Selectivity studies in the analytical separation of oligonucleotides using a TSKgel® SuperQ-5PW anion exchange column

TSKgel
APPLICATION NOTE

Introduction

Synthetic oligonucleotides are becoming increasingly popular as biotherapeutic agents. When their purification moves from analytical to manufacturing scale, the selectivity is expected to remain the same. Having the same bonding chemistry is therefore extremely helpful. TSKgel SuperQ-5PW analytical columns are designed with the same backbone chemistry and selectivity as their bulk process scale resin counterparts, TSKgel SuperQ-5PW and TOYOPEARL® SuperQ-650.

This application note shows a one-step analysis of a 20-mer DNA-based oligonucleotide, using a TSKgel SuperQ-5PW, 10 µm, 7.5 mm ID × 7.5 cm column. The effect of pH, temperature, and sample load on the selectivity and resolution, particularly in reference to the separation of N-1 and N+1 peaks from the main peak, are discussed.

The aligned selectivity of this anion exchange column with its process scale counterparts makes the TSKgel SuperQ-5PW column extremely useful for the separation of oligonucleotides.

Experimental Conditions

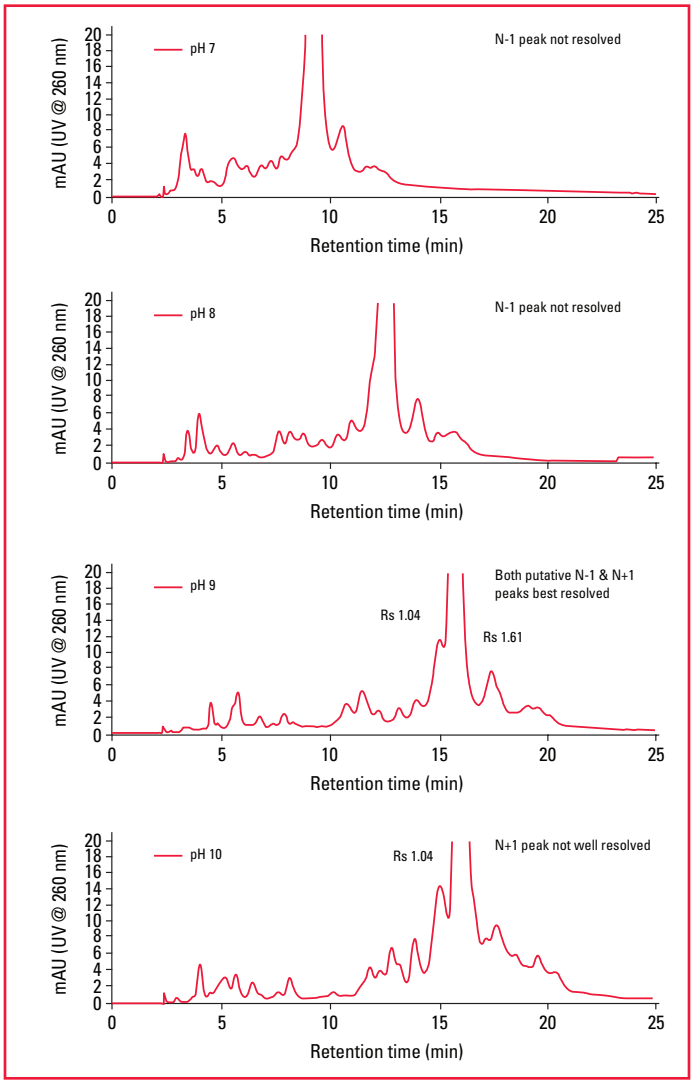
Columns:	TSKgel SuperQ-5PW, 10 µm, 7.5 mm ID × 7.5 cm (S0082-84NM)
Mobile phase:	A: 20 mmol/L Tris, pH 9.0 B: 20 mmol/L Tris, pH 9.0 + 1 mol/L NaCl
Gradient:	40-80% B over 30 minutes – an optimum gradient found to separate both N+1 and N-1 peaks of oligonucleotide
Flow rate:	0.9 mL/min
Detection:	UV @ 260 nm
Temperature:	ambient & 60 °C
Injection vol.:	15 µL
Samples:	phosphodiester deoxyoligonucleotide (20-mer) EcoRI sequence (Trilink Biotechnology, San Diego, CA): Lot# T34-C01A 5' - GAA TTC ATC GGT TCA GAG AC – 3' • purchased unpurified • extinction coefficient was 199.9 OD units/µmol • molecular weight of the free acid – 6140.9 Da • This sequence was chosen to minimize the amount of secondary structure effects during the purification experiments. • Reconstitution of oligonucleotide: for all of the experiments performed, the crude oligonucleotide was diluted into the equilibration buffer (mobile phase A) before loading onto the column. • stock concentration: 26.6 mg/mL; final dilution: 1:100 in mobile phase A • final concentration: 0.266 mg/mL = 0.266 µg/µL

Results and Discussion

Each of the three main species of oligonucleotide (N-1, N=20 and N+1) were separated under the optimum chromatographic conditions using both TSKgel SuperQ-5PW, 20 µm bulk resin and the TSKgel SuperQ-5PW column. The selectivity of the analytical column was the same as that of the bulk resin.

The effect of pH on selectivity and resolution was studied. The optimum pH value was found to be 9.0, as shown in the magnified view of the chromatograms in *Figure 1*.

Figure 1. Analysis of Crude Oligonucleotide Using TSKgel SuperQ-5PW Column at Different pH



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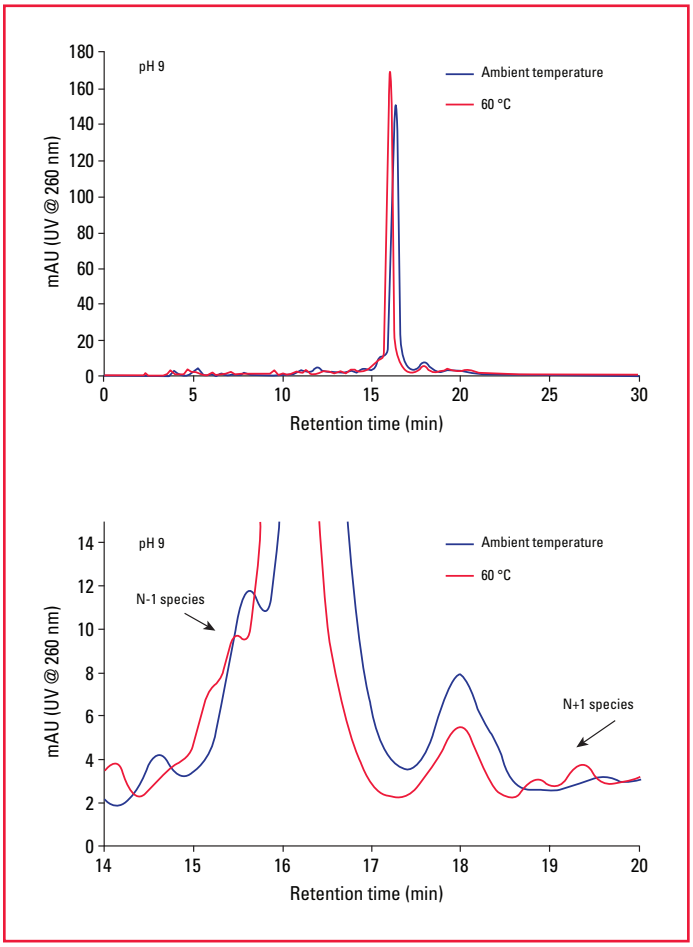
The effect of temperature on selectivity and resolution was also studied in reference to the separation of the N-1 and N+1 peaks from the main peak. As seen in *Figure 2*, better resolution could be obtained at 60 °C column temperature compared to ambient. Both the N-1 and N+1 peaks appear to be heterogeneous.

Finally, the effect of higher loading of crude oligonucleotide on the TSKgel SuperQ-PW column was measured (data not shown). Based on single injection data, the peak area analysis was linear within the experimental range of 3.98 µg to 79.68 µg. Higher loadings of crude oligonucleotide within this experimental range did not affect the peak purity percentage.

Figure 2. Analysis of Crude Oligonucleotide Using the TSKgel SuperQ-5PW Column at Different Temperature



Conclusions

These studies shows that a TSKgel SuperQ-5PW column can be used in the separation of oligonucleotides and the method can be useful for scaling up using TSKgel SuperQ-5PW bulk resin because of their shared selectivity and backbone chemistry.

Each of the three main species of oligonucleotide (N-1, N=20 and N+1) were successfully separated using the TSKgel SuperQ-5PW column at a pH of 9.0 and 60 °C, conditions determined to be optimal.

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