Purity analysis of mRNA vaccines by using CGE-LIF

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Introduction

This technical note describes a workflow that is reliable, flexible and can accurately assess the purity of mRNA during vaccine development and manufacturing. RNA vaccines are genetic vaccines that can develop immunity, and are safe and flexible. Compared with traditional vaccines, mRNA vaccines are able to trigger the immune system to produce balanced, long-lasting protection. During the manufacturing process of mRNA vaccines, incomplete fragments can be generated, which are present as impurities in mRNA vaccines. Therefore, the purity of mRNA vaccines needs to be evaluated with efficient analytical rigor.

The disadvantages of agarose gel electrophoresis in analyzing the purity of nucleic acids, such as complicated manual operation, lengthy analysis time, poor resolution, and imprecise quantification, led to the stepwise substitution of this method. Capillary gel electrophoresis (CGE) is a more efficient method for separating nucleic acid molecules and has several advantages, such as shorter isolation and analysis time, accurate quantification, and automation. It is suitable for the separation of nucleic acid molecules that are several hundred to several thousand nucleotides in length and can be effectively used for the detection and evaluation of mRNA vaccine purity.

The SCIEX PA 800 Plus Pharmaceutical Analytical System is a classic capillary electrophoresis instrument (Figure 1). In the purity analysis of mRNA vaccines, according to different lengths of the samples, different gel buffers can be designed, and appropriate parameters such as isolation voltage and capillary temperature can be selected.

Key features

- The SCIEX PA 800 Plus System can optimize the composition and concentration of the gel buffer. By changing the concentration of gel macromolecules, the analysis of nucleic acids with different lengths can reach the optimal resolution.

Figure 1. (1) PA 800 Plus Pharmaceutical Analysis System, (2) matched LIF detector (3) and nanoVial.

- **LIF detector:** as an ultra-high sensitivity detector, its baseline is smoother, and its sensitivity is higher, which is helpful for the accuracy of impurity detection and of integration
- **More accurate integration conditions:** the 32 Karat™ Software can set appropriate parameters such as width, threshold, and sensitivity, and the real data on mRNA vaccine purity is accurately integrated for reduction
- **Sample temperature control suitable for nucleic acid samples:** the instrument can set the storage temperature to 4–60° C. The mRNA vaccine samples are subject to denaturation when stored at room temperature. The low temperature storage environment can guarantee the stability of mRNA vaccine samples.
- **NanoVial for micro sample analysis:** nanoVial is suitable for micro sample injection analysis. The injection can be completed with just 5 µL of sample.
- **Optional instrument parameters:** the instrument can set a separation voltage between 1–30 kV and has a capillary temperature control of 15–60° C. The appropriate separation voltage and capillary temperature can be selected depending on the sample's peak time.

2. Reagents and methods

2.1. Reagents and samples

Polyvinylpyrrolidone (PVP, Sigma, PN 437190), 10 × TBE buffer (Sigma, PN 574795), urea (Sigma, PN U5378), SYBRTM Green II RNA Gel Stain (10000 concentrate in DMSO, Thermo, PN S7564), nuclease-free water (Ambion, PN AM9938)

The mRNA vaccine samples (with a length of about 4000 bp and a concentration of 1 mg/mL, dissolved in nuclease-free water) were provided by a domestic manufacturer.

2.2. Sample pretreatment method

The mRNA vaccine samples were diluted to 0.5 mg/mL with nuclease-free water; diluted samples were heated in a 65° C metal bath for 5 min, and then immediately placed on ice to cool down. 20 µL were transferred to a nanoVial (SCIEX, PN 5043467).

2.3. Configuration of gel buffer

a. Preparation of 30 mL 1 × TBE buffer: added 28 mLw nucle- ase-free water to 3 mL 10 × TBE buffer.

b. Preparation of 1 × TBE buffer solution containing 1% PVP and 4 M urea: 0.2 g PVP and 4.8 g urea respectively were dissolved with 1 × TBE buffer solution, and the volume was fixed to 20 mL after full dissolution.

c. Preparation of gel buffer containing dye: 2 µL of dye (1:100,000 dilution of dye) was added to 20 mL 1 × TBE buffer containing 1% PVP and 4 M urea, and mixed well as gel buffer.

2.4. Instruments and methods

SCIEX PA 800 Plus System was coupled with a laser-induced fluorescence detector (LIF). Conditions for fused silica capillary: 20/30.2 cm (effective/total length), 50 µm inside diameter (ID); isolation conditions: -5 kV, 30 min; injection conditions: - 5 kV, 3 s; capillary temperature: 25° C; sample chamber temperature: 8° C; LIF detector excitation wavelength: 488 nm, emission wavelength: 520 nm. Figure 2 shows the initial conditions for capillary activation and isolation of mRNA vaccine samples. Figure 3 shows the detector conditions for capillary activation and isolation.

New capillary activation: rinsed for 10 min at 50 psi with 0.1 mol/L NaOH solution, for 10 min at 50 psi with nuclease-free water, and for 10 min at 70 psi with the gel buffer. Figure 4 shows the sequence for capillary activation.

Capillary tubes were rinsed between every run for 1 min at 70 psi with nuclease-free water, and for 10 min at 70 psi with gel buffer. Figure 5 shows the sequence for isolation of mRNA vaccine samples.

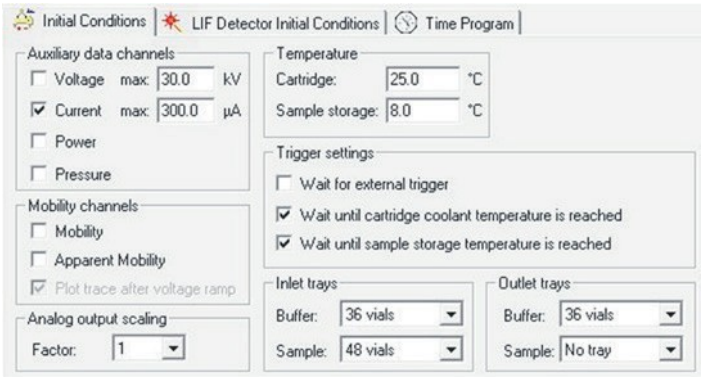


Figure 2. Initial conditions.

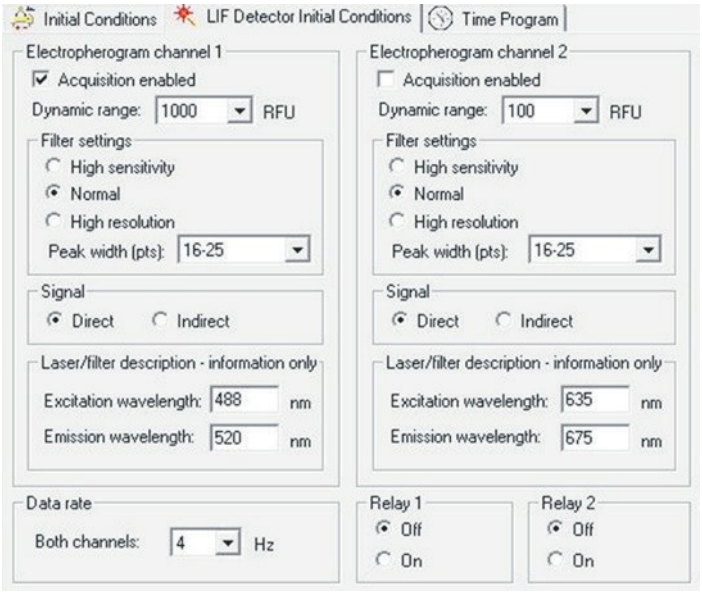
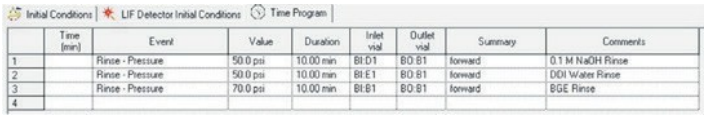


Figure 3. Detector conditions.



	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	50.0 psi	10.00 min	BI:D1	BO:B1	forward	0.1 M NaOH Rinse
2		Rinse - Pressure	50.0 psi	10.00 min	BI:E1	BO:B1	forward	DDI Water Rinse
3		Rinse - Pressure	70.0 psi	10.00 min	BI:B1	BO:B1	forward	BGE Rinse
4								

Figure 4. Sequence of capillary activation.

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	50.0 psi	1.00 min	BI:E1	BO:B1	forward	DDI Water Rinse
2		Rinse - Pressure	70.0 psi	10.00 min	BI:B1	BO:B1	forward	BGE Rinse
3		Wait		0.00 min	BI:A1	BO:A1		Water Dip
4		Inject - Voltage	5.0 KV	3.0 sec	SI:A1	BO:C1	Override, reverse polarity	Injection
5		Wait		0.00 min	BI:A1	BO:A1		Water Dip
6	0.00	Separate - Voltage	5.0 KV	30.00 min	BI:C1	BO:C1	0.17 Min ramp, reverse polarit	Separation
7								

Figure 5. Sequence for isolation of mRNA vaccine samples.

3. Results

The main peak and impurities of the mRNA vaccine samples could be detected under these conditions, as shown in Figure 6. With a flat baseline under the conditions of the LIF detector, smaller nucleic acid fragments were easily detected. After the integration processing by the 32 Karat™ Software, the purity of the mRNA vaccine sample was detected as 92.61%.

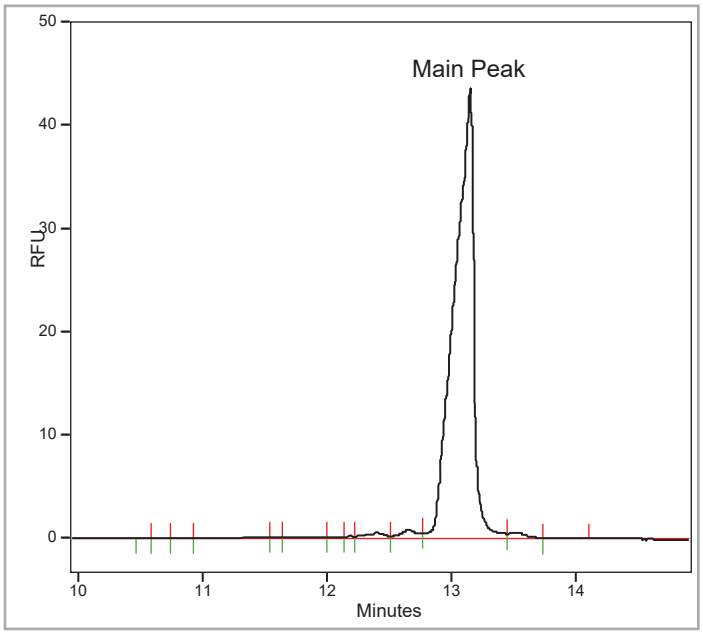


Figure 6. Electropherogram of purity of mRNA vaccine sample (approximately 4000 bp in length) analyzed by the CGE-LIF method.

4. Conclusion

In this paper, the purity of the mRNA vaccine sample (approximately 4000 bp in length) was assessed by using capillary gel electrophoresis (CGE) coupled with a LIF detector. The purity was determined to be 92.61% in a 1 × TBE gel buffer containing 1% PVP, 4 M urea, and 1:100,000 dilution of dye.

When assessing the purity of mRNA of different lengths (1000–5000 bp), the resolution of mRNA vaccines and their fragment impurities and the suitability of the method can be optimized by varying the concentration of PVP. Other gel buffers can also be tried to seek more optimal isolation conditions. Similarly, adjustable instrument parameters can also assist in reaching optimal mRNA isolation conditions.

While detecting the purity of mRNA vaccines, the capillary gel electrophoresis (CGE) can also identify peaks based on migration times of controls or standards to appraise mRNA vaccine products. It is an indispensable analytical tool in mRNA vaccine research and product quality control.

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