CE-LEDIF : Capillary Electrophoresis LED Induced Fluorescence Detector

Product Note Ref : PN 002 V1

Agilent Technologies CE 7100
Capillary Electrophoresis
Picometrics Zetalif™ LED
LED Induced Fluorescence Detector

This note describes how to interface the Picometrics LED Induced Fluorescence detector (LEDIF) with the Agilent Technologies 7100 Capillary Electrophoresis system (CE). The Zetalif LED detector can now be directly integrated in the Agilent cassette and allows for all functions of the Agilent HPCE.

The CE-LEDIF system provides high sensitivity and improved selectivity. It is possible to perform all modes of capillary electroseparation that are available with the Agilent Technologies CE.

Instrumental Set-up

The CE-LEDIF System consists of the following items:

- Agilent Technologies 7100 Capillary Electrophoresis System including all functional hardware for performing CE separation.

- Picometrics Zetalif LED Detector including a Detector, Flow Cell Kit, LEDIF Cassette, LED light source with the corresponding emission filter block, LIF Driver for Agilent Technologies Software.

About Capillary Electrophoresis

Capillary Electrophoresis (CE) is employed to separate molecules in the presence of an electric field.

CE can be used for the analysis of both large and small molecules, organic or inorganic. There are many application fields including environmental, clinical, forensic, biochemical and pharmaceutical analysis. CE has many advantages including superb efficiency (10 to 10 theoretical plates) and high resolution, fast analysis, low sample requirements (a few nL), low buffer consumption, ease of operation, and it can be completely automated.

Principle of CE (Figure 1)

CE employs a sample vial source and destination vial filled with a background electrolyte (BGE) (buffer). The ends of the capillary are immersed in the source and destination vials with the BGE. The migration of the analytes is initiated by an electric field that is applied between the source and destination vials and supplied to the electrodes by a High Voltage power supply.

The sample can be introduced in the capillary by electrokinetic injection or hydrodynamic injection.

The Agilent CE instrument contains all functional hardware for performing CE separations.

It includes :

- a HV power supply

- a Carousel for auto sampling (50-position) which can be thermostated simply by connecting an external water bath to the instrument (10 to 40 °C).

- Fraction collection: three different modes of collection: by pressure or electrokinetic elution or cIEF analysis (pressure & voltage can be applied simultaneously).

- Injection system: that can provide hydrodynamic or electrokinetic injection.

- Off-line buffer replenishment station: about 500 mL volume.

The instrument is able to employ an external gas pressure to perform CEC (Capillary ElectroChromatography) and CGE (Capillary in Gel Electrophoresis) and includes features that allow for a CE-MS interface. This provides the possibility to couple LIF detection (high sensitive quantitation) with MS detection (qualitative determination).

Capillary Thermostating

It is important to regulate the temperature of the environment around the capillary to ensure consistent separations.

This CE-LEDIF system allows temperature control by high-velocity recycling air flow (10 m/s) which is thermostatically regulated by a Peltier element.

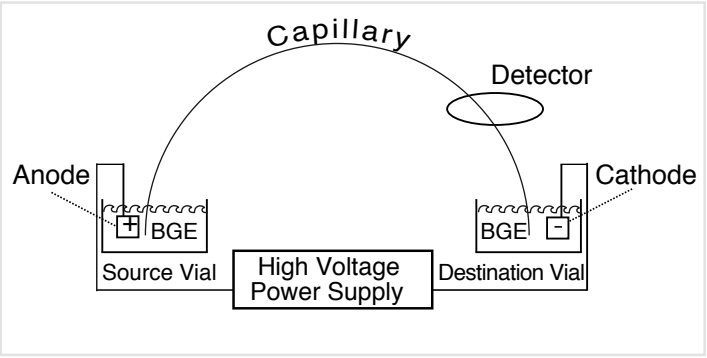


Figure 1 : Principle of CE separation

About LEDIF detection

LED induced fluorescence detection is “on capillary” detection: A ball lens in contact with the capillary has two functions: it focuses the light source beam into the capillary and collects the emitted fluorescence (Table1).

Capillary ID (µm)	Estimated* Cell Volume (nL)
25	0.1
50	0.4
75	0.9
100	1.6
150	3.5

Table 1 : Estimated LIF Cell Volume
* Based on 200 µm window length

Picometrics recently developed unique optical devices to allow a non-coherent light to be focused on a capillary size window.

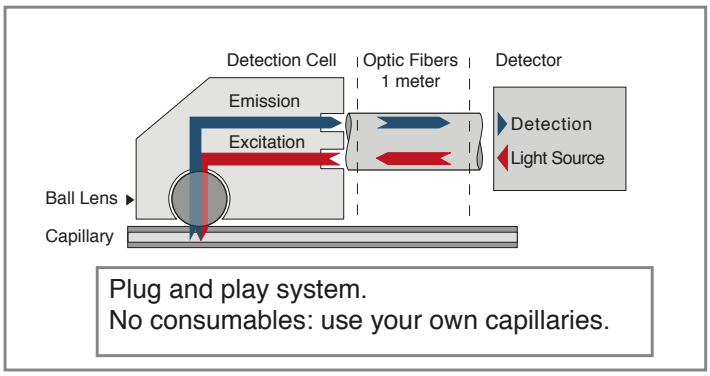


Figure 2 : LIF cell: new collinear optical arrangement

LEDIF Optical Arrangement: (Figure 2)

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 source such as MS.

The new LED collinear Induced Fluorescence Detector can now be used with any coated or non-coated capillary, from 25µm to 300µm ID (360µm 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.

Specifications of the Zetalif

External Communications:

- External Ethernet for Network communications.

- External event (detection of a relay state) function.

- A start/stop command port for an external event command (relay opened or closed)

- Analog output : Processed: 0-1V(DC) for the range 0-50 RFU, 0-100mV for the range 0-5 RFU

Power requirements:

100/240VAC, 47/63Hz, 1.5A

Operating conditions :

Temperature Range :10-40°C / 50-104°F

Relative humidity: <90% non-condensing.

Dimensions and weight : 43.0(H) x 23.0(W) x 34.0(D) cm / 16.9”(H) x 9.1”(W) x 13.3”(D) ; 12kg/26.4 lbs

LED light source range:

Available wavelengths: 450, 480, 530, 640nm. For other wavelengths, contact Picometrics.

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.

Interfacing the CE-LIF

The Agilent CE system is equipped with a LEDIF cassette provided by Picometrics. The LEDIF cassette integrates the LEDIF Flow Cell kit and the UV cell as shown in Figure 3.

The Picometrics driver allows for a full integration of all aspects of the fluorescence detector into the powerful Agilent software, including: full control of the LEDIF detector, storage of methods and a broad range of additional options (e.g. changing sensitivity during a run).

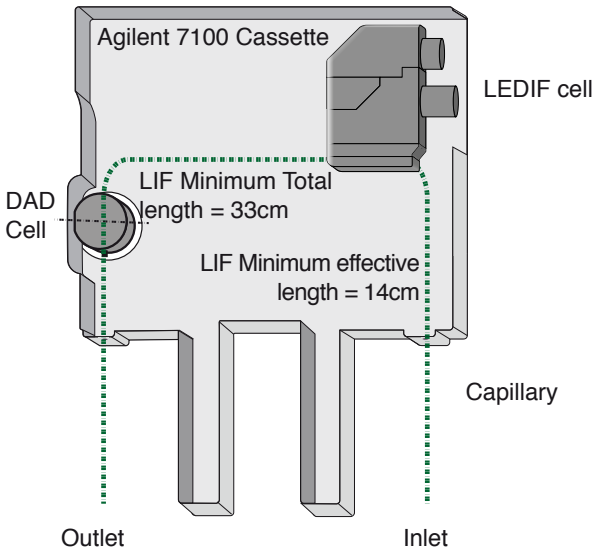


Figure 3: Inside View of the DAD/LEDIF Cassette

Labeling reactions

Some compounds (including many aromatic compounds) exhibit native fluorescence; in this case direct quantitation of the compound is easy and quite straightforward.

However, in most cases, the use of a fluorogenic reagent is required (a list of common reagents is presented in Table 2). The use of these reagents provides a high degree of sensitivity for the compound of interest.

Labeling Dyes	Excitation wavelength (nm)	Applications
FQ (3-(2-furoyl)quinoline-2-carboxaldehyde)	480	Proteins
5-TAMRA.SE (5-carboxytetramethylrhodamine, succinimidyl ester)	480 or 530	Proteins
NDA (naphthalene-2,3-dicarboxaldehyde	450	Neurochemistry/Proteins
FITC (fluorescein isothiocyanate)	480	Neurochemistry/Proteins
NDB-F (NDB-fluoride)	480	Neurochemistry
APTS (amino pyrenetrisulfonic acid)	450 or 480	Glycans
Luciferase yellow	450	Glycans
Oligreen	480	Oligonucleotides
Cy2, Cy3, Cy5	480, 530, 640	Oligonucleotides/Proteins
YoYo	480	Oligonucleotides
Bodipy FL EDA	480	Oligonucleotides
FM (fluorescien maleinimide)	480	Thiols/Homocysteine
IA (iodoacetamidofluorescein)	480	Thiols/Homocysteine

Table 2: Examples of Labeling Reagents

Some CE-LEDIF Applications

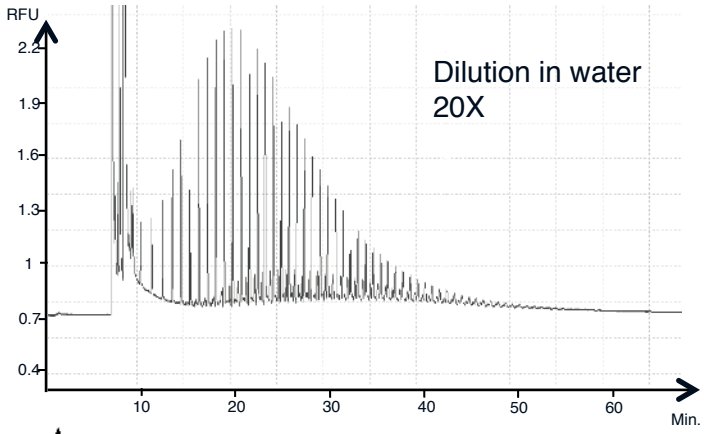
Carbohydrates Polysaccharides analysis

Application note Ref : AN 2.003-V2

9-Aminopyrene-1,4,6-trisulfonate (APTS) is a 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.

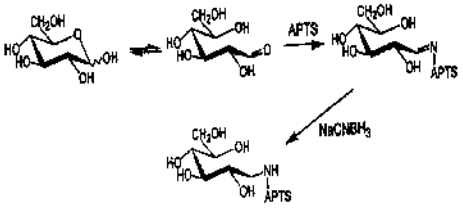
CE-L(ed)IF analysis



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

Sample: Dextran 5000 labeled with APTS

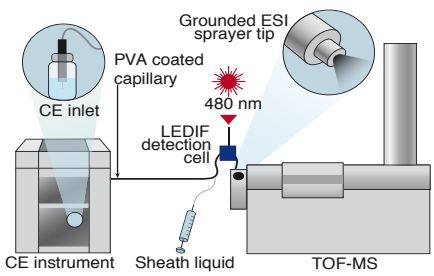
Labeling: 500 µg dextran 5000+ 15µL APTS solution (5mg in 75µL acetic acid and 425 µL water) + 5 µL cyanoborohydride 1M, heated at 55°C, 2 hours. After the reaction, the solution was diluted in water to get a final volume of 50µL. This solution was diluted in water 20x and 20 000x prior to CE/LEDIF analysis.



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

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

On-Line CE-LEDIF-MS for the Characterization of Antibody Glycosylation. CE performed with on-line LEDIF and on-line MS detectors in a single analysis.



Therapeutic Antibodies Monoclonal Antibodies (Mab's) analysis

Application note Ref: AN 2.001-V2

Antibody 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 (λmax. Exc.: 480nm; λmax. 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.

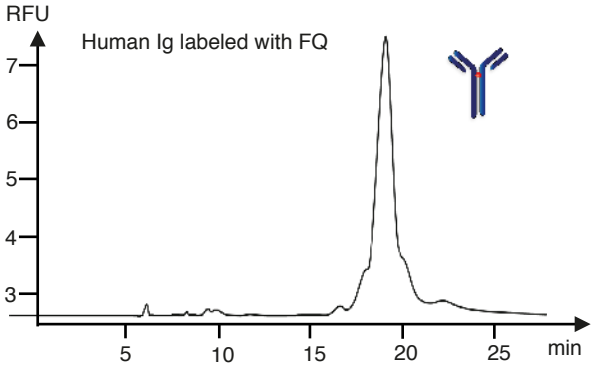


Figure 4: Analysis of human Ig labeled with FQ using a 480nm LED

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 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.

Method:
- Capillary: 33cm x 50 µm 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%.

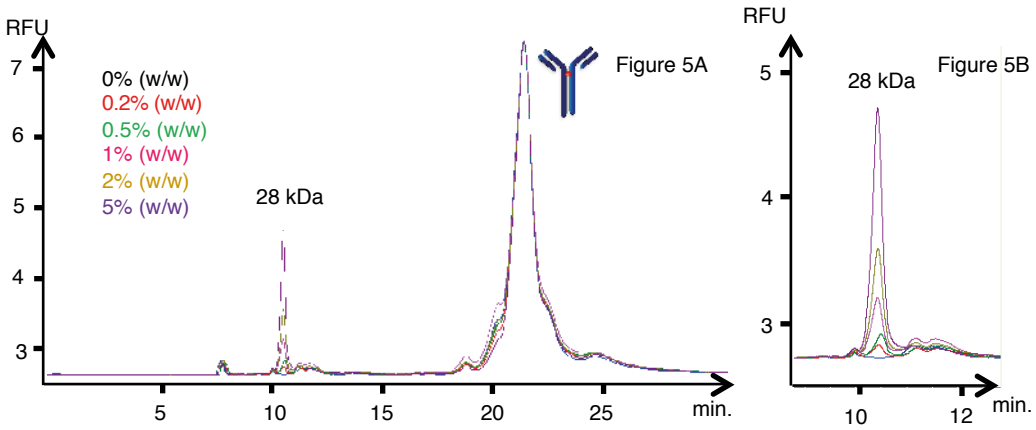


Figure 5 shows that the S/N ratio is 26 at 0.2%.
The extrapolation to a S/N ratio of 3 shows a limit of detection of 0.023% (w/w).

Figure 5A: Separation of human IgG labeled with FQ with five levels of contaminants via CE/L(ed)IF using a 480nm LED
Figure 5B: Extension of the egram of figure 5A between 9 and 12 minutes.

5-TAMRA.SE derivatization method

5-TAMRA.SE (λmax. Exc.: 550nm; λmax. 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 6 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 of 18.

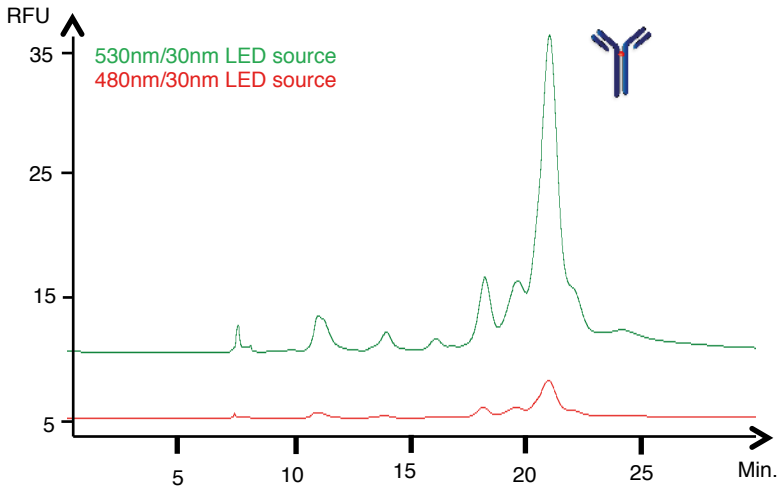


Figure 6: Analysis of human Ig labeled with 5-TAMRA.SE using a 480nm and 530nm LEDs

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

Other typical applications examples:

Peptides, Drugs, Food, Environmental, etc…
Many application notes may be accessed through the Picometrics website at http://www.picometrics.com

Conclusion :

CE-LEDIF provides a complete approach to the separation and detection of a broad range of complex samples. With the use of labeling reagents, Fluorescence is often the most sensitive detection for CE.

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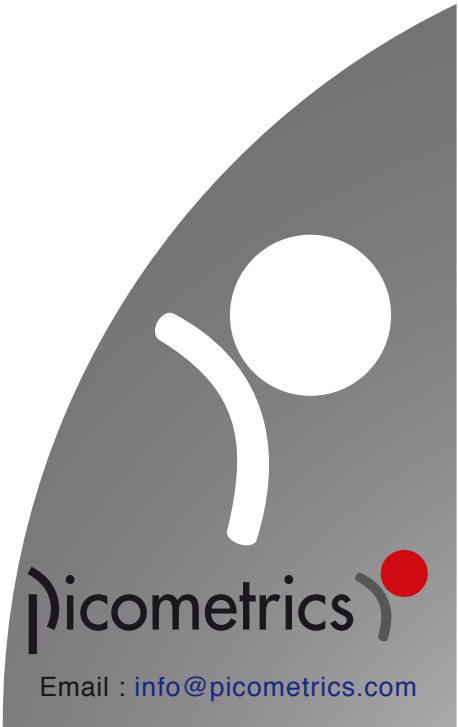
To learn more about Picometrics LEDIF Detectors, visit www.picometrics.com



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