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Oligonucleotide Purity Analysis by Capillary Electrophoresis

Introduction

Millions of oligonucleotides are synthesized each year for use as primers or probes in genetic analysis. Quality control of these preparations has traditionally been done by slab gel electrophoresis or HPLC, but these methods may require expensive reagents and laborious protocols, and may not provide desired quantitative precision or automation. Capillary electrophorsis (CE) using polymer solution buffers is being used increasingly for determination of oligonucleotide purity because of its high resolution, low consumption of sample and reagents, and ability to be fully automated. The dissolved polymers act as the sieving matrix and provide separations of oligonucleotides substantially equivalent to polyacrylamide and agarose gel electrophoresis.[1,2] This method is called dynamic sieving capillary electrophoresis (DSCE).

The CE Oligonucleotide Analysis Kit is optimized for use on the BioFocus® capillary electrophoresis system. It can be used to determine the purity of synthetic oligonucleotides with quantitative estimation of the amounts of product and failure sequences. Separations are achieved by DSCE. The polymer type and concentration have been selected to provide unit resolution of oligonucleotides in the 4–40mer range. Replacement of the sieving buffer in the capillary between each analysis provides reproducibility of migration time and peak area. The analysis employs capillaries coated internally with a novel polymer (polyAAEE, acryloylaminoethoxyethanol) which ensures high-resolution separations over many runs.

8 mer	GACTGACT
10 mer	GACTGACTGT
12 mer	GACTGACTGACT
14 mer	GACTGACTGACTGT
16 mer	GACTGACTGACTGACT
18 mer	GACTGACTGACTGACTGT
20 mer	GACTGACTGACTGACTGACT
22 mer	GACTGACTGACTGACTGACTGT
24 mer	GACTGACTGACTGACTGACTGACT
26 mer	GACTGACTGACTGACTGACTGACTGT
28 mer	GACTGACTGACTGACTGACTGACTGACT
30 mer	GACTGACTGACTGACTGACTGACTGACTGT
32 mer	GACTGACTGACTGACTGACTGACTGACTGACT

Fig. 1. Sequences of the synthetic oligonucleotides in the CE Oligonucleotide Size Standard.

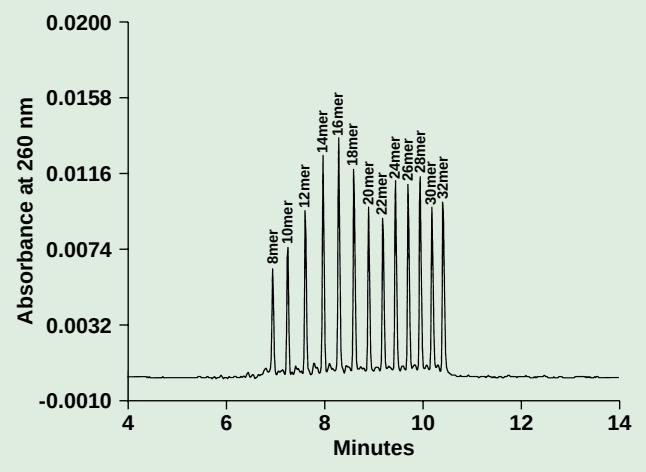

Fig. 2. Electropherogram of the CE Oligonucleotide Size Standards.

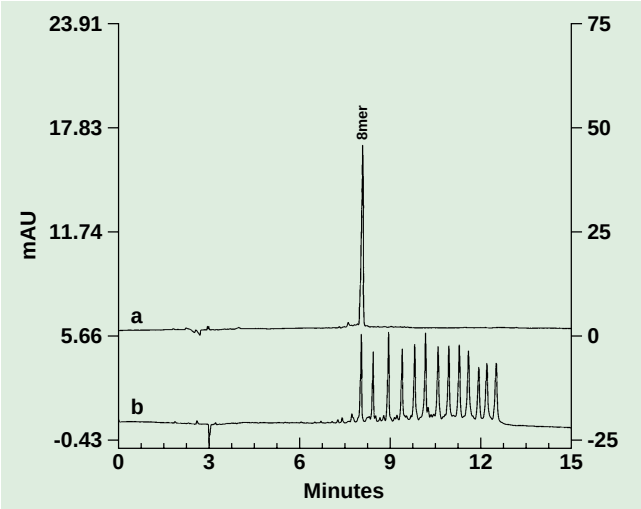

Fig. 3. Overlay of 8mer sample (a) and the Oligonucleotide Size Standard (b).

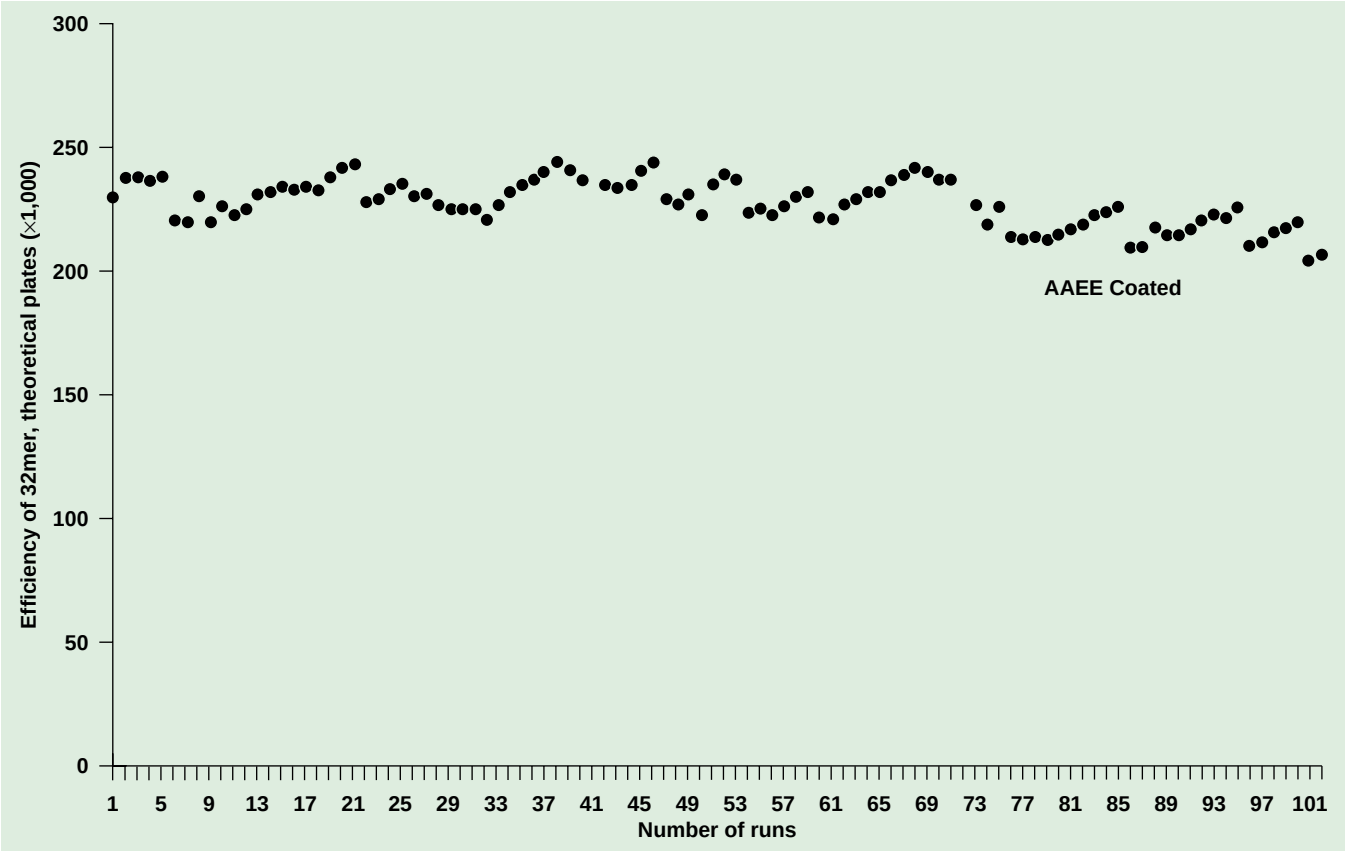

Fig. 4. Peak efficiency as a measure of capillary lifetime.

CE Oligonucleotide Kit Components

The kit contains all the reagents necessary to perform oligonucleotide separations using the BioFocus capillary electrophoresis system:

- Two fused silica capillaries, coated internally with poly(AAEE), 30 cm × 75 µm I.D. × 375 µm O.D., with detection window 8 cm from the end
- CE Oligonucleotide Run Buffer, 50 ml
- CE Oligonucleotide Size Standards 8-32mer, 13 peaks, total 0.5 OD units (see Figure 1).

Performance

Purity Results Fast

The BioFocus system provides automated analysis of up to 28 samples in one carousel setup. As Figure 2 illustrates, separations can be achieved in 10 or 12 minutes. The BioFocus Integration Software can be programmed to automatically integrate the results and print a quantitative report.

Accurate Size Estimation

The size standard can be used as an external standard to estimate the size of the sample oligonucleotide. Figure 3 shows an overlay of an 8mer synthysis product and the CE Oligonucleotide Size Standard.

High Resolution

Figure 2 shows an electropherogram of the CE Oligonucleotide Size Standard. Figure 4 shows that typical peak efficiency for the 32mer component is above 200,000 theoretical plates. Single base resolution is usually achievable out to 40mer. Resolution decreases with increasing size of the oligonucleotide.

Reproducibility

Table 1 shows the excellent reproducibility of the technique. Over a series of 12 successive runs, the average %RSD for area is 1.54% and 0.82% for migration time.

Key Benefits

- **Fast –** Results for a given sample are available in 12 minutes or less.
- **Automated –** Up to 28 samples can be automatically analyzed and reported.
- **Quantitative –** Direct on-line detection makes it possible to automatically collect, analyze and report data.
- **Low operating cost –** The automated system reduces the labor needed to run samples when compared to standard slab gel electrophoresis. Performing electrophoresis in the capillary format greatly reduces reagent consumption. Buffers are used in 0.5 ml quantities, and standards are used on nanoliter quantities.

Table 1. Precision Data on Migration Time and Area % for the 13 Oligonucleotide Standards

Migration Time (Minutes)

	8Mer	10Mer	12Mer	14Mer	16Mer	18Mer	20Mer	22Mer	24Mer	26Mer	28Mer	30Mer	32Mer
STDEV	0.0430	0.0462	0.0505	0.0533	0.0565	0.0574	0.0604	0.0626	0.0653	0.0673	0.0703	0.0727	0.0745
AVER	5.8017	6.0533	6.3500	6.6400	6.9008	7.1433	7.3850	7.6167	7.8217	8.0242	8.2217	8.4100	8.5875
RSD	0.74%	0.76%	0.79%	0.80%	0.82%	0.80%	0.82%	0.82%	0.84%	0.84%	0.86%	0.86%	0.87%
												av RSD	0.82%

Area %

	8Mer	10Mer	12Mer	14Mer	16Mer	18Mer	20Mer	22Mer	24Mer	26Mer	28Mer	30Mer	32Mer
STDEV	0.1150	0.0543	0.0616	0.0443	0.0591	0.1936	0.0717	0.2889	0.0889	0.0642	0.0453	0.2400	0.1413
AVER	6.5742	8.1025	7.1542	5.5983	11.1617	8.0383	9.3850	5.7392	6.1967	7.9042	6.8883	10.2325	7.0308
RSD	1.75%	0.67%	0.86%	0.79%	0.53%	2.41%	0.76%	5.03%	1.43%	0.81%	0.66%	2.35%	2.01%
												av RSD	1.54%

Ordering Information

Catalog Number	Product Description
148-4140	**CE Oligonucleotide Analysis Kit**
148-5026	**CE Oligonucleotide Run Buffer**
148-2014	**CE Oligonucleotide Size Standard**
148-3080	**BioCAP™ Oligonucleotide Analysis Capillary, 2 each**
148-3052	**BioFocus User Assembled Cartridge**

1. M. Zhu et al., J. Chromatography, **480** (1989) 311.
2. K. W. Talmadge et al., J. Chromatography A, **744** (1996) 347.

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