

Rapid Identification of Polysorbates 20 and 80 Directly Through Amber Bottles

Preserve the shelf-life of polysorbates using the Agilent Vaya handheld Raman spectrometer with SORS

Authors

Frédéric Prullière
Christopher Welsby
Agilent Technologies, Inc.

Introduction

Polysorbate 20 (PS 20) (Tween 20) and Polysorbate 80 (PS 80) are inactive ingredients that are often used in the formulation of biopharmaceuticals to help protect the active ingredient. Around 80% of commercial monoclonal antibody formulations contain polysorbates.

Polysorbates are susceptible to degradation through hydrolysis or auto-oxidation, so they require specific packaging and packaging conditions. To preserve product composition and shelf-life, PS 20 and PS 80 are packaged under an inert atmosphere and are typically stored in small to medium-sized amber glass bottles.

In accordance with ICH requirements on raw materials (1), bio-pharmaceutical and pharmaceutical manufacturers must identify polysorbates before use. Identification testing by FTIR or wet chemistry involves sampling from each bottle, which can impact the sterility of the sample, causing it to degrade.

Raman spectroscopy provides an alternative means of verifying the identification of raw materials, directly, without opening the sample bottle. However, many commercially available Raman spectrometers cannot collect reasonable quality spectra of polysorbate through amber glass bottles.

In this study, an Agilent Vaya Raman spectrometer with Spatially Offset Raman Spectroscopy (SORS) technology was used for the identification and differentiation of PS 20 and PS 80 through amber glass.

Non-invasive identification testing of raw materials



Figure 1. The Agilent Vaya Raman handheld spectrometer with SORS being used to identify materials through an amber bottle in a warehouse.

As shown in Figure 1, the Vaya Raman handheld spectrometer can be used to verify raw materials before they are used in biopharmaceutical and pharmaceutical formulations. The instrument performs a qualitative test directly through transparent and opaque containers at the point of need, within a few seconds. The non-invasive test method means that a single operator can easily receive, test, and release large batches of raw materials in a matter of hours. Without the need for a separate sampling booth, container opening, sample handling, and protective clothing, the Vaya instrument is faster, more efficient, and cost effective compared to using other techniques.

Spatially offset Raman spectroscopy

SORS uses light propagation through diffusely scattering materials theory, in combination with Raman spectroscopy to achieve through opaque container analysis.

SORS introduces a physical (spatial) offset between the region of the sample being excited by the laser light source and the region of the sample the detector is collecting information from. When analyzing a material through a container in the offset configuration, the Raman photons originate mostly from beneath the sample surface, providing a spectrum rich in “information” about the subsurface—i.e. the raw material. In contrast, the spectrum with no or “zero” physical offset yields a spectrum that is rich in the top layer “information” i.e. the container. The scaled subtraction of the container-rich “zero- offset ” spectrum from the raw material-rich “offset” spectrum provides a container-free raw material spectrum that can be used for identification verification purposes.

Unlike conventional Raman back-scattering spectroscopy, SORS can reliably perform identification tests through a variety of transparent and opaque containers. Examples include amber glass bottles, multilayer paper sacks, colored and transparent plastic liners, and opaque plastic containers made from polyethylene (PE), polyethylene terephthalate (PET), polypropylene (PP), or polycarbonate (PC).

Agilent SORS-based spectrometers are effective at conducting measurements through light filtering containers such as amber glass bottles for two main reasons:

- Sensitive CCD detectors capture the weaker (attenuated) offset signal and, therefore, the light that the amber glass lets through.
- An algorithm completely subtracts the zero-offset (container) signal including the container fluorescence, eliminating any fluorescence interference from the analyte signal.

Experimental

To demonstrate the identification (ID) of PS 20 versus PS 80 using the Vaya spectrometer, two ID verification methods were developed for PS 20 and 80 respectively. Each of the methods was generated using JT Baker branded PS, National Formulary (NF), multi-compendial grade products from Avantor Sciences (Radnor, PA, USA).

The methods developed on the Vaya used the built-in method development wizard and standard settings (specific glass containers) to generate the spectral data discussed in this application note. No additional data processing was performed beyond the automated baseline correction that is part of the normal analysis protocol for the Vaya system. Apart from information on the container type, which is provided by the operator, all the other acquisition parameters were set automatically by the Vaya system. A performance qualification test was performed before the acquisition of the SORS spectra.

Each of the developed methods was challenged 10 times for specificity using both PS samples. JT Baker bottles were selected because their darker color makes the analysis more challenging and they have been shown to display significant variability in their opacity, color, and thickness .

The same PS 20 and 80 samples were also analyzed using a conventional back-scattering Raman spectrometer fitted with a 785 nm laser. The acquisition parameters used to acquire the spectra were automatically set by the instrument and no additional data processing was performed on the spectra.

All measurements were performed in normal ambient light conditions (Sun LED lights) and at room temperature.

Results and discussion

Raman spectra for PS 20 and 80 acquired through JT Baker amber bottles using a conventional Raman spectrometer and a Vaya Raman spectrometer with SORS are shown in Figure 2. There is a clear difference between the spectra acquired by the two instruments. The Raman bands of the PS 20 and 80 samples are evident with the SORS instrument, showing that these two materials can be distinguished spectroscopically. The band at $\sim 1650\text{ cm}^{-1}$, which is characteristic of the monooleate group in PS 80, is in large part responsible for the differentiation of the two materials.

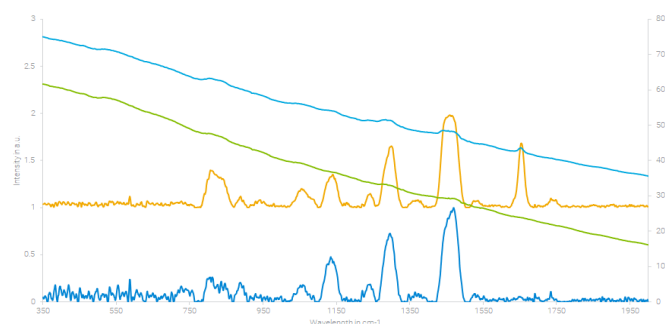


Figure 2. Raman spectra through amber glass of PS 20 and 80 using conventional handheld Raman and Vaya SORS spectrometers. The dark blue line is polysorbate 20 acquired with Vaya Raman, the green line is polysorbate 20 acquired with conventional Raman (785 nm laser), the orange line is polysorbate 80 acquired with Vaya Raman and the light blue line is polysorbate 80 acquired with conventional Raman (785 nm laser).

The PS spectra acquired with the conventional back-scattering Raman spectrometer using a laser at 785 nm display very weak Raman bands due to the intense fluorescence and light blocking from the amber glass bottle. The characteristic peak at 1650 cm^{-1} is no longer clearly distinguishable. Vaya mitigates the fluorescence of the container using a NIR wavelength laser (830 nm) and by subtracting the container contribution (zero position spectrum) using SORS.

Spectroscopy challenge matrices

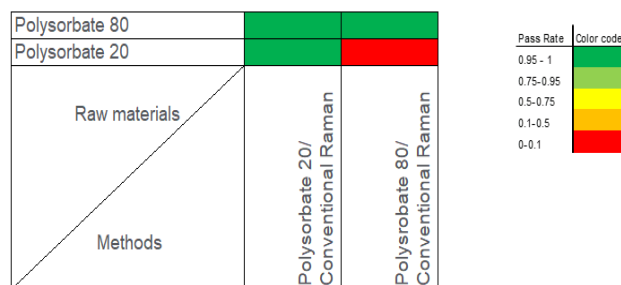


Figure 3. Identification of PS using a conventional Raman spectroscopy challenge matrix.

A challenge matrix graphically represents how an ID test differentiates and correctly verifies the identity of closely related structures. In a challenge matrix, an ID test is conducted for each of the analytes using the ID verification method developed for each analyte. An ideal challenge matrix will have a “pass rate” above 0.95 along the matrix diagonal, indicating that the method recognizes its corresponding material perfectly. Off the diagonal, the ideal matrix should display only pass score rates below 0.1 to indicate that the method correctly rejects incorrect analytes. An ideal matrix demonstrates that a group of methods has a low level of false positives, so that it can be used reliably in a warehouse environment. The PS challenge matrix for PS 20 and 80 that were analyzed using the conventional Raman spectrometer shows poor selectivity (Figure 3). The PS 20 model failed to differentiate between PS 20 and PS 80, while PS 80 passed against both PS 80 and PS 20.

In contrast, the PS methods developed on the Vaya SORS spectrometer showed excellent selectivity, as shown in Figure 4. The challenge matrix shows that PS 20 can easily be differentiated from PS 80.

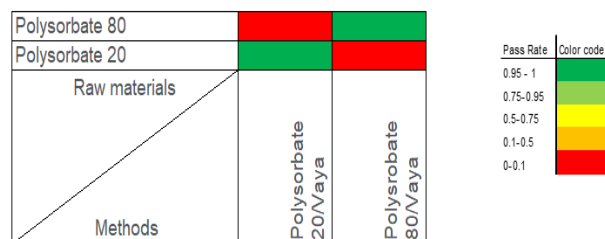


Figure 4. Identification of PS using a Vaya SORS challenge matrix.

Conclusion

The Agilent Vaya Raman spectrometer with SORS was used for the selective identification of PS 20 and 80 through amber glass bottles without the need to open the bottle for sampling.

Rapidly identifying raw materials through bottles and/or opaque containers is critical to an efficient, low-cost, and streamlined raw material receipt process in biopharmaceutical and pharmaceutical environments. It also promotes a safer environment for staff, as exposure to hazardous materials is reduced during the receipt process.

The direct testing method eliminates many of the steps required when using FTIR or conventional Raman spectroscopy. There's no need to clean the sampling booth, no transferring containers to and from the quarantine area for sampling or analysis, a reduced risk of spoilage and costs of disposing of reagents. The method also preserves the shelf-life of air sensitive materials.

The advantages of a SORS-based spectrometer can be extended to many other applications where the analyte/raw material has a low Raman scattering cross-section and is housed in a light filtering container.

Reference

1. ICH 7.30 Page 16 Of ICH Q7: ICH Harmonised Tripartite Guideline Good Manufacturing Practice Guide For Active Pharmaceutical Ingredients Q7. Accessed April 2021. <https://database.ich.org/sites/default/files/Q7%20Guideline.pdf>

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