

Picometrics Introduces: The Collinear LED induced Fluorescence Detector

„Plug and play” system.
No consumables: Use
your current capillaries.

Simplify Biomolecule
Detection of
Proteins,
Carbohydrates,
Oligonucleotides

For Capillary Electrophoresis, Capillary/NanoLC, MicroChip

- Simplify the use of Fluorescence detection in micro & nano liquid separation with the collinear optical technology.
- Work with the same sensitivity as the Laser Induced Fluorescence technology.
- Increase the lifetime and reduce the cost of replacing the Light Source.



Why LED instead of Laser based light sources?

Picometrics recently developed unique optical devices to allow a non-coherent light to be focused on a capillary size window. The LED light sources can mimic the Laser technology.

- Increase the lifetime and reduce the cost of replacing the source
- Reduce the energy consumption, less than 15W.

Why “Plug & Play”?

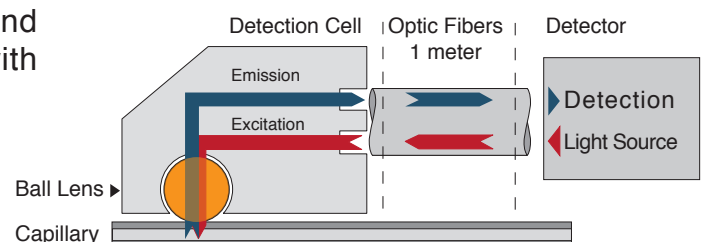
The new LED collinear Induced Fluorescence Detector can now be used with any coated or non-coated capillary, from 25 μ m to 300 μ m ID (360 μ m OD). The LED light sources simplify operations because of an ON/OFF system. Moreover, it eliminates the hazards of using Laser sources.

- One detector for any CE or LC.

What collinear optical arrangement means?

The collinear LIF technology was introduced by Picometrics 10 years ago. The recent development allows a super miniaturization of this patented technology. The detection cell size is now simplified and can be used externally; for example, integrated inside a CE cassette or implemented as close as possible to another detection sources such as MS.

The miniaturization, the simplification of the use and the superb sensitivity could easily be used with capillary LC or nanoLC and microChips.



LIF cell: New collinear optical arrangement

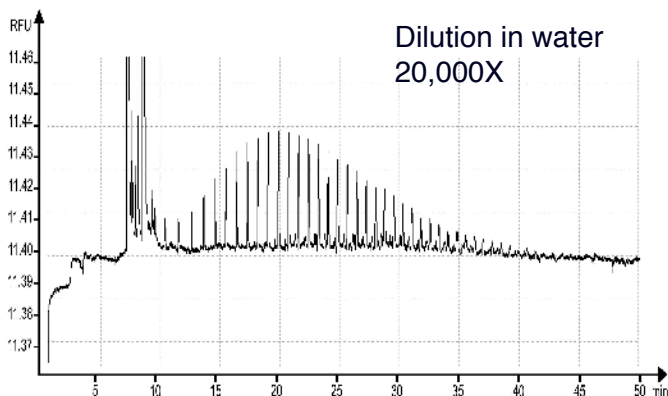
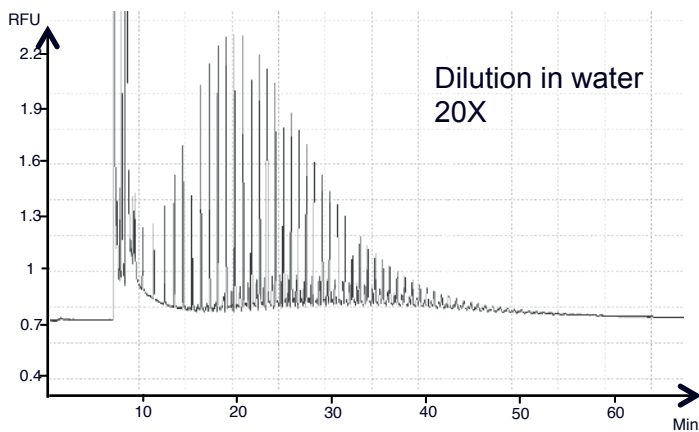
Polysaccharides analysis

Application note Ref : AN 2.003-V2

9-Aminopyrene-1,4,6-trisulfonate (APTS) is dye that is frequently used for the analysis of mono or oligosaccharides. The labeling of sugars involves a reductive amination of the reductive function of the mono or oligosaccharides followed by reaction with the dye.

APTS is routinely used in Capillary Electrophoresis separation. In this note, we analyze oligosaccharides labeled with APTS with a 480nm LED.

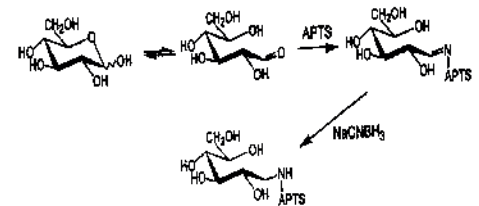
CE-L(ed)IF analysis



Instruments: Capillary electrophoresis: Agilent Technologies CE7100 Detector: Picometrics ZETALIF LED with LED 480nm/30nm

Sample: Dextran 5000 labeled with APTS

Labeling: 500µg dextran 5000 + 15µL APTS solution (5mg in 75µL acetic acid and 425 µL 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. This solution was diluted in water 20x and 20,000x prior to CE/LIF analysis.



Method:

- PVA coated capillary: 65 cm x 50 µm ID
- buffer 40mM ε-aminocaproic acid pH 4.5 adjusted with pure acetic acid glacial + 0.02% hydroxypropylmethylcellulose
- voltage : -20kV
- injection : 0.5psi, 10s
- temperature : 20°C.

References:

- [i] Frayse N, Jabbouri S, Treilhou M, Couderc F, Poinot V, *Glycobiology* 2002, 12, 741-8.
- [ii] Guttman A, Chen FT, Evangelista RA, *Electrophoresis* 1996, 17, 412-7.

Monoclonal Antibodies (Mab's) analysis

Application note Ref: AN 2.001-V2

Antibodies pharmaceuticals are therapeutics that play an important role in controlling a broad range of diseases such as cancer, allergy, inflammation, infectious and autoimmune diseases. Monoclonal antibodies have become a fast growing class of biopharmaceutical products.

To support analytical characterization in process development and quality control of therapeutic antibodies,

capillary electrophoresis-sodium dodecyl sulfate (CE-SDS) has been recognized as an important tool in place of SDS-Page because of the ease of use and the ability to automate.

The use of 5-carboxytetramethylrhodamine succinimidyl ester (5-TAMRA.SE) and 3-(2-furoyl)-quinoline-2 carboxaldehyde(FQ) as derivatizing agents improves the sensitivity and the reproducibility of the quantification.

FQ derivatization method

FQ (λ_{max} . Exc.: 480nm; λ_{max} . Emis.: 590nm) is a fluorogenic reagent : it becomes fluorescent only upon reaction with a primary amine.

The method of derivatization is rapid and no purification after labeling is necessary.

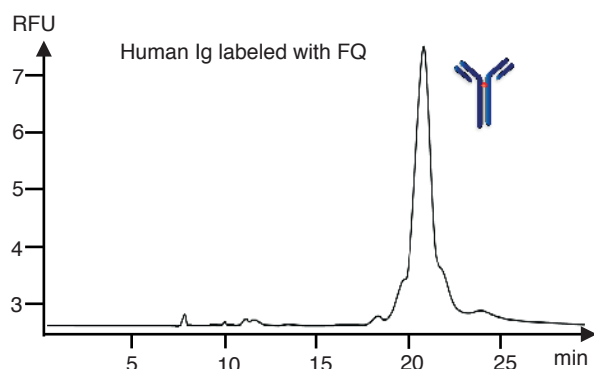


Figure 1: Analysis of human Ig labeled with FQ using a 480nm LED

Instruments: Capillary electrophoresis: Agilent Technologies CE7100
Detector: Picometrics ZETALIF LED with LED 480nm/30nm

Sample: Human Ig labeled with FQ

Labeling: rMAb (2mg/mL) was mixed in 35 μ L 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.

Method:

- Capillary: 33cm x 50 μ m ID (effective length 19cm)
- Buffer of commercial Beckman SDS kit
- Voltage: -16 kV
- Injection: -10kV, 10s
- Cassette temperature : 40°C

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

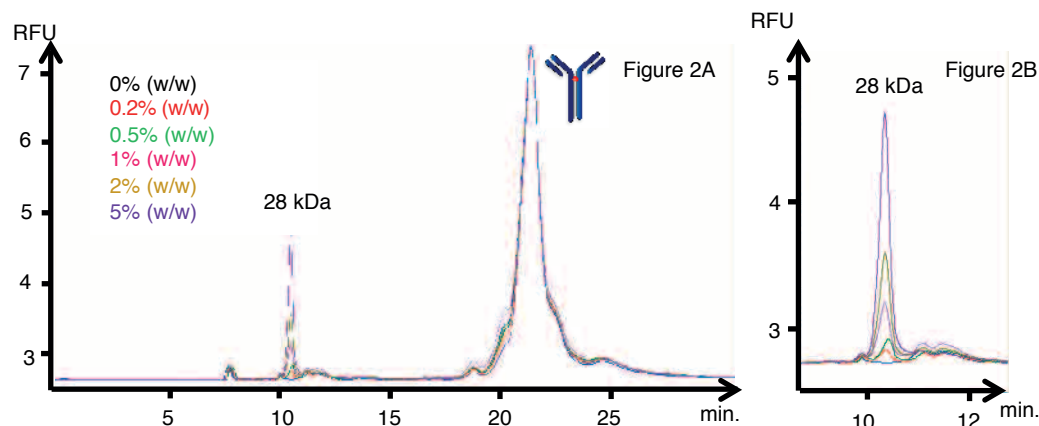


Figure 2A: Separation of human IgG labeled with FQ with five levels of contaminants via CE/L(ed)IF using a 480nm LED

Figure 2B: Extension of the egram of figure 2A between 9 and 12 minutes.

Figure 2 shows that the S/N ratio is 26 at 0.2%. The extrapolation to a S/N ratio of 3 shows a limit of detection of 0.023% (w/w).

5-TAMRA.SE (λ_{max} . Exc.: 550nm; λ_{max} . Emis.: 590nm) is commonly used as a labeling reagent of MAb's for fluorescence detection as it increases the sensitivity and maintains the profile of the analyte species.

Figure 3 shows the analysis of human Ig using two LEDs : 480nm and 530nm. Optimization of the excitation wavelength (530nm instead of 480nm) magnifies the S/N ratio by a factor 18.

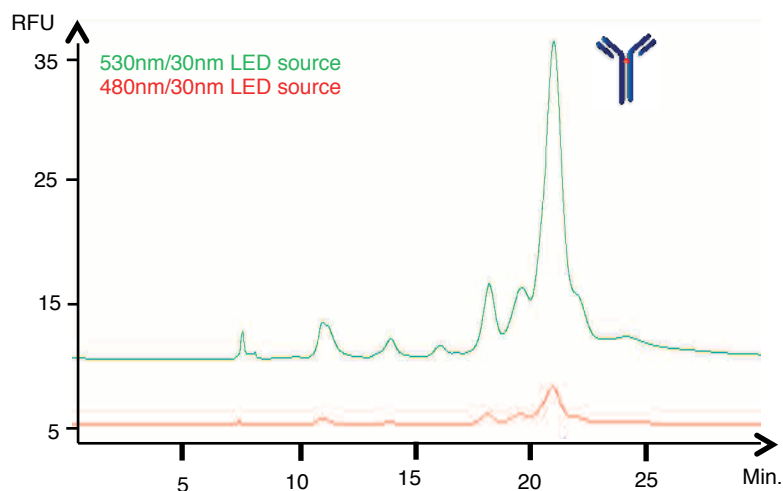


Figure 3: Analysis of human Ig labeled with 5-TAMRA.SE using a 480nm and 530nm LEDs

Instruments: Capillary electrophoresis: Agilent Technologies CE7100
 Detector: Picometrics ZETALIF LED with LED 480nm/30nm and 530nm/30nm

Sample: Human Ig labeled with 5-TAMRA.SE

Labeling: rMAb samples (2mg/mL) were buffer exchanged into 0.1 M sodium bicarbonate, pH 8.3, using a NAP-5 column. 10 μ L of 5-TAMRA.SE (1.4 mg/mL) dissolved in DMSO was then added to 190 μ L of rMAb solution and the resultant mixture incubated for 2 h at 30°C. After incubation, 190 μ L of the antibody-dye conjugate was loaded onto a second NAP-5 column and collected in 700 μ L of 0.1 M sodium bicarbonate, pH 8.3. Nonreduced SDS-rMAb conjugates were prepared by mixing 100 μ L of the rMAb-dye conjugate and 100 μ L of the CE-SDS sample buffer.

Method: same as FQ's method

	FQ derivatization	5-TAMRA.SE derivatization	
Wavelength (nm)	480	480	530
Derivatization method	- Fluorogenic reagent, no purification after labeling; - Rapid method of labeling (5min at 75°C)	- The excess of dye should be removed with NAP-5 column - Long period of time required for labeling and purification (2 hours at 30°C + purification)	
Ratio S/N on the main peak	3 700	1 400	25 800

Conclusion :

This note describes two method of derivatization of IgG with 5-TAMRA.SE and FQ. The CE-L(ed)IF is used to assess the purity and heterogeneity of IgG and its isoforms. The LED presents many advantages : less expensive, less energy consumable, and more stable.

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.