Application Note

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Fourier Transform Infrared Spectroscopy for Rapid Cleaning Verification of Mixing Vessels and Reaction Chambers

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Abstract

Rapid cleaning verification using Fourier transform infrared spectroscopy (FTIR) illustrates an exciting avenue for quick, low-cost cleaning verification with low technical barrier of entry. The Agilent 4300 handheld FTIR system was used to construct concentration models based on varying surface concentrations as the basis for rapid surface quantitation methods. These concentration models were able to recognize quantities of active pharmaceutical ingredient as low as 0.62 µg/cm^2, with potential to reduce this value based on the excellent linearity of the models.

Introduction

Contaminant detection has been at the forefront of manufacturing validation, verification, and cleaning for decades, spanning multiple scientific fields and generations of different technologies. At its core is a simple premise, namely the routine threshold detection or quantification of impurities in a manufacturing process. However, widely applicable contamination detection methods have a history of expense, time, and using compromises. These usually involve trading speed for greater accuracy, wide applicability for better limits of detection, and performance for portability.

In recent history, molecular spectroscopy has emerged as an exciting technology in the general field of detection. Shining light of various wavelengths onto samples to acquire analytical information lends itself well to the straightforward nature of contaminant detection: light travels quickly, is nondestructive towards samples, and requires little in the way of sample running costs. Various molecular spectroscopy techniques, such as UV-Vis and fluorescence are currently used for contaminant detection under very specific circumstances, which fulfill their niche roles. Nevertheless, the highly specific nature and broad chemical signatures of these techniques prevent widespread adoption in multiple manufacturing stages.

This is an area where vibrational spectroscopic techniques such as FTIR are applicable. The highly specific, unique chemical signatures present by vibrational modes of molecules in the infrared wavelength range allow critical identification and quantification to be performed even in the presence of complex spectra. With the advent of high-performance, portable, and handheld FTIR instruments, such as the Agilent 4500 portable and 4300 handheld FTIR spectrometers, respectively, contaminant detection is set to experience a whole new level of growth.

In recent years, the 4300 handheld FTIR spectrometer has experienced a rapid uptake by customers working in highly differing fields. Food purity and authenticity, nitrogen content in soil, and lightning-induced thermal damage to aircraft represent just a fraction of the growing list of applications perfectly suited to this instrument. Designed to be a handheld, battery-operated analyzer with no previous FTIR experience necessary, the 4300 can measure a wide variety of samples on many different surfaces, such as stainless steel and composites. It can be used for both quantitative and qualitative analysis of materials using the intuitive Agilent MicroLab mobile measurement software. The instrument itself is lightweight, weighing only 2 kg (4 lb), and is packed in a weather-resistant enclosure. Therefore, it is suitable for outdoor use, or in environments where cleaning of the instrument is required (rated IP54). Furthermore, it comes with two lithium batteries for up to four hours of operation, or it can be operated with a 110/220-volt AC power for extended use.

The 4300 provides versatility for use in a traditional analytical chemistry laboratory, manufacturing site, or even in the field. It can be configured in several different sampling configurations to accommodate the analysis range of different contaminated surfaces, as shown in Table 1. The sampling technologies (or "sampling modules") are easily interchangeable in the field and are automatically recognized by the system, which is convenient for nonexpert users during in-field or production environment screening.

Table 1. Agilent 4300 handheld FTIR spectrometer sampling modules.

Sampling Interface	Details
Diffuse Reflectance	The diffuse reflectance module provides the best signal intensity for rough samples and surfaces, such as artwork, soils, rocks and minerals, composites, rough plastics, fabrics, and corrosion on metal surfaces. In general, if the sample reflects little light, the diffuse reflectance interface will most likely be the sampling method of choice.
External Reflectance	The external reflectance module was designed for highly specular reflective surfaces, for example, films and coatings on reflective metal surfaces. The infrared energy passes through the film; reflects off the steel; passes back through the film, and is collected by the specular reflectance interface. The sampling module can be used in several applications, such as glasses; glazed or unglazed ceramic; polymer films; PE or PP pipes, and cables, to name a few. It is also used extensively for studying the cleanliness of metal or glass-lined vessels.
Grazing Angle	The grazing angle specular reflectance interface is similar in concept to the external reflectance interface, but with a much steeper cone half angle of 82 degrees. This makes it ideal for the analysis of thin (submicron-sized) films; the steep angle allows the IR light to interact with the film and has the secondary benefit of increasing the pathlength. The interface can be used to analyze anything the external reflectance interface can; however, the sample needs to be flat.

The different interfaces combined with the 4300 allow one instrument to service several different markets and applications. While implementing the 4300 into many fields is straightforward, some applications require a bit more care and method development upfront for operators to fully reap the long-term, cost-saving nature of the instrument. One of these fields is rapid cleaning verification of large mixing vessels and reaction chambers, which presents a host of unique technical challenges. These vessels are heavily used in the manufacturing of many different types of end products, from consumer products to pharmaceuticals. Therefore, maintaining a high threshold of cleanliness is imperative to avoid unwanted contamination between reagent types or batches. The current standard practice to verify the cleanliness of a vessel involves swabbing the vessel surface and sending the swab for analysis via HPLC to determine its cleanliness level.[1-3] The full process of cleaning verification can take time, from sampling all the way through to analysis, checking results and release of product. Our goal is to develop a rapid cleaning verification method based on handheld FTIR spectroscopy, with proposed advantages of result turnaround in several minutes, which is solvent-free and little to no sample running costs. The implications of this work could have rippling effects across multiple industries, reducing the number of required vessel-washing cycles, detergent, and water use; vessel downtime; the number of product recalls, and significant cost savings immediately.

Experimental

Preparation of steel coupons

Stainless steel was chosen as the substrate of interest, due to its highly reflective nature in the IR region and widespread use as the surface of many vessels used and routinely cleaned at customer sites. Several 5 × 5 cm stainless steel coupons polished to a number 7 finish ("mirror-like") were purchased from a metals manufacturing company. Each coupon was initially cleaned via a multistage process involving rinsing with solvent (water in most cases); contact washing with liquid soap; sonication in water with a drop of soap; subsequent solvent rinsing, and a final rinse with ethanol. The coupons were allowed to dry overnight prior to use.

Printing of analyte on steel coupons

Quantitation of analytes on solid substrates requires robust concentration models correlating IR absorbance with the amount of analyte present on the surface. Serial dilutions of the chosen analytes (acetaminophen, caffeine, clarithromycin antibiotic, CIP-92 detergent) were created in the concentration range of 1 to 4 g/L, with a mixture of water and ethanol as solvent, depending on the solubility of the analyte. These solutions were each "printed" (i.e. deposited by an automated solid deposition device, or "solid printer") on an individual coupon as 0.021 µL dots spaced 1 mm apart, thereby creating an array of analyte dots spanning an area of 30 cm^2. The printed coupons were left to dry overnight to ensure all solvent had been fully evaporated. The resulting surface concentration of the dot array pattern printed on the coupons was determined to be 1 to 5 $\mu g/cm^2$.

FTIR analysis of printed steel coupons

The 4300 handheld FTIR spectrometer was used to acquire spectra from each of the printed steel coupons. The 4300 was suspended 0.5 mm above each coupon to avoid contact, which might ruin the printed dot array pattern. Spectra were taken at 10 random locations on each coupon at 8 cm^{-1} resolution over 50 scans, resulting in a total acquisition time of roughly 15 seconds per spectrum. Background spectra were acquired using a blank, unprinted steel coupon prior to collecting spectra from each printed steel coupon.

Construction of concentration models

Once spectra for all coupons of a chosen analyte were acquired, the first derivative of each spectrum was taken. The purpose of this was to negate any rolling or shifting baselines, which are typical of solid surface mid-IR data collection at low signal intensities. The derived spectra were then supplied to a simple partial least squares (PLS) modeling algorithm to build the concentration model correlating analyte surface absorbance with surface concentration. Information at only one wavenumber (i.e. one latent variable) in the mid-IR fingerprint region (i.e. ~1,500 to 650 cm^{-1}) was used to construct these concentration models in efforts to keep said models as straightforward and widely applicable as possible. The correlation coefficient (R^2) threshold for model rejection was 0.95.

Results and discussion

The concentration models resulting analysis of printed coupons containing each analyte are displayed in Figure 1. Considering the inherent difficulty associated with solid-phase quantitation and the use of only one latent variable, the linearity of each model is outstanding and certainly holds much promise for a fully developed, rapid cleaning method based on FTIR spectroscopy. The high fidelity of each model indicates a good possibility that data taken directly from a similarly polished surface, contaminated with trace amounts of the same analyte, would result in a reliable quantitative result of surface contamination. This, in turn, could provide customers greater confidence in a threshold pass/fail criterion for surface cleanliness.

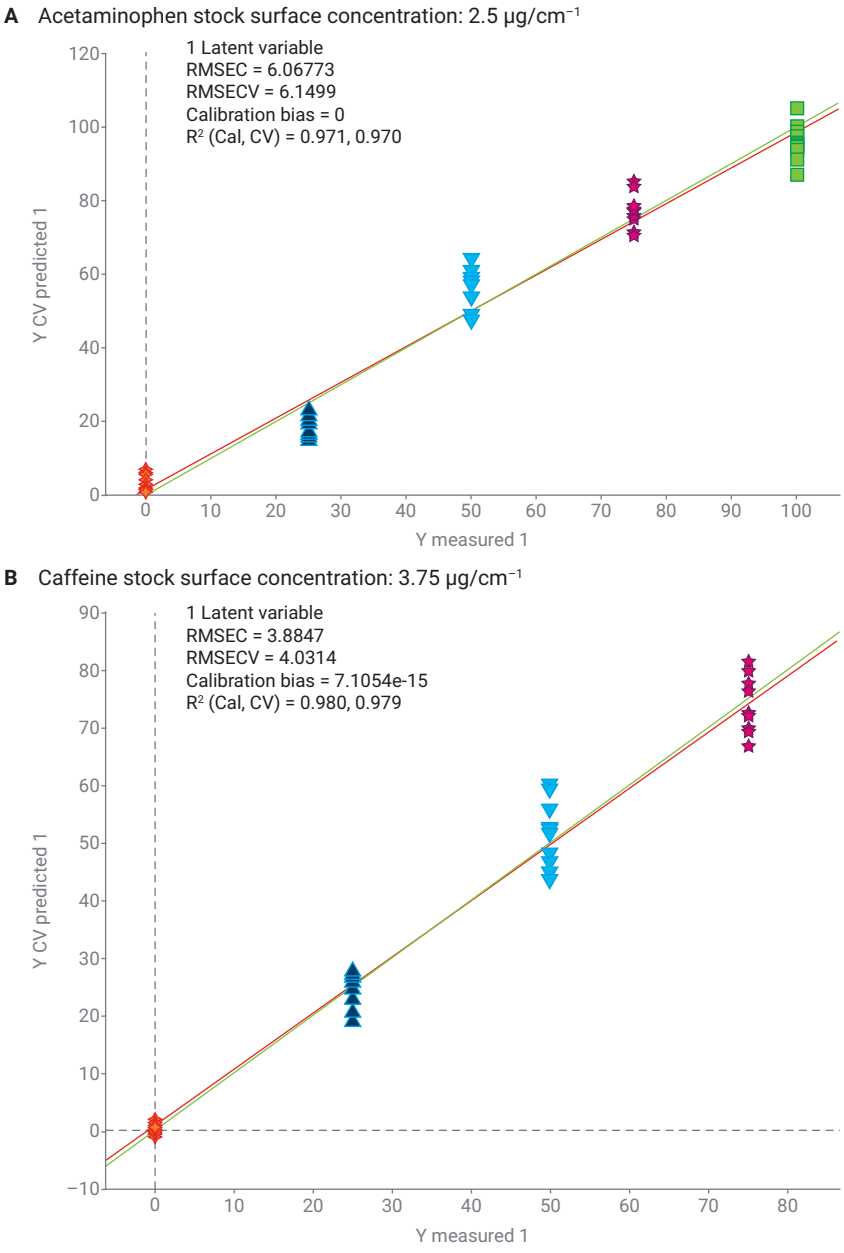
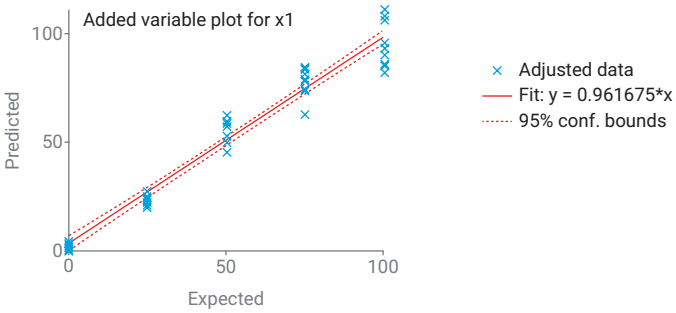


Figure 1. PLS models correlating analyte surface absorbance with surface concentration for chosen analytes: (A) acetaminophen, (B) caffeine, (C) clarithromycin antibiotic, (D) CIP-92 detergent. Axes denote the percentage of stock surface concentration. (Continued on the next page).

C Clarithromycin stock surface concentration: 5 µg/cm^{-1}



D CIP-92 stock surface concentration: 1% vol./vol.

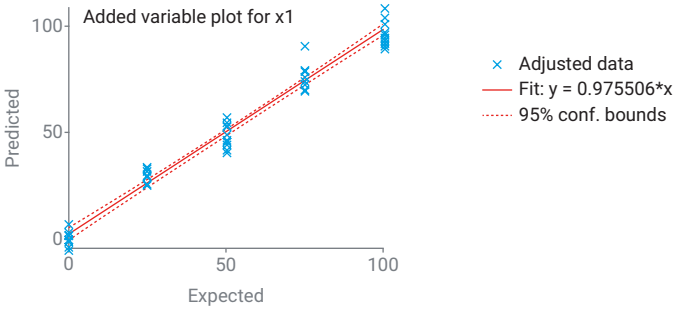


Figure 1. (Continued) PLS models correlating analyte surface absorbance with surface concentration for chosen analytes: (A) acetaminophen, (B) caffeine, (C) clarithromycin antibiotic, (D) CIP-92 detergent. Axes denote the percentage of stock surface concentration.

Much care was taken to randomize the 10 spots selected on each coupon for data acquisition, in order to average any reproducibility issues stemming from the coarseness of the dot arrays printed on each coupon. Real-world samples, consisting mostly of vessel surfaces contaminated with reagent remnants, would be much more akin to thin films deposited on metal surfaces rather than a neat array of dots. As such, any printing device selected to create the calibration standard coupons should print analyte dots as close as physically possible in order to most closely mimic the presence of a homogenous thin film akin to real-world samples. This would reduce the need to analyze as many randomized spots on each coupon as was done in the aforementioned section to build each concentration model, further streamlining model construction.

Conclusion

Overall, these results provide excellent support for the ability of the Agilent 4300 Handheld FTIR spectrometer to detect surface contaminant absorbance and relate these to surface concentration in cleaning verification assays. Future work in this area extends to validating each model through analysis of analyte surface concentration on a real vessel surface, rather than 5 × 5 cm coupons, with independent confirmation achieved through unrelated means, likely the standard USP swabbing and HPLC method. Additional studies on the impact of dot size and spacing on calibration coupon sets would provide insight into further parameter optimization during said calibration set preparation. Determining the influence of surface shininess on analyte signal quality would also comprise a worthwhile endeavor, allowing expansion of this potential rapid cleaning method to heavily worn surfaces with rougher, less polished appearances. Finally, although the 4300 is relatively compact, hard-to-reach surfaces in areas smaller than the instrument itself still present a challenge since direct measurement of these surfaces is not possible at this time.

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For any questions about this study, please feel free to contact the application scientist who conducted the analysis or the local Agilent Technologies sales representative.

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