Applications Of Electrophoretic Techniques For The Characterization Of Therapeutic Biomolecules

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Introduction

The characterization of therapeutic proteins such as monoclonal antibody (mAb) during different stages of manufacturing is crucial for timely and successful product release. Electrophoresis-based techniques and liquid chromatography (LC) either standalone or coupled to mass spectrometry (MS) are at the forefront for the in-depth analysis of protein purity, isoforms, stability, aggregation, posttranslational modifications, PEGylation, etc.

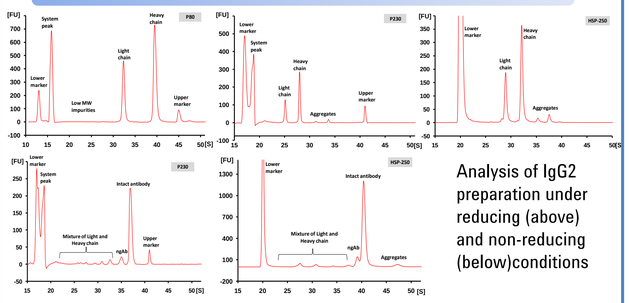
In this presentation, a combination of various electrophoretic techniques such as liquid-phase isoelectric focusing, microfluidic and capillary-based electrophoresis (CE) and combinations of those with mass spectrometry techniques will be discussed. We present a workflow based approach to the analysis of therapeutic proteins. In successive steps critical parameters like purity, accurate mass, aggregation, peptide sequence, glycopeptide and glycan analysis are analyzed. In brief, the workflow involved proteolytic digestion of mAb for peptide mapping, N-Glycanase and chemical labeling reaction for mAb glycan analysis, liquid-phase isoelectric focusing for enrichment of charge variants followed by a very detailed analysis using state of the art methods such as CE-MS and LC-MS. For the analysis of glycans, we use combinations of CE-MS and LC-MS to highlight the sweet spots of these techniques. CE-MS is found to be more useful in analysis of highly sialylated glycans (charged glycans) while nano LC-MS seems to be better adapted for analysis of neutral glycans. These two techniques can be used to get complementary data to profile all the glycans present in a given protein. In addition, microfluidic electrophoresis was used as a QC tool in initial screening for product purity, analysis of papain digestion fragments of mAb, protein PEGylation products, etc. The described workflow involves multiple platforms, provides an end to end solution for comprehensive protein characterization and aims at reducing the total product development time.

Instrumentation

- 2100 BioAnalyzer (P80, P230, HSP 250 protein assay kits)
- 3100 OFFGEL
- G7100 Capillary Electrophoresis (CE)
- G7100 Capillary Electrophoresis – 6520 QTOF Mass Spectrometry (CE-MS)

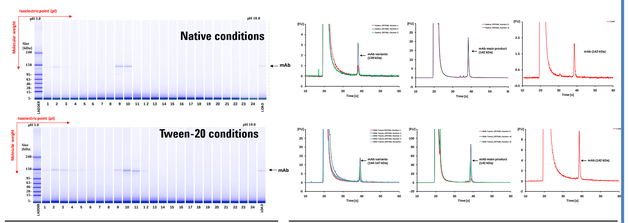
Result and Discussion

Therapeutic protein analysis with the microfluidic-based Bioanalyzer



Analysis of IgG2 preparation under reducing (above) and non-reducing (below)conditions

Analysis of antibody charge heterogeneity



Native conditions

+ Separation of different structural variants of mAb
  Major forms: fractions 9-10
  Charge variants: fractions 1-3
+ Fractions can directly be applied to downstream LC/MS analysis
–Lower protein recovery (< 50%)

Tween-20 conditions

+ Separation of different structural variants of mAb
  Major forms: fractions 9-11
  Charge variants: fractions 1-5
– Need to remove Tween-20 before downstream LC/MS analysis
+ Enhanced protein recovery (>70%)
+ Tween-20 enhances protein labeling efficiency with HSP-250 kit

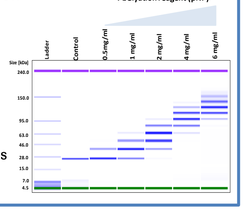
Characterization of PEGylated proteins

- The Bioanalyzer P230 Assay for Protein PEGylation

  √ Easy-to-use tool that provides high level of resolution

  √ Allows efficient optimization of PEGylation reaction conditions

  √ Fast and quantitative monitoring of production batches

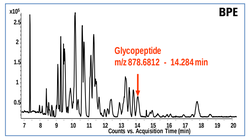


PEGylating reagents: Methoxy PEG p-nitrophenyl carbonate (mPEG pNP, MW 5000)

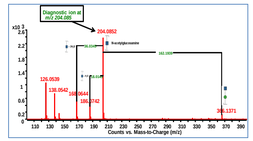
Result and Discussion

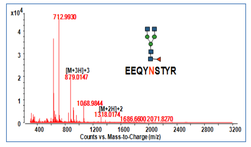
CE-QTOF MS analysis of glycopeptide - monoclonal antibody (mAb)

Base peak electropherogram (BPE)
Electrophoretic resolution of a BPE of trypsin digested mAb



CE-MS/MS of Glycopeptide
Glycopeptide was confirmed with intense sugar oxonium fragment ions

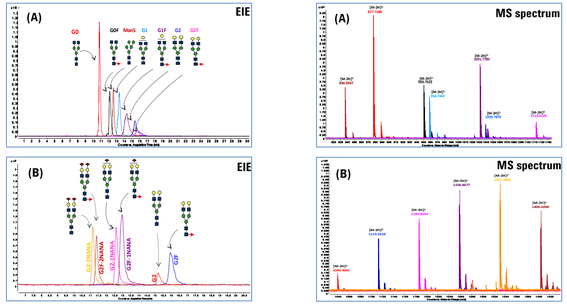




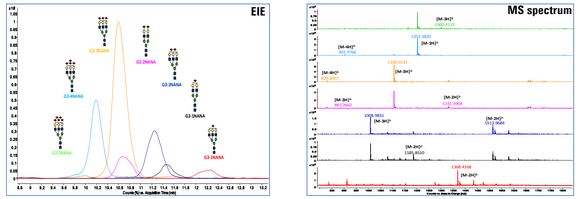
CE-MS spectrum of Glycopeptide
Peptide EEQYNSTYR with the assigned glycan structures

CE-QTOF MS analysis of glycans- Glycoprotein

Extracted ion electropherogram (EIE) and the representative MS trace from CE-MS analysis of APTS labeled neutral (A) and neutral/sialylated(B)glycans



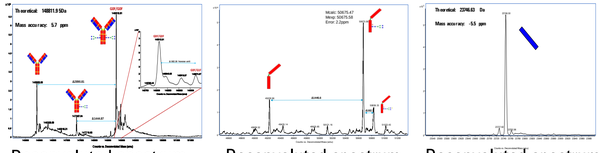
CE-MS analysis of released glycans from a glycoprotein



Result and Discussion

LC-MS analysis of monoclonal antibody (mAb)

Primary characterization of mAb

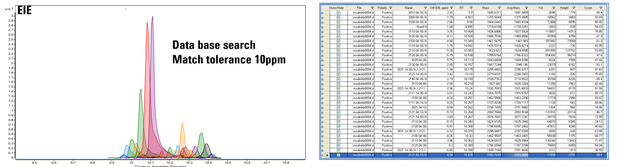


Deconvoluted spectrum of intact mAb

Deconvoluted spectrum of Heavy chain

Deconvoluted spectrum of Light chain

Glycan analysis using mAb-Glyco chip (HPLC-Chip/MS)



Overlay of all glycan structures found in the antibody

Compound list for the identified glycans

Conclusion

- Initial characterization of therapeutic protein/mAb is achieved using the electrophoretic techniques such as OFFGEL and microfluidc based electrophoresis. This sets further stage for detail analysis of mAb by advanced mass spectrometric techniques (CE-MS, LC-MS).
- The combination of CE with Q-TOF MS is a valuable tool for peptide mapping of small quantity biopharmaceuticals, especially in analysis of glycoproteins/peptides.
- Highly sialylated glycans was more suited when CE-MS was used as analysis tool while LC-MS seems to be better adapted for analysis of neutral glycans.
- Combination of various electrophoretic and LC techniques with mass spectrometry techniques was demonstrated for comprehensive protein characterization.

Acknowledgment

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