

Oligonucleotides—Fraction Collection from Capillary Gel Electrophoresis and Offline MALDI-TOF

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Abstract

Recent advances in biopolymer therapeutics has resulted in the development of antisense oligonucleotides to act as anti-viral and anti-cancer drugs.

Oligonucleotides cannot be successfully separated according to size by conventional capillary zone electrophoresis (CZE) since they have similar mobilities independent of their chain length. However, using a sieving-polymer-filled capillary, such analytes can be separated according to their chain length. For further analysis after separation it may be of interest to collect peaks and subject them to further offline analysis. This application brief reports on the collection of fractions from an oligonucleotide separation followed by offline MALDI-TOF determination of the analyte mass.

By using the Agilent oligonucleotide kit, antisense oligonucleotides can be separated using the Agilent Capillary Electrophoresis system. Agilent ChemStation software enables the fully automated collection of fractions using signal A as the peak sensor.

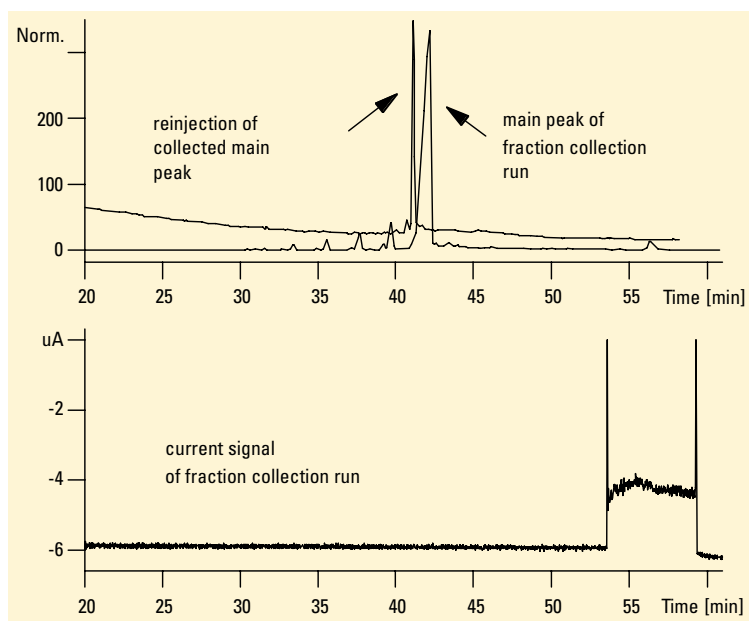


Figure 1
Automated fraction collection

Conditions

Buffer Oligonucleotide

Sieving Solution Polymer

Capillary PVA coated 24.5 cm left × 100 µm i.d. (order number G1600-60419)

Flush Regimen

Polymer flush from outlet -7.5 bar for 3 minutes

Injection 7 s at -10 kV

Run -25 kV, 30 °C

Detection 260 nm/8 nm DNA filter (order number G1600-62700)



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Experimental

All CE experiments were performed using the Agilent Capillary Electrophoresis system equipped with diode array detection and computer controlled via Agilent ChemStation software. Oligonucleotides were separated using the Oligonucleotide Kit (order number 5063-6530).

MALDI-TOF analysis was performed using the Agilent G2025A LD-TOF mass spectrometer. The matrix was 2,5 dihydroxyacetophenone and diammoniumhydrogen citrate in isopropanol/water. Laser energy was 4 μ J, negative polarity. Peaks mass data were determined from 50 shots.

Figure 1 shows the preparative separation of a 25-mer antisense oligonucleotide. The current trace indicates that the voltage is stopped at a given point, calculated by the software, to be when the peak of interest, in this case the main peak reaches the end of the capillary. The outlet vial is then replaced with a collection vial containing only a dilute buffer. The voltage is switched on, as indicated by the reestablishment of the current. The effluent of the column was collected for 5 minutes. Upon re-injecting from the collection vial it can be seen that a peak approximately corresponding to the migration time of the main peak is apparent. Using the same collected fraction this was then subjected to MALDI-TOF analysis using reversed polarity in order to determine its mass (figure 2). The results of this gave a determined mass of 3230.10.

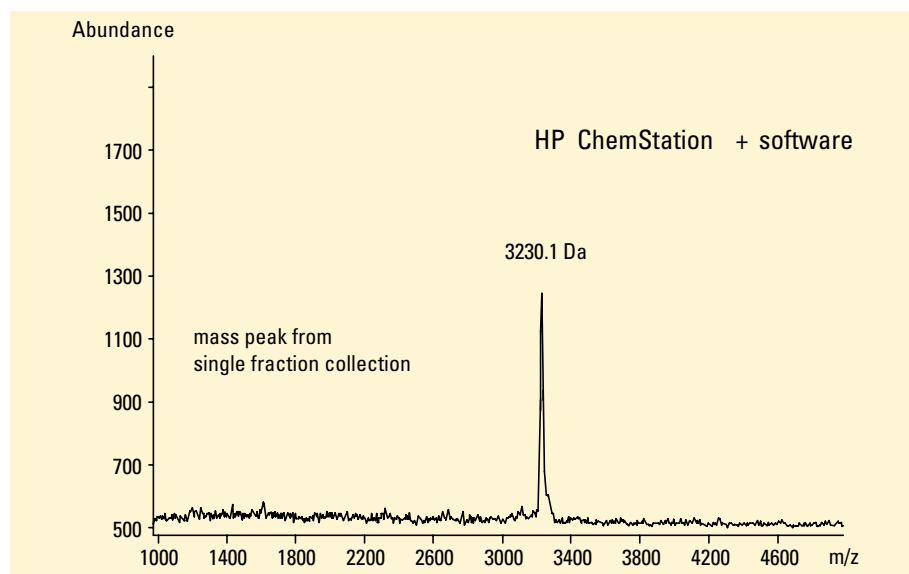


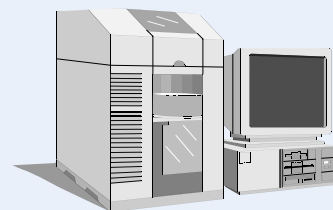
Figure 3
MALDI-TOF analysis from single fraction collection

Conclusions

The oligonucleotide analysis kit was used for the preparative separation of an oligonucleotide sample. A fraction was automatically collected using the Agilent ChemStation software into a collection vial. Sufficient sample was obtained to perform re-injection to confirm collection and to perform offline MALDI-TOF analysis of the oligonucleotide.

Equipment

- Agilent Capillary Electrophoresis system
- Agilent CE high-sensitivity detection cell
- Agilent G2025A LD-TOF MS
- Agilent ChemStation + software



The authors are application chemists at Agilent Technologies, Waldbronn, Germany.

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