

## CRITERIA FOR COMPARISON OF SPECTROFLUOROMETER SENSITIVITY

### 1. Introduction

Several methods have been proposed to measure (and compare) the sensitivity of spectrofluorometers. For years the minimum detection limit of quinine sulfate in 0.1N H<sub>2</sub>SO<sub>4</sub> has been the standard method<sup>1</sup>. Figure 1 shows a typical example of a quinine test (from 1977 JASCO FP-550 brochure).

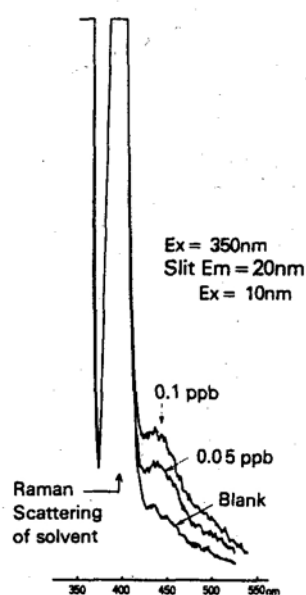


Figure 1. Estimation of the minimum detection limit of quinine sulfate in 0.1N H<sub>2</sub>SO<sub>4</sub>.

This is a good method since quinine is readily available and allows one to estimate sensitivity at very low levels.

The drawbacks to this evaluation method are:

- (a) That accurate sample preparation and storage of such dilute solutions may be difficult
- (b) That the nearby Raman band of the solvent may distort spectral data

Several years ago, most analytical instrument manufactures switched to the signal-to-noise ratio (SNR) of the Raman band of water for sensitivity specifications. One should clearly understand that this approach is completely different from the quinine experiment. In the first case, one estimates the detection limit of a fluorescent component, whereas, in the second case one has to prove how well a Raman band is detected!

There are many advantages to performing the Raman band of water experiment:

- (a) No sample preparation
- (b) The band is relatively weak (comparable to weak fluorescence bands)
- (c) The sample is very stable
- (d) High-purity water is typically available
- (e) It is possible to perform the test over the entire wavelength range

Almost all manufacturers are now specifying sensitivity using the water Raman band at  $\approx 397$  nm, with selected excitation is at 350 nm. Since there is no general consensus on a standard value for the monochromator's bandpass and photometer response time, a method to compare the data from various manufacturers is required. We suggest that the data may be somewhat correlated and compared by consideration of the following:

*Excitation energy is related to Ex bandpass: so passing from 5 to 10nm SBW you feed 4 times ( $2^2$ ) more photons on the sample. The same relationship holds for the emission side, so in passing to 10nm SBW here too you have another factor of 4, or in total  $\approx 4 \times 4 = 16$ . S/N increases with the square root of the light-flux, so by doubling both slit widths you may expect an improvement of  $\approx 4$ . Noise is proportional to the square-root of the response time, so switching from a response time of 2sec to 1sec. the noise level will increase by a factor of  $\approx 1.4$ . Finally one must keep in mind the relationship between the response and time constants: 1 TC  $\approx$  5 RES, so a response of 1 sec is equivalent to a time constant of 0.2 sec.*

## 2. Different approaches to measure the SNR using the Raman band of water

As stated above, 350nm excitation is an accepted standard. Nearly everybody also agrees that one should measure the band intensity not versus zero, but with respect to the average of the background. Unfortunately the ways to measure noise are so varied that it spoils any direct comparison as described below:

- 1) The noise is measured at the maximum Raman signal (either peak to peak or root mean square-RMS)  
The SNR is calculated by dividing the net peak height by the noise measured on a time-base, with intensity expressed as peak to peak ratio or RMS. The RMS value can be calculated using software (like Excel™) or tentatively estimated by dividing the peak to peak value by 5. This test is limited *since noise is part of the signal*, so a higher Raman band will mean higher sensitivity, but also higher noise. The standard method used by JASCO is the more conservative peak to peak ratio.
- 2) The noise is measured at a wavelength far from Raman band (peak to peak or RMS)  
The SNR is obtained dividing the net peak height by either P-P or RMS noise. This is a widely accepted procedure, and tries to emulate a blank measurement of water but does not consider the Raman effect!
- 3) The noise is measured with the emission detector at dark, by closing the emission side shutter, (again P-P or RMS). Any emission wavelength can be used.  
SNR is calculated as before. This is the easiest test to perform, but the values obtained are not representative of what a customer may expect with a normal sample, since the noise is coming only from the PM tube dark current and the photometer noise, not from light emission.
- 4) SNR can also be estimated measuring the noise directly from the water Raman spectra by expanding the scale around the base of the band. In this mode however it is difficult to properly measure the height, since it overlaps the shape of the peak. Time scan measurements of the noise do allow easy computation of its RMS value.
- 5) Another method that defines the SNR is the ratio between net peak intensity and the square root of the estimated background signal at 397 nm or at a wavelength far from Raman band. This method can barely be defined as a SNR calculation. In this method, it's imperative to use very good (fluorescence and bubble free) quality water and a properly cleaned cell, otherwise the slightest bit of contaminant will spoil the experiment. (Some fluorescence will be present at 397nm increasing the background level.) Use of wide slits, the only choice available in some instruments, will further limit this approach since elastic scattering can contribute to the background. In summary, this method has the same drawbacks as the old tests with quinine sulfate.

### 3. Experimental Results

Given the widely varying procedures, we decided to perform comparative tests using methodologies A, B and C. A JASCO spectrofluorometer Model FP-6500 (s/n C036460822) was used. A quartz cell (1cm path) was filled with deionized water purchased at a local store. Auto-zero was performed after the sensitivity selection (i.e. the high voltage applied on PM tube). The following parameters were used to collect the emission spectra:

Band width (Ex): 5 nm  
Band width(Em): 5 nm  
Response: 0.5 sec  
(equivalent to  $\cong 0.1$  sec time constant)  
Sensitivity: High  
Measurement range: 360 - 500 nm  
Data pitch: 0.1nm  
Excitation Wavelength: 350.0 nm  
Scanning speed: 100 nm/min

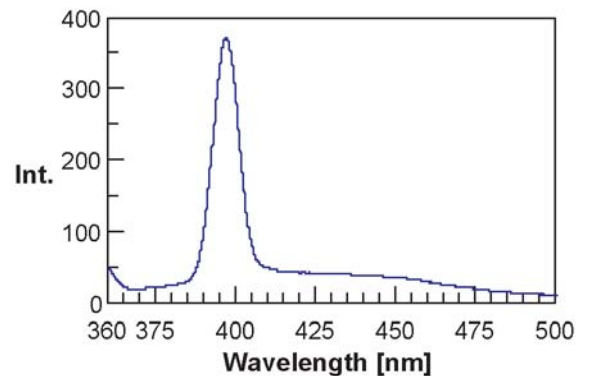


Figure 2. Emission spectrum of water.

In addition to the sharp Raman band at 397 nm one can see the presence of a weak, broad, fluorescence band. This is a very normal phenomenon when the quality of the water is questionable. Using data processing we were able to estimate its shape, shown in Figure 3:

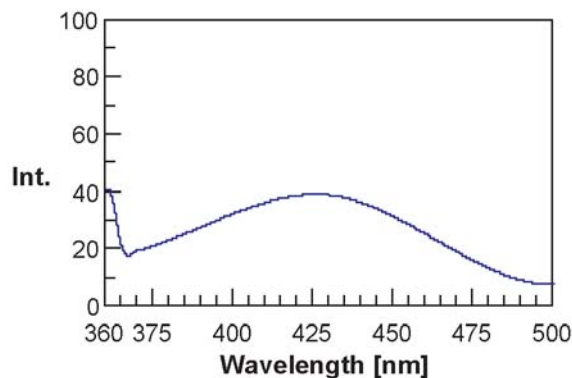


Figure 3. Processed emission data.

This band does not interfere with our evaluation since we can easily measure the net Raman band height by drawing a background line as demonstrated in Figure 4 (expanded view of Fig. 2):

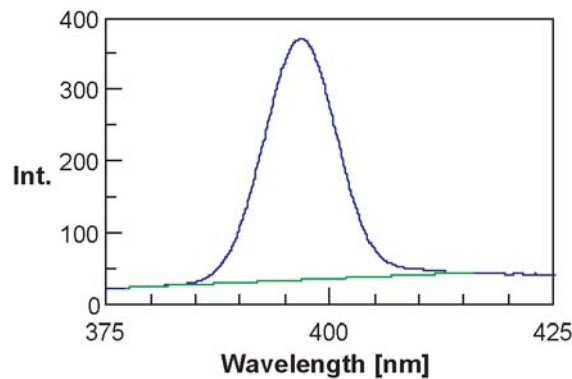


Figure 4. Peak height estimation.

The total band height at 397 nm is 372, while the net height used for further calculations is of 336.

Next, noise measurements were performed using the following acquisition parameters (475 nm was selected for method B eliminating the fluorescence band):

Measurement range: 0 - 50 sec  
 Data pitch: 0.1 sec  
 Data points: 501  
 Band width(Ex): 5 nm  
 Band width(Em): 5 nm  
 Response: 2 sec  
 (i.e.  $\cong 0.4$  sec time constant)  
 Sensitivity: High  
 Excitation Wavelength: 350 nm  
 Emission Wavelength: 397 nm, 475 nm  
 and emission shutter closed

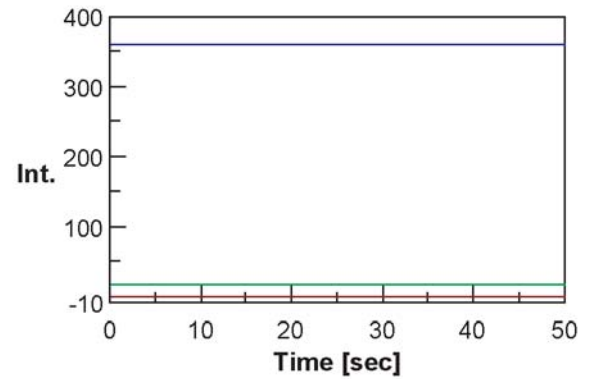


Figure 5. Noise measurement.

The results are shown in Figure 5:

Offsetting the data we obtained Figure 6:

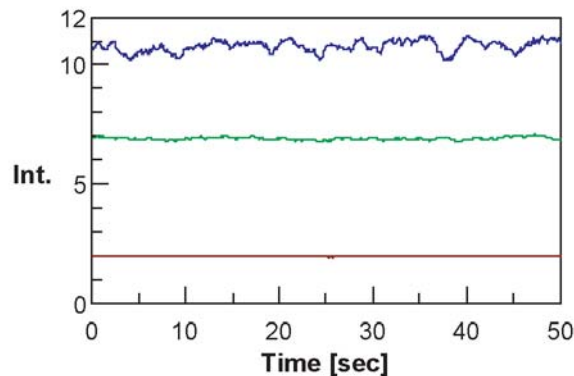


Figure 6. Data with offset.

Since the data was stored in graphical format as well as in .txt files, the P-P ratios were measured graphically and the RMS calculated by Excel™:

*Noise level at 397nm:*

peak to peak = 1.1  
 estimated RMS =  $1.1/5 = 0.22$   
 calculated RMS = 0.25

*Noise level at 475nm:*

peak to peak: 0.3  
 estimated RMS =  $0.3/5 = 0.06$   
 calculated RMS = 0.056

*Noise level at dark:*

peak to peak = 0.022  
 estimated RMS =  $0.022/5 = 0.0044$   
 calculated RMS = 0.0004

We have now all elements necessary to compute the SNR according to the methods listed above:

**Method A:**  $336/1.1 \cong 305_{p-p}$   
 $336/0.25 \cong 1344_{RMS}$

**Method B:**  $336/0.3 \cong 1120_{p-p}$   
 $336/0.056 \cong 6000_{RMS}$

**Method C:**  $336/0.022 \cong 15270_{p-p}$   
 $336/0.004 \cong 84000_{RMS}$

## 4. Conclusion

The sensitivity specifications of the FP-6500 found in the JASCO leaflet state:

Sensitivity: Signal to noise ratio of **200:1** or higher, using the Raman band of Water, peak to peak with slit widths of 5 nm on both EX and EM monochromators, and a 2 second response at 350 nm excitation.

Clearly JASCO is specifying the  $SNR_{p-p}$  using method A. Using this we obtained a SNR of 305:1, better than specified. But please note that  $SNR_{p-p}$  of 1120:1 and 15,720:1 are formally consistent with the specified definition, since (at least in the leaflet) JASCO is not indicating where noise is measured. If you add the fact that the other manufacturers may neglect not only to mention how the noise is measured, but also if it's considered as RMS, you'll understand why it's often impossible to compare the numbers listed in promotional material. So, when making comparisons, try to use homogeneous data on how/where noise is measured, while considering the bandpass of both monochromators as well as the response times.

Users may benefit from the sensitivity test to check performance of their unit from time to time. In this case the  $SNR_{p-p}$  used by JASCO is probably the easiest approach.

References :

- 1) ASTM Standard E 579-84, 1993, 142-143



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