

# Fast Isoelectric Focusing Method Development with imaged CIEF (*i*CIEF) for Protein Analysis

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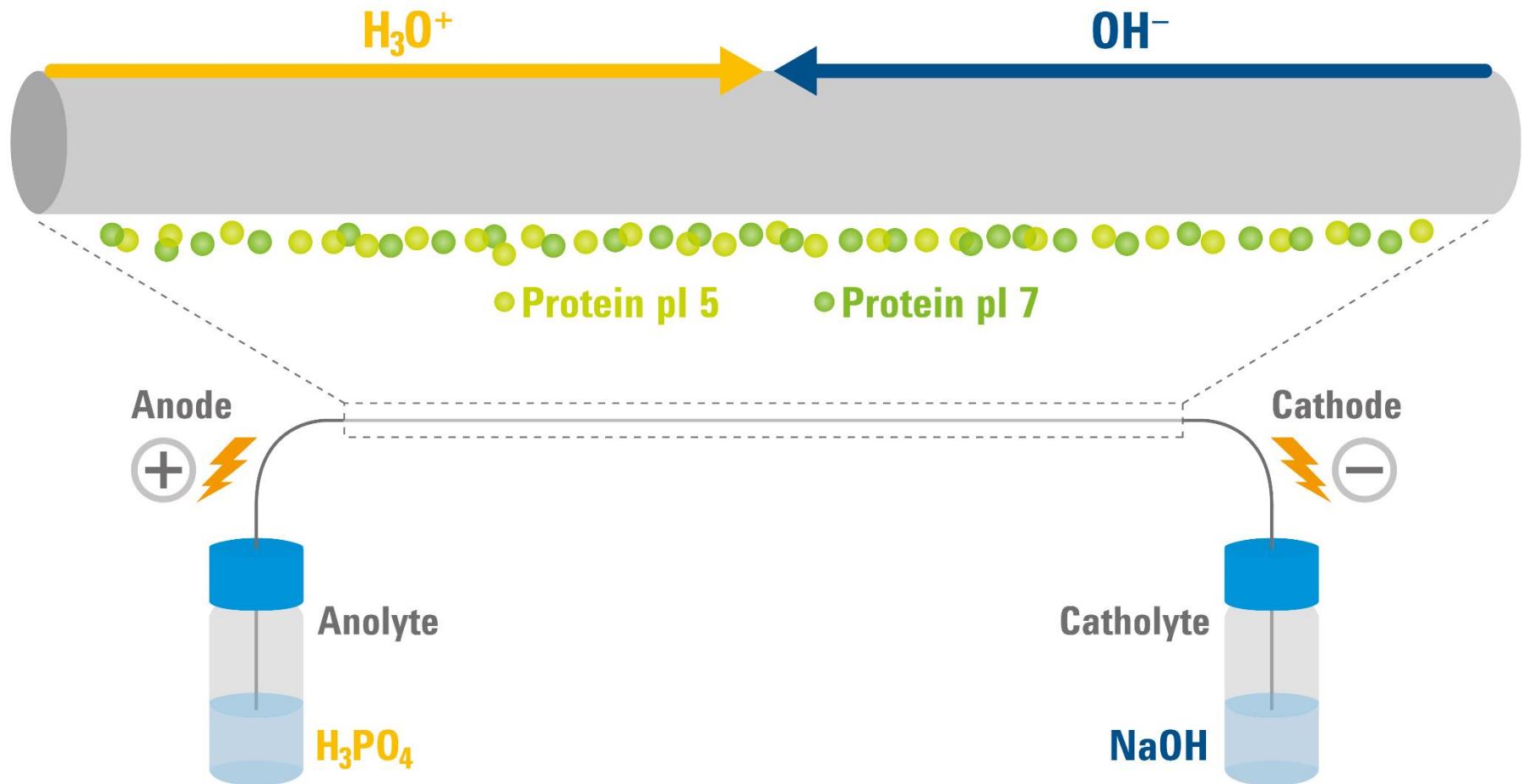
# Basic Principles of CIEF



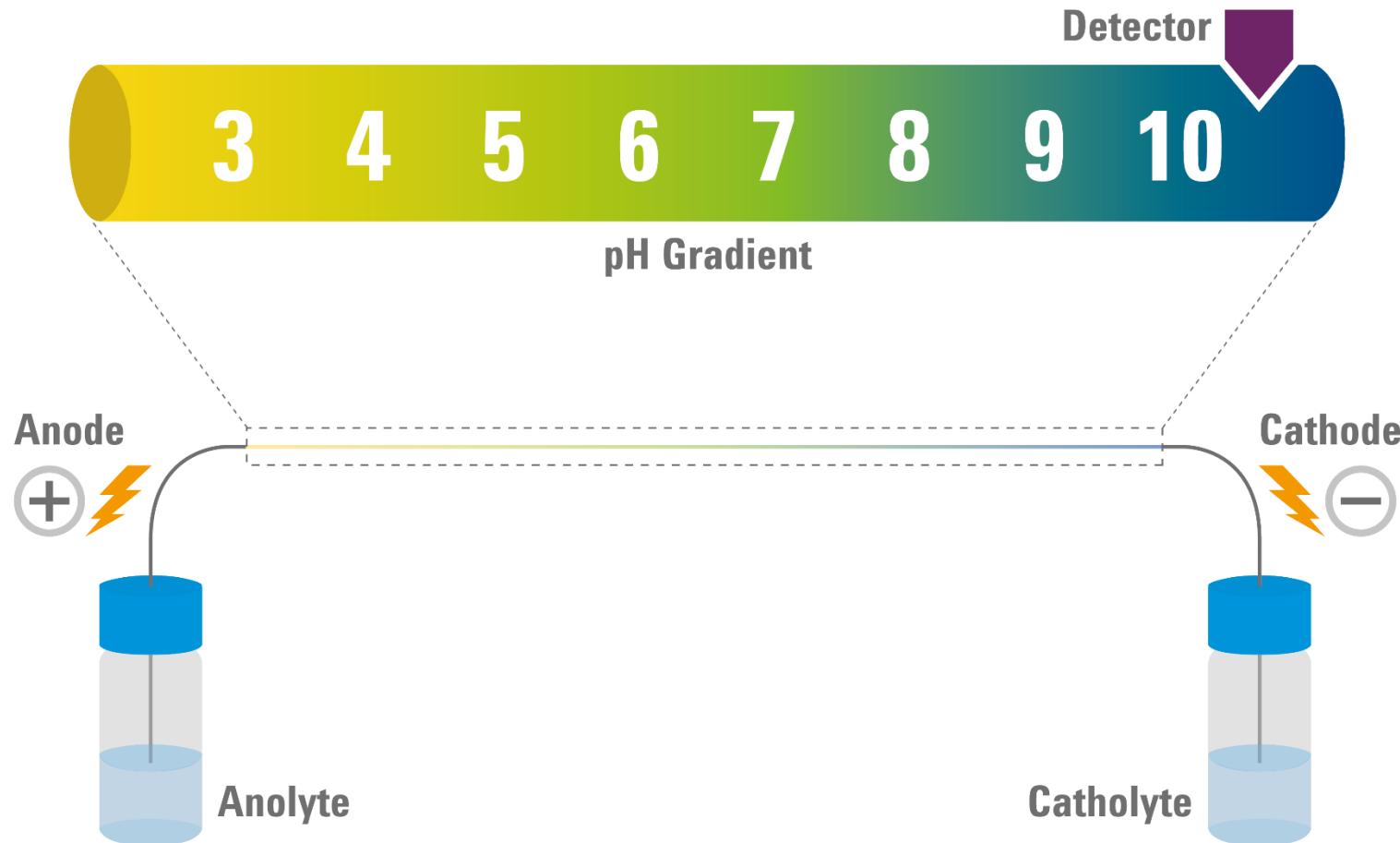
At the beginning of a CIEF run, the whole capillary (coated FS) is filled with a mixture of:

- Ampholytes (mixture of oligomers containing acidic and basic functional groups)
- Protein or peptide sample to be analyzed
- pI standards
- Polymers like e.g methyl cellulose to inhibit EOF in case a bare FS capillary is used
- Optional additives to increase solubility of proteins
- Sacrificing ampholytes that keep the focused zones before the detection zone
- The inlet low pH (anolyte); the outlet high pH (catholyte)
- High positive voltage in the anolyte vial

# Basic Principles of CIEF

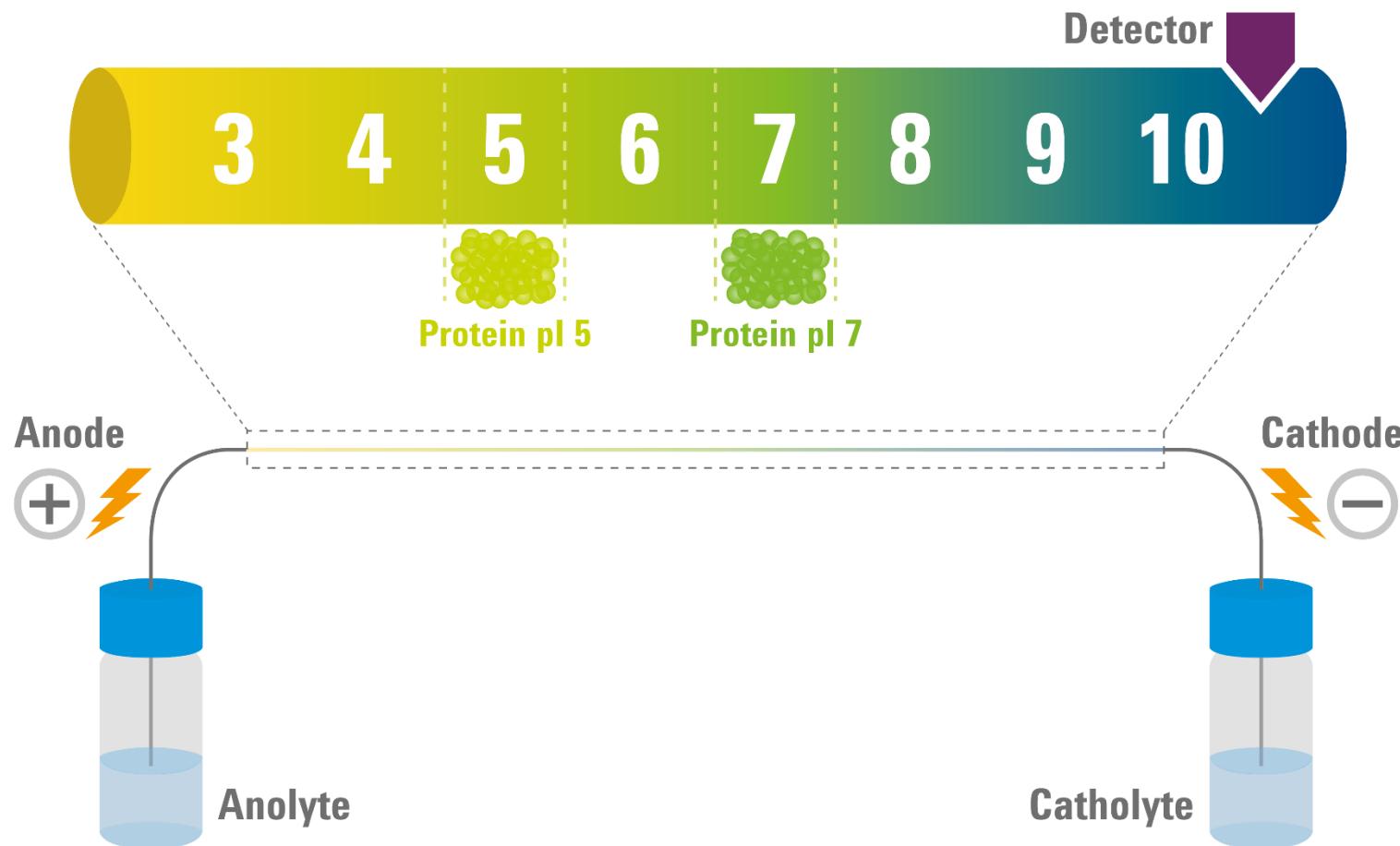


# Basic Principles of CIEF



Slide courtesy of Agilent Technologies

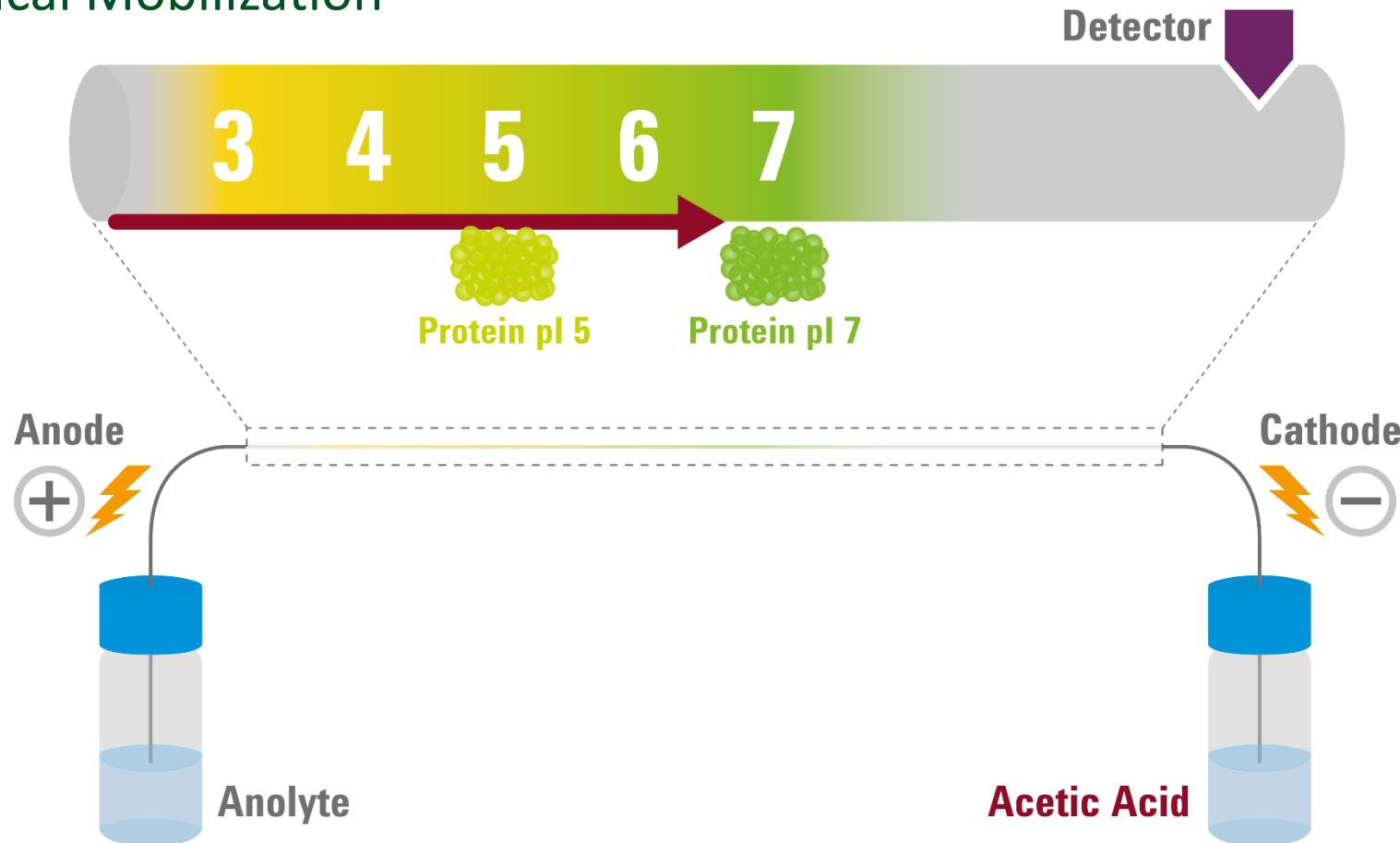
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Slide courtesy of Agilent Technologies

# Basic Principles of CIEF

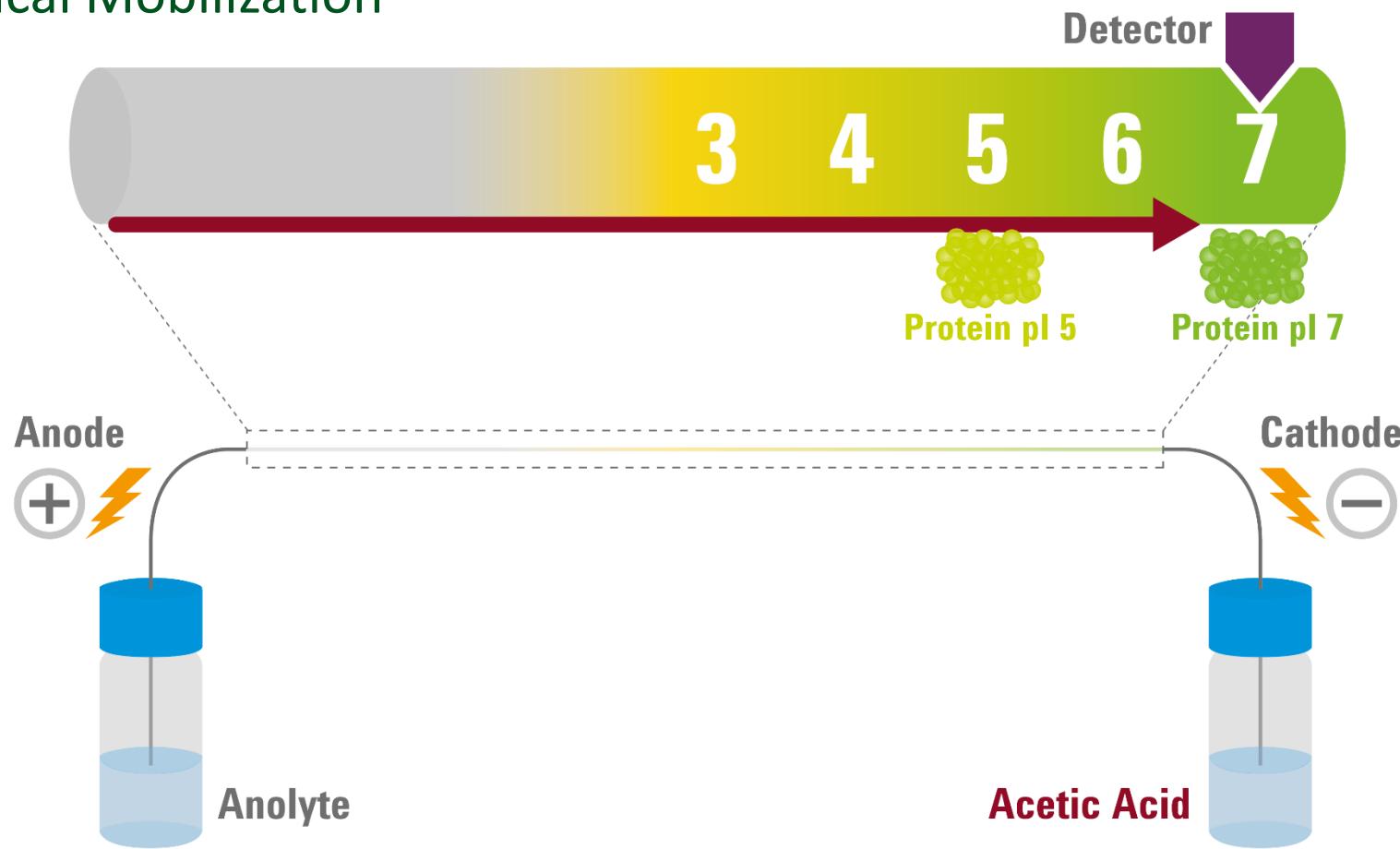
## Chemical Mobilization



Slide courtesy of Agilent Technologies

# Basic Principles of CIEF

## Chemical Mobilization



Slide courtesy of Agilent Technologies

# Conventional cIEF

## Pro's

- Uses existing commercial CE instruments
- Provides quantitative protein analysis in applicable concentration range (0.05 – 0.5 mg/mL)



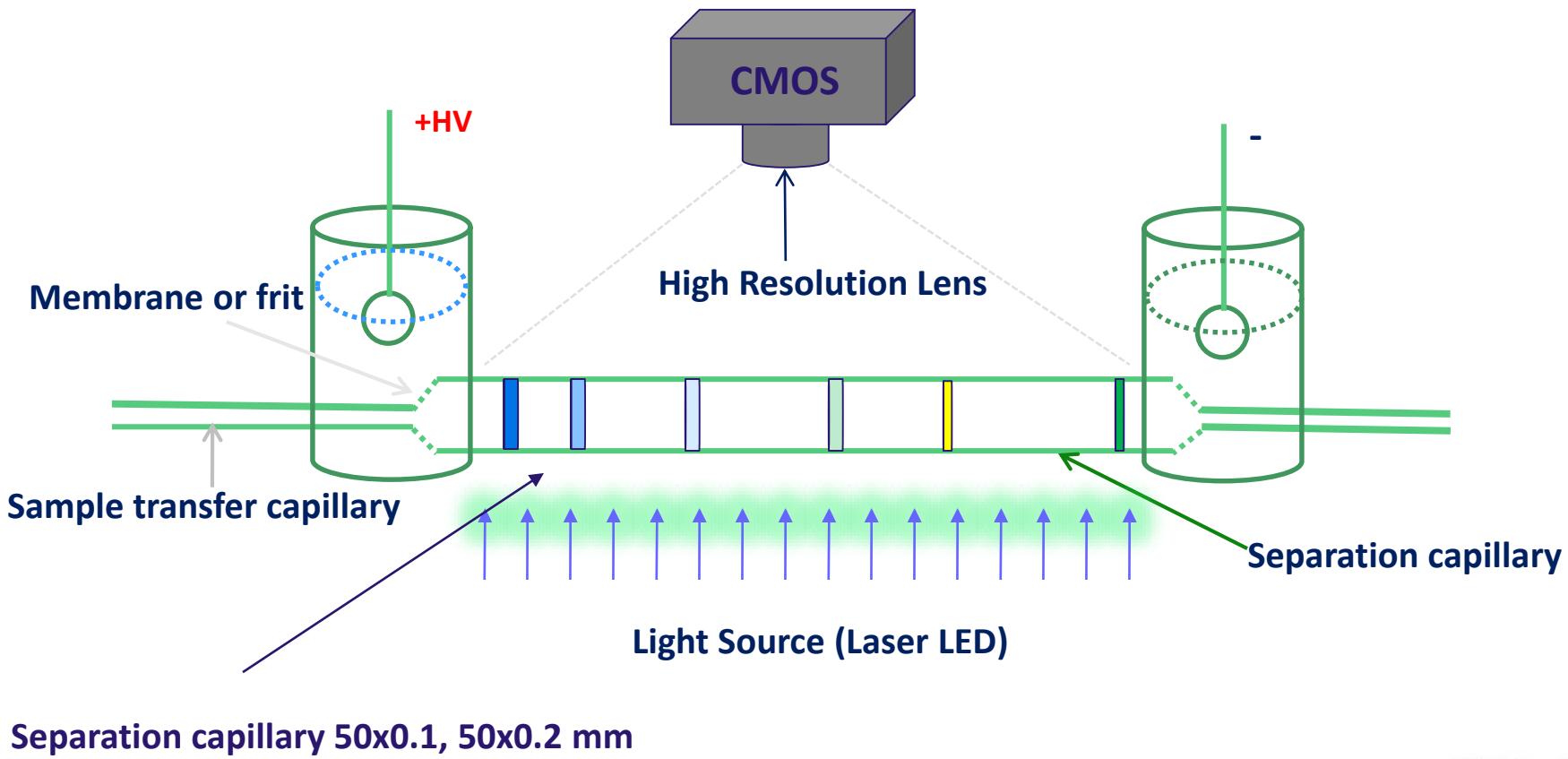
## Con's

- Long cycle time (focusing & mobilization takes about 1 run/hour)
- Time-consuming method development
- Additives to suppress anodic & cathodic drift
- Non-reproducible peak pattern
- Resolution loss during mobilization



CEInfinite

# Basic Principle of *i*CIEF



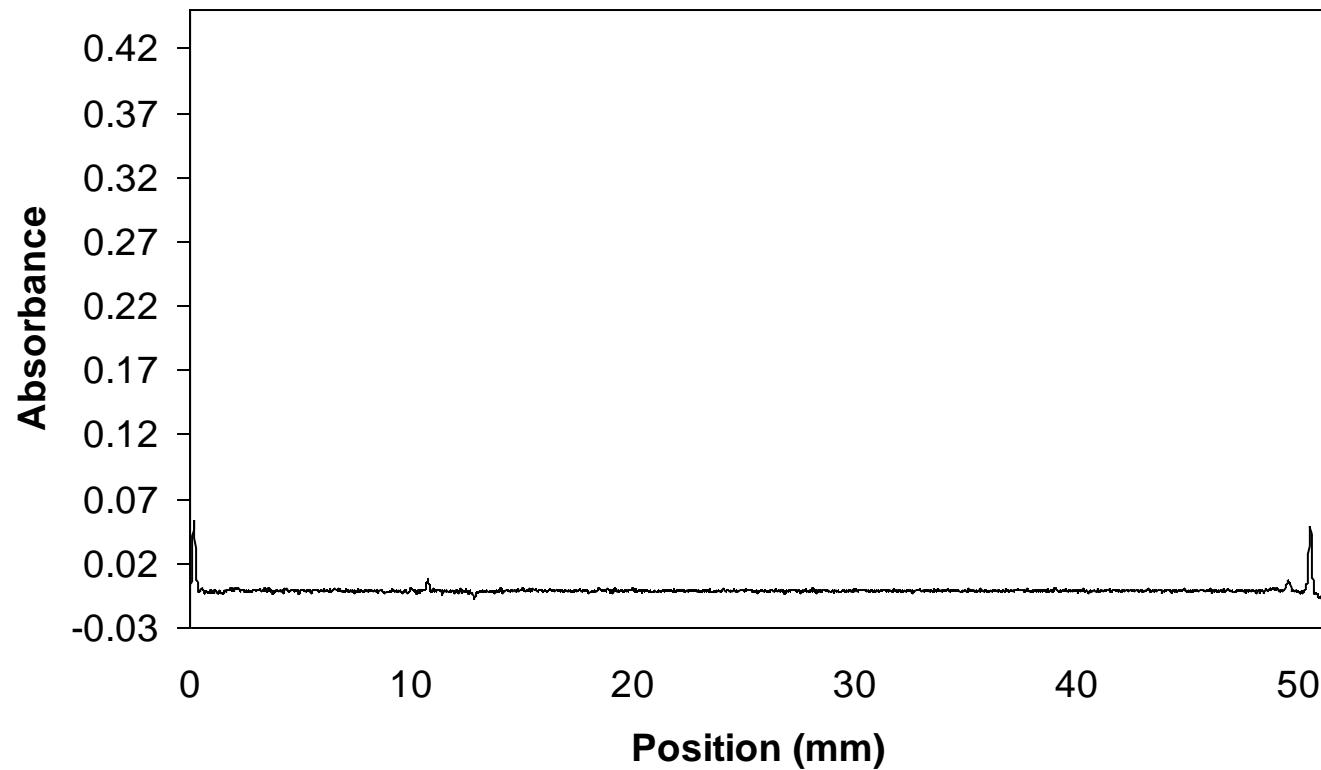
Separation capillary 50x0.1, 50x0.2 mm

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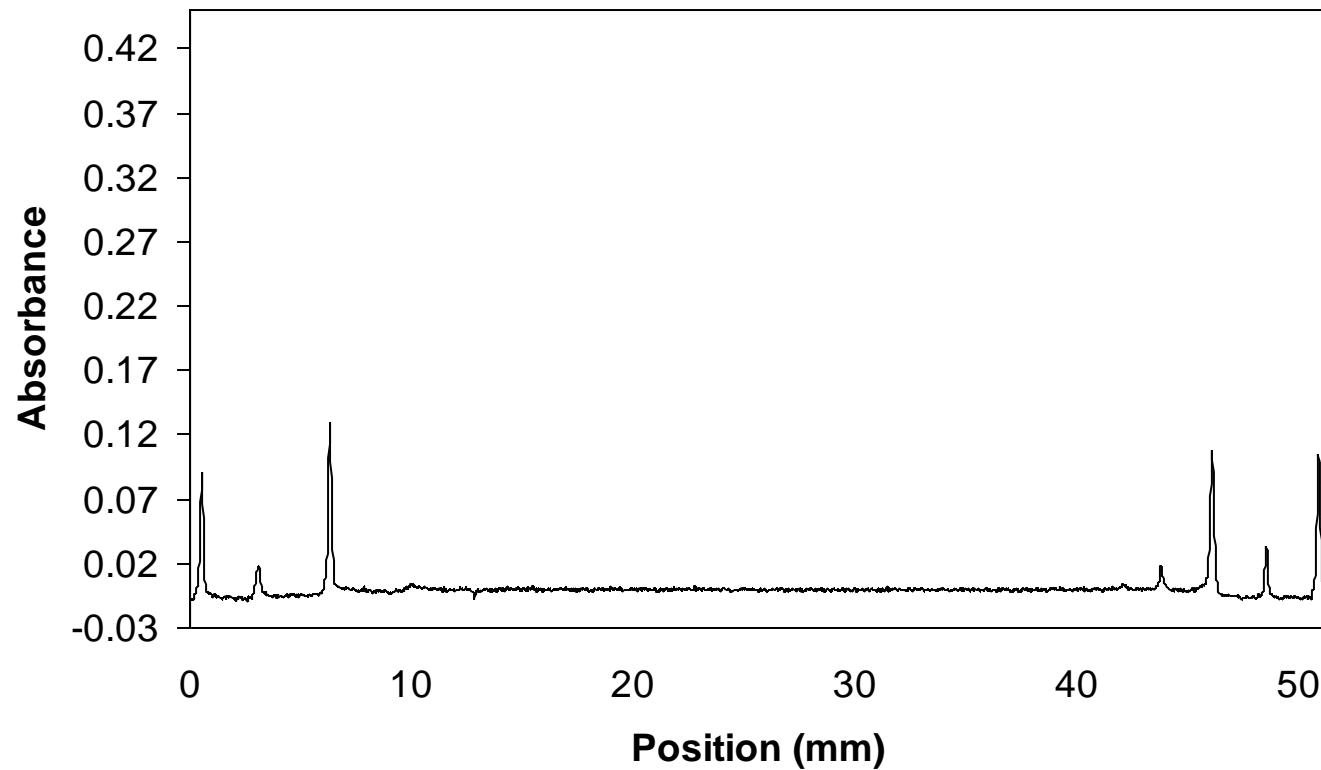
# Basic Principle of *i*CIEF

**Development of Separation of five *pI* Markers**

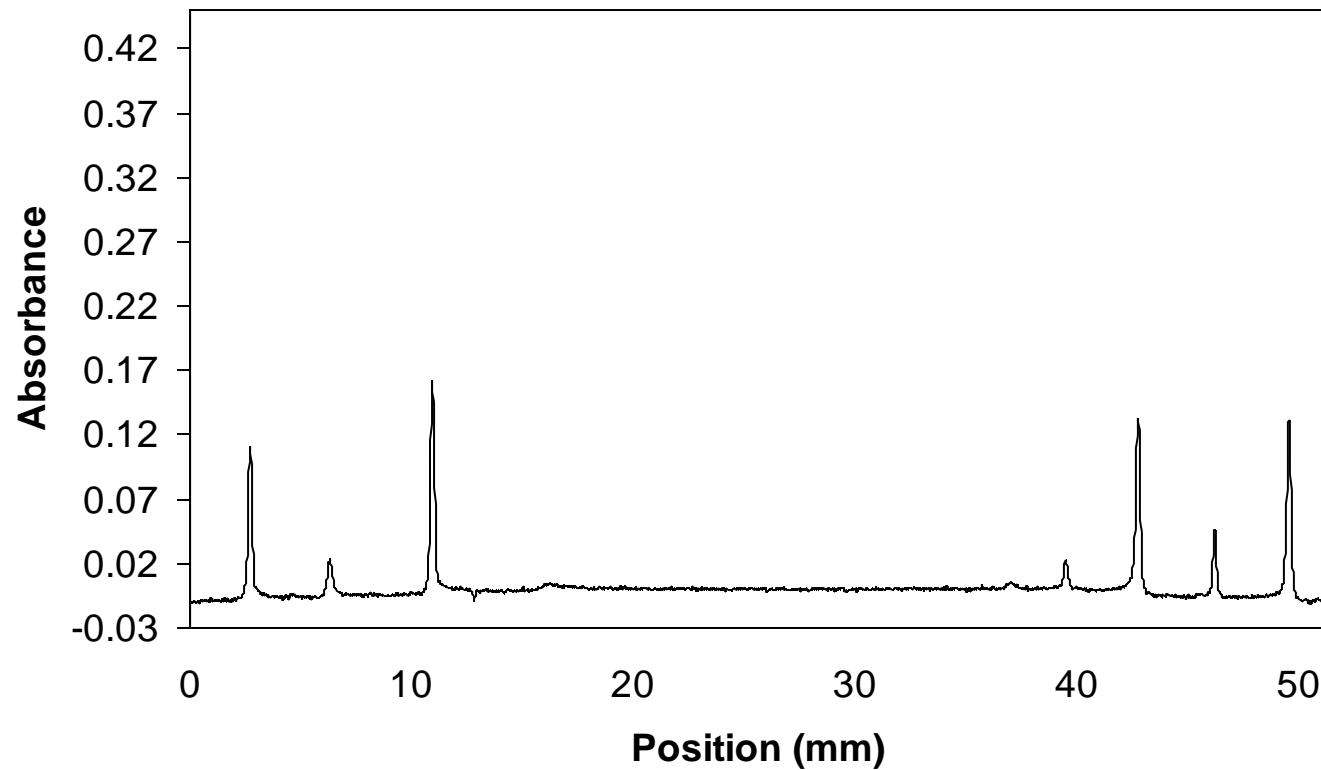
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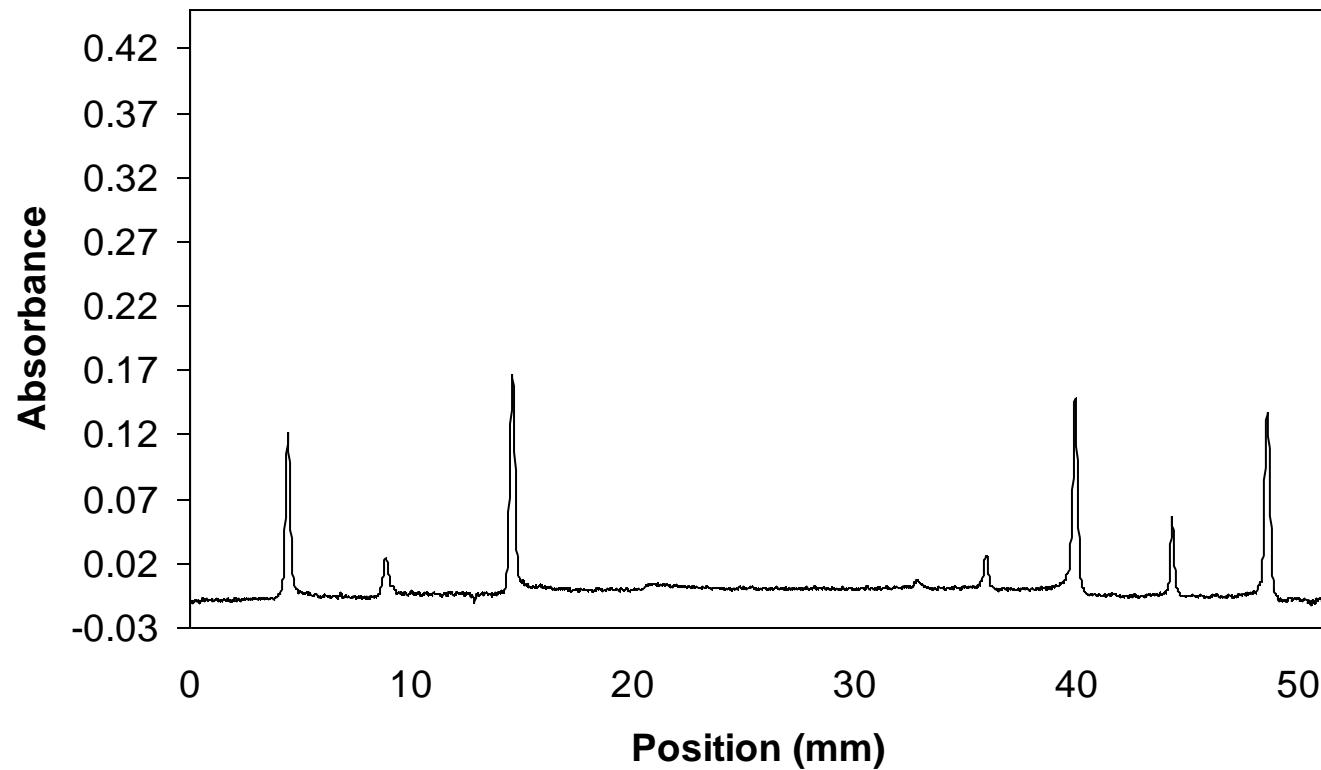
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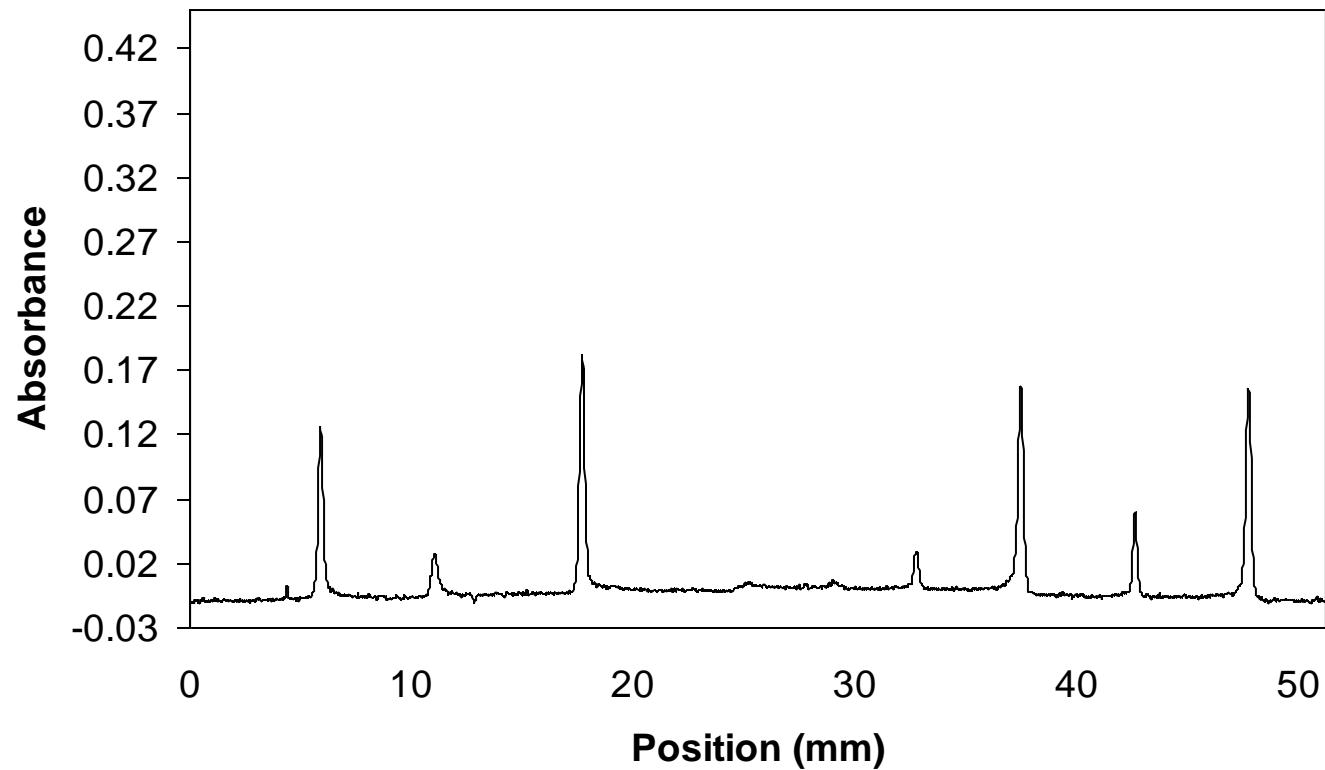
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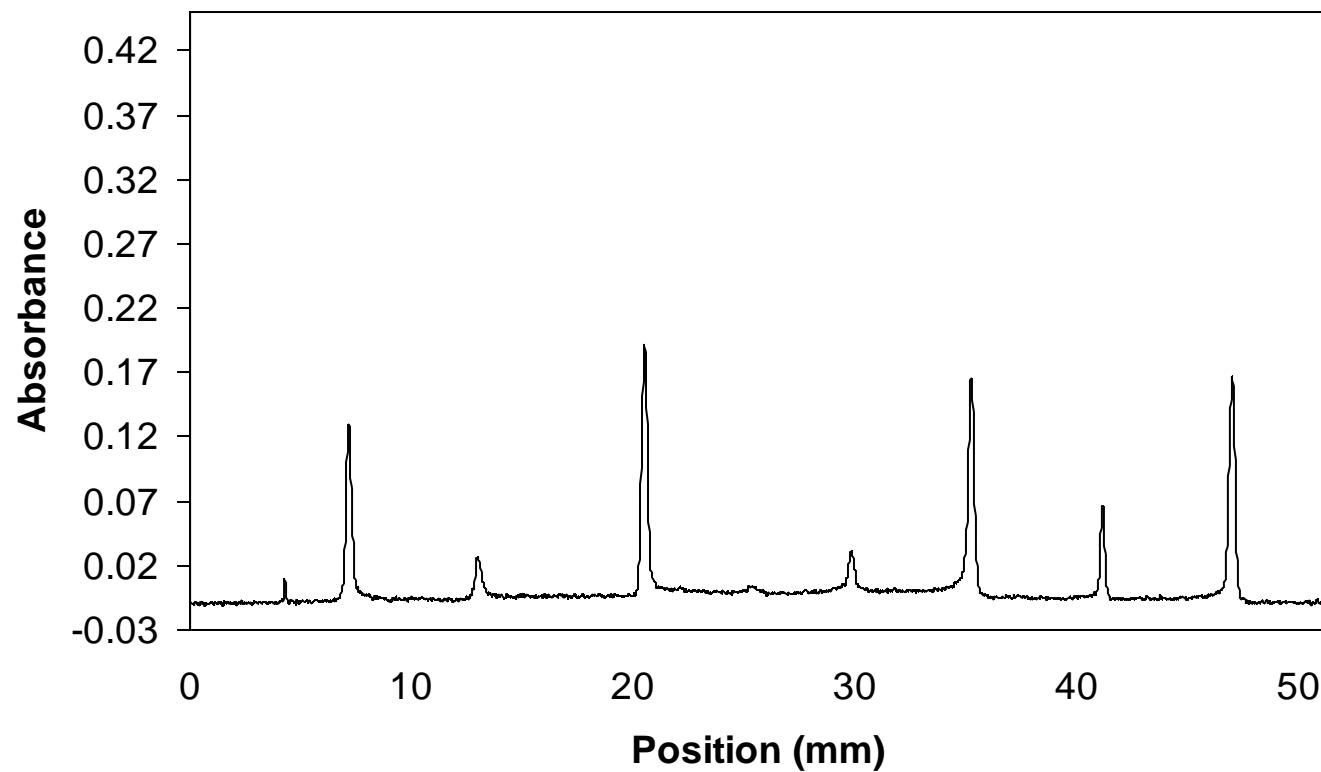
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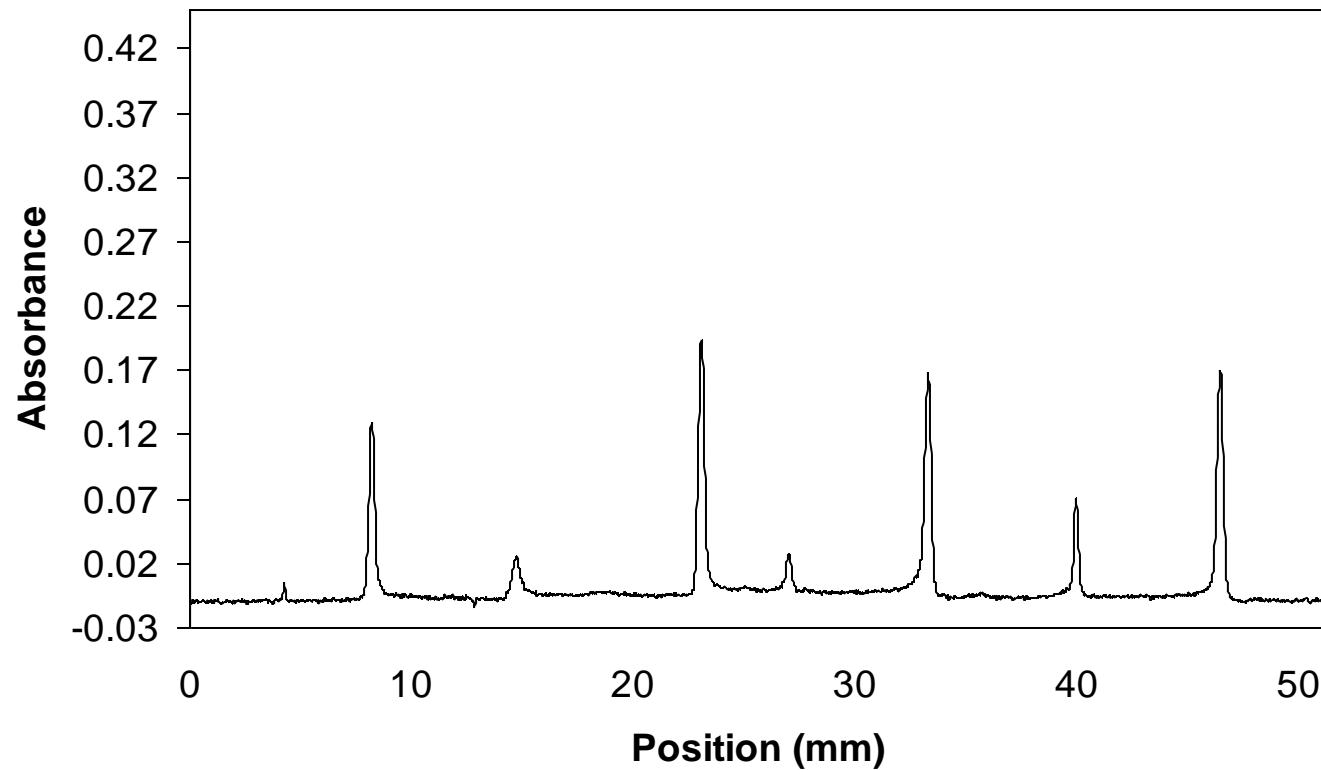
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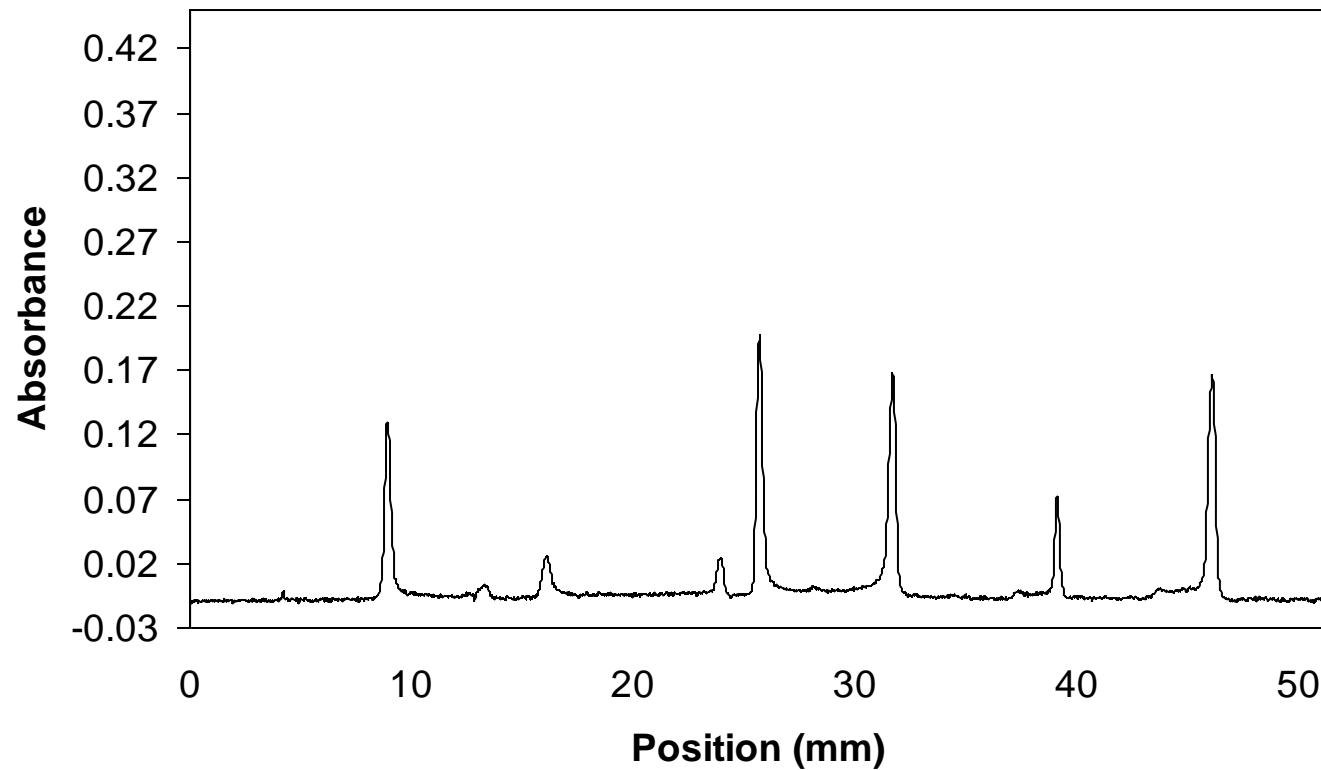
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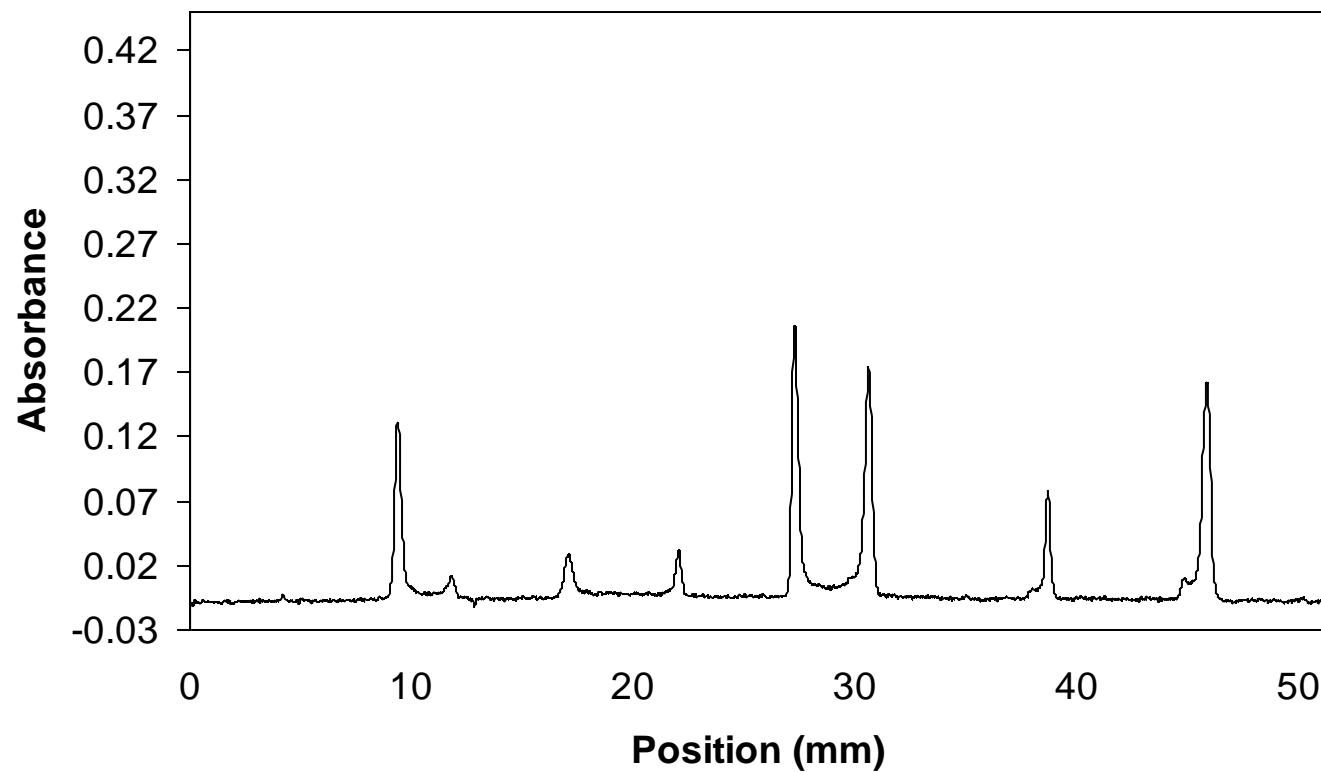
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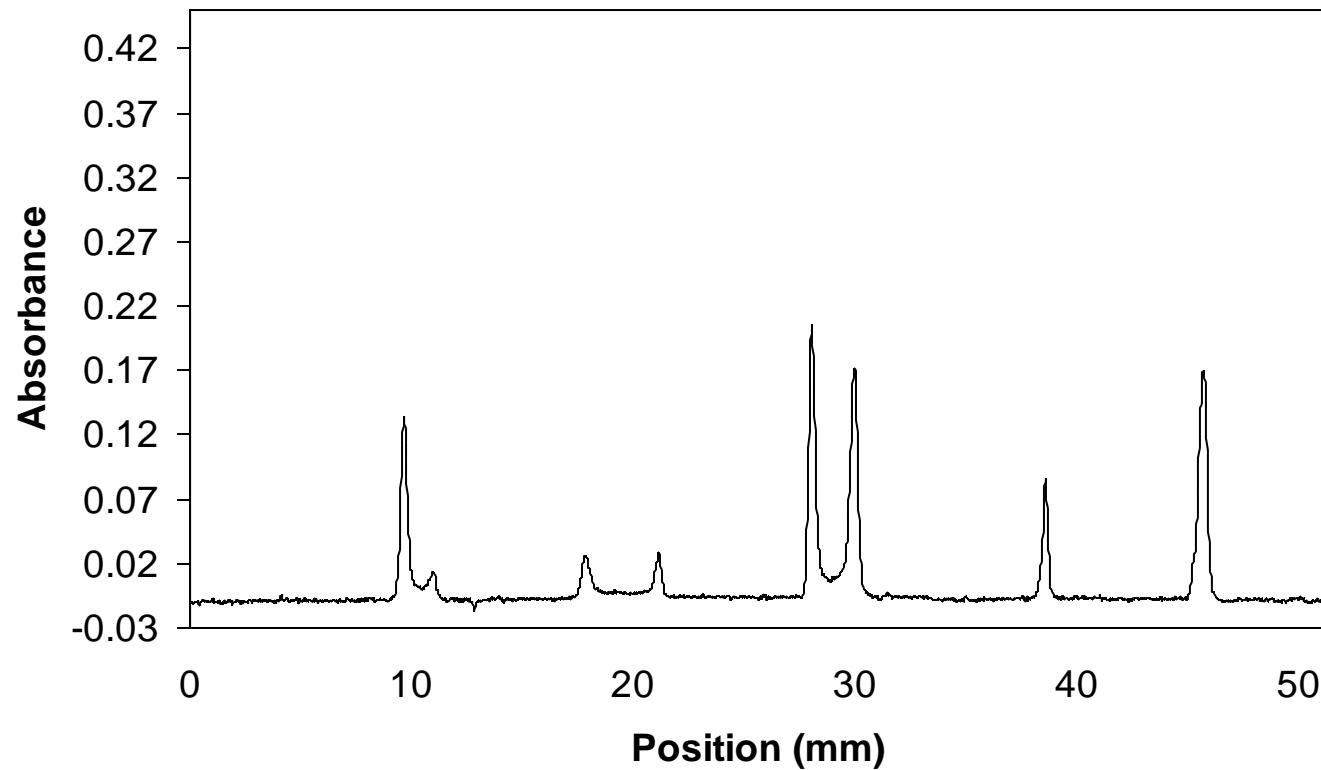
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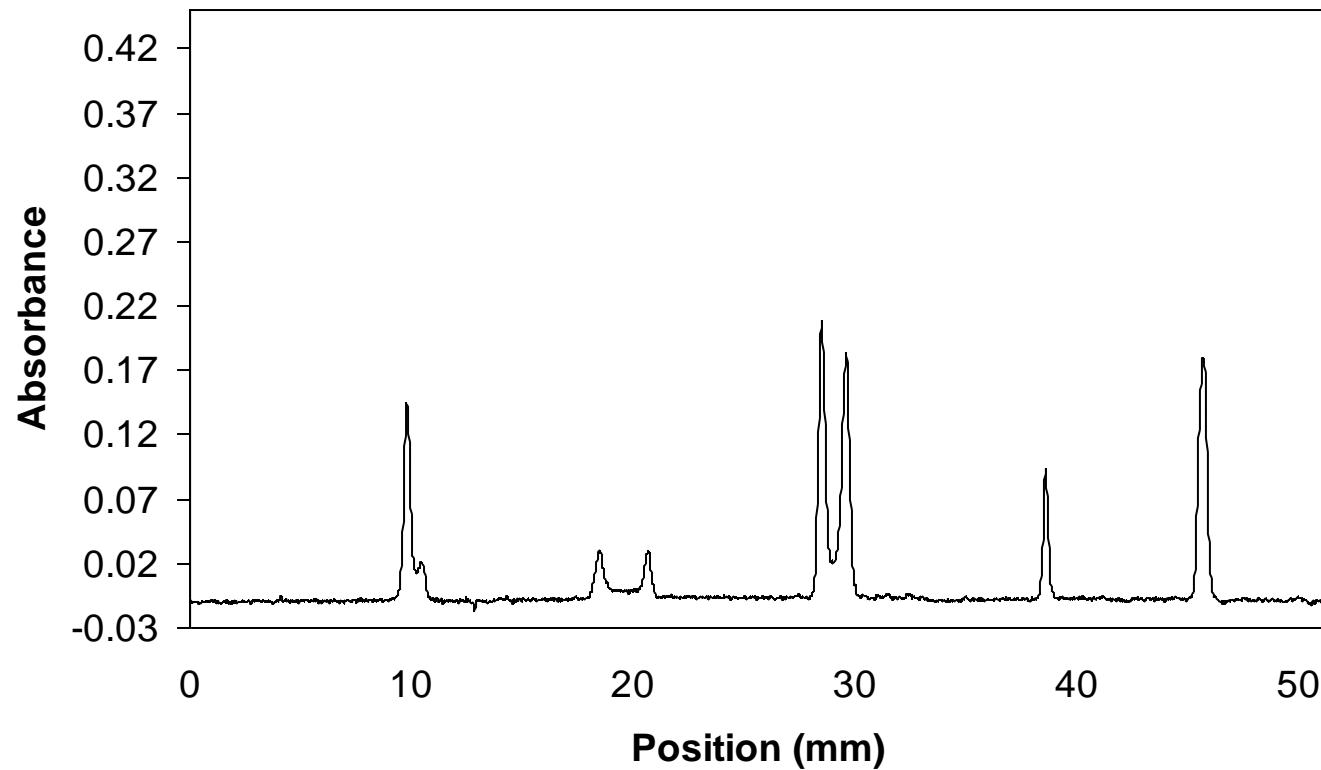
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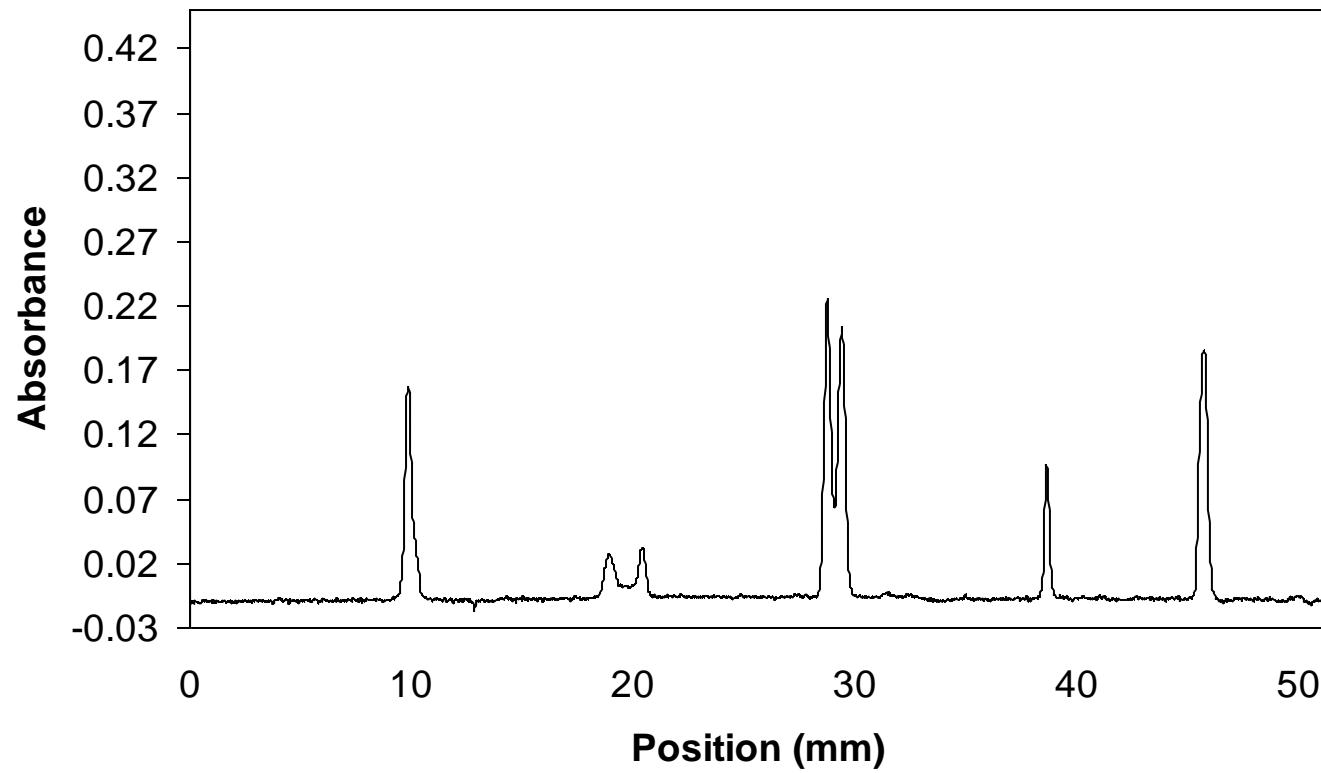
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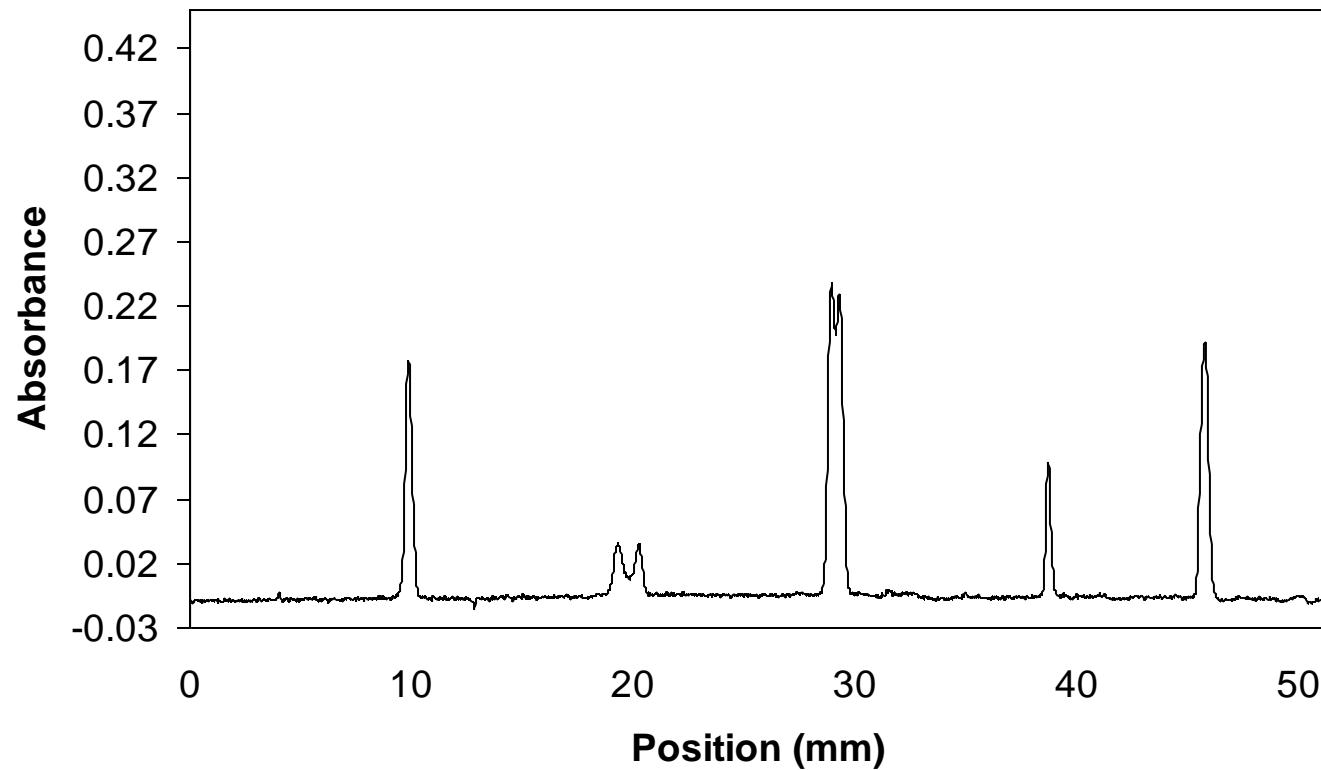
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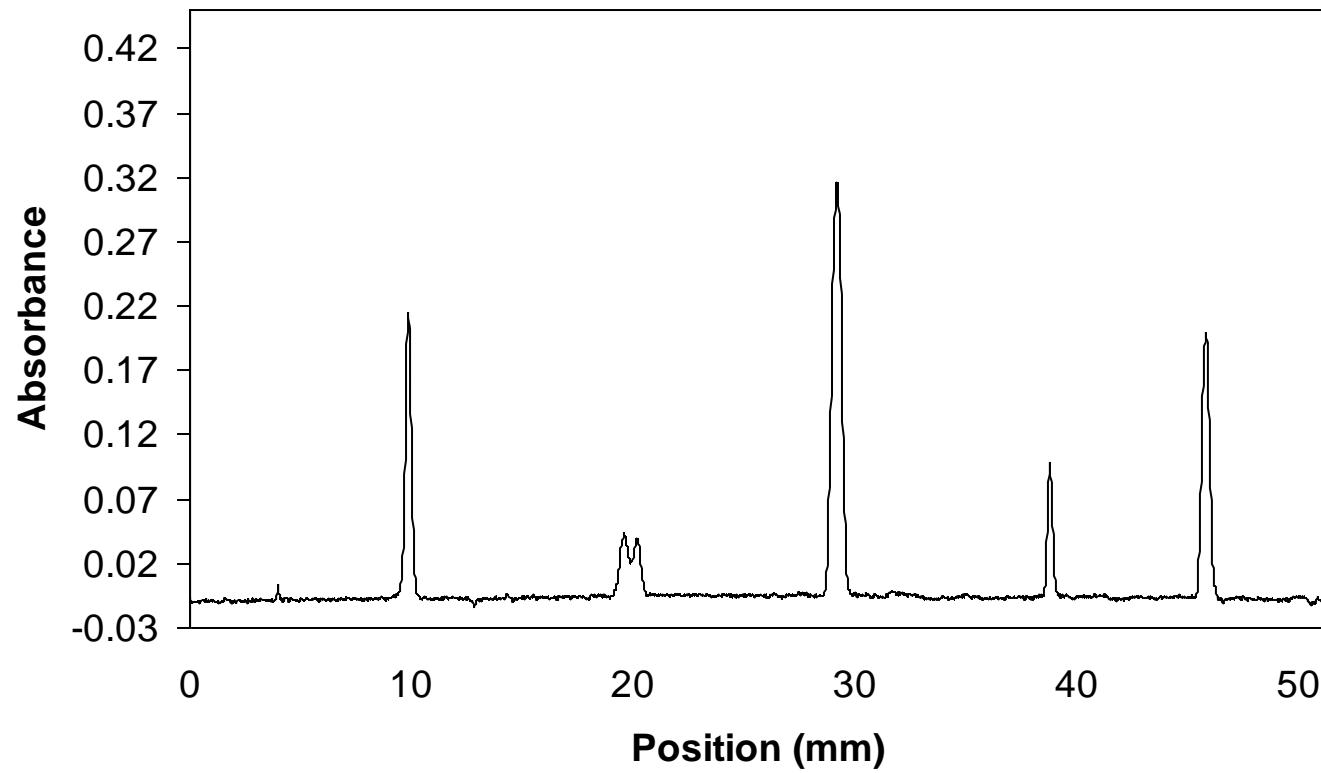
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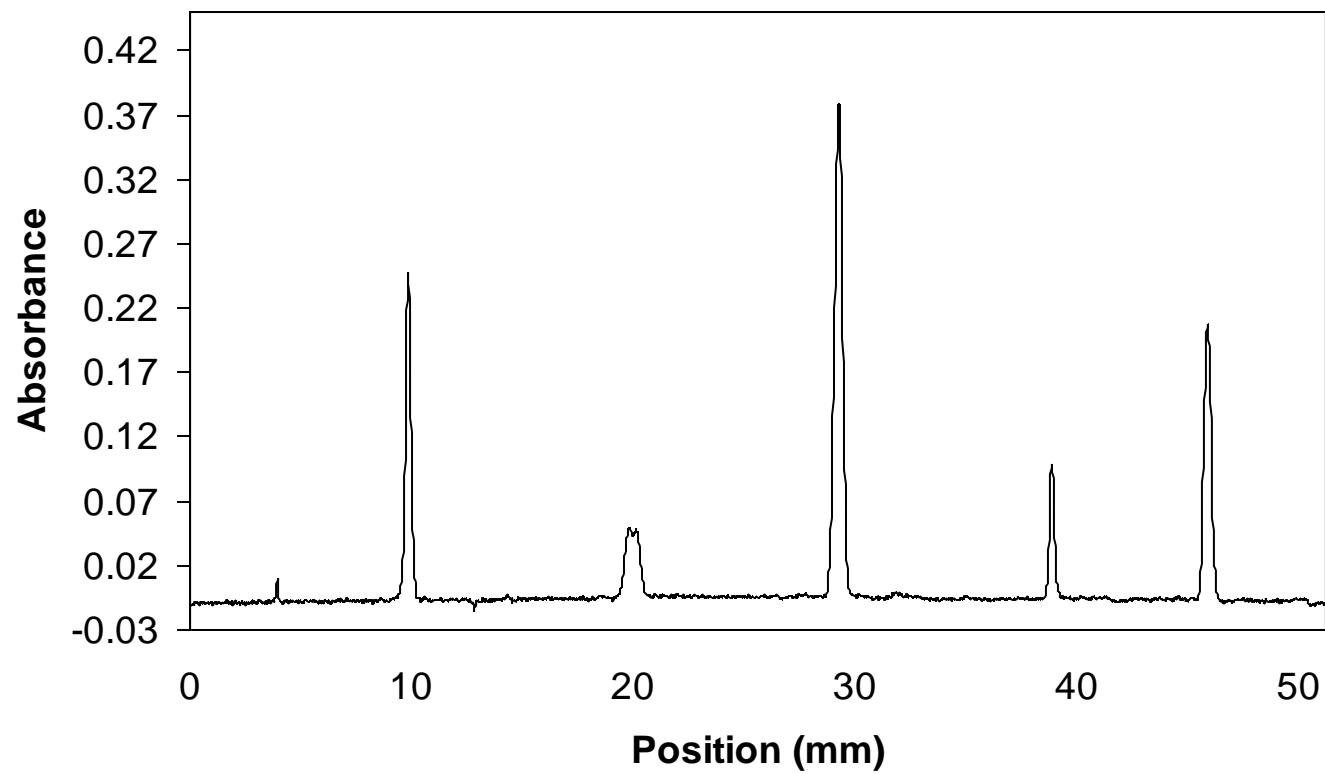
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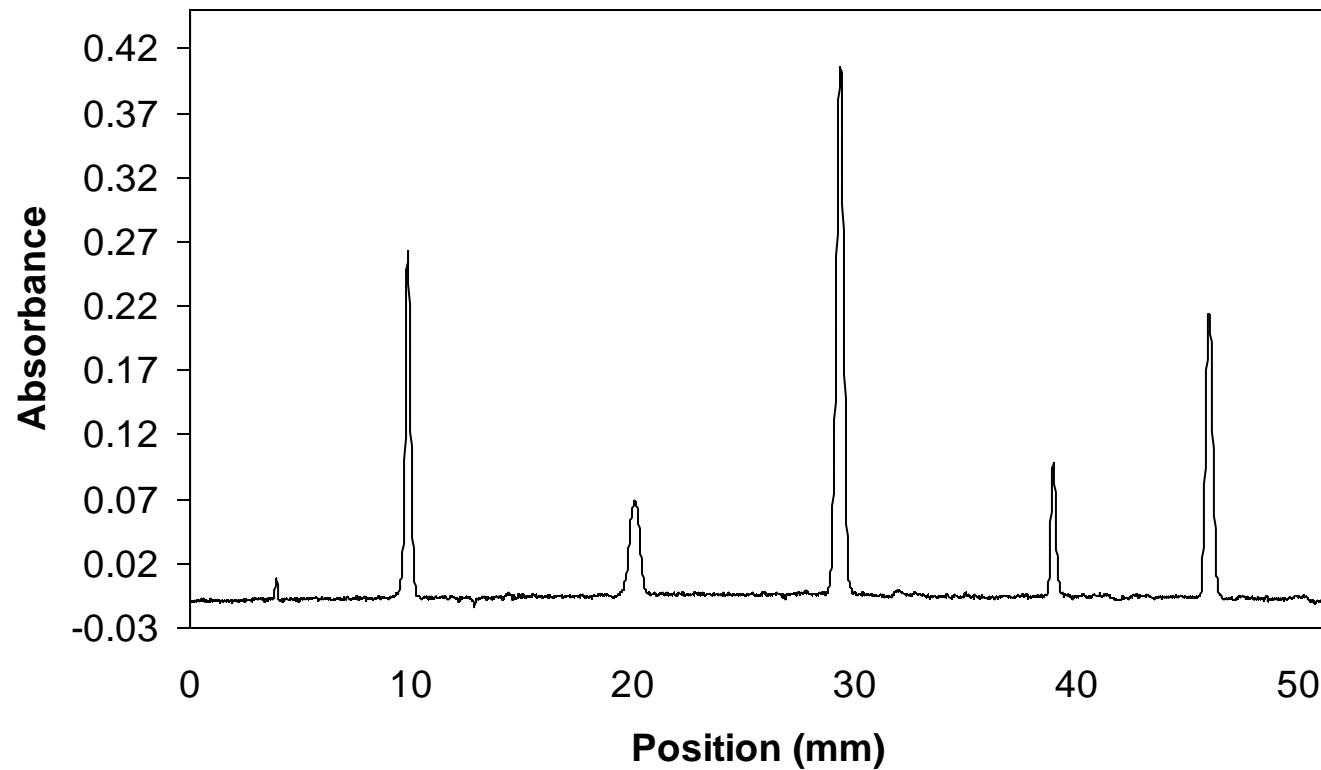
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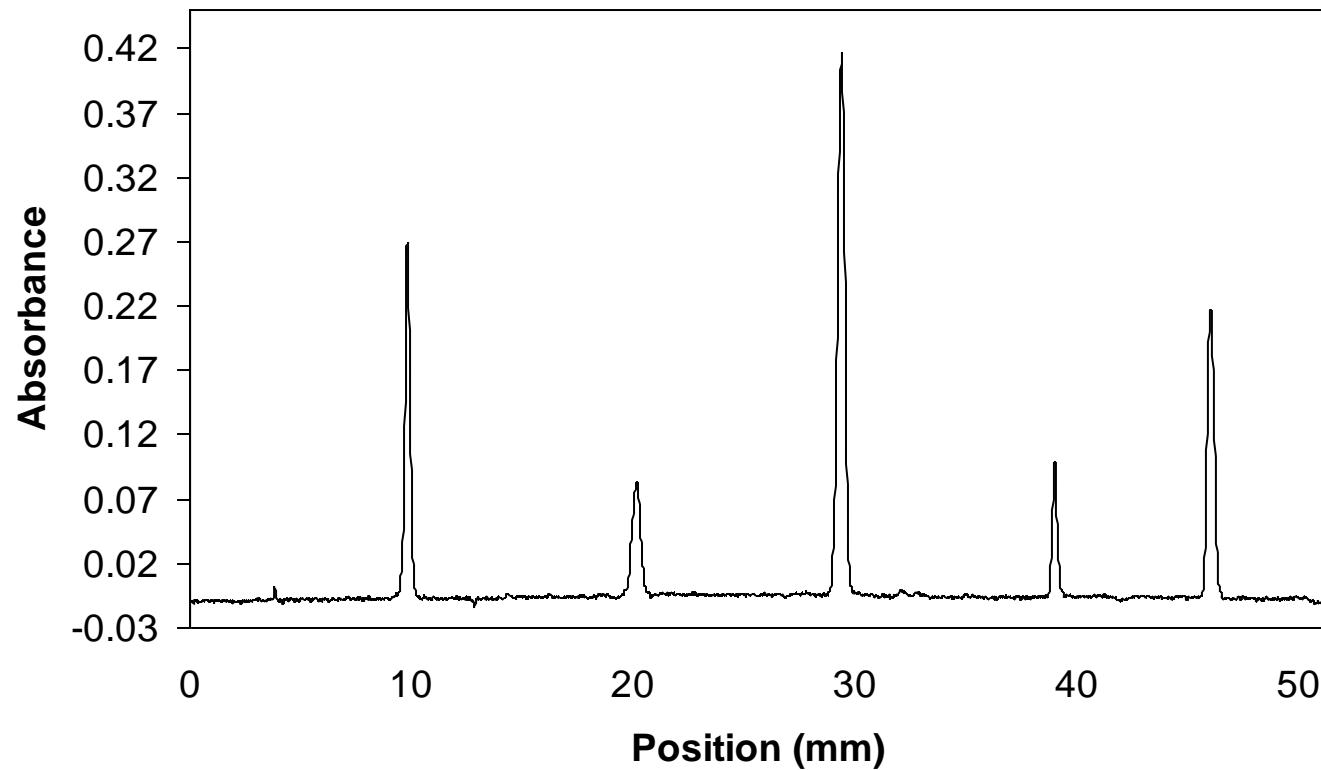
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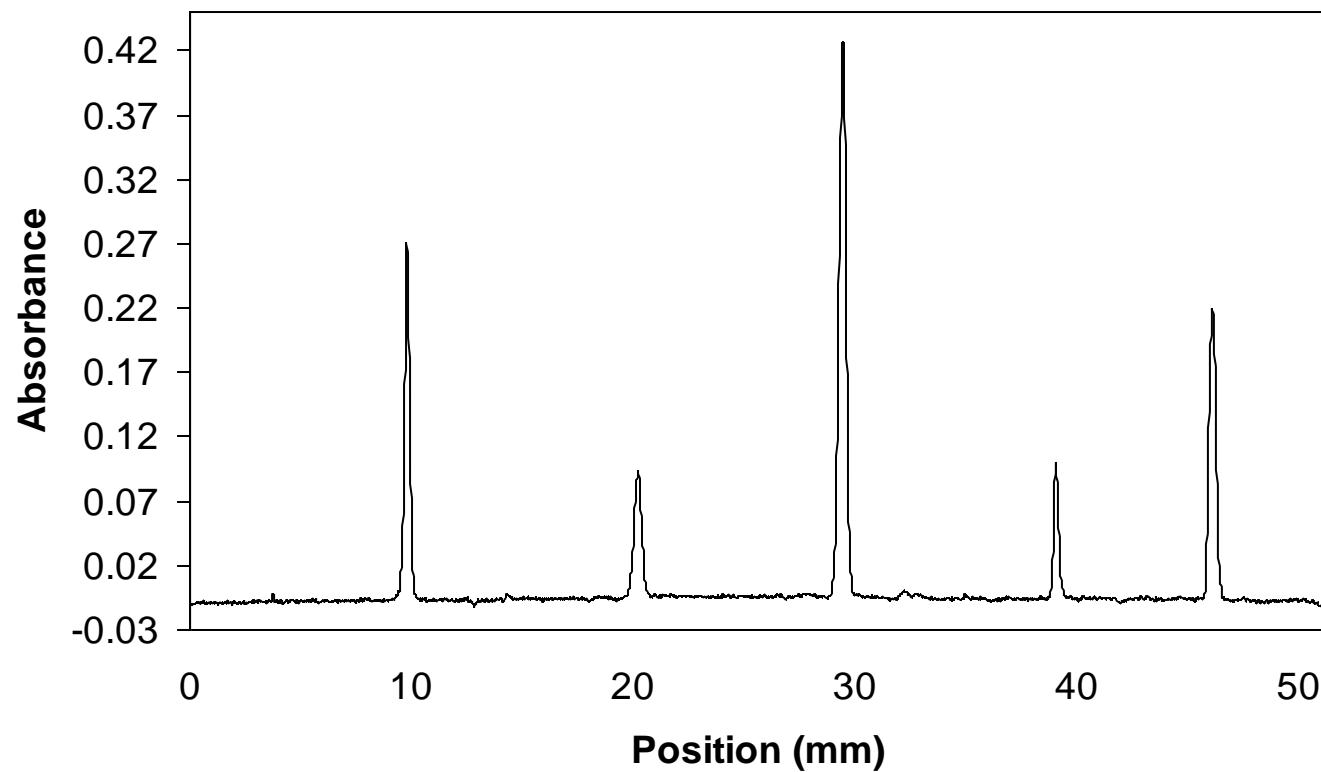
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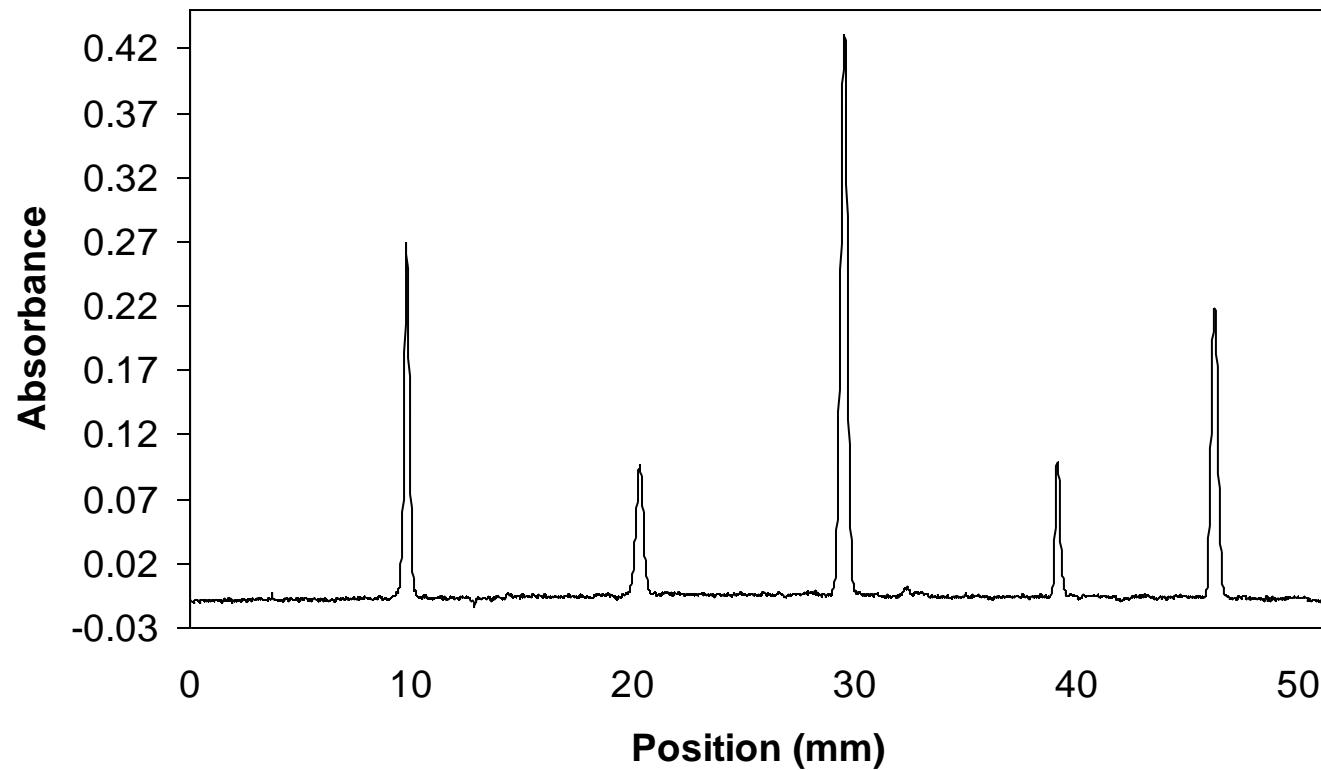
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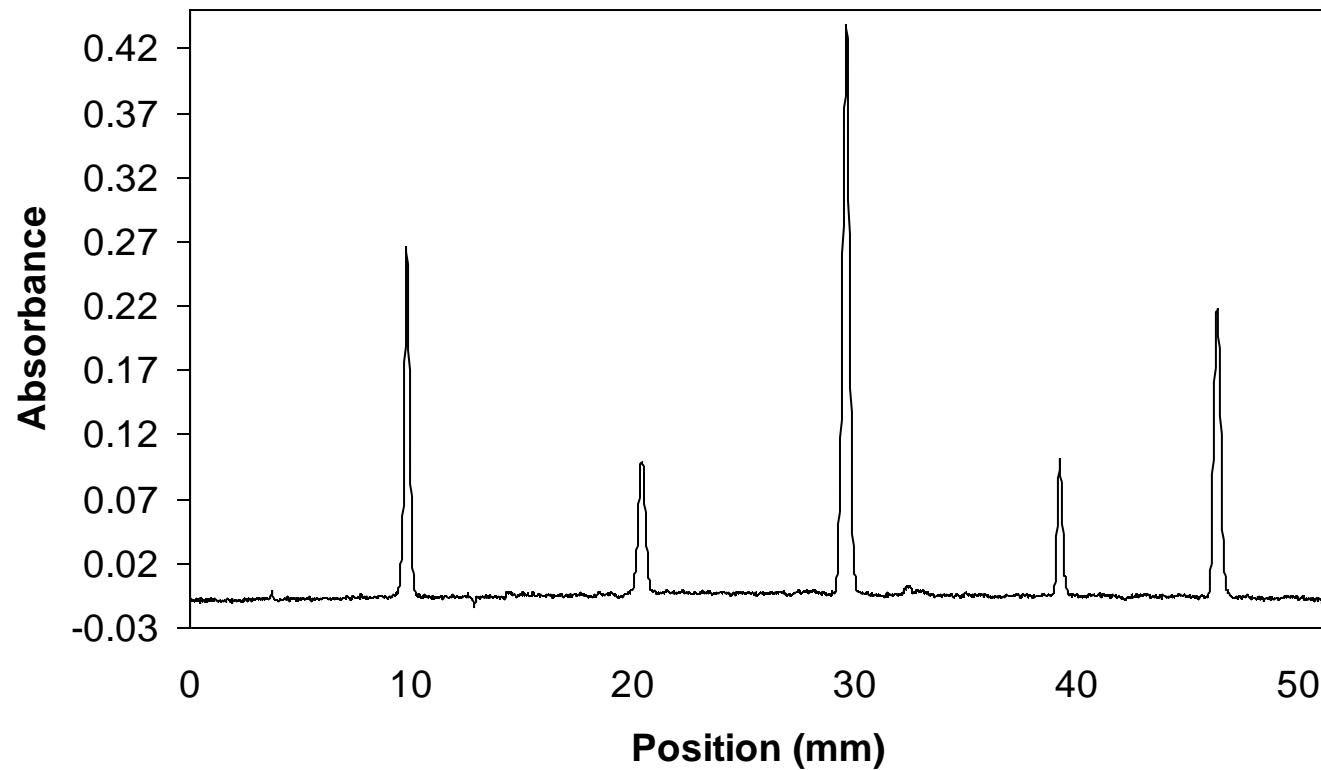
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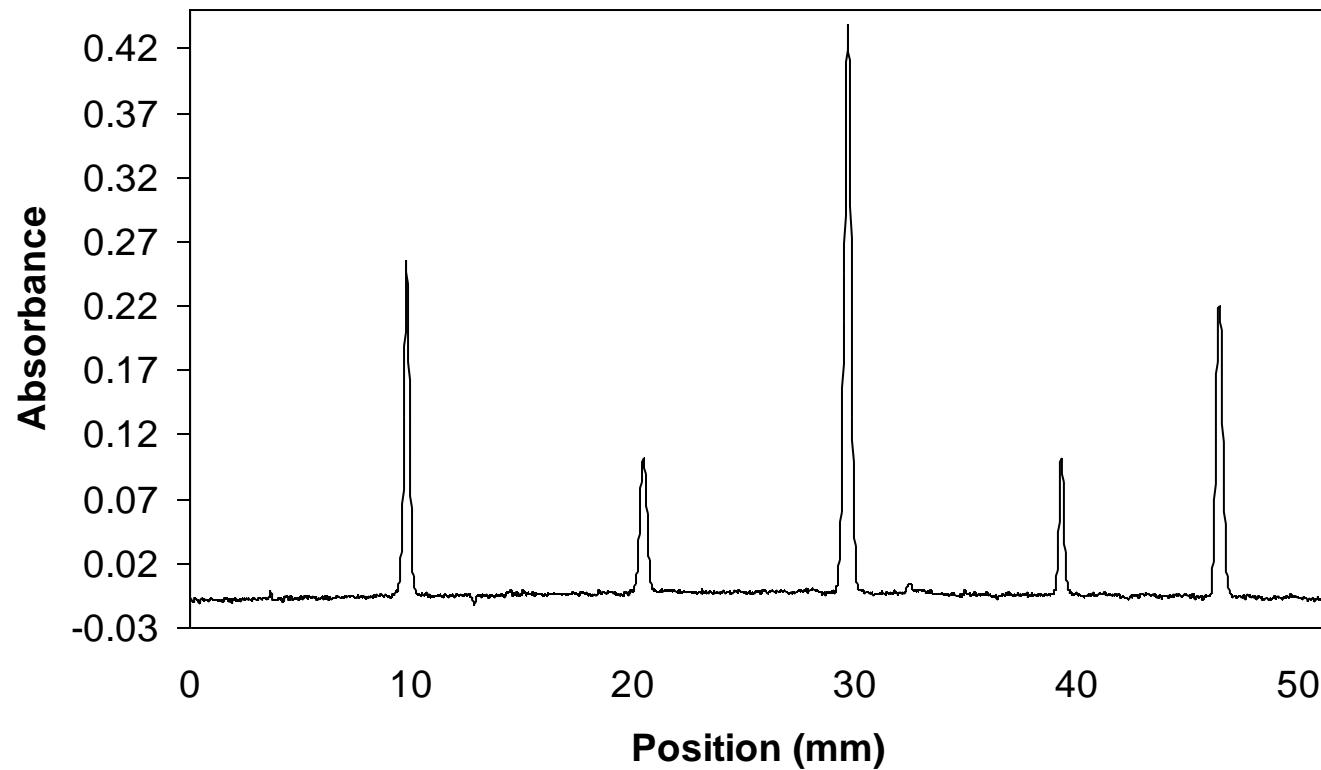
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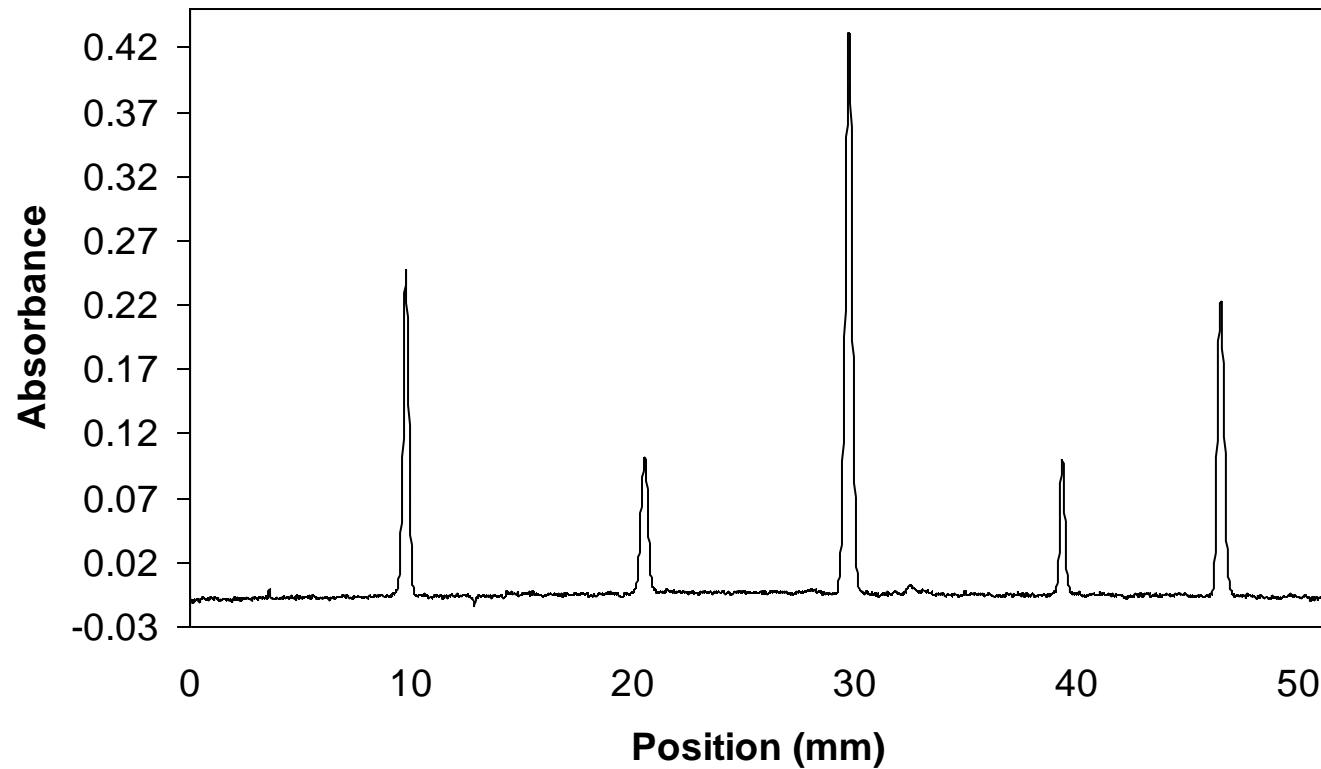
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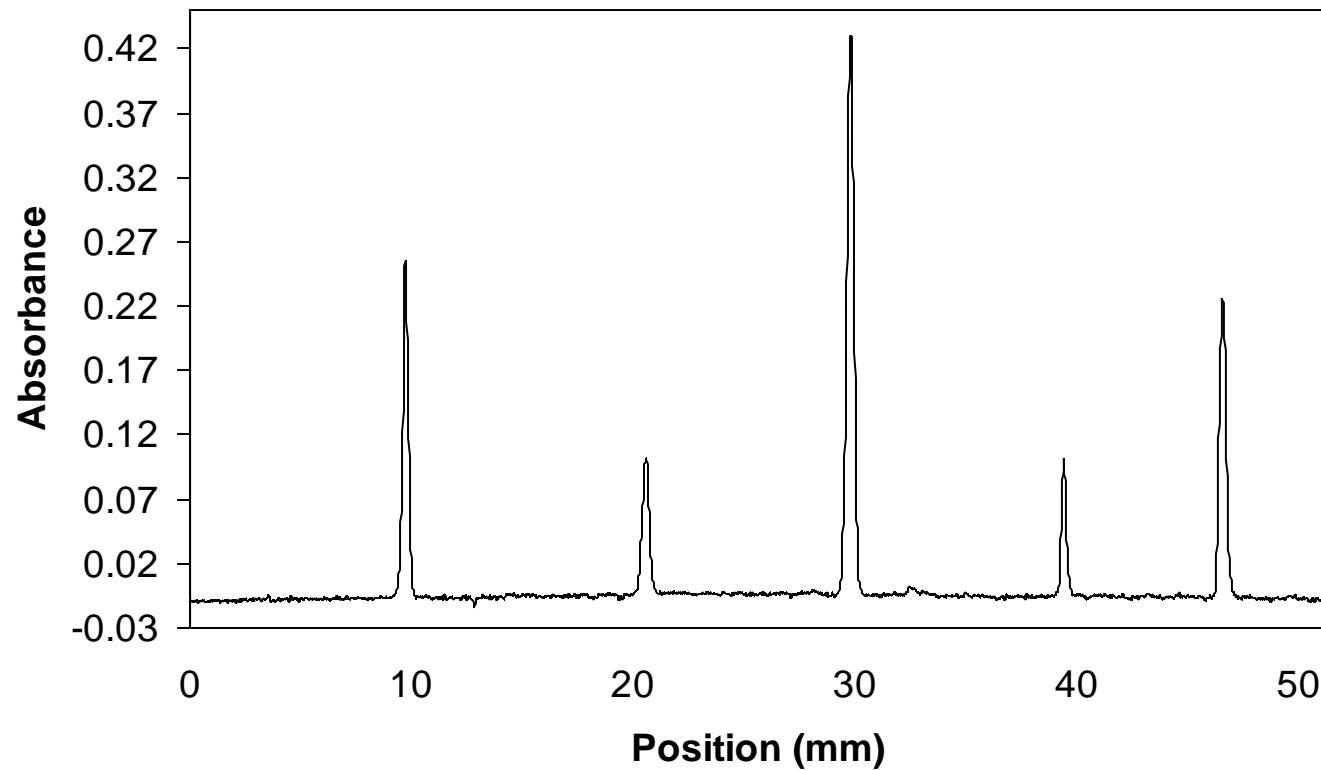
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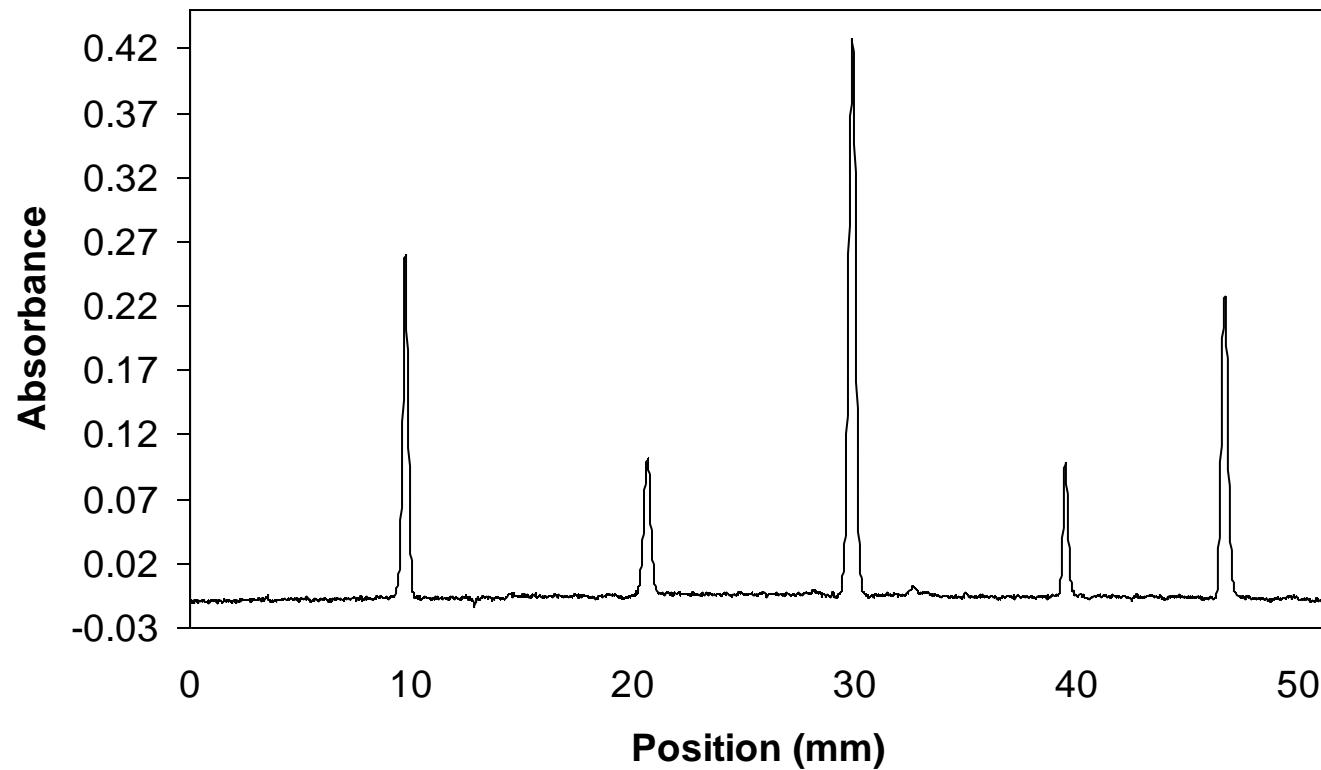
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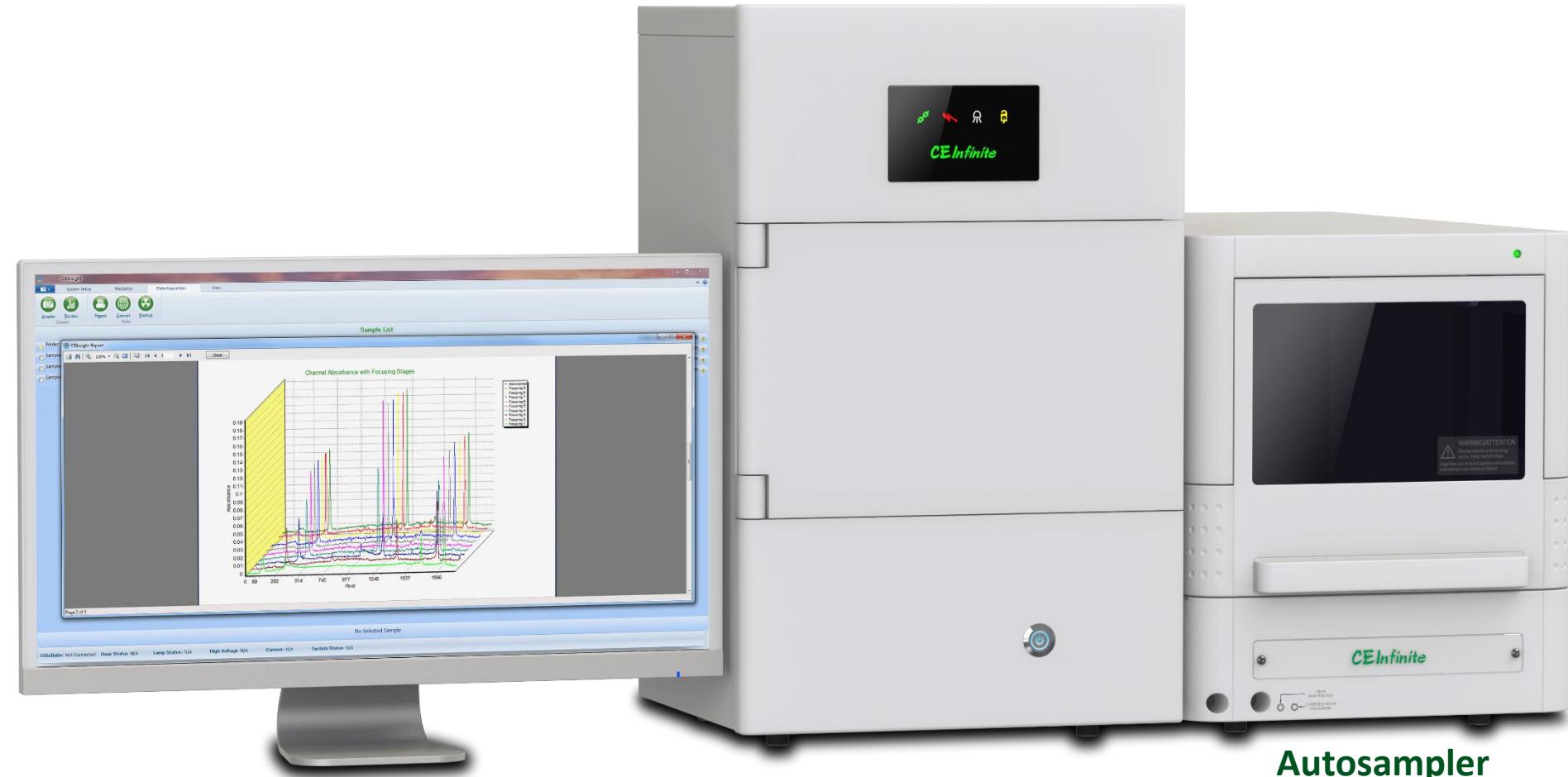


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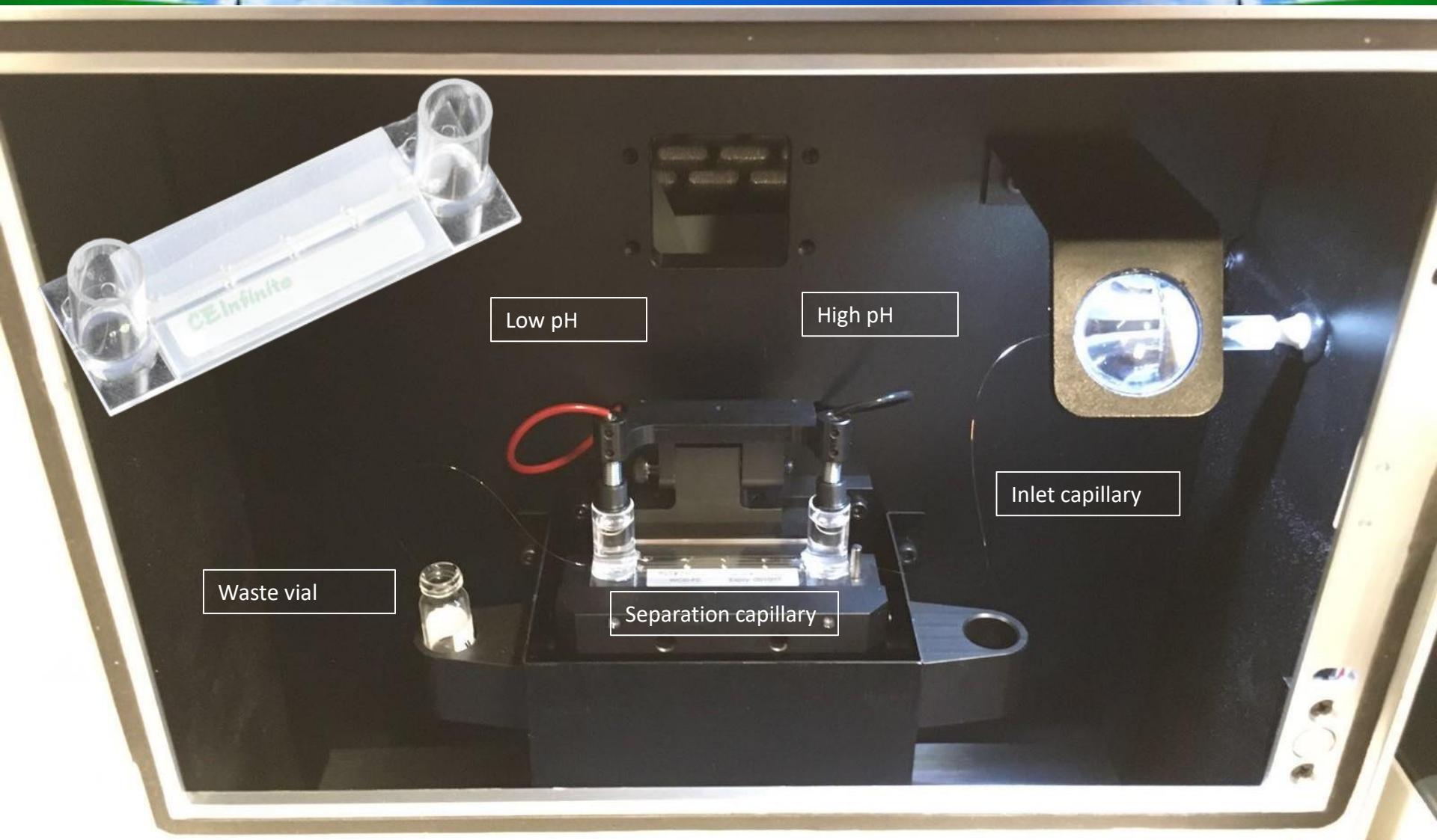




**Autosampler**

**CEInfinite**

# Basic Principle of *i*CIEF





**CE Infinite**

# CEInfinite by Advance Electrophoresis Solutions Ltd.

## Definition

- CE analyzer specially designed for cIEF – CEInfinite

## Advantages

- Provides quantitative protein analysis
- High resolution –  $\Delta pI \geq 0.02$  units
- Short analysis time (up to 6 runs/hour)
- Fast method development
- Reproducible peak pattern
- Proprietary pI markers and ampholytes with wide pI range (pH 2-11)
- Method development support and services

# *i*CIEF Method Development Guidelines

- Carrier ampholytes
  - 4% pH3-10 AESlyte
- Sample concentration
  - 0.1 mg/ml for single major peak sample, >0.1 mg for samples with multiple major peaks
- Additives
  - 0.35% methyl cellulose
- Focusing voltage and time
  - ~600 v/cm
  - 6-8 minutes for wide pH range carrier ampholytes
  - Always use pre focusing

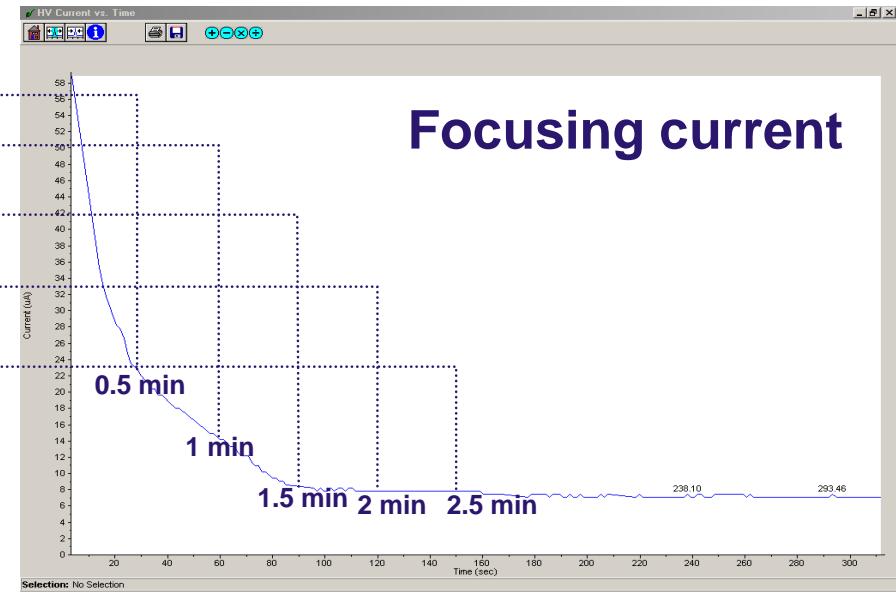
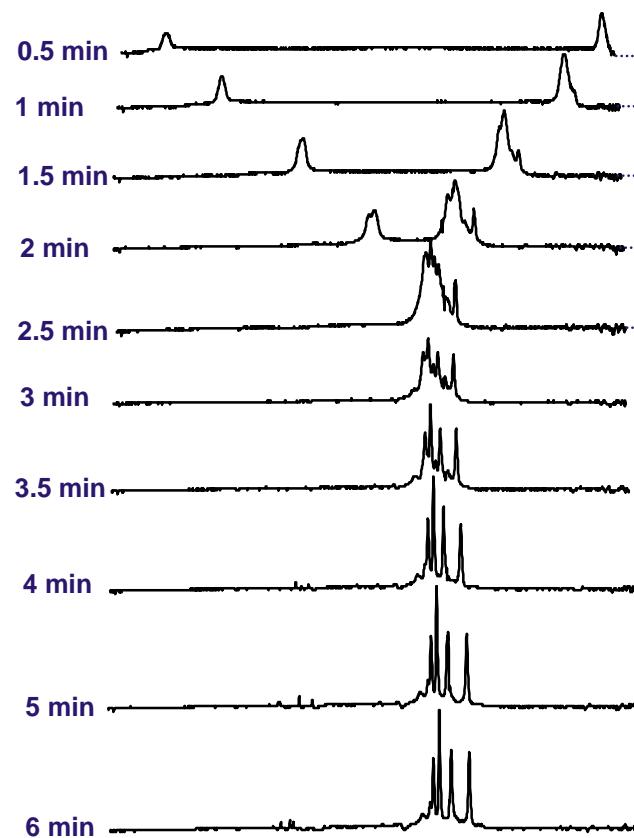
# Easy Focusing Time Optimization *iCIEF*



- Optimization of focusing time by a single run

- The whole-column detector monitors the IEF process within the separation column
- IEF process can be stopped at any time when the focusing is complete and the peak pattern is stable
- Sample aggregation and precipitation during IEF can be identified
- Additives are selected according to the identified problems

# Focusing Time



Focusing current

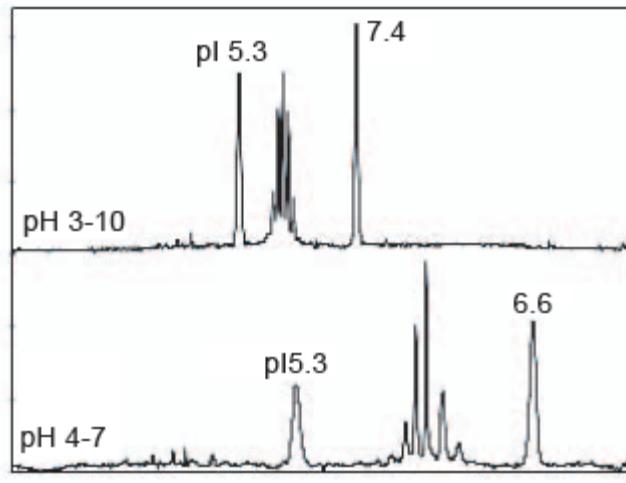
Sample: Hemoglobin A, F, S, C  
8% Pharmalyte 3-10  
600 V/cm

# Narrow pH Range Carrier Ampholytes

- Use narrow pH range carrier ampholytes to improve resolution
- The narrow pH range carrier ampholytes can be used alone or mixed with wide pH range carrier ampholytes

# Narrow pH Range Carrier Ampholytes

## Acidic Mab



**Sample:** Mab2

**Trace 1:** Pharmalyte 3-10  
6 minutes focusing

**Trace 2:** Servalyt 4-7  
12 minutes focusing

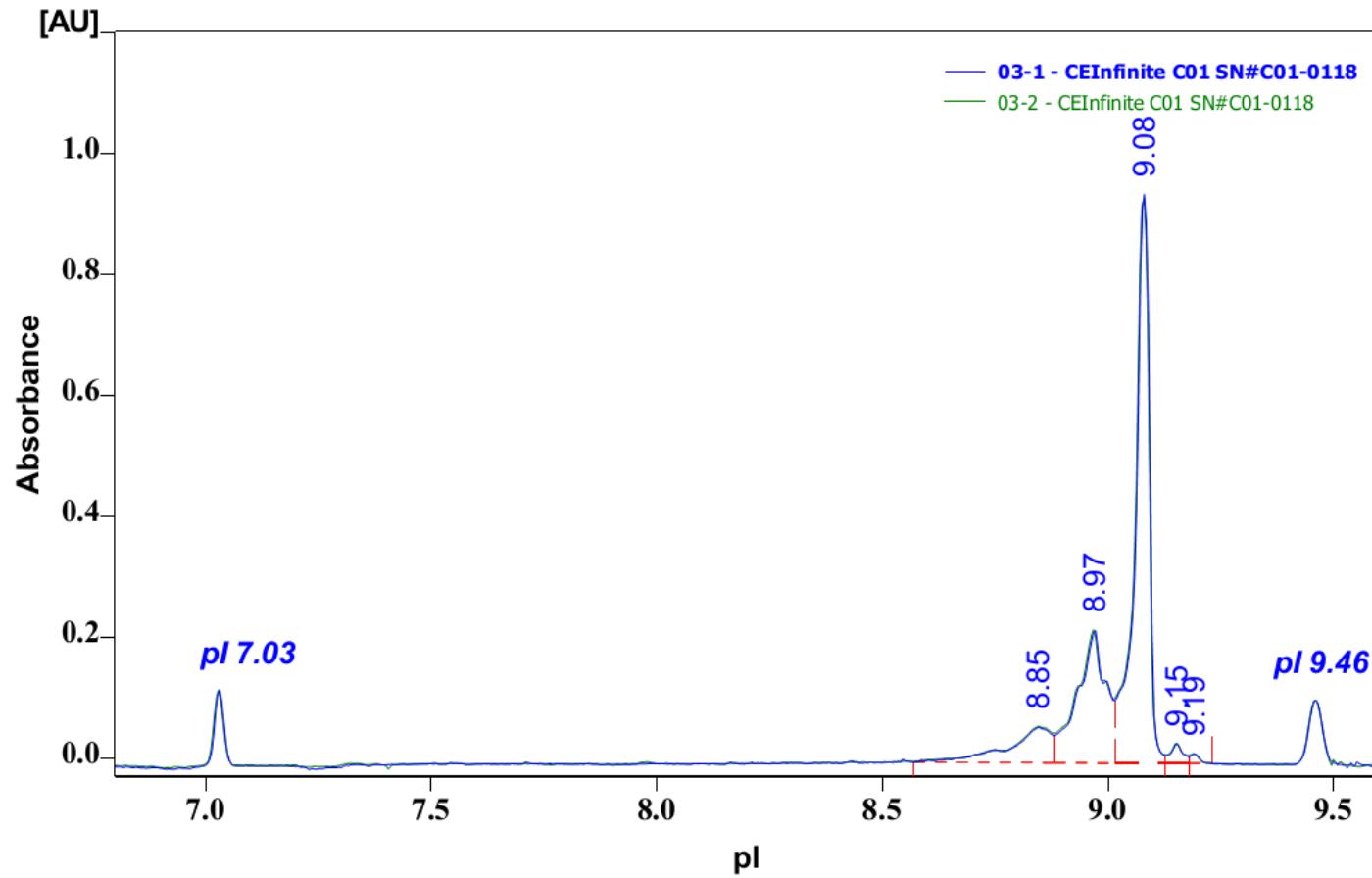
**Anolyte:** 80 mM H<sub>3</sub>PO<sub>4</sub>

**Catholyte:** 100 mM NaOH

# Recombinant Proteins

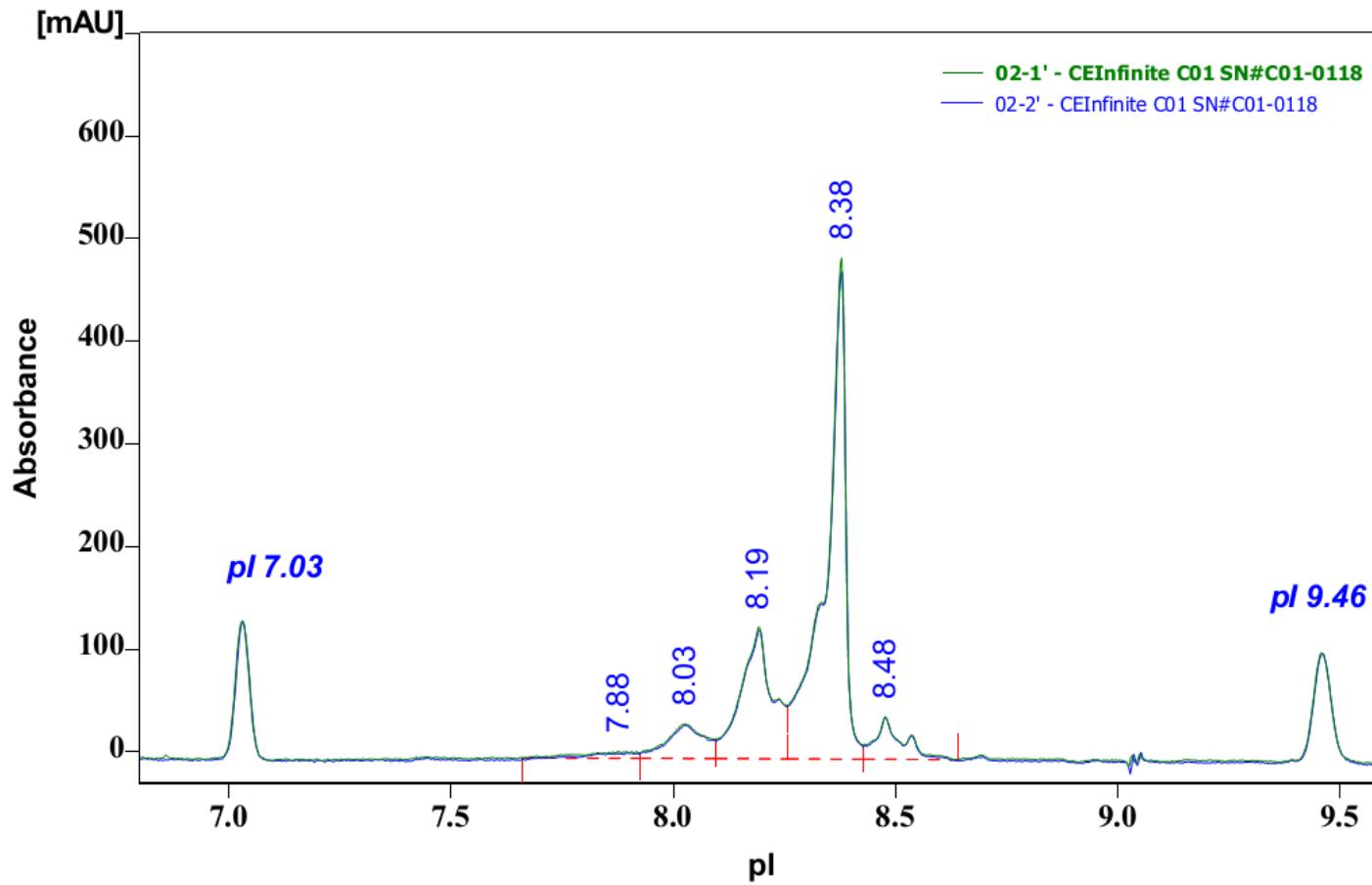
# Trastuzumab (Herceptin)

Overlay of 2 analyses



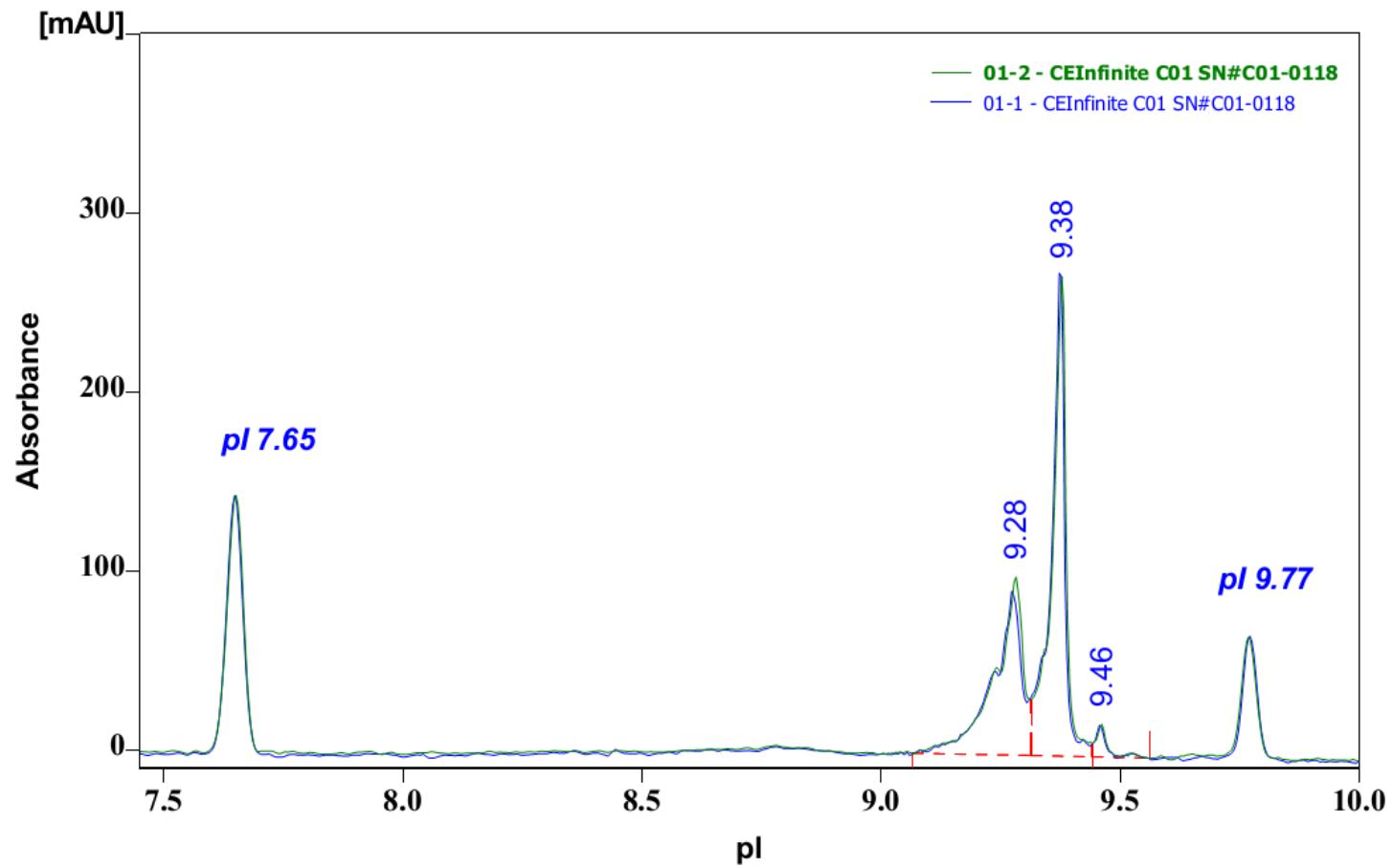
# Bevacizumab (Avastin)

Overlay of 2 analyses

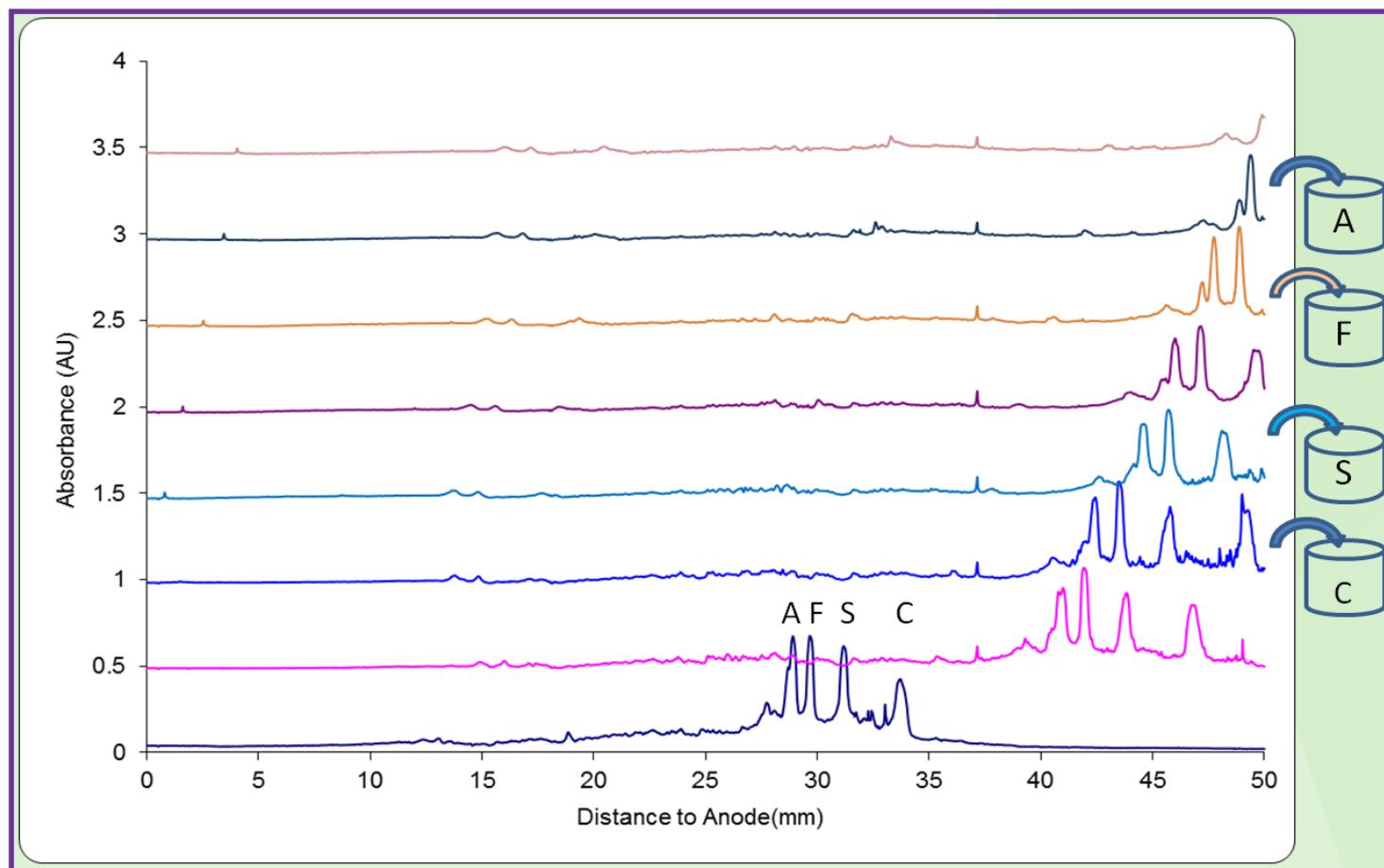


# Rituximab (Rituxan)

Overlay of 2 analyses

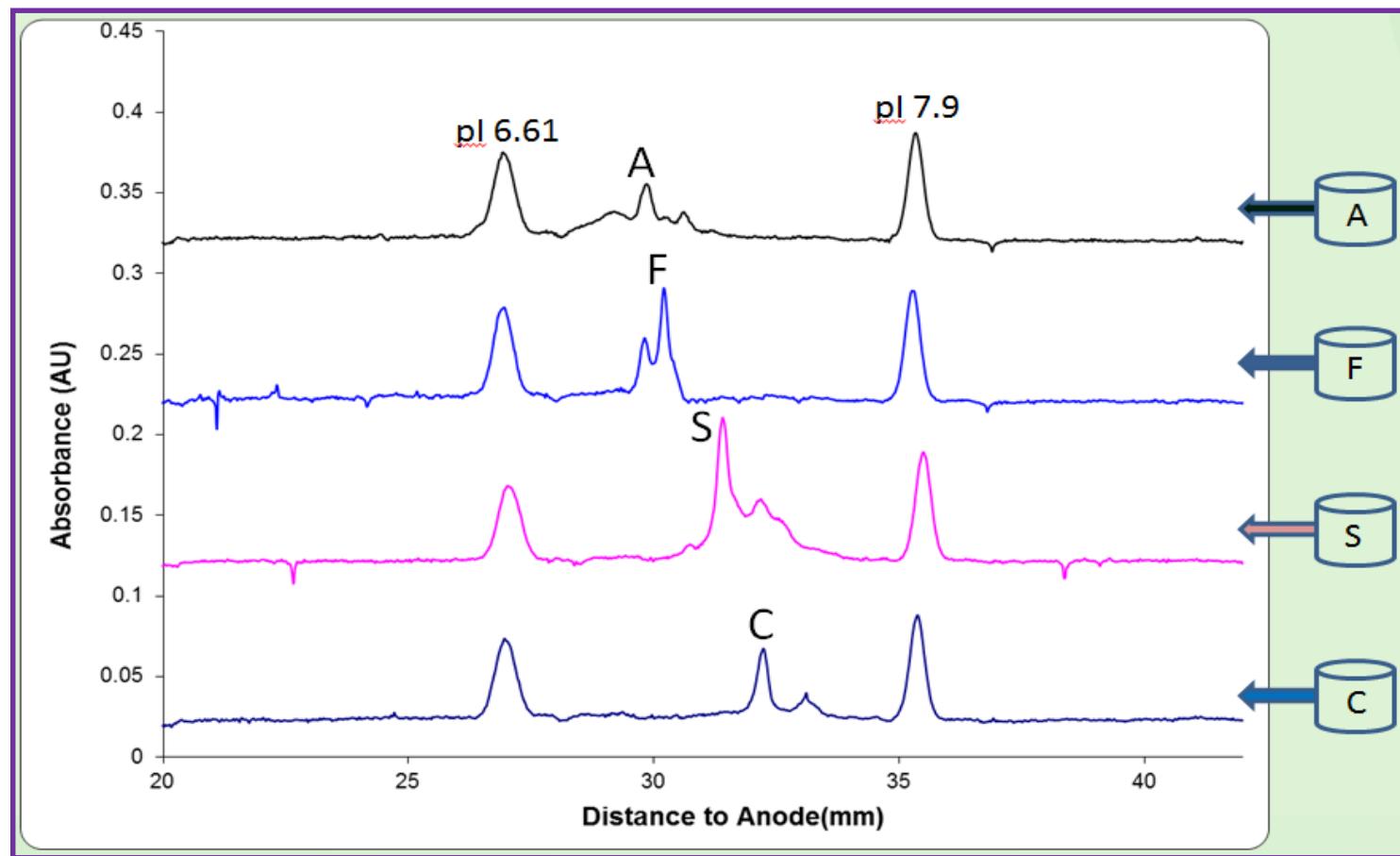


# Preparative *i*CIEF\*

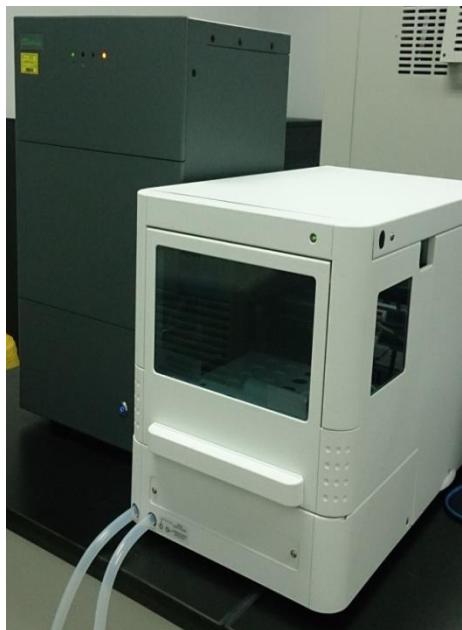


\*patent pending

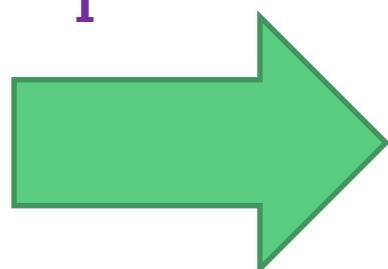
# Preparative *i*CIEF - Reinjection



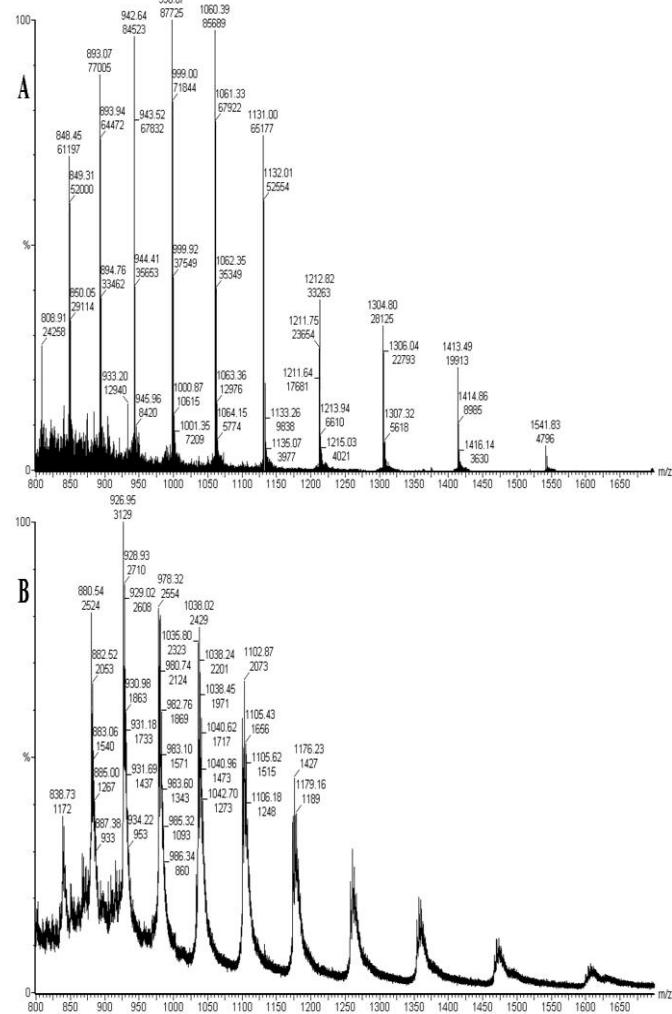
# *i*CIEF Coupling with MS



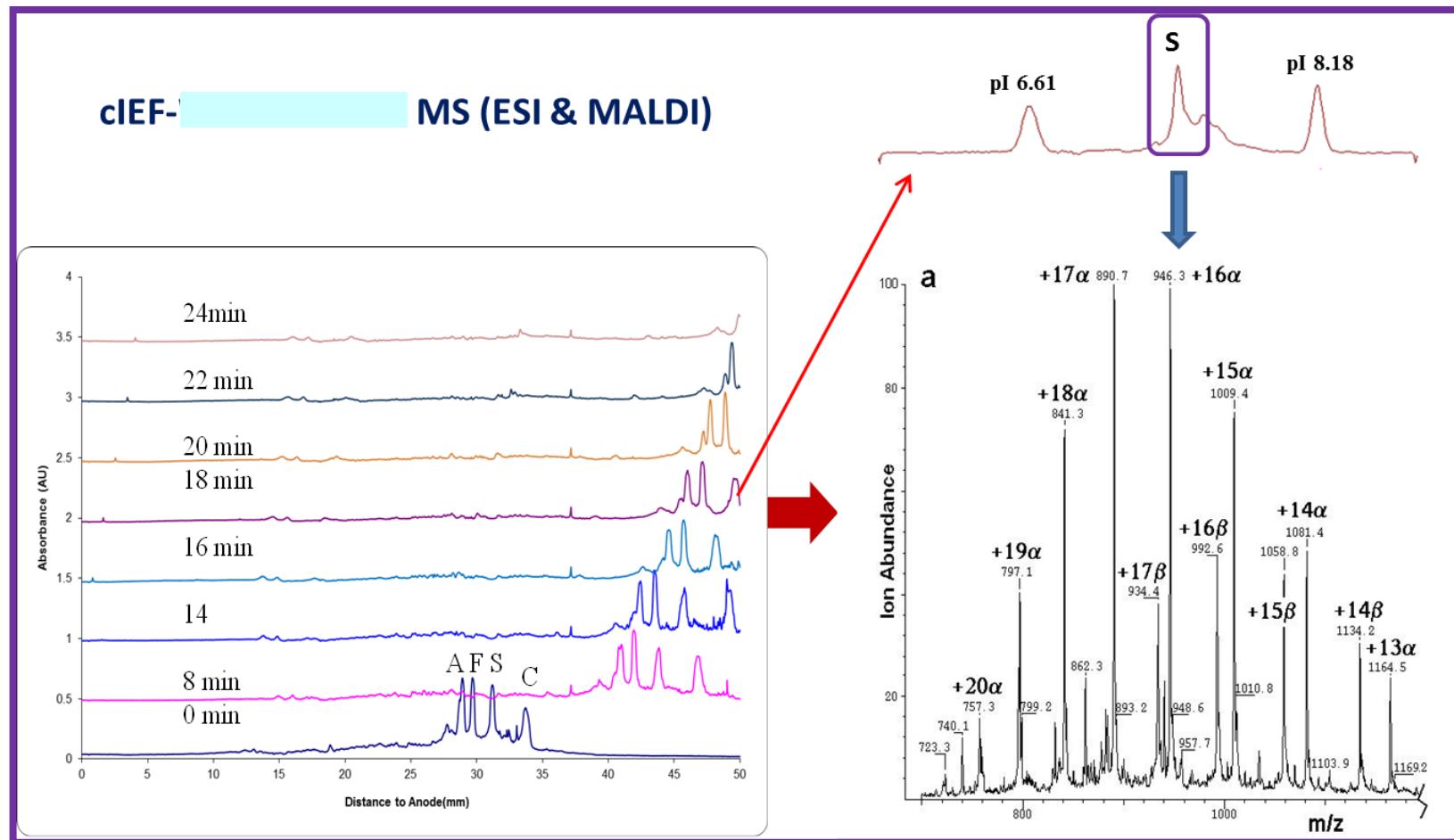
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ESI

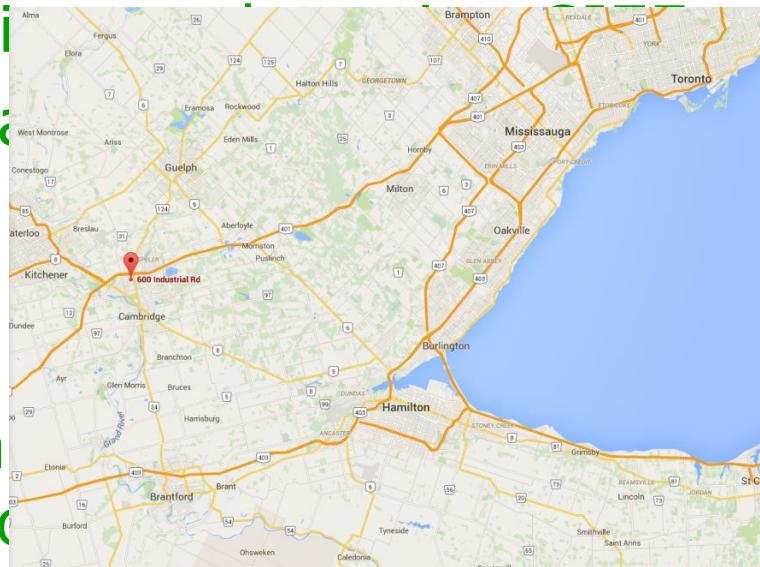


# iCIEF Coupling with MS



# About AES

- Founded in 2010 by former Convergent Bioscience employees.
- As the CEO, Dr. Huang is the (co-)inventor of patents for whole column iCIEF
- Currently based in Canada's Technology Triangle, Cambridge, Ontario
- Core technology WCID CE instrumentation, proprietary capillary cartridges, ampholytes, pI standards and buffers
- *i*CIEF based total solution provider for life science research and biopharmaceutical industry





Dedicated to providing the life science industry the most reliable, highest performance, and highest throughput whole column imaging detection capillary electrophoresis system for protein separation, quantification, and characterization

<http://www.ceinfinite.com>

# More Information and Contact

Product Website  
[ceinfinite.com](http://ceinfinite.com)

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