CE as an analytical tool in industrial biotechnology

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14 November 2013

On-Line and At-Line Analytical Technologies in the Industrial Biotechnology Sector

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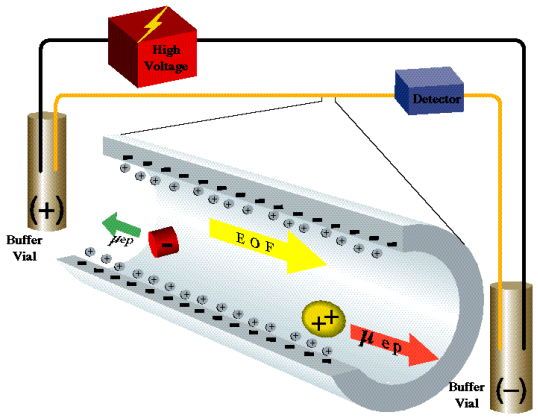
Coverage

- Modes of CE
- Industrial sectors for applications
- Companies and key markets
- Selected applications
- Myths and facts
- Conclusions

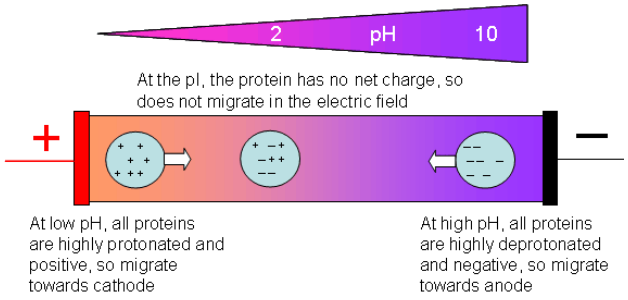
Modes of CE

Capillary zone electrophoresis (CZE)
for ions:
separation via differential migration in an electric field



Capillary gel electrophoresis (CGE)
for ions with same charge/size ratio (e.g. DNA, proteins denatured by SDS):
separation via differential migration through a gel network

Capillary isoelectric focussing (cIEF)
for proteins:
separation in an ampholyte pH gradient: proteins migrate to pH = pI



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Industrial sectors for applications

Biotechnology
- Protein therapeutics, R&D and QC

Pharmaceuticals
- Chiral separations; counter ion analysis

Food and beverage
- Small ions and vitamins

Health Care
- Clinical diagnostics, e.g. Haemoglobin variants

Forensics
- DNA sequencing

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Companies and key markets

Agilent Technologies

Automated Electrophoresis

Electrophoresis is used to separate, quantify, enrich and purify biomolecules which differ in their electrical charge or polarity. Agilent offers innovating electrophoretic separation solutions.

The Agilent 2100 Bioanalyzer system analyzes biomolecules or cells in microfluidic networks of channels and wells etched into glass chips.

The Agilent 2200 TapeStation system exhibits 96 well plate compatibility while performing rapid automated DNA, RNA, and protein electrophoresis.

The Agilent 3100 OFFGEL Fractionator resolves proteins or peptides by isoelectric point with liquid-phase recovery to achieve high resolution pI-based fractionation.

The Agilent 7100 Capillary Electrophoresis system is the most sensitive CE system on the market and seamlessly integrates with Agilent's MS systems.

7100 CE System

Key markets: biotechnology, chemicals, pharmaceuticals,

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Capillary Electrophoresis

From the highly sensitive multiplex gene expression capabilities of the GeXP Genetic Analysis System and the high-resolution applications of the PA 800 plus Pharmaceutical Analysis System, to the NEW exceedingly sensitive CESI 8000 High Performance Separation-ESI Module, we are determined to enhance and develop CE technologies that optimize your workflow efficiency. Our development initiatives encompasses hardware, software and chemistry, with a long list of advancements including our PA 800 plus, GeXP and CESI 8000.

Protein Characterization

Products

Robust, easy-to-use characterization for quantitative, automated protein analysis

GO

Applications

High-resolution, validated assays for purity & heterogeneity analysis

VIEW

PA 800 *plus* Pharmaceutical Analysis System

Key markets: biotechnology, pharmaceuticals, chemicals

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PerkinElmer
For the Better

LabChip® Separation

The LabChip GX family of instruments is the most advanced nucleic acid and protein separations system available today. Like its predecessor the LabChip 90, the GX utilizes PerkinElmer's innovative microfluidics technology to perform reproducible, high-resolution, eletrophoretic separations. Whether analyzing RNA integrity for better gene expression data or assessing protein quality in biological fermentation, the LabChip GX instrument accelerates your research and helps you generate more meaningful data, faster.

Key markets: biotechnology

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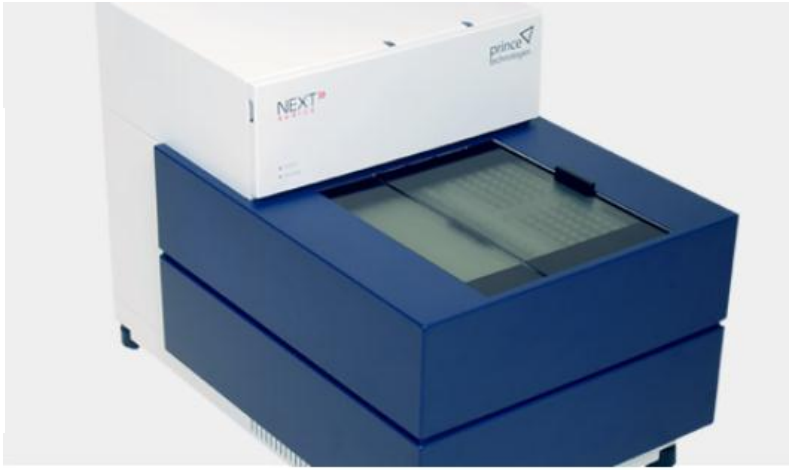
prince technologies

The CE specialists

We develop advanced capillary electrophoresis (CE) technologies and applications for use in research and development, industry, QC, education and the pharmaceutical market. Our mission is to develop tailor-made modular CE technologies that can be marketed by distributors worldwide, or which can be delivered to the market by OEM partners.



Introducing the PrinCE Next|800 platform for 2013

Prince Technologies are proud to announce the market introduction of this revolutionary new product, which we showcased at Pittcon 2013, in Philadelphia, USA.

Key markets: chemicals, food and beverage, pharmaceuticals, OEM

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proteinsimple

iCE3

Tomorrow's challenges are always looming so why not catapult ahead? iCE3 lets you move beyond the limits of traditional protein analysis.

Quick and simple method development gets you to product approval soon, or as we like to say: FDA, PDQ.

Capillary IEF :: iCE Systems

- Measure protein purity
- Charge heterogeneity protein analysis
- Method development
- Evaluate formulation development
- Screen cell lines

Key markets: biotechnology, pharmaceuticals

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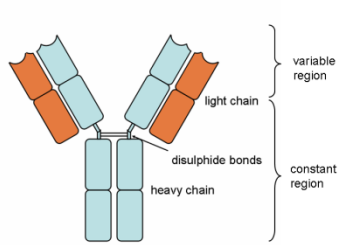
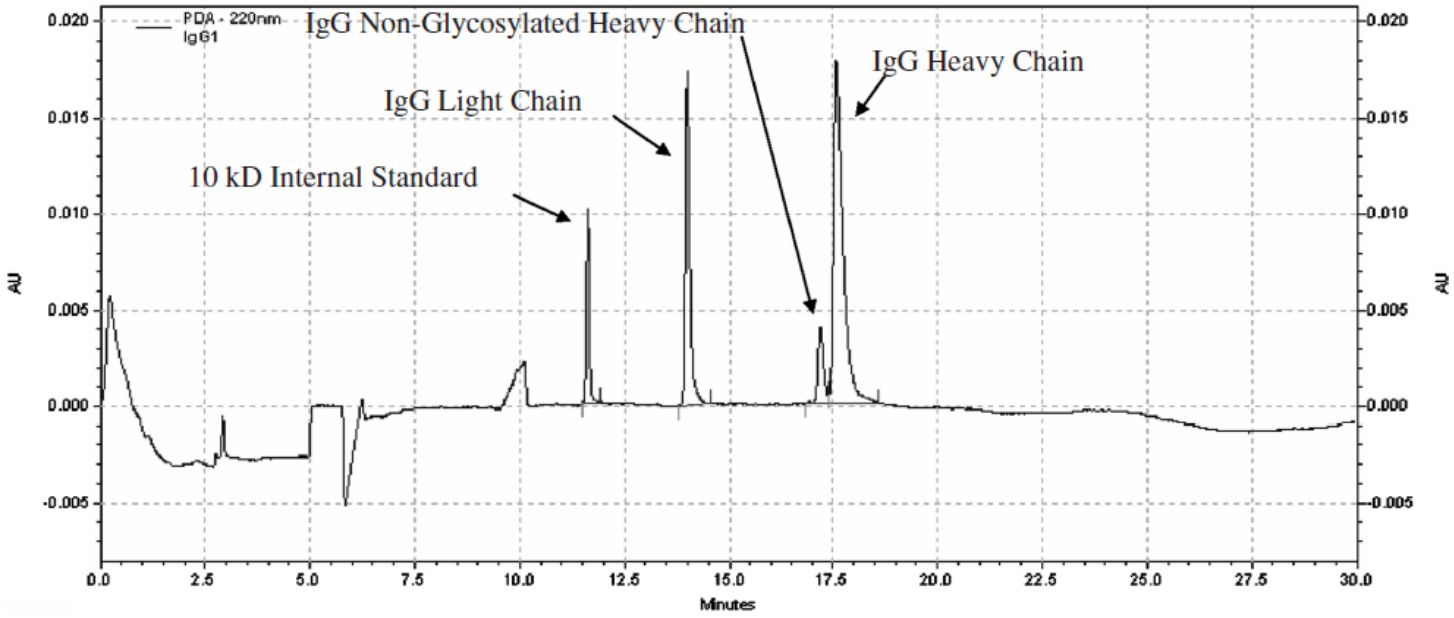
Selected applications

mAb and fragment analysis

CE mode: Capillary gel electrophoresis



- CE-SDS is an automated instrumental version of SDS PAGE
- Largest species has longest migration time



Nunnally et al, Chromatographia, 2006, 64, 359.

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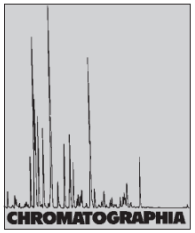
Intercompany study

A Series of Collaborations Between Various Pharmaceutical Companies and Regulatory Authorities Concerning the Analysis of Biomolecules Using Capillary Electrophoresis



2006, *64*, 359–368

B. Nunnally[1,✉], S.S. Park[2], K. Patel[2], M. Hong[3], X. Zhang[4], S.-X. Wang[4], B. Rener[5], A. Reed-Bogan[5] O. Salas-Solano[6], W. Lau[6], M. Girard[7], H. Carnegie[7], V. Garcia-Cañas[7], K.C. Cheng[8], M. Zeng[8] M. Ruesch[9], R. Frazier[10], C. Jochheim[11], K. Natarajan[11], K. Jessop[12], M. Saeed[12], F. Moffatt[12] S. Madren[1], S. Thiam[1], K. Altria[13]

In summary this report highlights that analytical characterisation of biomolecules by CE is a robust technology when the method is well described and controlled. This exercise supports and endorses the increased application of CE methodology within both development and QC laboratories within biopharmaceutical companies. It is anticipated that this exercise will facilitate both increased regulatory and industrial opinion of the use of CE within the biopharmaceutical application area.

	Relative Migration Time (RMT)			Peak Areas (%)		
	IgG Light Chain	IgG Non-glycosylated Heavy Chain	IgG Heavy Chain	IgG Light Chain	IgG Non-glycosylated Heavy Chain	IgG Heavy Chain
Company A Average	1.20	1.49	1.52	29%	8%	63%
Company B Average	1.19	1.44	1.47	30%	9%	62%
Company C Average	1.20	1.49	1.52	28%	9%	64%
Company D Average	1.20	1.48	1.52	35%	7%	58%
Company E Average	1.20	1.48	1.52	27%	9%	63%
Company F Average	1.20	1.48	1.52	30%	9%	61%
Company G Average	1.20	1.48	1.52	30%	9%	61%
Company H Average	1.20	1.48	1.51	29%	8%	62%
Average	1.20	1.48	1.51	30%	9%	62%
RSD	0.42	1.07	1.13	8.64	7.68	3.29

- **Note that this method is now well established in industry**

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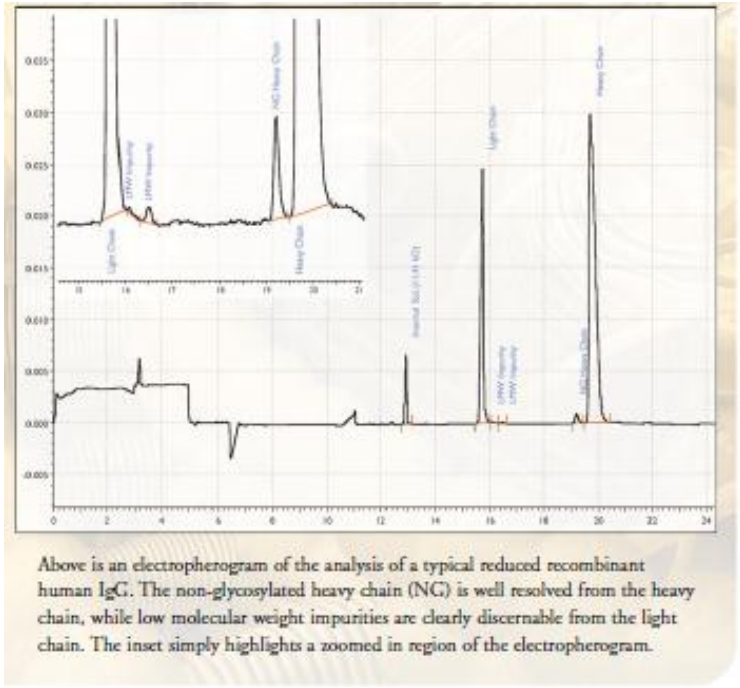
mAb and fragment analysis

Comparison of capillary and chip CE-SDS methods

CGE in capillary
(Beckman Coulter)
UV detection



Above is an electropherogram of the analysis of a typical reduced recombinant human IgG. The non-glycosylated heavy chain (NG) is well resolved from the heavy chain, while low molecular weight impurities are clearly discernable from the light chain. The inset simply highlights a zoomed in region of the electropherogram.

CGE on a chip
(Perkin Elmer)
Fluorescence detection

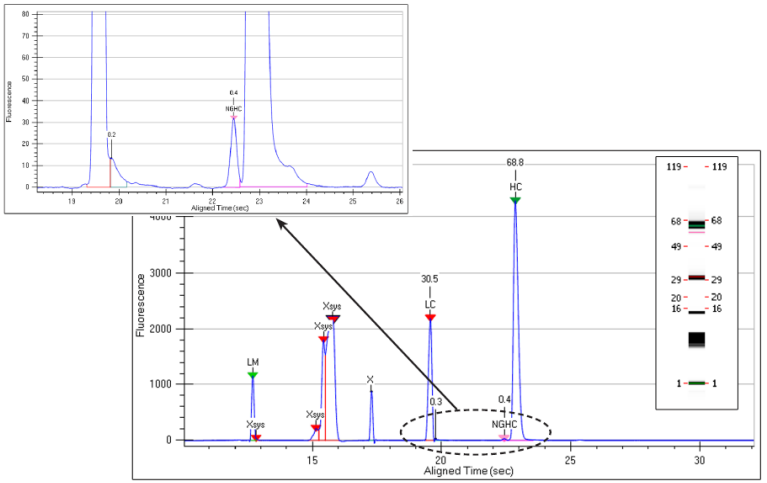


Figure 4. Reduced MAb electropherogram

Time to result: 41 s per sample

Note: Agilent also offer a chip based method

- **Large companies now use both chip and capillary based methods**

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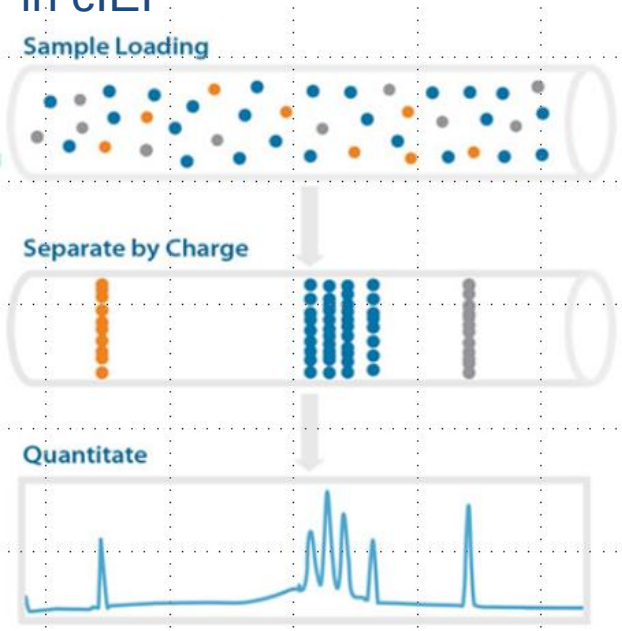
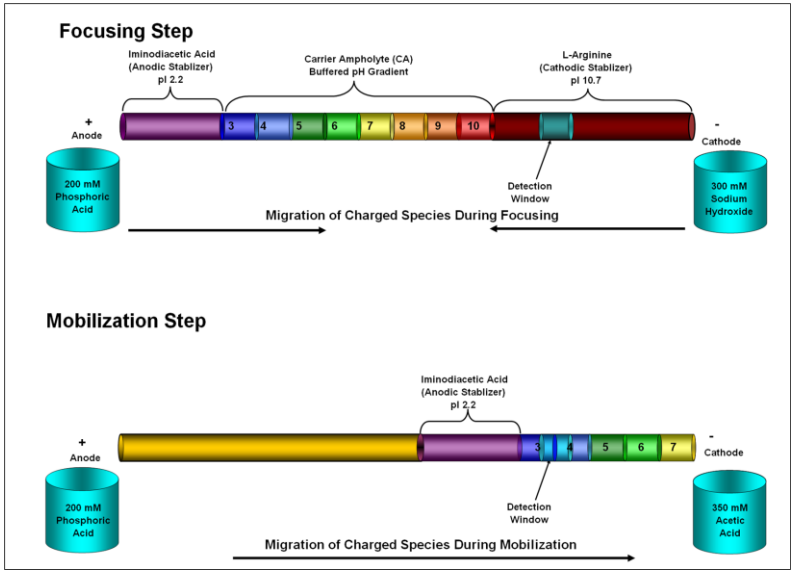
Charge variant analysis

CE mode: Capillary isoelectric focussing

Whole column imaging
(Protein Simple)
Avoids mobilization step in cIEF



Chemical mobilization
(Beckman Coulter)
Retains resolution of focussing step in mobilization step



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High-Resolution cIEF of Therapeutic Monoclonal Antibodies: A Platform Method Covering pH 4-10

Scott Mack, Ingrid D. Cruzado-Park, and Chitra K. Ratnayake
Beckman Coulter, Inc., Fullerton, CA USA

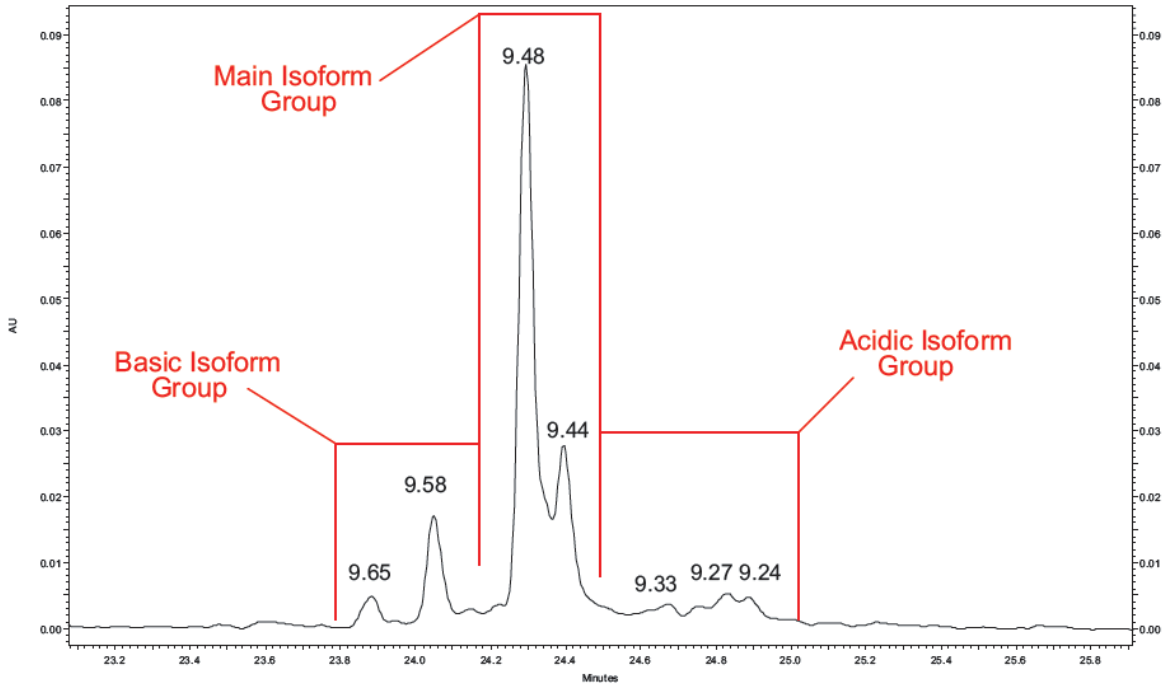

Figure 7: mAb (1) Peak Profile. A close up view of the mAb #1 cIEF separation.

n = 25

Calculated pI

Peaks	Average	Std Dev	CV
A	9.65	0.01	0.05%
B	9.58	0.01	0.06%
C	9.48	0.01	0.07%
D	9.44	0.01	0.09%
E	9.33	0.01	0.08%
F	9.27	0.01	0.07%
G	9.24	0.01	0.08%

Isoform Group Percent Composition

Group	Average	Std Dev	CV
Basic	13.94%	0.42%	3.04%
Main	71.97%	0.46%	0.64%
Acidic	14.09%	0.34%	2.38%

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A-12026A cIEF Monoclonal Antibodies pH 4-10.pdf

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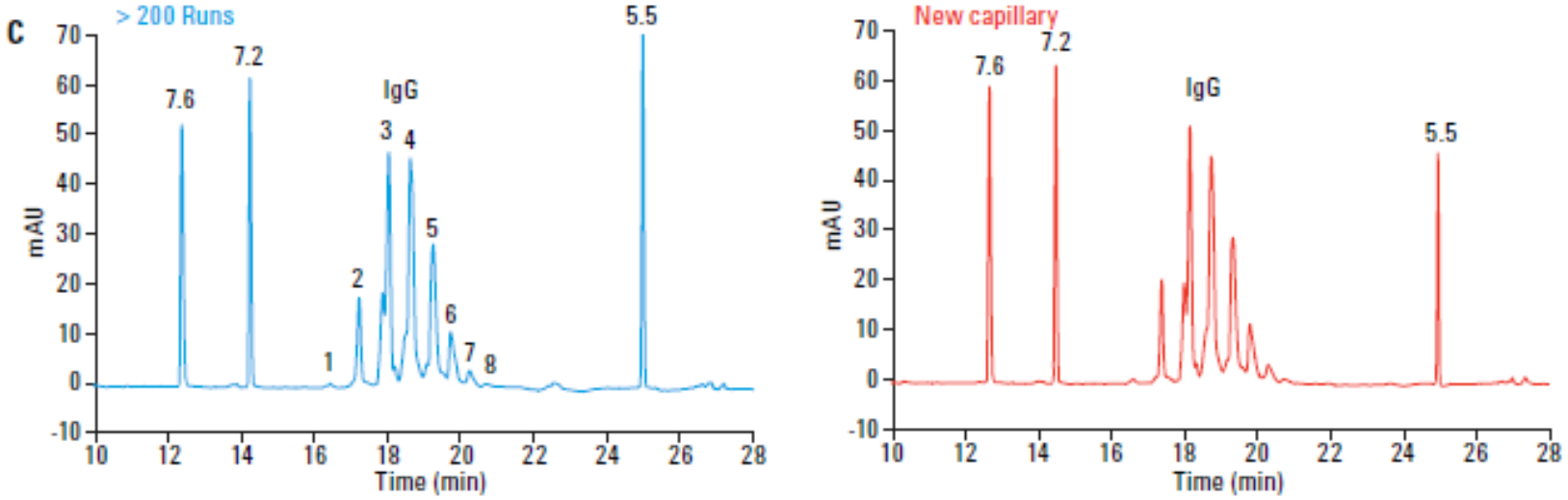
Monoclonal Antibody Charge Heterogeneity Analysis by Capillary Isoelectric Focusing on Fluorocarbon Coated Capillaries



- Intermediate precision measured on peak areas and pI.
- Robust and reliable performance demonstrated

http://www.chem.agilent.com/Library/applications/5991-2888EN.pdf

Agilent Technologies

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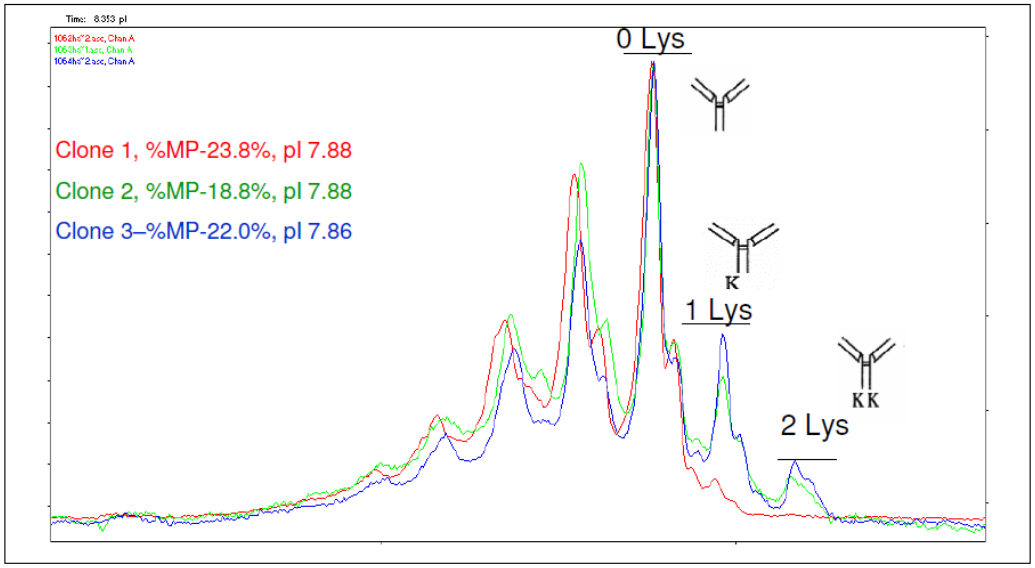
Use in cell culture development

Method Application

Imaged cIEF to Support Cell Culture Development



- Overall, some quality differences (charge isoforms) were observed between different clones.
- Clone 1 resulted in mAb molecules with less heterogeneity. The major isoform has no C-terminal lysine variants.

ZymoGenetics

Confidential

Conclusions

- High throughput and reproducible imaged cIEF assays were developed.
- Assays facilitated process development and are being used as characterization tools for lot characterization at ZymoGenetics

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ZymoGenetics

Presented at CE Pharm 2009

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Intercompany studies

Chromatographia (2011) 73:1137–1144
DOI 10.1007/s10337-011-2017-3

ORIGINAL

Intercompany Study to Evaluate the Robustness of Capillary Isoelectric Focusing Technology for the Analysis of Monoclonal Antibodies

Oscar Salas-Solano · Kunnel Babu · SungAe Suhr Park · Xinfeng Zhang · Li Zhang · Zoran Sosic · Boris Boumajny · Ming Zeng · Kuang-Chuan Cheng · Angelia Reed-Bogan · Stacey Cummins-Bitz · David A. Michels · Monica Parker · Paulina Bonasia · Mingfang Hong · Steven Cook · Margaret Ruesch · David Lamb · Dora Bolyan · Steffen Kiessig · Darren Allender · Brian Nunnally

Abstract Interlaboratory comparisons are essential to bringing emerging technologies into biopharmaceutical industry practice and regulatory acceptance. As a result, an international team including 12 laboratories from 10 independent biopharmaceutical companies in the United States and Switzerland was formed to evaluate the precision and robustness of capillary isoelectric focusing (CIEF) to assess the charge heterogeneity of monoclonal antibodies. The different laboratories determined the apparent pI and the relative distribution of the charge isoforms of a representative monoclonal antibody (rMAb) sample using the same CIEF method. Statistical evaluation of the data was performed to determine within and between-laboratory consistencies and outlying information. The apparent pI data generated for each charge variant peak showed very good precision between laboratories with percentage of RSD values of ≤0.5%. Similarly, the percentage of RSD for the rMAb charge variants percent peak area values are ≤4.4% across different laboratories with different analysts using different lots of ampholytes and multiple instruments. Taken together, these results validate the appropriate use of CIEF in the biopharmaceutical industry in support of regulatory submissions.

J. Sep. Sci. 2012, 35, 3124–3129

Research Article

Robustness of iCIEF methodology for the analysis of monoclonal antibodies: An interlaboratory study

An international team including 12 laboratories from 11 independent biopharmaceutical companies in the United States and Switzerland was formed to evaluate the precision and robustness of imaged capillary isoelectric focusing for the charge heterogeneity analysis of monoclonal antibodies. The different laboratories determined the apparent pI and the relative distribution of the charged isoforms for a representative monoclonal antibody sample using the same capillary isoelectric focusing assay. Statistical evaluation of the data was performed to determine within and between laboratory consistencies and outlying information. The apparent pI data generated for each charged variant peak showed very good precision between laboratories with RSD values of less than 0.8%. Similarly, the RSD for the therapeutic monoclonal antibody charged variants percent peak area values are less than 11% across different laboratories using different analyst, different lots of ampholytes and multiple instruments. These results validate the appropriate use of imaged capillary isoelectric focusing in the biopharmaceutical industry in support of process development and regulatory submissions of therapeutic antibodies.

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Charge variant analysis

CE mode: Capillary zone electrophoresis

J. Sep. Sci. 2011, *34*, 548–555

Yan He
Colleen Isele
Weiying Hou
Margaret Ruesch

Analytical R&D, Pfizer
BioTherapeutics R&D
Pharmaceutical Sciences,
Chesterfield, MO, USA

Research Article

Rapid analysis of charge variants of monoclonal antibodies with capillary zone electrophoresis in dynamically coated fused-silica capillary

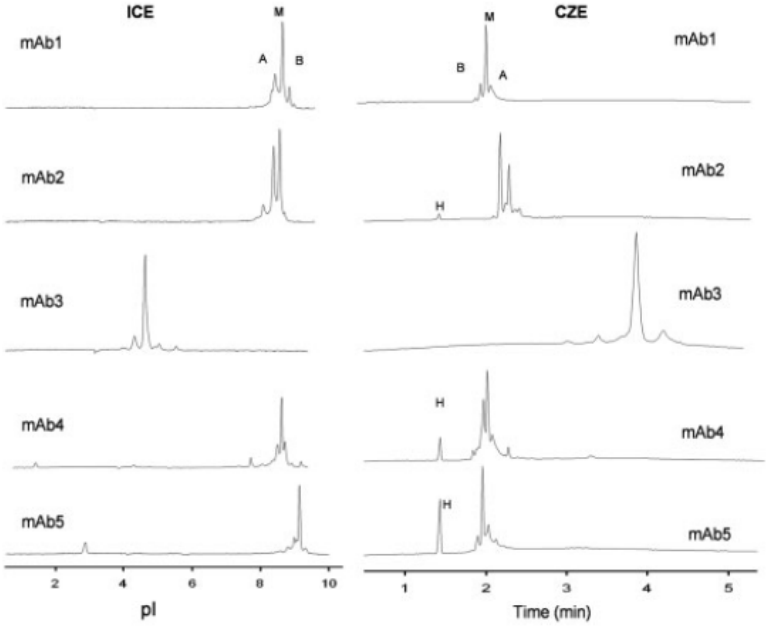

Figure 8. CZE versus iCE separation of charge variants of multiple mAbs. A, Acidic; M, main; B, basic; H, histidine in sample matrix. For iCE and CZE conditions, see Section 3.3.

- **Interlaboratory test of the Pfizer method currently underway**

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Pharmacopoeial methods

Table III: CE tests in the *European Pharmacopoeia (Ph. Eur.)* and the *US Pharmacopeia (USP)*

Monograph	*Ph. Eur.*	*USP*	Test	Description
Aprotinin	580	•	(Limit of) Des-Ala-aprotinin and des-Ala-des-Gly-aprotinin	A low-pH CZE system in which the polypeptide impurities are identified by their relative migration time and quantified by internal normalization.
Aprotinin concentrated solution	579		Des-Ala-aprotinin and des-Ala-des-Gly-aprotinin	CZE method similar to method for aprotinin substance.
Erythropoietin concentrated solution	1316		Identification	Isoform separation in a CZE system. Identification by comparison with reference solution. Quantification by internal normalization and comparison with specification table in pharmacopeia.
Galantamine HBr	2366	•	Enantiomeric purity	Chiral separation. Limit test in which the enantiomer peak in the sample solution is compared with the enantiomer peak in the diluted racemic reference solution.
Glutathione	1670		Related substances	A CZE system at pH 1.8 for the separation of related substances. Quantification by peak areas corrected for migration and response differences.
Levocabastine HCl	1484	•	Related substances and organic impurities	A CD-MEKC system at high pH for the organic impurities determination, including diastereoisomers. Quantification by internal normalization of the peak areas. Instead of a constant voltage, the current is controlled in gradient steps.
Ropivacaine HCl (monohydrate)	2335	•	Enantiomeric purity	A low-pH chiral system with triethanolamine as BGE co-ion and DM-β-CD as chiral selector. Quantification by corrected peak areas.
Somatropin	951		Identification: charged variants	Separation is performed on a pH 6.0 CZE system. Somatropin is identified by injecting a mixture of sample and reference solution, which should result in one peak. The deamidated charge variants of somatropin migrate with a relative migration between 1.02 and 1.11 compared to somatropin.
Somatropin concentrated solution	950		Identification: charged variants	Method similar to somatropin substance.
Somatropin for injection	952		Identification: charged variants	Method similar to somatropin substance.

Cari Sänger-van de Griend, LCGC North America, November 2012

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Myths and facts

Myth:
CE is not applicable to QC
Fact:
CE is no more difficult than other technologies but the typical QC analyst has limited capillary electrophoresis experience and requires proper training.
Myth:
CE cannot be validated
Fact:
CE methods have been successfully validated and implemented into QC by many pharmaceutical companies.
Myth:
Regulatory agencies do not accept CE methods
Fact:
The FDA as well as the EP is starting to request CE methods for replacement of conventional slab-gel methods.

Chantal Felten
Alpine Analytical Academy
Convergent Bioscience Webinar, 2010

Note that there are several consultancies providing courses and training in CE, including method development and validation.

Alpine Analytical Academy

Alpine Analytical Academy in the US

Kantisto Separation Sciences

Kantisto BV in Europe

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Conclusions

- Capillary electrophoresis is an excellent technique for use in industrial biotechnology.
- Pharmacopoeial and other well established and validated methods.
- Use in both QC and process development
- Instrumentation from a range of vendors.
- Courses and training available.

Acknowledgement

Cari Sänger-van de Griend, Kantisto BV

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