TOSOH

SEPARATION REPORT NO. 109

TSKgel STAT COLUMNS FOR HIGH PERFORMANCE ION EXCHANGE CHROMATOGRAPHY

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1. Introduction

Ion exchange chromatography is used as an analytical technique in various fields, including biopharmaceuticals, biochemistry and the food industry, as it can separate the components in biopolymer samples such as proteins and nucleic acids. Ion exchange chromatography comprises anion exchange chromatography, which takes place on a packing material that contains chemically-bonded quaternary or tertiary ammonium groups, and cation exchange chromatography, which is performed on a packing material to which sulfonic acid or carboxylic acid have been introduced. The optimal packing material can be selected based on the chemical properties, steric properties, or isoelectric point of the target protein, or its ability to selectively separate the target protein from contaminants. Nucleic acids are generally separated using anion exchange chromatography.

The TSKgel® line of ion exchange chromatography columns includes the 5PW series columns, which are based on a porous packing material (particle size: 10μm) and were developed for the purpose of laboratory-scale separation and purification, and the TSKgel NPR series columns, which are based on a non-porous packing material (particle size: 2.5μm) and were developed for high-speed, high resolution analyses. The separation conditions used with the laboratory-scale TSKgel 5PW series columns can be easily scaled up because the same packing material is also available in 20 and 30μm particle size fractions that are commonly used for high speed process-scale purification, and in the chemically similar 35, 65 and 100μm Toyopearl series of bulk resins, which are used for larger scale industrial purifications.

As mentioned, the TSKgel NPR columns are filled with a non-porous packing material (particle size: 2.5μm), which provides superior resolution and high throughput, and can be used for high resolution analysis. These small particle size NPR resins show excellent recovery at low peptide and protein concentrations.

The recently launched TSKgel STAT columns, which also use a non-porous packing material, provide the same or better resolution as the smaller particle size TSKgel NPR columns. As can be expected from their larger particle size, TSKgel STAT columns operate at lower column back pressure than TSKgel NPR columns. The basic characteristics and some application examples of the TSKgel STAT columns will be discussed in this report.

2. Basic Characteristics of Columns

2-1. Characteristics of Packing Materials

The TSKgel STAT series columns include the TSKgel Q-STAT and TSKgel DNA-STAT columns, both of which have a matrix comprised of a non-porous hydrophilic polymer (particle sizes: 10, 7, and 5μm) to which a quaternary ammonium group has been introduced, the TSKgel SP-STAT column with a hydrophilic polymer (particle size: 10 and 7μm) matrix to which a sulfopropyl group has been introduced, as well as the TSKgel CM-STAT column to which a carboxymethyl group has been introduced. The specifications and characteristics of each of these columns are shown in **Table 1**. The TSKgel STAT columns include 3.0mm ID x 3.5cm columns designed for high throughput analysis of 1 to 2 minutes (*particle size 10μm: high throughput columns*), and 4.6mm ID x 10cm columns designed for high resolution (*particle size 7μm: high resolution columns*). Eluents containing organic solvents can also be used because the matrix has a high level of mechanical strength and there is little shrinkage or swelling of the packing material in response to changing solvents. By adding organic solvents to the eluent, adsorption of highly hydrophobic samples can be suppressed and elution can be carried out more quickly. Moreover, contaminants that adhere to the column when real samples are analyzed can be cleaned and removed using an eluent containing an organic solvent.

Table 1 Characteristics of Packing Material of TSKgel STAT columns

Product name	**TSKgel Q-STAT**		**TSKgel DNA-STAT**	**TSKgel SP-STAT**		**TSKgel CM-STAT**	
Product No.	21960	21961	21962	21963	21964	21965	21966
Column size* (mm ID × cm)	3.0 × 3.5	4.6 × 10	4.6 × 10	3.0 × 3.5	4.6 ×10	3.0 × 3.5	4.6 ×10
Matrix	Non-porous hydrophilic polymer						
Particle size (μm)	10	7	5	10	7	10	7
Functional group	Quaternary ammonium			Sulfopropyl		Carboxymethyl	
Ion exchange capacity (μeq/g dry gel)	Approximately 250			Approximately 20		Approximately 100	
Counter ion	Chloride ion			Sodium ion			
Preloaded solvent	20% ethanol aqueous solution						

*: Column housing: Stainless steel (Frit: PEEK)

2-2 Column Pressure

Figure 1 shows column pressure in high resolution columns packed with various packing materials as a function of column temperature. Even at a low temperature of 5°C, column pressure is 10MPa or lower in each of these columns, at a standard flow rate of 1.0mL/min. This clearly shows that these columns can be used even in low pressure LC systems.

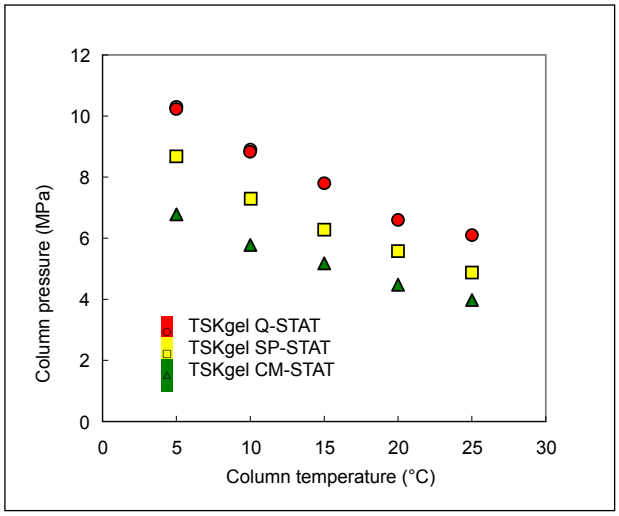

Figure 1 Relationship between column temperature and column pressure

Columns: TSKgel Q-STAT, 4.6 mm ID × 10cm
TSKgel SP-STAT, 4.6mm ID × 10cm
TSKgel CM-STAT, 4.6mm ID × 10cm
Eluent: 10mmol/L sodium acetate buffer, pH 5.0 for TSKgel SP-STAT and TSKgel CM-STAT
20mmol/L Tris-HCl buffer, pH 8.5 for TSKgel Q-STAT
Flow rate: 1.0mL/min

2-3 Separation of Standard Proteins

Figures 2 and **3** show a comparison of the separation of proteins using the high resolution TSKgel NPR columns (particle size: 2.5 µm). Due to improvements in surface-modification techniques, the TSKgel STAT columns provide stronger retention than the conventional TSKgel NPR columns, with high resolution and sharp peaks despite their larger particle size. It is also clear that the TSKgel SP-STAT and TSKgel CM-STAT columns differ with regard to their separation selectivity due to different ion exchange groups.

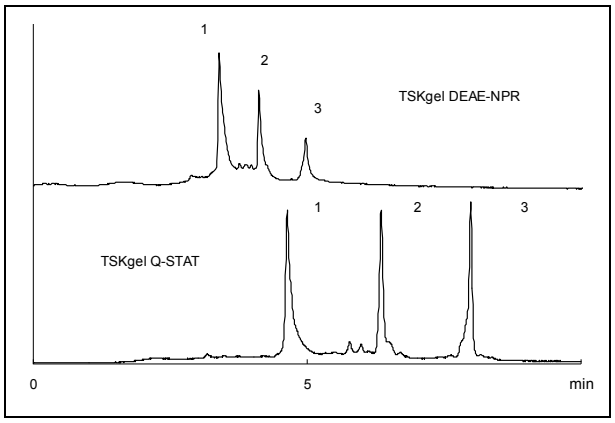

Figure 2 Comparison of anion exchange columns in separation of standard proteins

Columns: TSKgel DEAE-NPR, 4.6mm ID × 3.5cm (top)
TSKgel Q-STAT, 4.6mm ID × 10cm (bottom)
Eluent: A: 20mmol/L Tris-HCl buffer, pH 8.5
B: 0.5mol/L NaCl in 20mmol/L Tris-HCl buffer, pH 8.5
Gradient: 10min (100%B), linear
Flow rate: 1.0mL/min
Detection: UV@280nm
Temperature: 25°C

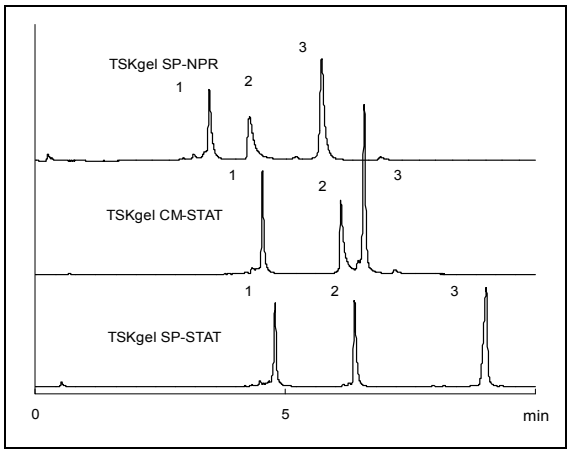

Figure 3 Comparison of cation exchange columns in separation of standard proteins

Columns: TSKgel SP-NPR, 4.6 mm ID × 3.5cm (top)
TSKgel CM-STAT, 4.6mm ID × 10cm (middle)
TSKgel SP-STAT, 4.6mm ID × 10cm (bottom)
Eluent: A: 10mmol/L sodium phosphate buffer, pH 6.2
B: 0.5mol/L NaCl in 10mmol/L sodium phosphate buffer, pH 6.2
Gradient: 10min (100%B), linear
Flow rate: 1.0mL/min
Detection: UV@280nm
Temperature: 25°C
Injection vol.: 10µg each
Samples: 1: α-chymotrypsinogen A
2. cytochrome C
3. lysozyme

Figures 4, **5** and **6** show high speed analysis data using high throughput columns packed with a packing material having a particle size of 10μm. Figure 4 contains data comparing separations on a TSKgel Q-STAT high throughput column with a monolith anion exchange column. The TSKgel Q-STAT high throughput column can separate three standard proteins within 1 minute and shows better peak shape than the monolith column. **Figure 5** contains data comparing separations on a TSKgel SP-STAT high throughput column with a monolith cation exchange column. The TSKgel SP-STAT high throughput column can separate three standard proteins within 1 minute and shows better peak shape than the monolith column.

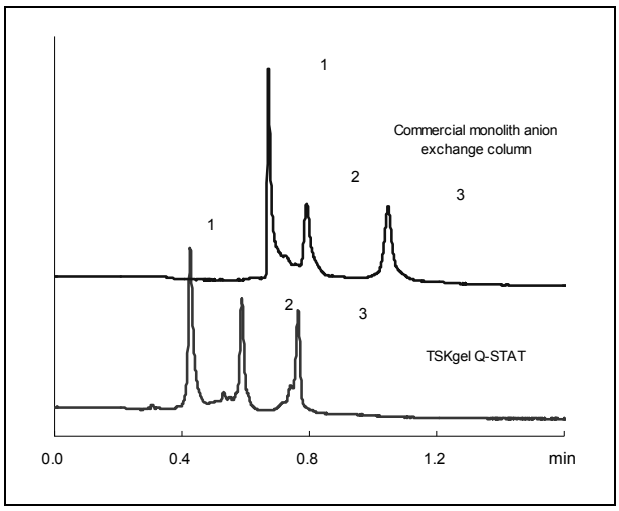

Figure 4 High throughput analysis of standard proteins using anion exchange columns

Columns: Commercial monolith anion exchange column, 5.0mm ID × 5cm (top)
TSKgel Q-STAT, 3.0mm ID × 3.5cm (bottom)
Eluent: A: 20mmol/L Tris-HCl buffer, pH 8.5
B: 0.5mol/L NaCl in 20mmol/L Tris-HCl buffer, pH 8.5
Gradient: 1min (100%B), linear
Flow rate: 2.0mL/min (monolith column)
1.0mL/min (TSKgel Q-STAT)
Detection: UV@280nm
Temperature: 25°C
Sample load: 3μg each
Samples: 1: conalbumin
2. ovalbumin
3. trypsin inhibitor
* using a micro mixer

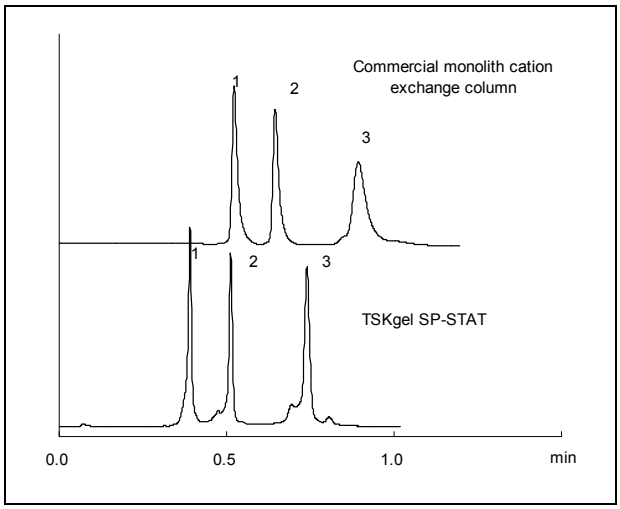

Figure 5 High throughput analysis of standard proteins using cation exchange columns

Columns: Commercial monolith cation exchange column, 5.0mm ID × 5cm (top)
TSKgel SP-STAT, 3.0mm ID × 3.5cm (bottom)
Eluent: A: 20mmol/L sodium acetate buffer, pH 5.0
B: 1.0mol/L NaCl in 20mmol/L sodium acetate buffer, pH 5.0 (TSKgel SP-STAT), 1.5mol/L NaCl in 20mmol/L sodium acetate buffer, pH 5.0 (monolith column)
Gradient: 1min (100%B), linear
Flow rate: 4.73mL/min (monolith column)
2.0mL/min (TSKgel SP-STAT)
Detection: UV@280nm
Temperature: 25°C
Injection vol.: 5μL
Samples: 1: α-chymotrypsinogen A (1g/L)
2. cytochrome C (1g/L)
3. lysozyme (1g/L)
* using a micro mixer

2-4 Separation of Nucleic Acids

Figure 6 shows a chromatogram on a TSKgel Q-STAT high throughput column to separate cytidine and 5 nucleotides within 1 minute.

The resolution between cytidine diphosphate (CDP) and cytidine triphosphate (CTP) was studied as a function of gradient time. **Figure 7** shows the results in a graph in which the resolution between these nucleotides is plotted against CTP elution time. Even with the high resolution column, resolution decreases as the gradient time becomes shorter, but it is clear that the resolution is better if the high throughput column is used when the gradient time is reduced to result in a very short CTP elution time (e.g., ≤ 2.5min in this case).

Figure 8 shows a chromatogram in which 15 nucleotides are separated in a single run on the high resolution column. Thus, with the TSKgel STAT columns, it is possible to select the column according to the analysis time and resolution required.

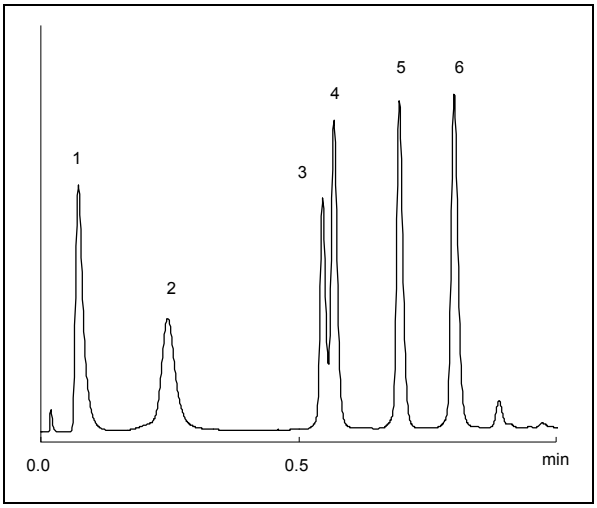

Figure 6 High-throughput analysis of nucleotides by an anion exchange column

Column: TSKgel Q-STAT, 3.0mm ID × 3.5cm
Eluent: A: 20mmol/L Tris-HCl buffer, pH 8.5
B: 0.5mol/L NaCl in 20mmol/L Tris-HCl
Gradient: 1min (100%B), linear
Flow rate: 2.0mL/min
Detection: UV@260nm
Temperature: 25°C
Injection vol.: 5µL
Samples: 1: cytidine (0.05g/L)
2. 3',5'-cyclic CMP (0.1g/L)
3. 2d-CMP (0.1g/L)
4. CMP (0.15g/L)
5. CDP (0.2g/L)
6. CTP (0.25g/L)

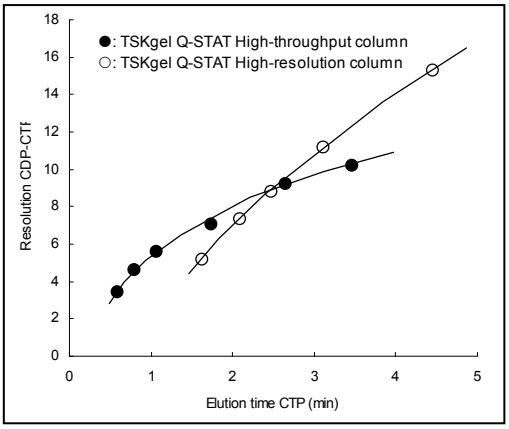

Figure 7 Use of high throughput column or high resolution column depending on analysis time

Columns: TSKgel Q-STAT, 3.0mm ID × 3.5cm
TSKgel Q-STAT, 4.6mm ID × 10cm
Eluent: A: 20mmol/L Tris-HCl buffer, pH 8.5
B: 0.5mol/L NaCl in 20mmol/L Tris-HCl buffer, pH 8.5
Gradient: time is varied
Flow rate: 2.0mL/min, 3.0mm ID column
1.5mL/min, 4.6mm ID column
Detection: UV@260nm
Temperature: 25°C
Samples: CTP, CDP

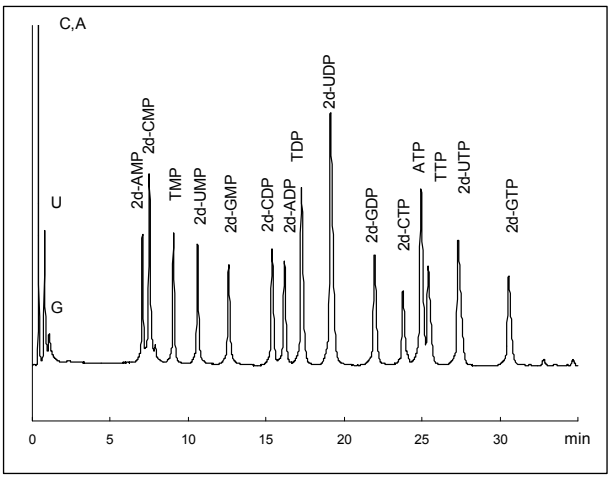

Figure 8 Analysis of nucleotides using anion exchange column

Column: TSKgel Q-STAT, 4.6mm ID × 10cm
Eluent: A: 20mmol/L Tris-HCl buffer, pH 8.5
B: 0.5mol/L NaCl in 20mmol/L Tris-HCl buffer, pH 8.5
Gradient: 60min (50%B), linear
Flow rate: 1.5mL/min
Detection: UV@260nm
Temperature: 25°C
Injection vol.: 5µL
Samples: nucleobases (0.05g/L each)
monophosphate nucleosides (0.1g/L each)
diphosphate nucleosides (0.15g/L each)
triphosphate nucleosides (0.2g/L each)

2-5 Sample Load

Generally speaking, the surface area of non-porous packing materials is much smaller than that of porous packing materials of the same particle size, sometimes as much as 100-fold, which is another way of saying that most of the surface area of porous particles is in the pores. Thus, since TSKgel STAT columns are based on a non-porous packing material, the surface area in the column is much smaller than that for ion exchange columns based on porous packing materials. As a result, the capacity of TSKgel STAT columns will be exhausted at lower sample loads than is usually the case for porous particle ion exchange columns. Capacity overload leads to a loss in resolution between two adjacent components when injecting a high sample concentration or a large sample volume. Figures 9 and 10 show the relationship between sample load and resolution. A sample load of not more than 10µg is appropriate for both the anion exchange column and the cation exchange column. Overloading of the column occurs at lower sample volume and lower sample concentration the shorter the column is or the narrower the internal diameter. The relationship between max amount and volume injected is roughly proportional to the column volume.

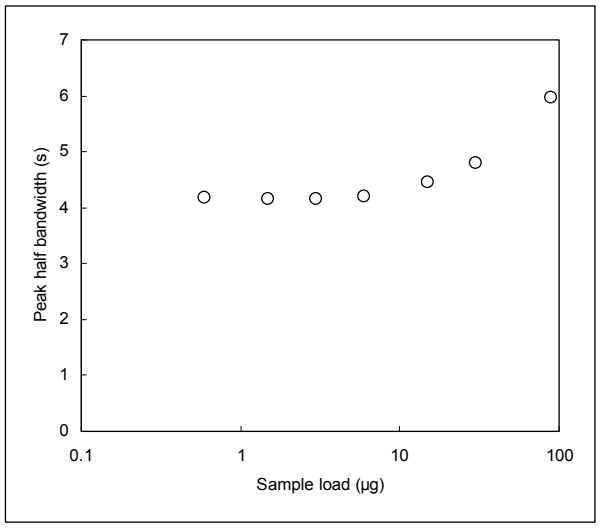

Figure 9 Relationship between sample load and peak half bandwidth

Column: TSKgel Q-STAT, 4.6mm ID × 10cm
Eluent: A: 20mmol/L Tris-HCl buffer, pH 8.5
B: 0.5mol/L NaCl in 20mmol/L Tris-HCl buffer, pH 8.5
Gradient: 10min (100%B), linear
Flow rate: 1.0mL/min
Detection: UV@280nm
Temperature: 25°C
Injection vol.: 5µL
Sample: ovalbumin

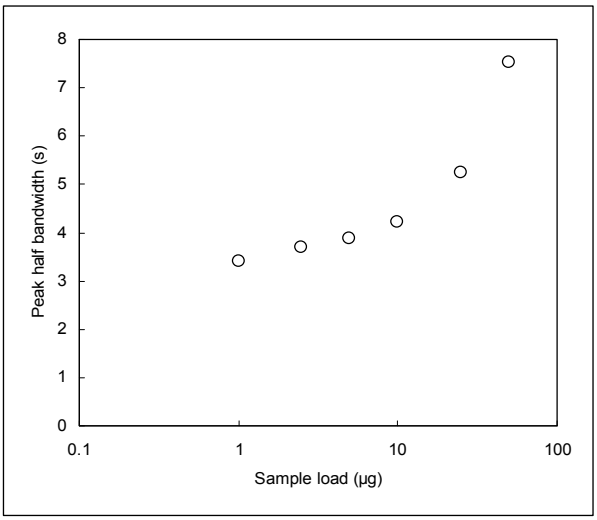

Figure 10 Relationship between sample load and peak half bandwidth

Column: TSKgel Q-STAT, 4.6mm ID x 10cm
Eluent: A: 20mmol/L sodium acetate buffer, pH 5.0
B: 0.5mol/L NaCl in 20mmol/L sodium acetate buffer, pH 5.0
Gradient: 10min (100%B), linear
Flow rate: 1.0mL/min
Detection: UV@280nm
Temperature: 25°C
Sample: cytochrome c

3. Anion Exchange Column Applications

Figure 11 shows the separation of commercial crude lipases. It is clear that with the TSKgel Q-STAT column, retention is stronger, resolution is better, and peaks are sharper, compared to the results on the TSKgel DEAE-NPR column. **Figure 12** compares the separation of a commercial BSA digestion standard. Clearly more peaks are separated on the TSKgel Q-STAT column when compared with the TSKgel DEAE-NPR column and a commercial WAX column, and fewer components are missed (inside circled areas in the figure).

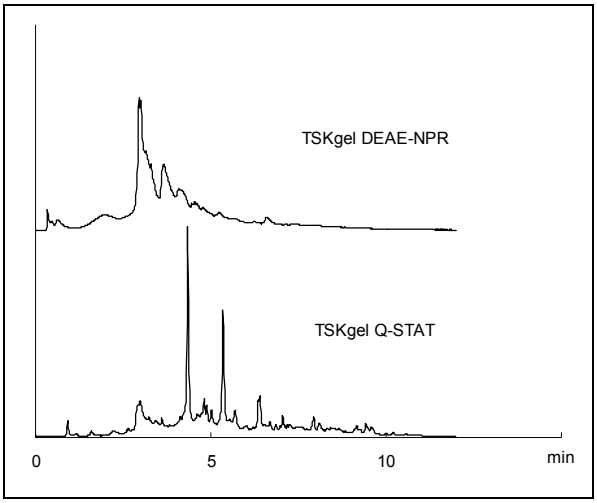

Figure 11 Separation of crude lipases

Columns: TSKgel DEAE-NPR, 4.6mm ID × 3.5cm (top)
TSKgel Q-STAT, 4.6mm ID × 10cm (bottom)
Eluent: A: 20mmol/L Tris-HCl buffer, pH 8.5
B: 0.5mol/L NaCl in 20mmol/L Tris-HCl buffer, pH 8.5
Gradient: 10min (100%B), linear
Flow rate: 1.0mL/min
Detection: UV@280nm
Temperature: 25°C
Injection vol.: 10µL
Sample: crude lipase

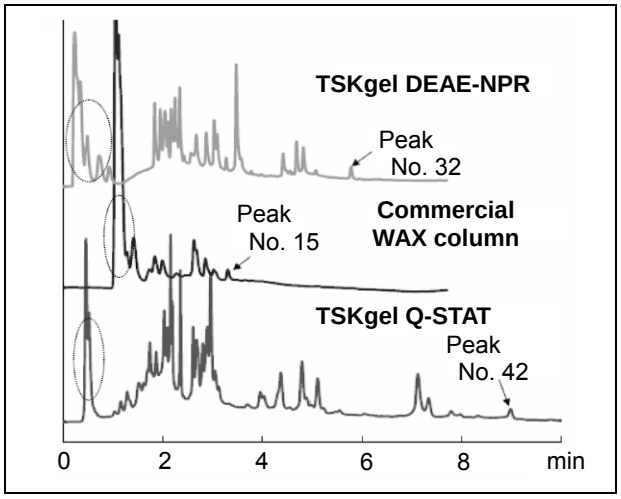

Figure 12 Separation of commercial BSA digest

Columns: TSKgel DEAE-NPR, 4.6mm ID × 3.5cm (top)
Commercial WAX column, 4.0mm ID × 25cm (middle)
TSKgel Q-STAT, 4.6mm ID × 10cm (bottom)
Eluent: A: 20mmol/L Tris-HCl buffer, pH 8.5
B: 0.5mol/L NaCl in 20mmol/L Tris-HCl buffer, pH 8.5
Gradient: 10min (100%B), linear
Flow rate: 1.5mL/min
Detection: UV@220nm
Temperature: 25°C
Injection vol.: 10µL
Sample: MassPREP BSA Digestion Standard, Waters, Co. [186002329] (10µL)

Figure 13 shows the separation of mouse ascites fluid containing a monoclonal antibody (top) and a partially purified monoclonal antibody (bottom) on a TSKgel Q-STAT column. The top chromatogram clearly shows that the antibody and albumin components are well separated. The bottom chromatogram shows that multiple peaks are present in the partially purified monoclonal sample. Figure 14 shows a chromatogram of the same partially purified mAb sample analyzed in Figure 13, now separated using a shallower salt gradient. It is well known that purified antibodies may contain multiple charge isomers due to incomplete removal of C-terminal lysine residues.

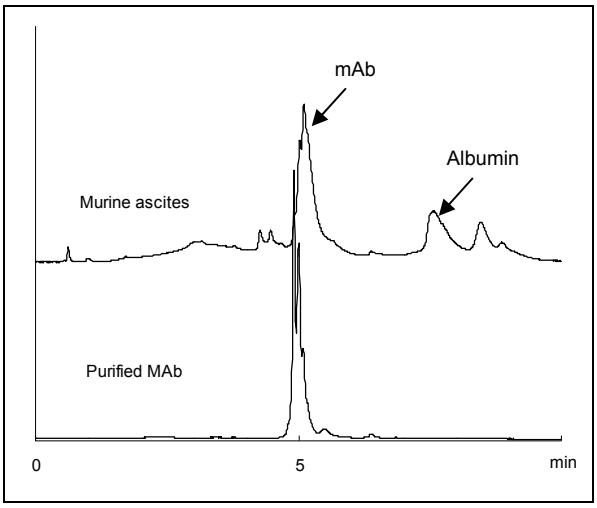

Figure 13 Separation of mouse ascites fluid containing monoclonal antibodies (top) and purified monoclonal antibodies (bottom)

Column: TSKgel Q-STAT, 4.6mm ID × 10cm
Eluent: A: 20mmol/L Tris-HCl buffer, pH 8.5
B: 0.5mol/L NaCl in 20mmol/L Tris-HCl buffer, pH 8.5
Gradient: 10min (100%B), linear
Flow rate: 1.0mL/min
Detection: UV@280nm
Temperature: 25°C
Injection vol.: 10µL
Sample: Top: 1/10 dilution of mouse ascites containing mAb
Bottom: purified mouse mAb
Sample diluted 10-fold with eluent A

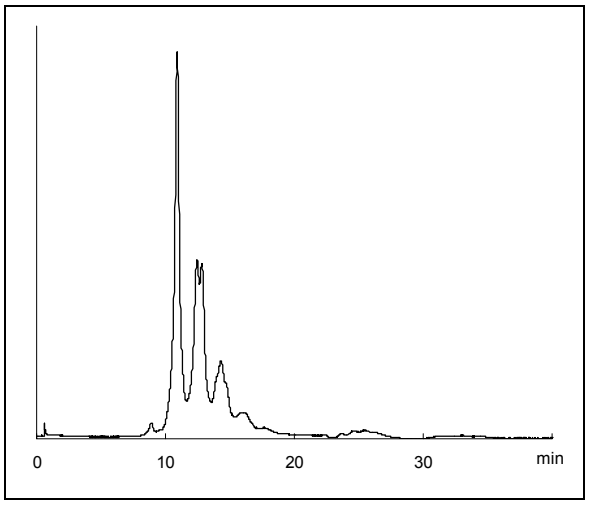

Figure 14 Separation of charge isomers of purified mouse monoclonal antibodies

Column: TSKgel Q-STAT, 4.6mm ID × 10cm
Eluent: A: 0.05mol/L NaCl in 20mmol/L Tris-HCl buffer, pH 8.5
B: 0.15mol/L NaCl in 20mmol/L Tris-HCl buffer, pH 8.5
Gradient: 0min (25%B), 30min (100%B), linear
Flow rate: 1.0mL/min
Detection: UV@280nm
Temperature: 25°C
Sample: purified mouse mAbs

Figures 15 and **16** demonstrate the applicability of TSKgel DNA-STAT columns for nucleic acid applications, in this example the analysis of a 1Kb DNA ladder. The sample contains DNA fragments of 23 different lengths from 75bp to 12,216bp. With the TSKgel DNA-NPR, separation conditions were not adequate for separating the 20th fragment and higher (≥9,126bp); however, with the TSKgel DNA-STAT, all fragments contained in the sample could be separated with a resolution of ≥1.5.

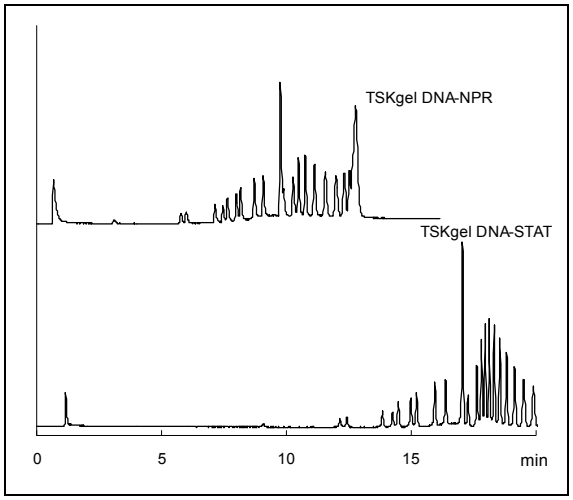

Figure 15 Separation of 1Kb DNA ladder

Column: TSKgel DNA-NPR, 4.6mm ID × 7.5cm (top)
TSKgel DNA-STAT, 4.6mm ID × 10cm (bottom)
Eluent: A: 20mmol/L Tris-HCl buffer, pH 9.0
B: 2.0mol/L NaCl in 20mmol/L Tris-HCl buffer, pH 9.0
Gradient: 0min (37.5%B), 20min (50%B), linear (TSKgel DNA-STAT)
0min (25%B), 20min (37.50%B), linear (TSKgel DNA-NPR)
Flow rate: 0.75mL/min
Detection: UV@260 nm
Temperature: 25°C
Injection vol.: 2µL
Sample: 1Kb DNA ladder

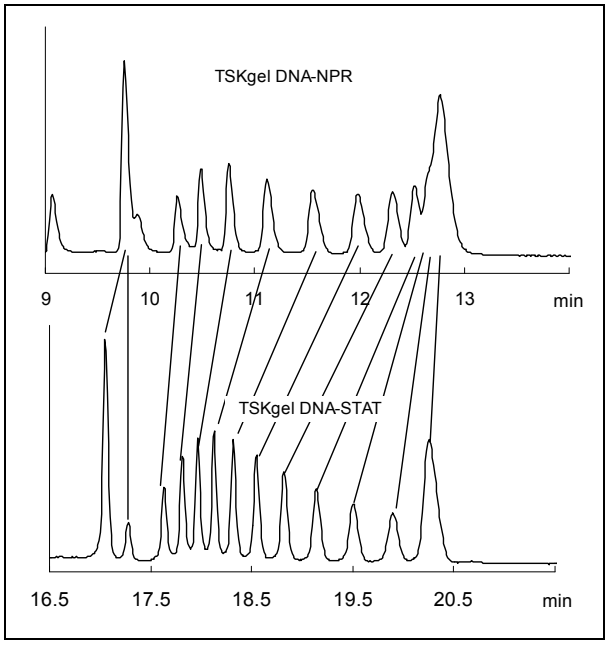

Figure 16 Enlargement of Figure 15

On the other hand, when analyzing short DNA fragments that differ in composition but are of the same length, either the TSKgel DNA-NPR column or the TSKgel DNA-STAT column may be selected for use. The data shown in Figure 17 demonstrates that both column types provide excellent resolution of four 26-mer primers, with the TSKgel DNA-NPR providing a shorter analysis time than the TSKgel DNA-STAT column.

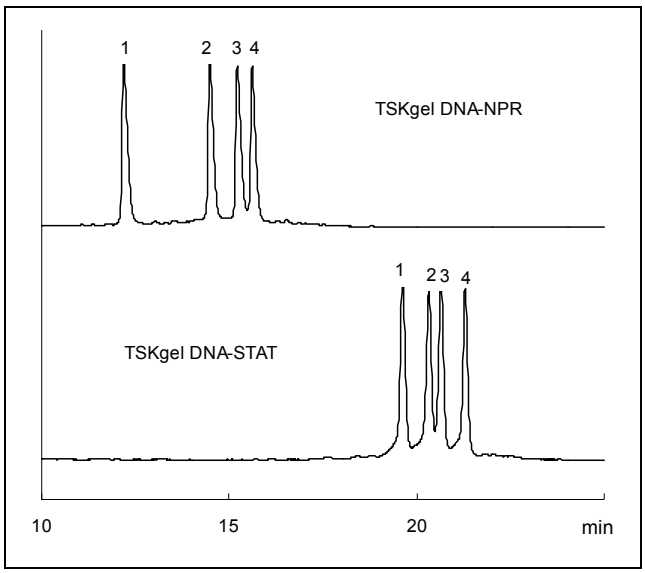

Figure 17 Separation of primers

Column: TSKgel DNA-NPR, 4.6mm ID × 7.5cm (top)
TSKgel DNA-STAT, 4.6mm ID × 10cm (bottom)
Eluent: A: 20mmol/L Tris-HCl buffer, pH 8.5
B: 0.75mol/L NaCl in 20mmol/L Tris-HCl buffer, pH 8.5
Gradient: 0min (50%B), 25min (75%B), linear
Flow rate: 0.8mL/min
Detection: UV@260nm
Temperature: 25°C
Injection vol.: 10µL
Sample: 26-mer primers
1. 5'-TAATTAAGGACTCCGTTCTTCTATAT-3'-NH_2
2. 5'-TCTTTACTTTAGTCACAAAGCGATAA-3'-NH_2
3. 5'-GACTCCGTTCTTCTATATTTTCGAGG-3'-NH_2
4. 5'-GGACGTGCTGGGTGTCTTCTCCGTCG-3'-NH_2
Sample concentration: 10µmol/L each

4. Cation Exchange Column Applications

Figure 18 shows an example of the separation of charge isomers of mAb using cation exchange columns. Isomers with slight differences in charge can be separated. For this sample the TSKgel CM-STAT column showed higher resolution with narrower peak width than the result that was obtained on a TSKgel SP-STAT column. In addition, as shown in **Figure 19**, the TSKgel CM-STAT column can also be used to separate charge isomers by pH gradient.

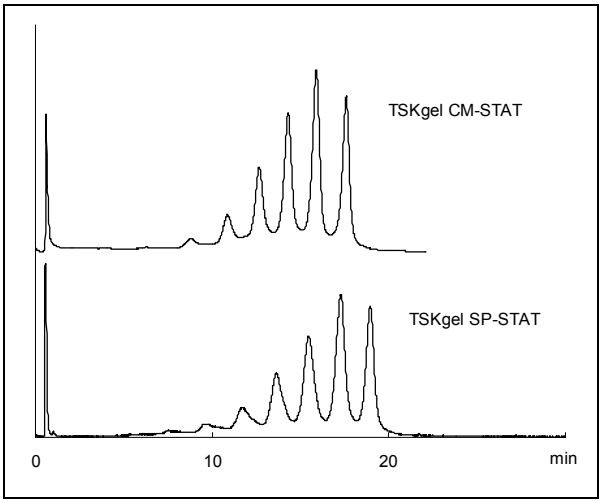

Figure 18 Separation of charge isomers of purified mAb using a salt gradient

Column: TSKgel CM-STAT, 4.6mm ID × 10cm (top)
TSKgel SP-STAT, 4.6mm ID × 10cm (bottom)
Eluent: A: 20mmol/L MES buffer, pH 6.0
B: 0.1mol/L NaCl in 20mmol/L MES buffer, pH 6.0
Gradient: 0min (25%B), 30min (55%B), linear (TSKgel SP-STAT)
0min (20%B), 30min (50%B), linear (TSKgel CM-STAT)
Flow rate: 1.0mL/min
Detection: UV@280nm
Temperature: 25°C
Injection vol.: 10µL
Sample: purified mAb
Sample concentration: 1g/L

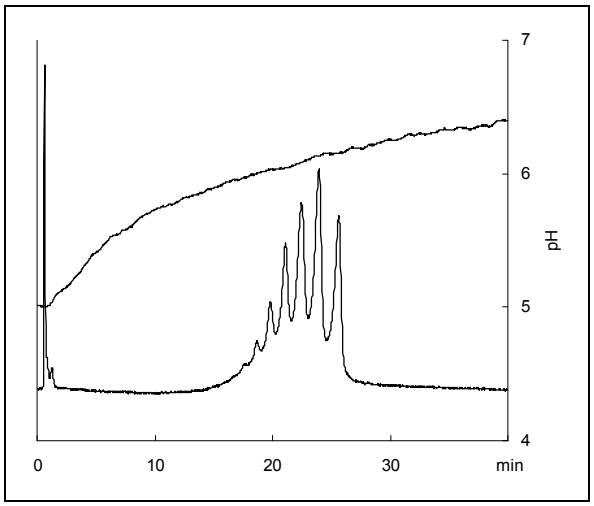

Figure 19 Separation of charge isomers of purified mAb using a pH gradient (chromatofocusing)

Column: TSKgel CM-STAT, 4.6mm ID × 10cm
Eluent: A: 50mmol/L sodium acetate buffer, pH 5.0
B: 30mmol/L sodium acetate buffer (pH not adjusted)
Column equilibrated with eluent A, the sample is injected, then eluted stepwise to 100% eluent B
Flow rate: 1.0mL/min
Detection: UV@280 nm
Temperature: 25°C
Injection vol.: 10µL
Sample: purified mAb
Sample concentration: 1g/L

Modification of proteins with polyethylene glycol (PEGylation) is being investigated with the objective of prolonging the in vivo half-life of proteins for medical use. **Figures 20** and **21** show how the PEGylation of lysozyme can be monitored on a high throughput TSKgel SP-STAT column. In **Figure 20**, superimposed chromatograms analyzing the reaction solution at set times after the start of the reaction are shown. **Figure 21** plots the percentage of each peak area in these chromatograms against the reaction time. Because high throughput TSKgel SP-STAT columns provide short analysis times, fast reactions such as PEGylation can be adequately monitored.

Also, as shown in **Figure 22**, very detailed information can be obtained when using the TSKgel SP-STAT high resolution column. The chromatogram clearly shows that multiple isomers of mono-, di- and tri-PEGylated proteins can be separated by extending the analysis time to about 10 minutes.

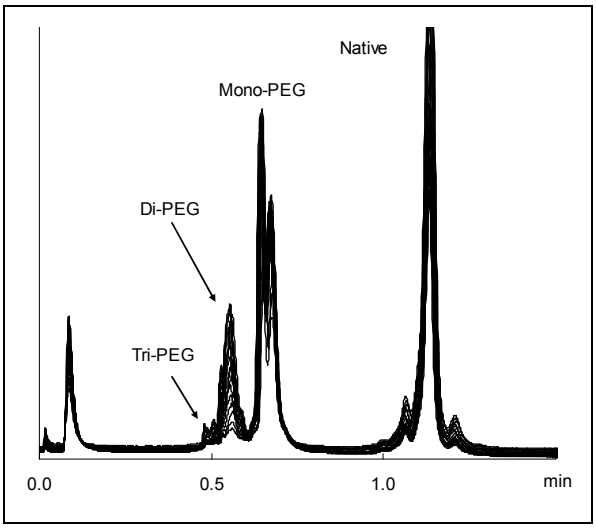

Figure 20 Superimposed chromatograms allow monitoring of lysozyme PEGylation reaction

Column: TSKgel SP-STAT, 3.0mm ID × 3.5cm
Eluent: A: 20mmol/L sodium acetate buffer, pH 5.0
B: 0.5mol/L NaCl in 20mmol/L sodium acetate buffer, pH 5.0
Gradient: 1.5min (100%B), linear
Flow rate: 2.0mL/min
Detection: UV@280nm
Temperature: 25°C
Sample: 5g/L lysozyme dissolved in phosphate buffer, and 3 times the molar equivalent of a PEGylation reagent (Sunbright ME-200CS, NOF Corp.) was added. Sampling was performed every 4 minutes and measurements were performed.

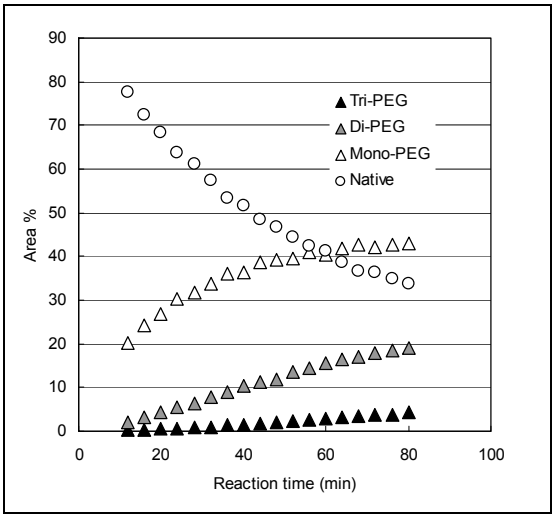

Figure 21 Chromatographic peak areas of PEGylated and native lysozyme as a function of reaction time

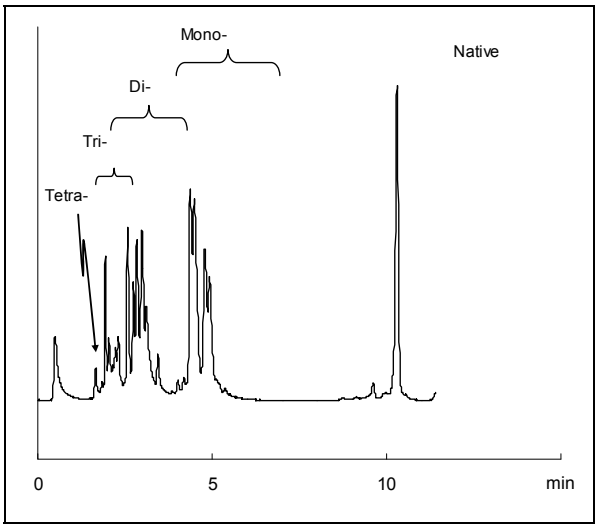

Figure 22 Chromatogram of PEGylated lysozyme

Column: TSKgel SP-STAT, 4.6mm ID × 10cm
Eluent: A: 20mmol/L sodium acetate buffer, pH 5.0
B: 0.5mol/L NaCl in 20mmol/L sodium acetate buffer, pH 5.0
Gradient: 10min (100%B), linear
Flow rate: 1.4mL/min
Detection: UV@280nm
Temperature: 25°C
Injection vol.: 5µL
Sample: Components from the final run of the reaction in Figure 20

Figure 23 shows a chromatogram of a commercial PEGylated protein drug. Each peak was sampled and analyzed by SDS-PAGE, the results pf which are shown in **Figure 24**. Although it is clear that almost all sampled fractions contain multiple components, the results of SDS-PAGE suggest that this method could be useful in the quality control of biopharmaceuticals.

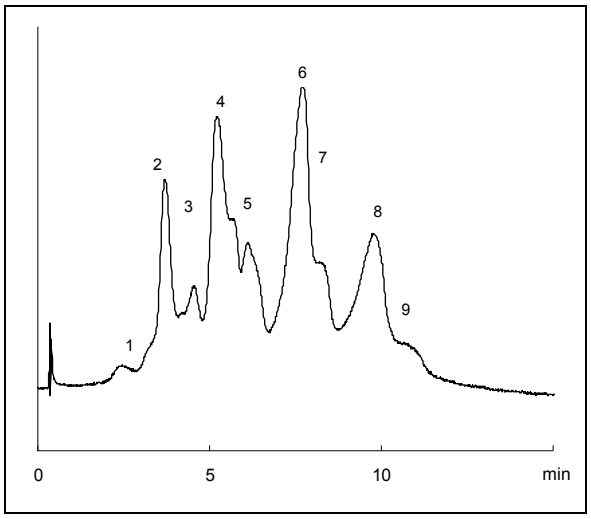

Figure 23 Separation of commercial PEGylated protein drug

Column:	TSKgel SP-STAT, 4.6mm ID × 10cm
Eluent:	A: 20mmol/L sodium acetate buffer, pH 4.2
	B: 0.2mol/L NaCl in 20mmol/L sodium acetate buffer, pH 4.2
Gradient:	20min (100%B), linear
Flow rate:	1.5mL/min
Detection:	UV@280nm
Temperature:	25°C
Sample:	commercial PEGylated protein drug

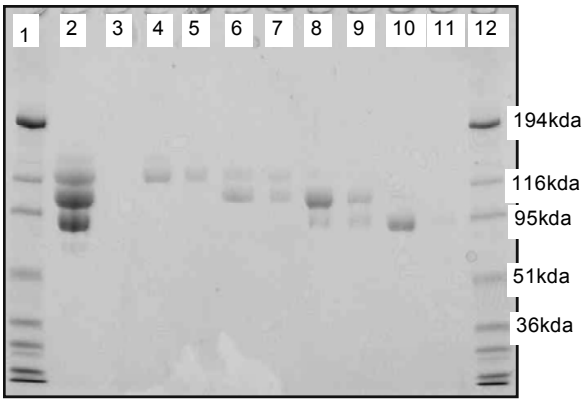

Figure 24 SDS-PAGE of elution fractions of commercial PEGylated protein drug

* Non-reducing SDS-PAGE
Lanes 1, 12: Markers
2: Commercial PEGylated protein drug
3 to 11: Peak fractions shown in Figure 22

Figure 25 shows chromatograms of a variety of antibody drugs on a TSKgel CM-STAT high resolution column. Variants for each antibody drug are well separated in a short period of time, clearly showing that the TSKgel CM-STAT column is suitable in the development and quality control of antibody drugs.

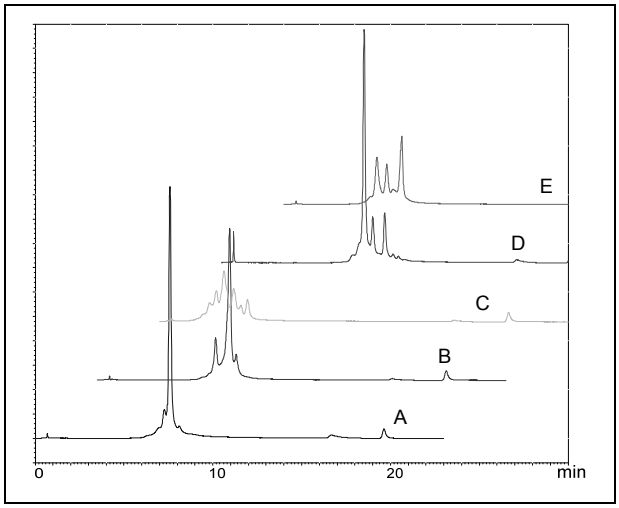

Figure 25 Separation of antibody drug variants

Column: TSKgel CM-STAT, 4.6mm ID × 10cm
Eluent: A: 20mmol/L MES buffer, pH 6.0
B: 0.5mol/L NaCl in 20mmol/L MES buffer, pH 6.0
Gradient: 0min (10%B), 15min (30%B), linear
Flow rate: 1.0mL/min
Detection: UV@280nm
Temperature: 25°C
Injection vol.: 20µL
Samples: A: human antibody, IgG_1
B: human antibody, IgG_1
C: chimera antibody, IgG_1
D: chimera antibody, IgG_1
E: chimera antibody, IgG_1
Sample concentration: 0.5g/L

* Courtesy of Professor Hitoshi Kakidani of the Sagami Chemical Research Institute

5. Conclusions

To date, the TSKgel NPR columns have been used to achieve high resolution in ion exchange chromatography of proteins and nucleic acids. However, there have been instances in which column pressure has been high and the peak shape of low molecular weight components has been poor. With the TSKgel STAT columns, due to improvements in matrix properties and surface modification techniques, the peak shape of low molecular weight components has been improved, and by optimizing particle size, high resolution in low pressure columns has been achieved. Moreover, it is expected that this line up of high resolution and high throughput TSKgel STAT columns will expand the range of applications for ion exchange chromatography.

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