Purification of DNA-Based Oligonucleotide at 60°C on TSKgel® SuperQ-5PW (20) Resin

Introduction

TSKgel SuperQ-5PW (20) resin is a 20µm 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 show data for an oligonucleotide separation at a temperature of 60ºC.

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 6.6mm ID x 15cm column was packed (as described in *"Packing and Use Guide, Toyopearl and TSKgel-5PW Instruction Manual"* available from Tosoh Bioscience) with TSKgel SuperQ-5PW (20).

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 1mg load, 38mL of crude oligonucleotide was diluted to 10mL 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: 20mmol/L Tris, 1mmol/L EDTA pH9.0
Buffer B: 20mmol/L Tris, 1mmol/L EDTA, 1mol/L NaCl pH9.0

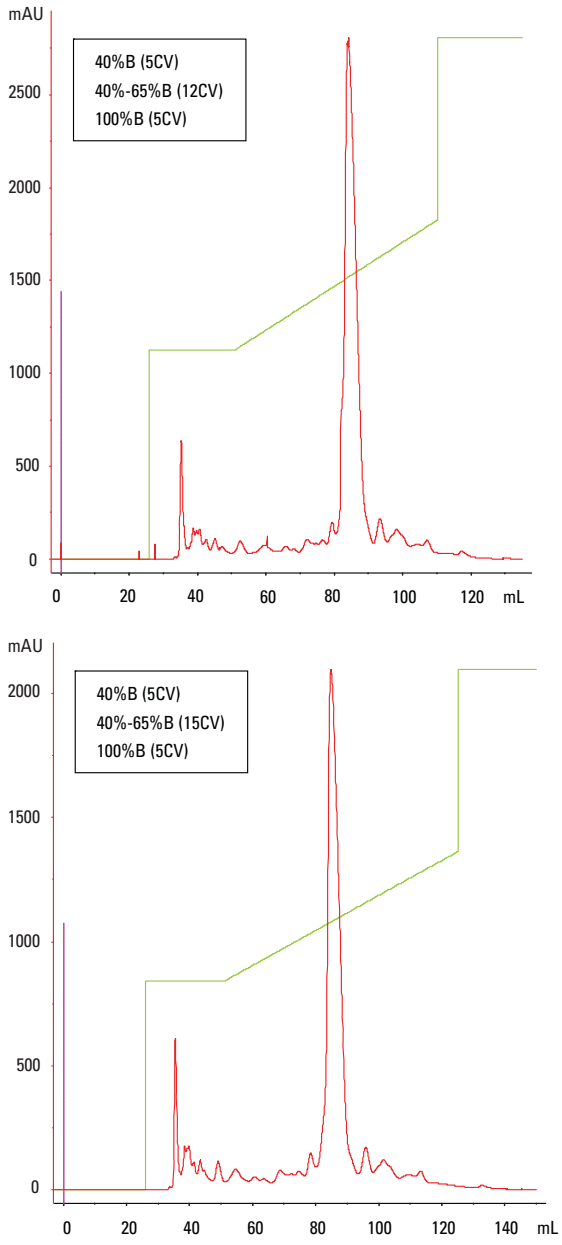
The gradient conditions selected for subsequent pH screening were:

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

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*.

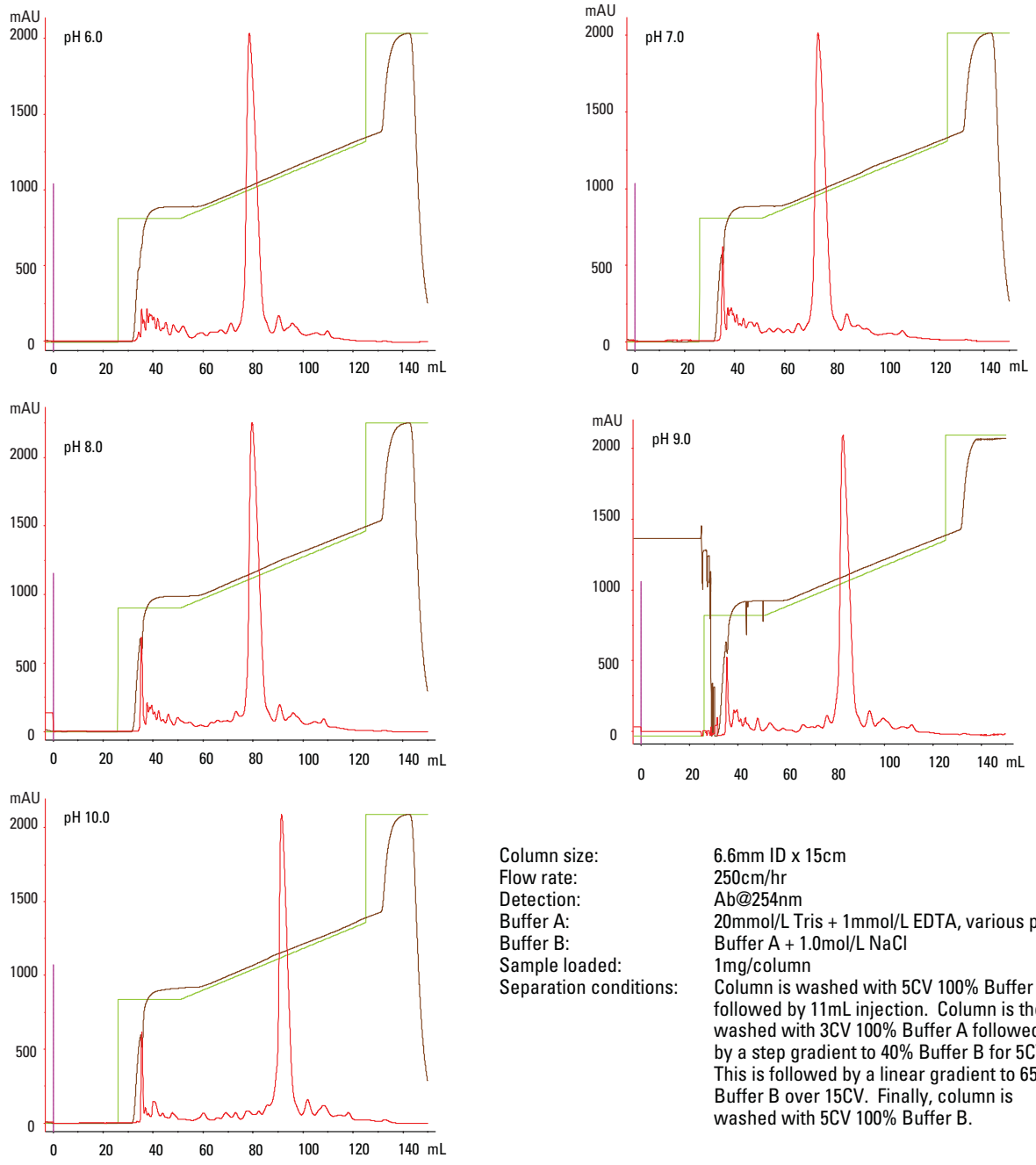
The peak purities and recoveries at the noted pH conditions are reported in *Table 1*.

Figure 1. TSKgel SuperQ-5PW (20) Resin using Different Gradients at 60ºC



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

Figure 2. Purification of Oligonucleotide at Various pH on TSKgel SuperQ-5PW (20) Resin at 60ºC



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

Table 1. Main Oligonucleotide Peak Purity and Recovery for TSKgel SuperQ-5PW (20) Resin for Various pH Values at 60ºC

pH Value	Main Peak Purity	Recovery
pH 6.0	92.7%	66.8%
pH 7.0	91.8%	65.2%
pH 9.0	96.1%	62.0%
pH 10.0	95.9%	50.7%

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 60ºC with varying pH conditions to successfully purify oligonucleotides. For this study pH9.0 was ideal.

For loading study information on TSKgel SuperQ-5PW (20) please refer to the technical presentation: ***"High Resolution Anion Exchange Chromatography Purification of Oligonucleotides"*** on the Tosoh Bioscience website.

Ordering Information

Part #	Description	Particle Size	Container Size
43383	**TSKgel SuperQ-5PW (20)**	15-25µm	25mL
18535	**TSKgel SuperQ-5PW (20)**	15-25µm	250mL
18546	**TSKgel SuperQ-5PW (20)**	15-25µm	1L
18547	**TSKgel SuperQ-5PW (20)**	15-25µm	5L

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