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

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OUTLINE

1. CE-SDS: Improvement Opportunities
   - Background
   - Challenges in applying CE-SDS to certain protein types
   - “CE-SHS”: Hydrophobic Detergents to Improve Data Quality

2. “CE-SHS” Method Development
   - Challenges
   - Solutions
CE-SDS: Capillary Gel Electrophoresis using Sodium Dodecyl Sulfate

Electrophoretic separation of analytes by size through a sieving (gel) matrix within a capillary:

At Bristol Myers Squibb it is used to:
- Quantify protein fragments/covalent aggregates
Proteins are denatured using SDS:

Why SDS? Rooted deep in method history; Developed around SDS (Tubes of gels → Slab gels → Capillaries)
- Uniform coating: 1.4 g SDS per g of Protein
- Harsh conditions sometimes necessary (70+ °C Heat)
  - Artifactual Fragmentation
- Reducing/Non-Reducing

Size separation through a sieving matrix
- Uniform m/z crucial
  - Different sized molecules essentially migrate at equal speeds…
- Sieving: Larger molecules move slower than smaller ones
Limited development options:
- Sample optimization: no problem
- Limited “Running Buffer” (Gel) commercial options
- Intimidating: HPLC Analogy is that the Gel = Solid/Mobile phase combined

- Reduced “mAb-1”

- %“Broad HC” + Post-HC unknowns = poor denaturation?
- % Unknowns highly dependent on [Sample]
CE-SDS: When the Data Looks Poor - Another Example

Fusion Protein ("RTP-1"): Impurity: Potential CQA

How Much Is There?

Expect LINEAR

No Assay Range: No Assay!
CE-SDS: When the Data Looks Poor – Try Changing the Detergent

Replacement or Addition of a Longer-Chain Detergent:

SDS (C12)  
SHS (C16)

“RTP-1”

“CE-SHS”

Main protein Peak

Impurity

Migration Time (Minutes)

Absorbance (AU)

Impurity Signal

Total Peak Area Signal

Relative % Area of Impurity

We Now Have an Assay!

3-fold increase in resolution
8-fold increase in plate count
Sensitivity Potential > 3-Fold
Detergent Tail Length Improved Peak Efficiency

- C11 – C16
- Samples/Sieving Gel buffers contain only the detergent shown:

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SHS (C16)  -SO4
STS (C14)  -SO4
SDS (C12)  -SO4
SUS (C11)  -SO4
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“RTP-1”
Peak Efficiency: SHS >> SDS Regardless of [SDS]

“RTP-1” Main Peak Shape Dependence on Gel Buffer Detergent Concentration:
Reduced “mAb-1”: Recovery of Expected Profile:
Typical “mAb-2” NR CE-SDS Profile

Non-Reduced “mAb-2” Improvements:

Typical “mAb-2” NR CE-SDS Profile

Improved Resolution
Reduction of Artifactual Species

We Now Have an Assay!

No Assay Range

CE-SDS → “CE-SHS”: Non-Reduced Example

0.5%
2.5%

Zoom-in
Intact mAb

CE-SDS

Non-Reduced “mAb-2” Improvements:

CE-SDS  “CE-SHS”:
Improved Resolution
Reduction of Artifactual Species

We Now Have an Assay!

No Assay Range
Further Improvement: Detergents in the Sample*

- Adding a Hydrophobic Detergent to the SAMPLE as well as the gel buffer further improved results by reducing post-HC artifacts under reducing conditions
  - STS (C14) was used due to SHS solubility issues

* Qian Guan, Poster P-110-T
Principles Behind SHS Performance
Hypothesis

Some Proteins can be “Resistant” to SDS binding*

- Add a hydrophobic detergent (SHS):

More uniformly denatured = better peak efficiency**

Important: More Hydrophobic = Higher Affinity

What Type of Protein Requires SHS for High Peak Efficiency?

Since Hydrophobicity of SHS >> SDS:

*Increase protein hydrophobicity, increase need for SHS?*

- Spacial Aggregation Propensity (SAP) Score: Measures surface hydrophobicity*
- SAP scores for select BMS mAbs shown below:

*Higher SAP score, more surface hydrophobic patches*

**Overall Protein Surface Hydrophobicity Not a Primary Determinant**

*Naresh Chennamsetty, Stanley Krystek (BMS)*

*mAbs (2009) 1, 580-582*
Is Surface Charge Important?

- RTP-1 Modeling: High “−” charge on the surface (Red)

Charge surface profile of the Variable Domain equivalent of RTP-1:

- SDS: electrostatic repulsion too great?
- SHS: Binding of more hydrophobic tail to the hydrophobic core overcomes the repulsion?

Surface Charge Appears to be an Important Determinant

What Type of Protein Requires SHS for High Peak Efficiency?

Is Thermal Stability Important?
- **RTP-1: Thermophilic***
  - E\textsubscript{2} Tm value > 80˚C
  - High energy of unfolding (DSC)

- **SHS**: Much less energy at lower concentration needed to denature Domain (E\textsubscript{2})

**Thermostability Appears to be an Important Determinant**
- “mAb-1” and “mAb-2”:
  - High Tm (> 85˚C)

Recent Industry Trend: Higher thermostability to extend expiries*

As $T_m$ rises industry-wide, can we expect an increase in the need for SDS alternatives?

Atypical (Includes alternative modalities: non-mAb; fusions, PEGylated, etc) examples**: 80:20

Alternative Detergent May Be the Solution for Some of These Modalities

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** Schwartz, M. Facilitator, CASSS CE-Pharm 2017, Table 8 Notes
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Primary Challenge:
**SHS-containing sieving gel matrix buffer**
- Recall HPLC Analogy: Gel = Solid/Mobile phase combined
  - Can we make this from scratch???

- Quality Control groups were nervous…
  - Reproducible?
  - Supply risk?

**GOAL:**
Provide robust Gel Buffer solution for multiple programs (mAbs 1 and 2, RTP-1)
“CE-SHS” Method Development Solutions

- Developed “from scratch” Production Protocol
  - Control over gel buffer components
  - Better understanding of component robustness/performance
  - Identified two vendors for each component

- Performed Multivariate DOEs Around Critical Parameters
  - Responses: Resolution, plate count, baseline, relative migration time

- Reproducible: 13 lots, 4 sites, 10 analysts
  - Responses: same as above
  - Stable: Two year expiry

MESSAGE: Don’t be afraid to “open the gel box” - High method optimization potential
Summary

- When CE-SDS doesn’t work: “CE-SHS”
  - Replacement/Addition of Sulfate detergents with longer alkyl chains
  - Better for thermophilic proteins?
  - Don’t be afraid to open the “gel box”

- Industry trend: Increasing thermophilicity – Expect more use of our “CE-SHS” method?

- When to use SHS? When to use SDS?
  - Hypothesis: peak efficiency depends on the state of denaturation
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