TOSOH

Application Note

PURIFICATION OF DNA-BASED OLIGONUCLEOTIDE AT 60°C ON TSKgel® SuperQ-5PW (20) RESIN

INTRODUCTION

TSKgel SuperQ-5PW (20) resin is a 20 µm particle size, strong anion exchange chromatographic resin used for large and small biomolecules. In downstream processing it can be used for intermediate purification and polishing steps. When used for oligonucleotides it does an excellent job of separating the oligonucleotide away from the "n-1" and "n+1" impurities.

The use of higher temperatures in a chromatographic separation can improve the resolution of the target molecule from closely eluting and similar chemistry impurities. In this report we compare data for an oligonucleotide separation at both ambient and 60 °C temperatures.

METHODS AND RESULTS

An unpurified, lyophilized, 20-mer oligonucleotide of the following sequence: 5' - GAA TTC ATC GGT TCAS GAG AC - 3' was purchased from Trilink Biotechnology, San Diego, CA. Two equivalent lots of crude oligonucleotide were used, one lot estimated at 64.9% purity by HPLC, and the second lot estimated at 61.6% purity by HPLC.

A 6.6 mm ID x 15 cm column was packed (as described in *"Packing and Use Guide, Toyopearl and TSKgel-5PW Instruction Manual"* available from Tosoh Bioscience)

The sample for injection was prepared by diluting the crude oligonucleotide into the column equilibration buffer (Buffer A) before loading onto the column. For a 1 mg load, 38 mL of crude oligonucleotide was diluted to 10 mL with Buffer A and loaded into the sample loop.

Two sets of gradient conditions (shown in Figure 1) were investigated for optimum target resolution using the following buffers:

- Buffer A: 20 mmol/LTris, 1 mmol/L EDTA pH 9.0
- Buffer B: 20 mmol/LTris, 1 mmol/LEDTA, 1 mol/L NaCl pH 9.0

The gradient conditions selected for subsequent pH screening were:

- 40% B (5 CV)
- 40%-65% B (15 CV)
- 100% B (5 CV)

TSKgel SuperQ-5PW (20) RESIN USING DIFFERENT GRADIENTS AT 60 °C

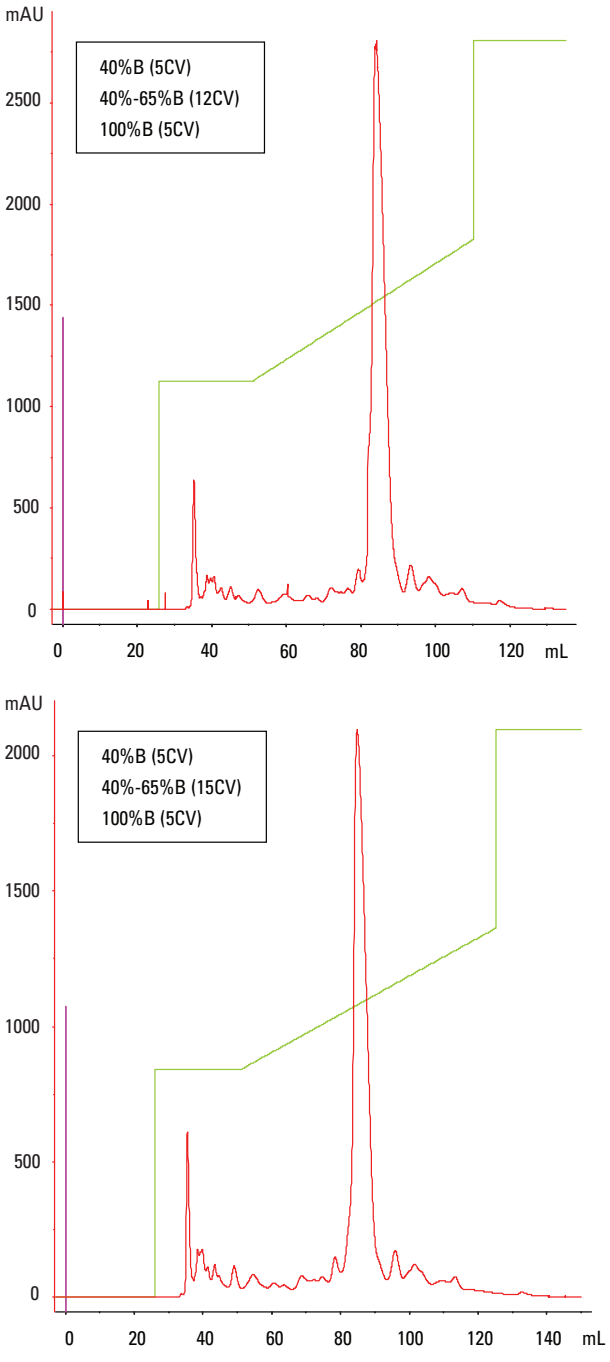

Figure 1

TSKgel SuperQ-5PW (20) visually resolved N-1 peak and N+1 peak from the main oligonucleotide peak at 60 °C. The gradient that best resolved these peaks was: Step to 40% B (5 CV), Gradient 40%-65% B (15 CV), Step to 100% B (5 CV)

PURIFICATION OF OLIGONUCLEOTIDE AT VARIOUS pH ON TSKgel SuperQ-5PW (20) RESIN AT 60 °C

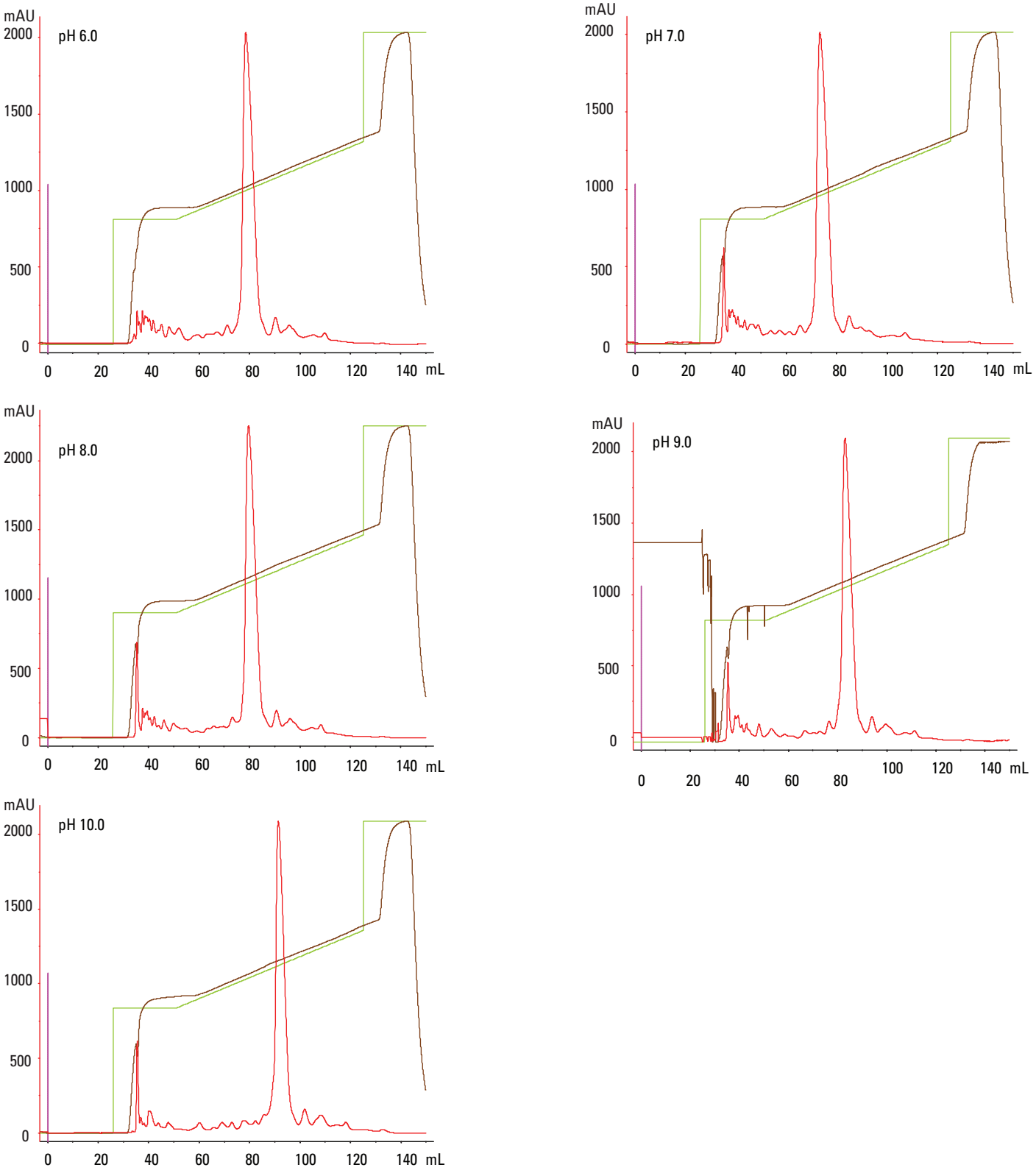

Figure 2

Column size: 6.6 mm ID x 15 cm; Flow rate: 250 cm/hr; Detection: Ab @ 254 nm; Buffer A: 20 mmol/L Tris + 1 mmol/L EDTA, various pH; Buffer B: Buffer A + 1.0 mol/L NaCl; Sample loaded: 1 mg/column; Separation conditions: Column is washed with 5 CV 100% Buffer A followed by 11 mL injection. Column is then washed with 3 CV 100% Buffer A followed by a step gradient to 40% Buffer B for 5 CV. This is followed by a linear gradient to 65% Buffer B over 15 CV. Finally, column is washed with 5 CV 100% Buffer B.

For the series of pH experiments, chromatographic runs were performed at pH values of 6.0, 7.0, 8.0, 9.0, 10.0. The operational conditions for each pH are as described in Figure 2. The peak purities and recoveries at the noted pH conditions are reported in Table 1.

MAIN OLIGONUCLEOTIDE PEAK PURITY AND RECOVERY FOR TSKgel SuperQ-5PW (20) RESIN FOR VARIOUS pH VALUES AT 60 °C

pH VALUE	MAIN PEAK PURITY	RECOVERY
pH 6.0	92.7%	68.8%
pH 7.0	91.8%	65.2%
pH 9.0	96.1%	62.0%
pH 10.0	95.9%	50.7%

Table 1

All pH levels except pH 8.0 showed an ability to purify oligonucleotides adequately. The purification performed at pH 9.0 showed the best purity and recovery of those values that were evaluated on the HPLC. The purification performed at pH 8.0 was not included due to poor separation between the main oligonucleotide peak and the N-1 peak seen by HPLC analysis.

CONCLUSION

The data shows that TSKgel SuperQ-5PW (20) resin can be used at 60 °C with varying pH conditions to successfully purify oligonucleotides. For this study pH 9.0 was ideal.

Ordering Information

TSKgel SuperQ-5PW (20)

Part-No	Description	Resin volume	Pore size	Particle size
0043383	TSKgel SuperQ-5PW (20)	25 mL	100 nm	20 µm
0018535	TSKgel SuperQ-5PW (20)	250 mL	100 nm	20 µm
0018546	TSKgel SuperQ-5PW (20)	1 L	100 nm	20 µm
0018547	TSKgel SuperQ-5PW (20)	5 L	100 nm	20 µm

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