



## Fluorescence of Quinine Sulfate

**GMP**

### Background:

Fluorescence spectroscopy analysis is a great tool for investigational research and analytical science applications. It is used often in biochemical, chemical, pharmaceutical, and medical applications, in addition to mineralogy, fluorescent labeling, sensors, and forensics applications. It is also used to aid in the identification of proteins, organic compounds, oils, and dyes, and used for environmental monitoring and laser induced chlorophyll fluorescence for crop yield assessments.

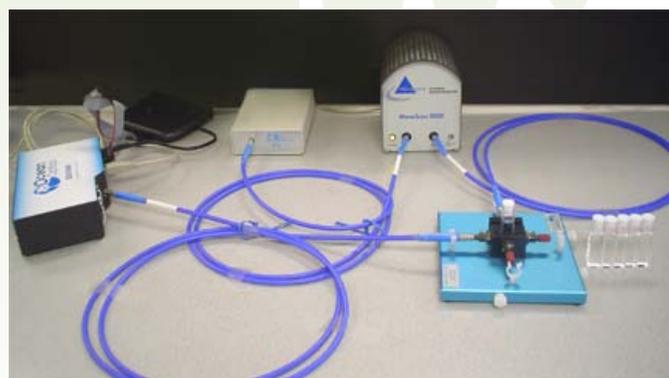
This type of spectroscopy focuses on the vibrational states of a sample. Certain substances can be excited to a higher electronic state by using a specific frequency. An particular excitation may deliver an emission or fluorescence peak.

Ocean Optics offers many options of ready to use fluorometry systems with different resolutions, off-the-shelf configurations, and time-gating options. Cuvette holders, LVF low pass and high pass filters, a fiber optic scanning monochromator, and variety of excitation sources are available. Our fluorescence spectrometers can detect fluorophores in liquids and powders, as well as from surfaces.

Our USB2000-FLG in our EDS2000 System has been used to detect anthrax. As well as to detect fluorescence in coral, fruit, and other flora and fauna.

### Experimental:

Standard stock solutions of quinine sulfate solution in methanol and sulfuric acid were prepared with approximate concentrations: 1, 20, 40, 60, 80, and 100 ug/mL. Smaller concentrations of quinine sulfate stock solutions of 0.50, 0.25, 0.06, 0.03, 0.01, and 0.00 were also created. These varying concentrations of quinine sulfate standard stock solutions were measured for fluorescence using CVFL-Q-10 quartz cuvettes in a CUV-ALL 4-way cuvette holder. These measurements were performed using a QE65000 spectrometer (grating #HC1, 200  $\mu\text{m}$  slit, range 349.2 nm – 1143.5 nm, optical resolution 6.4 nm (FWHM), PX-2 light source, HR4-BREAKOUT Breakout Box, MonoScan2000 scanning monochromator, and SpectraSuite software. Three QP1000-2-UV-VIS fibers were used to connect the PX-2 light source to the MonoScan2000, the MonoScan2000 to the CUV-ALL cuvette holder, and the CUV-ALL cuvette holder to the QE65000 spectrometer. Fibers were attached to the cuvette holder at 90 degrees. See *Figure 1*.



*Figure 1. Equipment set up for fluorescence.*

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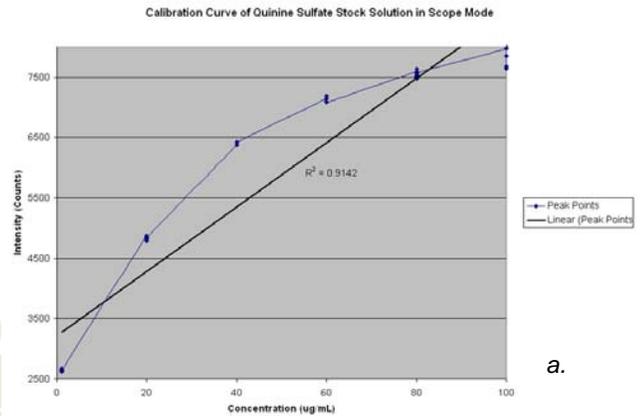
Fibers were taped down to reduce attenuation from movement. The PX-2 light source was warmed-up for 15 minutes prior to measurements. A new dark measurement was stored (and subtracted) between every change in sample and/or every 15 minutes to minimize error and drift. Spectra were recorded between 375-600 nm. Measurements were recorded using both scope mode and relative irradiance mode.

Scope mode data is unprocessed with the instrument response function not factored out. This may result in emission peaks not at the exact same wavelength as reported in the literature, variable intensity shifts, and curves having different shapes.)

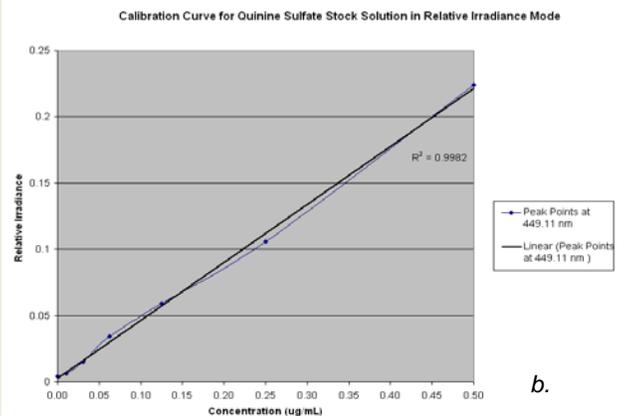
Relative irradiance measurements were performed using the same equipment, but with the addition of a LS-1 tungsten halogen light source that was used as a black body reference with known color temperature.

## Results

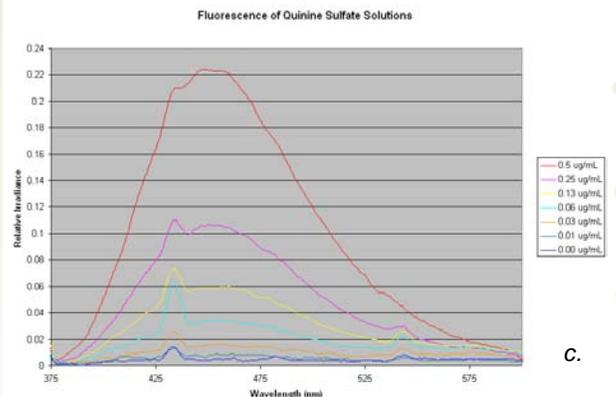
Emission, or fluorescence, peak spectra were collected from various concentrations of quinine sulfate stock solutions in both scope and relative irradiance modes. Replicate measurements were taken to create calibration curves. Peak locations varied slightly (more so in Scope Mode) from the reported 450 nm maximum fluorescence peak for quinine sulfate. In scope mode concentrations from 20-100% solutions peaked at 457.84 nm. The one percent solution replicates were the most variable in the measured peak location. The peak height locations for these replicate measurements were averaged and rounded to the nearest nanometer for reporting on the calibration curve. The measured peak for fluorescence in relative irradiance was found to be 449.11 nm for all concentrations. Calibration curves were created for two different concentration ranges. See Figures 2 and 3.



a.



b.



c.

Figure 2. a. Calibration curve of quinine sulfate from fluorescence peak points in scope data at concentrations from 1- 100 ug/mL . b. Calibration curve of quinine sulfate from fluorescence peak points at 449.11 nm from relative irradiance data. This graph illustrates the linear range at much smaller concentrations. c. Emission spectra of quinine sulfate solutions.



## Conclusions:

The most linear part of the calibration curve is in the small concentrations range under 1  $\mu\text{g/mL}$  (or 1 ppm or 1000 ppb). As expected, at greater concentrations of fluorophore, due to the inner filter effect, the excitation drops off after reaching a maximum intensity.

There was good linearity on the calibration curve for the smaller concentrations of quinine sulfate. The  $R^2$  value for linearity of the curve was 0.998 using the relative irradiance data.

Relative irradiance data proved to be more consistent over all and was much closer to the reported fluorescence peak locations of quinine sulfate in published literature.

A quinine calibration curve is suitable for checking quinine concentrations in liquids for quantitative analysis.

The QE65000 Scientific-grade Spectrometer is a sensitive system great for low-light level applications such as fluorescence. Since the QE65000 can achieve up to 90% quantum efficiency with high signal-to-noise and rapid signal processing speed, this would be the preferred spectrometer for fluorescence applications.

## Related References:

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