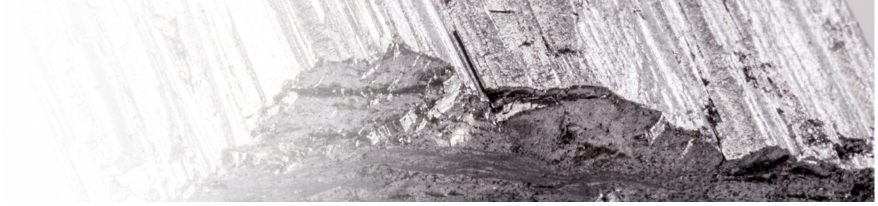

Lanthanide Quantification with Fluorolog-QM™ using Time-Resolved SSTD Mode

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Fluorescence
Technical Note
Fluorolog-QM

Introduction

Photoluminescence spectroscopy (PL) has often been used to identify and quantify molecules of interest in solution. The main advantage of PL is its inherent high sensitivity as compared to other optical techniques. However, when used in its basic steady-state mode, the detectability limit depends on the interference from fluorescent impurities, as well as stray light generated by the instrument itself. Once the PL signal of interest approaches the level of the background, the limit of detectability is established. However, for some classes of PL materials, especially lanthanide and actinide ions, which exhibit fluorescence lifetimes in the microsecond-to-millisecond range, the detectability limit can be vastly expanded by using the time-resolved fluorescence technique. Using pulsed excitation, the long-lived PL decays of these materials can be readily measured while gating out the interference from the short-lived impurity fluorescence and stray light.

In this note we demonstrate the advantage of the time-resolved approach over the steady-state for a series of dilutions of Eu^{3+} ions in aqueous medium, spanning three orders of magnitude of concentration.

Materials and Methods

The experiments were carried out with the Fluorolog-QM-75-21 operating in both the steady-state and the time-resolved SSTD mode. A 75W Xe arc lamp and a pulsed microsecond Xe lamp were used as excitation sources for the steady-state and SSTD, respectively. A TE-cooled, multimode PMT detector operating either in the photon counting or SSTD mode (software-switchable) was used for both types of measurements. In the SSTD mode, both fluorescence decays and time-resolved spectra were measured.

Steady-State Measurements

Fluorescence spectra for the Eu^{3+} sample with concentration ranging from 100 µM to 0.1 µM are presented in Fig. 1. Even at the highest concentration of 100 µM, the Eu^{3+} spectrum is distorted by strong intrinsic fluorescent impurity. With subsequent dilutions the Eu^{3+} and impurity emissions decrease until both reach the solvent background level and the Eu^{3+} spectrum is no longer detectable at 0.1 µM. While the spectrum is discernable down to ~ 1 µM concentration, any quantification is impractical due to the presence of strong background.

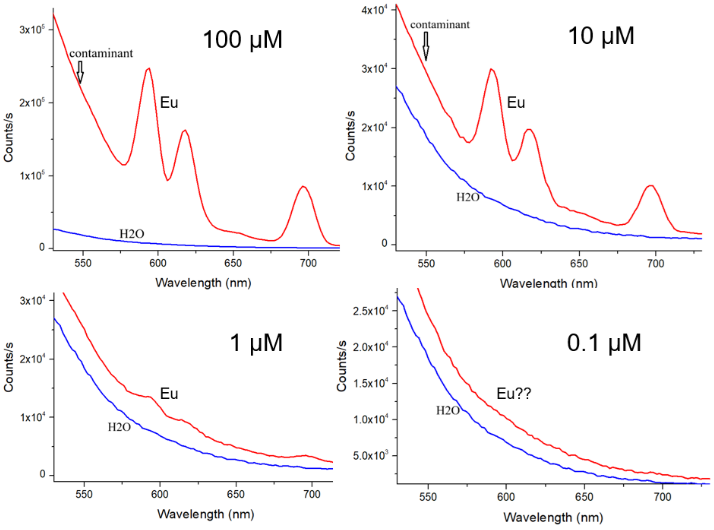

Fig. 1. Steady-state fluorescence spectra of Eu^{3+} samples at varying concentrations (λ_{exc} = 394 nm)

Time-Resolved SSTD Measurements

Fluorescence decays and time-resolved spectra of the same Eu^{3+} samples, obtained with the pulsed Xe lamp excitation and the SSTD mode of the Fluorolog-QM are shown in Fig. 2. The SSTD technique measures luminescence decays in real time, which allows for a very rapid averaging of thousands of lamp pulses and can generate useable data for low concentrations.

The decays in Fig. 2 show two lifetime components, the fast one corresponding to the background contaminants, followed by the slower decay of Eu^{3+} ion. The time-resolved spectra (TRES) measured by integrating the slow component and gating out the fast decay clearly show the uncontaminated emission from Eu^{3+}.

To quantify the concentration of Eu^{3+} one can, in principle, use either the decay or the TRES measurement. However, the decay measurement at a single wavelength is more practical, as one can accumulate good quality data much faster than with the TRES. For example, at the lowest concentration of 0.1 µM (Fig. 2), a usable decay was obtained after averaging 100K shots (~ 3 min). To get a comparable quality of TRES data would not be practical, as it would require extensive averaging at many wavelengths across the spectrum. Because of the above consideration, the total fluorescence intensities, calculated from the numerical fitting of the long decay component for each Eu^{3+} concentration, were used to quantify Eu^{3+} ion concentrations in the series of dilutions.

The total fluorescence intensity F is proportional to the integral of the decay function yielding a product of the pre-exponential factor A and the lifetime τ

$$F = A \int_0^\infty exp(-t/\tau)dt = A\tau$$

The results obtained from the single-exponential tail-fit of the decays in Fig. 2 are included in Table 1. The (A τ) vs Eu^{3+} concentration dependence exhibits nearly perfect linear relationship (Fig. 3) thus proving that the SSTD method can be successfully used for lanthanide quantification down to at least 0.1 µM (~ 15 ppb) and possibly even lower if a more extensive decay averaging is applied.

Conc (µM)	A	τ(µs)	A•τ
100	0.307	112.9	34.7
10	0.0306	106.5	3.26
1	0.00302	114.7	0.346
0.1	0.000363	99.5	0.0361

Table 1. Results of single-exponential fit to the long-lived component of Eu^{3+} decays at different concentrations

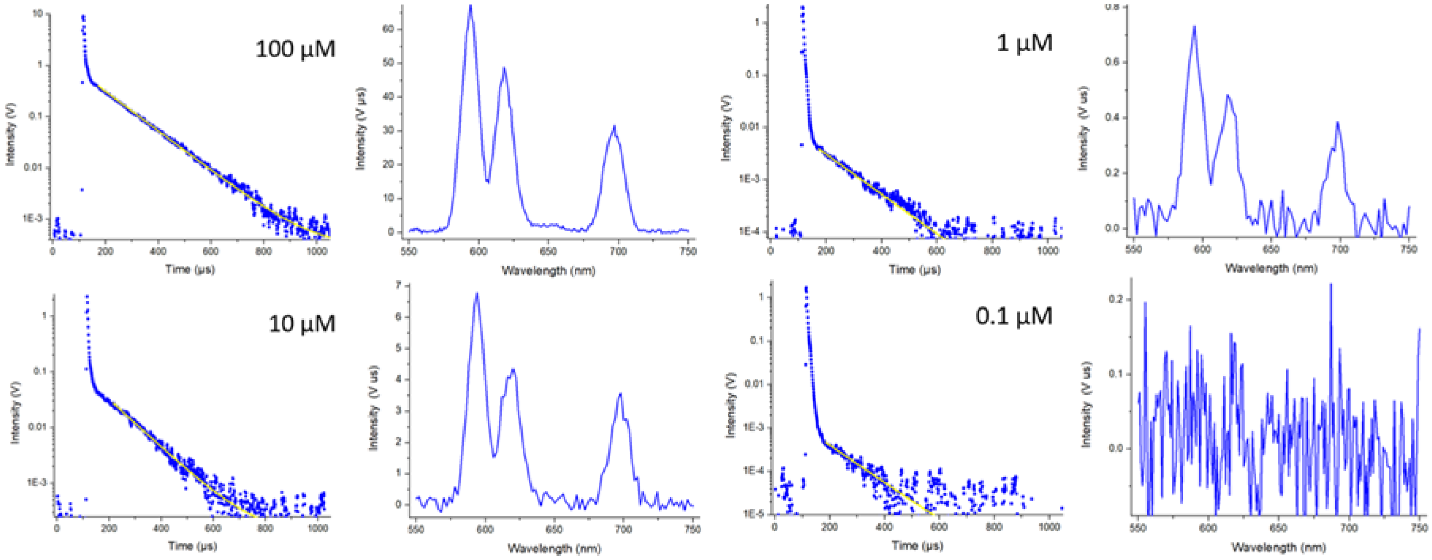

Fig. 2. Fluorescence decays (λ_{exc} = 394 nm, λ_{em} = 589 nm) and time-resolved spectra (TRES) of Eu^{3+} samples at varying concentration. TRES traces were measured by integrating intensity within the 150-850 µs time gate

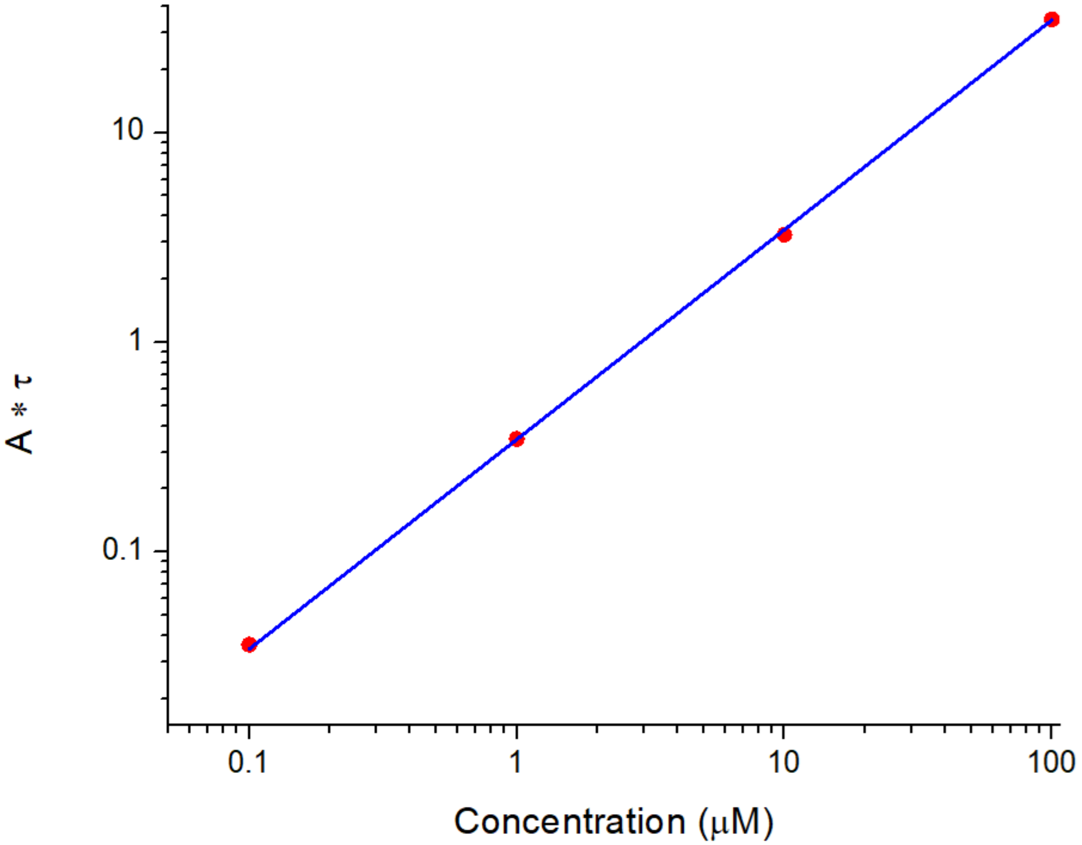

Fig. 3. Integrated intensities of Eu^{3+} fluorescence (A τ) calculated from 1-exponential fit of the long-lived decay component as a function of concentration

It should be emphasized that the sensitivity estimate presented in this study is obtained with the microsecond pulsed Xe lamp delivering energy of about 0.3 μJ/pulse at the sample. By using optional laser sources available for the Fluorolog-QM in the SSTD mode, such as Q-switched, broadly tunable OPO (~ 1 mJ/pulse) or modulated DPSS (~ 20 μJ/pulse, pulse width dependent) lasers, it is possible to enhance detectability threshold by 2-4 order of magnitude (down to ~ 0.001 - 0.1 ppb) depending on the laser source.

Conclusion

The Fluorolog-QM, with its time-resolved SSTD mode, is an excellent platform for detecting and quantifying lanthanides, actinides and other long-lived photoluminescent materials whose steady-state emission is distorted or obscured by fluorescent contaminants and scattered/stray light. We demonstrate that with the standard pulsed microsecond Xe lamp, the Eu^{3+} ions can be detected at the $\leq$15 ppb level. The detectability concentration threshold can be further enhanced by up to 4 orders of magnitude by using optional laser sources.

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