Gene Therapies, Vaccine and AAV CE Applications

Abstract:

This roundtable focuses on the role of capillary electrophoresis in the field of nucleic acid related therapeutic products and its recent applications. From the initial explorative phase, CE matured to be a valuable tool for characterization of gene therapy products, but it offers still potential for growth. The roundtable wants to offer a discussion point to understand the contribution of CE and its recent and future challenges in gene therapy and vaccines.

Questions for Discussion:

1. Nucleic acid integrity and identity are critical attributes to be addressed in gene therapy applications. However, when talking about therapeutic nucleic acids we mean a wide range of long and short, double, and single stranded, RNA and DNA molecules.

- Which one is your experience on this topic? Do you use CE or other technology?

- How do you approach the different sizes and nature of the nucleic acids? In particular, how do you approach larger nucleic acids of more than 2000 bases/base pairs?

2. Plasmid DNA in supercoiled conformation is generally considered the desirable form in gene therapy and methods are required to understand the topoisomers of a plasmid DNA.

- Do you use CE or other technology for plasmid DNA analysis?

- Which problems or challenges have you faced regarding kits variability, self-prepared gels, restriction enzymes, separation performance, labeling reagents, etc.?

3. mRNA encapsulated in lipid nanoparticles are especially known from the “Covid-19 vaccines”. Recently, other mRNA-based vaccines have gained a lot of interest in several clinical applications.

- Where do you see CE playing a role for mRNA-based vaccines?

- Regarding LNP-mRNAs, which strategy do you use to evaluate the encapsulation efficiency? Which LNP degradation conditions were the best to perform subsequent RNA analysis?

4. Apart from LNPs, Adeno-associated virus (AAV) or adenovirus (Ad) vectors are a popular choice as gene delivery systems. For AAV, critical quality attributes as genome integrity and genome content/titer, capsid composition, purity, and content, and loading efficiency or empty/full ratio are being studied and evaluated by CE.

- Do analytical techniques of viral systems play an important role in your company? Which method do you apply?

- In general, well-characterized standards are difficult to find for viral vector vaccines, however some applications are being developed where AAV reference material is used for quantification purposes. How do you approach the loading efficiency or empty/full ratio for AAV? Do you use TEM, AUC, AEX, or CE? How do you apply CE?

- Do you have experience with CZE/CIEF for (intact) AAV or Ad analysis? Which scope do you apply intact analysis for (i.e. stability, content, purity)?

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Notes:

- **Which technique/s do you apply for nucleic acids?**

AEX and IP-LC were the first method for some participants, but CGE is now the main technique.

Empty/Full AAV comes fast as a topic which raises the interest of the participants. It is not clear that the technology nowadays can really show and solve the problem of the E/F ratio in a simple way and how the partially full can be shown.

Regarding size limitations in CGE, low resolution for mRNA is mentioned by some participants and a need for higher resolution is expressed by some participants. CGE limitations are also related with keeping resolution in different size ranges in one sample.

It is mentioned that ladders are not consistent, they differ from what it is offered because ladders are mainly shown on agarose gel by the vendors. For dsDNA, the ladders are mentioned to be well stablished. For ssDNA the problem of annealing is mentioned.

- **For these new molecules, from all CE modes, which modes are expected to be important?**

CIEF for encapsulation efficiency in LNP-RNA systems with Maurice instrument is a topic for discussion. CGE is important for size separation, it provides perfect resolution. The roundtable agrees that CIEF and CGE will continue to expand. LC-MS is mentioned for RNA peak identification.

- **Which strategy is used for peak identification in nucleic acid analysis?**

MS is considered useful but specialized/trained people and technology is required. So other solutions should be also considered and not only MS.

It is considered that MS could be easier to apply if done in direct collaboration with the suppliers.

- **Is CE going to be the primary technique for gene therapy?**

A general statement from the participants is that CGE provides the highest resolution in this field, compared to any other technique, and therefore is considered as a key technique. Some participants

comment that they use CGE for the small range and SEC for the higher range. For AAV capsid proteins, CGE is commonly used together with LC-(MS) but stability of VP proteins is discussed as a problem. At this point, charge detection mass spectrometry (CDMS) enters the discussion. It is mentioned as not commercially available, but also that Megadalton Solutions and Waters are working on this. CDMS is considered slow and expensive but quite interesting for gene therapy applications.

- **Is there another technique for Empty/Full?**

Participants agree in AUC as the main technique for this topic. DLS is also mentioned.

- **Nucleic acids and CGE - range against resolution, how you can balance this problem?**

Some participants say to use different concentration and different buffers. But several participants agree that method development is challenging. The knowledge of how to "play" with the parameters for method development is missing and there are limitations regarding time and resources.

- **Challenges from the participants in the topic:**

Peak ID: the problem with peak identification is based on the large size of therapeutic nucleic acids and the availability of MS. Sequence and size are required for peak ID.

Agarose gel to CGE: method transfer is a big challenge for some participants. A discussion starts about the differences between both techniques. Spin filters is mentioned as an idea to isolate the peaks from the gel.

Peak ID: it is an important topic in the roundtable. One idea proposed for this is to analyze RNA with and without the polyA tail to see which part of the peak is related to the polyA distribution. Other idea mentioned is to test peptide nucleic acids (PNAs) as a possibility for ID but it is not clear for most participants how to apply them.

- **Critical quality attributes (CQA) for mRNA with CE**

Integrity/purity, PolyA. Identity is also mentioned.

- **CQA for AAV with CE**

VP ratio, VP purity. Titer is mentioned but associated with sensitivity problems. Genome purity. PTM characterization of the VP is also mentioned, and a short discussion starts about deamidation. CIEF-MS is considered useful for this purpose.

- **How to make MS more accessible or easy to use?**

MS is not considered GMP or a technique that can run in a "validated" environment. MS is used in the companies of most attendees only for characterization. The interaction between groups with different roles inside the companies is mentioned.

- **Intact AAV characterization in terms of charge:**

CIEF is considered possible but the interpretation of the results difficult. The application is missing for some participants, but it is discussed that for formulation development could be quite useful. Sensitivity problems are mentioned but Maurice with native fluorescence detection can be applied to gain sensitivity.

- **For small nucleic acids:**

LC and CE together are mentioned as a strategy to go. It is mentioned that some participants have more experience with LC in their labs.

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Notes:

Would like to have methods that span the space of development but finding with gene therapies this may not be the case.

Kits?

- Sometimes not amenable to method development
- CE - Gels are key but often proprietary
- Method development for gels are limited – dilutions for different types of separations

AAV – methods for evaluating partial/full/empty (required by FDA)

- FDA requires full/empty characterization
- LC – AEX method for resolution of partial/full/empty (intact) but resolution is not great
    - Better for some serotypes
    - Inner DNA is not strong enough to affect separation
- AUC is gold standard but not everyone has it, QC friendly?, not robust?
- Mass photometry – camera – put samples on microscope side and camera measure how they move around (REFYNE) but very sensitive, must sit on electronic stabilizer

What reference material is used for capsid and DNA material?

- Commercial standards may be too different and not behave the same
- Larger genome and better separation
- AAV8 serotype (paper in process) that can be used for other serotypes
- LNP – can use encapsulated and non-encapsulated standards to see total concentration

Plasmid isoforms

- Concern that fluorescence detection (through intercalation) may interact differently with different isoforms and lead to inaccuracy
- Compared with LC methods and comparable percentages were observed

Evaluating mRNA purity and LNP encapsulation efficiency

- Free siRNA stuck on the service of LNPs
- Washing LNPs but don't want to wash too much – salts, detergents, different ionic strengths

- Use coated capillary
- Ribo green assay is sensitive but buffer dependent.
- Use enzymes to treat LNPs and AAVs to remove surface genomic material but will this be QC friendly?
- UV 260nm
- Can we calibrate external genomic vs internal genomic DNA with extinction coefficients?
- Different pIs – use icIEF will have different pIs between LNP and LNP-siRNA
- Also an issue with on-off equilibrium to be able to reproduce data
- CQA is encapsulated efficiency