Table 4: New CE Frontiers - AAV, Vaccine Applications, Opportunities and Limitations for CE in DNA/RNA Applications

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Scope:

CE (e.g SDS-CGE, ICE) has been widely used as better alternative to conventional slab gel based SDS-PAGE and IEF in the analysis of size and charge variants of mAb, ADC, etc in past two decades. Today, there is a drive in the development of gene therapy products, for more targeted approaches to treat rare diseases. Gene therapy products often use a viral vector (capsid) as a carrier to transfer DNA/RNA to cells, and these products provide a high level of complexity so profiling and analyzing such products has posed new challenges for analytical chemists. This round table is focused on discussing how CE has been and will be used to profile and test the Gene Therapy product. What are the opportunities, limitations and challenges of developing and applying CE methods in a gene therapy product? These include the testing of intact virus particles (full/empty), capsid proteins and DNA/RNA.

Questions for Discussion

1. What methods do you use for intact AAV analysis (full/empty particle, charge heterogeneity), capsid protein purity in size and charge.
2. Have you developed CE method(s) for characterization of AAV? What CE methods?
3. What method do you use for plasmid DNA and genomic DNA purity analysis in gene therapy products? Do you use CE method(s) for plasmid DNA analysis?
4. What method do you use for RNA analysis in vaccines? Do you use CE method for RNA analysis?
5. What is the challenge of developing and adopting CE method(s) for AAV, DNA/RNA analysis?
6. Any other frontiers in CE application?

Discussion Notes:

I. Discussion for Q1 &Q2:

• SCIEX used cIEF for evaluation of full/empty ratio of AAV and CE-SDS for AAV purity assay Low concentration of AAV sample and high salt sample matrix pose challenge in sensitivity. For CE-SDS UV method, we explored online sample stacking to increase sensitivity (5- 8 fold) for low conc. samples analysis. Also looked at dye labeling workflows (CE-SDS LIF) to greatly increase sensitivity (over 100 fold). CE-SDS LIF and CE-SDS UV show similar value of VP protein ratio.

- Initially we tried CZE for full/empty ratio, but turned out not so straight forward, we switched to CIEF methods, which worked in a sense for some serotypes, but not for other serotypes. Since it's pI-based assay, the charge heterogeneity of capsid protein adds complexity in separation.

- CZE assays by Luuk for titer of adenovirus, using coated capillary. Would uncoated capillary a better choice? Could be. Coated capillary usually has limited shelf-life, esp. for dirty materials like in-process samples. Bare fuse capillary is also easy to clean.

Suggestion:

- Could vendors explore possibilities to provide commercial CZE buffer kits for AAV and AV titer ? SCIEX or Agilent?

- Currently SCIEX offers a General CZE kit including capillary cleaning

II. Discussion for Q3 &Q4

A. Plasmid DNA

- For DNA topology, we (Pfizer) use agarose gel methods (AGE) for purity (linear, circle, supercoil form, concatemers, etc.)

- At Genentech, we use purity methods tracking DNA isoforms (targeted for supercoiled), using SCIEX CE kits for these isoforms. Others used LC methods, CEX, AEX. We also looked at AEX methods but had carry-over issues.

- What are other people's experiences? We do not have carry-over issues with CE methods (using CGE type of method). People also tried SEC, but SEC is not platform.

- Pfizer collaborate with West Virginia University to explore the use of bare fused capillary, CGE-LIF method for purity quantitation (topology analysis of DNA)

o A paper capturing the collaboration outcome will be published soon. However, this CGE method is currently for research only at Pfizer. We also used AEX, but not generic, while CGE is generic.

o CGE-LIF gel preparation and storage: SCIEX gel buffer is lyo powder and need to be recon and stirred overnight prior to use. Self-prepared gel can be stored at 5 C for 1 to 2 month. We also had the option of freezing the aliquot of gel buffer and thaw it prior to use.

o We will start to compare the AGE and CGE methods for DNA purity in Pfizer, aiming at platform, broad spectrum (size or charge), easy to use, robust, ready for QC.

- Tried different dyes? Any changes? And why?

o Cyber gold or cyber green are used in commercial kit from Agilent and Sciex

o EtBr dye is used in self-prep CGE gel buffer at Pfizer. Cyber green dyes provide much higher sensitivity, but worse separation with self-prep gel buffer

o EtBr dye is also used in AGE., cyber green,

B. mRNA, NLP.

- We Pfizer use Agilent FA (CGE-LIF) for mRNA purity. We are also evaluating SCIEX CGE-LIF.
- PA800+ has been used in Genentech for mRNA.
- Multiple companies used Agilent Fragment Analyzer for mRNA purity. Gel based CE methods to support mRNA integrity/purity test.
- Purity is tied with efficacy. Not any LC methods for mRNA purity, due to limited resolution etc. CE seems to be the only option.
- Some linearity, reproducibilityand accuracy issues, when CGE assay used for mRNA. % main peak is consistent, but total peak intensity decreases over the sequence
  - Maybe due to salt related electrokinetic injection? Pressure injection might help. Do sandwich injections, injecting buffer-sample-buffer solutions.
  - Use low pressure and longer injection time to increase consistency to gain a balance of resolution decrease and consistency improvement.
  - Could increase dye strength help with consistency? We used 1 ppm EtBr dye in self-prep gel buffer, no information on dye concentration is provided in Sciex and Agilent kit. Long sequence trends to decrease signal, due to depletion of dye or dye stability. .
  - When prep linearity samples, we can use the formulation buffer to compensate sample concentration and improve linearity. Including everything in the matrix to prep linearity samples to maintain the ionic strength .
  - Capillary ttemperature could alter the signal intensity, due to degradation of dye. For EtBr dye, it is observed that optimal separation is achieved at 45 oC. Sensitivity is significantly reduced when capillary temperature is raised to 60 oC.

III. Discussion for Q5&Q6,

See notes under Q1-4.