Roundtable Session 1 – Table 8:

Role of CE for Therapeutic Nucleic Acids: Gene Therapy and Vaccines

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Abstract:

AAV and LNP based gene therapy and vaccines have been increasingly used to treat or prevent various diseases. The complexities of AAV and mRNA LNP drug product pose significant challenges to analytical quality control. The roundtable will discuss the opportunities and challenges in the application of CE to the characterization of therapeutic nucleic acids in AAV gene therapy and mRNA LNP vaccines.

Discussion Questions:

a) Abstract derived questions

1) What CE technologies are currently explored and/or implemented in the characterization of DNA, mRNA integrity in AAV and LNP in your labs?
2) What are the advantages and disadvantages of CE methodologies vs other techniques in the analysis of therapeutic nucleic acids ? Give specific examples in comparison.
3) What is your experience of using CE for plasmid DNA topology analysis? As compared to AGE, AEX, what is the advantage and disadvantage of CGE-LIF?
4) What CE methods do you use for analysis of mRNA integrity in LNP vaccine?
5) Lipid-mRNA adducts adversely affect potency of mRNA LNP vaccine. Do you use CE to monitor lipid -mRNA adducts? Have you explored other CE modes to investigate lipid-mRNA adducts?

Notes:

- CGE-LIF has been used for genomic DNA in AAV using PA800 in some labs: LIF calibration is essential for good sensitivity, CCF(calibration correction factor) needs to be in certain range to avoid signal saturation, manual setting is preferred using Sciex manual advise. In addition, appropriate filter alignment, if installed properly (direction) including aperture (cartridge) is required.
- mRNA: CGE-LIF based methods exist on Sciex instrument and Agilent chemistry(ladder, buffer). Methods developed on different platforms, e.g. transferred from Agilent instrument with chemistry and then run on PA800.

- AAV: LOQ set by S/N>10 for designing cut-off for peak integration, below disregarded. Variability of noise may impact detection of peaks and hence results, taking into account the impact of detector calibration.
- Hardware: Agilent fragment analyzer vs. Sciex kit(s) – head-to-head comparison (RNA/DNA) should be made (round-robin study?) to describe pro's and con's by data, e.g. regarding separation, robustness, etc.
- Bioanalyzer and LabChip (RNA/DNA) has been tested in sizing for this context, results were published (https://www.sciencedirect.com/science/article/pii/S0731708524003923).
- Integration during comparison of systems might be a problem due to setting baseline and peak separators consistently, i.e to avoid bias of the comparison.
- Denaturation step during sample preparation is strongly dependent on rather the temperature than on the buffer composition and/or pH to obtain ssRNA.
- Sciex kits can be used interchangeably for DNA and RNA analysis, decision is on the anticipated size range.
- RNA/DNA kits: Gels were developed for sequencing purpose and are now used for characterization. Improvement is in the responsibility of the vendor since they have control over the quality and development of their product. Hence method development from a kit becomes a problem, contradicting to ICH Q14 which requires for the full understanding of the method. This is not the case for kits due to the proprietary properties of components, e.g. gels. Using kits might be a risk since unknown content and limited if not even stopped supply by a single vendor is considered a business risk.
- Late migrating species for mRNA (when analyzing lipid particles): CE and HPLC have different separation efficiency. Post-main peak adduct visible in CE size dependent. IP-RP-HPLC is used to detect this RNA adduct, gives better separation than CE. Root-cause of peak: lipid adduct chemical as modification of RNA by lipid impurity.
  MEKC might give comparable results, but was not tested so far. Other micelle types e.g. mixed micelles mixed be needed to be adapted in size.
- Quality of batches of analysis kits is QC'd like for HPLCs. However, unknown composition makes optimization difficult, i.e. composition cannot be changed. CE-SDS kit has minimum information publicly available by the patent (Sciex only).
- Plasmid DNA by CE for topoisomers. Bigger plasmids go by IE-HPLC (e.g. DNA Pac column).
- Sciex RNA/DNA kits have changed from covalent (old kit) to dynamic coating (new kit). New kit comparison to IEX to be performed for characterization of pro's and con's by data.
- ProteinPac HPLC separation for empty/full determination of virus particles. cIEF was proposed by Sciex some time ago, but needs optimization per particle type (e.g. changes to the ampholyte composition) and is not a platform method. CZE should be applicable to separate AAV empty/full since DNA provides charge difference. Method(s) to be developed. Consider also new technology in this context: resistive pulse sensing, based on different mobilities of the particles, porous material.
- AAV about 20nm, AV approx. 90nm. Order of AAV is not as organized as considered, rather chaotic.
- Plasmid separations do not really required a gel, have different hydrodynamic ratio (e.g. SC vs. OC) and should be separated by CZE. So far almost all published methods use gels still. Development needed.

- Transient vs. steady-state mobility might be significant for AAV etc. (which is not for small analytes), overcoming friction in solution when starting electrophoresis. Potential application but no technical solution/instrumentation yet.