CE in biopharma science: it’s more than GCE and cIEF
Unexploited applications from the sewing box

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Introduction It’s more than GCE and cIEF

Feasibility 1
Characterization of PEGylated protein by CZE.

Feasibility 2 Site specific identification of deamidation site using deamidation specific digest and CZE.

Feasibility 3 Separation of glycated from non-glycated proteins by affinity CE.

Rough estimate of projects at Solvias
**PEGylation**

**Introduction**

- Disappearing/decreasing signals (in peptide map of PEGylated protein) indicate that the respective peptides are PEGylated completely/partially
- The number of new signals correlates with the number of PEGylation sites
- **Problem:** New signals not observed in HPLC peptide mapping

Lys-C peptide map (HPLC) of a PEGylated (top trace) and non-PEGylated protein (bottom trace)
PEGylation Peptide map of a PEGylated protein by CZE

PEGylated peptides

1000 – 5000 Da

> 30000 Da

p6, p7 = glycosylated, non-glycosylated
PEGylation Study heterogeneity on PEGylated peptides

Feasibility 1

Native PEGylated

desialylated

deglycosylated
PEGylation Summary

Summary
• The separation of both PEGylated and non-PEGylated peptides can be achieved within one CE run ranging from 1’000 to >30’000 Da.
• PEGylation site localization possible.
• Detection of PEGylated peptide allows additional characterization of glycans and sialylation on those peptides.
• Estimation of the overall PEGylation based on the A% of PEGylated signals to non-PEGylated signals.

Outlook
• Distinguish between PEGylation site on the same peptide.
• Further investigation will be needed in order to precise the individual PEGylation sites/degree.
Deamidation Current methods

Why does deamidation occur:
- One of the degradation pathway of biopharmaceuticals.
- Occurs under thermal and pH stress.
- Changes mass only minimally and charge by 1, but unspecifically.
- Can significantly affect structure and function of the protein.

Analytical problem:
- Determine the deamidation degree site specifically?

Current methods:
- ISOQUANT detection kit: Quantification of isoaspartate on protein level.
  - isoAsp specific.
- Peptide mapping: relative and site specific quantification of deamidation
  - Prone to sample preparation artifacts due to digestion at neutral or basic pH.
- Charge based methods: quantification of deamidation on protein level.
  - Charge variants not related to deamidation might interfere.
Deamidation Specific digest approach (Asp-N)

- Asp-N cleave before Asp and sometimes before Glu.
- Deamidation on an Asn generates an new cleavage site -> two new peptides
- Asp-N does not cleave at isoAsp sites so the deamidated peptide is still present.
- Asp-N also cleaves under acidic conditions allowing to minimize sample preparation artifacts due to digestion at neutral or basic pH.
Deamidation Specific digest (Asp-N)

- Significant degradation of p1 and p2.
- Detection of peptides expected to occur after Asn deamidation.
- Minor deamidation under high pH or low pH conditions at RT within 6d.
Deamidation Outlook

Summary
- Asp-N can be used as a deamidation specific digest.
- Peptides specific for each deamidation site are created.
- Can distinguish between closely positioned deamidation sites.
- Deamidation specific peptides increase when stressed.
- Based on the deamidation specific signals either an overall or a site-specific deamidation degree can be calculated.

Outlook
- Study more complex analytes.
- Develop a combined method to study Asn deamidation and Asp isomerization.
- Establish workflow with Glu-C to monitor glutamine deamidation.
What is glycation:
- Non-enzymatic glycosylation on protein amine groups. Occurs when protein is incubated with reducing sugars (e.g. glucose, galactose, fructose).
- Can further degrade to advanced glycation end product (AGE), which are highly reactive and toxic to some cell types.
- Consequently, glycation is a critical quality attribute.
- It may also happen during storage when reducing sugars are present in the formulation buffer.

Boronate affinity chromatography (BAC):
- Non-specific interaction have to be minimized by a shielding reagent.
- Problem:
  - Method has to be fine tune for each new analyte.
  - Retained peak is not pure
- The retained peak purity has to be assessed by an orthogonal method.
- Alternatively methods: charge based separation and LC-MS.
Glycation CE Approach-Principle

Boric acid interactions with vicinal diols of sugar: BGE pH > 8 (strong interaction, if pH > pI protein, negatively charged proteins)
Glycation Results

Impact on CE profile:
- Width of the peak correlates with the width of the glycation distribution.
- Reactivity (reaction enthalpy) and glycation rate
- Number of potential glycation sites not sterically hindered
Glycation Results

- Changed $\mu_{\text{eff}}$ and profile of protein signal upon glycation stress
- Different behavior between glucose and fructose due to different dissociation constant.
- Up to 14 hexoses per mAB molecule detected by LC-MS upon stress.
Glycation  Conclusion

Summary:
• Transferred BAC from a HPLC setup to a CE setup.
• Separation of glycated peak proportionally to the degree of stress.
• Separated two different molecules with the same method.
• Separated the same molecule with glycated with two different sugars.

Outlook:
• Focus on separation on only minimally glycated biopharmaceuticals.
• Assess the separation of different sugars.
• Boronate CE on a digested sample be it subunits or peptides.
Conclusion

It doesn’t always have to be gel CE or cIEF.

CZE allows to monitor very **small and very large peptides** simultaneously.

AspN is an interesting approach identify site **specific deamidation** sites.

**Boronate affinity chromatography** can adapted to CE settings.
Thank you

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