APPLICATION NOTE

EXPLORING THE FLUORESCENCE EMISSION OF RHODAMINE 6G IN THE NANOMOLAR RANGE WITH OUR FLEX SERIES

INTRODUCTION

Most fluorescence measurements requiring high sensitivity use xanthene dyes, which absorb and emit light in the visible region (from 400 to 750 nm). Owing to their structural rigidity, xanthene dyes display high fluorescence quantum yields and exhibit a small Stokes shift (around 20-30 nm) [1,2].

Rhodamine 6G, also known as Rhodamine 590, belongs to the xanthene group and is one of the most common active mediums used in liquid dye lasers [2,3]. With two amino groups partially alkylated and incorporated in the six-membered rings of the xanthene moiety, Rhodamine 6G (see **Figure 1**) exhibits a very high fluorescence quantum yield (0.95 in ethanol) and a small fluorescence lifetime (3.8 ns in ethanol) [2,3].

In addition to being used as a medium in lasers, Rhodamine 6G can also be used as a fluorescent tracer to help evaluate the development and course of liquid flows, or in imaging, to produce fluorescent images of living cells with low background noise and high resolution [4,5].

Keywords:

Fluorescence Spectroscopy;

LED Light Source;

FLEX Spectrometer;

Rhodamine 6G;

Multipurpose Cuvette Holder;

Nanomolar Range;

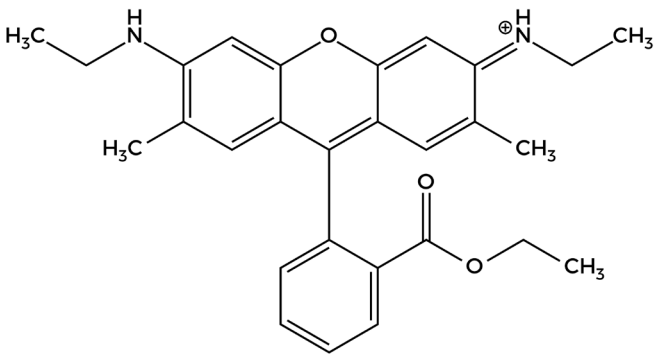

Figure 1 – Molecular structure of Rhodamine 6G [3].

In this Application Note, we explore the fluorescence detection capability of our FLEX series using different concentrations of Rhodamine 6G in ethanol.

MATERIALS & METHODS

Reagents

- Rhodamine 6G ($C_{28}H_{31}N_2O_3Cl$; MW ≈ 479.02 g/mol; Dye Content ~ 95 %);
- Ethanol (CH_3CH_2OH, ~ 96 %);

Instruments and Accessories:

- LED Light Source;
- 525 nm LED Slide (excitation wavelength);
- Optical Fibers with 1000 µm of diameter (both 1 m long);
- Multipurpose Cuvette Holder (with signal enhancing mirrors);
- FLEX RES+ Spectrometer (Slit: 200 µm; Grating: 600 grooves/500 nm; Detector: Toshiba 3648 pixel; Improved Sensitivity);
- Two fluorescence quartz cuvettes with a pathlength of 1 cm;

(1) (2) (3)

Figure 2 – LED Light source (1), FLEX spectrometer (2), and multipurpose cuvette holder (3) were used explore the fluorescence detection capability of our FLEX series.

EXPERIMENTAL PROCEDURE

1. Prepare a stock solution of Rhodamine 6G in ethanol (the concentration of the stock solution was determined through absorption, using a LS-DW and the multipurpose cuvette holder set for absorption measurements);

2. Using the stock solution, prepare a number of standard solutions with concentrations ranging from 250 nM to 0.80 nM (solutions with lower concentration are expected to have a greater volume);

3. Fill a fluorescence cuvette with an ethanol solution and use it to perform the baseline;

4. The instrument's parameters used in the LightScan software were changed according to the solution measured;

RESULTS

The fluorescence spectrum of the Rhodamine 6G stock solution, for excitation at 525 nm, is represented in **Figure 3**.

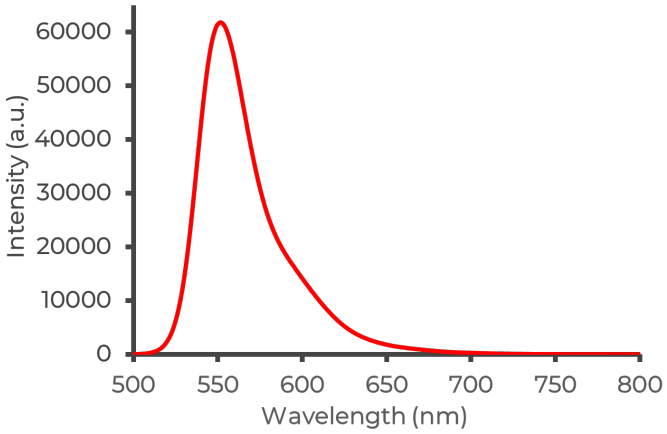

Figure 3 – Fluorescence spectrum of a Rhodamine 6G stock solution.

The fluorescence spectrum of Rhodamine 6G in ethanol shows a maximum peak around 550 nm. Both structure and maximum peak are in accordance with literature [6].

The signal enhancing mirrors installed on the Multipurpose Cuvette Holder are able to improve the signal detection on the FLEX spectrometer around 5 times. This allows the user to work with lower integration times, which makes it possible to increase the averages and yield spectra with less noise. Despite significantly improving the sensitivity of the spectrometer, the signal enhancing mirrors also increase the inner filter effect.

The relation of the fluorescence intensity with the concentration of the Rhodamine 6G solutions is displayed in **Figure 4**.

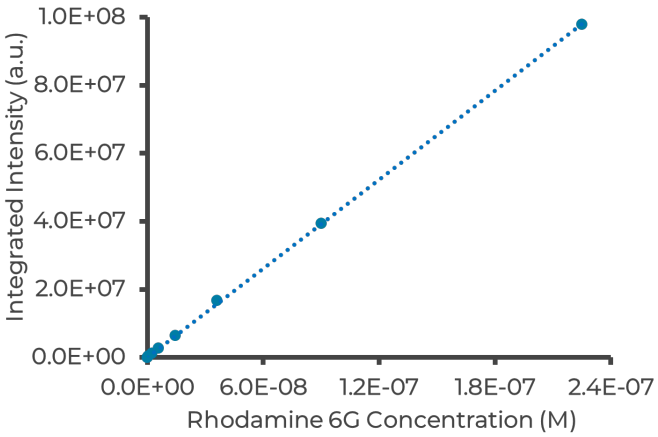

Figure 4 – Integrated fluorescence intensity as a function of the concentration of the standard solutions of Rhodamine 6G in ethanol. The results given here are corrected for the baseline with ethanol and the integration time selected on the LightScan Software.

The integrated fluorescence intensity of Rhodamine 6G displays an excellent linear relation (R2 ≈ 0.999) with the concentration of the solutions and was detected within a range of 250 to 0.8 nM. For lower concentrations solutions (bellow 6 nM), baseline correction and high integration times (within the second range) were required.

The lowest concentration at which fluorescence emission was measured was 0.8 nM (see **Figure 5**).

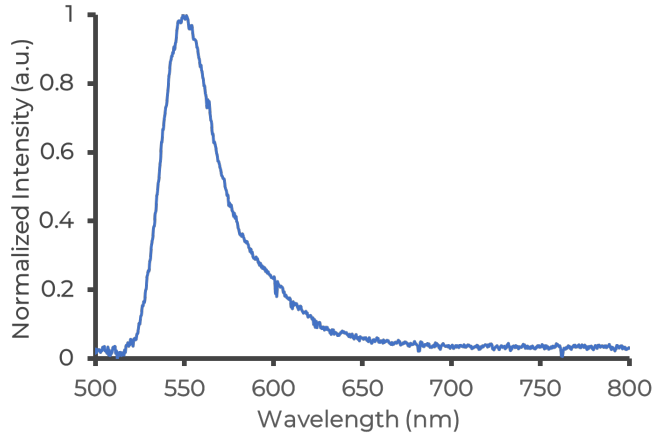

Figure 5 – Fluorescence spectrum of a 0.80 nM Rhodamine 6G solution. This spectra was corrected for the baseline with ethanol and the integration time selected on the LightScan Software. Integration Time: 43 s.

CONCLUSIONS

In this Application Note, we demonstrate the detection capability of our FLEX series when configured for fluorescence measurements. Using the setup presented here, it is possible to measure the fluorescence emission of a Rhodamine 6G solution with a concentration of 0.80 nM.

The signal enhancing mirrors installed on the multipurpose cuvette holder are essential to improve the sensitivity of this fluorescence setup. In addition to great sensitivity, our FLEX series also provides fast measurements, great accuracy, and large flexibility regarding fluorescence measurements.

Furthermore, this setup is an ideal solution for research groups that require instruments with a small-footprint but demand fluorescence measurements in the nM range.

REFERENCES

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