



APPLICATION NOTE AN-SPEC-1054

Aseptic Verification of Polysorbates in Amber Bottles with 1064 nm Raman

Reliable, through-container material identification without opening containers

Identification of polysorbates through amber glass bottles is challenging due to fluorescence interference under traditional Raman wavelengths. This application note demonstrates the effectiveness of 1064 nm handheld Raman spectroscopy for direct, through-container verification of Polysorbate 20 (PS20) and Polysorbate 80 (PS80). Using this approach, high-

quality spectra with minimal fluorescence were obtained, enabling clear differentiation between the two materials. The method provides a rapid, aseptic, and contamination-free solution for raw material identification in pharmaceutical and biopharmaceutical manufacturing environments.

INTRODUCTION

Polysorbate 20 (PS20) and Polysorbate 80 (PS80) are widely used excipients in pharmaceutical and biopharmaceutical manufacturing, most commonly supplied in amber bottles to reduce light driven degradation. While this packaging protects material quality, it presents significant challenges for handheld Raman analysis. Under 785 nm excitation, polysorbates exhibit strong fluorescence, and amber glass contributes additional background signal, often overwhelming the Raman features needed for identification.

Raman excitation at 1064 nm minimizes fluorescence from both the sample and its container, enabling direct through-bottle identification that supports aseptic material handling workflows. The system is fully compliant with the Raman chapters of major pharmacopeias, and together with its records management software, it meets FDA 21 CFR Part 11 requirements with a complete audit trail.

Although PS20 and PS80 share similar chemical structures, PS80 contains a higher proportion of oleate esters that produce a distinguishing Raman

band near $\sim 1650\text{ cm}^{-1}$. Using the NanoRam 1064 and the bottle adapter, clear spectra can be collected directly through sealed amber glass, allowing reliable differentiation and streamlined PASS/FAIL verification.

COMPARISON MEASUREMENT: 785 NM LASER

A reference spectrum of PS80 collected with a 785 nm handheld Raman system shows an intense fluorescence background that obscures multiple Raman features and reduces the interpretability of the spectrum. This response is typical of polysorbates and similar excipients under shorter wavelength excitation and is further intensified by the amber bottle.

In contrast, the NanoRam1064 produces spectra with minimal fluorescence and well-defined Raman structure, even when measurements are taken directly through amber glass. This comparison clearly highlights the practical limitations of 785 nm Raman for polysorbate analysis and underscores the value of 1064 nm excitation for reliable through-container verification

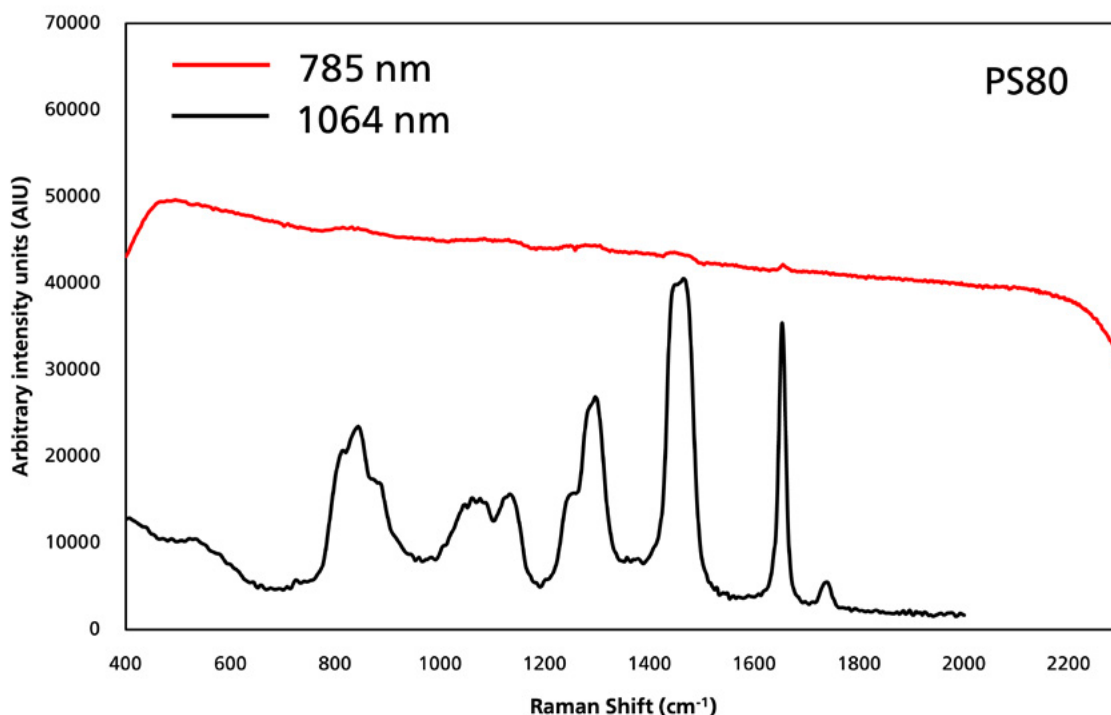


Figure 1. PS80 measured at 785 nm versus 1064 nm, illustrating reduced fluorescence at 1064 nm. Spectra are offset for visibility.

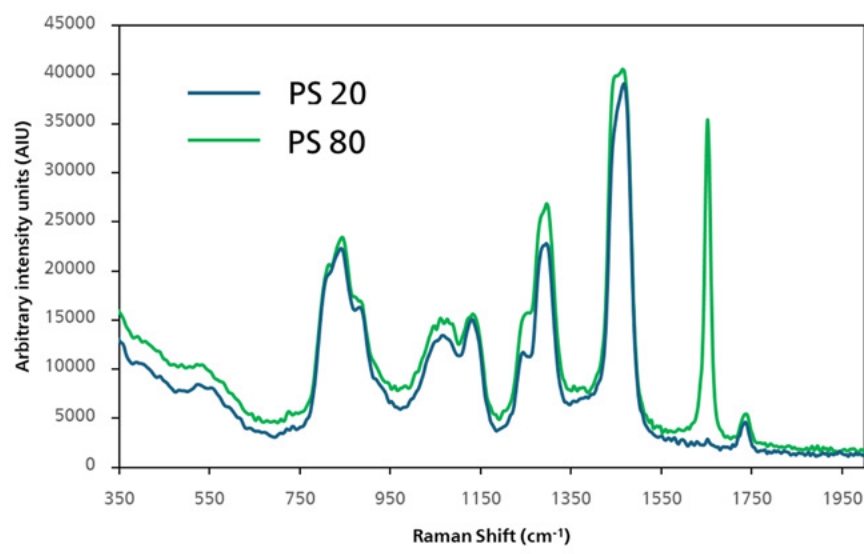


Figure 2. Overlay of PS20 and PS80 spectra measured through amber glass, highlighting the distinct PS80 feature near $\sim 1650\text{ cm}^{-1}$.

SPECTRAL ANALYSIS THROUGH AMBER GLASS

Spectra of PS20 and PS80 were collected directly through their amber bottles using the bottle adapter. Figure 2 shows the spectral overlay. Despite absorption and attenuation from the container, the NanoRam1064 produced well-resolved spectra with minimal fluorescence, consistent with the improved clarity expected from 1064 nm excitation for materials prone to fluorescence interference. A clear distinguishing feature appears near approximately 1650 cm^{-1} , where PS80 exhibits a band associated with oleate ester groups. This peak provides a reliable basis for differentiating PS80 from PS20 in both visual assessment and algorithmic analysis.

The NanoRam1064 was used to develop PS20 and PS80 identification methods directly on the device, including all model building and cross correlation steps. For material discrimination, the NanoRam 1064 applies a patented reduced variable multivariate (RVM) analysis rather than traditional PCA (Principle Component Analysis). This method focuses only on the most informative regions of the spectrum, (1) improving robustness for closely related and fluorescent materials such as polysorbates, and (2) reducing the number of spectra required to produce a multivariate model. More details about the RVM analysis method can be found in the application note “Reduced Variable Multivariate Analysis for Material Identification with the NanoRam-1064”.

Using these on-device tools, simple PASS/FAIL methods were created for PS20 and PS80. Each accepted only its own material and rejected the other, and a correlation matrix further supported the selectivity. (Table 1) The diagonal values (material vs. itself) show strong reproducibility, while off-diagonal values remain distinctly lower, confirming statistically meaningful separation. Together, these results show that the NanoRam1064 provides an efficient, through-container solution for verifying polysorbates in sealed amber bottles. A brief explanation of the algorithm and its advantages can be found in the NanoRam-1064 Method Development Technical Note.

Table 1.

Sample \ Method	Polysorbate 20	Polysorbate 80
	Polysorbate 20	Polysorbate 80
Polysorbate 20	PASS	FAIL
Polysorbate 80	FAIL	PASS

CONCLUSION

This study demonstrates that the NanoRam1064 enables reliable, aseptic identification of Polysorbate 20 and Polysorbate 80 directly through amber bottles using the bottle adapter. The 1064 nm excitation produced high quality, low fluorescence spectra that supported the development of robust RVM identification methods requiring only a limited number of training spectra. Clear spectral differences, including the PS80 band near $\sim 1650\text{ cm}^{-1}$, allowed each method to distinguish its target material with strong specificity, and correlation results provided additional confirmation of selectivity. Together, these findings show that the NanoRam1064 offers an efficient and contamination free approach for raw material verification of polysorbates in pharmaceutical and biopharmaceutical environments.