TOSOH

Application Note

PURIFICATION OF OLIGONUCLEOTIDES ON TOYOPEARL GigaCap® Q-650S

TOYOPEARL GigaCap Q-650S, a high capacity/high resolution anion exchange resin for process scale applications, was recently introduced by Tosoh Corporation. This resin, with dynamic binding capacities approaching 190 g/L for bovine serum albumin (BSA), is the newest member of the TOYOPEARL® product line.

TOYOPEARL GigaCap Q-650S maintains the high capacity of our popular TOYOPEARL GigaCap Q-650M and the 35 µm particle size provides high resolution for improved separation of process impurities and aggregates.

The purification of oligonucleotides using anion exchange chromatography has traditionally fallen to resins such as TSKgel® SuperQ-5PW (20) that offer high resolution and selectivity in conjunction with excellent mechanical stability at very high column pressures. TOYOPEARL GigaCap Q-650S resin offers a low pressure alternative to oligonucleotide purification while preserving the selectivity, resolution and yields of those higher pressure processes.

TOYOPEARL and TSKgel products are hydroxylated methacrylic polymer resins and are made commercially in many different pore sizes and particle diameters. TOYOPEARL resins vary from TSKgel resins by having a lower degree of crosslinking. Lower crosslinking makes available a larger number of resin sites for ligand immobilization when producing TOYOPEARL resins. This lower degree of crosslinking also makes for a less rigid bead. Therefore a functionalized TOYOPEARL resin will have a lower pressure rating than the corresponding TSKgel material.

Because similarly functionalized TSKgel and TOYOPEARL resin types have the same backbone polymer chemistry, the selectivity for proteins, oligonucleotides and their attendant impurities remains the same. TOYOPEARL resin products can be used at high linear velocities and withstand operating pressures up to 0.3 MPa while TSKgel resins can withstand operating pressures of up to 2.0 MPa.

Table 1 shows the comparative properties of TOYOPEARL GigaCap Q-650S and TSKgel SuperQ-5PW (20) resins and dynamic binding capacities for the oligonucleotide used in these experiments. The following experiments detail the purification of an oligonucleotide using TOYOPEARL GigaCap Q-650S and TSKgel SuperQ-5PW (20) resins.

Oligonucleotides are short, linear sequences of deoxyribonucleic acid or ribonucleic acid that are generally manufactured by chemical synthesis. Because of the unique structure of these molecules and the way they are synthesized, oligonucleotides require special consideration during chromatographic purification.

During the synthesis of the oligonucleotide, there are a small percentage of sequences where a segment may either be deleted or have more than one segment attached (N-1 and N+1 respectively are the common nomenclature).

PROPERTIES OF TSKgel SuperQ-5PW AND TOYOPEARL GigaCap Q-650S

	TSKgel SuperQ-5PW (20)	TOYOPEARL GigaCap Q-650S
Particle size (µm)	20	35
Pore diameter (nm)	100	100
Ion exchange capacity (eq/L resin)	0.14	0.17
DBC oligo (g/L resin)	46.4	36.8
Max pressure	2.0 MPa	0.7 MPa

Table 1

TSKgel SuperQ-5PW (20), 1.0 mg LOAD

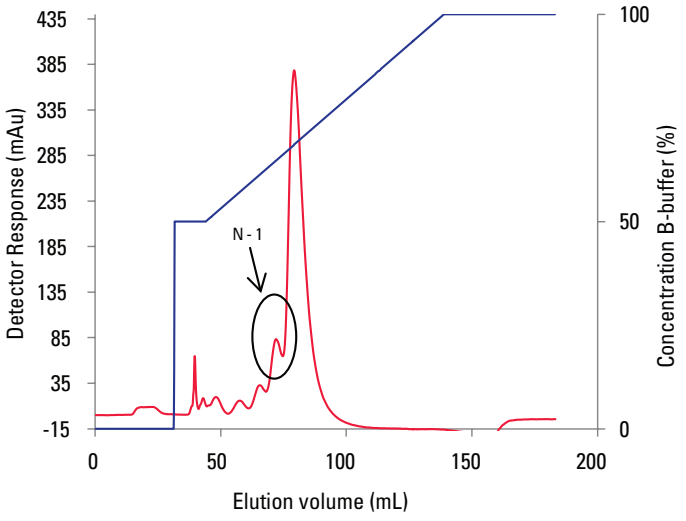

Figure 1

Resin: TSKgel SuperQ-5PW (20); Column size: 6.6 mm ID × 18.5 cm (6.3 mL); Mobile phase: A: 20 mmol/L NaOH; B: 20 mmol/L NaOH, 3.0 mol/L NaCl; Gradient: 50% B (2 CV); 50-100% B (15 CV); 100% B (2 CV); Flow rate: 200 cm/hr(1.14 mL/min); Detection: UV @ 254 nm; Sample load: 1.0 mg; Sample: crude phosphorothioate deoxyoligonucleotide

TOYOPEARL GigaCap-Q-650S, 1.0 mg LOAD

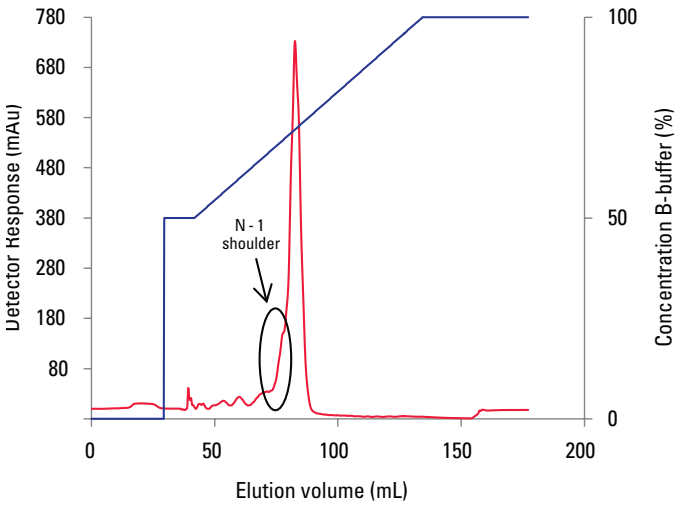

Figure 2

Resin: TOYOPEARL GigaCap Q-650S; Column size: 6.6 mm ID × 18.5 cm (6.3 mL); Mobile phase: A: 20 mmol/L NaOH; B: 20 mmol/L NaOH, 3.0 mol/L NaCl; Gradient: 50% B (2 CV); 50-100% B (15 CV); 100% B (2 CV); Flow rate: 200 cm/hr (1.14 mL/min); Detection: UV @ 254 nm; Sample load: 1.0 mg; Sample: crude phosphorothioate deoxyoligonucleotide

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.

EXPERIMENTAL CONDITIONS / RESULTS

The data presented here demonstrate the similar capabilities of TOYOPEARL GigaCap Q-650S and TSKgel SuperQ-5PW (20) resins to purify a phosphorothioate deoxyribonucleotide (24-mer).

Experiments were carried out on 6.6 mm ID × 18.0 ± 0.5 cm columns packed with TOYOPEARL GigaCap Q-650S and TSKgel SuperQ-5PW (20) resins. The columns were first under-loaded with a 1.0 mg sample of crude oligonucleotide to better visualize resin performance, Figures 1-2. As can be seen from these chromatograms, the N-1 peak was slightly better resolved with the TSKgel SuperQ-5PW (20) than with the TOYOPEARL GigaCap Q-650S, perhaps due to the smaller particle size of the TSKgel resin. HPLC analysis of fractions taken across the peaks (data not shown) revealed that both resins were able to adequately resolve the full length oligonucleotide.

After optimizing the elution gradient, the performance of the resins was then compared at 80% of each resin's respective dynamic binding capacity for this oligonucleotide, Figures 3-4. As can be seen in the chromatograms, there was a visible N+1 peak that was resolved from the largest oligonucleotide peak in addition to the N-1 peak. Many of the low molecular weight impurities are visually resolved as well.

PURIFICATION OF OLIGONUCLEOTIDE AT 80% DBC ON TSKgel SuperQ-5PW (20) RESIN

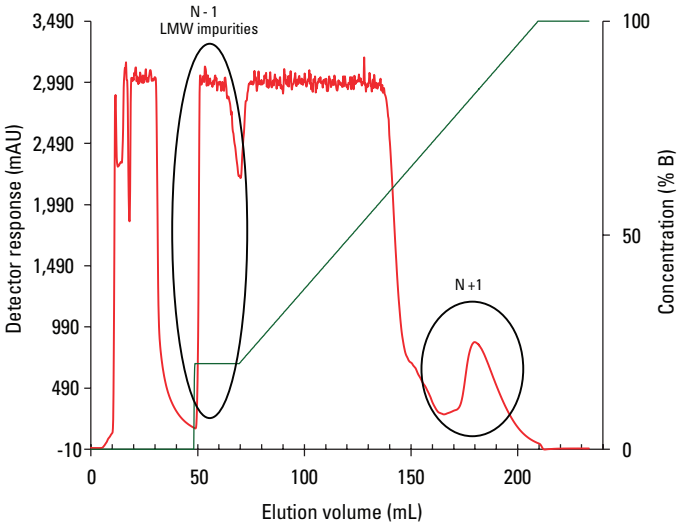

Figure 3

Resin: TSKgel SuperQ-5PW (20); Column size: 6.6 mm ID × 18.5 cm (6.3 mL); Mobile phase: A: 20 mmol/L NaOH; B: 20 mmol/L NaOH, 3.0 mol/L NaCl; Gradient: 20% B (2 CV), 20-100% B (20 CV); 100% B (2 CV); Flow rate: 200 cm/hr (1.14 mL/min); Detection: UV @ 254 nm; Sample load: 235 mg; Sample: crude phosphorothioate deoxyoligonucleotide

PURIFICATION OF OLIGONUCLEOTIDE AT 80% DBC ON TOYOPEARL GigaCap Q-650S RESIN

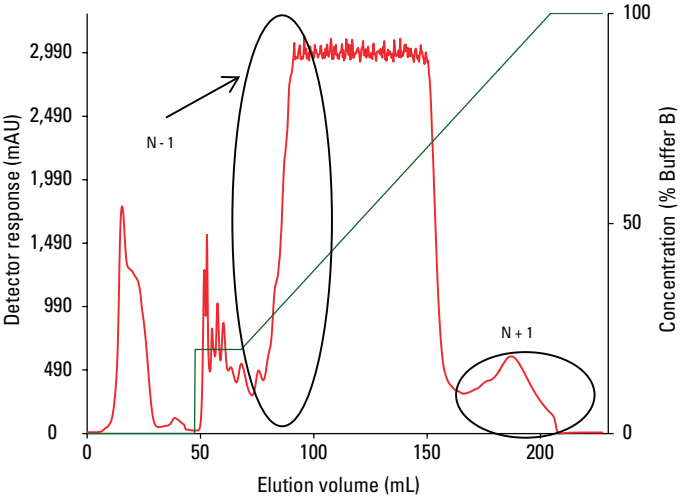

Figure 4

Resin: TOYOPEARL GigaCap Q-650S;
Column size: 6.6 mm ID × 18 cm (6.16 mL);
Mobile phase: A: 20 mmol/L NaOH; B: mobile phase A + 3.0 mol/L NaCl;
Gradient: step to 20% B (2 CV); 20% - 100% B (20 CV); 100% B (2 CV);
Flow rate: 200 cm/hr (1.14 mL/min); Detection: UV @ 254 nm;
Injection vol.: 181.4 mg;
Sample: crude phosphorothioate deoxyribonucleotide

Though the chromatograms in Figures 3 and 4 went off scale for UV, the general shape of the chromatograms is unchanged from that of the corresponding chromatogram when only 1.0 mg was loaded. HPLC analysis of fraction purity (data not shown) indicates that selectivity and resolution are maintained even at 80% DBC loading conditions.

TSKgel SuperQ-5PW (20) RESIN: 80% DBC ELUTION PEAK WITH FRACTION PURITY HISTOGRAM

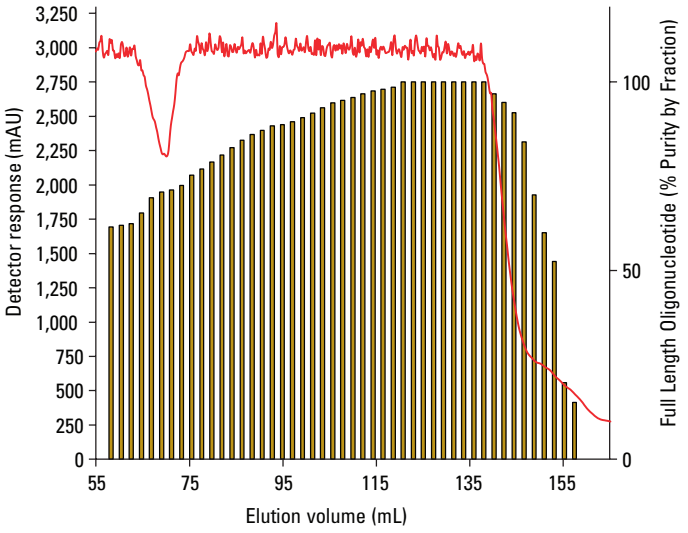

Figure 5

Resin: TSKgel SuperQ-5PW (20); Column size: 6.6 mm ID × 18.5 cm (6.3 mL);
Mobile phase: A: 20 mmol/L NaOH; B: 20 mmol/L NaOH, 3.0 mol/L NaCl;
Gradient: 20% B (2 CV), 20-100% B (20 CV); 100% B (2 CV);
Flow rate: 200 cm/hr (1.14 mL/min); Detection: UV @ 254 nm; Sample load: 235 mg; Sample: crude phosphorothioate deoxyoligonucleotide

TOYOPEARL GigaCap Q-650S RESIN: 80% DBC ELUTION PEAK WITH FRACTION PURITY HISTOGRAM

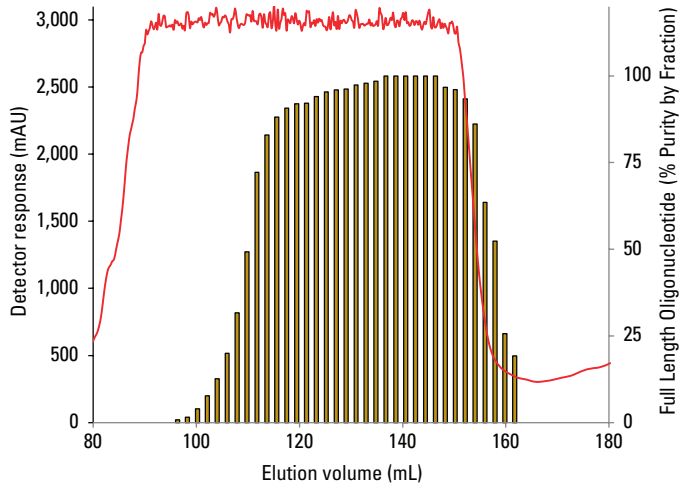

Figure 6

Resin: TOYOPEARL GigaCap Q-650S;
Column size: 6.6 mm ID × 18 cm (6.16 mL);
Mobile phase: A: 20 mmol/L NaOH; B: mobile phase A + 3.0 mol/L NaCl;
Gradient: step to 20% B (2 CV); 20% - 100% B (20 CV); 100% B (2 CV);
Flow rate: 200 cm/hr (1.14 mL/min); Detection: UV @ 254 nm;
Injection vol.: 181.4 mg;
Sample: crude phosphorothioate deoxyribonucleotide

OLIGONUCLEOTIDE PURITY AND YIELD FROM 80% DBC PURIFICATIONS

Resin	Crude Oligo Purity	Final Oligo Purity	% Yield
TSKgel SuperQ-5PW (20)	66.5%	96.4%	72.5%
TOYOPEARL GigaCap Q-650S	66.5%	96.9%	81.3%

Table 1

An enlarged image of the main oligonucleotide peak, overlaid with a histogram showing HPLC results for fraction purity, highlights the chromatographic separation of the full length oligonucleotide, Figures 5-6. At 80% DBC, the TSKgel SuperQ-5PW (20) resin had some breakthrough of the full length product from the main peak into the N-1 peak while the TOYOPEARL Q-650S did not. This indicates that the TOYOPEARL Q-650S was better able to maintain resolution at 80% DBC loading conditions.

After pooling fractions of purified oligonucleotide, the yield and purity of the final product was determined for each resin, Table 2. The TSKgel SuperQ-5PW (20) and TOYOPEARL GigaCap Q-650S generated very high purity full length oligonucleotide (96.4% and 96.9% respectively) from crude synthesis material. The yield of full length oligonucleotide was almost 9% greater on the TOYOPEARL GigaCap Q-650S than the yield from the TSKgel SuperQ-5PW (20).

Product yield is affected by the amount of crude material loaded onto the column. In general, as column loading approaches saturating conditions, yield will decrease. This phenomenon appears to be more pronounced with the TSKgel SuperQ-5PW (20) resin than with the TOYOPEARL GigaCap Q-650S resin.

Recovery was determined by comparing the amount of full length oligonucleotide present in the crude sample loaded onto the column with the amount of full length oligonucleotide present in the fraction pool.

CONCLUSION

TOYOPEARL GigaCap Q-650S is capable of delivering oligonucleotides of comparable purity to that seen with the TSKgel SuperQ-5PW (20) resin and at slightly higher process yields under the same loading conditions but at lower pressures. This capability allows chromatographers to purify oligonucleotides without the added expense of purchasing high pressure manufacturing equipment.