Grazing-Angle Fiber-Optic IRRAS for in Situ Cleaning Validation

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Abstract:
Grazing-angle Fourier transform infrared reflection−absorption spectrometry (IRRAS) using a fiber-optic accessory has been investigated as a potential in situ technique for the detection and quantification of contamination by active pharmaceutical agents on glass and metal surfaces. Two methods were used for contamination preparation: one based on smearing a known amount of sample, in solution, onto the substrate and the other by spraying the substrate with an aerosol of the analyte in a volatile solvent. Chemometric calibrations using partial least-squares (PLS) regression are presented and evaluated for acetaminophen on aluminum and glass, and ibuprofen on aluminum and stainless steel. The results indicate that surface loadings of 0.05 μg/cm^2 is a readily achievable limit of detection for the IRRAS technique.

Introduction

Clean equipment is vitally important in the pharmaceutical industry, and cleaning validation of surfaces is therefore critical to many processes for the manufacture of pharmaceuticals. Ex situ analytical methods such as swabbing/HPLC and rinse-water analysis have been the most commonly used approaches, but they can be time consuming and therefore expensive. Consequently, there has been considerable interest in new methods for rapidly checking the cleanliness of equipment before its release for use.[1] In this regard, direct, in situ methods potentially have very significant advantages.

Fourier transform infrared reflection−absorption spectroscopy (IRRAS) at a grazing angle is a well-established method for analyzing thin films on solid surfaces.[2,3] Commercial reflectance accessories designed to fit inside the spectrometer sample chamber are obviously of limited use in an industrial setting where the ability to take the spectrometer to the measurement region is important. However, the combination of a grazing-angle IRRAS sampling head with a flexible, fiber-optic cable[4] provides a convenient way to collect mid-IR spectroscopic data from contaminated surfaces in situ. Preliminary work with aluminum surfaces has shown that quantitative data can be obtained that compare favorably with the results of an industry-standard swabbing/HPLC method.[5]

This contribution presents the results of investigations of a spectroscopic method for cleaning validation that uses IRRAS to detect and quantify active pharmaceutical ingredients (APIs) on aluminum, stainless steel, and glass surfaces. This work uses a Remspec fiber-optic grazing-angle head,[4] which permits measurements to be performed on samples outside the spectrometer body, thereby providing the sampling flexibility required for in situ applications.

Experimental Section

Materials. The solvents used were Milli-Q water, methanol (ACS or doubly distilled solvent grade), ethanol (solvent grade), acetone (solvent grade), and tetrahydrofuran (ACS grade). *O*-Acetylsalicylic acid (aspirin), 4-acetamidophenol (acetaminophen), and 4-isobutyl-α-methylphenylacetic acid (ibuprofen) were obtained from Avocado Research Chemicals Ltd. and Sigma Aldrich. Sodium dodecyl sulfate (SDS) was obtained from BDH Laboratory Supplies, dried at 60 °C for 2 days and then stored in a desiccator prior to use. Metal test coupons were cut from 0.75-mm-thick aluminum and 316 stainless steel stock. Glass coupons were cut from 3-mm-thick window glass and were roughened on one side to prevent reflection from the back face.

Instrumentation. A schematic of the IRRAS instrument is given in Figure 1. The spectra were collected by using a BrukerOptics Vector 22 FTIR spectrometer with OPUS data-collection software and Quant2 chemometrics package from BrukerOptics. A prototype Remspec SpotView grazing-angle head was connected to the external beam port of the spectrometer by a ∼1.5-m, 3-mm diameter, 19-fiber chalcogenide-glass optical bundle, which transmits from 5000 to 900 cm^{-1} with the exception of a strong H−Se absorption band at ∼2200 cm^{-1} (Figure 1 inset). Off-axis parabolic

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(1) See, for example: Amer, G. Ensuring Successful Validation: the Logical Steps to Efficient Cleaning Procedures. *BioPharm (Duluth, Minn.)* **2001**, *14*, 26.
(2) Umemura, J. Reflection−Absorption Spectroscopy of Thin Films on Metallic Substrates. In *Handbook of Vibrational Spectroscopy*; Chalmers, J. M., Griffiths, P. R., Eds.: Wiley: Chichester, UK, 2002; Vol. 2, pp 982−998.
(3) Kattner J.; Hoffmann, H. External Reflection Spectroscopy of Thin Films on Dielectric Substrates. In *Handbook of Vibrational Spectroscopy*; Chalmers, J. M., Griffiths, P. R., Eds.: Wiley: Chichester, UK, 2002; Vol. 2, pp 1009−1027.
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(5) Mehta, N. K.; Goenaga-Polo, J.; Hernandez-Rivera, S. P.; Hernandez, D.; Thomson, M. A.; Melling P. J. Development of an In Situ Spectroscopic Method for Cleaning Validation Using Mid-IR Fiber-Optics. *BioPharm* (Duluth, Minn.) **2002**, *15*, 36.

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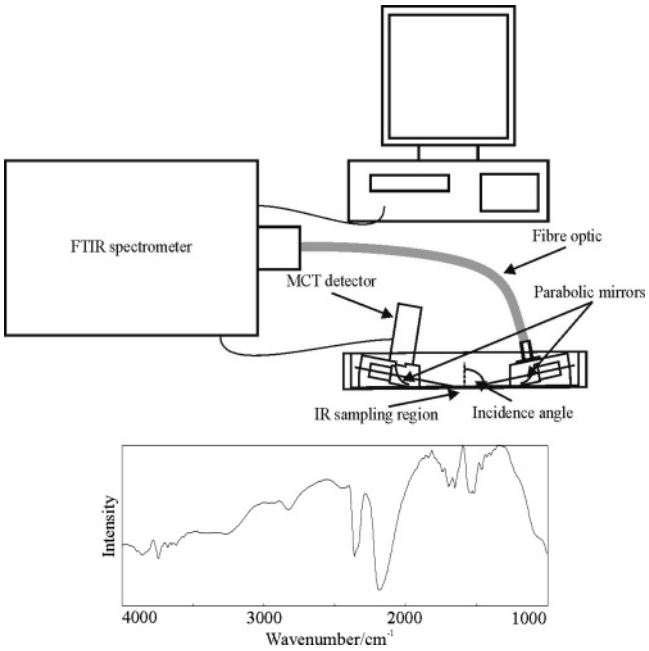

Figure 1. **Schematic diagram of the instrument used for the measurement of the IRRAS of surfaces contaminated with organic compounds. (Inset)"Single beam" response of the system including the chalcogenide glass used in the fiber bundle.**

mirrors collimate the beam onto the sample (at an incidence angle of 80° to the normal) and focus the reflected light to an integrated Infrared Associates MCT detector. The IR footprint produced by the grazing-angle probe is elliptical, with the intensity decaying from the middle toward the edges.

Sample Preparation. Two methods were used to prepare samples.

(1) Smear Method. A known volume (~1 mL) of an accurately prepared standard solution of the target compound in a quick-drying solvent was dispensed onto the surface of a test coupon and then spread as evenly as possible across the surface during drying, using the straight edge of a soft plastic spatula. This method is rapid and easily executed without specialized equipment. The amount of applied material is readily controlled and can be calculated without the need for an independent analysis. However, coverage is typically uneven due to beading and puddling of the solution, and it is possible for some of the applied loading to be removed during the spreading operation.

(2) Spray Method. A Paasche double-action, internal-mix airbrush (operated with a small compressor and ballast tank) was used to spray the test coupons with 5−10 mL of a solution (typical concentration ~0.5 g/L) of the target compound in a volatile solvent. By this method, it was possible to generate samples with more uniform coverage, and the surface loading (amount per unit area) could be varied by changing the concentration of the solution or the number of passes of the spray across the sample surface. Samples could be loaded with more than one compound either by spraying separate solutions consecutively or by spraying a mixed solution.

Since the precise amount of material deposited by the spray method is difficult to predetermine, an independent method was used to establish the loading after the IRRAS measurements. The coupons were washed using 25−100 mL of an appropriate solvent (water for SDS, ethanol for APIs) to remove the entire amount of the target compound that had been applied. APIs have strong UV absorbance bands (acetaminophen ϵ_{249} 11 4000 L mol^{-1} cm^{-1}; aspirin ϵ_{225} 19 000 L mol^{-1} cm^{-1}; ibuprofen ϵ_{219} 18 300 L mol^{-1} cm^{-1}); thus, a GBC-920 UV/visible spectrophotometer was used colorimetrically to measure the amount of each compound in the API washings, and the surface loadings were back-calculated. Since SDS has no UV/visible chromophore, an alternative method was required to measure the concentrations in the aqueous washings; one obvious choice, HPLC, was ruled out as the available instrument used UV spectroscopic detection. Of several other techniques investigated, quantitative ^{1}H NMR spectroscopy gave the best combination of sensitivity and reproducibility. One hundred microliters of dioxane (internal standard) in phosphate buffer was added to 400 μL of rinsate to give 0.5 mL containing 100 μmol L^{-1} of dioxane at pH 7.5. This solution was placed in a 5-mm NMR tube with a 3-mm D_2O insert. Thirty-two transients were collected in 9 min from an Oxford Instruments AS500 spectrometer using 8.1-μs, 90° radio frequency pulses, with 1.89-s acquisition times and 15-s delays between pulses. Each transient comprised 30 272 data over a sweep width of 8 kHz. The 4.6-ppm H_2O proton singlet was suppressed by using a double-pulse field-gradient spin−echo (DPFGSE) technique. The transients were zero filled to 128 k data prior to Fourier transformation, and phasing and baseline corrections were carried out manually using Varian VNMR software, version 6.1 C. The integrals of unresolved SDS CH_2 resonances at ~1.12 ppm and the dioxane resonance at ~3.55 ppm were determined. A calibration curve was produced by using standardized SDS solutions, and a standard solution was included with each set of samples to ensure that the calibration remained valid.

Data Collection. As implemented here, IRRAS is a single-beam technique requiring sequential measurement of background and sample spectra. The spectral range was 990−4000 cm^{-1}, and the resolution was 4 cm^{-1} (glass substrate) or 8 cm^{-1} (metal substrates). Single-channel background spectra (R_0) were obtained from clean test coupons of the appropriate substrate by averaging over 100−200 interferometer scans at 35 or 40 kHz scanning speed. Five to seven sample spectra (R) were collected from different regions of each loaded coupon by averaging over 25−100 scans. The IRRAS spectrum was calculated as $\log(R_0/R)$.

Results and Discussion

IRRAS is an open-path method where the IR beam passes through the atmosphere before and after the sample. This means that fluctuations in the composition of the ambient atmosphere that are rapid on the time scale of the experiment can lead to the appearance of features due to atmospheric components that absorb in the mid-IR To illustrate this problem, the lowest trace in Figure 2 shows a spectrum obtained from a glass coupon loaded with 0.21 μg/cm^2 of acetaminophen. The fine-structured bands above ~1400 cm^{-1} arise from the bending vibration of water vapor. These and other features due to atmospheric species (particularly the

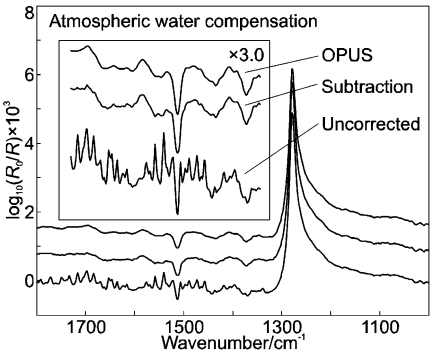

Figure 2. **IRRAS of 0.21 μg/cm^2 acetaminophen on glass. (Lower Trace) Fine-structured spectral features above ~1400 cm^{-1} due to atmospheric H_2O. (Middle and Upper Traces) Results of auto-subtraction of a reference H_2O spectrum and application of the Bruker OPUS atmospheric compensation, respectively. Both treatments reveal the underlying API bands.**

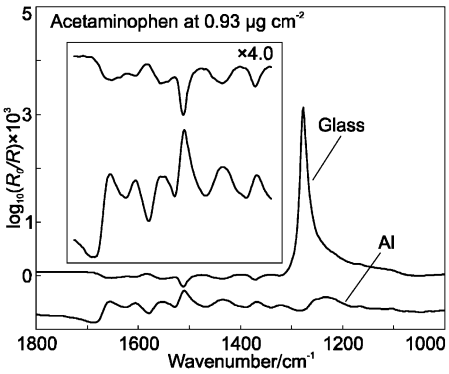

Figure 3. **IRRAS (after correction for atmospheric H_2O) of acetaminophen deposited at a loading of 0.93 μg/cm^2 on glass (upper trace) and aluminum (lower trace) coupons using the spray and smear methods, respectively.**

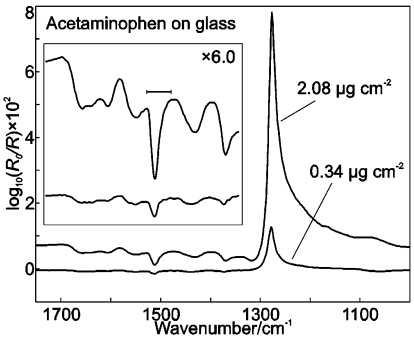

Figure 4. **IRRAS spectra (after correction for atmospheric H_2O) of acetaminophen on glass at loadings of 0.34 and 2.08 μg/cm^2. (Inset) Enlargement of the region containing the peak used for univariate calibration and showing the limits of integration.**

stretching modes of CO_2 at ~2350 cm^{-1} and H_2O in the region 4000−3400 cm^{-1}) were nearly ubiquitous in our experiments, even with minimal periods between the collection of the background and sample spectra. The H_2O bands are especially problematic since they occur in the regions that contain the majority of features from the target surface contaminants and their intensities are relatively large at the sensitivities required to detect surface contaminations at loadings relevant to pharmaceutical requirements.

One way of addressing this problem is to apply software corrections. The most straightforward method is to subtract an appropriately scaled independently measured reference spectrum. Alternatively, the Bruker OPUS package includes an "atmospheric compensation" option that ameliorates interferences due to H_2O and/or CO_2 by using an undocumented algorithm. While not perfect, either of these methods, carefully applied, can remove the gross effects of the atmospheric species, as shown in upper traces of Figure 2. The subtraction-based correction, using a H_2O spectrum measured under similar conditions, was applied to the spectra presented in Figures 3−5.

IRRAS spectra depend on the nature of the substrate surface from which the radiation is reflected as well as those of the incident medium and the surface film through which the radiation is transmitted. Broadly, substrates can be classified as metal or dielectric, with the two groups having vastly different optical properties. Both are used in chemical reactors and hence are relevant to this study. An illustration of the differences is presented in Figure 3 for acetaminophen at the same loading (0.93 μg/cm^2) on aluminum and glass. The spectrum from aluminum (lower trace) has an appearance similar to that of an absorption spectrum and is dominated by features characteristic of acetaminophen. In contrast, in the spectrum from the glass coupon (upper trace) the acetaminophen features are inverted and have intensities that are much reduced in comparison with the equivalent features from the aluminum spectrum. In addition, the dominant feature in the glass spectrum is a strong band at ~1250 cm^{-1} due to Si−O modes of the substrate. Discussion of the reasons for these differences is beyond the scope of this report, and a detailed treatment can be found in refs 2 and 3.

High-wavenumber API bands, such as those due to O−H and N−H stretches, are prominent in IR absorption and diffuse-reflectance powder spectra and would normally be regarded as candidates for quantitative analysis. However, they are much weaker in the IRRAS and could be detected (at acceptable signal-to-noise ratios) only at loadings substantially greater than those relevant to pharmaceutical cleaning protocols. Instead, we have examined the C═O and aromatic C−C stretching regions.

Expansions of the appropriate (~1800−1000 cm^{-1}) range of the IRRAS of acetaminophen and aspirin on glass are shown in Figures 4 and 5, respectively. The API features have wavenumbers similar to those for the bands in the diffuse-reflectance powder spectra, but they are inverted and have quite different relative intensities. As with all spectra obtained from glass coupons, the region is dominated by the ~1250-cm^{-1} Si−O feature, whose intensity is strongly dependent on surface loading and whose profile changes from that of an absorption-like band to a dispersive specular-reflectance band as the loadings are increased.

The conventional method of using spectroscopic data for quantitative analysis is to assume a direct functional relationship (such as Beer's law) between signal intensity (peak heights or band area) and sample concentration. We have examined this type of univariate approach for several APIs

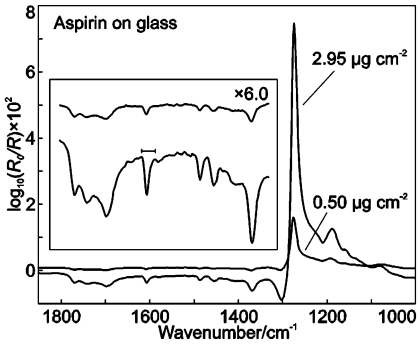

Figure 5. **IRRAS spectra (after correction for atmospheric H_2O) of aspirin on glass at loadings of 0.50 and 2.95 μg/cm^2. (Inset) Enlargement of the region containing the peak used for univariate calibration, showing the limits of integration.**

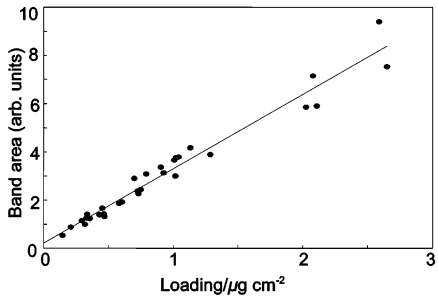

Figure 6. **Plot of acetaminophen IRRAS band integral (1485−1527 cm^{-1}) versus loading as determined by UV absorption colorimetry. Slope: 3.1 ± 0.1; Ordinate intercept: 0.2 ± 0.1; R^2 = 0.962.**

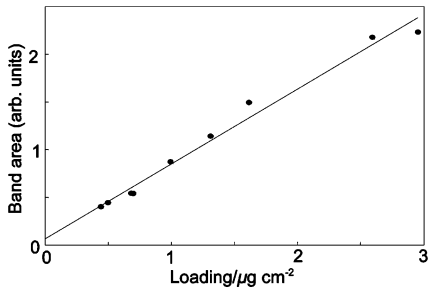

Figure 7. **Plot of aspirin IRRAS band integral (1587−1618 cm^{-1}) versus loading as determined by UV absorption colorimetry. Slope: 0.79 ± 0.04; Ordinate intercept: 0.21 ± 0.06; R^2 = 0.984.**

and SDS on glass and aluminum and present here the cases of the acetaminophen and aspirin on glass, whose spectra are shown in Figures 4 and 5. The intensity parameters were chosen to be the absolute integrals of single, well-distinguished bands centered at ∼1510 cm^{-1} for acetaminophen and at ∼1605 cm^{-1} for aspirin. The ranges of integration are indicated by horizontal bars in the insets to Figures 4 and 5. To account for baseline variations between spectra, a straight line was subtracted to make the endpoints of the integration range zero.

Figures 6 and 7 show that the univariate peak-integral data are well represented by straight-line fits at loadings between ∼0.3 and 3 μg/cm^2. The results clearly establish a quantitative relationship between IRRAS intensity data and levels of these contaminants on glass surfaces, thereby extending results reported previously for metal surfaces.[5] With these data, acetaminophen loadings of 0.5 μg/cm^2 and aspirin loadings of 1.0 μg/cm^2 can be quantified with a 95% confidence interval of ∼25%. Improvements in precision could be achieved through the collection of larger data sets, but the lower detection limit is largely dictated by limitations of the water-vapor corrections rather than the instrumentation.

Despite the apparent success of the analyses presented above, more sophisticated approaches are required in more general circumstances, including those that are relevant to cleaning validation in industrial settings.

First, linear behavior in IRRAS is more the exception than the rule. IRRAS features of thin films on surfaces frequently show strongly nonlinear behavior, including variations of relative intensities and wavelength shifts as the surface loading changes. In the most general sense, the complexities of, and interactions between, the physical phenomena that underpin IRRAS are such that linear behavior should not be anticipated (except approximately over restricted ranges) even with the most simplifying assumptions about the nature of the materials at the surface interfaces. At low surface loadings, these considerations will be compounded by molecular effects including surface packing and consequent changes in the orientation of the molecular dipole moments as the loading approaches a monolayer.[3]

Second (and as documented above), interferences from the atmospheric species (especially H_2O) occur in the same spectral ranges as features from the target compounds. This problem can sometimes be circumvented (as above) by employing compensation procedures. However, these work best under the conditions that prevail in a research laboratory—namely where spectra are obtained using model samples over a short time interval and with minimal changes in all other variables.

Finally, in industrial settings, surface contamination generally will involve several components with overlapping IR spectra. Under these conditions, the IRRAS intensity in any given region cannot be unequivocally assigned to just one component.

As previously demonstrated,[5] chemometric modeling methods based on factor analysis and partial-least-squares (PLS) fitting can be used to correlate IRRAS with surface loadings of organic contaminants with the advantages that (a) they are intrinsically multivariate approaches and can therefore be used to quantify more than one contaminant simultaneously, (b) they can automatically accommodate nonlinear relationships between spectra and surface concentration data, and (c) the presence of variable interfering components (including atmospheric H_2O and CO_2) can be accounted for by segregating their effects into separate factors in the analysis. The remainder of this contribution examines aspects of the application of chemometric methods to the quantification of surface loadings of APIs on glass and metal substrates using IRRAS. The samples, preparation conditions, and loadings are summarized in Table 1. The chemometric modeling was performed over the spectral analysis ranges listed in Table 1 by using the OPUS Quant2 package without atmospheric corrections or other preprocessing, except for the default mean-centering in Quant2.

Table 1. **Sample preparation data and Quant2 calibration parameters for the chemometric models of single-component surface contamination by acetaminophen and ibuprofen on glass or metal substrates[a]**

sample set	Ac:Al	Ac:glass(A)	Ac:glass(B)	Ib:Al	Ib:SS	Ib:Al + Ib:SS(A)	Ib:Al + Ib:SS(B)
API	acetaminophen	acetaminophen	acetaminophen	ibuprofen	ibuprofen	ibuprofen	ibuprofen
substrate	aluminum	glass	glass	aluminum	316-stainless steel	Al or 316-SS	Al or 316-SS
prepn method	smear	spray/UV	spray/UV	smear	smear	smear	smear
loadings/μg/cm^2	0.70−1.64	0. − 1.14	0.042 −0.58	0.10−1.00	0.01−1.05	0.01−1.05	0.05−1.05
number of coupons	8	24	13	5	6	11	4
spectral range/cm^{-1}	1870−990	1801 −1398	1801 −1398	3835−2720; 2276−995	3835−2720; 2276−995	3835−2720; 2276−995	3835−2720; 2276−995
RMSECV/μg/cm^2	0.1	0.05906		0.08	0.07	0.09	
RMSEP/μg/cm^2			0.052				0.2
rank	10	6	6	6	8	9	9
R^2	0.962	0.959	0.894	0.980	0.980	0.970	0.887

[a] API = active pharmaceutical ingredient; RMSECV = root-mean-square error of cross validation; RMSEP = root-mean-square error of prediction

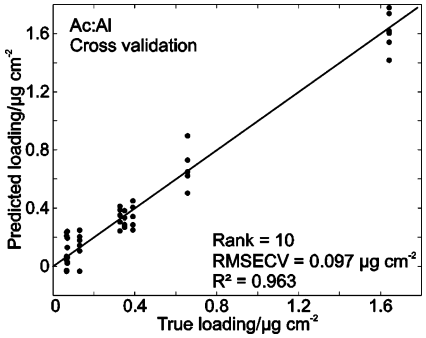

Figure 8. **Predicted versus true surface loadings of acetaminophen on aluminum (data set Ac:Al) from a leave-one-out cross validation using Quant2. The samples were prepared by the smear method, and the true loadings were calculated from the initial solution concentrations.**

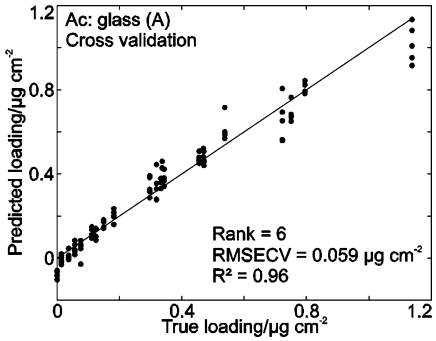

Figure 9. **Predicted versus true surface loadings of acetaminophen on glass (data set Ac:glass(A)) from a leave-one-out cross validation using Quant2. The samples were prepared by the spray method, and the true loadings were determined by UV colorimetry.**

Figures 8 and 9 show the results of standard leave-one-out cross validations of data sets for acetaminophen on aluminum (Ac:Al) and glass (Ac:glass(A)) respectively prepared by the smear and spray/UV methods. The spectral analysis region was approximately the same as that shown in Figures 4 and 5, but truncated at the low wavenumber end for Ac:glass(A) to exclude the Si−O feature. The cross validation results, especially the low values of root-mean-square errors of cross validation (RMSECV < 0.1 μg/cm^2), are encouraging and indicate that good chemometric models can be developed from these types of spectra. The correlation

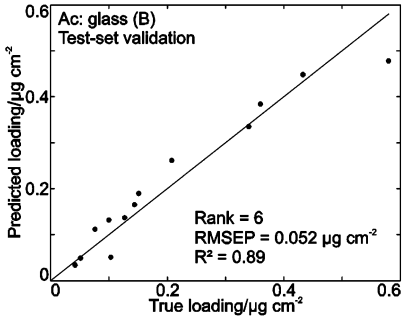

Figure 10. **Robustness test of the Quant2 chemometric model for surface loadings of acetaminophen on glass. The points indicate the average predicted surface loadings for data set Ac:glass(B) determined by using the calibration model based on set Ac:glass(A) applied to five spectra measured from each sample; RMSEP and R^2 are calculated from the raw predictions. The true loadings of Ac:glass(B) were determined by UV colorimetry.**

factors are similar, but the RMSECV is lower for the glass data (RMSECV = 0.059 μg cm^{-2} for Ac:glass(A) versus 0.097 for Ac:Al), perhaps reflecting improved uniformity of the spray preparation method.

To test the robustness of such models, a second sample set of acetaminophen on glass (Ac:glass(B), see Table 1) was prepared independently by the spray/UV method. The chemometric model developed from Ac:glass(A) was then used to predict the surface loadings from the Ac:glass(B) spectra. The results are shown graphically in Figure 10; they have a correlation factor of $R^2 = 0.89$ and a root-mean-square error of prediction of RMSEP = 0.052.

These and similar results for aspirin, ibuprofen, and SDS indicate that a combination of IRRAS and chemometric modeling will work for a range of compounds on both metal and glass surfaces. Ideally, it would be possible to build a single calibration for a particular compound or set of compounds on a number of different, but related, substrates. This is likely to be a challenging goal for dielectric substrates that have distinct absorption bands in the mid-IR but should be feasible for metals.

To test this prediction, two sets of test coupons were prepared by the smear method using ibuprofen as the target compound: one with aluminum as the substrate (Ib:Al) and

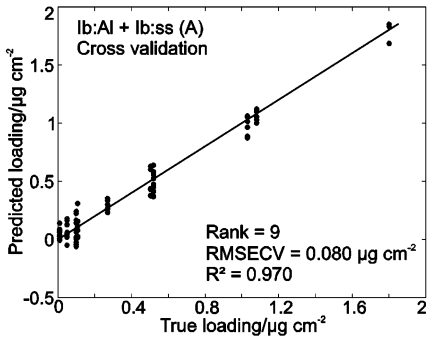

Figure 11. **Predicted versus true surface loading of combined data for ibuprofen on aluminum and stainless steel (data set Ib:Al+Ib:SS(A)) from a leave-one-out cross validation using Quant2. The samples were prepared by the smear method, and the true loadings were calculated from the initial solution concentrations.**

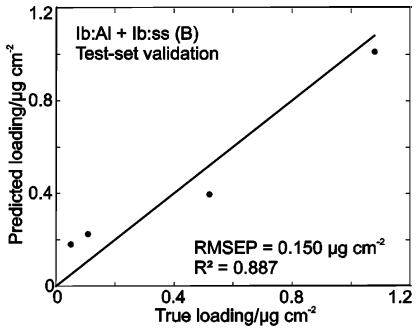

Figure 12. **Robustness test of the Quant2 chemometric model for surface loadings of ibuprofen on aluminum and stainless steel. The points indicate the predicted surface loadings for data set Ib:Al+Ib:SS(B) determined by using a calibration model based on set Ib:Al+Ib:SS(A). The samples were prepared by the smear method, and the true loadings were calculated from the initial solution concentrations.**

the other using 316 stainless steel (Ib:SS). Quant2 chemometric models were built using a broad spectral range (3835−995 cm^{-1}) but excluding the 1720−2276 cm^{-1} window, which contains the atmospheric CO_2 band but no features of the target API. The models for Ib:Al and Ib:SS individually were of a similar quality to those above for acetaminophen, and the associated parameters are given in the Table 1. The combined set (Ib:Al+Ib:SS(A)) was then processed to yield a new model with the leave-one-out cross validation shown graphically in Figure 11 and the corresponding parameters summarized in the right-most column of Table 1. These compare very favorably with those for the individual models, indicating that the single calibration is sufficient for both substrates and providing encouraging evidence that it might be possible to treat metal surfaces (at least) together as a single class.

A robustness test of the combined Ib:Al+Ib:SS calibration model was carried out by collecting six further IRRAS spectra from each of four additional coupons prepared by the smear techniques; two on aluminum and two on stainless steel substrates (data set Ib:Al+Ib:SS(B)). The average loadings obtained from the model are plotted against the true loadings in Figure 12. The overall correlation for the predictions ($R^2 = 0.887$, and RMSEP = 0.2) indicates that usable, robust calibrations can be built for use on more than

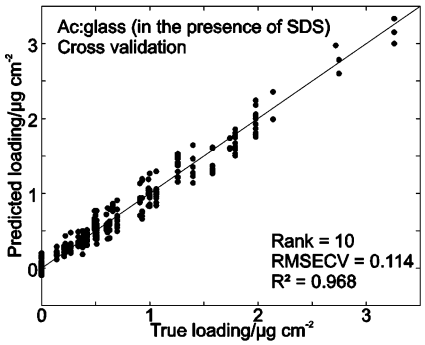

Figure 13. **Predicted versus true surface loading of acetaminophen on glass in the presence of varying amounts of sodium dodecyl sulfate (SDS), from a leave-one-out cross validation using Quant2. The samples were prepared by the spray method. True loadings for acetaminophen and SDS were determined, respectively, by UV colorimetry and quantitative ^{1}H NMR spectroscopy.**

one type of metal surface.

As a technicality, it should be noted that the aluminum coupons used in this experiment had a dull finish, while the stainless steel ones had a mirror finish. The effect of surface finish on the present application of IRRAS spectra has not yet been the subject of a systematic investigation but is expected to be a factor that must be considered when data sets are being built for calibration.

The results presented thus far pertain to single APIs on otherwise clean surfaces. An important issue for practical applications of the IRRAS method will be the detection of contaminants in the presence of other materials, such as excipients and cleaning materials. This is a more complex problem than those addressed above, but one that we have begun to investigate using model systems.

Figure 13 shows the results of a leave-one-out cross-validation of a Quant2 calibration model built from 333 IRRAS spectra taken from glass coupons spray coated with independently varied amounts of acetaminophen and SDS, a component of many pharmaceutical cleaning agents. The high correlation factor ($R^2 = 0.968$) and the comparatively low RMSECV of 0.1 $\mu g/cm^2$ indicate that it will be possible to develop useful calibration models that take account of varying amounts of additional materials in addition to the APIs that are of quantitative interest. Work on this question and the related problem of simultaneously quantifying two or more different materials is continuing, and the results will be presented elsewhere.

Conclusions

The work presented in this contribution has shown that the combination of IRRAS and chemometric modeling has significant promise for the in situ quantification of contamination of glass and metal surface by APIs, even in the presence of interference from atmospheric absorption bands. The case for single-component APIs is firmly established, while preliminary results for binary mixtures on glass are encouraging.

For the single-component contaminants on glass and metal surfaces, the calibration models described here have RMS errors of cross validation (RMSECV) and prediction (RM-

SEP) near, or below, 0.1μg/cm^2. The lowest surface loadings investigated were 0.01 and 0.05 μg/cm^2 for ibuprofen on stainless steel. After correction for absorption by water vapor, the 0.05 μg/cm^2 spectrum exceeds the "three-times-baseline-noise" criterion for the limit of detection (LOD), while the 0.01-μg/cm^2 spectrum barely passes the same test. For practical purposes, loadings of 0.05 μg/cm^2 can therefore be taken as being a readily achievable LOD for the IRRAS technique applied in the manner outlined in this report. Further developments are likely to realize lower limits soon.

To be of genuine utility in industrial applications such as pharmaceutical cleaning validation, the IRRAS method must be shown to be more generally applicable. The range of substrates must be broadened to include other metals, enamels, and certain plastics, and investigation of the dependence of the spectra on the surface finish. More chemically complicated contaminant mixtures representative, for example, of those used in industrial reactor-cleaning protocols must also be investigated. These issues are the focus of current investigations in our laboratories and will be the subject of publications presented at a later date.

Although further work is needed to determine the limits of the technique, it is becoming clear that IRRAS has the sensitivity and versatility required for in situ detection and quantification of pharmaceutical compounds and other materials at surface concentrations well within the range of interest for pharmaceutical cleaning validation and other similar applications.

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