Deep UV Raman and fluorescence spectroscopy for real-time in situ process monitoring

W. F. Hug*, Q. Nguyen, M. Reid, K. Sijapati, & R.D. Reid
Photon Systems, Inc., 1512 Industrial Park St., Covina, CA 91722

Abstract

Raman spectroscopy has become an increasingly common analytical method for real-time, on-line, in-line, and off-line in situ monitoring of product quality in a variety of pharmaceutical, chemical, and biological manufacturing environments, including wastewater quality. The major shortcomings of Raman spectroscopy conducted in the near UV, visible, and IR are that: 1) highly efficient fluorescence emissions from targeted and surrounding materials within the excitation volume of a complex sample often obscures or alters the Raman signature of the materials of interest; 2) essential and informative fluorescence features of many organic and biological materials are not excited when excitation occurs at wavelengths longer than 250 nm; and 3) Raman signal strength is diminished due to Rayleigh Law and lack of resonance effects. This is especially true of simple organic compounds and biological materials such as amino acids, proteins, peptides, and whole microbial organisms as well as a wide range of active pharmaceutical ingredients as well as their presence in other manufacturing environments and environmentally in wastewater.

Unless excitation occurs at wavelength less than about 250 nm, there is significant overlap between Raman and native fluorescence spectral regions from a wide array of organic and biological materials including active pharmaceutical ingredients (APIs) and excipients. This overlap obscures weak Raman emissions and alters the emission spectra of fluorescence emissions due to strong CH and OH Raman bands, both of which reduce the fidelity of spectral classification. This overlap is considerably worse for excitation above 260 nm.

Raman emissions provide information about the chemical bonds within the mixtures present in the excitation volume of detection. Fluorescence emissions provide complementary information about the overall electronic configuration of the targeted material. Together, Raman and fluorescence information more fully describe the chemical compounds of interest. Simultaneous acquisition of both forms of emissions coupled with chemometric analysis enables detection and characterization of a wide range of organic and biological material not possible when excitation occurs in the near UV, visible, or IR.

We will describe two, new, compact, low cost, instruments employing deep UV excitation to address these growing applications: the DUV Raman PL 200, and the TraC-X. The DUV Raman PL 200 is a portable, 7”x 8”x 25”, 22 lb instrument, which is fully self-contained including a 248 nm laser and controller, a spectrometer with two, computer controlled, holographic gratings, and a multi-stage thermo-electrically cooled 2048x122 element, back thinned, back illuminated, high quantum efficiency detector. And we will also describe the TraC-X sensor, a 3”x 3.5”x 7.5”, < 2 lb, fully self-contained, deep UV excited autofluorescence-only instrument, with built-in deep UV source, low spectral resolution spectrometer, detectors, and microprocessor for analyzing spectral results and providing processed information. As examples, we will discuss applications for real-time in situ monitoring of APIs during continuous liquid and powder manufacturing in the pharmaceutical industry and measurement of nitrates and nitrites in wastewater treatment plants.

Keywords: pharmaceutical process monitoring; liquids, powders; real-time; deep UV Raman; native fluorescence; detection; classification, wastewater, trace contaminants.

1. OPTICAL DETECTION IN PROCESS ANALYTICAL TECHNOLOGY

Present process analytical technology optical detection methods have limitation have limitations in measuring key components of their process, whether it is measuring the concentration of active pharmaceutical ingredients (APIs) in continuous flow manufacturing the Pharma or other continuous flow manufacturing

applications, or real-time measurement of trace contaminants in municipal wastewater treatment plants. The common problem is making measurements in real-time in materials that have complex backgrounds resulting in fluorescence obscuration or interference of Raman spectra as well as alteration or elimination of fluorescence spectra due to ineffective excitation wavelengths.

2. ADVANTAGES OF DEEP UV RAMAN & FLUORESCENCE DETECTION

Deep UV Raman and photoluminescence or fluorescence optical methods offer the ability to detect and identify material at much lower concentrations with higher specificity than is possible with near-UV, visible, or IR methods due to the higher energy photons associated with UV excitation. At longer excitation wavelengths many materials are simply not detectable at all using Raman or fluorescence optical methods. Excitation below 250 nm enables fluorescence-free Raman where the spectral regions of Raman and fluorescence occur in different spectral regions and there is no overlap and resulting interference. Since fluorescence is so much more efficient than Raman emissions, the slightest amount of fluorescence from an API or anything surrounding the API within the laser beam excitation spot can easily obscure Raman emissions. In addition, excitation below 250 nm also enables fluorescence detection of small organic molecules not possible with excitation at longer wavelengths since these molecules, including many pharmaceutical APIs, emit at wavelengths as short as 270 nm and would not exhibit fluorescence if excited at longer wavelengths. Similar advantages occur for detection of trace contaminants in wastewater treatment plants.

The primary advantages of detection in the deep UV below 250 nm include:

a) Clear Raman spectra with no obscuration or alteration by native fluorescence.
b) Detection of fluorescence spectra not possible at longer wavelengths & without spectral alteration by strong Raman bands.
c) Simultaneous detection of Raman and fluorescence emissions, providing more information about a substance.
d) Enables detection at much lower concentrations with higher specificity than near UV, visible, or IR methods.
e) Enables real-time API detection of APIs at < 0.04% wt/wt in Pharma continuous flow manufacturing.
f) Enables much higher specificity using fluorescence alone when excitation is below 250 nm

The first three advantages are illustrated in Fig. 1 below, where the Raman and fluorescence bands of over 50 different materials is illustrated with excitation at 248.6 nm. The first 4000 cm-1 region of Raman shift occupy the spectral region from the laser wavelength at 248.6 nm and about 270 nm. Above that is the fluorescence region of organic materials, where simple, single phenyl ring compounds emit at the shortest wavelengths, and larger ring structure organics emit an increasingly longer wavelength. The envelope of the fluorescence region of all natural materials is nominally between 270 nm and 950 nm, with few exceptions.

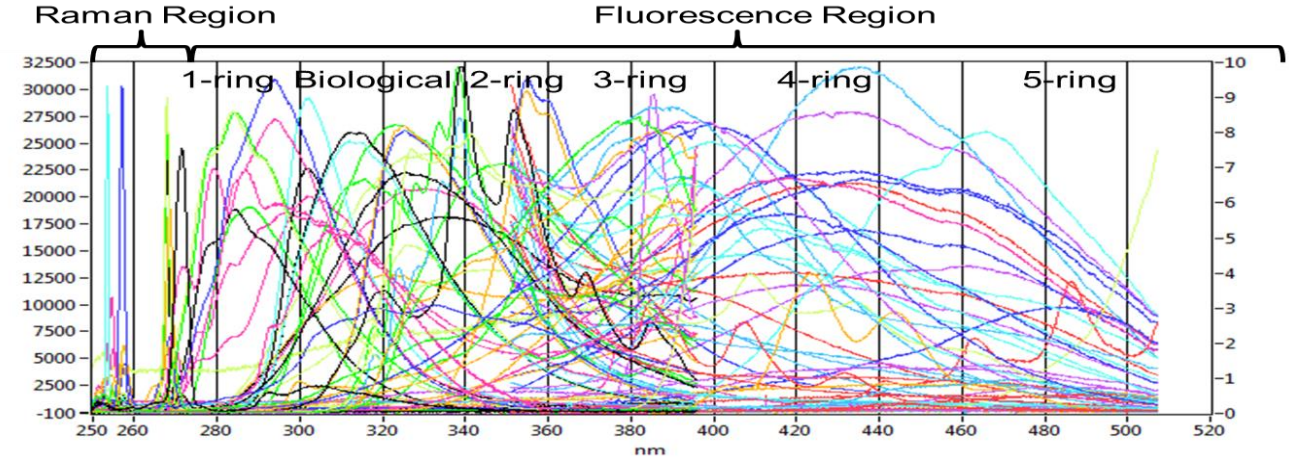


Figure 1. Spectral regions of Raman and fluorescence for a wide variety of materials.

The broad rainbow "fluorescence region" of Fig. 2 illustrates the envelope of the fluorescence of all natural materials shown in Fig. 1, along with the excitation wavelength of common laser types, in black, and the Raman shift spectral region associated with 3500 cm-1 of Raman shift for each of these laser wavelengths, in red.

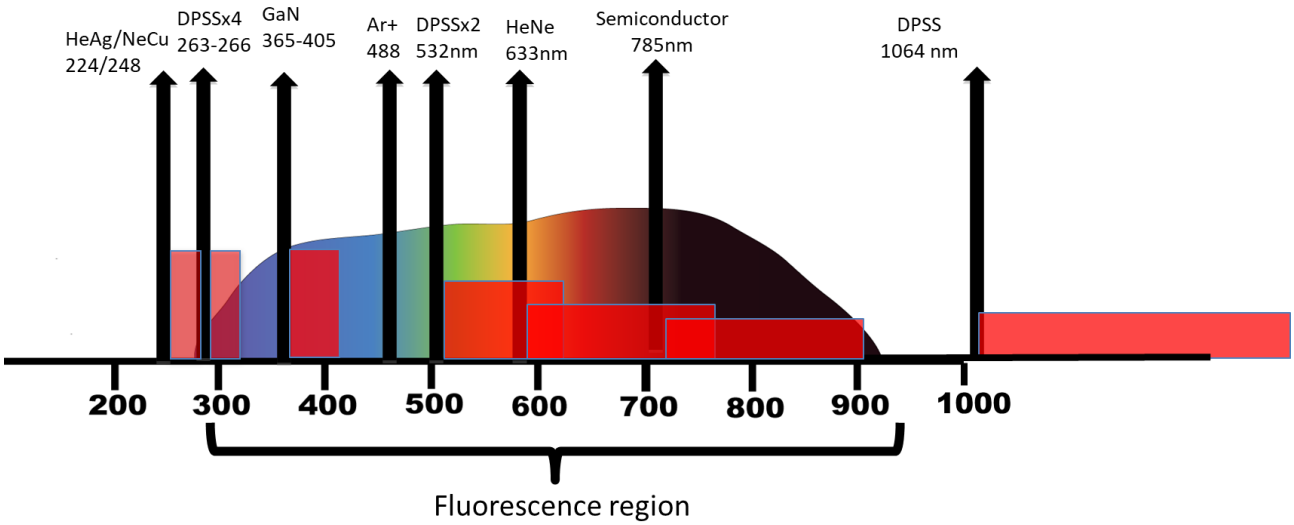


Figure 2. Relationship between laser excitation wavelength and 3500 cm-1 of Raman shift, compared with the fluorescence regions of many organic materials.

Fluorescence is the most sensitive method of optical detection with typically between 1E5 to 1E8 times more sensitivity than Raman, providing for detection at lower concentrations and longer standoff distances than Raman detection. Limits of detection for deep UV excited fluorescence are typically in the low fg range with concentrations in the low pg/cm^2 range. Limits of detection for Raman are typically in the low ng range with concentration in the µg/cm^2 range. Fluorescence detection requires an excitation wavelength sufficiently shorter than the lowest fluorescence emission wavelength so that alteration of the fluorescence spectrum is not altered by strong Raman bands, typically from OH or CH resonances. Raman spectra provide information about the chemical bonds and functional group of an analyte. Fluorescence spectra provide information about the overall electronic structure of an analyte. Although Raman is well respected for providing information about an analyte, fluorescence also provides significant information. The combination of both methods of detection provide expanded understanding of an analyte under investigation. An example of this is shown below in Fig. 3.

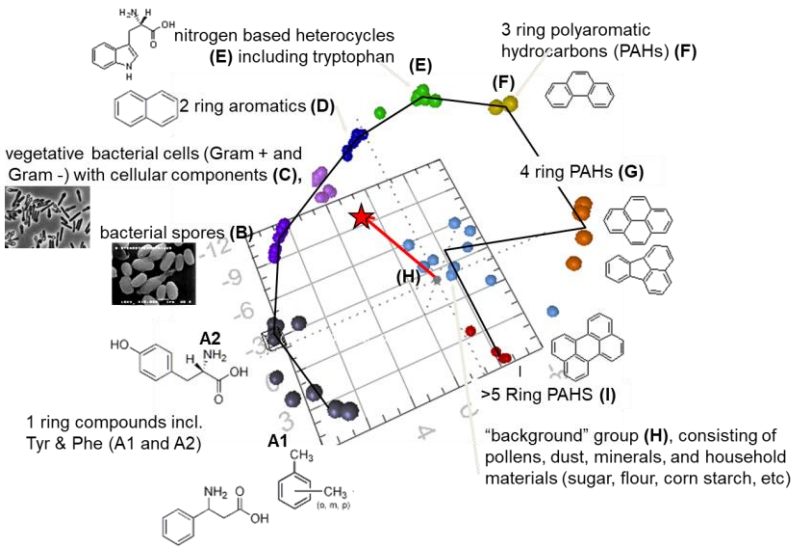


Figure 3. Chemical differentiability using autofluorescence alone.

Figure 3 illustrated the ability of fluorescence alone to differentiate a variety of compounds and composite materials, such as bacterial spores and cells. This figure employs principal component analysis (PCA) to analyze the spectra of Fig. 1 and illustrate the clustering of different types of material when viewed in PCA this space. When excitation occurs above 250 nm, this clustering of materials disappears and similar material begin to increasingly overlap or disappear, as excitation wavelength becomes too long to excite fluorescence in many of these materials.

A major advantage of deep UV fluorescence is the very clear ability to differentiate various organic compounds, where, with Raman alone, these materials would be very difficult to differentiate except with very high spectral resolution spectrometer needed to separate the nuances of the CH stretch region of these different organic compounds. Also, because fluorescence is so sensitive, the identity of a compound can be easily found by any of several methods, including PCA, as illustrated in Fig. 4, but other algorithms also, at requiring a very low exposure dose. In this case, differentiation was accomplished in less than 40 µs. The red star in Fig. 3 illustrates an example of detection of an unknown compound can be detected and differentiated using Euclidian distance from known compounds or composite materials, such as bacterial spores and cells. We have also demonstrated that it is possible to zoom into any specific region of this PCA diagram and further differentiate or identify material in very fine detail, including biological material [1].

3. LIQUID DETECTION EXAMPLES FOR PHARMA APPLICATIONS

The following discussion is for detection of liquid API ingredients suspended in a continuous flow manufacturing application where the excipients and additives offer a complex medium in which to detect the APIs. Figures 4 through 6 illustrate the ability of deep UV Raman methods to detect clear Raman spectra of APIs in complex pharma backgrounds for use in continuous flow manufacturing of drugs. The first illustration, in Fig. 4 below, shows Raman spectra of an over the counter (OTC) drug called Benylin with Raman excitation wavelengths at 1064 nm, 785 nm, and 248 nm. For each of these Raman spectra, the Raman shift is registered so show the region between 200 cm-1 and 2000 cm-1, with the 248 nm Raman spectrum also extending to over 3600 cm-1. The 248 nm spectrum is raw data with no baseline subtraction or compensation.

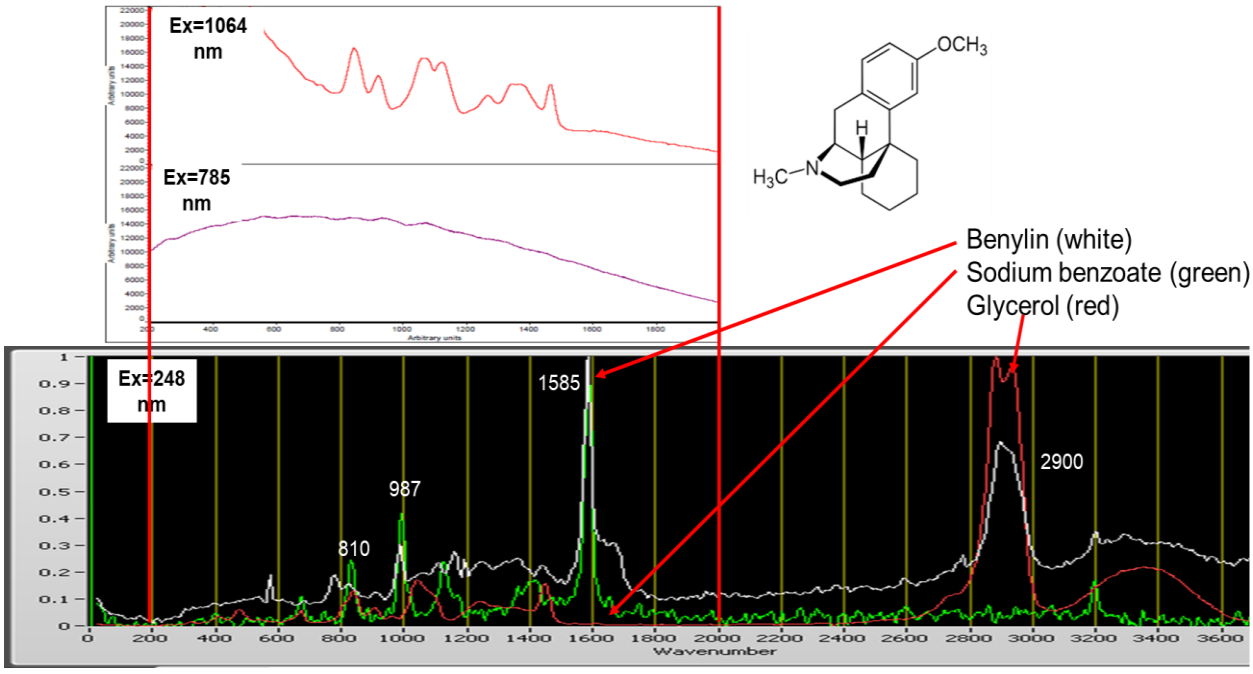


Figure 4. Raman spectra of OTC Benylin with excitation at 1064 nm, 785 nm, and 248 nm.

As illustrated above, fluorescence from the ingredients in this product obscured the Raman signature of the API when excited at 1064 nm and 785 nm, but clear Raman spectra of the API are observed when excitation is below 250 nm, in this case at 248 nm.

Other examples are below of ibuprofen and acetaminophen embedded in a mixture of excipients and other ingredients. Figure 5 below show the deep UV Raman spectrum of OTC Children's Motrin with bubblegum flavor. The Raman spectra in the fingerprint region are very clear, and the fluorescence emissions can be seen ramping up at the longer Raman shifts due to natural fluorophores within ibuprofen as well as bubblegum flavorants.

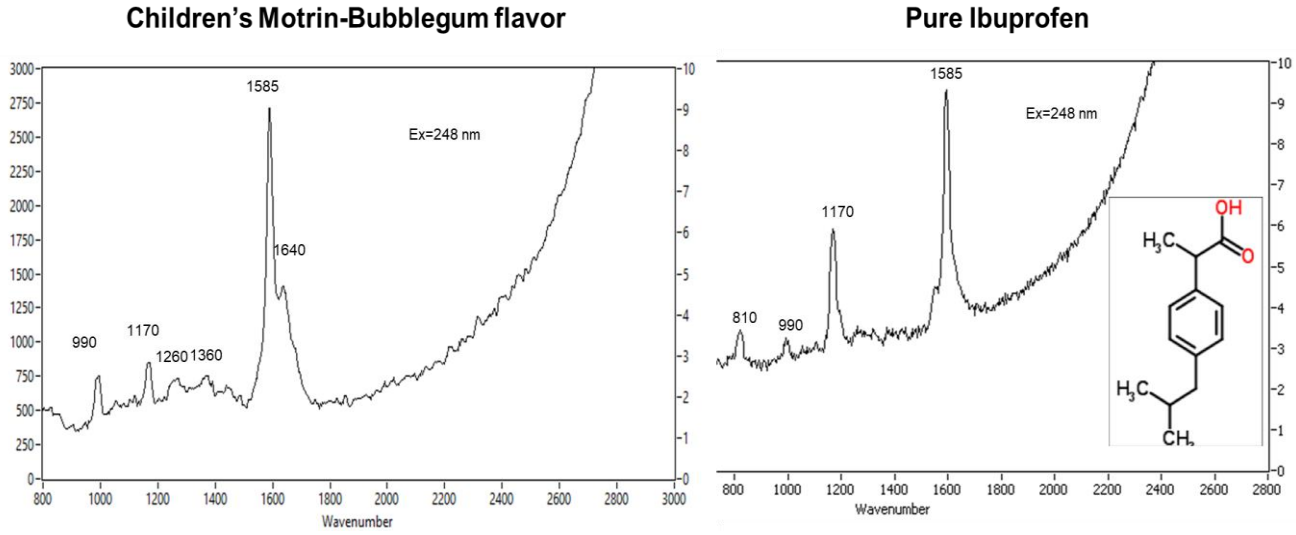


Figure 5. Raman spectra of pure ibuprofen and OTC Children's Motrin with bubble gum flavor

OTC Children's Tylenol deep UV Raman spectra are shown in Fig. 6 below with a wide range of flavorants, where the fluorescence contribution to the spectra are outside the Raman fingerprint region, enabling clear Raman spectra with no obscuration of interference.

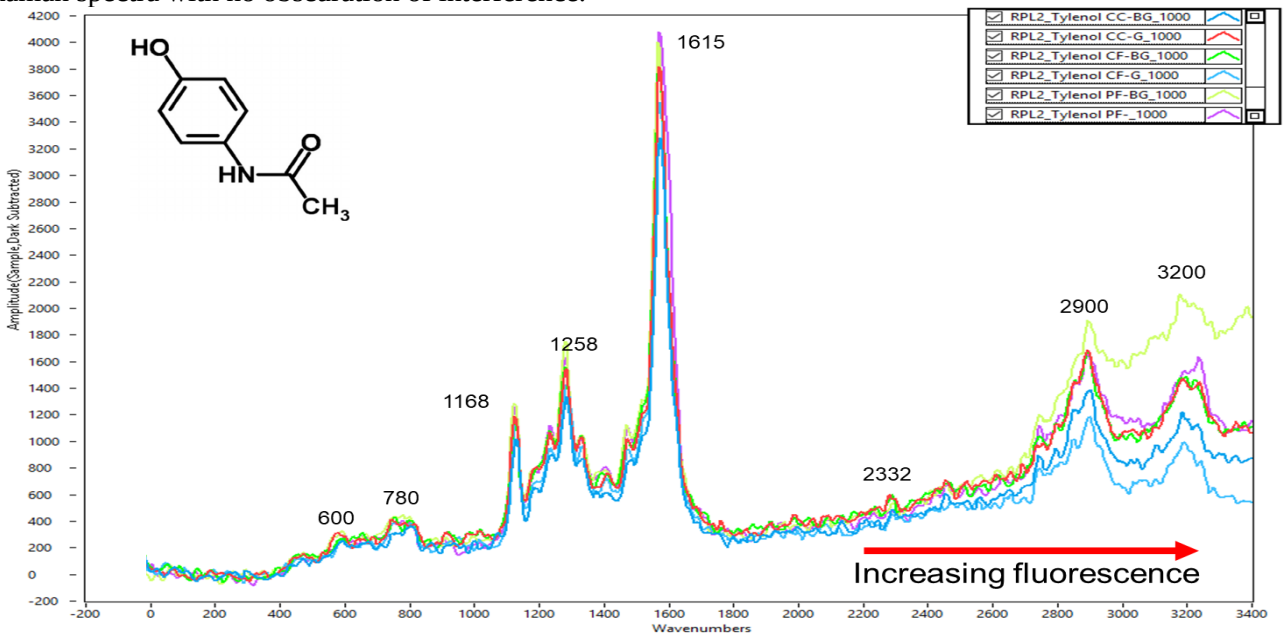


Figure 6. Raman spectra of OTC Children's Tylenol with the clearly visible acetaminophen Raman bands.

4. POWDER DETECTION EXAMPLES FOR PHARMA APPLICATIONS

In the case of detection of powder mixtures in continuous manufacturing of **high potency drugs**, the problem is that NIR and FITR techniques are limited in most powder mixtures to > 2% bulk ratios. In addition, absorption techniques and excipient interference limits dynamic range. Again, the goal is to explore techniques focused on Deep UV spectroscopy (fluorescence and Raman) to extend in process control of high potency drugs to lower than 0.1% bulk ratios. Less than 0.04% has been demonstrated with deep UV detection.

An example of this is detection of APIs embedded with excipients and other materials in powder form directly in final feed frame prior to pill formation. Figure 7 below illustrates a typical powder feed frame where the powders are blended. Detection is accomplished through a window into the powder mixture as it is being blended. The choice of detection can be either deep UV Raman or fluorescence depending on the best results. Again, this process done with NIR has limits of detection above 2%, where the goal is less than 0.1% for high potency drugs.

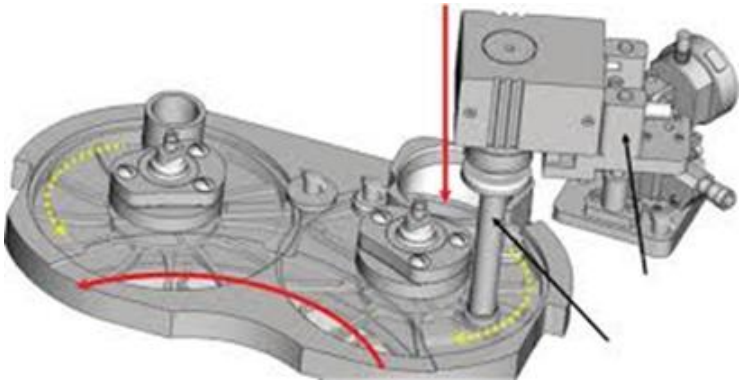


Figure 7. Typical final feed frame for continuous manufacture of drugs from powder mixtures.

An example of powder detection was the use of Saccharin as analog material, where powder-powder mixtures ranging from 3% to 0.1% Saccharin was detection were prepared by a customer in excipients including MCC, Lactose, Crospovidone, sodium stearyl, and magnesium stearate. An autofluorescence spectra of these components is shown below in Fig. 8, with excitation at 248 nm, where emission from Saccharin is clearly very different from any of the other excipients which are at least 10X lower in signal strength.

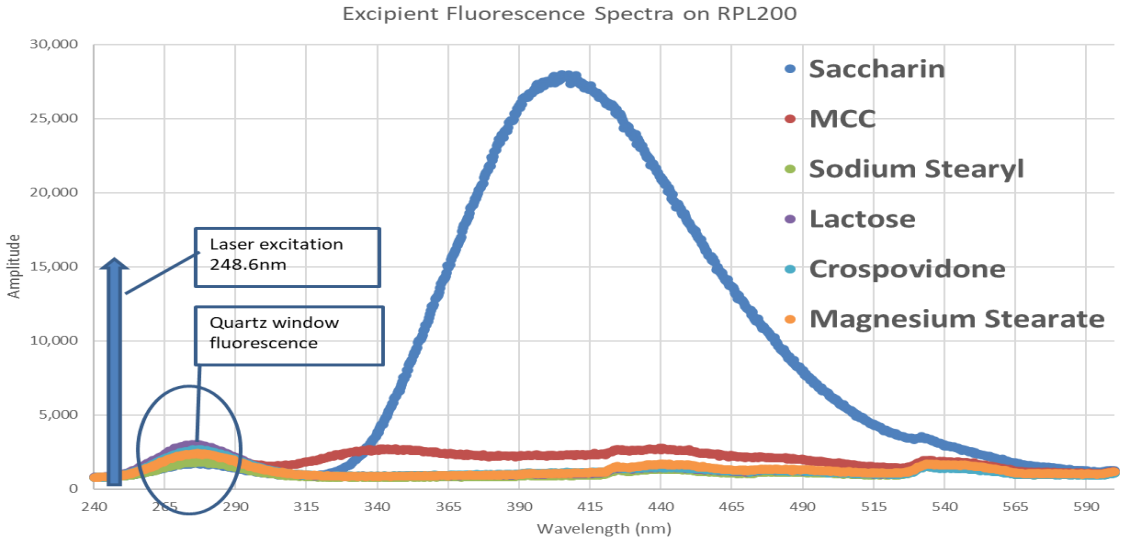


Figure 8. Autofluorescence spectra of powder-powder mixtures of Saccharin and excipients.

The above spectra were taken during a single, 40 µs, laser pulse using the DUV Raman PL 200 instrument with a 300 mm focal length objective lens.

Autofluorescence date were also taken on the same powder-powder mixtures using the TraC-X instrument. For these measurements, a signal strength about 6 billion A/D counts were measured at the peak of the Saccharine and nearby wavelengths, where the Saccharine signal to MCC background was 2570:1, and the worst signal to background was for magnesium stearate at 727:1. These results are shown in Fig. 9 for 3 detection bands near the Saccharine peak, with the inset being a photo of the detection end of the TraC-X instrument focused into the powder-powder mixture fixture.

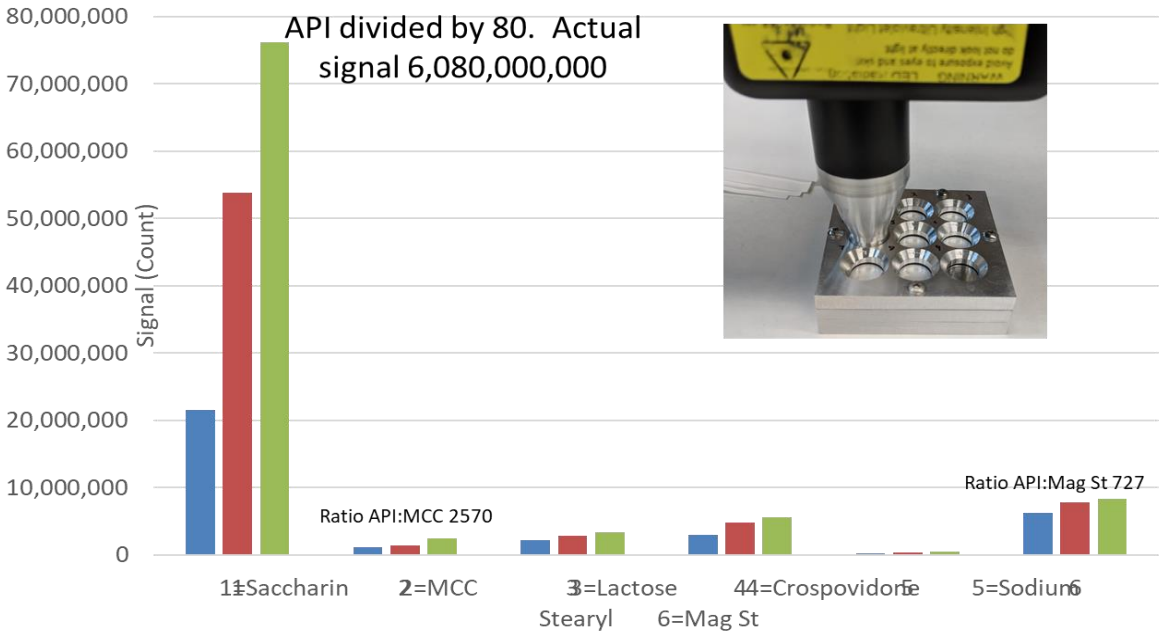
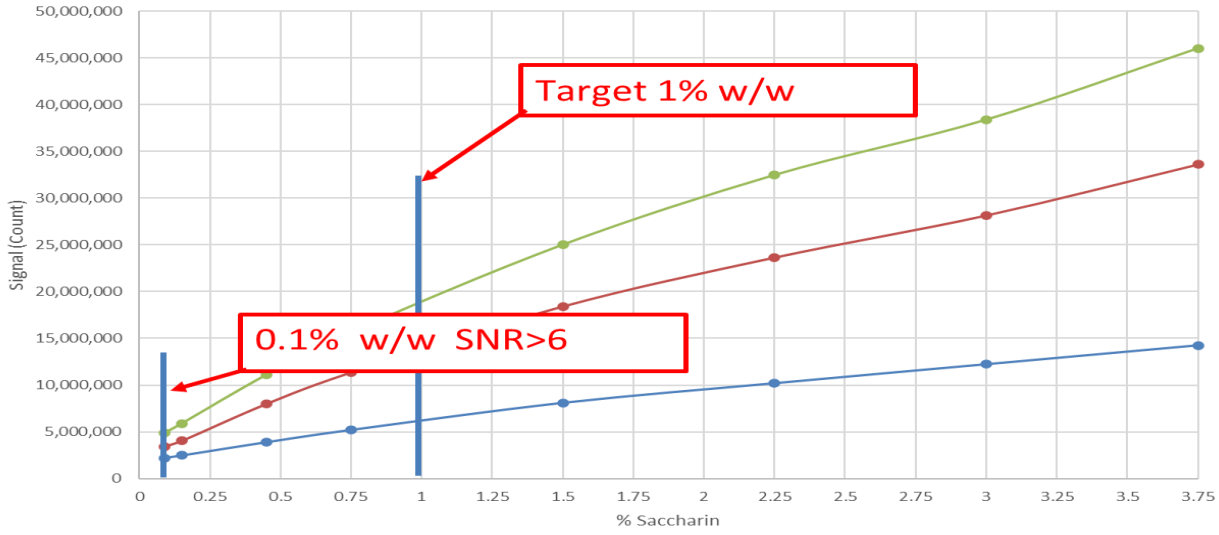


Figure 9. Detection results of Saccharine/excipient powder-powder mixtures with TraC-X sensor.

The peak autofluorescence signature strength of Saccharine is shown below vs concentration in Fig. 10 below using a dramatically reduced excitation energy, about 135X, to eliminate signal saturation at the higher concentrations. However, even at this reduced excitation, the LOD for Saccharine would be about 0.5% at a SNR of 3 or potentially much higher SNR or lower LOD with available increase in excitation energy.



or

Figure 10. Autofluorescence signal strength versus Saccharine concentration in powder/powder mixture.

These results are clearly excellent, and hold promise of measuring even lower concentrations of Saccharine or other potential high potency APIs. The same prospect is not so excellent using Raman spectroscopy, where there was significant spectral interference between the Raman bands of Saccharine and several of the excipients.

5. REAL TIME MEASUREMENT OF KEY WASTEWATER CONTAMINANTS

As an example of the benefits of deep UV Raman and fluorescence detection methods, detection of contaminants in wastewater is becoming more important, as the effluent of modern wastewater treatment plants (WWTPs) is increasingly mixed with "fresh" water to enhance potable water supplies. New regulations on potable water are increasing the need to detect increasingly smaller trace concentration of an increasingly wider range of contaminants. And the need to make measurements in near-real-time is important to ensure that regulated limits are not exceeded. The weekly testing at remote laboratories is rapidly becoming insufficient to manage our potable water needs.

An example of key contaminants are nitrates and nitrites, which are highly regulated and have significant economic benefits for real-time measurement. Deep UV Raman spectroscopy has been shown to enable quantitative detection of these materials in wastewater, even in water highly contaminated by organic compounds and debris which obscure Raman detection when conducted at wavelengths above 250 nm. The fluorescence signatures of wastewater itself enable detection of a wide range of organic materials, which also needs to be removed from WWTPs. And, as described earlier, these measurements provide significant specificity and well as breadth of description of these contaminants.

6. DEEP UV RAMAN AND FLUORESCENCE INSTRUMENTATION

Presently, Photon Systems, Inc. offers two primary products to address these PAT applications: The DUV Raman PL 200 and the TraC-X instruments. These are the instrument featured in the discussion and results above.

The DUV Raman PL 200 is a fully integrated deep UV laser-based Raman and autofluorescence instrument which includes a 248.6 nm laser and laser power supply, a 200 mm focal length Czerny-Turner spectrograph with two computer controlled gratings and a thermoelectrically cooled, high quantum efficiency, 2048 pixel CCD array camera, an imbedded microprocessor and communication system to provide for built-in test and automated spectral calibration for both Raman and fluorescence, as well as data storage. The DUV Raman PL 200 system provides a Raman spectral range from about 300 cm-1 to 4000 cm-1 with nominal resolution below 15 cm-1 and as low as 8 cm-1, depending on grating and slit width selection, and a fluorescence spectral range from 250 nm up to 650 nm, depending of grating selection up to about resolution of 1 nm or less, depending on the grating selection.

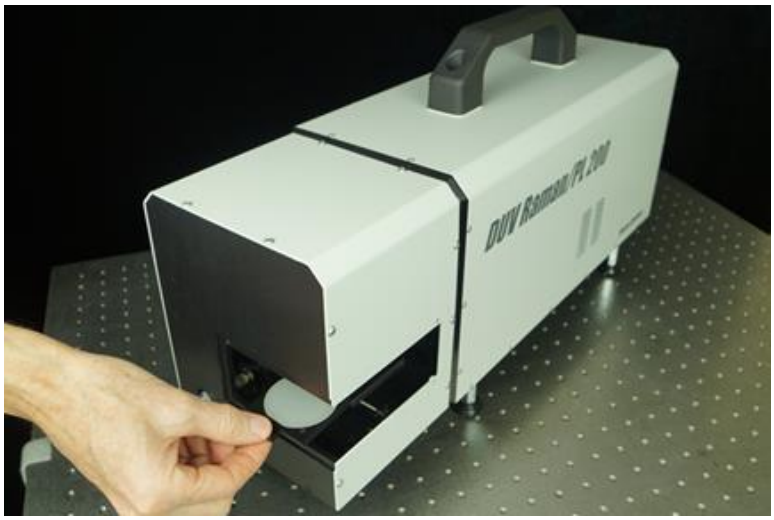


Figure 11. DUV Raman PL 200 instrument

There are a wide range of sample interface options, including the one shown in Fig. 11, which has a computer controlled XY stage for mapping the Raman or fluorescence spectra over a surface up to about 50 x 50 mm. Or there are fluid flow cell interface options. The size of the DUV Raman PL 200 is 7" wide by 8" high by

24” deep for the configuration shown. Without the computerized XY stage front end, the instrument is only 16” deep. Weight is less than 25 lbs, and is powered by 85 to 270 VAC or 24 VDC, with maximum power draw of 150 W.

The TraC-X instrument is a fully integrated deep UV autofluorescence instrument which provides detection in 4 deep UV spectral bands in the spectral range from 280 nm to 450 nm. The TraC-X has an embedded microprocessor for sensor control, data processing and storage, and display. It can take and record time-stamped full spectrum data at rates up to about 10 Hz. It has high sensitivity, with limits of detection in the ng/cm2 range, depending on the analyte.

The TraC-X is an intrinsically safe sensor with dimensions of 3” by 3.5” x 7.5” and weight less than 2 lbs, including battery for over 40 hours of continuous use. It has a working distance of 2 to 4 cm.

Figure 12. TraC-X instrument

7. SUMMARY

Deep UV Raman and fluorescence methods with excitation below 250 nm have been shown in many publications and over many years, beginning with the work of Prof. Sanford Asher at U. Pittsburgh and Prof. Wilford Nelson at U. Rhode Island, beginning in the 1970s, to provide detection capabilities unsurpassed by near UV, visible, and IR methods. The impediment to widespread use has been the size, weight, and cost of prior deep UV systems. This paper illustrates the benefits of deep UV methods for just a few examples in pharmaceutical continuous manufacturing and wastewater treatment. There are many more examples in ultra-sensitive detection of trace contamination on surfaces or in water or other liquids for a broad range of applications including chemical, biological, and semiconductor manufacturing, environmental testing including wastewater, first responder situational awareness, etc., using simple, low cost instruments such as those offered here.

8. ACKNOWLEDGEMENTS

We acknowledge the efforts of the entire staff at Photon Systems, Inc, and the assistance and encouragement of several government agencies via various methods of support, but especially from NASA and DoD.