Application Note

Biopharma/Pharma

Agilent

Trusted Answers

Differentiating Biopharmaceutical Raw Materials Using Spatially Offset Raman Spectroscopy

Measuring raw materials without sampling—directly through their containers

Authors

Frédéric Prullière
Christopher Welsby
Agilent Technologies, Inc.

Introduction

Ensuring a continuous supply of compliant biopharma raw materials is an industry challenge. Growing global demand led to a major tragedy in the pharmaceutical/biopharmaceutical industry. In 2008, a scarcity of suitable pigs due to a swine flu outbreak, combined with a 100% increase in heparin crude price, led to the contamination of heparin by oversulfated chondroitin sulfate, which made its way through the supply chain (*1*). In the aftermath of this scandal, regulatory agencies worldwide strongly encouraged biopharmaceutical manufacturers to improve existing controls, and, implement process analytical technology (PAT) based solutions for better control of bioprocesses. Among these controls, screening or identity testing of raw materials at reception is an effective first line of defense to prevent the introduction of non-compliant raw materials. However, current identification test protocols, often based on Fourier transform infrared (FTIR) or Raman spectroscopy, are ill-suited to meet the need for quality and speed, as demanded by biopharmaceutical production.

Biopharma upstream processes often operate continuously. Medium preparation tanks and reactors can consume 800 kg to 1600 kg of growth media, carbohydrates, buffers and amino acids in a single cycle. This creates a demand for raw materials that can overwhelm warehouse supply processes. Opening containers of raw materials to take samples for identification and quality testing purposes is time consuming and reduces the ability of the warehouse to meet production demands.

This study aimed to demonstrate the use of a handheld Agilent Vaya Raman instrument to correctly differentiate raw materials from one another. The Vaya can measure materials through a container. This capability offers significant time savings for the reception and release of raw materials into biopharma upstream processes.

Agilent Vaya Raman system for identification testing

Figure 1. The Agilent Vaya Raman handheld spectrometer being used to measure materials through a paper sack in a quarantine warehouse.

The Agilent Vaya Raman handheld spectrometer (Figure 1) speeds up the identity verification of biopharmaceutical raw materials for biologics. It enables non spectroscopists to perform an identification test through transparent and non-transparent containers anywhere where identification of contents is required. Using Spatially Offset Raman Spectroscopy (SORS), a Vaya instrument performs an ID test in a matter of seconds and delivers a PASS/FAIL answer. A single operator can easily receive and release raw materials to Production in a matter of hours. A sampling booth, container opening, PPE, and logistical movements to and from the sampling booth are no longer required.

Spatially offset Raman spectroscopy

Raman spectroscopy is based on the excitation of the analyte to a virtual electronic state by a monochromatic light and the subsequent probing of vibration modes of the covalent bonds. The subsequent de-excitation typically shifts the monochromatic light (i.e. LASER) up or down to yield a Raman spectrum with bands (shifts) specific to substructures in the molecule.

In conventional Raman spectroscopy (Figure 2), analysis through transparent containers is made possible by optically positioning the laser focal spot and maximizing Raman signal, at an offset to the spectrometer aperture, to mostly excite the content inside the container rather than the container itself.

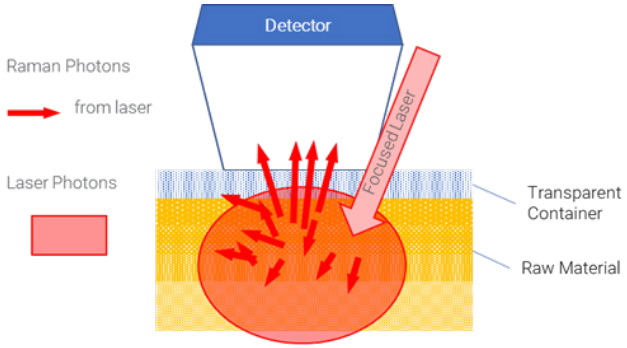

Figure 2. Conventional Raman spectroscopy configuration.

SORS differs from conventional Raman spectroscopy. It combines the property of light propagation through diffusely scattering materials and Raman spectroscopy to achieve true through-container analysis.

Unlike conventional Raman spectroscopy, where the Raman signal is collected from the laser illumination zone, SORS uses near point illumination and collection areas that are mutually displaced by a physical offset (Figure 3).

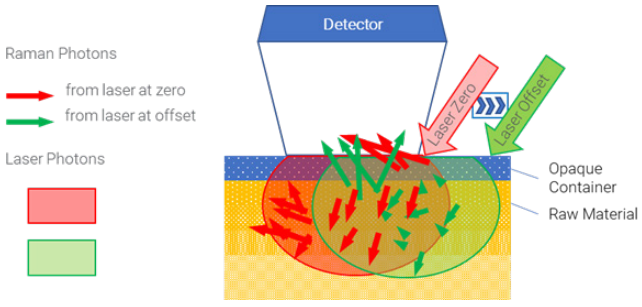

Figure 3. SORS spectroscopy configuration.

In this offset configuration, with the propagation of the laser inside the analyte, the Raman photons collected in the detection area originate mostly from beneath the sample surface. This configuration yields a spectrum rich in subsurface "information". In contrast, a spectrum generated with no or "zero" physical offset yields a spectrum rich in the top layer "information". To identify a raw material through a container, the container-rich "zero offset" spectrum can be subtracted from the raw material-rich "offset" spectrum. The resultant spectrum corresponds to a container-free raw material spectrum that can be used for ID verification purposes.

Unlike conventional Raman spectroscopy, SORS can reliably perform identification tests through a variety of transparent and opaque containers. It can successfully identify materials inside amber bottles, FIBCs, multi-layer paper sacks, colored and transparent plastic liners, and opaque colored polyethylene containers.

SORS based spectrometers are particularly effective at identifying materials with weak to low Raman cross section through transparent containers. SORS spectrometers use very sensitive CCD detectors to capture the much attenuated offset signal and therefore the weak signal from a low scatterer in a zero configuration.

Experimental

To demonstrate the ability of a Vaya instrument to differentiate biopharmaceutical raw materials from one another, a number of common raw materials were purchased. All of the samples were reagent grade from Sigma Aldrich and were supplied in transparent and non-transparent containers. The samples were measured as received. The samples represented five classes of biological raw materials: amino acids, biological buffer, cell culture media, surfactants and inorganic salts. The samples were:

- **Biological buffers:** HEPES a Good's Buffer or 2-[4-(2-hydroxyethyl) piperazin-1-yl] ethane sulfonic acid, CHES or 2-(Cyclohexylamino) ethane sulfonic acid, TRIS or 2-Amino-2-(hydroxymethyl) propane-1,3-diol. All three buffers were received in white polyethylene containers.
- **Surfactants:** Triton X100[l] or 2-[4-(2,4,4-trimethylpentan-2-yl) phenoxy] ethanol, PEG or Polyethylene Glycol and Polysorbate 80 (PS 80). Triton X100 and PEG were received in white polyethylene containers. Polysorbate 80 was received in an amber bottle.
- **Amino acids:** L-Alanine, L-Phenylalanine, Glycine. All three amino acids were received in white polyethylene containers.
- **Cell culture media:** HAM's F10 and RPMI-1640. Due to small quantity, cell culture media were transferred into glass vial and small translucent PE container (no color).
- **Inorganic salts:** Potassium Dihydrogen Phosphate, Dipotassium Hydrogen Phosphate, Calcium Phosphate (TCP). All three salts were received in white polyethylene containers.

For each class of raw material, the Raman spectrum of each material was acquired directly through the container using the Agilent Vaya Raman. The spectra of each material in a class were then overlaid for differentiation purposes. The measurements of the five classes of materials were performed on the containers as received from the manufacturer (unless noted otherwise), as illustrated in Figure 4.

Figure 4. Analysis of raw materials through a white polyethylene container by the Vaya instrument and examples of the containers the raw materials (biological buffers) were received in.

The analytical conditions used to acquire each scan were automatically set by the instrument with no intervention from the operator. All of the samples were run at room temperature and under ambient light (Sun LED lights). Each scan took no longer than 35 seconds. A performance qualification test was performed prior to the acquisition of the Raman and SORS spectra.

Results & discussion

Raman spectra for HEPES, CHES, and TRIS buffers are shown in Figure 5. All three spectra show distinct and specific Raman bands which can be used for the identification and speciation of these buffers prior to release for use into upstream processes. In particular, HEPES and CHES can easily be differentiated using the alicyclic bands in the 600 to 1300 cm^{-1} region despite a partially shared structural backbone.

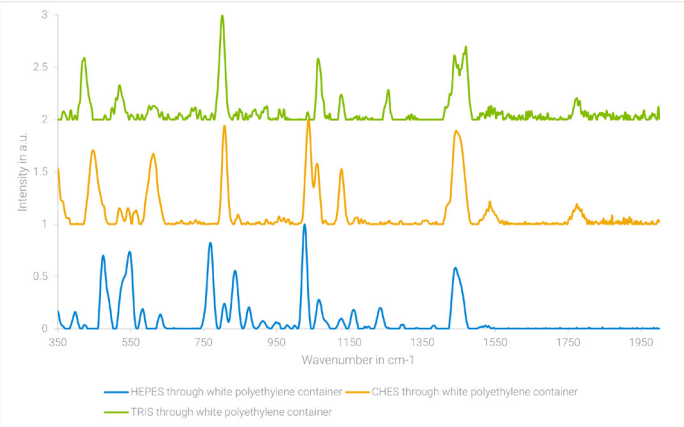

Figure 5. The overlaid Raman spectra of biological buffers, obtained directly through each white polyethylene container.

Raman spectra for Triton X100, PEG and PS 80 surfactants are shown in Figure 6. The Triton X100, PEG, and PS 80 can easily be differentiated from each other. The band at ~1650 cm^{-1} in the PS 80 Raman spectrum is characteristic of the presence of the monooleate group, and, is in large part responsible for the differentiation with the two other surfactants. The band at 1615 cm^{-1} in the Triton X100 spectrum is indicative of the presence of an aromatic ring and contributes to the differentiation of the three surfactants.

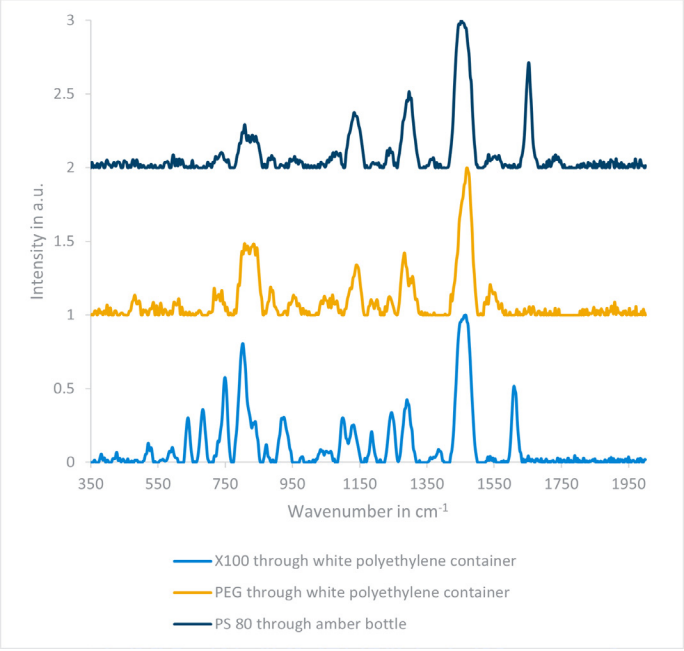

Figure 6. Overlaid Raman spectra of the surfactants, obtained directly through each container.

Figure 7 shows the overlay of L-Alanine, L-Phenylalanine and Glycine amino acids. These three amino acids can easily be distinguished by their specific markers (2). For Alanine, the presence of a strong band at 852 cm^{-1} is a strong differentiator compared to other aliphatic amino-acids. For Glycine, the presence of two bands at 894 cm^{-1}(Cα–C; OCO; COO−symmetric stretch) and 1327 cm^{-1} (N–Cα–H; NH_3-asymetric rock;NH_3-asymetric rock; N–Cα–Hα) enables this speciation. For Phenylalanine, the band 1005 cm^{-1} (δ ring) for ring breathing enables an easy speciation versus other aromatic amino acids and simpler aliphatic amino-acids.

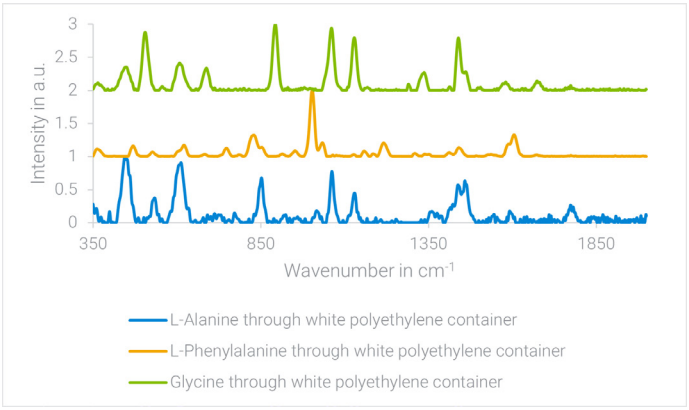

Figure 7. Overlaid Raman spectra of the amino acids, obtained directly through each container.

Figure 8. Overlaid Raman spectra of the cell culture media, obtained directly through each container.

Figure 8 shows the spectral overlay for RPMI-1640 and Ham's F10 cell culture media. SORS spectroscopy, either through clear vial or through a translucent polyethylene container, can differentiate these two dry powder media (DPM). DPM are mostly made of inorganic Raman inactive ionic salts like sodium chloride and Raman active materials like sugar and buffers (ionic and organic). Low mass percent of Raman active materials like amino-acids, vitamins, and inorganic salts complete these DPM. The acquired Raman spectra are therefore partially reflective of the DPM composition and differentiation is mostly based on the high mass percent Raman active and/or High Raman scattering cross section (σ) components. Although, SORS cannot be used as a mean to accurately check the composition, it can be a rapid method to differentiate incoming shipments for DPM based on high mass% or high cross section components of the DPM.

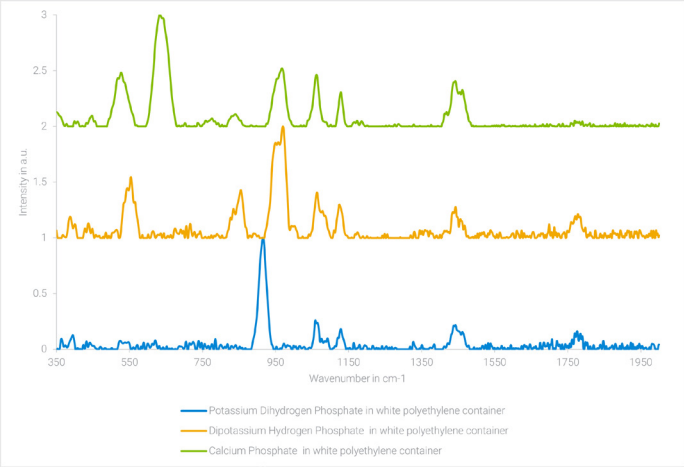

Figure 9. Overlaid Raman spectra of the inorganic salts, obtained directly through each container.

Figure 9 shows the spectral overlay for commonly used inorganic salts. SORS spectroscopy can differentiate all three phosphate derivatives regardless of the protonation level of the "phosphate" core and the counterion.

Conclusion

SORS spectroscopy, provided by the handheld Agilent Vaya Raman instrument, was successfully able to differentiate biopharmaceutical raw materials, without opening their containers.

Sufficiently good quality spectra of each raw material, through the container, was obtained in less than 35 seconds. The spectra could be used to differentiate the raw material from another in the same material class.

Even materials inside translucent containers, such as the dry powder media in this study, could be measured. These results demonstrate the effectiveness of SORS for container subtraction for a wide variety of materials used in upstream processes.

With fast analysis times and the ability to measure through transparent and opaque containers, the Agilent Vaya is ideally suited for use with large volumes of biopharmaceutical raw materials. The raw materials can be identified in quarantine immediately after receipt. The Vaya Raman instrument reduces or eliminates many of the required steps when using FTIR or conventional Raman spectroscopy—no sampling booth cleaning, no transferring containers from and to the quarantine area for sampling/analysis etc. In addition, it preserves raw material sterility and reduces associated spoilage costs.

References

1. Chess EK, Bairstow S, Donovan S, Havel K, Hu P, Johnson RJ, Lee S, McKee J, Miller R, Moore E, Nordhaus M, Ray J, Szabo C, Wielgos T. Case study: contamination of heparin with oversulfated chondroitin sulfate. Handb Exp Pharmacol. **2012**;(207):99-125. doi: 10.1007/978-3-642-23056-1_6. PMID: 22566223.
2. Raman Spectra of Amino Acids and their aqueous solutions. *Spectrochimica Acta Part A* 78, **2011**, 1187-1195.

www.agilent.com/chem

DE44321.2635532407

Printed in the USA, June 2, 2021
5994-3534EN

Agilent
Trusted Answers