no features from the substrate. Recent results[4] have shown that individual compounds can be distinguished and quantified in the presence of varying amounts of additional or “interfering” compounds such as pharmaceutical exipients. The effect of varying surface roughness is a potential factor in the examination of working reactors, etc., and a recent study[5] shows that this can usually be accommodated in a carefully built calibration model. Glass and various plastic surfaces can be successfully analyzed as well. The lower (red) trace in Figure 2 shows the spectrum of acetaminophen on a glass surface. Note that the features are inverted (this is typical of dielectric surfaces in this spectral region) and that a strong Si–O feature from the glass is visible near 1260 cm^{-1}. The fingerprint region of spectra collected from glass surfaces is readily identifiable, and distinctive features can be picked out of the spectra taken from mixtures of compounds (see *Figure 3*), making it possible to distinguish active compounds from materials such as detergents[6] and from other active compounds.[7]

Plastic surfaces such as silicone, fluoropolymer, and ethylene propylene diene monomer (EPDM) are all used in pharmaceutical reactors and may therefore be subject to cleaning validation requirements. Like glass, these materials are dielectric and the fingerprint region of the contaminant spectrum is inverted. In addition, spectral features from the plastic itself may be observed, and may vary depending on the degree of surface soiling. It is usually possible to

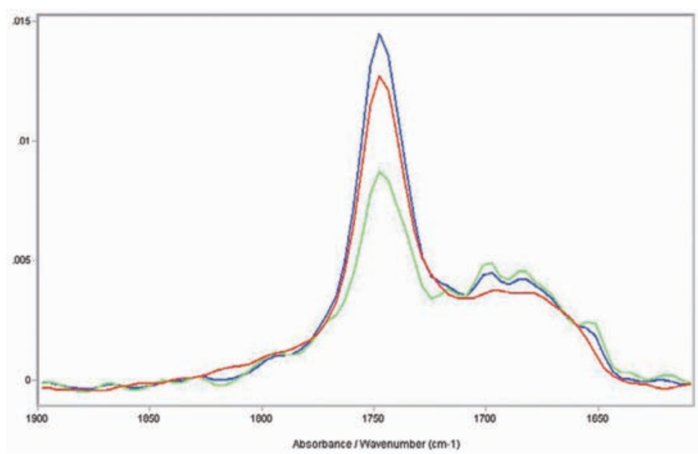

Figure 5 SpotView spectra of peanut oil on a stainless steel surface at concentrations of 0.34 (green line), 0.67 (red line), and 1.11 µg/cm^2 (blue line).

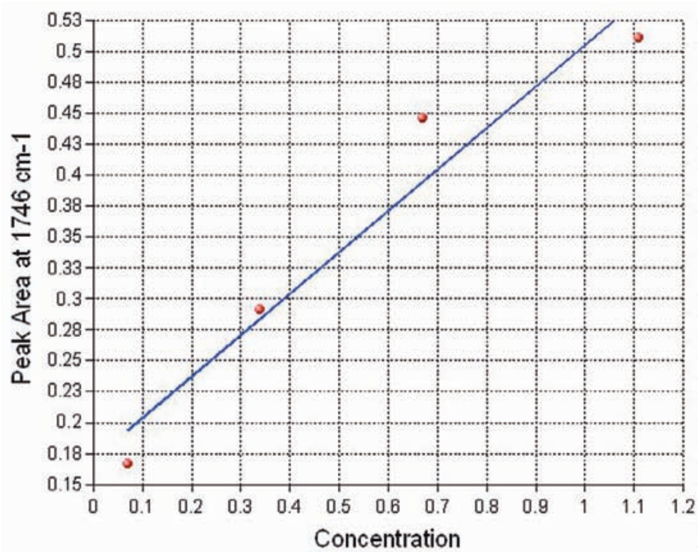

Figure 6 Graph of peak area at 1746 cm^{-1} (averaged over six spectra for each point) versus surface concentration of peanut oil on a stainless steel surface.

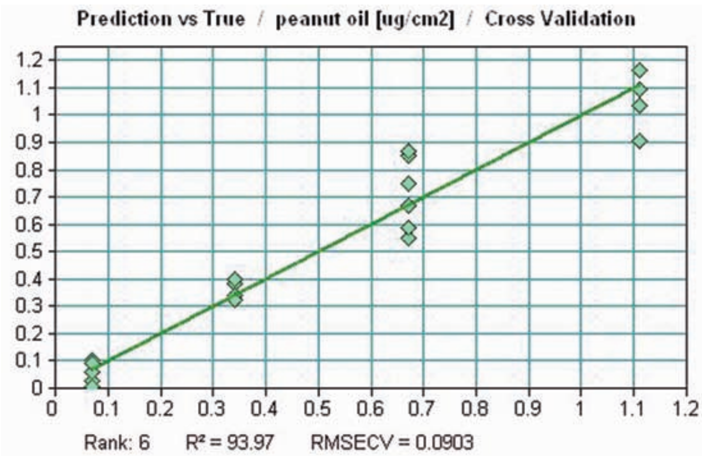

Figure 7 Graph of the results of a leave-one-out cross-validation of a PLS1 model based on six SpotView spectra taken at each of four concentrations of peanut oil on stainless steel (calculations performed using **Bruker Optics** [Billerica, MA] Quant2 chemometrics package).

identify features in the spectrum that can be calibrated. For example, *Figure 4* shows two spectra taken from an EPDM surface that had been exposed to aqueous solutions of bovine serum albumin. The characteristic amide I and amide II peaks of the protein are clearly visible, along with minor features from the EPDM itself.

Proteins and incompletely characterized materials

Industries with an interest in cleaning validation extend beyond traditional pharmaceuticals to include biotechnology and some areas of the food and personal products industries. In these areas, the contaminating materials are likely to be proteins, vegetable oils, and similar substances. As the spectra in Figure 4 show, proteins are detectable and quantifiable even on somewhat intractable surfaces such as EPDM.

Peanut oil is another example of a poorly characterized material that can be successfully measured using SpotView. *Figure 5* shows a feature from the SpotView spectrum of peanut oil on a stainless steel surface. It is apparent by eye that the size of the feature varies with surface concentration, and this trend is confirmed when the average peak area (six spectra for each point) is calculated and graphed against the surface concentration of the oil (see *Figure 6*). In favorable cases, simple peak height or peak area measurements can be used to calibrate SpotView measurements. However, it is usually necessary to collect a statistically defined set of spectra and develop a chemometrics model (see *Figure 7*) to ensure that compounds are correctly identified and accurately measured at very low loadings, particularly in cases in which complex mixtures of materials are present. The chemometrics approach can compensate for the presence of interfering spectral features such as those from atmospheric water, and for the changes that are often observed in mid-IR spectra due to direct analyte–surface interactions when surface loadings are very low.

Conclusion

SpotView offers an entirely new approach to surface analysis and cleaning validation. Results compare well with those from established swabbing methods.[8] The system promises to reduce equipment downtime significantly and give a direct, real-time answer to the question: Is this surface clean?

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