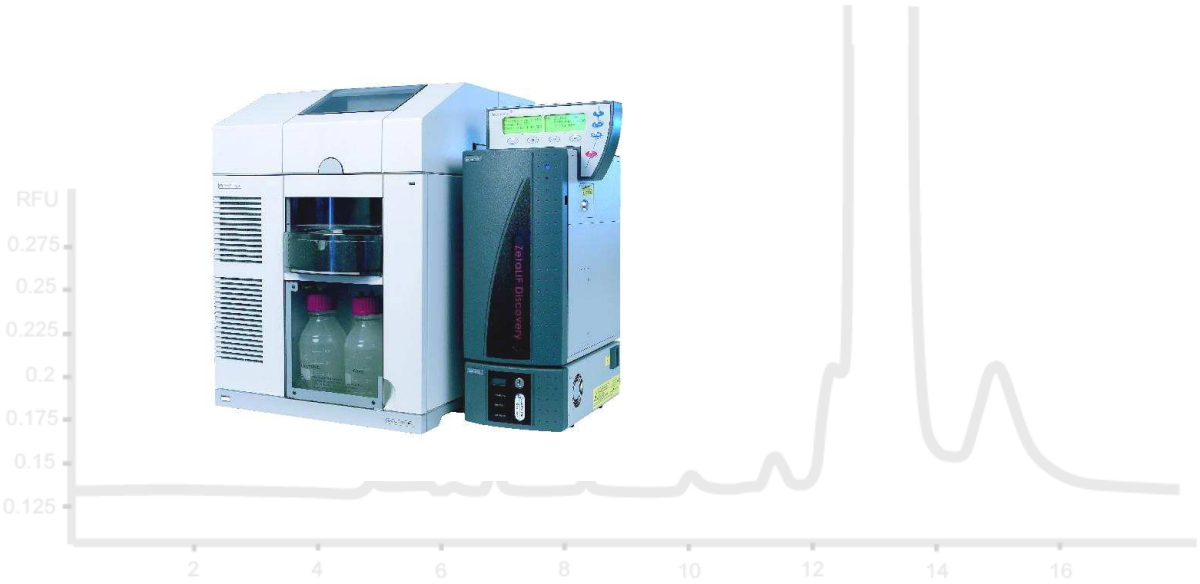
Agilent Technologies
Channel Partner

Protein analysis

Application note Ref : AN 066

Monoclonal Antibodies (Mabs) analysis

Capillary Electrophoresis with Laser Induced Fluorescence Detection



Abstract:
Antibody pharmaceuticals is a category of therapeutics that plays an important role in controlling many types of diseases such as cancer, allergy, inflammation, infectious or autoimmune diseases. Monoclonal antibodies have become a fast growing class of biopharmaceutical products.
There is an increased demand in the pharmaceutical industry for tools applicable to large scale production and there is an increasing need for product characterization and control of the manufacturing process.

In the biopharmaceutical industry, to support analytical characterization, process development, and quality control of therapeutic antibodies, capillary electrophoresis-sodium dodecyl sulfate (CE-SDS) has been recognized as an important alternative tool of SDS-Page because of the ease of use and the ability to automate.

UV absorbance detection is widely used in CE-SDS. In contrast to standard Liquid Chromatography where proteins are usually detected at 280nm, detector signal in a CE system is not satisfactory due to a very short detection path length (25 to 75µm). Although the absorbance of protein at 210nm is greater than at 280nm, detection in the low UV region is not very sensitive.
Alternatively, CE derivatization techniques for Laser Induced Fluorescence detection have been tested to increase detection sensitivity of proteins but the sample preparation is still very restrictive.

In this application note, we present 2 different LIF detections: Fluorescence derivatization and native fluorescence.

Fluorescence Derivatization in CE SDS with LIF

CE derivatization techniques for Laser Induced Fluorescence detection is highly sensitive in protein analysis. But the sample preparation requires purification of labeled MAbs and the derivatizated sample form is unstable.

FITC labeled IgG

Experimental conditions:

LIF Capillary:
Internal Diameter 50µm
Total length: 33 cm
Effective length: 14 cm

Method:
Injection: sample : 25 seconds at -15kV
Analysis condition: -20kV
Temperature (cassette and sample): 25°C

Sample:
Mouse IgG2a FITC conjugate

Instruments:
Agilent HP3D CE
Picometrics ZETALIF Discovery detector.
Laser: 488 nm (excitation)

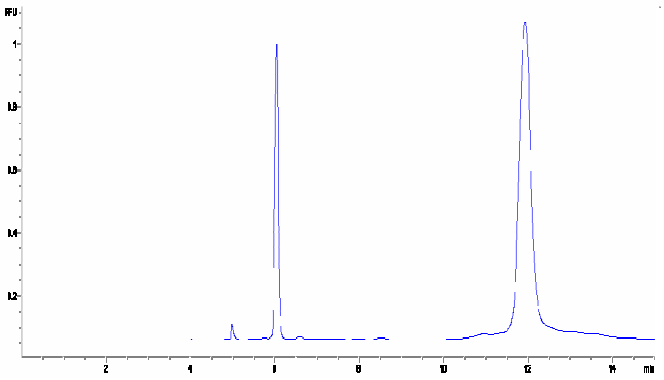


Figure 1: Non reduced Sample at 100 ng/mL

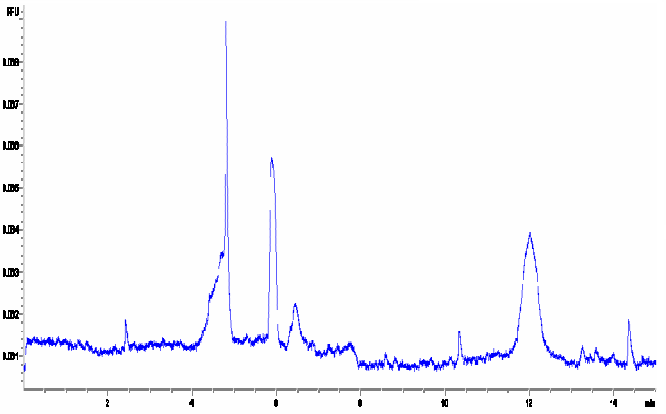


Figure 2: Non reduced Sample at 0.5 ng/mL; S/N=10

LOD* obtained for FITC labeled IgG is: **150 pg/mL**

TAMRA labeled IgG

Experimental conditions:

LIF Capillary:
Internal Diameter 50µm
Total length: 33 cm
Effective length: 14 cm

Method:
Injection: sample : 25 seconds at -15kV
Analysis condition: -20kV
Temperature (cassette and sample): 25°C

Sample:
Tetramethylrhodamine goat anti-mouse IgG

Instruments:
Agilent HP3D CE
Picometrics ZETALIF Discovery detector.
Laser: 532 nm (excitation)

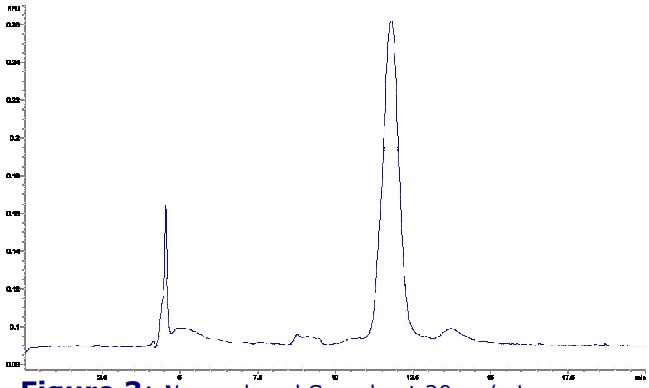


Figure 3: Non reduced Sample at 20 ng/mL

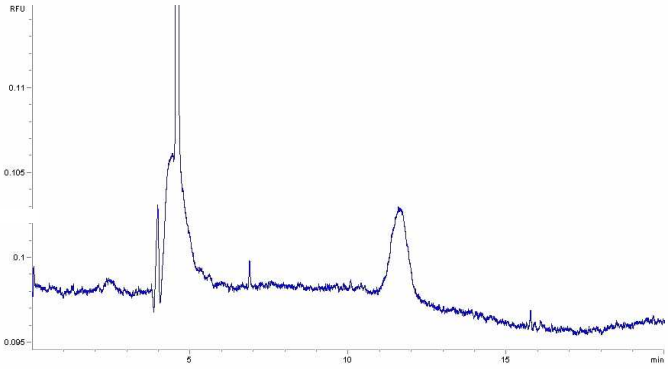


Figure 4: Non reduced Sample at 200 pg/mL; S/N=12

When the sample is labeled with TAMRA, preparation and purification of the samples requires many steps [1]: Buffer exchange using NAP-5 columns, Incubation with TAMRA, Buffer exchange (dye removal) using NAP-5 column.

LOD* obtained for TAMRA labeled IgG is: **50 pg/mL**

* LOD calculated for a ratio S/N=3

[1] Anal.Chem. 2006, **78**, 6583-6594

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Native Fluorescence in CE SDS with LIF

A deep UV laser allows excitation of the tryptophan and tyrosine contained in proteins

Like the UV absorbance detection, native LIF does not require a sample preparation step.
In CE SDS, The DAD detector provides an LOD* of µg/mL range. Native LIF detection demonstrates sensitivity nearly equivalent to silver stained gels (10ng/ml)[2] when using LIF detection.

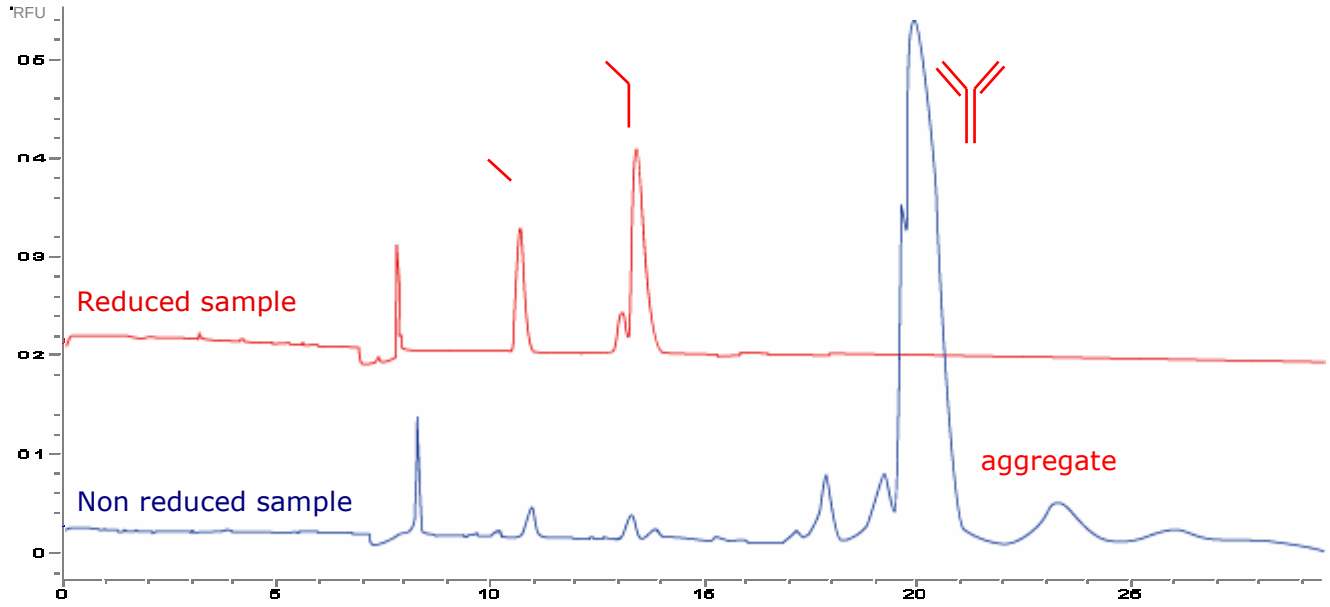

Figure 1: Sample injection at 1 mg/mL;

Experimental conditions:

Sample:
IgG Standard

Instruments:
Agilent HP3D CE
Picometrics ZETALIF Discovery detector.
Laser: 266 nm (excitation)

LIF Capillary:
Internal Diameter 50µm
Total length: 33 cm
Effective length: 14 cm

Method:
Injection: water : 1000mbars, 0.2min
sample : 15 seconds at -15kV
wait 0.2min in vials of water
Analysis condition: -15kV
Temperature (cassette and sample): 15°C

The figure 1 shows the impurity profile of a reduced and non reduced IgG standard, even apparently pure antibodies .

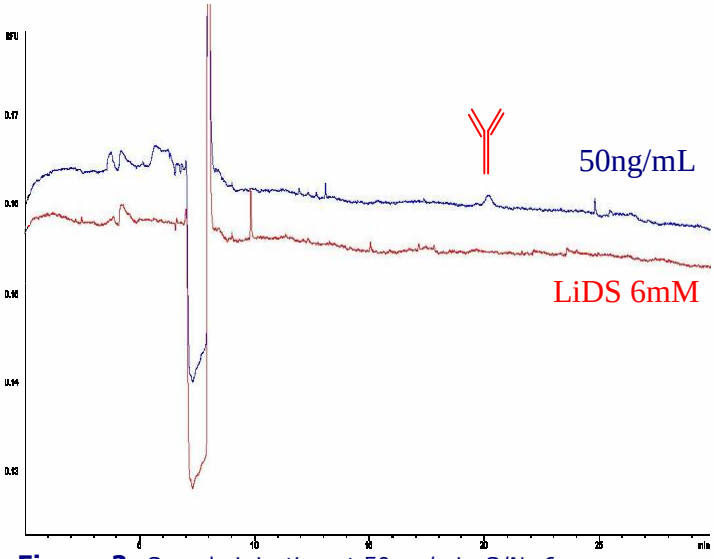

Figure 2: Sample injection at 50 ng/mL; S/N=6

Native fluorescence detection with Laser Induced Fluorescence is a simple and sensitive solution for MAbs production like impurities assay thanks to the good resolution obtained. Antibody degradation, molecular instability or manufacturing inconsistency can be easily followed with this method.

LOD* obtained with native fuorescence IgG is: **25 ng/mL**

[2] Hunt G, Nashabeh W (1999) Anal Chem.**71**, 2390–97.

* LOD calculated for a ratio S/N=3

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Comparison of the different detection in CE-SDS

- UV Absorbance
- Native Fluorescence
- Fluorescence derivatization

CE-SDS	UV Absorbance	Native Fluorescence	Fluorescence Derivatization	
Wavelength nm	210 -220	266	488	532
LOD	500ng/ml[2] to 1µg/ml	25ng/ml	150pg/ml	50pg/ml

Email : info@picometrics.com
Web site: www.picometrics.com

[2] Hunt G, Nashabeh W (1999) Anal Chem,71:2390–97.