Oligonucleotide analysis by capillary gel electrophoresis

Application Note

Nucleic Acid Analysis

Authors

Gordon Ross,

Ulrike Jegle,

Agilent Technologies,

Waldbronn, Germany

Abstract

Oligonucleotides cannot be successfully separated according to size using conventional capillary zone electrophoresis (CZE) because they have similar mobilities independent of their chain length. This is due to the addition of equivalent unit charge for every additional nucleotide, and nucleotides themselves have similar masses. This means that their charge to mass ratio remains very similar. Such analytes can be separated according to their chain length by using a sieving matrix. In order to perform this separation the capillary must be filled with a sieving matrix so that smaller oligonucleotides move faster through the capillary than larger, slower moving analytes. Oligonucleotides can be separated using the Agilent Capillary Electrophoresis system. In order to correctly identify analytes by their size, a high degree of reproducibility is also necessary between standards and samples, which is achieved using a PVA coated capillary. It is possible to analyze single- and double-stranded DNA oligonucleotides, single-stranded RNA oligonucleotides and antisense oligonucleotides. The seiving polymer solutions have been optimized for separating oligonucleotides with a chain length 5 to 80 nucleotides.

Chromatographic conditions

Buffer:	Oligonucleotide kit neutral buffer (order number 8500-6886)
Sieving Solution:	Polymer solution A: 27 (w/v)% Polyethylenglykol 35000 (PEG) in 200 mM BisTris Polymer solution B: 27 (w/v)% Polyethylenglykol 35000 (PEG) in 200 mM BisTris buffer solution, 20 (w/v)% acetonitrile
Capillary:	PVA coated 24.5 cm eff x 100 µm id (G1600-60419)
Flush Regimen:	High pressure flush from outlet -7.5 bar for 3 min
Injection:	7 sec at -10 kV
Run:	-25 kV, 30 °C
Detection:	260 nm/8 nm DNA filter (G1600-62700)
Sample:	Oligonucleotide pd(A) 12–18

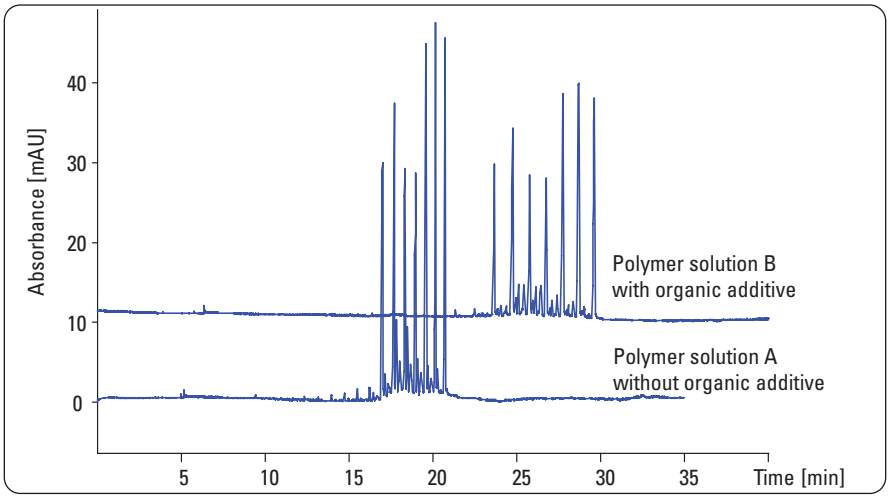

Figure 1
Separation of oligonucleotide standard pd(A) 12–18 using polymer solution A and polymer solution B.

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Experimental

All experiments were performed using the Agilent Capillary Electrophoresis system equipped with diode array detection and Agilent ChemStation software.

Figure 1 shows the separation of an oligonucleotide standard pd(A) 12–18 using polymer solution A and polymer solution B. In this case the organic additive in polymer solution B enhances the resolution of the separation. Reproducibility and robustness are demonstrated in figure 2 by the highly reproducible migration times taken from an exemplary 6 runs at 0.35 % RSD over 73 runs. The kit can be used for checking the purity of oligonucleotide preparations (figure 3) with the benefit of being able to tailor the polymer solution to the needs of the analysis. In this case using solution A—without organic additive—provides a better resolution of the 15–mer failure sequence in this 16–mer oligonucleotide.

Equipment

- Agilent Capillary Electrophoresis system
- Agilent CE high-sensitivity detection cell
- Agilent ChemStation

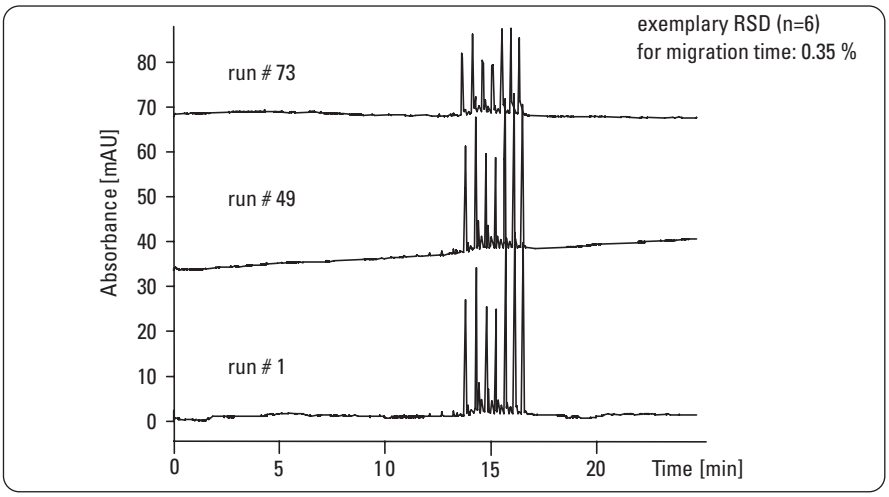

Figure 2
Reproducibility of migration time.

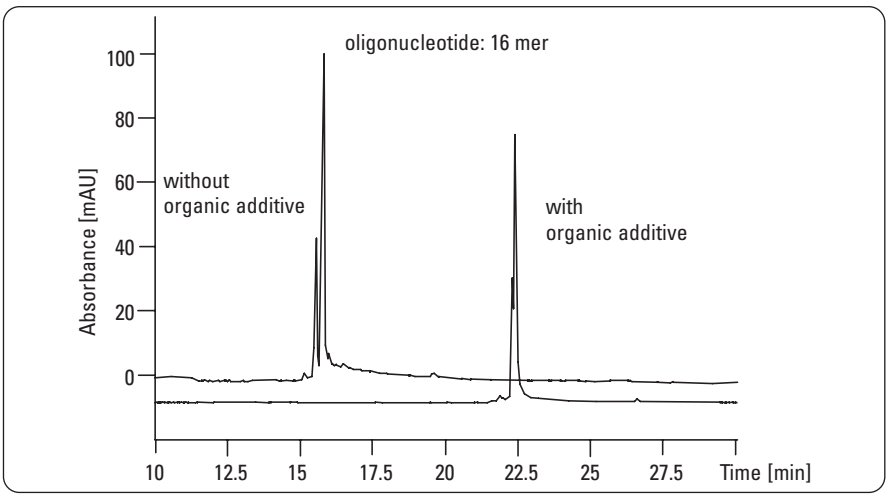

Figure 3
Optimal polymer solution for purity check.

Conclusion

The Agilent CE system is suitable for the analysis of oligonucleotides in the range 5 to 80–mers. Migration time reproducibility can be achieved at less than 1 % RSD for both the polymer A and polymer B solutions.

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