Novel Ion-Exchange Products for the Biopharmaceutical Industry

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Overview

Brief introduction on Tosoh

TSK-GEL® columns for Ion Exchange (STAT columns)
- Applications
- Conclusions

TSK-GEL process media for Ion Exchange
- Applications
- Conclusions

Answer to your questions
Who We Are

Tosoh Corporation is a diversified global chemical company with a strong reputation in basic chemicals, petrochemicals, and specialty products. Since its founding in 1935, Tosoh has expanded into high-value-added businesses, such as organic chemicals, bioscience, quartzware, and advanced electronic materials.
Tosoh Logo: Corporate Philosophy, History

Curved lines “the realization of happiness”
The right-angle cut at the top portrays an image of contributing to society.
Red corporate color – the “Tosoh Spirit”

<table>
<thead>
<tr>
<th>Tosoh Corporation:</th>
<th>1937</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tosoh SID:</td>
<td>1971</td>
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<tr>
<td>TosoHaas:</td>
<td>1987</td>
</tr>
<tr>
<td>Tosoh Biosep:</td>
<td>2000</td>
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<tr>
<td>Tosoh Bioscience:</td>
<td>2001</td>
</tr>
</tbody>
</table>

TSK-GEL® brand Chromatographic Media

TSK – Toyo Soda Kogyo
- Clinical Diagnostics
- Hemoglobin A1c analysis for diabetes
- Pre-packed columns for analytical separations
- Process chromatography resins
- GPC Instrument for polymer separations

Click here and navigate through our website
## Ion Exchange Products: Columns

<table>
<thead>
<tr>
<th>Anion Exchange Columns (type, matrix)</th>
<th>Cation Exchange Columns (type, matrix)</th>
</tr>
</thead>
</table>
| - Strong anion, methacrylic  
  - TSKgel BioAssist Q  
  - TSKgel SuperQ-5PW  
  - Strong anion, silica  
  - TSKgel QAE-2SW  
  - Strong anion, polymer  
  - TSKgel Q-STAT  
  - Strong anion, polystyrene  
  - TSKgel Sugar AXI  
  - TSKgel Sugar AXG  
  - TSKgel SAX  
  - Weak anion, methacrylic  
  - TSKgel DEAE-5PW  
  - TSKgel DEAE-NPR  
  - TSKgel DNA-NPR  
  - Weak anion, silica  
  - TSKgel DEAE-2SW  
  - TSKgel DEAE-3SW  
  - Strong anion, polymer  
  - TSKgel DNA-STAT | - Strong cation, methacrylic  
  - TSKgel BioAssist S  
  - TSKgel SP-5PW  
  - TSKgel SP-NPR  
  - Strong cation, silica  
  - TSKgel SP-2SW  
  - Strong cation, polymer  
  - TSKgel SP-STAT  
  - Strong cation, polystyrene  
  - TSKgel SCX  
  - Weak cation, methacrylic  
  - TSKgel CM-5PW  
  - TSKgel OApak-A  
  - Weak cation, silica  
  - TSKgel CM-2SW  
  - TSKgel CM-3SW  
  - Weak cation, polymer  
  - TSKgel CM-STAT |

*TSK-GEL Q-STAT  
TSK-GEL DNA-STAT  
Anion Exchange Columns  

*TSK-GEL CM-STAT  
TSK-GEL SP-STAT  
Cation Exchange Columns  

Packed with non-porous resin particles, these columns enable high speed and high resolution analysis, as well as isolation, of biomolecules.
• Very efficient chromatography
  • for high as well as low MW solutes
    • novel bonding chemistry and the absence of micro-pores
• High speed and high resolution analysis of biomolecules
• Higher adsorption capacities and lower pressures compared with competitive non-porous columns
• 7 or 10μm particles for SP and CM chemistries
# Basic Properties of TSK-GEL Q-STAT and DNA-STAT Anion Exchange Columns

<table>
<thead>
<tr>
<th>Property</th>
<th>TSK-GEL Q-STAT</th>
<th>TSK-GEL DNA-STAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base material</td>
<td>Cross-linked hydrophilic polymer (mono-disperse particles)</td>
<td></td>
</tr>
<tr>
<td>Pore size</td>
<td>Non-porous</td>
<td></td>
</tr>
<tr>
<td>Functional group</td>
<td>Quaternary ammonium</td>
<td></td>
</tr>
<tr>
<td>Particle size</td>
<td>7µm</td>
<td>10µm</td>
</tr>
<tr>
<td>Column size</td>
<td>4.6mm ID x 10cm</td>
<td>3mm ID x 3.5cm</td>
</tr>
<tr>
<td>Application</td>
<td>High resolution protein separation</td>
<td>High resolution protein separation</td>
</tr>
</tbody>
</table>
Protein Separations on Non-Porous Anion Exchange

Improved protein peak shapes on TSKgel Q-STAT vs. non-porous WAX column.

Columns:
- A: TSKgel Q-STAT, 7µm, 4.6mm ID x 10cm
- B: Brand A, Non-porous WAX, 4mm ID x 25cm

Eluent:
- A: 20mmol/L Tris-HCl (pH8.5)
- B: 0.5mol/L NaCl in buffer A

Gradient:
- 0% B (0min), 100% B (10min)

Flow rate: 1.0mL/min

Detection: UV@280nm

Samples:
1. conalbumin
2. ovalbumin
3. trypsin Inhibitor
The protein mixture was completely separated within 1 minute and with higher resolution on the TSKgel Q-STAT column in comparison to the monolithic WAX column.
Low molecular weight nucleotides were separated with excellent peak shape, demonstrating the absence of micro-pores on the TSKgel Q-STAT column.
TSK-GEL Q-STAT series columns show higher resolution over a wide range of sample mass compared with the TSKgel DEAE-NPR column.
Static Binding Capacity (SBC) of BSA on Non-Porous TSK-GEL Anion Exchange Resin

Despite the fact that surface area decreases with increasing particle size, the larger TSK-GEL Q-STAT particles have higher binding capacities than the smaller TSK-GEL DEAE-NPR particles.

The novel bonding chemistry used in the preparation of TSK-GEL Q-STAT resin resulted in a dramatic increase in static binding capacity, more than compensating for the loss in external surface area of the larger particles.

<table>
<thead>
<tr>
<th>Property</th>
<th>TSK-GEL DEAE-NPR</th>
<th>TSK-GEL Q-STAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle size</td>
<td>2.5µm</td>
<td>5µm</td>
</tr>
<tr>
<td>Static binding capacity (mg BSA/mg dry gel)</td>
<td>9.1</td>
<td>38.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>27.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20.9</td>
</tr>
</tbody>
</table>
Separation of DNA on TSK-GEL DNA-STAT Columns
Four 26-mers of different composition were separated successfully on a TSKgel DNA-STAT column.

Column: TSKgel DNA-STAT, 5μm, 4.6mm ID x 10cm
Eluent: A: 20mmol/L Tris-HCl (pH8.5)
B: 0.75mol/L NaCl in buffer A
Gradient: 50% B (0min), 75% B (25min)
Flow-rate: 0.8mL/min
Detection: UV@260nm
Samples:
1. 5’-TAATTAAGGACTCCGTTCTTCTATAT-3’-NH2
2. 5’-TCTTTACTTTAGTCACAAGCGATAA-3’-NH2
3. 5’-GACTCCGTTCTTCTATATTTTCGAGG-3’-NH2
4. 5’-GGACGTGCTGGGTGTCTTCTCCGTGC-3’-NH2
Comparing the Resolution of DNA Fragments on Non-Porous Anion Exchange Columns

Large DNA fragments containing more than 10kb were successfully separated on a TSKgel DNA-STAT column packed with 5μm particles.

Columns: A: TSKgel DNA-STAT, 5μm, 4.6mm ID x 10cm  
B: TSKgel DNA-NPR, 2.5μm, 4.6mm ID x 7.5cm

Eluent: A: 20mmol/L Tris-HCl (pH8.5)  
B: 1.0mol/L NaCl (pH8.5) in buffer A

Gradient: A: 75% B (0min), 100% B (20min)  
B: 50% B (0min), 75% B (20min)

Flow-rate: 0.5mL/min  
Detection: UV@260nm  
Sample: 1kb ladder
Separation of Oligonucleotides under Different Conditions on TSKgel DNA-STAT Column

- **Column:** TSKgel DNA-STAT, 5µm, 4.6mm ID x 10cm
- **Eluent:**
  - A) 20mmol/L Tris-HCl (pH 8.5)
  - B) 1.0mol/L NaCl in A (pH 8.5)
- **Gradient:** B) 0 to 100% (30min)
- **Flow-rate:** 0.5mL/min
- **Detection:** UV@260nm

**Graphs:**
- **20mer**
  - Elution time: 60 minutes
  - UV peak: 25 minutes

- **48mer**
  - Elution time: 60 minutes
  - UV peak: 25 minutes
Comparing the Resolution of DNA Fragments on Non-Porous Anion Exchange Columns

Columns:  
A: TSKgel DNA-STAT, 5μm, 4.6mm ID x 10cm  
B: TSKgel DNA-NPR, 2.5μm, 4.6mm ID x 7.5cm

Eluent:  
A: 20mmol/L Tris-HCl (pH8.5)  
B: 1.0mol/L NaCl (pH8.5) in buffer A

Gradient:  
A: 75% B (0min), 100% B (20min)  
B: 50% B (0min), 75% B (20min)

Flow-rate: 0.5mL/min
Detection: UV@260nm
Sample: 1kb ladder
Applications of TSK-GEL Q-STAT and TSK-GEL DEAE-NPR Columns
Three peaks were isolated from a TSKgel Q-STAT column and assigned as F(ab’)2, pFc and intact IgG by SDS-PAGE.
Purification of mAb2 from Mouse Ascites on a TSKgel Q-STAT Column

Analysis results from the isolated fraction (yellow band in the upper chromatogram) suggest a single component.
An amplified DNA sample was successfully separated from primer and polymerase in less than two minutes on a TSKgel Q-STAT column.
Conclusions

- Tosoh Corporation developed novel, non-porous anionic exchange resins, TSK-GEL Q-STAT and TSK-GEL DNA-STAT, with high loading capacities and a low operating pressure by adopting larger particle sizes (5μm TSK-GEL DNA-STAT, 7 and 10μm TSK-GEL Q-STAT) and by grafting functional chains onto the non-porous surface.

- The short (3.5cm) TSKgel Q-STAT column packed with 10μm particles yielded high throughput analyses with separations within a few minutes.

- Higher resolution of proteins and DNA samples were obtained on the 10cm TSK-GEL DNA-STAT column packed with 5μm particles and the TSK-GEL Q-STAT column packed with 7μm particles compared to a TSKgel DNA-NPR, 2.5μm non-porous column.
Conclusions

- The sample loading capacity of a TSKgel Q-STAT, 10μm column was twice that of a TSKgel DEAE-NPR, 2.5μm column.

- The new surface modification improves not only chromatographic performance but also sample capacity.

- Improved DNA fragment separations were obtained using a TSKgel DNA-STAT column with 5μm particle size.

- For small molecules such as nucleotides, sharper peak shapes were attributed to the absence of very small pores on the STAT particles.

- The absence of micro-pores and by grafting a novel bonded phase structure resulted in very efficient chromatography on the non-porous anion exchange TSK-GEL STAT columns.

- The new column line is very useful for separating proteins and DNA samples from small to large molecular weights with high throughput and high resolution.
In this comparison of protein separations on various cation exchange columns, different selectivities were observed for each set of proteins on all three columns. The TSKgel SP-STAT column shows excellent resolution for cytochrome C and lysozyme.
The fast separation of standard proteins was investigated using short cation exchange columns. A TSKgel SP-STAT column shows superior resolution, better peak shape, and a shorter analysis time (<60 seconds) compared to a monolithic ST-type column.
Antibody Separation Profiles on TSK-GEL STAT Series Cation Exchange Columns

Five different antibodies were separated on TSKgel SP-STAT and TSKgel CM-STAT with high resolution cation exchange columns. The TSKgel CM-STAT column provided better peak shape, higher resolution and shorter analysis time than could be obtained on the TSKgel SP-STAT column.

Columns:  
A: TSKgel SP-STAT, 7μm, 4.6mm ID x 10cm
B: TSKgel CM-STAT, 7μm, 4.6mm ID x 10cm

Eluent:  
A: 20mmol/L MES (pH6.0)
B: 20mmol/L MES + 0.5mol/L NaCl (pH6.0)

Gradient:  
10% B (0min), 30% B (30min), 100% B (30min), 100% B (32min), 10% B (32min), 10% B (36min)

Flow-rate: 1.0mL/min
Temp.: Ambient
Detection: UV@280nm
Inj. Vol.: 20μL
The analysis profiles for the five antibodies on a TSKgel CM-STAT column were compared with the profiles obtained on a competitive non-porous type cation exchange column. Similar or higher resolution profiles were obtained on the TSKgel CM-STAT column in approximately half the time.
In-Process Analysis of Pegylated $\beta$-Lactoglobulin on High Throughput TSKgel SP-STAT Column

A sample of $\beta$-lactoglobulin (5 mg/mL) was reacted with polyethylene glycol (5 kDa) in a pH 6.5 phosphate buffer. The formation of pegylated proteins was monitored in 5 minute intervals on a 3.5cm TSKgel SP-STAT column. Peak areas of mono-, di-, and tri-pegylated proteins increased with reaction time, while the area of unreacted $\beta$-lactoglobulin declined.
A monoclonal antibody sample (5mg/mL) was reacted with polyethylene glycol (5kDa) in a pH 6.5 phosphate buffer. The formation of pegylated antibodies was monitored in 5 minute intervals on a 3.5cm TSKgel SP-STAT column. Peak areas of pegylated antibodies increased with reaction time, while the peak area of native antibody decreased.
High resolution analysis of a monoclonal antibody can be successfully performed on a TSKgel CM-STAT, 10cm column.
A pharmaceutical preparation containing pegylated protein was analyzed on a 10cm TSKgel SP-STAT column. Label information claims that the pegylated protein sample consists of a mixture of 4, 5 and 6 PEG molecules attached to a 192 amino acid protein. As expected, molecular weights as determined by non-reduced SDS-PAGE are much higher than actual molecular weights for the various fractions. None of the fractions were further analyzed.
Fast Separation of Protein Standards using a TSKgel SP-STAT Column

Columns: A: TSKgel SP-STAT, 10μm, 3.0mm ID x 3.5cm  
B: ProSwift SCX-1S Monolith, 4.6mm ID x 5cm

Eluent:  
A: 20mmol/L sodium acetate (pH5.0)  
B: 1.0mol/L NaCl in buffer A (pH5.0) for column A  
1.5mol/L NaCl in buffer A (5.0) for column B

Gradient: 0% B (0min), 100% B (1min)
Flow rate: A: 2.0mL/min  
B: 4.73mL/min
Detection: UV@280nm
Samples: 1. alpha-chymotrypsinogen A  
2. cytochrome C  
3. lysozyme
Conclusions

- Two new cation exchange columns, TSK-GEL SP-STAT and TSK-GEL CM-STAT, were evaluated for the analysis of biological samples.

- Short 3.5cm long columns, packed with 10μm particles, were very useful for high throughput separations requiring less than one minute analysis time, while, as expected, higher resolution protein separations were obtained on 10cm columns packed with 7μm particles.

- TSK-GEL CM-STAT and TSK-GEL SP-STAT cation exchange columns show excellent resolution and fast separations of protein samples compared to other non-porous and monolithic CIEC columns.
Conclusions

- TSK-GEL CM-STAT columns showed sharper peaks for mAb samples compared with other non-porous cation exchange columns.
- Pegylated proteins were analyzed on a short TSKgel SP-STAT column.
- The conversion ratios of pegylated and native proteins can be monitored by following the reaction using 5 minute intervals.
- TSK-GEL CM-STAT and TSK-GEL SP-STAT cation exchange columns can be powerful tools for fast and high resolution separations of proteins.
**Toyopearl GigaCap** is a registered trademark of Tosoh Corporation

Toyopearl GigaCap S-650M is the first in a family of ion exchange resins optimized for high throughput chromatography of IgG.

The high capacity for lysozyme shows that Toyopearl GigaCap S-650M will be an excellent resin for smaller proteins as well.

*Toyopearl GigaCap is a registered trademark of Tosoh Corporation*

### Typical Properties of Toyopearl GigaCap S-650M

<table>
<thead>
<tr>
<th></th>
<th>Toyopearl SP-650M</th>
<th>Toyopearl SP-550C</th>
<th>Toyopearl GigaCap S-650M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle size (µm)</td>
<td>40 - 90</td>
<td>50 - 150</td>
<td>50 - 100</td>
</tr>
<tr>
<td>Ion exchange capacity (meq/mL resin)</td>
<td>0.13 - 0.17</td>
<td>0.14 - 0.18</td>
<td>0.1 - 0.2</td>
</tr>
<tr>
<td>Binding capacity (mg/mL gel)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lysozyme @ 212cm/hr</td>
<td>48</td>
<td>81</td>
<td>167 (280cm/hr)</td>
</tr>
<tr>
<td>IgG @ 212cm/hr</td>
<td>43</td>
<td>14</td>
<td>145</td>
</tr>
</tbody>
</table>
Toyopearl GigaCap S-650M: Product Highlights

Structure:

\[ \text{HW-65} - O-R' - \text{SO}_3^- \]
strong cation exchanger
(Note: \( R' \) = proprietary polymer)

Product Attributes:

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pore size (mean)</td>
<td>1,000Å</td>
</tr>
<tr>
<td>Particle size (mean)</td>
<td>75μm (M-grade)</td>
</tr>
<tr>
<td>Pressure rating</td>
<td>3 bar</td>
</tr>
<tr>
<td>Shipping buffer</td>
<td>20% ethanol</td>
</tr>
<tr>
<td>pH stability</td>
<td>3-13</td>
</tr>
<tr>
<td>Shelf life (estimated)</td>
<td>10 years</td>
</tr>
</tbody>
</table>
The new Toyopearl GigaCap S-650M resin featured very high binding capacities with excellent binding kinetics and almost quantitative recoveries (data not shown).
Toyopearl GigaCap S-650M Alkaline Stability

**Graph:**
- **Toyopearl GigaCap S-650M Alkaline Stability**
- **Y-axis:** DBC (mg/mL-gel) 0 to 160
- **X-axis:** Week 0 to 6

**Table:**
- **Conditions:** Toyopearl GigaCap S-650M is stored in 1mol/L NaOH solution at room temperature.

<table>
<thead>
<tr>
<th>Evaluation Period (Soaking)</th>
<th>Week</th>
<th>0</th>
<th>1</th>
<th>3</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>IEC Capacity</td>
<td>mcg/mL</td>
<td>0.159</td>
<td>0.157</td>
<td>0.158</td>
<td>0.156</td>
</tr>
<tr>
<td>hlgG-DBC (10% breakthrough)</td>
<td>mg/mL-gel</td>
<td>143</td>
<td>144</td>
<td>140</td>
<td>135</td>
</tr>
<tr>
<td>hlgG-recovery</td>
<td>%</td>
<td>99</td>
<td>101</td>
<td>100</td>
<td>99</td>
</tr>
</tbody>
</table>

**Toyopearl GigaCap S-650M CIP-Alkaline Stability**

<table>
<thead>
<tr>
<th>Prior to NaOH</th>
<th>After 50 Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toyopearl GigaCap S-650M</td>
<td>167mg/mL-resin</td>
</tr>
</tbody>
</table>
Toyopearl GigaCap S-650M was packed into a 9cm Millipore Moduline column to measure the pressure/flow characteristics. The resin had similar profiles for both water and 1.0mol/L NaCl.
Conclusions

- Toyopearl GigaCap S-650M is an ideal cation exchange resin for the chromatographic purification of biotherapeutic products.
- Both large and small molecules have increased binding capacities, making it a versatile resin for all proteins.
- With the high binding capacities the need for very large columns and massive buffer consumption are minimized, particularly for those proteins expressed at higher levels as a result of upstream improvements.
- The new Toyopearl GigaCap S-650M chromatography resin is based on the same base bead as the other Toyopearl resins and, therefore, will have similar pressure-flow and chemical stability characteristics.
Toyopearl GigaCap Q-650

Structure:

\[ \text{HW-65} - O - R' - N^+ - (\text{CH}_3)_3 \]

strong anion exchanger

(Note: \( R' \) = proprietary)

Product Attributes:

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pore size (mean of base bead)</td>
<td>1,000Å</td>
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<td>Particle size (mean)</td>
<td>75µm (M-grade)</td>
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<td>Pressure rating</td>
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<tr>
<td>Shipping buffer</td>
<td>20% ethanol</td>
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<tr>
<td>pH stability</td>
<td>3-13</td>
</tr>
<tr>
<td>Shelf life (estimated)</td>
<td>10 years</td>
</tr>
</tbody>
</table>
Toyopearl GigaCap Q-650

It exhibits typical dynamic binding capacities in the vicinity of 175mg/mL for BSA (66kD) and 49mg/mL for thyroglobulin (660kD).
Less peak tailing is often observed resulting in more concentrated elution fractions with lower buffer volumes than agarose-based media.
Conclusions

- Toyopearl GigaCap Q-650 exhibits typical dynamic binding capacities in the vicinity of 175mg/mL for BSA (66kD) and 49mg/mL for thyroglobulin (660kD).

- This new polymer product maintains high capacity at increased linear velocities, has good pressure flow characteristics and will withstand back pressures up to 3 bar.

- The new higher capacity and back pressure stability of Toyopearl GigaCap Q-650 resin creates opportunities for increased throughput in all anion exchange purification steps.

- In addition to the high DBC and good pressure flow characteristics, Toyopearl GigaCap Q-650 media displays excellent binding and elution kinetics, which results in a more concentrated protein fraction collected during the elution step.
Thank You for your attention!

Please visit our website:
www.tosohbioscience.com

Atis Chakrabarti
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