Microfluidic Electrophoresis Assays for Rapid Characterization of Protein in Research and Development

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Microfluidic Electrophoresis Assays for Rapid Characterization of Protein in Research and Development

14 November, 2012

Participating Experts:

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For the Better
Microfluidic Assays for High Throughput Screening of Protein Quality in Bioprocess Development

Bahram Fathollahi

Microfluidics R&D

November 14, 2012
What is a Microfluidic Device?

- An integrated device with controlled transport of sample and reagents in microchannels to carry out bio/chemical analysis

Advantages

- Miniaturization
- Fast analysis/high throughput
- Small sample volume
- Integration
- Automation
Microfluidic Chip Format and Fabrication

- **Planar Chip**
  - Single use
  - Samples added to wells manually

- **Sipper Chip**
  - Reusable
  - Samples introduced automatically from microtiter plate

**Chip Fabrication**
- Chips manufactured using photolithography and wet etching technology
- Quartz and glass substrate
- Highly reproducible and precise control of channel geometry
  - Typical depth - 5 µm to 25 µm
  - Typical width - 10 µm to 100s µm
Electrophoretic Separation in Microfluidic Chips

SAMPLE LOAD AND INJECTION

SEPARATION

DETECTION

Buffer Reservoirs
Sample Reservoirs

B
Sample Load

C

A
Sample Injection

D
Waste Reservoir

LIF detection

Sample Separation

A

ΔV

ΔV

ΔV

ΔV
Commercial Microfluidic Electrophoresis Platforms

Bioanalyzer 2100

Experion

Planar chip format

Higher Throughput

- RNA & DNA analysis
- Proteins
- N-Glycans (LabChip Only)
- Digital information
- Easy-to-use
- Time and cost savings

LabChip | DX/GX/GXII

Sipper chip format
Protein Characterization Assays on LabChip GX II

Multiple HT Screening Assays on a Single Microfluidic Platform

1) Microchip CE-SDS – 40 sec/sample
   - Protein expression optimization
   - Assess purity
     - Reduced and non-reduced
   - Titer
   - Quantify impurities
     - Product- and process-related
   - Monitor fragmentation and Ab assembly

2) N-Glycan Profiling – 60 sec/sample
   - Measure relative amounts of major \(N\)-linked glycans

3) Charge Variant Profiling – 68 to 90 sec/sample
   - Identify and measure relative amounts of basic, main, acidic variants
Microchip CE-SDS Assay Workflow

Crude or purified protein sample (2µL)

Add Sample Buffer
Heat denature ~15min

Reusable chip
Up to 400 samples

Prepare Gel/Dye-Solution and Ladder

Add Reagents to chip
Place Ladder and Buffer on GXII

~ 40 sec per sample
~ 75 min for 96-well plate

Plate View
Gel View
E-gram

Sizing, Conc., and Purity

Identified expected protein peaks
**Attributes**

<table>
<thead>
<tr>
<th>Assay</th>
<th>Instrument time</th>
<th>Microchip CE-SDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>ProA HPLC, RP HPLC</td>
<td>2-5 min, ~20 min</td>
</tr>
<tr>
<td>Assembly, covalent aggregation</td>
<td>nrCE-SDS</td>
<td>30-50 min</td>
</tr>
<tr>
<td>Fragmentation, Degree of N-glycosylation</td>
<td>rCE-SDS</td>
<td>30-50 min</td>
</tr>
</tbody>
</table>
HT N-Glycan Profiling Sample Prep Workflow

1. **Denature and Reduce mAb in Denaturing Buffer**
2. **Add Denatured Reduced mAb to Enzyme Plate**
3. **Heat (Denatured mAb + Enzyme) at 37 °C for ~1h**
4. **Transfer to Dye Plate**
5. **Heat Assay Plate at 55 °C for ~2h**
6. **Dilute with separation Buffer**
7. **Read 96-well plate on LabChip GXII ~1.5h**
Digested and Labeled N-Glycan Profile
Methods of Characterizing Charge Heterogeneity

- Three methods of separation used in the biotherapeutics industry:
  - **Ion Exchange Chromatography (IEC)**
    - Extension of HPLC systems
    - Proteins interact with charged resin, based on pI and hydrophobicity
  - **Capillary Zone Electrophoresis (CZE)**
    - Based on Capillary Electrophoresis (CE) systems
    - Proteins migrate at different speeds, based on pI and hydrodynamic drag
  - **Isoelectric Focusing (icIEF)**
    - Based on imaged CE systems
    - Proteins migrate to a particular position within a pH gradient, based on pI

- Limitation of current methods: **Lack of speed**

<table>
<thead>
<tr>
<th>Analysis Time</th>
<th>IEC</th>
<th>icIEF</th>
<th>CZE</th>
<th>Microchip-CZE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per Sample</td>
<td>30 - 90 min</td>
<td>15 - 20 min</td>
<td>8 - 30 min</td>
<td>1 - 2 min</td>
</tr>
<tr>
<td>Per 96-Well Plate</td>
<td>48 - 144 hrs</td>
<td>24 - 32 hrs</td>
<td>16 - 48 hrs</td>
<td>1.8 - 2.9 hrs</td>
</tr>
</tbody>
</table>
**Variant Detection**

- Conventional methods use absorbance at 280 nm
- LabChip detects via LIF
  - Dye must be conjugated to protein variants
  - Dye must absorb & emit at wavelengths that are compatible with instrument optics
  - Dye must conserve charge of protein variants

**From icIEF analysis**

- **Charge profile does not change**

---

![Dye conjugation diagram with Lys and NHS ester reactions.](image)

**Relative Amount (%)**

<table>
<thead>
<tr>
<th></th>
<th>Acidic Variants</th>
<th>Main Variant</th>
<th>Basic Variants</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Before Labeling</strong></td>
<td><img src="image" alt="Graph" /></td>
<td><img src="image" alt="Graph" /></td>
<td><img src="image" alt="Graph" /></td>
</tr>
<tr>
<td><strong>After Labeling</strong></td>
<td><img src="image" alt="Graph" /></td>
<td><img src="image" alt="Graph" /></td>
<td><img src="image" alt="Graph" /></td>
</tr>
</tbody>
</table>

**Notes:**

- Δt = 10 min.
- T = 22 °C
- pH > 8.0
Direct Comparison With Alternative Methods

- **Microchip-CZE**
  - 60 sec
  - Basic
  - Acidic

- **icIEF**
  - 15 min
  - Acidic
  - Basic

- **Conventional CZE**
  - ~10min
  - Main isoform: 8.810
  - Acidic Basic Acidic Basic Acidic

- **Relative Amount (%)**
  - Basic
  - Main
  - Acidic

- Time (sec): 25 to 60
- Fluorescence: 0 to 200

- Relative Amount: 0 to 80

- Times:
  - Microchip CZE: 60 sec
  - icIEF: 15 min
  - Conventional CZE: ~10 min

- Values:
  - 8.585
  - 8.712
  - 8.948
  - 9.044
  - 8.00
  - 8.20
  - 8.40
  - 8.60
  - 8.80
  - 9.00
  - 9.20
  - 9.40
  - 9.60

- Units:
  - Minutes
  - Fluorescence
  - Relative Amount

- Graphs show comparison between Microchip CZE, icIEF, and Conventional CZE in terms of analysis time and relative amount of basic and acidic isoforms.
Higher demand of product quality requires multi-factorial DOE studies in upstream and downstream process development.

- Clone selection/cell culture optimization
- Sample prep/µscale purification
  - BioTx workstation
  - µscale chromatographic purification
- Analytics for determination of critical quality attributes
  - Purity Assessment
  - N-Glycan profiling
  - Charge Variant
- Informatics
- Optimization and Scale-up
  - Process parameter optimization
  - Process understanding and decisions
Acknowledgements

▶ Microfluidics R&D
  ▪ Tobias Wheeler
  ▪ Lucy Sun
  ▪ Rajendra Singh
  ▪ Mai Ho
  ▪ Hui Xu
  ▪ Melissa Takahashi
  ▪ Roger Dettloff

▶ Chip and Reagent Fabrication
  ▪ Joan McAuliffe
  ▪ Ken Summers
  ▪ Trang Le
  ▪ Khushroo Gandhi

▶ Software
  ▪ Advit Bhatt
  ▪ Dan Camporese
  ▪ Renil Das

▶ Marketing & App Support
  ▪ Rick Bunch
  ▪ Krystyna Hohenauer
  ▪ Nate Cosper
  ▪ Seth Cohen
  ▪ Linda Gary
  ▪ Marsha Paul

Thank you!
Microfluidic Electrophoresis Assays for Rapid Characterization of Protein in Research and Development

Process Science Germany, 2012
Project Timelines
Focus on Critical Bottlenecks in Development

Overall Project Timeline

- Exploratory Research
- Preclinical Development
- Clinical Dev. Phase I,II
- Phase IIb/III Registration
- Market Supply

Bottleneck to Clinic

- Cell line
- Process Development/optimization
- non GMP scale up, Tox material
- GMP Pilot Plant PhI - Scale up

- Critical bottleneck is time to the clinic
- Reduce time to clinic to shorten time to market
- Often is data on the critical path in development
- Early process understanding critical to efficiency and safety

More data → Better Decisions → Efficient Development → Better Products

- Fast track development by applying “platform technologies”
- Material supply: 1 g - 2 kg
Project Timelines
Impact of Miniaturization and Automation

Standard Development Outline

- Process development starts after Clone Phase.
- Cell line development on Critical Path (Bottleneck).
- Material supply limiting in early cell line development.
- Small amounts of material available during Pool and Clone development to test product quality.
- This material can be used to begin process development with miniaturization and automation.
Standard Development Outline

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BI-PurEx: Lean Development Strategy
Improving on Speed and Process Understanding

Platform Process

- Capture
- Acid Treatment
- Polishing 1
- Polishing 2
- Virus filtration
- Formulation

Novel Technology Platform

BI-PurEX™

Excellence in Downstream Development

- time
- process economy
- process knowledge
- customer requirements
- flexibility
- intrinsic factors
BI-PurEx: Lean Development Strategy
Improving on Speed and Process Understanding

Optimized Process

- Capture
- Acid Treatment
- Polishing 1
- Polishing 2
- Virus filtration
- Formulation

BI-PurEx – Purification Excellence

- Preferred Process Steps
- Integrated decision tree
- HTAnalytical Methods
- Automation and Miniaturized screening tools
- Design of Experiments
- Novel Analytical Tools

Quality by Design (QbD)
BI-PurEx: Lean Development Strategy
Understanding the product & the process

Product & Process are critical

- Cell line development is on critical path
- Rate limiting step is cell growth, very little material with which to gain knowledge
- Choosing the correct cell line for each product is most critical
- Impacts product quality, functionality, expression levels, comparability...
- Early knowledge and correct decisions will have a significant impact on efforts and timeline throughout the development process.
- Initial challenges in Glycosylation
- Mainly controlled by cell line decision
- Purification normally has very little impact on glycans
- Impacts comparability and functionality of molecule
- Critical to get early knowledge and impact cell line development decisions!
• Using Microfluidic Assay – all pools are analyzed for glycan map prior to cell line decision.
**Product Understanding**

**High Throughput Glyco Analysis**

**HT - Microfluidic Assay**
- High Throughput
- Very reproducible
- Comparable to standard Methods.

<table>
<thead>
<tr>
<th>DS01 [5mg/mL]</th>
<th>Inj. 1</th>
<th>Inj. 2</th>
<th>Inj. 3</th>
<th>Mean</th>
<th>SD</th>
<th>VK</th>
<th>Std. Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Man5</td>
<td>4,05</td>
<td>4,27</td>
<td>4,24</td>
<td>4,19</td>
<td>0,12</td>
<td>2,85</td>
<td>4,52</td>
</tr>
<tr>
<td>NGA2</td>
<td>2,93</td>
<td>2,89</td>
<td>2,83</td>
<td>2,88</td>
<td>0,05</td>
<td>1,75</td>
<td>2,73</td>
</tr>
<tr>
<td>NGA2F</td>
<td>59,61</td>
<td>57,5</td>
<td>59,1</td>
<td>58,79</td>
<td>1,10</td>
<td>1,87</td>
<td>60,48</td>
</tr>
<tr>
<td>NA2G1F</td>
<td>14,2</td>
<td>13,8</td>
<td>13,82</td>
<td>13,94</td>
<td>0,23</td>
<td>1,62</td>
<td>20,6</td>
</tr>
<tr>
<td>NA2F</td>
<td>1,7</td>
<td>1,83</td>
<td>1,43</td>
<td>1,65</td>
<td>12,34</td>
<td>12,3</td>
<td>1,9</td>
</tr>
</tbody>
</table>
Product Understanding
Product Based Impurities

- Product purity influenced by purification process development
- Microfluidic Assay helps point out critical impurities to focus later development efforts and to track in cell line decisions.

<table>
<thead>
<tr>
<th></th>
<th>Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>97.1% Purity</td>
</tr>
<tr>
<td>Clone 1</td>
<td>94.1% Purity</td>
</tr>
<tr>
<td>Clone 2</td>
<td>94.6% Purity</td>
</tr>
<tr>
<td>Clone 3</td>
<td>95.1% Purity</td>
</tr>
<tr>
<td>Clone 4</td>
<td>94.3% Purity</td>
</tr>
<tr>
<td>Clone 5</td>
<td>94.2% Purity</td>
</tr>
</tbody>
</table>

Electropherogram from reduced mAb sample
Product Understanding
Product Purity – Charge Heterogeneity

Qualitative analysis no longer enough
- Impact on safety and efficacy

Critical to Comparability
- Impacted by cell line
- Impacted by fermentation process
- Impacted by purification process

High throughput method required to look at all samples
Product Understanding
Product Purity – Charge Heterogeneity

Sensitivity very high

Ability to detect differences between clones

Ability to detect differences to reference material.

iCE

WCX

R&D 1

Clone Z
Fully automated purification screening platform
- From resin and plate preparation to step elution
- Screen 96 variables and generate of up to 500 sample per day
- Fully automated sample preparation and analytical assays
- Automated homogeneous assays to screen critical output parameters
Process Understanding
High-Throughput Parallel Chromatography

- gaining process knowledge earlier
- in a shorter timeframe
- with less material demand

- Microfluidic Assay allows analysis of impurities profile for each of the samples generated.
### Sample Name | % Purity | Type
--- | --- | ---
Standard | 0.22 | Unknown
AT Prod Pool | 0.88 | Unknown
DF Prod Pool | 0.87 | Unknown
AEX Prod Pool | 0.88 | Unknown
CEX Prod Pool | 0.76 | Unknown
Bulk Prod Pool | 0.79 | Unknown

- Assay set-up not complex, carried out within development lab
- Data delivered within hours, not days
- Can take next step to test fractionation of individual process steps
- IMPACT on real time decisions!
Acquity UPLC H-Class:

- Shorten analysis time by factor of 2.5
- Increase resolution through smaller particle diameter
- Lower injection volumes
- Low carry over
- Applicable to WCX, RP….
Analytical techniques with increased throughput. Understand impact of each step on product quality and contaminant removal.
Thanks to many colleagues at Boehringer Ingelheim especially:
to H. Kaufmann, D. Ambrosious
to J. Stolzenberger, S. Combe, O. Haas, S. Müller, D. Dieterle

Thanks for your attention!!!
The Use Of Microfluidics Technology in the Pharmaceutical Industry:

MicroChip Electrophoresis as a High Throughput Process Analytical Platform to support the Development of Therapeutic Monoclonal Antibodies

Tim Blanc and Qinwei Zhou
ImClone Systems. Branchburg, NJ 08876
Introduction

Rapid analytical separations is an important application stemming from the development of Microfluidics. One such separation technique is MicroChip Electrophoresis (MCE). MCE can be performed in several modes to provide valuable insights to the relationship between bioprocess parameters and the quality attributes of biopharmaceutical products.

Recombinant Monoclonal antibodies commonly exist as heterogeneous mixtures of product variants. Several factors contribute to product variants, one such factor is glycosylation. Using Glycosylation as the example, it is important to understand what the relationship is between process parameters and glycan distribution expressed on the antibody. An effective Quality by Design (QbD) approach uses design of experiments (DOE) to determine the most important process parameters, termed Critical Process Parameters (CPPs). However, the number of samples required for the experimental design can be high. Testing this number of sample to gain the most thorough process understanding is time and cost prohibitive by standard methods.

Discussed will the regulatory and biopharmaceutical issues that have accelerated the need for rapid analytical separation. Then, will be discussed application on MCE that have been developed to address those needs.
The New Quality Paradigm

• ICH Q8 (R2)  Pharmaceutical Development
• ICH Q9  Quality Risk Management
• ICH Q10  Pharmaceutical Quality Systems
• ICH Q11  Development and Manufacture of Drug Substance

ICH Q11: “Risk management, and scientific knowledge are used more extensively to identify and understand process parameters and unit operations that impact critical quality attributes (CQA’s) and develop appropriate control strategies applicable over the lifecycle of the drug substance.”
Quality Risk Management:
Linking Process Knowledge, Product Knowledge and Quality Systems

Ref.: Quality Attributes of Recombinant Therapeutic Proteins: An Assessment of Impact on Safety and Efficacy as part of a Quality by Design Development Approach
Eon-Duval, A. et.al BIOTECHNOL. PROG., 2012, Vol.00, No. 00
Good Business too

Process Development and Scale-up (Changes)

Comparability Studies- Linking Commercial scale product back to original material use in Tox. & PK Studies

Assure QCA’s are maintained within acceptable ranges (QTPP), Quality Target Product Profile, throughout Process Development. Maintain link to Tox. & PK studies.
Heterogeneity of Monoclonal Antibodies

Therapeutic Mab Product Contents

Product Variants

Process Related Impurities
- Trace amounts (ppb-ppm)
  - Residual Host Cell DNA,
  - Residual Host Cell Protein,
  - Residual Protein-A.

Product Related Impurities
- 1-5%
  - Antibody Fragments,
  - Aggregates

Product Variants
- Post-Translational Modifications:
  - Glycan Variants,
  - C-Terminal Truncation,
  - Deamidation, etc.

Critical Quality Attributes (CQA’s)
Heterogeneity of Monoclonal Antibodies

Therapeutic Mab Product Contents

Product Variants

Multiple Sources give rise to Product Variants
Critical Process Parameters (CPP)

- CPPs are independent process parameters most likely to affect the quality attributes of a product or intermediate.
- CPPs are determined by analytical testing, scientific judgment and on research, scale-up or manufacturing experience.
- CPPs are controlled and monitored to confirm that the impurity profile is comparable to or better than historical data from development and manufacturing.
- Quality attributes related to CPPs include:
  - Product Variants Distribution (Glycosylation is a major contributor)
  - Product-Related Impurities
  - Process-Related Impurities
Glycosylation
Implications of Glycosylation Variants

- The greatest source of Mab Product Variants is Glycosylation
- Glycosylation is very closely tied to process parameters
- Aspects of glycosylation will frequently be CQA’s

<table>
<thead>
<tr>
<th>Glycan Structural Characteristic</th>
<th>Implication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fucose:</td>
<td>Effector Function (e.g. ADCC)</td>
</tr>
<tr>
<td>Sialic Acids (NANA, NGNA)</td>
<td>PK modulation (up or down)</td>
</tr>
<tr>
<td>NGNA</td>
<td>Immunogenicity</td>
</tr>
<tr>
<td>α-Galactose</td>
<td>Immunogenicity</td>
</tr>
</tbody>
</table>
MCE and Product Variants

- Sample prep designed in microtiter plate format with immobilized enzyme to reduce sample prep from 2 days to ½ day (Glycans).

- Rapid electrophoretic separation on Microchip with sensitive laser-induced fluorescence detection. (<60 seconds per sample)

- Analytical separations in under 60 seconds for determinations of:
  - Size Heterogeneity
  - Charge Heterogeneity
  - Glycosylation Heterogeneity

- Throughput and Accuracy for thorough QbD testing, CPP determination and Successful Control Strategy efforts
Data is all numerical for statistical comparison.
MCE Platform: Size Heterogeneity

MCE-SDS < 60 SECONDS per Samples

CE-SDS ≈ 60 Minutes Samples
MCE Platform For QbD: Charge Heterogeneity

MCE < 60 SECONDS per Samples

IEC >60 MINUTES per Sample
MCE Platform: Glycan Heterogeneity

MCE < 60 SECONDS per Samples

HPLC ≈ 60 Minutes Samples
Conclusions

• Rapid Analysis time and easily automated.

• Allows more samples tested and better insight to Impact of process parameters.

• CPP’s determined and detailed process understanding.

• Effective Control Strategy.

• Relevant Drug Substance Specification (JOS).
Science Webinar Series

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14 November, 2012

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San Francisco, CA

To submit your questions, type them into the text box and click Submit.

Q&A

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