DEMONSTRATION OF FLUORESCENCE BACKGROUND REMOVAL IN THE PRESENCE OF FD&C BLUE NO. 1

APPLICATION NOTE RAMAN-011 (US)

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Abstract

The topic of this brief application note is the correction of fluorescent background during the application of Raman spectroscopy to personal care products, some of which are colored with dyes similar to those used in scientific endeavors to stain microbial samples and add fluorophores to antibody analyses, such as flow cytometry.

In this brief application note, TSI describes Raman measurements of commercially available fluorescent solutions with both 532 and 785 nm laser excitation. Predictably, the green laser system experienced more issues with fluorescence in the samples than did the 785 nm system. The background removal system in the Raman Reader program was able to deal well with both, even though because of the large amount of fluorescence generated with the 532 nm laser constrained the acquisition times to very short ones, and the associated Raman intensities were lower than at 785 nm.

Samples

Samples were acquired from a local drugstore and included Colgate® Minions® Bello Bubble Fruit® Mouthwash, a bright blue aqueous solution. The active ingredient is listed as sodium fluoride 0.02% (0.01% w/v fluoride ion). This is an anti-cavity agent. The inactive ingredients (concentrations not listed, but presumably in order of decreasing concentration: water, glycerin, propylene glycol, sorbitol, poloxamer 338, poloxamer 407, flavor, PEG-40 castor oil, phosphoric acid, sodium saccharin, disodium phosphate, potassium sorbate, cetylpyridinium chloride, sucralose, FD&C blue no. 1.

Measurements and Results

Two TSI ChemLogix Raman instruments were used to acquire spectra on a variety of personal care products. An EZRaman-I with a spectral range from 100–4000 cm^{-1} was used to acquire spectra of a variety of personal care products, including the spectrum of the mouthwash shown below. The 532 nm laser in this system was run at 50 mW because of the presence of high fluorescence signal that rose smoothly from 100 cm^{-1} to 25,000 counts at 3500 cm^{-1}. An acquisition time of 0.5 s (and 20 averages) were used to obtain this data. The samples were presented to the system in a vial housed in the vial/cuvette sampling accessory. Raw and baseline corrected spectra are shown in Figure 1. The inset in this Figure is a detailed view of the corrected fingerprint region, showing low intensity signals, but the spectrum is clear enough for assignment, even after having been corrected from 25,000 counts of fluorescence.

A TSI ChemLogix ProRaman-L was also used to make this measurement. This instrument used a 785 nm laser for excitation and had a spectral range of 100–3300 cm^{-1}. TSI exposed the sample to about 150 mW of laser power in this case, because there was much less of an issue with fluorescence at the longer excitation wavelength. Acquisition times of 15 s and a 5x average were employed. The sample was presented in a quartz cuvette using the vial/cuvette holder accessory.

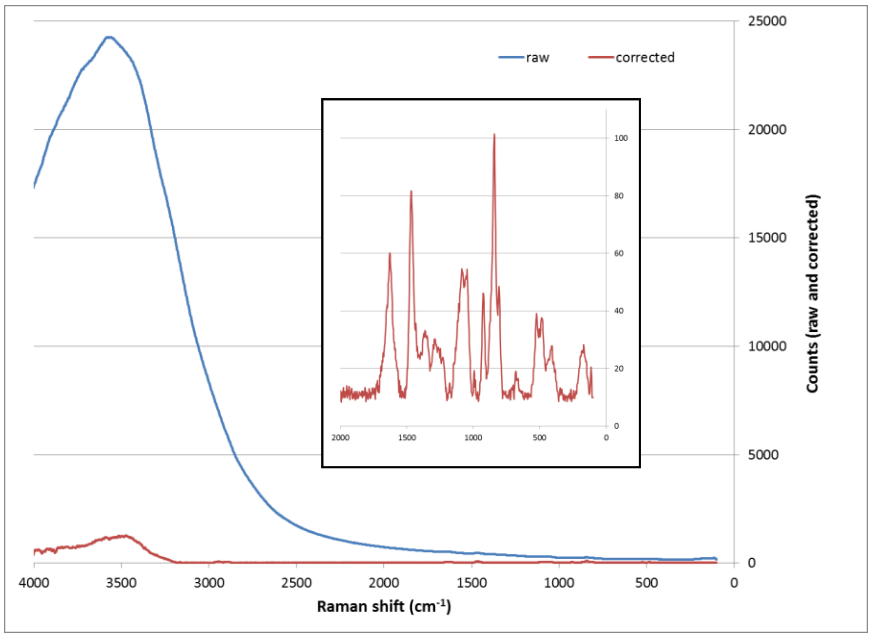

Figure 1. Raman spectra of mouthwash containing FD&C Blue no. 1, excited with 532 nm laser. Inset is background corrected fingerprint section.

Figure 2 shows that the raw spectrum excited with 785 nm exhibits a much less severe fluorescence background than does the one excited with 532 nm. Because of that, TSI was able to employ longer acquisition periods with more laser power, and therefore the Raman signals were higher, in comparison to the background. Background correction still yielded better results than the raw data, but in this case, the background correction perhaps was not absolutely required to obtain a spectrum clear enough to assign.

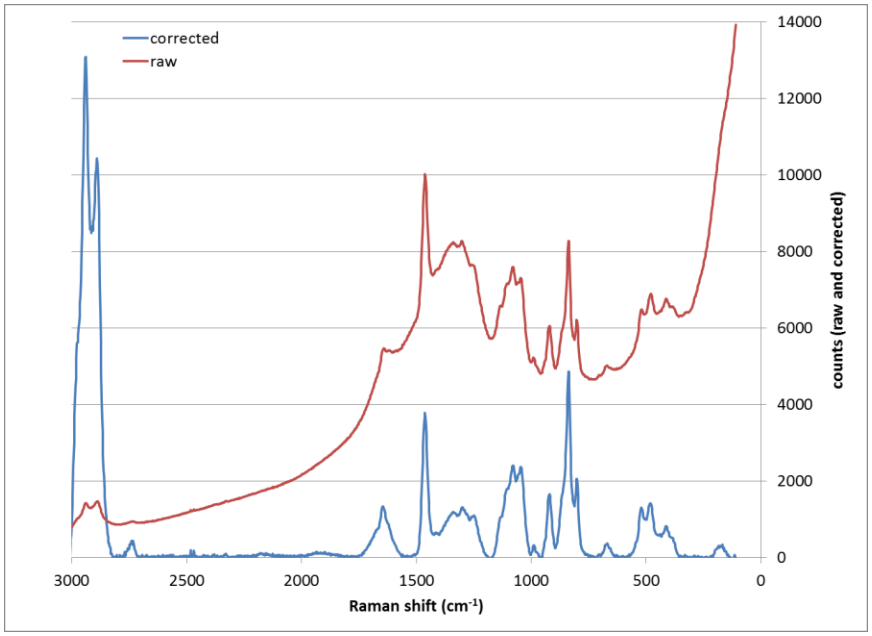

Figure 2. Raw and background corrected spectra of mouthwash containing FD&C Blue no. 1, excited with 785 nm laser.

Brilliant blue has an absorption maximum near 600 nm, but is still absorptive to exhibits a much less severe x and clearly produces a fluorescent background when excited by either wavelength, even though there is likely to be only some tens-hundreds of PPM present in the mouthwash. This background is seen in both of the raw spectra of the mouthwash.

These fluorescent backgrounds were in both cases readily removed with the Raman Reader software. The resulting spectra is as clean as literature examples of one of the primary components. This is only one example of ways in which the TSI ChemLogix Raman products remove broad-band backgrounds to reveal useful Raman spectra.

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