Obtaining the Best Signal-to-Noise Ratio

Introduction

HORIBA Scientific uses the water-Raman peak at 397 nm as a standard for determining *S*/*N* on our spectro-fluorometers. Thus the importance of clean water on the water-raman signal-to-noise ratio (*S*/*N*)—and all fluorescence spectra in general—cannot be underestimated. Water purity is often measured via total organic carbon (TOC) levels. TOC describes the overall amount of organic contaminants in water. Water may contain a variety of organic compounds and materials originating from natural and artificial sources. Decomposition of leaves creates humic acids and tannins; bacteria and their products are organic, and man-made sources may include polymers, pharmaceutical compounds, and plasticizers. This Technical Note demonstrates why highly purified water is crucial to obtaining the best fluorescence spectra using your instrument. A comparison is made between tap water and laboratory-purified water.

Method

Local tap water (Edison, NJ) was the sample. A modular Fluorolog®-3-22 spectrofluorometer (Fig. 1), incorporating double-grating mono-chromators in excitation and emission positions, and a thermoelectrically cooled R928P photomultiplier tube PMT), recorded the spectra. Excitation light in all cases (λ = 350 nm) was pro-vided by the standard 450-W ozone-free Xe lamp. TOC levels in tap water were ~

Fig. 1. *Fluorolog® modular spectrofluorometer.*

1 part per million. Tap water was compared with samples from a PURELAB® Ultra water purifier taken from the 25-L reservoir after the reverse-osmosis unit (Option-S), and from the final polishing unit (Mk2). Fig. 2 compares water-Raman scans using the three samples of water. The black curve

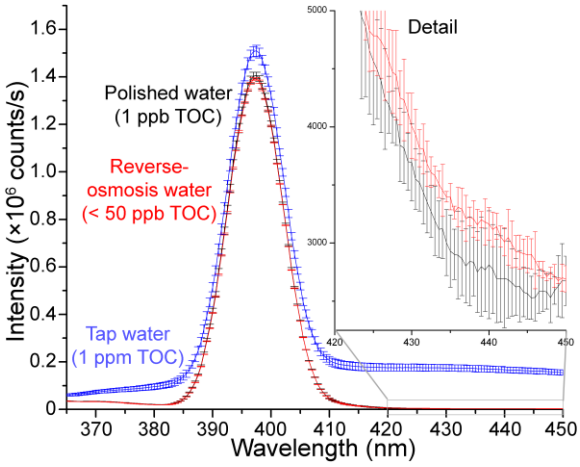

Fig. 2. *Water-Raman peaks using (blue) tap water, (red) reverse-osmosis water, and (black) polished water. The detail expands the scale to reveal the difference between the two purified water samples.*

shows the polished and the red the reverse-osmosis, with the blue indicating the tap water. The reverse-osmosis unit removes the vast majority of the TOC (needed to get to the final < 2 parts per billion) with the polisher.

Raman *S*/*N* values (from Eq. 1 below) are indicated in Fig. 3.

$$\frac{S}{N} = \frac{S_{peak} - S_{background}}{\sqrt{S_{background}}} \qquad \text{Eq. 1}$$

Note that tap water gives a *S*/*N* < 3500:1 but the purified water samples both have *S*/*N* > 25 000:1.

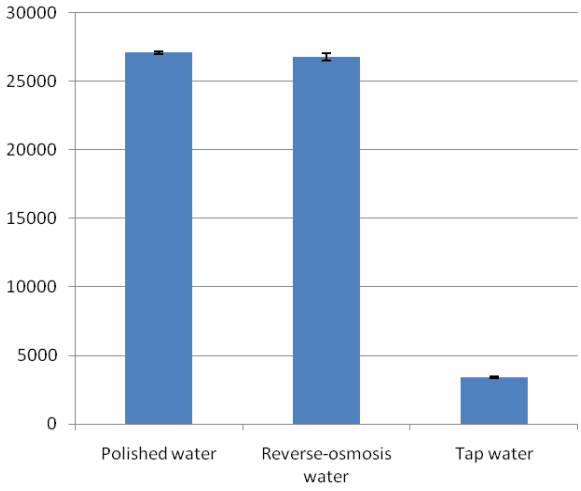

Fig. 3. S/N *for water-Raman scans using various samples. The black error-bars are standard deviations. Precise* S/N *values are: Polished water = 27 100; reverse-osmosis water = 26 800; tap water = 3430.*

In addition, three different Fluorolog®-3 spectrofluorometers with different monochromator configurations were tested, measuring the Raman peak of the polished water. Peak and baseline values are provided in Table 1.

Table 1. S/N *for water-Raman scans using Fluorolog®-3 spectrofluorometers with different monochromator configurations.*

Monochromator types	Raman peak (counts/s)	Baseline, 450 nm (counts/s)	*S*/*N*
Single-grating exc. and em.	1.26×10^6	6291	15 900
Double-grating exc.; single-grating em.	1.19×10^6	4692	17 300
Double grating exc. and em.	1.34×10^6	2378	27 400

Using even single-grating monochromators in excitation and emission positions offers far better *S*/*N* (15 900) than plain tap-water in a spectrofluorometer with double-grating monochromators in excitation and emission positions (3430).

Conclusions

These results demonstrate that removing TOC is extremely important for optimal signal-to-noise ratios for your fluorescence spectra during the lifetime of your instrument.