Fluorescence detection in CE-SDS: comparison between laser and Light Emitting Diode (LED) for FQ antibody analysis

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Introduction : Laser induced fluorescence (LIF) detection is routinely used as a powerful tool with Capillary Electrophoresis for the characterization and quantification of Mab's.
The use of 3-(2-furoyl)-quinoline-2 carboxaldehyde (FQ) as a labeling reagent of proteins, improves the sensitivity, sample preparation and the robustness of the quantification [ii]. Purification of labeled product is not necessary since FQ is a fluorogenic reagent which becomes fluorescent only upon reaction with a primary amine.

A comparative study was performed with a 7100 Agilent Technologies CE and two Fluorescence light sources: a 488nm solid state laser and a Light Emitting Diode (LED). This recently developed detector employs an original optical arrangement to optimize the light beam and meet the usual LIF performance.
We observed an extremely low detected concentration (LOD) with the 488nm solid state laser and blue LED. The LOD for minor species were determined from spiked contaminant of known concentration at 0.2%, 0.5%, 1%, 2%, 5%.

Collinear laser or LED induced fluorescence detector

Figure 1 presents the collinear optical arrangement for LIF and L(ed)IF detection.

The specificity of this detector is the use of 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.

This detection cell can be used with coated and non-coated capillary from 25µm to 300µm ID (360µm OD). This cell can be integrated in the cassette of the capillary electrophoresis or implemented as close as possible to the MS source.

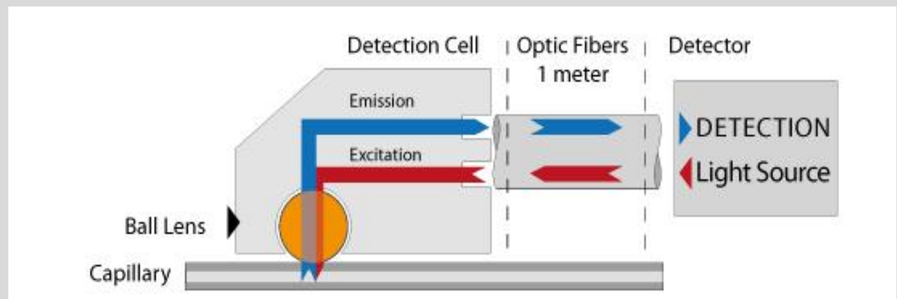


Figure 1: Collinear laser or LED induced fluorescence detector

Analysis of FQ labeled antibody using a 488nm Laser

LIF detection uses a high intensity light source (typically a laser) to excite molecules. In this work, we used a solid state laser at 488nm to characterize and quantify antibodies.

System : CE Instrument: Agilent 7100 CE; Detector: Picometrics - Laser source: 488 nm (20 mW) Capillary : 33 cm total length ; 19 cm effective length.
Method : Buffer of commercial Beckman SDS kit
Injection : -10kV, 10s, Separation : -16kV
Sample : Human Ig labeled with FQ at 2 mg/mL

Preparation of FQ-Labeled rMAbs. rMAb (2mg/mL) was added to 35 µL of 0.1 M citrate-phosphate (pH 6.5), 2% SDS, 10 mM NEM, 1 mM KCN, and 25 nmol FQ; Labeling reactions were incubated for 5 min at 75°C.

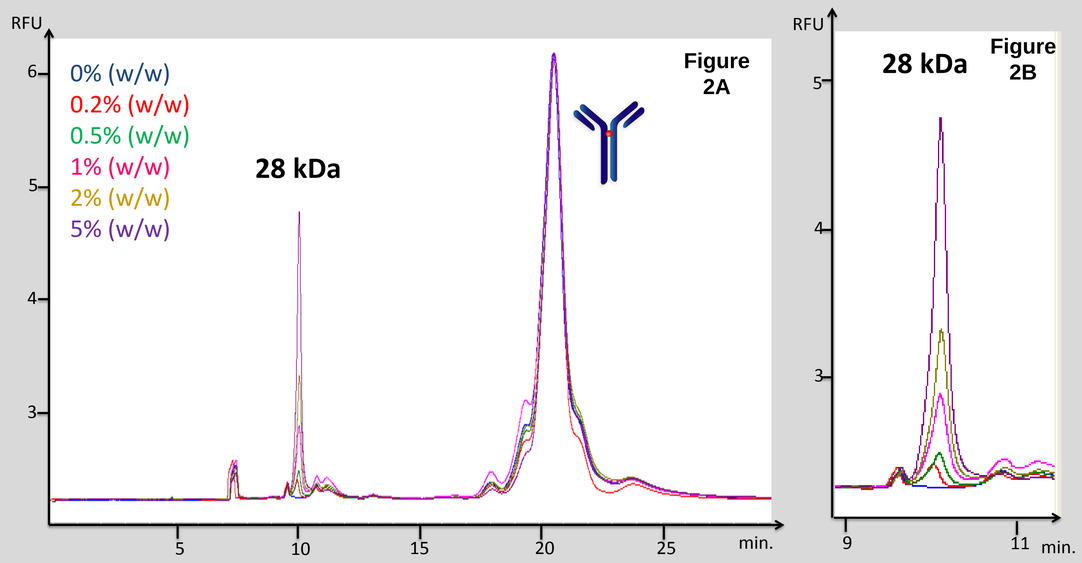
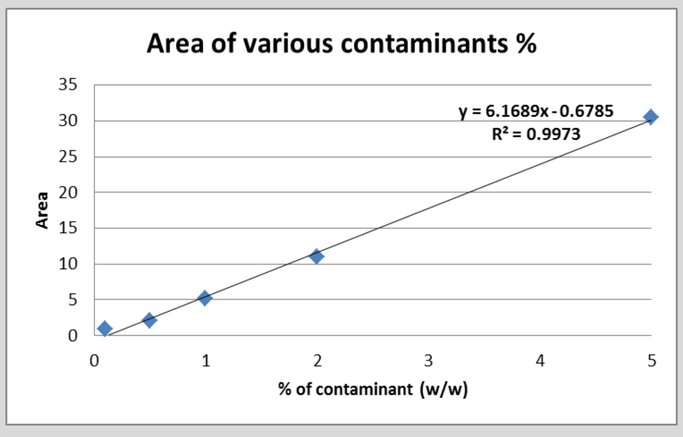


Figure 2A presents the separation of human IgG labeled with FQ with five levels of contaminants via CE/LIF using a 488nm solid state laser.

Figure 2B is an extension of the egram of figure 2A between 9 and 11 minutes.



To measure the limit of detection, rMAb sample was spiked with a 28kDa protein size standard at different levels as shown in Figure 2A : 0.2%, 0.5%, 1%, 2%, 5%.

Figure 3 : Different levels of 28kDa protein depending on spike area

Figure 3 is a plot of the spike level in fonction of the area. At 0.2%, the ratio S/N is 47.
The extrapolation to a S/N ratio of 3 shows a limit of detection of 0.013% (w/w).

Analysis of FQ labeled antibody using a LED

This section describes the analysis using a Light Emitting Diode source for the excitation. The LED presents a very high divergent light beam. This excitation beam was focused on the capillary due to the original patented optical arrangement from Picometrics .

In this work, we studied the performance of this set-up on the analysis of FQ labeled antibodies compared to the 488nm solid state laser.

System : CE Instrument: Agilent 7100 CE; Detector: Picometrics LED source : 480nm/30nm Capillary : 33 cm total length ; 19 cm effective length.
Method : Buffer of commercial Beckman SDS kit
Injection : -10kV, 10s, Separation : -16kV
Sample : Human Ig labeled with FQ at 2 mg/mL

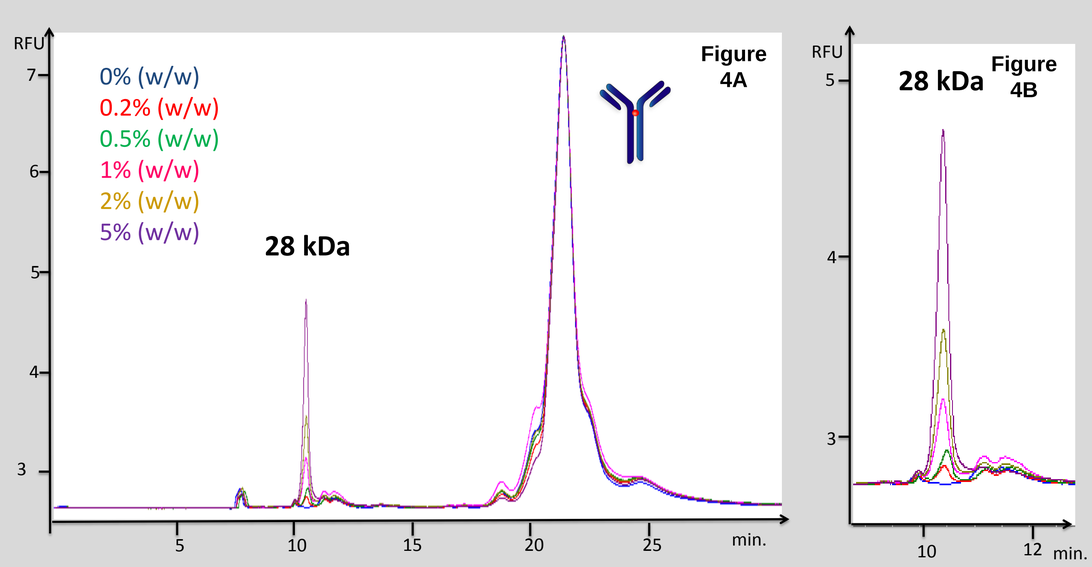
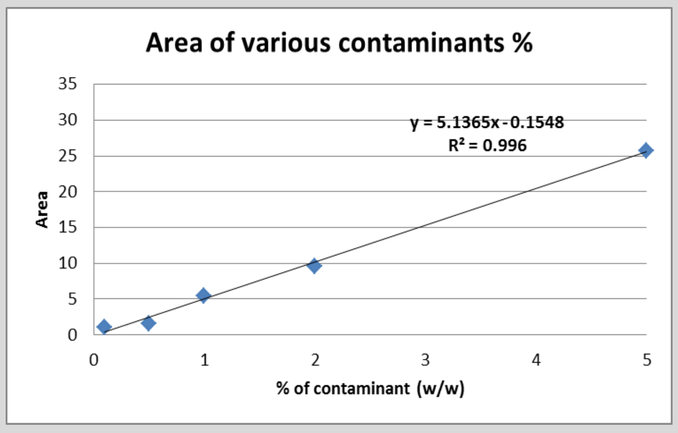


Figure 4A shows the performance of the new optical arrangement with a LED source on the CE/LIF analysis of human IgG labeled with FQ with a 28kDa spike at 5%, 2%, 1%, 0.5%, 0.2%.

Figure 4B is an extension of the 28kDa area.



With the L(ed)IF detection, the ratio S/N at 0.2% (w/w) is 26.
The extrapolation to a S/N ratio of 3 shows a limit of detection at 0.023% (w/w).

Figure 5 : Different levels of 28kDa protein depending on spike area

Conclusion: LIF based on LED technology provides similar sensitivity to that obtained from conventional LIF technology using a laser. The high divergent light beam of the LED is focused on the capillary due to the original optical arrangement from Picometrics.

The performance of the two light sources were performed with the analysis of FQ antibodies. The 488nm laser and the LED source permit to reach a low limit of detection (0.02% (w/w) contaminant level).
This high sensitivity is necessary to detect intact and reduced antibodies and especially the low level impurities including aggregates.

References :
[i] Hunt G, Nashabeh W. Anal. Chem. 1999, 71, 2390-2397
[ii] Michels DA, Brady LJ, Guo A, Balland A. Anal. Chem. 2007, 79, 5963-5971
[iii] Salas-Solano, O.; Tomlinson, B.; Du, S.; Parker, M.; Strahan, A.; Ma, S. Anal.Chem. 2006, 78, 6583-6594.