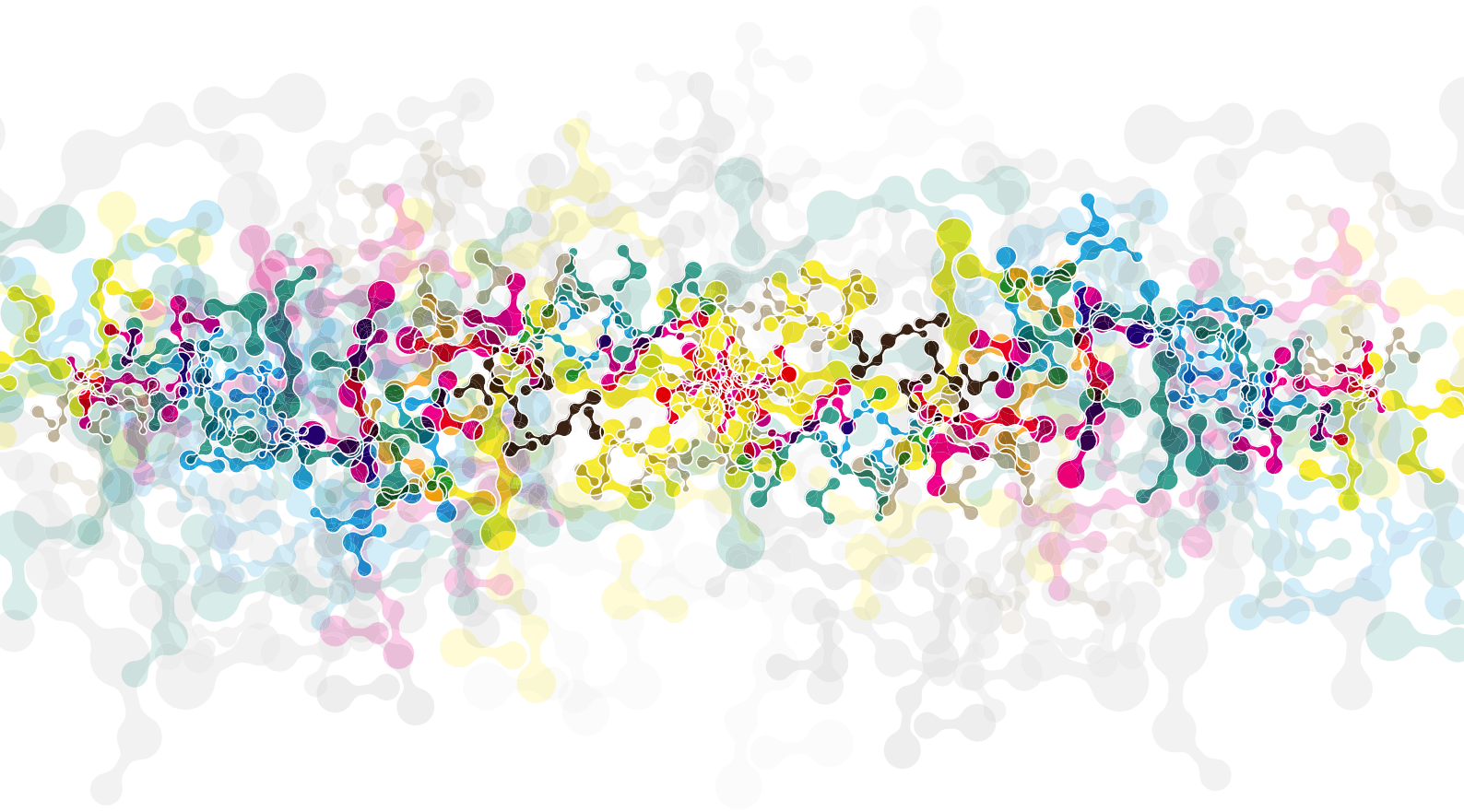
TOSOH

# Oligonucleotide Purification and Analysis



## SEPARATION OF OLIGONUCLEOTIDES FROM ANALYTICAL TO PROCESS SCALE

The oligonucleotide therapeutics field has seen remarkable progress over the last few years. The first antisense drug was approved and clinical trials using siRNA or splice switching oligonucleotide show promising developments.

The analytical separation of oligonucleotides as well as their purification following synthesis require special considerations. These have been traditionally met by anion exchange chromatography. Specifically designed resins offer high resolution and selectivity and allow specific oligonucleotide extraction, analysis, purification and polishing.

Tosoh Bioscience offers different product lines for analytical and process scale applications that enable either specific isolation and analysis of oligonucleotides or the process scale purification thereof.

**TOSOH BIOSCIENCE**

## SOLUTIONS FOR ALL YOUR SEPARATION TASKS FROM LAB TO PRODUCTION

Tosoh Bioscience is a leading supplier of chromatographic columns, media and sophisticated gel permeation instruments. By bringing the best in quality products, services and technologies from Tosoh Corporation, Tosoh Bioscience is dedicated to pioneering solutions used to solve complex separation tasks.

For more than 25 years, Tosoh Bioscience is dedicated to develop cutting-edge solutions that meet the needs of customers developing and producing new medicines including plasma products, monoclonal antibodies, recombinant proteins, peptides, vaccines and oligonucleotides. We provide support during the development of a validated HPLC assay or in preparation for a filing of a new drug with the regulatory agencies.

## TSKgel® CHROMATOGRAPHY MEDIA FOR PURIFICATION OF OLIGONUCLEOTIDES

### TSKgel SuperQ-5PW (20)

This TSKgel resin is based on hydroxylated methacrylic polymers with 20 µm particle size, and derivatized with strong anion exchange groups. It can be used for the process scale purification of oligonucleotides.

It offers high resolution and selectivity, as shown in Figure 1 in conjunction with excellent mechanical stability at rather high column pressures (up to 2.0 MPa).

The use of higher temperatures in a chromatographic separation can improve the resolution of the target molecule from closely eluting and similar chemistry impurities. Purity and recovery of a 20mer oligonucleotide separation at 60°C have been evaluated at different pH values (see table 1).

**Resin of choice for highest resolution for your oligonucleotide**

**TSKgel SuperQ-5PW**

**MAIN OLIGONUCLEOTIDE PEAK PURITY AND RECOVERY FOR TSKgel SuperQ-5PW (20) AT 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

## TOYOPEARL® CHROMATOGRAPHY MEDIA FOR PURIFICATION OF OLIGONUCLEOTIDES

### TOYOPEARL GigaCap Q-650S

This TOYOPEARL resin is a high capacity/high resolution anion exchange resin for process scale applications. If pressure is the limitation for convenient oligonucleotide purification, GigaCap Q-650S allows chromatographers to purify oligonucleotides without the added expense of purchasing high pressure manufacturing equipment.

Though also consisting of hydroxylated methacrylic polymers (35 µm particles), this TOYOPEARL resin varies from its TSKgel counterpart by having a lower degree of crosslinking. The lower degree of crosslinking results in a less rigid bead. Therefore, a functionalized TOYOPEARL resin will have a lower pressure rating than the corresponding TSKgel material (up to 0.3 MPa) and can be used at high linear velocities. The selectivity, even if lower than with the TSKgel SuperQ-5PW (20), is still sufficient for reaching purities above 95 % (see figure 2).

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.

**Resin of choice for oligonucleotides purification with lowest pressure**

**TOYOPEARL GigaCap Q-650S**

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

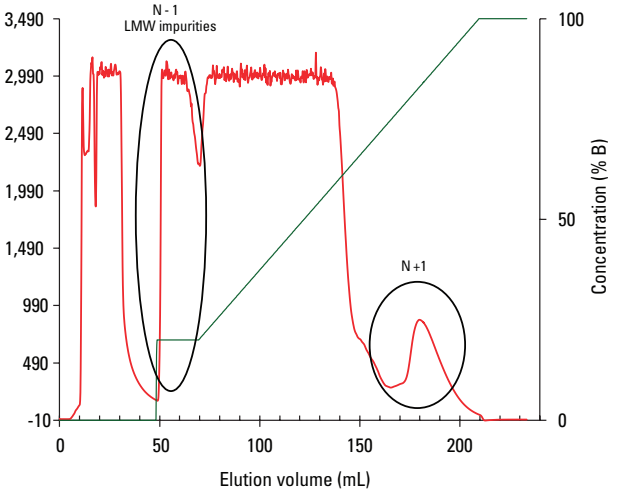



Figure 1

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

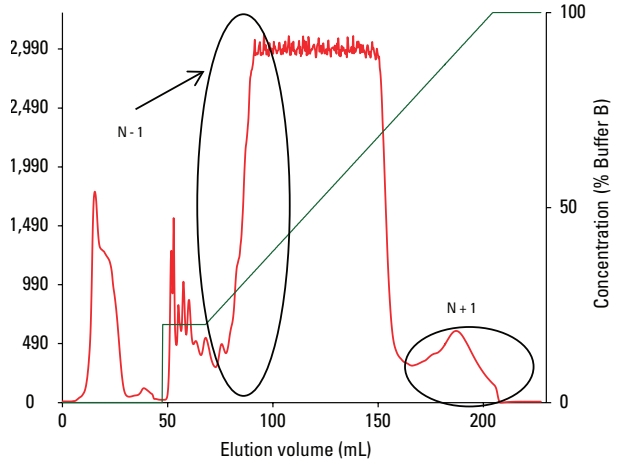



Figure 2

## CHROMATOGRAPHY COLUMNS FOR ANALYSIS OF OLIGONUCLEOTIDES

### TSKgel Q-STAT and TSKgel DNA-STAT

The anion exchange columns of the STAT product lines are well-designed for chromatographic separation of oligonucleotides. They offer very efficient separation and provide high speed and high resolution analyses.

The columns are packed with mono-disperse, non-porous resin particles, the surface of which consists of an open access network of multi-layered anion exchange groups.

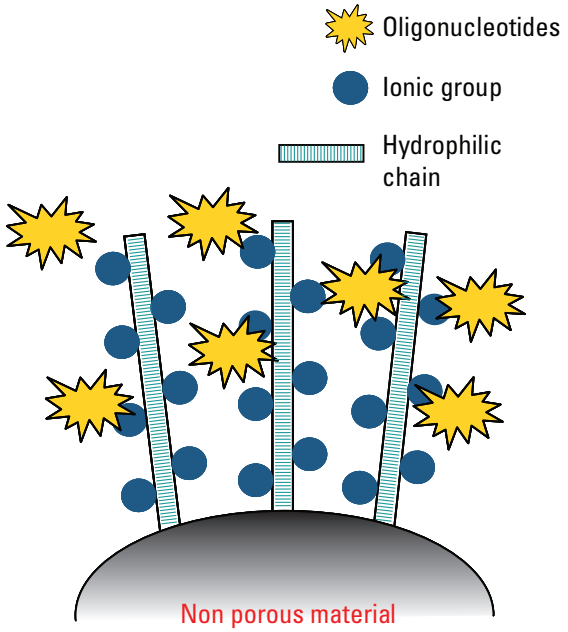
The particle size is relatively large, 7 or 10 µm for TSKgel Q-STAT and 5 µm for TSKgel DNA-STAT. These characteristics combined with the innovative bonding chemistry result in a respectable loading capacity and a low operating pressure together with high resolution and short analysis times, qualities not found in traditional columns.

Mono-, di-, and tri-nucleotides were separated with excellent peak shape on a TSKgel DNA-STAT column. The narrow, symmetrical peaks, as shown in Figure 4, demonstrate the absence of micro-pores on this new generation of non-porous resin columns. TSKgel DNA-STAT columns are also, as the name implies, first choice for large nucleic acid fragments.

**Columns of choice for your oligonucleotides analysis**

**TSKgel Q-STAT and DNA-STAT**

**OPEN ACCESS NETWORK OF IONIC GROUPS ACCOUNTS FOR FAST AND EFFICIENT SEPARATIONS**



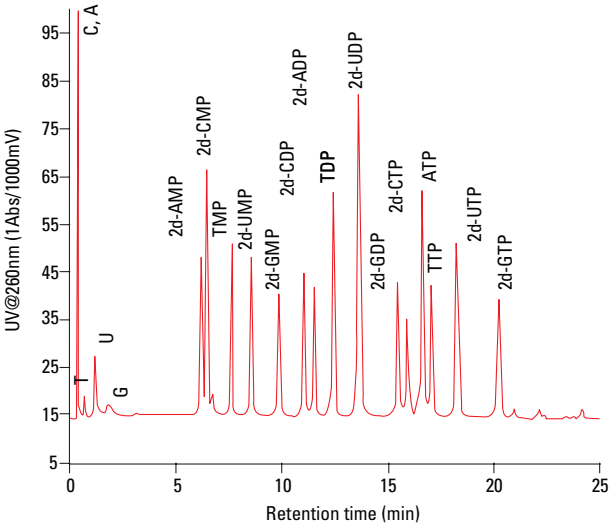




Figure 3

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WWW.TOSOHBIOSCIENCE.DE I WWW.TSKGEL.COM I WWW.TOYOPEARL.COM I WWW.TOYOSCREEN.COM I WWW.ECOSEC.EU