CE-L(ed)IF: fluorescence excitation wavelength optimization for polysaccharides analysis

François Couderc[1] ; Pierre Naccache[2]; Audrey Boutonnet[2]; Jacques Fabre[2]; Bernard Feurer[2]
1. Laboratoire des IMRCP, Université Paul Sabatier, Université de Toulouse, 31062 Toulouse, France
2. Picometrics, 18 rue Sainte Cécile, 31100 Toulouse, France

Introduction : 9-Aminopyrene-1,4,6-trisulfonate (APTS) is a dye that is frequently used for the analysis of mono or oligo saccharides (Fraysse et al Electrophoresis. 2003, 24, 3364).
APTS is routinely used in Capillary Electrophoresis separations with a 488nm laser. The process to label sugars is very well known, it consists of a reductive amination of the reductive function of the mono or oligosaccharides. These reactions can be done on very small samples (as small as 5µL).

However, 488nm is not the maximum excitation wavelength of the APTS. In this poster we use different excitation wavelengths of LEDs (450nm and 480nm) to monitor the mono or oligo saccharides and report the lowest detected (LOD) concentration of mono or oligo saccharides. We show that the LOD also depends on the optimization of the excitation wavelength and on the stability of the light source.

Comparison of the noise level using a LED and a Laser

Figure 1 shows the baseline for 10 minutes with two light sources: 488nm laser source (figure 1A) and 480nm LED (figure 1B).

Based on the ASTM E 685-93 noise determination, the peak-to-peak measurement noise during a short time dt is 3mRFU with a LED and 2.5mRFU with a laser. During the t period, the noise is 4mRFU for the LED and 7mRFU for the laser. The LED has a smaller long noise and bigger short noise than a laser. With the LED, the baseline is more stable with rapid and regular oscillations.

The rise time value is an electronic noise reduction used to attenuate all the peaks having a rise time lower than the selected value. This function is more effective with the LED .

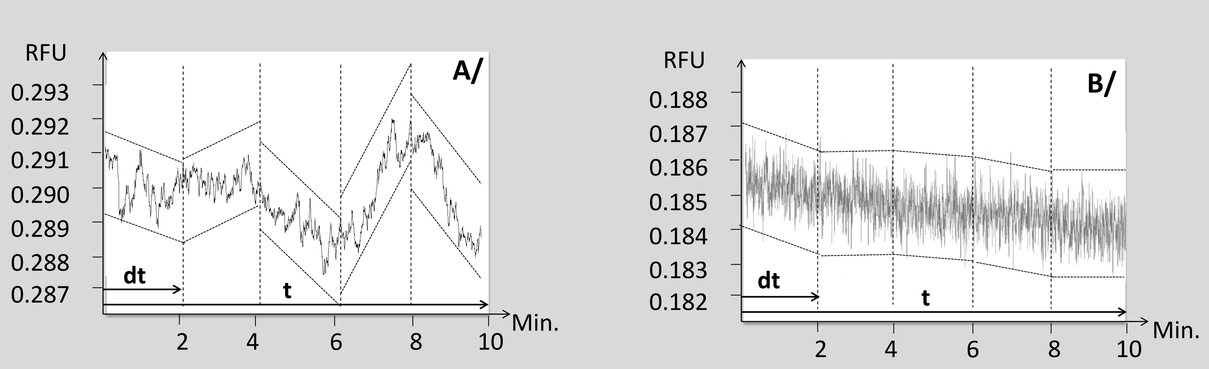

Figure 1: Baseline for 10 minutes A/ with 488nm laser; B/ with 488nm LED:
dt is a selected time with random variations of the signal (short noise) and t is a time range of 5dt (long noise).

CE/L(ed)IF and CE/LIF for the analysis of APTS-dextran

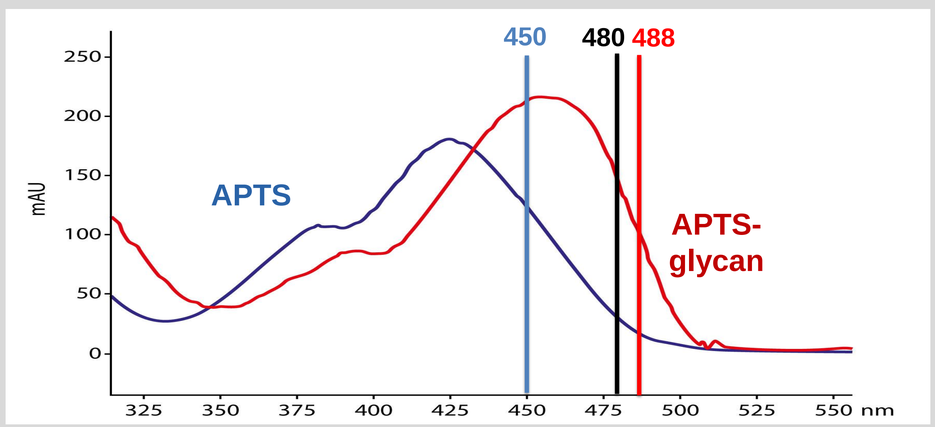

Figure 2: Excitation spectra of APTS and APTS-glycan

Figure 2 shows the excitation spectra of APTS and APTS-glycan.
The maximum excitation wavelength is 455nm. 488nm is the useful wavelength (wavelength of the argon-ion laser).

The use of LED instead of a laser permits the use of a excitation wavelength extremely close to the maximum excitation wavelength of APTS-glycan.
At 450nm, the signal of APTS-glycan is greater than at 488 nm.

The analysis of a dextran labeled with APTS was performed using 3 light sources: a 488nm solid state laser, a 450nm and a 480nm LED (figure 3).

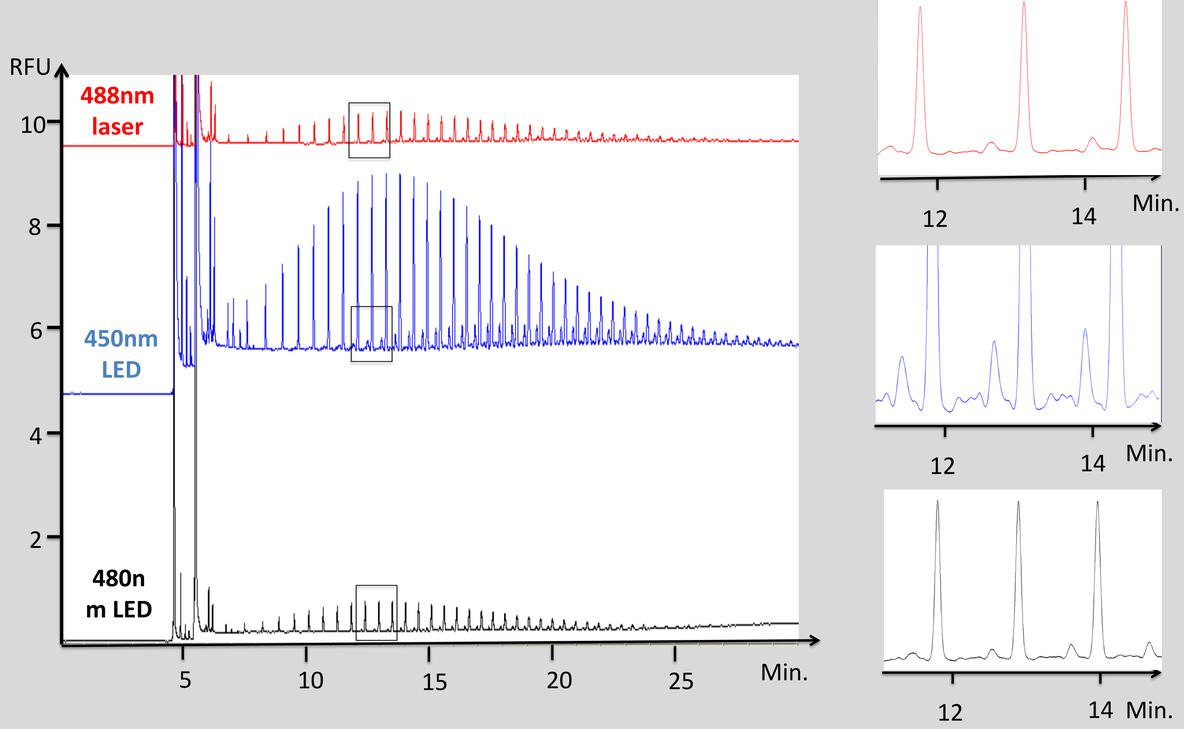

Figure 3: CE/LIF of dextran 5000 labeled with APTS with three ligth sources: 488nm laser, 450nm LED, 480nm LED. The inset is an extension between 12 and 14 minutes

Instrumentation and method:

- **System:** CE Instrument: Agilent 7100CE ; Detector: Picometrics

The Picometrics detector uses a ball lens which focuses the light source beam into the inner diameter of the capillary, and collects the fluorescence with a high numerical aperture.

- **CE separation:** Capillary: 50µm ID, 65cm total length, 50cm effective length, Buffer 40mM ε-aminocaproic acid pH 4.5 adjusted with pure glacial acetic acid + 0.02% hydroxypropylmethylcellulose, voltage : -20kV, injection : 0.5psi, 10s, temperature : 20°C.

- **APTS labeling:** 500µg dextran 5000 + 15µL APTS solution (5mg in 75µL acetic acid and 425µL water) + 5µL cyanoborohydride 1M, heated at 55°C, 2 hours. After the reaction, the solution was diluted in water to get a final volume of 50µL. The sample is diluted in water by a factor 20 before the injection.

Profile of the dextran is the same with the three light sources : 488nm laser, 450nm LED and 480nm LED.

The sensitivity obtained with the 480nm LED and the 488nm laser are similar. Optimization of the excitation wavelength (450nm instead of 480nm) magnifies the ratio S/N by a factor 4 (table 1).

	488nm Laser	450nm LED	480nm LED
Short Noise (mRFU)	2.5	3.5	3
Long Noise (mRFU)	7	5	4
Signal (RFU)	0.57	3.4	0.57
Signal/noise peak	228	971	190
Area peak	1.4	8.4	1.4

Table 1: Ratio signal/noise and area of the 10mer obtained with three ligth sources: 488nm laser, 450nm LED, 480nm LED

Conclusion: The highly divergent light beam of the LED is focused on the capillary due to the original patented optical arrangement developed by Picometrics. LIF based on LED technology provides a similar sensitivity as conventional LIF technology using a laser.
Optimizing the wavelength (nearer to the maximum of the dye excitation) and using emission filters can provide a significant increase in sensitivity. For APTS, 450nm is the most appropriate excitation wavelength.
The adaptation of the excitation wavelength increases the sensitivity by a factor 4.

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