Increased Load of DNA-Based Oligonucleotide on TSKgel SuperQ-5PW (20) Resin

Introduction

Oligonucleotides are entering clinical trials in increasing numbers. In many ways their entry into the clinic mirrors the development of protein therapeutics in the late 1980's and early 1990's. On the other hand, the purification process for an oligonucleotide therapeutic is very different than that for a protein. Typically one high resolution, high load step is used rather than a train of capture, intermediate purification, and polishing columns.

TSKgel SuperQ-5PW (20) resin, a 20µm particle size, anion exchange media is becoming the "go-to" product for oligonucleotide purification. This paper demonstrates the performance of the resin at higher loads while maintaining the resolution and purity of the target molecule.

Oligonucleotides are short, linear sequences of deoxyribonucleic acid or ribonucleic acid that are generally manufactured by chemical synthesis. Due to the unique structure of these molecules and the way they are synthesized, oligonucleotides require special considerations during chromatographic purification. During the synthesis of the oligonucleotide, there are a small percentage of sequences where a particular nucleotide may either be deleted or have more than one segment attached (N-1 and N+1 respectively are the common nomenclature). Taken collectively, these synthesis errors may produce measurable amounts of impurities. The similarity in the impurities to the target molecule requires a high resolution technique to adequately isolate the target molecule.

This report shows the power of TSKgel SuperQ-5PW (20) anion exchange media to purify synthetic DNA based oligonucleotides.

Objective

The objective of the study is as follows:

- To determine the ability of TSKgel SuperQ-5PW (20) to adequately purify crude oligonucleotide at loads greater than 1mg/5.13mL column.

Experimental Methods

The phosphodiester deoxyoligonucleotide (20-mer) used in this study had the following sequence:

5' - GAA TTC ATC GGT TCA GAG AC - 3'

This oligonucleotide was purchased unpurified (estimated at 64.9% purity by HPLC) in lyophilized form from Trilink Biotechnology, San Diego, CA. The extinction coefficient was 199.9 OD units/µmol and the molecular weight of the free acid was 6140.9 Da. This sequence was chosen to minimize the amount of secondary structure effects during the purification experiments.

TSKgel SuperQ-5PW (20µm) resin was packed in a 6.6mm ID x 15cm column. The column was performance tested with NaCl (1% CV injection of 2mol/L NaCl with a mobile phase of 1mol/L NaCl) and found to be acceptable for use in these experiments.

For all of the experiments performed, the crude oligonucleotide was diluted into the column equilibration buffer (Buffer A) before loading onto the column. For a 1mg load, 52µL of crude oligonucleotide was diluted to 10mL with Buffer A and loaded into the sample loop. For a 2mg or 5mg load, 104µL and 260µL respectively were used. All experiments in the 6.6mm ID columns were run at 250cm/hr.

The buffers chosen for this set of experiments were as follows:

Buffer A: 20mmol/L Tris, 1mmol/L EDTA pH 9.0

Buffer B: 20mmol/L Tris, 1mmol/L EDTA, 1mol/L NaCl pH 9.0

Buffer A served as the column equilibration and sample dilution buffer and Buffer B served as the gradient elution buffer.

A modified step gradient previously established for purifying oligonucleotide was used on the TSKgel SuperQ-5PW (20) column. The established gradient for the resin is as follows and used throughout the experiments conducted for this report:

Step to 35%B (5CV)

Linear Gradient 35%-53%B (10CV)

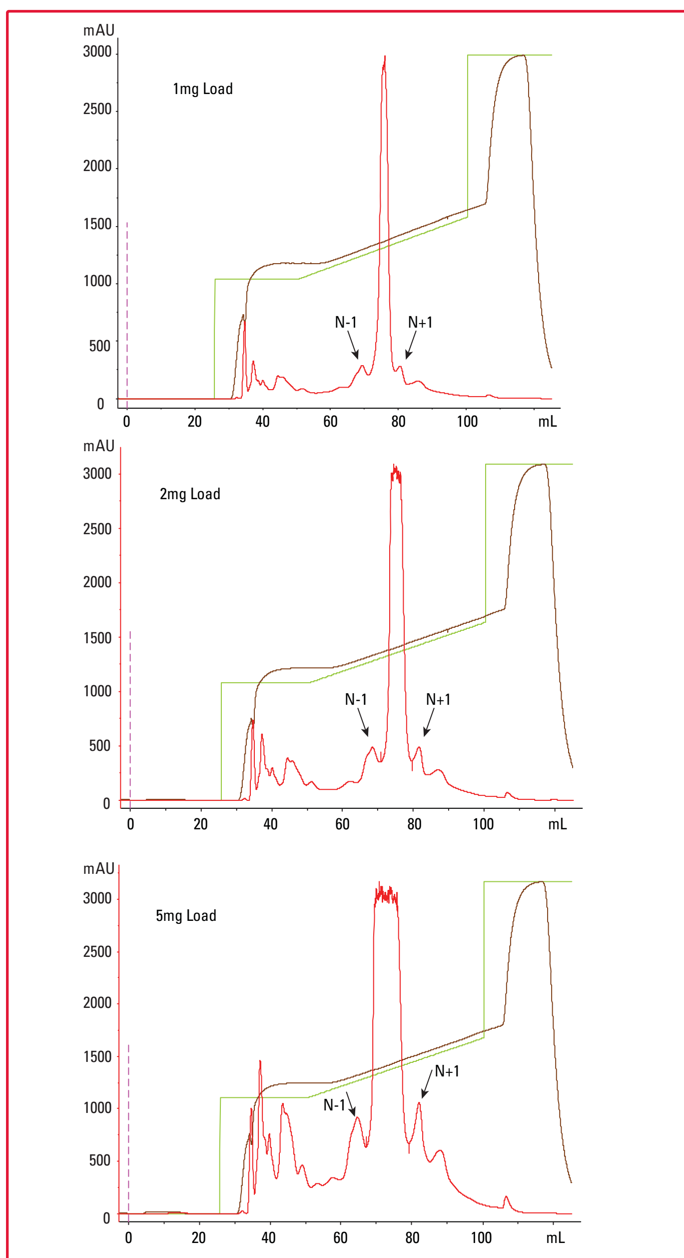
Step to 100%B (5CV)

Fractions were collected along the N-1 peak, main peak and N+1 peak region and were analyzed using a TSKgel DNA-NPR HPLC column (4.6mm ID x 7.5cm) to check for peak purity. Fractions of the main oligonucleotide peak that were greater than 85% pure were pooled together. This pool was then analyzed for overall peak purity and recovery. Recovery of pure oligonucleotide is determined by multiplying the total (mg) amount of material (N-1, Oligonucleotide, N+1) in the pooled fractions by the percentage of pure oligonucleotide in that pool as determined by TSKgel DNA-NPR HPLC.

Results

1.) TSKgel SuperQ-5PW (20) resin visually resolved N-1 peak and N+1 peak from the main oligonucleotide peak at 1, 2 and 5mg loads *(Figure 1)*.

Figure 1. TSKgel SuperQ-5PW Resin at Different Loads of Crude Oligonucleotide



2.) The 97.04% purity of the main peak indicates that at a 5mg load the resin is not yet being loaded to its operational capacity *(Table 1)*.

Table 1. TSKgel SuperQ-5PW (20) Resin at Different Loads of Crude Oligonucleotide

Data	TSKgel Super-Q 5PW (20) resin
Load (mg)	1mg
Crude Oligo Purity	64.86%
Main Peak Purity	94.12%
Main Peak Oligo Recovered	.452mg
Main Peak % Yield	70%
Load (mg)	2mg
Crude Oligo Purity	64.86%
Main Peak Purity	94.45%
Main Peak Oligo Recovered	.916mg
Main Peak % Yield	70.61%
Load (mg)	5mg
Crude Oligo Purity	64.86%
Main Peak Purity	97.04%
Main Peak Oligo Recovered	2.33mg
Main Peak % Yield	72%

Conclusions

- TSKgel SuperQ-5PW (20) resin can separate oligonucleotides at loads greater than 1mg and potentially greater than 5mg / 5.13mL column.
- TSKgel SuperQ-5PW (20) maintains excellent main peak purity even at higher loads.

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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