

**CE in the Biotechnology &
Pharmaceutical Industries:
20th Symposium on the Practical
Applications for the Analysis of
Proteins, Nucleotides and Small
Molecules
(CE Pharm 2018)**

Symposium Co-Chairs:

David Michels, *Genentech, a Member of the Roche Group*
Joel Welch, *CDER, FDA*

September 9-12, 2018
Hilton San Francisco Union Square
San Francisco, CA

Organized by



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Welcome to CE Pharm 2018: CE in the Biotechnology and Pharmaceutical Industries: 20th Symposium on the Practical Applications for the Analysis of Proteins, Nucleotides and Small Molecules

We are pleased to welcome you to CE Pharm 2018, a symposium devoted to the practical concerns that will strengthen the use of CE within the biotechnology and pharmaceutical industries. The goal of this symposium is to provide a forum for the discussion of recent developments in the analysis and characterization of protein therapeutics, nucleotides and small molecules by CE and related techniques. The symposium will feature presentations from leading experts within industry and regulatory agencies from around the world. Applications will highlight the use of CE in various areas of product development including high-throughput screening, process development, product characterization, formulation studies, validated lot release and stability testing. Attendees will have the opportunity to discuss the use of CE with regulatory agencies. In addition, CE troubleshooting approaches will be presented, and instrumentation companies will show advances in CE instruments, sensitivity and reagents. The symposium will allow for open discussions aimed at improving and increasing the use of CE for analysis of proteins, small molecules, carbohydrates, metabolites, and other molecules, with a focus on validation and qualification, new technology and QbD.

The success of this symposium will depend not only on the outstanding cast of experienced and knowledgeable speakers and workshop leaders, but also on the interactions and open discussions that take place among the attendees. We encourage you to participate whole-heartedly in the discussion sections that have been designed to stimulate the exchange of ideas and information.

We would like to thank the speakers who are generously giving their time and resources and also you for your attendance, which will make this endeavor a success.

We gratefully acknowledge the generosity of our exhibitors and program partners: 908 Devices Inc., Advanced Electrophoresis Solutions Ltd., Amgen Inc., BiOptic Inc., CMP Scientific, Biogen, Eli Lilly and Company, Genentech, a Member of the Roche Group, F. Hoffmann-La Roche Ltd., PerkinElmer Inc., Pfizer, Inc., ProteinSimple, a Bio-technique brand, ProZyme, A part of Agilent, Regeneron Pharmaceuticals, Inc., Sanofi, SCIEX and Thermo Fisher Scientific.

We are thankful for the expert assistance of CASSS and the audiovisual expertise of Michael Johnstone from MJ Audio-Visual Productions. Their experience and guidance in the preparation of this symposium have been invaluable.

THE SCIENTIFIC ORGANIZING COMMITTEE

Tim Blanc, *Eli Lilly and Company*

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SungAe Suhr Park, *Samsung Bioepis*

Richard Rustandi, *Merck & Co., Inc.*

Maria A. Schwarz, *Solvias AG*

Zoran Susic, *Biogen*

Ewoud van Tricht, *Janssen Vaccines and Prevention*

Joel Welch, *CDER, FDA (Co-chair)*

CE Pharm Award History and Qualifications

Objective:

Recognize and award an individual for **sustained** and **significant** contribution to the practical application of CE to the analysis of biotechnology and pharmaceutical products.

Qualification for Award:

- a. **Advocate for CE from biotechnology and pharmaceutical industry**
- b. Technical advancement or considered as a leader in developing or implementing various CE applications, such as:
 - New CE Application for R&D
 - CE Method Qualification
 - CE Method Validation
 - CE Method Transfer
- c. Technical reputation, in terms of number of presentations, publications, and patents
- d. Dedication to CE Pharm meeting as speaker, tutor, poster presenter or committee member
- e. Mentor, advisor and advocate of industrial-based CE practitioners in other industrial applications such as food chemistry, forensics and clinical

Past Recipients of the "CE Pharm Award" include:

2006 - Norberto Guzman – *Johnson & Johnson*
2007 - Kevin Altria – *GlaxoSmithKline*
2008 - Anthony Chen and Wassim Nashabeh – *Genentech, Inc.*
2009 - Stacey Ma – *Genentech, Inc.*
2010 - SungAe Suhr Park – *Amgen Inc.*
2011 - Oscar Salas-Solano – *Seattle Genetics, Inc.*
2012 - Franka Kálmán – *University of Applied Sciences Western Switzerland*
2013 - András Guttman – *Northeastern University*
2014 - Michel Girard – *Health Canada*
2015 - Cari Sängler - van de Griend – *Kantisto BV*
2016 - Sarah Kennett – *CDER, FDA*
2017 - Margaret Ruesch – *Pfizer, Inc.*
2018 - Winner will be announced Wednesday at 10:30

Do you think we are missing someone influential? Add your suggestion to the list.

Suggestions for next year's award can be submitted with your post-meeting evaluation.

CASSS CE Pharm Student Travel Grants

CASSS is pleased to provide a limited number of student travel grants for students who present applicable posters at CE Pharm 2018. PhD students or post-doctoral fellows conducting research in academia or industry throughout the world are eligible.

Why you should apply:

This symposium gives insight into the current topics and issues under discussion within the pharmaceutical and biotech industry and, as such, gives attendees the opportunity to bridge between industry, academia and regulatory agencies. The presentations and workshops will be devoted to practical concerns that strengthen the use of CE within the biotechnology and pharmaceutical industries. Applications will highlight uses of CE in various areas of product development, including high-throughput screening, formulation studies, process development, product characterization and validated lot release and stability testing. As a participant, you will have an excellent opportunity to meet, network and participate in exchanging knowledge for mutual education with other CE practitioners.

Requirements are:

- Present a poster on a CE topic
- Proof of studentship/post-doc status
- Recommendation from the supervisor/advisor

CASSS has awarded student travel grants to the following individuals:

Separation of Protein and Peptides using Styrene/Nphenylacrylamide Porous Layer Immobilized Open Tubular Capillary Column

Ashraf Ali, *Inha University, Incheon, South Korea*

Microfluidic Western Blotting with Improved Throughput and Sensitivity

Natalie Arvin, *University of Michigan, Ann Arbor, MI USA*

Size-based and Charge-based Separations for Biopharmaceutical Analysis – Comparative Performance Studies with Various Electrophoretic and Chromatographic Techniques

Julia Kahle, *Technical University Braunschweig, Braunschweig, Germany*

A Nanofluidic Device for Continuous Online Monitoring of Antibody Quality in Continuous Biomanufacturing

Taehong Kwon, *Massachusetts Institute of Technology, Cambridge, MA USA*

Battling the Backlog: Novel CZE System for Forensic Separations

Sarah Lum, *University of Notre Dame, Notre Dame, IN USA*

The Scientific Organizing Committee gratefully acknowledges the following partners for their generous support of this Symposium:

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Welcome Reception

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The Scientific Organizing Committee gratefully acknowledges the following media for their promotional consideration of CE Pharm 2018:

Media Program Partners

American Laboratory/Labcompare
American Pharmaceutical Review
The Analytical Scientist
Genetic Engineering & Biotechnology News
LCGC
The Pathologist
Pharmaceutical Outsourcing
Technology Networks

CE Pharm 2018

Scientific Final Program Summary

Sunday, September 9, 2018

08:00 – 09:00 **Breakfast** (for course attendees ONLY) in Union Square 21 (4th Floor)

08:00 – 13:30 **Registration** (for course attendees ONLY) in the Golden Gate Foyer (Lobby Level)

09:00 – 17:00

Short Course in Union Square 22 (4th Floor)
Applications of Capillary Electrophoresis to the Analysis of Protein Therapeutics

Short Course Facilitators:

David A. Michels, *Genentech, a Member of the Roche Group, South San Francisco, CA USA*
and Cari Sanger - van de Griend, *Kantisto B.V., Baarn, The Netherlands*

12:30 – 13:30 **Hosted Lunch** (for course attendees ONLY) in Union Square 21

16:30 – 19:00 **Registration CE Pharm 2018** in the Golden Gate Foyer (Lobby Level)

17:30 – 17:35 **Welcome and Introductory Comments** in Plaza Room B
David A. Michels, *Genentech, a Member of the Roche Group, South San Francisco, CA USA*

17:35 – 19:00

An Evening Social with CE Experts: A Panel Perspective on Past, Present and Future
in Plaza Room B

Moderator: Tim Blanc, *Eli Lilly and Company, Branchburg, NJ USA*

Panel Members:

Norberto Guzman, *Princeton Biochemicals Inc., Princeton, NJ USA*

Amy Herr, *University of California, Berkeley, Berkeley, CA USA*

Susan Lunte, *University of Kansas, Lawrence, KS USA*

Wassim Nashabeh, *Genentech, a Member of the Roche Group, South San Francisco, CA USA*

SungAe Suhr Park, *Samsung Bioepis, Incheon, South Korea*

Michael J. Ramsey, *University of North Carolina, Chapel Hill, Chapel Hill, NC USA*

19:00 – 21:00 **Welcome Reception** in the Plaza Room A

Monday, September 10, 2018

- 07:30 – 08:30 **Breakfast** in the Golden Gate Ballroom
- 07:30 – 17:45 **Registration** in the Golden Gate Foyer (Lobby Level)
- 08:30 – 08:45 **Welcome and Introductory Comments** in Plaza Room B
David A. Michels, *Genentech, a Member of the Roche Group, South San Francisco, CA USA*

Keynote I Session in Plaza Room B
Session Chair: David A. Michels, *Genentech, a Member of the Roche Group, South San Francisco, CA USA*

- 08:45 – 09:30 **Protein + Isoform Profiling in Single Cells: Electrophoretic Cytometry**
Amy Herr, *University of California, Berkeley, Berkeley, CA USA*
- 09:30 – 09:45 **Discussion**
- 09:45 – 10:15 **Networking Break** – Visit the Exhibits and Posters in the Golden Gate Ballroom

Complex Molecules and/or Formats Session in Plaza Room B
Session Chairs: Nathan Lacher, *Pfizer, Inc., Chesterfield, MO USA*
and Ewoud van Tricht, *Janssen Vaccines and Prevention, Leiden, Netherlands*

- 10:15 – 10:40 **CE Methods for the Quantitation of Adeno-Associated Virus (AAV) Capsid Purity in Support of Gene Therapy**
Emily Menesale, *Biogen, Cambridge, MA USA*
- 10:40 – 11:05 **Use of Capillary Electrophoresis in the Development of Bispecific Fusion Proteins**
Jonathan Gilroy, *Aptevo Therapeutics, Seattle, WA USA*
- 11:05 – 11:30 **Application of Affinity Capillary Electrophoresis for Charge Heterogeneity Profiling of Biopharmaceuticals**
Jan Stracke, *F. Hoffmann-La Roche Ltd., Basel, Switzerland*
- 11:30 – 11:45 **Discussion**

Monday, September 10, 2018 continued

11:45 – 12:30

CE Pharm Partner Showcase in Plaza Room B
Facilitators: Mei Han, *Amgen Inc., South San Francisco, CA USA*
and Nomalie Jaya, *Seattle Genetics, Inc., Bothell, WA USA*

12:30 – 12:45 **Lunch for Technical Seminar Attendees** – Please take lunch and return to Plaza Room B for the “Lunch and Learn”

12:45 – 13:45 **Technical Seminar/Lunch and Learn**

More “iCE” for Your Biologics
Kevin Strozyk, *Seattle Genetics, Inc., Bothell, WA USA*

Sponsored by ProteinSimple, a Bio-Techne brand

Plaza Room B

13:45 – 14:00 **Moving Break**– Please make your way to the Franciscan Ballroom on the Ballroom Level

14:00 – 15:00 **Roundtable Session I** in the Franciscan Ballroom

15:00 – 16:30 **Poster Session I** – Visit the Exhibits and Posters in the Golden Gate Ballroom

Novel Methods and Applications Session in Plaza Room B
Session Chairs: Tara Enda, *Bristol-Myers Squibb Company, Pennington, NJ USA*
and C. Mark Lies, *SCIEX, Brea, CA USA*

16:30 – 16:55 **Capillary Electrophoresis Sodium Hexadecyl Sulfate (CE-SHS): A Novel Approach to Characterizing the Purity of Certain Therapeutic Proteins**
Jeff Beckman, *Bristol-Myers Squibb Company, Devens, MA USA*

16:55 – 17:20 **Detection and Quantification of Homodimer Impurities in Bispecific Antibodies by Affinity Capillary Electrophoresis**
Kelsey Dent, *Genentech, a Member of the Roche Group, South San Francisco, CA USA*

17:20 – 17:45 **Contamination Control of Bound Species Determination by iCE for a Metal-bound Protein**
Xiaoping He, *Pfizer, Inc., Chesterfield, MO USA*

17:45 – 18:00 **Discussion**

Monday, September 10, 2018 continued

18:00 – 18:10

Kevin Altria Memorial Presentation

18:10 – 19:10

Exhibition Reception – Visit the Exhibitors in the Golden Gate Ballroom

Tuesday, September 11, 2018

- 07:30 – 08:30 **Breakfast** in the Golden Gate Ballroom
- 07:30 – 08:30 **New Member Breakfast** in Union Square 23 & 24 (4th Floor)
- 08:00 – 18:00 **Registration** in the Golden Gate Foyer
- 08:30 – 09:30

Troubleshooting Workshop in Plaza Room B

Workshop Facilitators: Tim Blanc, *Eli Lilly and Company, Branchburg, NJ USA*
and Cari Sanger - van de Griend, *Kantisto B.V., Baarn, Netherlands*

- 09:30 – 10:00 **Networking Break** – Visit the Exhibits and Posters in the Golden Gate Ballroom

Regulatory Session in Plaza Room B

Session Chairs: SungAe Suhr Park, *Samsung Bioepis, Incheon, South Korea*
and Zoran Susic, *Biogen, Cambridge, MA USA*

- 10:00 – 10:25 **Counterfeit: Strategies for the Testing of Monoclonal Antibodies. A Regulator’s Perspective**
Wolf Holtkamp, *Paul-Ehrlich-Institut, Langen, Germany*
- 10:25 – 10:50 **A Regulatory Perspective on CE Method Validation During Product Development**
Jacek Cieslak, *CDER, FDA, Silver Spring, MD USA*
- 10:50 – 11:15 **Korea FDA's Past, Present and Future on Regulation of Biopharmaceuticals & A Consideration of Microbiome Metabolomics**
Suenie Park, *Hallym University, Chuncheon, South Korea*
- 11:15 – 11:30 **Discussion**
- 11:30 – 11:45 **Lunch for Technical Seminar Attendees** – Please take lunch and return to the Plaza Room B for the “Lunch and Learn”

11:45 – 12:45 **Technical Seminar/Lunch and Learn**

Novel Approaches to Improving CE Performance for Purity and ADC Analyses

Richard Brown, *LifeArc, Stevenage, United Kingdom*

Samuel Shepard, *MedImmune Limited, Cambridge, United Kingdom*

Sponsored by SCIEX

Plaza Room B

Tuesday, September 11, 2018 continued

12:45 – 14:15 **Poster Session II** - Visit the Exhibits and Posters in the Golden Gate Ballroom

Probing Protein Stability Session in Plaza Room B

Session Chair: Mei Han, *Amgen Inc.* and Kathir Muthusamy, *Regeneron Pharmaceuticals, Inc.*

14:15 – 14:40 **Using CE-SDS-LIF and CE-MS to Characterize Enzyme Induced Degradation of Protein Therapeutics**
Yunan Wang, *Amgen Inc., South San Francisco, CA USA*

14:40 – 15:05 **Application of SDS-CGE to the Purity and Stability Assessment of Vaccine Antigens**
Michael Leach, *Sanofi Pasteur, Toronto, ON Canada*

15:05 – 15:30 **There is More CZE for Proteins!**
Cari Sanger-van de Griend, *Kantisto B.V., Baarn, Netherlands*

15:30 – 15:45 **Discussion**

15:45 – 16:45 **Technical Seminar**

Host Glycomic Determinants of Interferon- α -Mediated Reduction of HIV Persistence *in vivo*

Mohamed Abdel-Mohsen, *The Wistar Institute, Philadelphia, PA USA*

Sponsored by Thermo Fisher Scientific

Plaza Room B

16:45 – 17:00 **Networking Break** – Please make your way to the Franciscan Ballroom on the Ballroom Level

17:00 – 18:00 **Roundtable Session II** in the Franciscan Ballroom

18:00 – 19:00 **Networking Reception**

Wednesday, September 12, 2018

07:30 – 08:30 **Breakfast** in the Golden Gate Ballroom

08:00 – 16:15 **Registration** in the Golden Gate Foyer

Method Development and Validation Considerations Session I in Plaza Room B
Session Chairs: Nomalie Jaya, *Seattle Genetics, Inc., Bothell, WA USA*
and Richard Rustandi, *Merck & Co., Inc., West Point, PA USA*

08:30 – 08:55 **Do You CE What I CE? Lessons Learned from Method Transfers**
Theresa Hassett, *Seattle Genetics, Inc., Bothell, WA USA*

08:55 – 09:20 **Method Puberty; Adenovirus Quantification CZE Method Implementation Challenges**
Lars Geurink, *Janssen Vaccines and Prevention, Leiden, Netherlands*

09:20 – 09:45 **Supporting the Success and Consistency of Analytical Methods in Multiple Laboratories: A Case Study using Microfluidic Capillary Gel Electrophoresis**
Michael Smith, *GlaxoSmithKline, King of Prussia, PA USA*

09:45 – 10:00 **Discussion**

10:00 – 10:30 **Networking Break** – Visit the Exhibits and Posters in the Golden Gate Ballroom

10:30 – 10:45 **Presentation of the CE Pharm Award**

Method Development and Validation Considerations Session II in Plaza Room B
Session Chairs: Steffen Kiessig, *F. Hoffmann-La Roche Ltd., Basel, Switzerland*
and Maria A. Schwarz, *Solvias AG, Kaiseraugst, Switzerland*

10:45 – 11:10 **Evaluating the Transition from CE to MCE in the Quality Control Laboratory**
Timothy Riehlman, *Regeneron Pharmaceutical, Inc. Rensselaer, NY USA*

11:10 – 11:35 **Development of a CE-SDS-based Method using the Quality by Design Approach**
Philippe Simeoni, *Novartis Pharma AG, Basel, Switzerland*

11:35 – 12:00 **Validation Strategies and What Method Parameters to Focus on**
Angelina Rafai, *Solvias AG, Kaiseraugst, Switzerland*

12:00 – 12:15 **Discussion**

Wednesday, September 12, 2018 continued

12:15 – 12:30 **Lunch for Technical Seminar Attendees** – Please take lunch and return to Plaza Room B for the “Lunch and Learn”

12:30 – 13:30 **Technical Seminar/Lunch and Learn**

LabChip Electrophoresis: A Robust, Sensitive and Intelligent Platform for Bi-therapeutics Characterization

Anubhar Tripathi, *Brown University, Providence, RI USA*

Sponsored by PerkinElmer

Plaza Room B

13:30 – 13:45 **Mini Break**

Young Scientist Session in Plaza Room B

Session Chairs: Richard Rustandi, *Merck & Co., Inc., West Point, PA USA*
and Ewoud van Tricht, *Janssen Vaccines and Prevention, Leiden, Netherlands*

13:45 – 13:55 **A Nanofluidic Device for Continuous Online Monitoring of Antibody Quality in Continuous Biomanufacturing**

Taehong Kwon, *Massachusetts Institute of Technology, Cambridge, MA USA*

13:55 – 14:05 **Size-based and Charge-based Separations for Biopharmaceutical Analysis – Comparative Performance Studies with Various Electrophoretic and Chromatographic Techniques**

Julia Kahle, *Technical University Braunschweig, Braunschweig, Germany*

14:05 – 14:15 **Microfluidic Western Blotting with Improved Throughput and Sensitivity**

Natalie Arvin, *University of Michigan, Ann Arbor, MI USA*

Unexpected Uses in Characterization Session in Plaza Room B

Session Chairs: Tim Blanc, *Eli Lilly and Company, Branchburg, NJ USA*
and Göran Hübner, *Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany*

14:15 – 14:40 **CE in Biopharma Science: It's More than GCE and cIEF. Unexploited Applications from the Sewing Box**

Samuel Bader, *Solvias AG, Kaiseraugst, Switzerland*

14:40 – 15:05 **Approaches to Developing a Robust icIEF Charge Variant Method**

Elisabeth Krug, *Eli Lilly and Company, Indianapolis, IN USA*

Wednesday, September 12, 2018 continued

15:05 – 15:30 **Applications of Zip Chip CE-MS for Monitoring of Product Quality During Manufacturing Process**
Pamela Feng, *Biogen, Cambridge, MA USA*

15:30 – 15:45 **Discussion**

15:45 – 16:00 **Mini Break**

<p>Keynote II Session in Plaza Room B Session Chair: Joel Welch, <i>CDER, FDA, Silver Spring, MD USA</i></p>

16:00 – 16:45 **CE in Drug Development: Where Have We Been and Where Are We Going?**
Barry Karger, *Professor Emeritus Northeastern University, Boston, MA USA*

16:45 – 17:00 **Discussion**

17:00 – 17:15 **Closing Comments** in Plaza Room B
Joel Welch, *CDER, FDA, Silver Spring, MD USA*

NOTES:

An Evening Social with CE Experts: A Panel Perspective on Past, Present and Future

Moderator:

Tim Blanc, *Eli Lilly and Company, Branchburg, NJ USA*

Panel Members:

Norberto Guzman, *Princeton Biochemicals Inc., Princeton, NJ USA*

Amy Herr, *University of California, Berkeley, Berkeley, CA USA*

Susan Lunte, *University of Kansas, Lawrence, KS USA*

Wassim Nashabeh, *Genentech, a Member of the Roche Group, South San Francisco, CA USA*

SungAe Suhr Park, *Samsung Bioepis, Incheon, South Korea*

Michael J. Ramsey, *University of North Carolina, Chapel Hill, Chapel Hill, NC USA*

This year marks the 20th anniversary of the CASSS CE Pharm Conference and the organizing committee have arranged a special tribute to some of the key pioneers and innovators of capillary electrophoresis (CE) whose work has greatly shaped and influenced the way CE technology is utilized and regulated within our industry. This event will showcase a diverse group of panelists who possess a wealth of historical insights and experiences and will provide attendees the opportunity to engage in an open dialogue with some of the most well-recognized CE contributors from the past few decades. In addition, this evening event will create a unique and memorable way to kick-off the meeting. Timothy Blanc, who is a long-time expert and veteran of the CE organizing committee, will graciously host this panel discussion and lead a format that invites each panelist to share a short story related to their personal CE journey. The panelist stories will then be followed by a 25-minute open Q&A session.

NOTES:

NOTES:

Oral Abstracts

Protein + Isoform Profiling in Single Cells: Electrophoretic Cytometry

Amy Herr

University of California, Berkeley, Berkeley, CA USA

From fundamental biosciences to applied biomedicine, high dimensionality data is increasingly important. In single-cell measurement tools, microfluidic design has underpinned the throughput, multiplexing and quantitation needed for this rich data. Genomics and transcriptomics are leading examples. Yet, measurement of proteins lags. While proteins and their dynamic forms are the downstream effectors of function, the immunoassay remains the *de facto* standard (flow cytometry, mass cytometry, immunofluorescence). We posit that to realize the full potential of high-dimensionality cytometry, new approaches to protein measurement are needed. I will describe our ‘electrophoretic cytometry’ tools that increase target selectivity beyond simple immunoassays. Enhanced selectivity is essential for targets that lack high quality immunoreagents – as is the case for the vast majority of protein forms (proteoforms). I will share our results on highly multiplexed single-cell western blotting and single-cell isoelectric focusing that resolves single charge-unit proteoform differences. In fundamental engineering and design, I will discuss how the physics and chemistry accessible in microsystems allows both the “scale-down” of electrophoresis to single cells and the “scale-up” to concurrent analyses of large numbers of cells. Particular emphasis will be placed on precision control of fluids and materials transport in passive systems, with no pumps or valves. Precise reagent control allows for integration of cytometry with sophisticated sample preparation – the unsung hero of measurement science. I will discuss compartment-specific, single-cell western blotting for nucleo-cytoplasmic profiling, which eliminates the need for complex image segmentation algorithms. Lastly, I will link our bioengineering research to driving cytology needs, including understanding the role of protein signaling and truncated isoforms in development of breast cancer drug resistance and understanding protein signaling in individual circulating tumor cells. Taken together, we view microfluidic design strategies as key to advancing protein measurement performance needed to address unmet gaps in quantitative biology and precision medicine.

NOTES:

CE Methods for the Quantification of Adeno-Associated Virus (AAV) Capsid Purity in Gene Therapy

Emily Menesale

Biogen, Cambridge, MA USA

Recombinant adeno-associated virus (AAV) is a promising platform for human gene therapy. AAV capsids are comprised of three viral proteins: VP1, VP2, and VP3, with different ratios reported for several serotypes. Assessment of capsid purity is necessary for release testing and product development to ensure product quality and process consistency. SDS-PAGE has traditionally been used to assess capsid protein purity, but it is labor-intensive and produces semi-quantitative results. Capillary electrophoresis (CE) is widely used in analyzing product purity for biologics products. Here, we discuss our development and optimization of a CE-SDS method to assess AAV capsid purity. This method can quantitate the AAV capsid ratio and detect minor impurity species. It has also demonstrated promises in analyzing stability samples from different stress conditions. This robust CE-SDS method is a significant improvement over traditional gel-based methods, and it can be used to support process development and release testing.

NOTES:

Use of Capillary Electrophoresis in the Development of Bispecific Fusion Proteins

Jonathan Gilroy

Aptevo Therapeutics, Seattle, WA USA

Analytical methods that will accurately characterize the critical quality attributes of a molecule need to be developed to ensure that the molecule has a suitable safety profile for conducting animal toxicology studies and clinical trials. Since no single attribute can completely demonstrate the safety and product quality of a molecule, many of the attributes of the molecule must be measured using a combination of analytical methods. Capillary electrophoresis provides several useful techniques for characterizing these therapeutics to better understand the product quality of these molecules. We present the utility of capillary gel electrophoresis (CGE) and capillary isoelectric focusing (cIEF) methods in process development, drug product release, and stability testing of ADAPTIR™ bispecific fusion proteins. CGE is discussed as a useful tool for monitoring size variants for drug product release and stability testing. cIEF is a widely used technique to monitor the charge distribution of proteins. We show increased resolution and recovery for representative ADAPTIR proteins with cIEF relative to other analytical methods. The impact of sample preparation, denaturant, and non-specific interactions are discussed. Overall, these methodologies enable efficient monitoring and characterization of size and charge variants for complex biotherapeutics.

NOTES:

Application of Affinity Capillary Electrophoresis for Charge Heterogeneity Profiling of Biopharmaceuticals

Bernd Moritz¹, Andrei Hutanu², Steffen Kiessig¹, Andrea Bathke¹, Cecile Avenal¹, Markus Wild¹, Jan Stracke¹

¹*F. Hoffmann-La Roche Ltd., Basel, Switzerland,* ²*Technical University of Munich, Munich, Germany*

Charge heterogeneity profiling represents an important part in quality control (QC) of biopharmaceuticals. Due to the increasing complexity of these therapeutic entities, the development of new analytical techniques is needed. In this work, affinity capillary electrophoresis (ACE) was established as a method for the analysis of a mixture of two similar monoclonal antibodies (mAbs) that is typical for novel co-formulation approaches. The addition of a specific ligand/antigen results in the complexation of one mAb in the co-formulation, thus changing its migration time in the electric field. Consequently, the charged variants of the not shifted mAb can be characterized without interferences. Additionally, partial-filling ACE (PFACE) was implemented for further improvement of the method. This approach requires only very small amounts of ligands and was completely comparable to capillary zone electrophoresis (CZE) results of the same non-co-formulated mAb without antigen additive. In parallel, sample preparation is simplified. Applicability of the approach for routine QC testing has been demonstrated.

NOTES:

Capillary Electrophoresis Sodium Hexadecyl Sulfate (CE-SHS): A Novel Approach to Characterizing the Purity of a Variety of Therapeutic Proteins

Jeff Beckman

Bristol-Myers Squibb Company, Devens, MA USA

Capillary Gel Electrophoresis using sodium dodecyl sulfate (CE-SDS) is used commercially to provide quantitative purity data for therapeutic protein characterization and release. Acceptable performance of this method would yield protein peaks that are baseline resolved and symmetrical. Nominal CE-SDS conditions and parameters were not optimal for several therapeutic proteins including two mAbs and a fusion protein, where acceptable resolution and peak symmetry were not achieved. The application of longer alkyl chain detergents, such as sodium hexadecyl sulfate, in the running buffer matrix increased peak resolution and plate count by several fold compared to a traditional SDS-based running gel matrix. At BMS we developed and qualified viable “CE-SHS” methods for characterization and release using an SHS-containing running buffer matrix. The mechanism by which these detergents achieved these results is discussed. This work underscores the potential of detergents other than SDS to enhance the resolution and separation power of CE-based separation methods.

NOTES:

Detection and Quantification of Homodimer Impurities in Bispecific Antibodies by Affinity Capillary Electrophoresis

Kelsey Dent, Toby Reichenberg, David Fischer, David Michels

Genentech, a Member of the Roche Group, South San Francisco, CA USA

Bispecific antibodies (BsAbs) have attracted widespread therapeutic interest in recent years due to their unique ability to recognize two distinct antigen targets. At Genentech, these BsAbs are primarily generated via knob-into-hole technology, whereby complementary mutations are made in the CH3 domain of the heavy chains to form “knob” and “hole” structures. Production of knob-into-hole BsAbs can result in a unique set of product-related impurities, including both knob-knob and hole-hole homodimer species. These homodimer variants can be challenging to quantitate as they may be present at low levels and have physicochemical characteristics that are highly similar to the intended BsAb product. Depending on the BsAb target(s), homodimer species may pose a risk to patient safety and consequently must be accurately and precisely quantitated. To insure product quality, these homodimers, if formed, must be cleared during purification and must be controlled over the product’s shelf life.

We present a highly specific affinity capillary electrophoresis (ACE) method for detection of homodimers in bispecific products. In this method, the specificity and affinity of the BsAb target to the antigen are exploited to achieve a separation by CZE based on differences in electrophoretic mobility. Details of method development and implementation of this novel assay to support process and product development are described.

NOTES:

Contamination Control of Bound Species Determination by iCE for a Metal-bound Protein

Xiaoping He

Pfizer, Inc., Chesterfield, MO USA

Imaged capillary electrophoresis (iCE) was used to determine the contents of the metal bound species and the metal free species for a protein component in a vaccine that targets multiple antigens. Inconsistent measurements of %bound species among analysts and laboratories have been observed over times, which prevented the iCE method from being validated. Investigations on potential causes were carried out and the results demonstrated that variations in %bound species were neither product nor process associated, and were caused by metal contaminations in pI markers and other assay reagents. Based on the findings, proper controls over metal contamination were established for the method, which will enable a successful validation of the iCE method for the purpose of measuring the contents of metal bound and metal free species of the protein of interest.

NOTES:

Counterfeit: Strategies for the Testing of Monoclonal Antibodies. A Regulator's Perspective

Wolf Holtkamp

Paul-Ehrlich-Institut, Langen, Germany

There is growing universal concern regarding falsified medicines not only because of major economic damage but more importantly as a serious risk to human health. Monoclonal antibodies (mAb) are one of the largest growing product classes for the treatment of a variety of diseases, with combined world-wide sales around \$125 billion. Due to their high cost mAbs became a preferred target for counterfeiting. This leads to the need to develop new analytical techniques that can be employed in control laboratories to assess the safety, quality, and identity of these molecules. In Germany the Paul-Ehrlich-Institut (PEI) is responsible for the safety and efficacy of vaccines and biomedicines and regulates the admission to the German market. In the course of this process PEI is also testing mAbs within the CAP-Testing program organized by the European Directorate of the Quality of Medicines & HealthCare (EDQM). As a national control authority PEI is also informed about potential counterfeit products detected on the market and, where appropriate, is responsible to take measures. To be able to fulfill this function and to be prepared for the testing of counterfeit products it is important to develop general methods for the identification of mAbs that are specific, robust, and valid. We set out to establish a cIEF-method that can routinely be used in control laboratories to identify a brought range of mAbs due to their charge heterogeneity. This provides important information about product quality, stability, and the identity of the respective molecules. Together with other techniques such as peptide mapping analysis, ion exchange chromatography or SDS-PAGE analysis, cIEF provides the tools to identify counterfeit medication to ensure patient-safety and to reduce public-health risks.

NOTES:

A Regulatory Perspective on CE Method Validation During Product Development

Jacek Cieslak

CDER, FDA, Silver Spring, MD USA

Development and validation of the capillary electrophoresis (CE) methods used for testing of product quality during product development is a continuous and evolving process. Developing and implementing CE assays suitable to assure patient safety in early clinical stages and transitioning to validated assays that can support a commercial license application may present unique development and regulatory challenges. This presentation will provide regulatory expectations for qualification and validation of CE methods at different stages of product development, including discussion of case studies.

NOTES:

Korea FDA's Past, Present and Future on Regulation of Biopharmaceuticals & A Consideration on Microbiome Metabolomics

Suenie Park

Hallym Univerisity, Chuncheon, South Korea

Korea FDA (currently named as Ministry of Food and Drug Safety, MFDS) has strengthened its capacity of regulating biopharmaceuticals with tremendous efforts to harmonize to the advanced regulatory organization, including EU and US-FDA during last 2 decades. In this period, I have been working related to many innovative programs carried out KFDA(MFDS) for app. 15 years. Last 5 years, I have been working in private sector. Still, I am also serving as a global advancing division chief of private sector of Dynamic Bio program (Bio-industry Supporting Strategic Planning Board) nominated from Korea FDA(MFDS) since 2016. Notably, based on last two decades' efforts KFDA(MFDS) became to function as a WHO collaborating center, a WHO education center, a WHO testing Lab., and a Chairman country on Biosimilar Regulation in International Pharmaceutical Regulators Programme(IPRP) in 2013 in addition to join to PIC/S in 2014 and obtain ICH Regulatory member country in 2016. Based on professional and effective communication between regulatory personnel and biopharmaceuticals developers, several leading companies from Korea have enlisted their products successfully to EMA and FDA. Those companies are such as Celltrion, Samsung Bioepis and Medipost etc on Biosimilar products and Stem cell therapeutics. This year, KFDA(MFDS) will open the third international symposium on Biopharmaceutical (GBC2018) during June 26~29 in Seoul, Korea. In this talk, I will introduce some on this Global Bioconference including MFDS history, several systems with which MFDS has worked for supporting for Biocompanies for the above Korea companies' successes and current strategies as for 2018 based both on my public and private sector experiences. I will be also discussing a few examples on Method validation processes such as HPLC including CE for assessing the specification and experimental methods validation of biopharmaceuticals on biopharmaceuticals and microbiome metabolomics for precision medicine.

NOTES:

Using CE-SDS-LIF and CE-MS to Characterize Enzyme Induced Degradation of Protein Therapeutics

Yunan Wang, Mei Han, Nikita Patel, Dan Rock, Brooke Rock

Amgen Inc., South San Francisco, CA USA

Proteins constitute an expanding class of therapeutic molecules. These include novel molecules such as cytokines fused to either N- or C- terminal of an antibody or the Fc- portion of the antibody to extend the molecule's *in vivo* half-life. These novel fusion molecules can be susceptible to proteolytic degradation *in vivo*. Therefore, characterizing the protein stability with enzyme induced degradation prior to *in vivo* studies can reduce, replace, and refine the use of animals, as well as improve efficiency of lead candidate selection.

Capillary electrophoresis sodium dodecyl sulfate (CE-SDS) as a sensitive, robust and reliable separation and quantification method with high molecular weight resolution has been widely used for protein purity and impurity assessment in the biopharmaceutical industry. Here, we utilize the CE-SDS with laser-induced fluorescence detection (CE-SDS-LIF) to separate and quantify the various truncated fragments of the Fc-fusion protein along with their intact form over the time course in the *in vitro* enzymatic induced degradation studies.

Immunoaffinity capture capillary electrophoresis–mass spectrometry (IA-CE-MS) is a powerful tool for intact mass analysis of specific proteins in complex matrices [1]. It extracts both the intact therapeutic protein and potential catabolites from the matrix. Coupling the separation power of CE to a time-of-flight (TOF) mass spectrometry using the nanoflow sheath-liquid interface provides an accurate identification of the truncated fragments. In this work, we used this process to identify the truncated forms from enzymatic induced degradation *in vitro* studies.

[1] Mei Han, Josh T. Pearson, Yunan Wang, Dwight Winters, Marcus Soto, Dan A. Rock, Brooke M. Rock *Analytical Biochemistry* 539 (2017) 118–126

NOTES:

Application of SDS-CGE to the Purity and Stability Assessment of Vaccine Antigens

Michael Leach, Elena Newman, Bruce Carpick

Sanofi Pasteur, Toronto, ON Canada

The assessment of protein antigen purity and stability under thermal stress are key elements in an analytical strategy for control and characterization of protein-based vaccines both during their clinical development and during their production post-licensure. In Sanofi Pasteur, we have developed purity and stability-indicating SDS-CGE methods to support multiple vaccine projects, including a bacterial multi-subunit toxin, a bacterial outer member virulence factor, and the Tuberculosis Hyvac (H4) antigen, which is a fusion protein of Tuberculosis antigens Ag85B and TB10.4 developed by the Statens Serum Institut, Denmark. Such antigen proteins have traditionally been tested for purity and stability using SDS-PAGE. Our general approach to development of SDS-CGE methods on Sciex PA800+ CE systems is to optimize sample preparation factors (reducing agent, heating temperature, sample load) and instrument parameters (injection mode and conditions, running conditions), in order to enhance peak resolution for purity methods and relative peak area precision for stability-indicating methods. This approach yields CE methods suitable for use in GMP release of clinical antigen Drug Substance as well as characterization of antigen stability under thermal stress. Our general method development approach and method performance results for select purity and relative antigen content assays will be presented.

NOTES:

There is More CZE for Proteins!

Cari Sanger-van de Griend

Kantisto B.V., Baarn, Netherlands

CZE is regaining interest in the biopharma field with the implementation of the “aminocaproic acid method” for monoclonal antibodies [1,2]. However, you can do much more! In this lecture we will look at other examples such as the use of CZE to study protein folding and the protein higher order structure as well as preliminary results for protein mapping.

[1] Y He, C Isele, W Hou and M Ruesch, Rapid analysis of charge variants of monoclonal antibodies with capillary zone electrophoresis in dynamically coated fused-silica capillary, *J Sep Sci* 34 (2011) 548 – 555

[2] B Moritz, V Schnaible, S Kiessig, A Heyne, M Wild, C Finkler, S Christians, K Muller, L Zhang, K Furuya, M Hassel, M Hamm, R Rustandi, Y He, O Salas Solano, C Whitmore, S Park, D Hansen, M Santos, M Lies, Evaluation of capillary zone electrophoresis for charge heterogeneity testing of monoclonal antibodies, *J Chromatogr B* 983-984 (2015) 101 – 110

NOTES:

Do You CE What I CE? Lessons Learned from Method Transfers

Theresa Hassett, Tami Wu

Seattle Genetics, Inc., Bothell, WA USA

Capillary Electrophoresis methods are commonly utilized for lot release and stability testing of biopharmaceutical products. Method transfers are important aspects in the lifecycle of a product that have the potential of impacting product quality and patient safety. There are published guidance and requirements for method transfer by regulatory agencies, however each transfer situation is unique. In this presentation, we will discuss challenges and lessons learned from method transfer between Sending Lab (SL) to Receiving Lab (RL). Factors such as differences in instrument, technique or interpretation of the procedure can lead to transfer failures. Elements of the transfer process will be presented as well as case studies from successful and challenging method transfers.

NOTES:

Method Puberty; Adenovirus Quantification CZE Method Implementation Challenges

Lars Geurink¹, Ewoud van Tricht¹, Cari Sanger-van de Griend²

¹*Janssen Vaccines and Prevention, Leiden, Netherlands,* ²*Kantisto B.V., Baarn, Netherlands*

As in real life, any analytical method implemented progresses from a child in development to a mature grown up. The maturation of a test method comes with a lot of changes and discussions. We developed and implemented a CZE method for the quantification of the adenovirus concentration.

The implementation of the CZE method was performed according to analytical quality by design (AQbD) principles. First, a control strategy was defined by means of a Failure Mode Effect Analysis (FMEA) to mitigate all risk related to the method of transfer and operation. Operators were trained, and equipment and materials were purchased. After implementation method performance was measured by trending of critical performance indicators and by keeping an issue log. In parallel with the method implementation, additional features were requested and developed.

Since the method was implemented, nearly 600 assay runs (1500 samples), except for 1, were analyzed at the planned date. Nonetheless, a hyper-care period with trending, issue logging and recurring meetings was needed. To date, the test method was validated for virus seed release, in-process control testing and DS / DP release and stability testing and the method is carried out by about 15 trained operators at 5 different sites.

Although the implementation was directed towards success through careful preparation and close monitoring, the adenovirus quantification CZE method maturation was turbulent and full of lessons learned.

NOTES:

Supporting the Success and Consistency of Analytical Methods in Multiple Laboratories: A Case Study using Microfluidic Capillary Gel Electrophoresis

Mike Smith

GlaxoSmithKline, King of Prussia, PA USA

When progressing biotherapeutic products into late phase development, analytical methods are often transferred to commercial testing sites or external contract research organizations (CROs). Managing the performance of modern analytical methods in these laboratories is often a challenging task due to their sophistication and complexity. High invalid assay rates, misalignment of assay best practices, and inconsistent training practices at the testing site are some of the potential undesirable effects that require consistent monitoring. Successful implementation of complex analytical technologies and consistency of method performance at commercial testing laboratories requires proactive, collaborative, and innovative support from technical subject matter experts throughout the late-phase lifecycle of a product.

Microfluidic capillary gel electrophoresis m(CGE) is an analytical technique used for purity testing of biotherapeutics at release and on stability. Since 2010, through extensive collaboration between multiple R&D and QC lines, GSK has industrialized the use of this CGE format for commercial products. MCGE was first introduced as a replacement for SDS-PAGE analysis, and subsequently developed as a platform method for early-phase monoclonal antibodies. Validated methods for two late-phase products were transferred to the commercial testing sites, and since then the products have been commercialized and the mCGE methods are currently in use for batch release and stability testing. This talk will discuss the strategies and challenges involved in these method implementations. Additionally, several examples of collaborative approaches to improving and sustaining method performance within the commercial testing sites will be discussed.

NOTES:

Evaluating the Transition from CE to MCE in the Quality Control Laboratory

Timothy Riehlman

Regeneron Pharmaceutical, Inc., Rensselaer, NY USA

Implementation of robust, reproducible, user-friendly technology is critical to meet the testing demands for biological products placed on today's Quality Control (QC) laboratories. Upgrades in technology are necessary to facilitate increased output, while continuing to generate quality analytical data and attempting to minimize the number of invalid test results and instrument-related investigations. While electrophoresis has historically been used in QC for product purity and fragmentation analysis, the methodology has transitioned from gel, to capillary, and more recently, to the microchip. Microchip Capillary Electrophoresis (MCE) allows for dramatically reduced sample analysis times, while maintaining the performance and reproducibility standards required for QC analysis. Here, we evaluate the development of the MCE assay for implementation in the QC lab, assess critical assay attributes, and examine instrument performance and invalid rates.

NOTES:

Development of a CE-SDS-based Method using the Quality by Design Approach

Philippe Simeoni

Novartis Pharma AG, Basel, Switzerland

Capillary electrophoresis (CE-SDS) under reduced conditions is a well-established analytical method to assess the purity of Biologics such as antibodies (mAbs). Although a platform method with generic electrophoretic conditions is established, the electrophoretic conditions have to be confirmed with every mAb and occasionally might have to be adapted to ensure a proper purity determination. Applying the generic method to our mAb resulted in a non-tolerable heavy chain peak tailing. To overcome this issue, a method development using the Quality by Design approach was performed. Required performance criteria to control product variants or impurities were defined in an Analytical Target Profile (ATP). Then, numerical method performance output criteria to measure method performance with respect to the ATP were defined. A screening Design of Experiment (DoE) demonstrated which potential input parameter impacts the performance of the method. Optimization of the electrophoretic conditions was performed using an optimization DoE. Finally, all information was combined into a method design space. The acquired method understanding helped us to move an inadvisable buffer peak arising from buffer degeneration out of the region of interest. The enhanced method understanding helped achieving the predefined analytical performance and robustness.

NOTES:

Validation Strategies and What Method Parameters to Focus on

Angelina Rafai, Maria A. Schwarz

Solvias AG, Kaiseraugst, Switzerland

Analytical method validation is a crucial part of the method life cycle with respect to pharmaceutical products. In general, method validation is performed according to the ICH guideline Q2(R1) “Validation of Analytical Procedures: Text and Methodology”. The guideline describes the procedure for the evaluation of required validation characteristics for identity tests, impurity tests (quantitative and limit) and assays. However, considering all analytical test methods applied in routine analysis of pharmaceutical products, particularly biopharmaceuticals (release and stability testing) it becomes evident that a high number of these methods are based on the determination of relative peak areas, i.e. area percentage methods. Since these methods cannot be allocated to any of the method types mentioned in the ICH guideline, they are usually treated as quantitative methods. As such, validation characteristics like linearity, accuracy and LOQ are usually assessed based on absolute peak areas rather than relative. Since the absolute peak areas do not correspond to the analytical result of area percentage methods, the common validation procedures somehow reveal gaps. The presentation will discuss on current validation procedures, which validation parameters to focus on and suitable suggestions for area percentage methods.

NOTES:

A Nanofluidic Device for Continuous Online Monitoring of Antibody Quality in Continuous Biomanufacturing

Taehong Kwon, Ko Sung Hee, Jean-François Hamel, Jongyoon Han

Massachusetts Institute of Technology, Cambridge, MA USA

Biologics are highly sensitive to manufacturing, handling, and storage conditions due to large size and complex structure. Undesirable physical, chemical, and biological changes in biologics may reduce drug efficacy and compromise patients' safety by inducing immune responses. Thus, real-time continuous assessments of critical quality attributes (*e.g.*, purity and bioactivity) of therapeutic proteins at all stages of manufacturing process is crucial for maintaining product quality and making rapid decision concerning the manufacturing process for ensuring patients' safety.

In this context, we introduce a novel nanofluidic device for continuous-flow multivariate protein analysis with high detection sensitivity, resolution, and analysis speed for real-time critical quality assessments. Periodic and angled nanofilter arrays served as molecular sieving structure were fabricated to achieve continuous-flow size-based analysis of therapeutic proteins in relatively simple operation, allowing for real-time analysis. Different configurations of nanofilter arrays enabled both (SDS-based) size separation for purity analysis and homogeneous binding assay for activity analysis.

Considering that there is a growing trend toward continuous biomanufacturing to improve product quality and reduce manufacturing cost, we implemented fully automated continuous online monitoring of purity of monoclonal antibodies (IgG₁) produced from small-scale (350 mL) perfusion culture of Chinese Hamster Ovary (CHO) cells using a membrane-less cell retention device. The cell culture supernatant containing IgG₁ was continuously fed into the online sample preparation system (buffer exchange, labeling, denaturation) after culture clarification, and antibody purity was analyzed by the nanofluidic device.

The system developed here can be extended to perform nanofluidic homogeneous binding assay, thus enabling continuous online multiparameter analysis of antibody quality during continuous biomanufacturing. The nanofluidic device is expected to complement the existing bench-type conventional analysis tools, such as CE-SDS, SDS-PAGE, and binding assay equipment, to meet quality assurance requirements of current and future biomanufacturing systems.

NOTES:

Size-based and Charge-based Separations for Biopharmaceutical Analysis – Comparative Performance Studies with Various Electrophoretic and Chromatographic Techniques

Julia Kahle, Kai Jorrit Maul, Holger Zagst, Hermann Wätzig

Technische Universität Braunschweig, Braunschweig, Germany

In an inter-instrument approach, the current performance and limits of CE-SDS were tested [1]. Next to general performance parameters like precision, linearity and resolution, ease of use, reliability and sustainability were considered. Here, the determination of intermediate precision with long measurement series was the central focus. RSD values down to 0.16 % (relative migration times, n=120) and 1.7 % (%areas, n=52) were achieved. Comparable measurements were conducted with size exclusion chromatography (SEC).

Next, several options for the analysis of charge variants were evaluated. Especially (imaged) capillary isoelectric focusing (cIEF) and ion exchange chromatography (IEX) are well-established tools for their determination. Additionally, capillary zone electrophoresis (CZE) significantly gained in importance [2]. It is not completely clarified which of these separation techniques is most suitable for which specific analytical issue. Therefore, three test molecules (NISTmAb, Infliximab and Erythropoietin) were measured with the four above stated techniques. Here, precision (RSD down to 0.9 % for the %areas, n=60), resolution/peak count (up to 10 variants for NISTmAb), analysis time and ease of use were selected as the most important parameters.

Method development was found to be sophisticated particularly for cIEF. Therefore, a design of experiments (DOE) approach and multidimensional regression was conducted for the isoelectric focusing methods. Main goal was to achieve a deeper understanding of the adjustable parameters ampholyte concentration (2 to 8 %), ampholyte ratio (1:1 to 1:0), L-arginine (0 to 10 mM) and urea (2 to 4 M) amounts. A downscaled version of this design can be used for fast and accurate method adjustments.

[1] Kahle J., Maul K. J., Wätzig H., *The next generation of capillary electrophoresis instruments: Performance of CE-SDS protein analysis*, Electrophoresis 2018, 39, 311-325.

[2] Kahle J., Wätzig H., *Determination of protein charge variants with (imaged) capillary isoelectric focusing and capillary zone electrophoresis*, Electrophoresis 2018, accepted article.

NOTES:

Microfluidic Western Blotting with Improved Throughput and Sensitivity

Natalie Arvin, Mohamed Dawod, Robert Kennedy

University of Michigan, Ann Arbor, MI USA

Western blotting is a widely used protein assay platform for the analysis and detection of proteins in complex biological samples. The technique, in its conventional form, involves the separation of proteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transfer of separated proteins from the gel to a binding membrane, and immunoassay for detection of target proteins. We have reported a microfluidic Western blotting system that achieves 100-fold reduction in sample volume consumption with 10-fold reduction in separation time. Microchip electrophoresis separations are directly captured on protein-binding membranes by dragging the microchip outlet across the membrane. The overall throughput of the microfluidic Western blot was still limited, however, by the immunoassay step which traditionally requires 4-20 h to complete. We developed a microscale immunoassay with direct deposition of reagents to further improve the throughput and miniaturization of the Western blot. Syringe-flow deposition of antibodies at low microliter/min flow allows for entire immunoassay to be completed in 1 h with comparable sensitivity to incubation steps requiring 20 h. A 40-fold reduction in antibody consumption is also achieved. Detection of GAPDH and β -Tubulin in A431 cell lysate has been demonstrated. To increase the detection sensitivity of the microfluidic Western blot, we implemented electrokinetic supercharging (EKS) preconcentration with constant volume injection of sample. EKS preconcentration combines field amplified sample injection (FASI) and transient isotachopheresis (tITP). FASI is implemented by diluting the sample in a low conductivity matrix such that when sample is electrokinetically injected preconcentration stacking against the higher conductivity background gel electrolyte occurs. A second preconcentration stacking step is done by tITP where the sample is sandwiched between a leading electrolyte and a system induced terminating electrolyte. A 100-fold enhancement in signal intensity for a protein-ladder has been demonstrated by implementing EKS preconcentration for the microfluidic Western blot.

NOTES:

CE in Biopharma Science: It's more than GCE and cIEF - Unexploited Applications from the Sewing Box

Samuel Bader, Maria A. Schwarz

Solvias AG, Kaiseraugst, Switzerland

GCE-SDS and cIEF are powerful and well accepted analytical techniques for a wide series of methods. With the rise of biopharmaceuticals such as ADCs or pegylation or the trend to monitor characteristics such as glycation or post-translational modification, the challenge to separate differently modified proteins and peptides variants has increased. While some generally do not cause problems, for others it is necessary to have a deep look into the sewing box for a suitable separation strategy.

While HPLC and the above-mentioned CE techniques are power horses for standard test, there are several problem and limitation for them. With increasing demand for more detailed analyses, either as a specific customer request or because of a lack of an analytical method, the need for special applications also increases.

In this talk we will present some examples showing “alternative” CE methods. For each of these examples we will outline the specific underlying problem for either HPLC or the most commonly used CE method and why CZE is a suitable technique. For instance, pegylated peptides are notoriously difficult to detect using HPLC techniques. However, they are readily observable by CZE. Another especially interesting and versatile technique is the combination of an affinity reagent in the background electrolyte. The affinity reagent causes a different migration behaviour and thus separation from the unmodified species. This strategy was applied to determine the amount of the sialic acid or glycation in a monoclonal antibody sample. It is known that CE could also be strong in peptide mapping generating a q/r depending profile, combining these features with a deamidation specific digest, deamidation can be monitored in a highly specific and efficient way suitable for UV detection.

This showcasing of different CE applications should spark the innovation for novel analytical methods.

NOTES:

Approaches to Developing a Robust icIEF Charge Variant Method

Elisabeth Krug

Eli Lilly and Company, Indianapolis, IN USA

Developing robust but selective analytical methods is one of the key deliverables for the analytical organization in pharmaceutical development. Effective method development ensures that laboratory resources are optimized, while methods meet the objectives required at each stage of drug development. Phase-appropriate method development is critical to balance time and cost, while still ensuring product quality. Charge variant distribution is one of the attributes tested for biopharmaceuticals throughout their life cycle. To meet the elements of cost and time, icIEF is often the technique of choice. The presentation will focus on how to develop a robust icIEF method for mAbs, point out the factors to consider, and lastly explain, why it may not pay off to shortcut the development of this assay at early stages.

NOTES:

Applications of ZipChip CE-MS for Monitoring of Product Quality During Manufacturing Process

Pam Feng

Biogen, Cambridge, MA USA

Rapid and sensitive process and product quality analysis is important during biopharmaceutical development to ensure consistency of manufacturing process. A low level of protein concentration and complex sample matrices can often present challenge for product quality characterization at early stages of cell line and process development support. ZipChip CE-MS, an emerging technology, allows highly efficient separations and handles sample matrices that are often problematic for LC, leading to more reliable identification and characterization of both cell culture media and mAb product quality attributes. Briefly, microfluidic ZipChip CE-MS approach was developed to monitor glycosylation profile, titer and metabolites in cell culture harvest samples with minimal sample preparation. The analysis of time-course bioreactor cell culture mAb samples under reducing conditions provided comparable, yet faster N-glycosylation characterization vs traditional rpLC-MS approach. Details surrounding different applications of ZipChip CE-MS for improved product understanding will be presented in this talk

NOTES:

CE in Drug Development: Where Have We Been and Where Are We Going?

Barry Karger

Professor Emeritus Northeastern University, Boston, MA USA

On the 20th anniversary of CASSS CE-Pharm, this lecture will present a perspective of where CE has been and where it is heading. We will first look at CE of the 1990s and the advances that led to the establishment of the technology in the pharm and biotech fields. Important lessons were learned in the early days that remain true today, e.g. there is a high hurdle to replace established methods. A continual issue from the beginning has been – how does CE compare to LC? As evidenced by the 20 years of this meeting, CE has found its place with well-established applications, for example, glycan and protein analysis. CE is practiced today in both a column and microfluidic format and both have their specific advantages. CE was held back over the years by a lack of a robust interface to ESI-MS. This is now changing, and there are already exciting applications using both the column and microfluidic formats. Improvements in native MS instrumentation, especially in high mass/resolution, should lead to CE advances in intact biopharmaceutical and protein complex characterization, given the open tube nature of CE and the limited or absence of solute-surface interaction. Further, new therapeutic approaches in gene and cell therapy open up exciting opportunities for the application of CE to various levels of product characterization. It is easy to envision that the CE Pharm meeting will be scheduled in 2038, twenty years from now.

NOTES:

Troubleshooting Workshop

Tuesday, September 11

08:30 – 09:30

Plaza Room B

Facilitators:

Tim Blanc, *Eli Lilly and Company, Branchburg, NJ USA*

Cari Sanger-van de Griend, *Kantisto B.V., Baarn, Netherlands*

Scribes:

Nomalie Jaya, *Seattle Genetics, Inc., Bothell, WA USA*

Steffen Kiessig, *F. Hoffmann-La Roche Ltd, Basel, Switzerland*

Ewoud van Tricht, *Janssen Vaccines and Prevention, Leiden, Netherlands*

Analytical methods subject matter experts (SMEs) play an important business role by ensuring the success of technologies in labs supporting characterization and GMP testing. As a community of Capillary Electrophoresis SMEs, sharing expertise among the industry is one of the primary objectives of the CE Pharm Meeting and is the focus in our annual troubleshooting workshop. Each laboratory makes unique distinctions about common problems and devises clever solutions to mitigate such problems within their organization. Some have affectionately coined this acquired information as “Tribal Knowledge.” Internally, it may be viewed as too trivial to publish, even though it is critical to the performance of important methods. The goal of this workshop is to share and harness such tribal knowledge across our CE community.

While lively and informative discussions are the goal, a picture (or electropherogram) can provide a much higher level of clarity to the discussion. If your lab is struggling with a troubleshooting issue, here is an opportunity to bring it to a larger forum of CE users and SMEs with diverse troubleshooting experiences. You can submit your electropherograms anonymously without any identifying information if you like; just a brief description of the issue is enough. Four years ago, CASSS and the CE Pharm Committee began soliciting examples for this workshop and the response has been impressive. Reports from the past workshops can be found at <http://www.casss.org/page/CEPharmTroubleshoot>. A report of this year’s troubleshooting session will be published on this page as well.

NOTES:

NOTES:

Roundtable Discussion

Session I

Monday, September 10

14:00 – 15:00

Session II

Tuesday, September 11

17:00 – 18:00

There are 12 roundtable topics. The plan is for these to be active discussions, not presentations or lectures. To create useful discussion, we are going to try and limit each topic to 10 attendees. **Seating will be on a first come, first serve basis.** These discussions will include a facilitator, whose role is to help assist the discussion and ensure a lively exchange, and a scribe, whose role is to make general, anonymous notes about the discussion that will be posted on the CE Pharm 2018 website.

Listed below is a quick view of the Roundtable Topics, Facilitators and Scribes:

Table 1: CE-MS: Unexploited Opportunities in Process Development

Session I

Facilitator: Mei Han, *Amgen Inc.*

Scribe: Yunan Wang, *Amgen Inc.*

Session II

Facilitator: Göran Hübner, *Boehringer Ingelheim Pharma GmbH & Co. KG*

Scribe: Tara Enda, *Bristol-Myers Squibb Company*

Table 2: CE in New Emerging Therapeutic Areas (Gene Therapy, Oligonucleotides)

Session I

Facilitator: Richard Rustandi, *Merck & Co., Inc.*

Scribe: Xiaoping He, *Pfizer, Inc.*

Session II

Facilitator: SungAe Park, *Samsung Bioepis Co., Ltd.*

Scribe: Ashraf Ali, *Inha University*

Table 3: Real Time Release/PAT, How Does CE Fit In?

Session I

Facilitator: Lars Geurink, *Janssen Vaccines and Prevention*

Scribe: Ewoud van Tricht, *Janssen Vaccines and Prevention*

Session II

Facilitator: Lars Geurink, *Janssen Vaccines and Prevention*

Scribe: Ewoud van Tricht, *Janssen Vaccines and Prevention*

Table 4: CE Troubleshooting: Lessons Learned

Session I

Facilitator: Tom Niedringhaus, *Genentech, a Member of the Roche Group*

Scribe: Sarah Lum, *University of Notre Dame*

Session II

Facilitator: Kathir Muthusamy, *Regeneron Pharmaceuticals, Inc.*

Scribe: Theresa Hassett, *Seattle Genetics, Inc.*

Table 5: Unmet Needs in Analytical Characterization: Chances for CE (non MS applications)

Session I

Facilitator: Hermann Wätzig, *University of Braunschweig*

Scribe: Gordon Freckleton, *Celgene Corporation*

Session II

Facilitator: C. Mark Lies, *SCIEX*

Scribe: Nathan Lacher, *Pfizer, Inc.*

Table 6: CE Methods Meet Contract Labs - Challenges and Successes

Session I

Facilitator: Abbie Esterman, *Bristol-Myers Squibb Company*

Scribe: Nomalie Jaya, *Seattle Genetics, Inc.*

Session II

Facilitator: David Fischer, *Genentech, a Member of the Roche Group*

Scribe: Elisabeth Krug, *Eli Lilly and Company*

Table 7: Strategies for Moving CE-based Charge Methods into Late Development or Commercial Facilities

Session I

Facilitator: Nathan Lacher, *Pfizer, Inc.*

Scribe: Eric Ong, *Regeneron Pharmaceuticals, Inc.*

Session II

Facilitator: Tim Blanc, *Eli Lilly and Company*

Scribe: Nomalie Jaya, *Seattle Genetics, Inc.*

Table 8: Validation Strategies and What Method Parameters to Focus on

Session I

Facilitator: Maria A. Schwarz, *Solvias AG*

Scribe: Steffen Kiessig, *F. Hoffmann - La Roche Ltd.*

Session II

Facilitator: Maria A. Schwarz, *Solvias AG*

Scribe: Steffen Kiessig, *F. Hoffmann - La Roche Ltd.*

Table 9: Platform Methods - How Much Optimization Should be Necessary?

Session I

Facilitator: Elisabeth Krug, *Eli Lilly and Company*

Scribe: Tim Riehlman, *Regeneron Pharmaceuticals, Inc.*

Session II

Facilitator: Kevin Strozyk, *Seattle Genetics, Inc.*

Scribe: Abbie Esterman, *Bristol-Myers Squibb Company*

Table 10: How Does the Emergence of the Multi-Attribute Methods (MAM) Impact the Role of CE?

Session I

Facilitator: Göran Hübner, *Boehringer Ingelheim Pharma GmbH & Co. KG*

Scribe: Zoran Sobic, *Biogen*

Session II

Facilitator: Zoran Sobic, *Biogen*

Scribe: Jonathan Gilroy, *Aptevo Therapeutics*

Table 11: Experience with Native Fluorescence-based ICIEF: Pro & Cons

Session I

Facilitator: Kevin Strozyk, *Seattle Genetics, Inc.*

Scribe: SungAe Park, *Samsung Bioepis Co., Ltd.*

Session II

Facilitator: Tom Niedringhaus, *Genentech, a Member of the Roche Group*

Scribe: Julia Kahle, *University of Braunschweig*

Table 12: Vendor Engagement and Communication: Striving Towards Successful Collaborations and Information Sharing

Session I

Facilitator: David Fischer, *Genentech, a Member of the Roche Group*

Scribe: Theresa Hassett, *Seattle Genetics, Inc.*

Session II

Facilitator: Jeff Beckman, *Bristol-Myers Squibb Company*

Scribe: Handy Yowanto, *SCIEX*

Technical Seminars

Technical Seminar: Lunch and Learn

Monday, September 10

12:45 – 13:45

Plaza Room B

More “iCE” for Your Biologics

Kevin Strozyk¹, Xin Jiang²

¹ Seattle Genetics, Inc., Bothell, WA USA, ²ProteinSimple, a Bio-Techne brand, San Jose, CA USA

Imaged capillary isoelectric focusing (icIEF) by iCE™ systems has been a standard method for analyzing charge variants of therapeutic proteins. The newest Maurice™ system affords expanded capabilities with the addition of CE-SDS mode, and sensitive native fluorescence detection, to bring a lot more “iCE” to your biologics analysis.

Antibody-drug conjugates (ADC) are complex biopharmaceutical products that require several analytical methods to assess product quality, yet few methods are available to detect drug-linker properties when attached to ADCs. At this seminar, Kevin Strozyk (Seattle Genetics) presents a fluorescence-based approach to detect intact drug-linker distribution on ADC charge variants using icIEF. Samples possessing pyrrollobenzodiazepine dimer (PBD-dimer) payloads display a unique fluorescence spectrum that allows selective detection to separate from the antibody backbone. This presentation covers the results and experiences from a thorough evaluation, and discussion of the potential applications to ADCs.

Several novel applications of Maurice for biologic analysis will also be presented. The seminar is interactive to encourage discussion with speakers, the ProteinSimple team; and to share your own experiences.

NOTES:

Technical Seminar: Lunch and Learn
Tuesday, September 11
11:45 – 12:45
Plaza Room B

Novel Approaches to Improving CE Performance for Purity and ADC Analyses

Richard Brown¹, Samuel Shepherd²

¹LifeArc, Stevenage, United Kingdom, MedImmune Limited, Cambridge, United Kingdom²

Dr. Richard Brown (LifeArc) will demonstrate how to utilize the power of CE with optical detection for easy DAR calculations. The Drug-to-Antibody Ratio (DAR) of Antibody-Drug Conjugates (ADCs) can be easily determined with simple Capillary Electrophoresis (CE) analysis. In this presentation, we will show how CE can detect modifications of 0.2–6kDa from thermally stressed samples of lysine ADCs and will show how this approach is being applied to cysteine ADCs. DAR values obtained are comparable to those obtained via alternative methodologies such as liquid chromatography (LC).

Dr. Samuel Shepherd (Medimmune) has developed a novel approach to simplify CE-SDS data analysis by eliminating migration time drifts. The applications of a few drops of mineral oil in your CE-SDS analysis has been demonstrated to eliminate shifts when analyzing multiple samples, which leads to greater reproducibility and easier data analysis.

NOTES:

Technical Seminar: Lunch and Learn
Tuesday, September 11
15:45 – 16:45
Plaza Room B

Host Glycomic Determinants of Interferon- α -Mediated Reduction of HIV Persistence *in vivo*

Leila B Giron¹, Florent Colomb¹, Karam Mounzer², Sherry Wang³, Jay R. Kostman², Pablo Tebas⁴, Una O'Doherty⁴, Livio Azzoni¹, Emmanouil Papasavvas¹, Luis J Montaner¹, Mohamed Abdel-Mohsen¹

¹*The Wistar Institute, Philadelphia, PA USA*, ²*Philadelphia FIGHT, Philadelphia, PA USA*,
³*Thermo Fisher Scientific, South San Francisco, CA USA*; ⁴*University of Pennsylvania, Philadelphia, PA USA*

Interferon- α (IFN α) therapy was associated with significant suppression of HIV viremia and reduction in levels of HIV DNA during suppressive antiretroviral therapy (ART). Cytokines modulate host glycosylation machinery, and glycosylation plays a critical role in mediating several antibody immunological functions. We assessed the impact of pegylated IFN α 2a (Peg-IFN α 2a) immunotherapy on isolated IgG glycomes of 18 HIV-mono-infected individuals on suppressive ART, using the GlycanAssure™ HyPerformance™ assay with capillary electrophoresis analysis. Peg-IFN α 2a treatment was associated with 1) a significant increase in the proportion of the pro-inflammatory and pro-ADCC, non-sialylated bisecting GlcNAc glycan structures (G0FB, G1FB, G2FB); and 2) a significant decrease in the proportion of the anti-ADCC total fucosylated glycans. IFN α immunotherapy in HIV+ ART-suppressed individuals is associated with glycomic alterations that have previously associated with higher inflammation and higher ADCC activities. Our data indicate a potential role for circulating glycans and lectins in informing and/or determining host immune response and HIV persistence during IFN- α treatment.

NOTES:

Technical Seminar: Lunch and Learn
Wednesday, September 12
12:30 – 13:30
Plaza Room B

LabChip Electrophoresis: A Robust, Sensitive and Intelligent Platform for Bio-therapeutics Characterization and Analysis

Anubhav Tripathi

Brown University, Providence, RI USA

To succeed in today's competitive environment, significant efforts are being made by biopharmaceutical companies to produce recombinant protein products with high yield and more critically high quality to ensure therapeutic protein product safety and efficacy. For protein purity analysis, microfluidic molecular separation offers distinct advantages over traditional capillary electrophoresis in sample consumption, ease of use, and speed of analysis. We use a smart microfluidic platform for high throughput quantitation and quality screening of protein products. The method utilizes a microchip which utilizes real-time electro-hydrodynamic optimization to eliminate variability in reported results, i.e. run-to-run and day-to-day variability, which may be caused by micro-scale fluctuations in the physical properties of chips, reagents, or instrument components. The Labchips' SMART OPTIMIZATION algorithm uses migration time data and a known ratio of high concentration and low concentration standard protein, VeriMAb® to achieve high sensitivity, accuracy and repeatability for a diverse variety of samples. The high-resolution microchip utilizes small sample plug sizes of proteins for quantification of purity. The results show time of arrival reproducibility of $\pm 2\%$ over 96 samples with $< 1\%$ CV for the main proteins peaks and $< 20\%$ CV for the major impurity peaks. In dilution series we observe linearity with R^2 values always above 0.99 and percent recovery consistently above 70% for concentrations as low as 10 ng/uL. Since the smart microfluidic feedback platform is automatable, quantitative, robust, and capable of high throughput analyses, it provides both great analytical capacity and a wide spectrum of product coverage for both protein product development and downstream production processes in biopharmaceutical industry.

NOTES:

Poster Abstracts

New Questions for Known Molecules

P-101-M

Separation of Protein and Peptides Using Thin Porous Polymer Layer Immobilized Open Tubular Capillary Column

Ashraf Ali

Inha University, Incheon, South Korea

A copolymer immobilized OT-CEC column has been developed in the current study for the separation of different peptides present in a tryptic digest of cytochrome C with enhanced separation efficiency and improved peak capacity which is better than those of the previous studies. Long copolymer chains were immobilized on the inner surface of the capillary via reversible addition-fragmentation chain transfer (RAFT) polymerization. Pretreated silica capillary column (50 μm internal diameter and 120 cm length) was chemically modified with 4-(Chloromethyl)phenyl isocyanate in the presence of dibutyltin dichloride as a catalyst. The terminal halogen (Cl) of the bound ligand (4-(Chloromethyl)phenyl isocyanate) was reacted with sodium diethyldithiocarbamate to incorporate the initiator moieties. A thin polymer layer was made on the inner surface of the capillary by reversible addition-fragmentation transfer polymerization upon the initiator moieties using a mixture of styrene, N-phenyl acrylamide, and methacrylic acid. The copolymer immobilized open tubular capillary column was used for the separation of the synthetic mixture of five peptides and tryptic digest of cytochrome C sample in capillary electrochromatography. Very high separation efficiency (Ca. 1440,000 plates/m) was obtained for synthetic peptides while (Ca. 320,000 plates/m) for some of the peptides in a tryptic digest of cytochrome C under optimized elution conditions.

NOTES:

P-103-M

Enhanced CE Imaging in icIEF for Application in Bioanalysis

Katelyn Cousteils, Victor Li, Tiemin Huang

Advanced Electrophoresis Solutions Ltd., Cambridge, ON Canada

Capillary isoelectric focusing (cIEF) has become an indispensable tool in life science research and in the development of new biopharmaceutical products. cIEF is the preferred method for study of post translational modifications, for protein degradation studies, variability in glycosylation, formulation of biopharma and for instance in profiling and authentication of food products. Capillary isoelectric focusing with whole column imaging detection (icIEF) was introduced on the bioanalysis market some years ago. This technique resolves these issues by providing faster method development, simpler practical operation, better reliability and robustness, and a higher analytical throughput than cIEF.

Based on this technology, AES (Advanced Electrophoresis Solutions Ltd) has made some important breakthroughs such as the 200 μm inner diameter (ID) separation capillary, high throughput onboard sample mixing, and proprietary carrier ampholytes (CAs) and pI standards optimized for cIEF. A commercially available enzyme was used as the sample for testing.

When compared to a standard 100 μm ID cartridge, detection sensitivity is increased by 100% with the 200 μm ID CEInfinite cartridge. Onboard mixing shows consistent peak shape and size for each injection of a combined sample. CEInfinite CAs increase resolution relative to other commercially available CAs.

High resolution, high sensitivity icIEF separations with CEInfinite system have demonstrated increased sensitivity, resolution, and reproducibility. The applications of this instrument can easily be applied to therapeutic proteins such as antibody drug conjugates (ADC), monoclonal antibodies (mAbs), and fusion proteins with little method development beyond traditional cIEF preparations. AES has commercialized and released these improved technologies in the new CEInfinite icIEF instruments and supporting consumables.

NOTES:

P-105-M

Demonstration of Real-time Imaged cIEF-MS with a Novel Microchip System for Analysis of Intact Biopharmaceuticals

Erik Gentalen, Barry Clerkson, Scott Mack

Intabio, Inc., Portola Valley, CA USA

The complexity of biologic drug production demands that multiple measurements are required to characterize proteins throughout development and manufacturing. Capillary isoelectric focusing “cIEF” is routinely used as the analytical platform for initial isoform assessment because it provides exquisite resolution, high precision and is robust and easily reproducible across laboratories. Mass spectrometry “MS” provides precise molecular characterization of an isoform and how it has been modified. However, up to weeks of scale up, chromatographic method development and sample isolation can separate the first detection of an isoform by cIEF from the MS analysis required to identify the nature of the protein modification.

Intabio has developed a proprietary protein analytics platform, Blaze™ to seamlessly integrate cIEF with MS analysis, thereby reducing the time delay and infrastructure needs required to identify protein variants. Blaze utilizes a proprietary microchip technology to integrate (1) charge heterogeneity analysis by isoelectric focusing, (2) whole-column imaging by 280nm for detection and quantitation, and (3) MS sample preparation and delivery by electrospray into an adjacent mass spectrometer to identify each isoform. We will demonstrate the imaged cIEF-MS assay for the NIST monoclonal antibody, recombinant proteins, and monoclonal antibody samples. Blaze has the flexibility to run in 2 modes: as a standalone for cIEF only, or connected to a mass spectrometer for cIEF-MS.

We will outline the sample requirements and workflow for both operational modes.

NOTES:

P-107-M

Highly Efficient Evaporative Fluorophore Labeling of Carbohydrates

Andras Guttman¹, Balazs Reider², Marton Szigeti³

¹SCIEX, Brea, CA USA, ²Horvath Csaba Laboratory of Bioseparation Sciences, Veszprem, Hungary, ³University of Debrecen, Debrecen, Hungary

As analytical glycomics became to prominence, newer and more efficient sample preparation methods are being developed. Albeit, numerous reductive amination-based carbohydrate labeling protocols have been reported in the literature, the preferred way to conduct the reaction is in closed vials. Here we report on a novel evaporative labeling protocol with the great advantage of continuously concentrating the reagents during the tagging reaction, therefore accommodating to reach the optimal reagent concentrations for a wide range of glycan structures in a complex mixture. The mild conditions of the evaporative labeling process minimized sialylation loss, otherwise representing a major issue in reductive amination-based carbohydrate tagging. The approach was applied in conjunction with an improved PNGase F digestion mediated N-glycan release process, where the effect of enzyme/protein ratio was optimized. In addition, balanced dispersion of dry samples was accommodated by supplementing the low volume labeling mixtures (several microliters) with extra solvent (e.g., THF). Evaporative labeling also makes glycan labeling automation-friendly, suitable for standard open 96 well plate format operation.

NOTES:

P-109-M

Laser Beam Mediated Fluorescence Imaging Detection of Fluorophore Labeled Analytes in CESI-MS

Andras Guttman¹, Mate Szarka², Marton Szigeti³

¹SCIEX, Brea, CA USA, ²Horvath Csaba Laboratory of Bioseparation Sciences, Veszprem, Hungary, ³University of Debrecen, Debrecen, Hungary

Capillary electrophoresis in conjunction with laser induced fluorescent detection is a sensitive high-performance bioanalytical technique. Coupling capillary electrophoresis with mass spectrometry gives an additional dimension to generate information for full structural elucidation of the sample components. However, due to the possibility of in-source degradation of labile residues during the ionization process in the electrospray, simultaneous use of an optical detection system, such as fluorescence is highly advisable. In this poster, a novel and easily applicable laser beam mediated fluorescence imaging detection is introduced, which is capable of providing high sensitivity simultaneous detection of fluorophore labeled molecules. Detection occurs practically at the same time when the analyte molecules enter the MS orifice. Validation was carried out through capillary electrophoresis with laser beam mediated fluorescence imaging and electrospray ionization mass spectrometry (CESI-LBMFI-MS) analysis of fluorophore labeled linear and complex carbohydrates as well as amino acids, peptides and proteins in picomolar concentration range.

NOTES:

P-111-M

Analysis of Highly Sialylated Samples on the GlycanAssure™ System

Shaheer Khan, Jenkuei Liu, Zoltan Szabo, Peter Bell

Thermo Fisher Scientific, South San Francisco, CA USA

Glycosylation plays many important roles in biological processes involving function, pharmacokinetics, stability, and immunogenicity, which is why it is important to monitor recombinant protein heterogeneity to ensure safety, effectiveness, as well as consistency in manufacturing for biopharmaceuticals. The most widely used method in laboratories today for glycan analysis has the disadvantage in that sample prep is time consuming with overnight N-Glycan release steps, additional purification steps to remove excess salts, excess labeling reagents, large protein input, and difficulty producing data on highly sialylated glycans.

Because sialylated glycans elute at the back end of the LC profile, analysis and accurate quantitation of highly sialylated glycans consistently is a challenge in LC methods. CE offers an advantage in analysis as sialylated glycans migrate at the front end of the electropherogram, making it an ideal platform to analyze and accurately quantitate highly sialylated glycans. CE also offers an opportunity to analyze samples with low glycoprotein input as sample requirement is very low, in comparison to typical LC methods.

In this study, we evaluated APTS-labeling conditions on highly sialylated glycans, specifically Fetuin. Furthermore, we also tested 3500xl CE separation capability for analysis of biantennary, triantennary, and tetraantennary glycans labelled with APTS dye. Results reinforce the robustness of the GlycanAssure workflow, even with shorter labeling time. The 3500xl CE platform achieves quantitative, high resolution glycan analysis, with high-sensitivity and high-reproducibility.

NOTES:

P-113-M

Method Optimization and Evaluation for RNA Purity Analysis using CE-LIF Technology

Tingting Li, Mukesh Malik, Handy Yowanto

SCIEX, Brea, CA USA

Recent advances in gene therapy research have gained promise in the utility of gene therapeutics compounds. The ability to quantify RNA purity and quality is critical to ensure the safety and efficacy of these molecules. Comparing to traditional slab-gel based electrophoresis methods, CGE offers superior resolution, shorter analysis time, automated operation and exceptional sensitivity when combined with a laser induced fluorescence detector. Compared to chip-based CE systems, the PA800 Plus provides open chemistry, which enables flexibility for method modification and optimization to generate optimal results for each specific project.

In this study, a CE-LIF fast separation method which was used to evaluate total RNA quality was optimized to achieve higher resolution; and the method was then evaluated for RNA purity determination. The resolution of this method was optimized for RNA size ranging from 200 bases to 6583 bases and with superior sensitivity. The optimized method demonstrates good resolving power, MT and CPA% repeatability, excellent linearity from 0.33ng/mL to 333 ng/mL for 1.2kb RNA Marker and LOQ as low as 0.33ng/mL. This method is a platform method for RNA analysis which could also be further modified to suit more specific user criteria.

NOTES:

P-115-M

Native Analysis of Monoclonal Antibodies by Microchip Capillary Electrophoresis-ESI-MS

Scott Mellors, Erin Redman

908 Devices Inc., Boston, MA, USA

The characterization of intact monoclonal antibodies by mass spectrometry is challenging due to the size and complexity of the molecules. The ability to separate charge variants in a separation that is directly coupled to the mass spectrometer greatly improves the MS analysis by minimizing spectral overlap of similar variants, while also providing a charge variant separation which aids in identification of the species. We have previously demonstrated the ability to do such an analysis using microfluidic capillary electrophoresis – ESI-MS under “near native conditions”. This current work extends that capability by operating under fully native conditions which maintain the folded structure of the molecules both during the CE separation and through the ESI transition into the gas phase.

NOTES:

P-117-M

Battling the Backlog: Novel CZE System for Forensic Separations

Sarah Lum, Norman Dovichi

University of Notre Dame, Notre Dame, IN USA

The national backlog in sexual assault cases is estimated to be between tens of thousands to a half million untested rape kits. Current methods are time and labor intensive, requiring overnight procedures and delivering a lower than 40% success rate. The primary challenge crime labs face in analyzing these cases is the separation of purified male DNA from the mixture of primarily female DNA from gynecological swabs. Effective elution of the sample from the swab and efficient separation of intact sperm cells from epithelial and other cellular debris allow for a successful analysis of the perpetrator DNA. Capillary zone electrophoresis (CZE) is a promising tool to perform the cell separation and has three major advantages over alternative technologies: a small amount of sample is consumed, which allows for replicate analyses of limited available evidence; rapid separation time compared to standard methods; and single cell detection and collection when interfaced with an automated fraction collector developed in-house. In this work, simulated sexual assault samples are eluted from cotton swabs and the mixture is directly electrokinetically injected into a novel CZE system where intact cells and lysed cellular matrices are separated by their unique electrophoretic properties. Sample eluted from the distal end of the capillary is collected into individual wells on a microtiter plate which correspond to a CZE migration time interval. Light microscopy is used to confirm the separation and collection of intact sperm cells at designated time points in under 20 minutes. Quantitative PCR results verify the isolation of purified male DNA for short tandem repeat (STR) analysis and perpetrator identification.

NOTES:

P-119-M

Method Development for the Separation of Stereoisomers in Drug-linker Intermediates by Capillary Electrophoresis

Richard Moran

Seattle Genetics Inc., Bothell, WA USA

Separation and identification of chiral isomers is an important aspect in pharmaceutical and biopharmaceutical drug development. Liquid and gas chromatography are commonly used techniques for separation of these isomers prior to analysis, but method development can be laborious and expensive, and often does not produce satisfactory results. Capillary electrophoresis is an established technique that has become widely used in the pharmaceutical industry for chiral separations due to its flexibility and comparatively low cost. In this work, we develop and evaluate a CE method for the separation of chiral drug-linker isoforms that have proven difficult to separate by LC. The results show that CE is a promising tool for monitoring chiral isoforms during drug-linker production.

NOTES:

P-121-M

Comparison the N-Glycan of Different Source's Recombinant Human Erythropoietin by using Capillary Electrophoresis with Laser-induced Fluorescence Detector

Tingjun Ren

SCIEX, Shanghai, China

Recombinant human erythropoietin (rhuEPO) has been extensively used as a pharmaceutical product for treating anemia in clinic. The rhuEPO is a typical glycoprotein, and the glycosylation is up to 40%. So, glycosylation of rhuEPO is of great importance and could affect the biological activity, immunogenicity and pharmacokinetics. In this study, by using effective glycan capture by magnetic microparticles, we can realize the glycan releasing and labeling in 60 mins, and analysis in conjunction with a rapid high-resolution capillary electrophoresis analysis method for the prepared samples using laser induced fluorescence detection (CE-LIF) in 5 mins. Also, the GU software can identify the glycan structure automatically. As far as we know, this is the first time to compare different company's glycan of rhuEPO qualitatively and quantitatively by using CE-LIF. The proposed method is fast and has good reproducibility, which allowed the inter-day RSD of migration time and peak area were around 0.2% and 2.6% (n=5), respectively. Hence, this method has great significance of studying the glycan glycosylation of rhuEPO.

NOTES:

P-123-M

Increasing the Sensitivity of IgG Purity and Heterogeneity Assay on PA 800 Plus by using Fluorescent Labels

Marcia Santos

SCIEX, Brea, CA USA

When tasked with the assessment of critical quality attributes of protein biologic products, many analytical characterization approaches are currently available. Separation based techniques such as size exclusion chromatography, Analytical Ultracentrifugation, capillary isoelectric Focusing, Ion-Exchange Chromatography and CE-SDS are common.

CE-SDS has been successfully employed for the quantification of clipped products, host cell proteins, and aggregation products, glycosylation site occupancy analysis, monomer purity analysis, to name a few. Typically, CE-SDS is performed using UV absorbance detection at 214 or 220 nm. However, the LOD of this assay in most commercial instruments is only 2.5 ug/mL and many impurities may fall below this range and impose a risk on product safety and efficacy. Therefore, there is an unmet need to improve the sensitivity of CE-SDS based assays.

This work demonstrates how the use of Laser Induced Fluorescence detection to detect monoclonal antibodies derivatized with primary amine reactive fluorophores can increase the sensitivity of CE-SDS using the PA800 Plus.

Using the NIST antibody reference material as a surrogate, we investigated two amine reactive dyes; 3-2-(furoyl quinoline-2-carboxaldehyde (FQ) and 5-Carboxytetramethylrhodamine-succinimidyl ester (5-TAMRA.SE). Workflow parameters pertaining to sample preparation and cleanup, ease of labeling, reagent toxicity, peak shape, resolution, labeling induced artifacts were used to compare these two labeling strategies.

NOTES:

P-125-M

Detecting Drug-Linker on ADC Charge Variants using Imaged Capillary Isoelectric Focusing (icIEF) with Novel Fluorescence Detection

Kevin Strozyk, Oscar Salas-Solano, Nomalie Jaya

Seattle Genetics Inc., Bothell, WA USA

Antibody-drug conjugates (ADC) are complex biopharmaceutical products that require several analytical methods to measure product quality. New technologies constantly strive to improve sensitivity, efficiency, and robustness for monitoring quality attributes; however, few methods are available to detect drug-linker properties when attached to ADCs.

Here we present a fluorescence-based approach to detect drug linker across the charge variant distribution of an ADC. Samples possessing pyrrollobenzodiazepine dimer (PBD-dimer) payloads display a unique fluorescence spectrum that allows selective detection separate from the antibody backbone. We present the results and experiences from a thorough evaluation and discuss the potential applications to ADCs.

NOTES:

P-127-M

The Development of a Bromide Quantification Method for Viral Vaccine Development

Bojana Pajic¹, Lars Geurink¹, Cari Sanger – van de Griend², Ewoud van Tricht¹

¹*Janssen Vaccines and Prevention, Leiden, Netherlands,* ²*Kantisto B.V., Baarn, Netherlands*

Domiphen bromide (DB) is one of the components used for virus purification during the downstream process (DSP) in viral vaccine production. DB is added to an intermediate from the production process to bind and precipitate the host cell DNA. Bromide has recently been identified as a potential critical impurity and therefore a bromide specific assay was required to demonstrate bromide clearance throughout the vaccine production process. For this purpose, a capillary electrophoresis method was developed based on the publication from Sanger - van de Griend et al. [J. of Chrom A, 977 (265-275), 2002]. The bromide content was measured using a bare fused silica capillary with direct UV detection at 200 nm. The background electrolyte (BGE) contained 100 mM methanesulphonic acid, 74 mM triethanolamine and 60% v/v acetonitrile to improve the selectivity and minimize the electromigration dispersion of the bromide.

The biggest challenge during the assay development was the high chloride concentration in the sample formulations (75 mM or 420 mM chloride) which caused impacted the accuracy of the bromide determination. Two approaches were evaluated to reduce the interference from chloride on the bromide quantification: 1) transient isotachopheresis with iodide as leading electrolyte and 2) dilution of the sample matrix. The method was validated in accordance with ICH guideline Q2 (R1) and proved to be suitable for its intended use. The assay range for the formulations #1 and #2 (75 mM chloride) was 5 – 100 μ M bromide and for formulation #3 (420 mM chloride) was 10 – 100 μ M bromide. The trueness was 97 – 107% spiked recovery. For all formulations the precision was within 10% RSD, except for the lowest concentration level in formulation #3 (420 mM). The assay showed to be suitable for quantifying bromide in all three tested formulations.

NOTES:

P-129-M

Separation of Somatropin using the Optimized European Pharmacopoeia Method on the PA800 plus Pharmaceutical Analysis System

Fang Wang

SCIEX, Brea, CA USA

This poster describes sample preparation, instrument setup and data analysis of Somatropin charge variant distribution analysis using CZE method with the PA800 *plus* Pharmaceutical Analysis System.

Recombinant human growth hormone (somatropin, hGH) is a 191 amino acid protein of 22kDa molecular weight used in the treatment of a number of childhood growth disorders and growth hormone deficiency. Control and release methods in QC have focused on physical-chemical parameters including the charge variants. The European Pharmacopoeia (Ph. Eur.) monographs Somatropin, Somatropin bulk solution and Somatropin for injections first called for analysis of charged variants in Somatropin by isoelectric focusing (IEF). In January 2006, following a interlaboratory collaborative study, which concluded that capillary zone electrophoresis (CZE) was more sensitive and reproducible, Ph. Eur replaced IEF with CZE for the control of charge related impurities. In 2009, a Pharmeuropa Scientific Notes (2009-1) published an optimized CZE condition with complete verification data. The optimized condition used more available detector (200 nm) and showed improved reproducibility in peak migration time.

Note: For Research Use Only; not for use in diagnostic procedures.

NOTES:

P-131-M

Analysis of Cas9 by Capillary Electrophoresis Sodium Dodecyl Sulfate (CE-SDS) and Imaged Capillary Isoelectric Focusing (icIEF) using Maurice from ProteinSimple

Jiaqi Wu, Chris Heger, Annegret Boge

ProteinSimple, a Bio-Techne brand, San Jose, CA USA

CRISPR/Cas9 has become a revolutionary tool for precise genome editing in a wide variety of prokaryotes and eukaryotes, and multiple Cas9 variants have been engineered to further broaden its functionality. Purity, heterogeneity and stability are critical properties in the biophysical characterization, chemical modification and structural investigation of Cas9 and its variants. At the forefront of techniques to monitor these properties are capillary electrophoresis sodium dodecyl sulfate (CE-SDS) and capillary isoelectric focusing (cIEF). Maurice™ from ProteinSimple enables both CE-SDS and imaged cIEF in a single unit, with several key advantages over other CE-SDS and cIEF methods such as ease-of-use and speed. The highly sensitive native fluorescence whole column detector affords analysis of low concentration samples. In this study, Cas9 samples were obtained from three vendors and analyzed by CE-SDS and cIEF on Maurice. For all three samples, purity and charge heterogeneity of the Cas9 samples were evaluated. The impact of stress on sample stability was also evaluated. These results demonstrate that Maurice is a powerful tool for monitoring purity, heterogeneity and stability of Cas9.

NOTES:

P-133-M

High-throughput Quantification of a Process-related Protein Impurity: Combine the Power of Automated CE based Western Immunoassay using Wes and Automated RoboColumn Purification using Tecan

Chen Zhang¹, Pankti Shah², Naichi Liu¹, John Henstrand¹

¹*BioMarin Pharmaceutical, Novato, CA USA*, ²*University of Calgary, Calgary, AB, Canada*

Analysis of process-related protein impurities is critical to ensure process robustness and patient safety. Innovative and high-throughput analytical technologies for protein impurities monitoring are highly needed to support current rapid biopharmaceutical development and manufacturing process. Traditional Western blot is widely used for characterization and identification of process-related protein impurities in biopharmaceutical industry, from early stage development, process optimization to manufacturing process monitoring. However, the labor-intensive procedures result in limited throughput and poor reproducibility, and also difficulty of achieving accurate quantitation. Here we demonstrate the application of a fully automated, high-throughput capillary electrophoresis (CE) based Western blot platform (Wes) for quantitation of a process-related protein. First, we successfully developed a Wes assay for the target protein impurity in clarified samples, which showed high sensitivity, great reproducibility, high dynamic linear range, and high-throughput analytical ability (for 25 sample analysis in parallel, sample preparation takes ~ 2 hrs and instrument run takes ~ 2.5 hrs). However, for non-clarified samples such as bioreactor cell culture harvest and ultra-filtrate, it is particularly challenging to quantify the low abundant protein impurities directly in the very complex matrix. To address this problem and also keep high-throughput workflow, we further developed a sample clean-up method based on Tecan liquid handling workstation together with RoboColumn system, which enabled fully automated column chromatography up to eight columns in parallel. The protein impurity response was significantly increased on Wes after sample clean-up and achieved better quantitation capability. Compared to batch purification approach, this automated setup removed all manual steps; compared to AKTA system, it saved ~ 90% time for cleaning up 8 samples. Here, our automated platform combining the power of both CE-Western blot and Tecan RoboColumn provides a strategy to meet increasing need of fast analytical support for process development and process monitoring.

NOTES:

P-135-M

AAV Sample Analysis by CE-SDS with Sample Stacking Method

Libin Zhu, Ian Parsons

Charles River Laboratories, Woburn, MA, USA

CE-SDS method to determine the Adeno Associated Virus (capsid) particle's integrity and product purity was developed. As a widely utilized delivery tool for gene therapy, Adeno associated viruses (AAVs) are also used as drugs for various ailments. The AAV particle (capsid) consists of three highly homologous proteins VP1, VP2 and VP3 (1:1:10 ratio). Structural integrity of the capsid is critical for functional performance of the AAV vector. Therefore, a method to assess AAV capsid integrity as well as product purity is needed. Capillary gel electrophoresis using sodium dodecyl sulfate (CE-SDS) is used for purity analysis of AAV particles. The commonly used CE-SDS method is able to determine the purity of AAV sample of 4×10^{12} particles/mL with at least 50 μ L of sample volume. The developed sample stacking method is able to decrease the sample amount of 5-fold and increase the method sensitivity. The assay specificity, linearity, precision and repeatability were investigated. The sample reducing conditions and sample process methods were studied as well.

NOTES:

New Techniques and New Targets

P-102-T

Separation and Isolation of NISTmAb Charge Variants with CEInfinite Preparative iCIEF

Katelyn Cousteils, Jennifer Hu, Victor Li, Tiemin Huang

Advanced Electrophoresis Solutions Ltd., Cambridge, ON Canada

In the biopharmaceutical industry, capillary isoelectric focusing (CIEF) has earned its place as a standard in laboratories across the world. Whole column imaging detection capillary isoelectric focusing (iCIEF) has been shown to have many advantages including increased resolution of charge-based protein separation. The charge variants of a protein can be associated with pathways of protein degradation. Characterization of the exact differences between these variants provides valuable information for stability studies, formulation, and processing control. Using advanced preparative iCIEF technology to produce single charge variant protein samples would be beneficial for characterization and numerous other assays.

Advanced Electrophoresis Solutions Ltd. (AES) developed a proprietary column diameter transformation cartridge (patent pending), which has a large inner diameter (ID) separation capillary, and a small ID transfer capillary. Once the proteins are focused and separated, they are forced into the transfer capillary with minimal peak remixing. Additionally, acrylamide derivative (AD) coatings eliminate the need for the use of methylcellulose, which can interfere with downstream analyses. High resolution preparative iCIEF is illustrated with iCIEF separation, individual peak collection, and iCIEF of the collections. The reverse-polarity capability of the CEInfinite Preparative instrument was utilized to obtain high purity fractionation of basic and acidic protein isomer peaks.

High efficiency iCIEF fractionation of the NISTmAb and other protein samples was performed using the CEInfinite Preparative iCIEF instrument. The isolated fractions were collected into individual vials containing carrier ampholytes and reinjected into iCIEF. Charge variant peaks with a pI difference of 0.1 were collected with 60% purity or greater.

The CEInfinite Preparative iCIEF instrument, in conjunction with the proprietary cartridges, is capable of explicit charge variant separation of the NISTmAb monoclonal antibody. This technique can be applied to other proteins of therapeutic potential.

NOTES:

P-104-T

Efficient Analysis of Monoclonal Antibodies by Capillary Electrophoresis

Caleen Dayaratna, Mark Li

Patheon, part of Thermo Fisher Scientific, Princeton, NJ USA

The use of monoclonal antibodies (mAbs) as therapeutic agents is constantly increasing. It is therefore critical to have methods that can measure the purity of these mAbs. The Beckman PA800plus is the benchmark for capillary electrophoresis testing, however, the sample throughput is low due to a separation time of about 35 minutes per sample. In an effort to decrease the time for in-process sample testing, microchip capillary electrophoresis (MCE) using the LabChip® GXII Touch was compared to the Beckman PA800plus for nine mAbs under reduced and non-reduced conditions. The electrophoretograms generated by both methods were comparable with non-reduced MCE showing acceptable linearity, accuracy and precision (RSD \leq 4%) for mAb1. These results suggest that MCE provides a high-throughput approach to accelerate sample testing and reduce turnaround times for the biopharmaceutical industry.

NOTES:

P-106-T

A Modernistic Approach to Charge Variant Profiling for mAb Combinations

Abbie Esterman

Bristol Myers Squibb Company, Pennington, NJ USA

Clinical benefits of combining doses of two or more therapeutic agents, such as mAb/small molecule and mAb/mAb treatments have emerged in recent years, Co-administration of injectable therapeutic agents is also considered beneficial from a patient convenience perspective. It is important to characterize these combinations, especially biologic agents, to ensure compatibility. Therefore, developing tools to accurately characterize and quantify these mixtures is key to ensure compatibility. The analytical challenge is that these mixtures may contain a wide concentration range of the actives, where one component will be within the linear range of the method while the other one could be either below the limit of detection (LOD) or saturating the detector. Development of key analytical methods using electrophoretic and chromatographic techniques are needed to characterize the wide range of actives in these combinations.

In this study charge variant profiling of monoclonal antibody mixtures was explored using a multi-detector approach. The data shows that this novel approach is able to accurately quantify mixtures that span nearly 40-fold differences in ratio.

NOTES:

P-108-T

A Multipurpose Adenovirus CZE Method

Lars Geurink¹, Ewoud van Tricht¹, Cari Sanger-van de Griend²

¹*Janssen Vaccines and Prevention, Leiden, Netherlands*, ²*Kantisto B.V., Baarn, Netherlands*

We developed a CZE method for rapid, precise and accurate quantification of the adenovirus concentration to decrease production process intermediate hold time. However, during development it was shown that adenovirus peak changes, adenovirus peak migrationtime shifts, and new peak appearances could be linked to product purification progression and adenovirus particle stability and identity. Consequently, the CZE adenovirus method was used in several investigations and developed for process and product development, process and product characterization and eventually clinical trial material production, release and stability.

NOTES:

P-110-T

Minimize CE-SDS Method Induced High Molecular Weight Artifacts in Purity Determination of mAbs under Reducing Condition by Novel Application of Alternative Detergents in Gel and Sample Buffers

Qian Guan, Jeff Beckman, Sergey Voronov, Julia Ding, Nesredin Mussa

Bristol-Myers Squibb Company, Devens, MA USA

At Bristol Myers Squibb, Capillary Gel Electrophoresis using Sodium Dodecyl Sulfate (CE-SDS) is utilized to assess the purity of Therapeutic Proteins. For the two IgG 1 type of monoclonal antibodies mAb-1 and mAb-2, it was found that artificial high molecular weight (HMW) species are generated under both typical non-reducing and reducing CE-SDS conditions. This presentation will focus on developing custom sample buffer using analytical quality by design (AQbD) approach in reduced CE-SDS in combination with the SHS-containing gel buffer (discussed in a separate talk) that minimizes HMW artifacts. It was discovered that the HMW artifacts very likely are due to incomplete denaturation of heavy chain of mAbs, leading to underestimated purity results of protein products. The levels of these artifacts demonstrate high dependency on the concentrations of protein, SDS of sample buffer and reducing agent 2-mercaptoethanol. HMW also increased over time during sample on-board stability testing. The fundamentals of the novel custom gel invention is covered separately, and this talk will highlight the establishment of custom sample buffer with built-in robustness via AQbD approach, in which one factor at a time (OFAT) studies and design of experiments (DoE) were applied for buffer component optimization and method performance robustness verification. In conjunction with our custom gel, this new sample buffer developed following AQbD approach produced satisfactory results in qualification studies and analysis of real samples of mAb-1 and mAb-2.

NOTES:

P-112-T

Automation of Glycan Sequencing for Biophtherapeutics

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¹SCIEX, Brea, CA USA, ²University of Debrecen, Debrecen, Hungary

Sequence analysis of the N-linked carbohydrates of therapeutic glycoproteins is increasingly gaining interest in the biopharmaceutical industry. Monosaccharide residue and linkage specific enzymes are routinely used for exoglycosidase digestion-based carbohydrate sequencing. Although introduced decades ago, this method is still mostly practiced by following tedious and time-consuming manual processes. Here we describe an automated carbohydrate sequencing approach using the appropriate exoglycosidase enzymes in conjunction with the utilization of some of the existing features of a capillary electrophoresis (CE) instrument in a pre-programmed fashion. The enzymatic reactions were accomplished within the temperature-controlled sample storage compartment of the capillary electrophoresis unit and the separation capillary was also utilized for accurate delivery of the exoglycosidase enzymes. CE analysis was conducted after each digestion step obtaining in this way the glycan sequence information in 60 and 128 minutes using the semi- and the fully-automated methods respectively, in contrast to the conventional methods that may take up to days to accomplish the same task.

NOTES:

P-114-T

Peak Characterization of IgG1 Antibody Sodium Dodecyl Sulfate-capillary Gel Electrophoresis Methods – A Routine Task that Might Still Afford Surprises

Göran Hübner, Marion Bäumlisberger

Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany

SDS-cGE is a substantial tool for the characterization and quality control of biotherapeutics. Depending on the cQAs addressed, it may be conducted under reducing and non-reducing conditions. The usage as characterization method, and especially as quality control method for product release and stability testing, requires a sound method qualification and characterization. This also includes the identification of the detected variants. Even though technically possible, peak fractionation of CE-based methods is usually still not suitable for peak characterization tasks. Furthermore, CE-based methods for release and stability testing of biotherapeutics are generally also not compatible with coupling to mass spectrometry (MS). In the case of e.g. SDS-cGE, replacement of CE with PAGE partially overcomes the fractionation issue. Bands observed in a gel migrate comparably and can be digested, cut out, and analyzed by MS in order to identify underlying variants. Recovery of intact proteins by e.g. electroblotting or electroelution allows for obtaining additional important information by intact protein MS analysis, but is challenging or impossible for large proteins. However, obtained samples are either denatured or even digested, excluding their subsequent analysis in bio- or binding assays for the assessment of any impact on e.g. efficacy. Another approach for the peak characterization is the indirect deduction of their identity by usage of information gathered from degradation studies and characterization studies of variants observed in different liquid chromatography methods. Analysis of samples from these studies not only allows for identification of the peaks, but usually also allows for assessment of their functional impact using bio- and binding assays. In this work, examples of indirect peak identification including functional impact are given. The identification of main peaks and minor species revealed well-known and expected variants. It also revealed unexpected variants and indicated that SDS-cGE might be a useful tool to monitor them.

NOTES:

P-116-T

Impact of Sample Handling During Size Variant Characterization of a Therapeutic Antibody – A Case Study

Natalie Jones, Oscar Salas-Solano, Nomalie Jaya, Sunnie Kim

Seattle Genetics Inc., Seattle, WA USA

Monoclonal antibodies (mAbs) and related products such as antibody-drug conjugates (ADCs) constitute an expanding class of therapeutics. During drug development the size heterogeneity of such products is routinely monitored by multiple methods including Size Exclusion Chromatography (SEC) and Capillary Electrophoresis Sodium Dodecyl Sulfate (CE-SDS) assays. The size variants monitored include aggregates (also known as high molecular weight (HMW)) and fragments of the monomer species.

Aggregation, of mAbs and ADCs is considered a Critical Quality Attribute (CQA) with potential impacts to pharmacokinetics, efficacy and immunogenicity. Additionally, aggregate species are heterogeneous including a range of species from covalent and non-covalent dimers to higher order structure oligomers. Characterization of product specific aggregation is done to determine the identity and biological activity of aggregate species, which can inform product specifications and stability.

During size variant characterization of a mAb intermediate and subsequent ADC, peak enrichment for aggregation species was achieved through Size Exclusion HPLC (SE-HPLC) fractionation. Non-reducing CE-SDS (NR-CE-SDS) was then used to quantitate levels of covalent dimer versus non-covalent dimer in the HMW species. During these studies it was observed that SE-HPLC fractionation can artificially enrich for covalent dimer due to disassociation of non-covalent dimer during fractionation. For the final ADC characterization strategy of the HMW species we detail the precautionary steps taken in order to ensure the enrichment of the HMW maintained the inherent levels of covalent and non-covalent dimer. Representative HMW species was then used to evaluate biological activity.

We demonstrate the importance of understanding species enrichment techniques thoroughly so that analysis of data is not misguided resulting in erroneous conclusions.

NOTES:

P-118-T

Development of a Rapid APTS Sample Preparation Workflow for N-Glycan Release & Labeling

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¹*ProZyme, A part of Agilent, Hayward, CA USA*, ²*Bristol-Myers Squibb Company, Redwood City, CA USA*

The structure of N-glycans on biotherapeutics can impact immunogenicity, pharmacokinetics and pharmacodynamics, making N-glycan characterization an essential part of the development process. A common approach is to derivatize enzymatically-released N-glycans with a fluorescent dye for detection and separate the glycan mixture to assess relative distribution of glycan species. For separation by capillary electrophoresis (CE), the charged dye APTS (8-aminopyrene-1,3,6-trisulfonate) is frequently used to enable migration of neutral glycan species. Using traditional methods, preparation of labeled N-glycans often requires numerous hours or days to complete.

We present an APTS workflow as an addition to our Gly-X sample preparation platform, where APTS-labeled N-glycans are ready for CE in ~2.5 hours. A 5-minute in-solution deglycosylation with PNGase F releases N-glycans and is enabled by a denaturation reagent that enhances exposure of N-glycosylation sites for rapid enzymatic cleavage at an elevated temperature. Following conversion of glycosylamines (-NH₂) to the free reducing end form (-OH), glycans are loaded onto a cleanup matrix. APTS labeling by reductive amination (1 hour) is performed on-matrix, eliminating the lengthy dry down step of traditional APTS workflows. A 96-well vacuum plate cleanup is used to remove >99% of free APTS label and other reaction components, and preserves sialylated N-glycan species. The APTS workflow is supported by a range of APTS-labeled migration standards, individual N-glycans and N-glycan libraries.

NOTES:

P-120-T

Fully Automated GlycanAssure AutoXpress N-Glycan Sample Preparation for N-glycan Analysis on UPLC and CE

Nan Liu

Thermo Fisher Scientific, South San Francisco, CA USA

The glycans or polysaccharides attached to proteins after protein post-translation modification play critical roles in eukaryotic cell protein functions, such as protein assembling and folding stability, signal transduction, ligand binding, protein interaction, etc. In the therapeutic immunoglobulin, the N-glycosylation on amide nitrogen of asparagine is a critical quality attribute in the pharmacology, affecting immunogenicity, pharmacokinetics and pharmacodynamics. Glycan sample preparation (deglycosylation and dye labeling) is a key step for glycan analysis.

We have developed a fully automated platform and workflow for N-glycan sample preparation using Thermo Fisher GlycanAssure reagents. The magnetic beads-based, automated procedure provides streamline workflow for glycoprotein denaturation and deglycosylation, APTS labeling of the released glycans and cleaning of the excess free dyes. The hand-on time for 13 samples is as short as ~5 minutes because the operators only need to add their glycoprotein to micro-tubes before they start the automated run. The setup of the instrument is simple and easy as all of the reagents are prefilled in the prefilled cartridges, which are stable at -20C for at least one year. The total sample preparation time on the instrument is 1 hour and 45 min and the APTS labeled glycans are ready to be analyzed on UPLC and CE instruments. The fully automated platform is demonstrated to result in high quality of glycan profiles analyzed on both UPLC and CE. The glycan profiles of NIST mAb, human serum IgG, RNase B and Fetuin are comparable to the GlycanAssure manual kits, as well as to other glycan sample preparation kits in the market.

NOTES:

P-122-T

High-Throughput and High-resolution Glycan and Data Analysis by Capillary Electrophoresis 3500xL Instrument

Jenkuei Liu, Shaheer Khan, Bharti Solanki-Nand

Thermo Fisher Scientific, South San Francisco, CA USA

A high throughput analysis of APTS-labeled glycans is demonstrated with 3500xL capillary electrophoresis system along with rapid and easy data analysis GlycanAssure software. The instrument can resolve glycans from 24 samples simultaneously in ~40 min or less than 2 min per sample. Analysis of 96 samples can be finished in ~2 hours and 40 minutes. The system has a high detection sensitivity of ≤ 0.02 fmole/ μ L of APTS-Labeled glycans.

The analysis system shows high reproducibility among 576 runs from 12 injections of 24 capillaries on two instruments. These electropherograms were nicely aligned and showed relative quantity of every peak with single digit CV%. Resolution was demonstrated by separation of sialylated glycans with 1 to 5 sialic acids of Fetuin and separation of $\alpha(2-3)$ and $\alpha(2-6)$ isoforms of sialylated bi-antennary, tri-antennary, and tetra-antennary glycans.

The GlycanAssure software can rapidly analyze 96 samples in 10 minutes, including alignment, relative quantity and CV%. The combination of glycan separation by 24-capillary 3500XL system and rapid data analysis GlycanAssure program provides a powerful high throughput tool in development of bioproduction processes.

NOTES:

P-124-T

A Novel Low Fragmentation CE-SDS Sample Buffer for Analysis of Therapeutic Antibodies

Will McElroy, Chris Heger, Annegret Boge

ProteinSimple, a Bio-Techne brand, San Jose, CA USA

A major category of biomolecules for the biotherapeutics industry is recombinant therapeutic antibodies (mAbs). A standard analytical technique for mAbs is Capillary electrophoresis-sodium dodecyl sulfate (CE-SDS). CE-SDS is used for size-based characterization and purity analysis for QC lot release. In general, CE-SDS analysis for each sample includes both reduced (R) and non-reduced (NR) analysis. The CE-SDS analysis requires heating the antibody diluted in an SDS sample buffer plus a reducing or alkylating reagent for reduced and non-reduced samples respectively. Several publications on CE-SDS analysis have reported unexpectedly high fragmentation in CE-SDS-NR samples. To address this issue customized low fragmentation CE-SDS buffers were developed for their samples. To date, however, these low fragmentation sample buffers typically suffer from lower sample injection efficiency and thus lower sensitivity than traditional sample buffers. In this study, a new CE-SDS sample buffer was developed which provides comparable peak profiles combined with high injection efficiency and very low fragmentation, compared to standard CE-SDS sample buffers.

During reagent development, several CE-SDS sample buffer candidates minimized fragmentation while retaining high injection efficiency. In a subset of the initial candidate buffers, the peak profiles for the internal standard and mAb size isoforms substantially changed. The candidate buffers that did not exhibit separation aberrations were further evaluated, and the final CE-SDS sample buffer was tested on innovator and biosimilar monoclonal antibodies to confirm performance.

NOTES:

P-126-T

Electrophoresis-based Characterization to Maximize the Efficiency of Capillary Electrophoresis Method to Monitor Critical Quality Attributes of Fc Fusion Protein

Roberta Russo

Merck Serono Spa, Rome, Italy

The use of Fc fusion proteins to improve pharmacological properties of therapeutic protein domains has increased significantly in the last decades. Method development, validation and characterization of this class of protein represents a unique analytical challenge due to the inherent heterogeneity of recombinant protein expression. Moreover, several physical and chemical degradation pathways can occur during manufacturing and storage that compromise protein integrity, leading to a potentially harmful, unstable product.

Capillary Electrophoresis (CE)-based separation techniques, such as Capillary Gel Electrophoresis CGE-SDS, provide versatile, efficient, and fast analyses. CGE-SDS method characterization is generally challenging due to the inability of collecting separated fractions in order to perform species identification by direct characterization approaches.

We present a CGE-SDS method applied to a complex Fc fusion protein, in a late stage of clinical program, developed to replace old methods for monitoring species (e. g. fragments, clipped species and truncated forms), under reducing and non-reducing conditions. The method was then successfully validated.

An in deep characterization was necessary to gain a thorough understanding of a complex profile. The work further extends the use of an electrophoretic fractionator system to separate various molecular species, demonstrating the high potential of combining CGE-SDS methods with SDS-PAGE and mass spectrometry, and showing the importance of establishing proper correlation of different techniques to characterize proteins.

The overall study has revealed the potentiality of combining different techniques in order to increase the analytic method capabilities in monitoring critical quality attributes in complex biopharmaceutical products.

NOTES:

P-128-T

A Comparison of UPLC and CE-based Approaches for Glycan Profiling of Biotherapeutic Proteins - Challenges for the Fast Characterization of New Biological Entities (NBEs) and Biosimilar Candidates

Thomas Schröter, Michaela Wachs, Roland Moussa, Martin Blüggel

Protagen Protein Services GmbH, Dortmund, Germany

The characterization of biopharmaceuticals such as new biological entities (NBEs) and their biosimilars is a challenging field due to their structural complexity. When implementing a manufacturing process, variations in the protein structure or post-translational modifications occur. Therefore, a characterization program is required according to the guidelines of national authorities. When characterizing proteins from scratch, glycosylation is a central modification that has to be taken into account, as it can affect the half-life and the stability of the protein.

As established standard UHPLC methods, such as HILIC with IPC or 2AB labeling are commonly used to detect different glycans in biopharmaceuticals. However, sample preparation takes up a large amount of the total analysis time, the method is not suitable for high-throughput analysis. In addition to that, sample analysis time takes up to 90 min per sample. In particular, for time sensitive development such as clone selections processes or assessing comparability of biosimilars to originators, those methods do not qualify for a quick screening process.

In this study, we evaluated a CE-based fast glycan labeling and analysis kit as a fast screening method to identify samples for subsequent detailed HILIC analysis. To assess the comparability the UHPLC method and CE method have been performed using the same sample. We analyzed a IgG market product and compared the obtained results with respect to identified glycans and the obtained relative peak areas.

As for the glycans identified by both methods, the relative areas were in the same value range with a certain degree of variance. All in all, the fast glycan analysis seems to be a suitable tool for fast screening of glycan composition and a decision support for selecting a reduced sample set for subsequent in-depth HILIC analysis.

NOTES:

P-130-T

Electrophoresis Strikes Back: Utilizing the Isogen CEInfinite System to Collect and Expedite Identification of Capillary Isoelectric Focusing Peaks

Kristin Schultz-Kuszek, Abigail Markle, Ben Niu, Jeremy Springall, Xiangyang Wang

MedImmune, A member of the AstraZeneca Group, Gaithersburg, MD USA

Charge variant heterogeneity is present in most biopharmaceutical protein products. Isolation and identification of charge variants separated using the specification method is an important part of product characterization and manufacturing control strategy development. For Capillary Isoelectric Focusing (cIEF), this is a major time commitment. Since peak collection using cIEF separation has not been possible, Ion Exchange Chromatography (IEC) has typically been used as an orthogonal technique to achieve charge variant enrichment for further characterization. Drawbacks of this approach include the time investment in developing the IEC method and that the two techniques do not use the same separation mechanism, so pure IEC fractions often result in a mixture of cIEF peaks, preventing definitive cIEF peak identification.

We evaluated an Isogen CEInfinite instrument for expediting cIEF peak identification. The CEInfinite performs the cIEF focusing, mobilizes each peak, and collects it inside a matrix of your choosing. MedImmune tested the CEInfinite with five projects: one peptide antibody fusion molecule, one fusion protein, one bispecific antibody, and two monoclonal antibodies. Over the course of five days, four projects had successful fraction collection runs with little cIEF method development time upfront. The CEInfinite collected 11 fractions from one degraded sample where pI differences between the fractions were only 0.1 pI units! From these fractions, nine contained enough protein for intact mass analysis with post translation differences of the molecules observed between the different fractions. The results demonstrate the power of the CEInfinite to expedite cIEF peak identification through on-line focusing and fractionation without the need of an accompanying IEC method.

NOTES:

P-132-T

Elimination of Migration Time Drift for Product Profiles by CE-SDS, Simplifying Data Analysis

Samuel Shepherd

MedImmune Limited, Cambridge, United Kingdom

Capillary electrophoresis SDS (CE-SDS) is an industry standard for fragment analysis of monoclonal antibodies and other novel large biologics. MedImmune uses the SCIEX PA 800 Plus system for this analysis which shows greater resolution and sensitivity over previous platform methodologies, such as the Bioanalyser and GXII. An industry know issue with CE-SDS is drift over time of the product peaks, giving rise to complications in data analysis, peak identification, and comparability. During each sequence it was observed that gel buffer vials are subject to evaporation, and this was believed to be correlated to the occurrence of profile drift. A collaboration between SCIEX and MedImmune undertook an investigation into the use of mineral oil layered on top of gel buffer solutions during system preparation. It was hypothesised that evaporation of water from the gel buffer during the course of the sequence leads to a concentration effect of the gel buffer components. This would result in changes to the migration of the product through the capillary during electrophoresis, giving rise to the observed drift. The addition of mineral oil was proposed as a mechanism to reduce gel buffer evaporation, providing consistent migration time of the various product peaks that would result in minimising the profile drift. NIST-mAb was used as a test molecule for this collaborative investigation. The use of mineral oil was assessed for any potential negative impact upon assay reproducibility, linearity, and accuracy under reducing and non-reducing conditions, both thermally stressed and unstressed. We have demonstrated that the addition of mineral oil significantly reduces evaporation of the gel buffer, resulting in a complete mitigation of profile drift whilst having no negative impact upon any of the above assay attributes assessed. It was also shown that signal response is not negatively impacted and that the use of mineral oil does not produce any atypical peaks or artefacts.

NOTES:

P-134-T

Measurement Uncertainty in ACE an Evaluation of the Experimental Design

Matthias Oliver Stein¹, Rob Haselberg², Mona Mozafari-Torshizi¹, Hermann Wätzig¹

¹TU Braunschweig, Braunschweig, Germany, ²Vrije Universiteit, Amsterdam, Netherlands

During all phases of drug development, ligand binding assays play a crucial role. Therefore, high quality methods for determining binding properties are necessary. The basic principle of the most ligand binding assays is mostly the same. Changes of a certain chemical response (e.g. mobility in ms ACE) are measured over a defined concentration range. The dissociation constant is calculated by nonlinear fitting of the chemical response and the corresponding ligand concentrations to the binding equation. The measurement uncertainty of the dissociation constant's determination was evaluated statistically by Design of Experiments (DOE) with a focus on four measurement parameters. The DOE revealed combinations of these parameters where the overall uncertainty increases dramatically. To avoid strong uncertainty propagation, all parameters should be higher than a certain limit. The normalized maximum response range (nMRR; quotient maximal response change/ primary measurement uncertainty) should be higher than 250. A minimum of 0.25 for both the data point range (DPR) and the data point position (DPP) should be aspired. More than one parameter near the lower threshold leads to an exorbitant amplification of the uncertainty. The number of different data points does not strongly influence the uncertainty of the K_D determination when there are at least a number of five measuring points (NoDP). Precision can be maximized when the nMRR is greater than 600, DPR covers at least 40 % of the binding hyperbola and the data points are set on the upper part of the binding-curve (DPP 0.45 – 0.95). Therefore, the data points are to be set within the range of K_D to a tenfold of its value. If that is complied, five or more different data points are enough to calculate the binding constants adequately. It is possible to enhance the precision (factor 80) by simply adjusting the measurement conditions.

NOTES:

P-136-T

The Implementation of a New Method Development Process

Ewoud van Tricht¹, Lars Geurink, Cari Sanger-van de Griend²

¹Janssen Vaccines and Prevention, Leiden, Netherlands, ²Kantisto B.V., Baarn, Netherlands

During the past years we have developed a way of working within Analytical Development that aims for delivering the right method at the right time. A process was implemented focusing on customer alignment, collaboration, risk management, multivariate design of experiments and method life cycle management. This resulted in high quality analytical tests to support process development and ensure quality and safety of our products.

NOTES:

P-138-T

Applications of Capillary Electrophoresis in Monitoring the Basic Charge Variants of Monoclonal Antibodies during Upstream Process Development

Lin Wang

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Various post translational modifications (PTMs) may induce charge heterogeneity of monoclonal antibodies (mAbs) during cell culture production. The charge variants could potentially affect the structure, stability and biological activity of mAbs. Thus it's critical to monitor the charge variants of mAbs in order to ensure the product quality and process consistency. In this study, imaged capillary isoelectric focusing (icIEF) has been used to monitor the impact of copper concentration in cell culture media on charge variants of mAbs. Copper concentration in cell culture media has been reported to influence process performance and product quality. We have found the basic peak levels increased as a function of copper concentration. Furthermore, the basic peak increase has been confirmed to be proline amidation by mass spectrometry analysis as the C-terminal lysine was not affected by copper concentration. Therefore, copper concentration needs to be carefully evaluated to determine the optimal range that can improve cell culture performance as well as maintain desired product quality.

NOTES:

P-140-T

Extending the Molecular Weight Range for Maurice CE-SDS

Jiaqi Wu, Yasef Khan, Chris Heger, Annegret Boge

ProteinSimple, a Bio-Techne brand, San Jose, CA USA

The CE-SDS mode of the Maurice™ platform has several critical advantages over traditional CE-SDS instrumentation including ease-of-use, faster analysis, and a flatter baseline. While most researchers utilize Maurice CE-SDS to examine the purity of monoclonal antibodies, in some cases the test samples can be outside the range of our current Molecular Weight standards. While a separation system can be optimized for proteins with various sizes, it is often more desirable if a single separation system can be applied to a diverse group of molecules. To address these points, we will show how to create an extended molecular weight range for Maurice CE-SDS using the current Maurice CE-SDS separation system and commercially available materials.

NOTES:

P-142-T

A Nanofluidic Device for Continuous Online Monitoring of Antibody Quality in Continuous Biomanufacturing

Taehong Kwon, Ko Sung Hee, Jean-François Hamel, Jongyoon Han

Massachusetts Institute of Technology, Cambridge, MA USA

Biologics are highly sensitive to manufacturing, handling, and storage conditions due to large size and complex structure. Undesirable physical, chemical, and biological changes in biologics may reduce drug efficacy and compromise patients' safety by inducing immune responses. Thus, real-time continuous assessments of critical quality attributes (*e.g.*, purity and bioactivity) of therapeutic proteins at all stages of manufacturing process is crucial for maintaining product quality and making rapid decision concerning the manufacturing process for ensuring patients' safety.

In this context, we introduce a novel nanofluidic device for continuous-flow multivariate protein analysis with high detection sensitivity, resolution, and analysis speed for real-time critical quality assessments. Periodic and angled nanofilter arrays served as molecular sieving structure were fabricated to achieve continuous-flow size-based analysis of therapeutic proteins in relatively simple operation, allowing for real-time analysis. Different configurations of nanofilter arrays enabled both (SDS-based) size separation for purity analysis and homogeneous binding assay for activity analysis.

Considering that there is a growing trend toward continuous biomanufacturing to improve product quality and reduce manufacturing cost, we implemented fully automated continuous online monitoring of purity of monoclonal antibodies (IgG₁) produced from small-scale (350 mL) perfusion culture of Chinese Hamster Ovary (CHO) cells using a membrane-less cell retention device. The cell culture supernatant containing IgG₁ was continuously fed into the online sample preparation system (buffer exchange, labeling, denaturation) after culture clarification, and antibody purity was analyzed by the nanofluidic device.

The system developed here can be extended to perform nanofluidic homogeneous binding assay, thus enabling continuous online multiparameter analysis of antibody quality during continuous biomanufacturing. The nanofluidic device is expected to complement the existing bench-type conventional analysis tools, such as CE-SDS, SDS-PAGE, and binding assay equipment, to meet quality assurance requirements of current and future biomanufacturing systems.

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Late Breaking

LB-01-M

Charge Method Evaluation and Development for a Unique Therapeutic Protein

Thomas Niedringhaus, Brian Roper, Belinda Tang, David Fischer, David A. Michels

Genentech, a Member of the Roche Group, South San Francisco, CA USA

For biopharmaceutical companies, the ability to monitor charge heterogeneity of a therapeutic protein is critical in determining manufacturing consistency, ensuring patient safety and therapeutic efficacy, and measuring protein stability. Several technologies are capable of measuring protein charge heterogeneity; however, it is often necessary to evaluate multiple methodologies to determine product-specific suitability. In the study presented, a complex and unique therapeutic protein requires an updated charge method to be added to the quality control system as part of ongoing lifecycle management. In order to provide a long-term solution, a charge method that consistently resolves charge variants, is stability indicating, and is sufficiently robust must be identified. This poster highlights the evaluation of several charge methods for the commercially manufactured therapeutic protein, including Capillary Zone Electrophoresis (CZE), Ion-Exchange Chromatography (IEC), and Imaged Capillary Isoelectric Focusing (iCIEF). Details of method evaluation and subsequent method development experiments are presented. Additional considerations are laid out to facilitate a charge method selection that will enable a long-term solution to the lifecycle management need.

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LB-02-T

Detection of AAV Structure: Capsid Proteins and Nucleic Acid Composition on LabChip® GXII Touch™ Platform

Natalia Rodionova, Erik Miller, Michael Nilsson

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For over fifty years, adeno-associated virus (AAV) has remained an elusive object in virology studies and gene therapeutics /1/. Many biotherapeutic companies are currently involved in recombinant AAV production with needs of viral particle characterization in terms of capsid structure and nucleic acid content to estimate therapeutic doses.

The PerkinElmer LabChip® GXII Touch™ HT platform is an ideal instrument to analyze proteins and nucleic acids from the same sample with high sensitivity and reproducibility. The instrument can distinguish differences of protein molecular weight as low as 1kDa, which can be crucial to characterize artificially synthesized biopolymers such as recombinant capsid proteins. Additionally, the fast sample run times from multi-well plates allow for high throughput experiments and can open opportunities to monitor kinetic processes such as breakage of virus particles or their interactions with receptors.

Extracted from multiple assays, our results show total analysis of all viral components after breaking apart of AAVs in serotype 2, 8 and 9. The established limit of detection (LOD) of LabChip® GXII Touch™ for DNA, RNA and protein molecules allows the accurate identification of all viral components and impurities. Constructs without DNA or RNA inside have been used as ‘null’ controls.

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LB-03-M

Automated Sample Preparation for CE-SDS LIF in QC

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Capillary electrophoresis sodium dodecyl sulfate with laser-induced fluorescence (CE-SDS LIF) detection is extensively used as a quantitative release and stability method to monitor product quality due to its high sensitivity and resolution. However, sample preparation in the CE-SDS LIF method is a complicated, labor intensive, multi-step process. As the biopharmaceutical industry increasingly demands ever greater productivity, we have been exploring the implementation of automation to meet this industrial trend.

An automated sample preparation script was developed for the Tecan Fluent 1080 liquid handler. This script was developed to be a like-for-like equivalent of the existing manual sample preparation methods. A single Tecan script was developed to automate the various CE-SDS LIF manual sample preparation methods currently within our control system. This script accommodates product-specific variations of the manual sample preparation methods, including protein concentration, reagent concentration, and reduced vs non-reduced preparation. Finally, this Tecan script was developed to facilitate batch testing of up to 24 samples in a single prep session.

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LB-04-T

Greater Sensitivity for Highly Charged Isoform Molecules Using Imaged Capillary Electrophoresis

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The common technologies for evaluation of charged isoforms for multiple commercial bio-products are based on capillary electrophoresis, Ion exchange chromatography, and traditional gel methodologies. Resin lot variability impacting chromatographic resolution and indirectly method precision creates a challenge with ion exchange methods for evaluation of critical charged attributes. On the other hand, traditional SDS-PAGE gels provide greater sensitivity through various staining techniques. However, disadvantages with this technique include higher analyst to analyst variability, extensive staining / destaining time for quantitation in addition to the process being more labor intensive. For these reasons, CE technologies provide advantages for charged variants analysis. Amgen evaluated an imaged capillary electrophoresis (iCE) system which provides sufficient method sensitivity with significant improvement in execution efficiency based on the MauriCE platform from Protein Simple. The platform uses an iCE system capable of detecting protein variants using intrinsic fluorescence. Therefore, this system would be expected to provide greater sensitivity for isoelectric focusing than the traditional capillary electrophoresis methods.

Amgen challenged the capabilities of the system with highly charged and low concentration products. The glycoproteins used have isoelectric points ranging from 2.0-4.3. These proteins were analyzed at different protein concentrations between 0.1 – 0.5 mg/mL. Method development data demonstrated that the methodology provides well resolved and precise assessment of the relative isoforms distribution. The results also indicated the iCE technology with intrinsic fluorescence detection provide greater sensitivity (LOD - 0.4µg/mL) than traditional methodologies. These results demonstrated that the imaged CE platform provides clear advantages over other charge profile techniques for biological therapeutics.

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LB-05-M

Comparison of the Three CE-SDS Platforms for Non-reduced and Reduced Applications for Monoclonal Antibodies and BiTEs®

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Capillary electrophoresis sodium dodecyl sulfate (CE-SDS) is an integral analytical tool in product quality determination used in the biotechnology and pharmaceutical industries. There are several CE-SDS platforms available in the market that are utilized for speed and better data quality, depending on fit for purpose. Three platforms have been identified and evaluated to assess the different product quality attributes in the various stages of process development.

PerkinElmer's The LabChip® GXII Touch™ provides the speed in large sample demand and is applied to the initial stages of clone selections in choosing the different constructs of the molecules. ProteinSimple® Maurice CE-SDS also delivers in a high throughput capability with a better resolution of the different peaks of interest. Sciex' PA 800 Plus CE-SDS is applied to the final pools in the selection process. Traditional monoclonal antibodies (mAb) and bispecific molecules (BiTEs®) have been analyzed with these three CE-SDS platforms, and all have generated comparable results that have a significant impact in drug design and delivery.

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