TOSOH

Efficient Purification of Short Oligonucleotides using AEX Resins

Your Challenge

- You need to increase the purity and recovery of your oligonucleotide.
- Conventional oligo purification is limited by low binding capacity of anion exchange resins.

Our Solution

TSKgel® SuperQ-4PW (20) resin

- Optimized pore size, exceptional purity, high recovery

What was done?

- Compared purification of short oligos using the novel TSKgel SuperQ-4PW (20) and commercially available TSKgel SuperQ-5PW (20).

What was the result?

- TSKgel SuperQ-4PW (20) resin showed superior binding capacity, purity, and recovery of short oligos.

TSKgel SuperQ-4PW (20) delivers up to 30 % higher binding capacity and improved recovery for oligos ≤50-mer, enabling high product yield without changing existing purification processes.

Your Benefit

Enhances oligo purity and recovery, thereby improving process efficiency.

Oligonucleotide Purification

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SEPARATION & PURIFICATION

CONNECTING MINDS.
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Application Note

Efficient Purification of Short Oligonucleotides using TSKgel® SuperQ-4PW (20) Resin

The purification of synthetic oligonucleotides requires chromatographic media that combines high resolution, strong binding capacity, and consistent performance. TSKgel SuperQ-4PW (20) is a newly developed anion exchange resin optimized for efficient purification of oligos shorter than 50 nucleotides. Its 50 nm pore size provides increased surface area for shorter oligonucleotides, resulting in higher binding capacity for these molecules. For purification of larger therapeutic nucleic acids, TSKgel SuperQ-5PW (20)—featuring the same ligand and bead size as TSKgel SuperQ-4PW (20), but with a larger 100 nm pore size—remains the industry's gold standard.

Experimental Conditions

Material:

The following resins were used for the experiments in this Application Note:

Property	TSKgel SuperQ-5PW (20)	TSKgel SuperQ-4PW (20)
Base Material	Polymethacrylate	
Ligand	Quaternary amine (strong anion exchange resin)	
Average Bead Size	20 µm	
Average Pore Size	100 nm	50 nm

Method: Dynamic binding capacity (DBC) determination

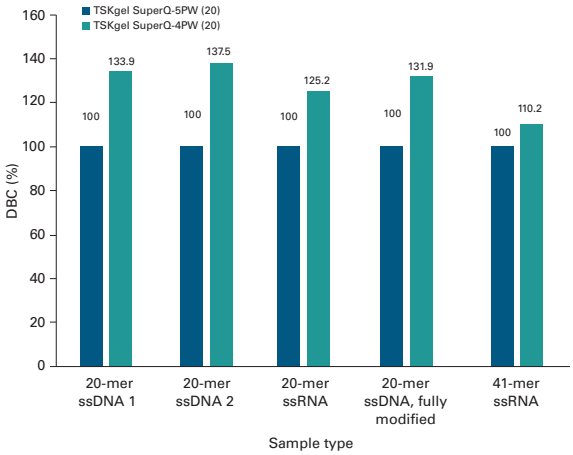
The DBC for various sequences in the range of 20 to 41 nucleotides was determined.

First, the maximum absorption of the sample was determined by measuring UV-absorption at 260 nm wavelength. Afterwards, the sample was loaded onto the columns until 10 % of the previously determined maximum absorption was detected in the column breakthrough. The load volume to reach the 10 % threshold was noted. By multiplying load volume with sample concentration, loaded mass could be determined. DBC was calculated by dividing loaded mass by column volume.

Results and Discussion: Dynamic binding capacity

Dynamic binding capacity was evaluated for various oligonucleotide sequences on the two tested resins and normalized against the capacity determined for TSKgel SuperQ-5PW (20) *(Figure 1)*.

Figure 1. Normalized DBC comparison for both AEX media using various oligonucleotides



TSKgel SuperQ-4PW (20) exhibits 25 % – 37 % higher dynamic binding capacity than TSKgel SuperQ-5PW (20) for 20-mer oligonucleotides and about 10 % higher for a 41-mer. While optimized for 20-mers, increased capacity is also observed for oligonucleotides in the 40–50 nucleotide range.

Method: Purification process of a 20-mer oligonucleotide

A 20-mer ssDNA oligonucleotide was used to determine purity and recovery during a purification process using TSKgel SuperQ-4PW (20) and TSKgel SuperQ-5PW (20) resins.

Conditions for the process:

Column:	Self-packed column (0.3 cm ID × 5.0 cm) BH = 0.35 mL CV)
Mobile phase:	A: 50 mmol/L Tris-HCl, pH 8.5 B: Buffer A + 0.6 mol/L sodium bromide
Flow rate:	85 cm/h
Gradient:	0 – 100 % B, 37 CV
Temperature:	25° C
Sample Type:	20-mer oligonucleotide ssDNA (unmodified)
Sample concentration:	1.0 g/L
Load Level:	Approx. 80 % DBC
Sample Loaded:	25 mg/mL for TSKgel SuperQ-5PW (20) 34 mg/mL for TSKgel SuperQ-4PW (20)

Method: Purification process of a 41-mer oligonucleotide

A 41-mer RNA oligonucleotide with a 2′-O-Methyl sequence was used to determine purity and recovery during a purification process using TSKgel SuperQ-4PW (20) and TSKgel SuperQ-5PW (20) resins.

Conditions for the process:

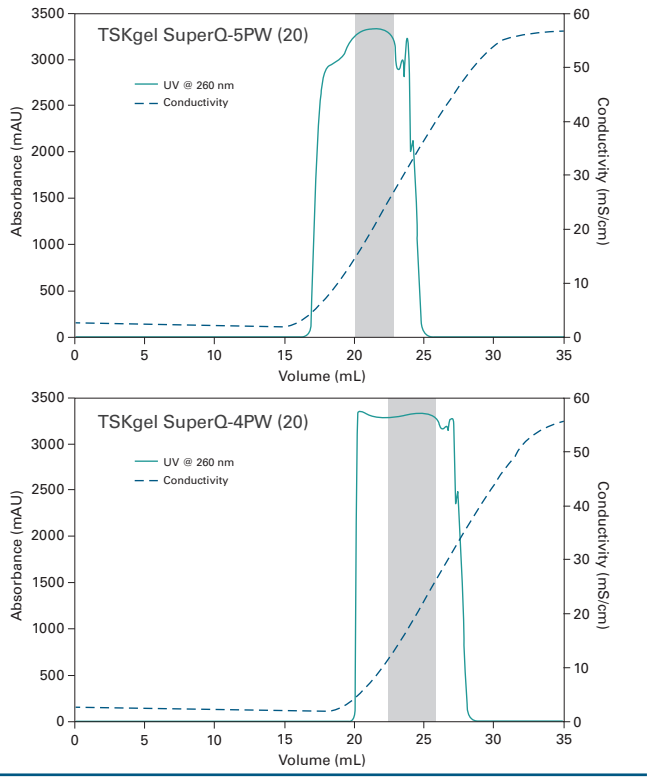
Column:	SkillPak 5 (0.8 cm ID × 10 cm BH = 5 mL CV)
Mobile phase:	A: 20 mmol/L sodium phosphate, pH 7.8 B: Buffer A + 1.4 mol/L sodium bromide
Flow rate:	120 cm/h
Gradient:	0 – 100 % B, 30 CV
Temperature:	60° C
Sample Type:	41-mer RNA oligonucleotide with 2′-O-Methyl modification
Sample Concentration:	1.2 g/L
Load Level:	Approx. 80 % DBC
Sample Loading:	32.5 g/L for TSKgel SuperQ-5PW (20) 35.1 g/L for TSKgel SuperQ-4PW (20)

Results and Discussion

TSKgel SuperQ-5PW (20) vs. TSKgel SuperQ-4PW (20) purification comparison with a 20-mer oligonucleotide

Two purification runs were performed to compare the purity and recovery of TSKgel SuperQ-5PW (20) and TSKgel SuperQ-4PW (20). A crude 20-mer ssDNA oligonucleotide with a purity of 67 % was loaded to 80 % of DBC onto both columns.

Figure 2. Process chromatogram of both resins for 20-mer oligonucleotide



The purity of all fractions was determined using a TSKgel DNA-NPR column. Fractions with a purity above 85 % (area in orange) were pooled.

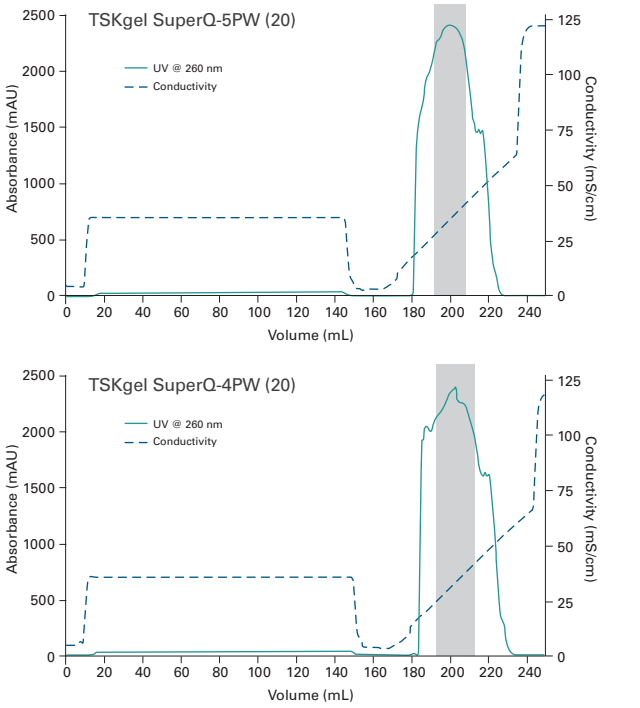
Property	TSKgel SuperQ-5PW (20)	TSKgel SuperQ-4PW (20)
Amount loaded sample	25 mg/mL resin (80 % DBC)	34 mg/mL resin (80 % DBC)
Pool Purity (AEX-HPLC)	94 %	94 %
FLP-Recovery	67 %	76 %

For a 20-mer ssDNA oligonucleotide, TSKgel SuperQ-4PW (20) shows 9 % higher recovery than TSKgel SuperQ-5PW (20), with identical purity. When considering the higher mass of loaded material and the increased recovery, an increase of 54.2 % of product at identical pool purity was achieved.

TSKgel SuperQ-5PW (20) vs. SuperQ-4PW (20) purification comparison with a 41-mer oligonucleotide

A crude 2′-O-Methyl modified 41-mer RNA oligonucleotide with 52.5 % purity was purified using both tested resins. 80 % of the previously determined total DBC was loaded onto the column, and elution was performed using a linear gradient for both resins.

Figure 3. Process chromatogram of both resins for 41-mer oligonucleotide



All fractions with a purity of >85 % were pooled (in grey in elution peak).

Property	TSKgel SuperQ-5PW (20)	TSKgel SuperQ-4PW (20)
Amount loaded sample	162.8 mg (32.5 mg/mL)	175.3 mg (35.1 mg/mL)
Pool Purity (AEX-HPLC)	88.9 %	91.3 %
FLP-Recovery	78.6 %	98.2 %

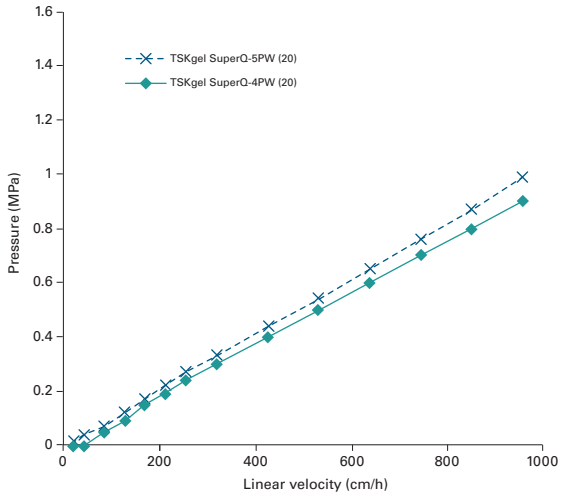
TSKgel SuperQ-4PW (20) demonstrated approximately 2 % higher purity and 20 % higher recovery than TSKgel SuperQ-5PW (20) in gradient elution experiments.

When considering the higher mass of loaded material and the increased recovery, using TSKgel SuperQ-4PW (20) resulted in a 35 % increase in recovered product per run at comparable pool purity.

Pressure Flow Stability

While the two resins differ in pore size, their base material and particle size are identical. As a result, their pressure-flow properties are very comparable.

Figure 4.



Conclusions

TSKgel SuperQ-4PW (20) resin stands out as a dependable and efficient choice for the purification of oligonucleotides (DNA or RNA) up to 50 nucleotides in length. With an average binding capacity 32 % higher than TSKgel SuperQ-5PW (20) resin, it ensures efficient processing even under demanding load conditions. Users can expect consistently high recovery rates and exceptional purity, without compromising pressure-flow performance. Its reliability and ease of integration into existing purification workflows make TSKgel SuperQ-4PW (20) a trustworthy resin in oligonucleotide purification.

Featured Products

Part #	Description
0045564	SkillPak 1 SuperQ-4PW (20), 5 × 1 mL col.
0045566	SkillPak 50 SuperQ-4PW (20), 50 mL col.
0045567	SkillPak 200 SuperQ-4PW (20), 200 mL col.
0045244	SkillPak 5 TSKgel SuperQ-5PW (20), 5 mL col.
0023588	TSKgel SuperQ-4PW (20), 25 mL
0030000	TSKgel SuperQ-4PW (20), 250 mL
0030001	TSKgel SuperQ-4PW (20), 1 L
0030002	TSKgel SuperQ-4PW (20), 5 L
0030003	TSKgel SuperQ-4PW (20), 25 L
0030004	TSKgel SuperQ-4PW (20), 50 L
0018249	TSKgel DNA-NPR, 2.5 µm, 4.6 mm ID × 7.5 cm