High Speed and High Resolution Anion Exchange Chromatography for Biological Samples on Non-Porous Packings

Hiroyuki MORIYAMA, Mutsumi SHIMADA, Kazuaki MURANAKA, Toshinao IWAEDA

Separation Center, TOSOH Corporation

www. tosohbioscience.com

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Introduction

- Mono-disperse and non-porous resins (NPR) provide high efficiency and rapid analysis of biological samples such as proteins and nucleic acid fragments. These columns play an important rule in the field of proteomics. However, due to their small surface area NPR columns have smaller loading capacities than porous resins. Also, 2.5μm particle size NPR columns require high operating pressures.
- Tosoh Corporation developed novel non-porous anionic exchange (AEX) resins, marketed as TSK-GEL Q- STAT (7 and 10μm) and TSK-GEL DNA-STAT (5μm), with a high loading capacity and a low operating pressure by adopting larger particle sizes (5-10μm) and by applying a novel bonding chemistry.
- The novel IEX resins show higher adsorption capacities and lower pressures compared with current non-porous columns of the same column dimension. Rapid separations of proteins were achieved within 1 minute on short columns packed with 10μm resin.
- High resolution analyses on 10cm columns packed with 7μm resin demonstrated high loading capacity.
- DNA oligomers and polymers were separated on a 10cm TSKgel DNA-STAT column packed with 5μm particles. Higher resolution of DNA fragments on the TSKgel DNA-STAT column was obtained in comparison to the TSKgel DNA-NPR column.
- The basic properties of the novel anion exchange columns and how they apply to the separation of proteins, DNA fragments and low molecular weight compounds are reported in comparison with commercially available monolithic and non-porous AIEX columns.

Experimental

- **HPLC columns - Tosoh Corporation**
  - **TSKgel Q-STAT, 10µm, 4.6mm ID x 5cm**
  - **TSKgel Q-STAT, 7µm, 4.6mm ID x 5cm**
  - **TSKgel DNA-STAT, 5µm, 4.6mm ID x 10cm**
  - **TSKgel DEAE-NPR, 2.5µm, 4.6mm ID x 3.5cm**
  - **TSKgel DNA-NPR, 2.5µm, 4.6mm ID x 7.5cm**

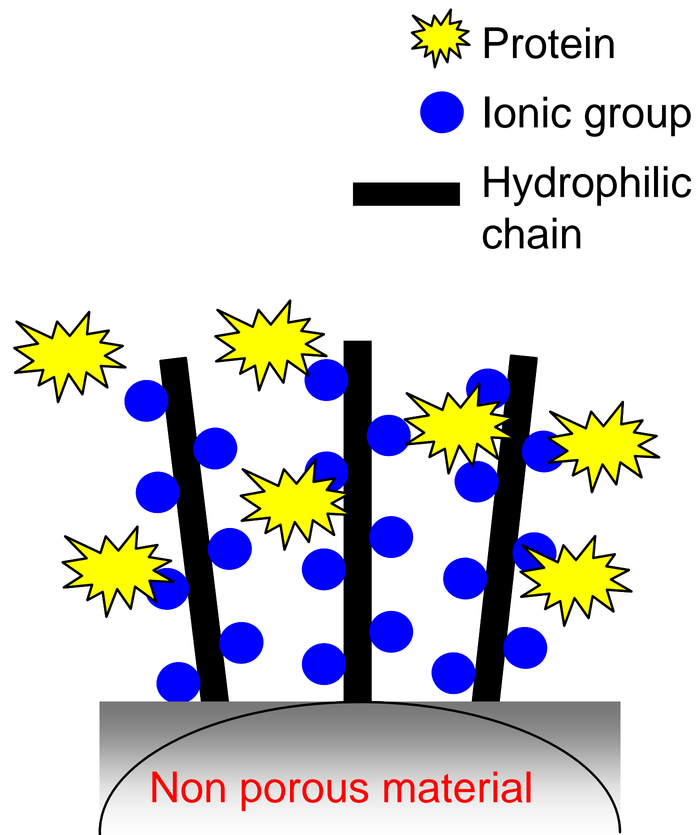
- **HPLC columns - Commercially available**
  - **Brand A: Non-porous WAX type, 10µm, 4mm ID x 25cm (Dionex)**
  - **Brand B: Monolithic WAX type, 4.6mm ID x 5cm (Dionex)**

- **Reagents**
  - **All proteins and DNA samples were purchased from Sigma. The Amplified DNA by PCR product was a gift from the Tokyo Research Center (TOSOH Co., Kanagawa). Other reagents were purchased from Kishida Chemicals (Osaka).**

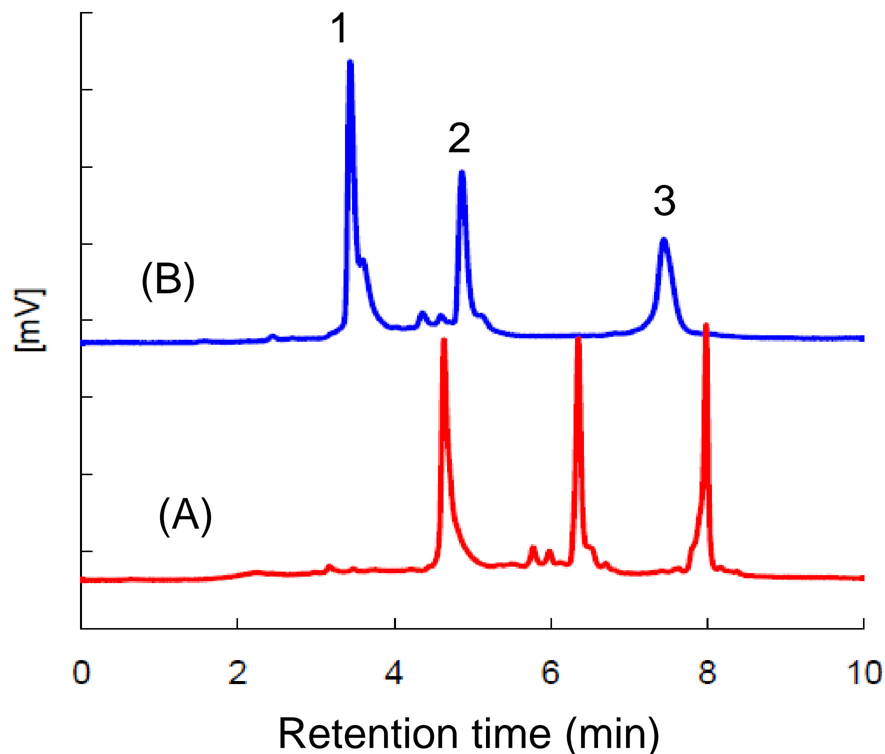
Basic Properties of TSK-GEL Q-STAT and DNA-STAT Anion Exchange Columns

Property	TSK-GEL Q-STAT		TSK-GEL DNA-STAT
Base material	Cross-linked hydrophilic polymer (mono-disperse particles)		
Pore size	Non-porous		
Functional group	Quaternary ammonium		
Particle size	7µm	10µm	5µm
Column size	4.6mm ID x 10cm	3mm ID x 3.5cm	4.6mm ID x 10cm
Application	High resolution protein separation	High resolution protein separation	High resolution DNA separations

Schematic Diagram of TSK-GEL STAT Series



Protein Separations on Non-Porous Anion Exchange Columns



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)

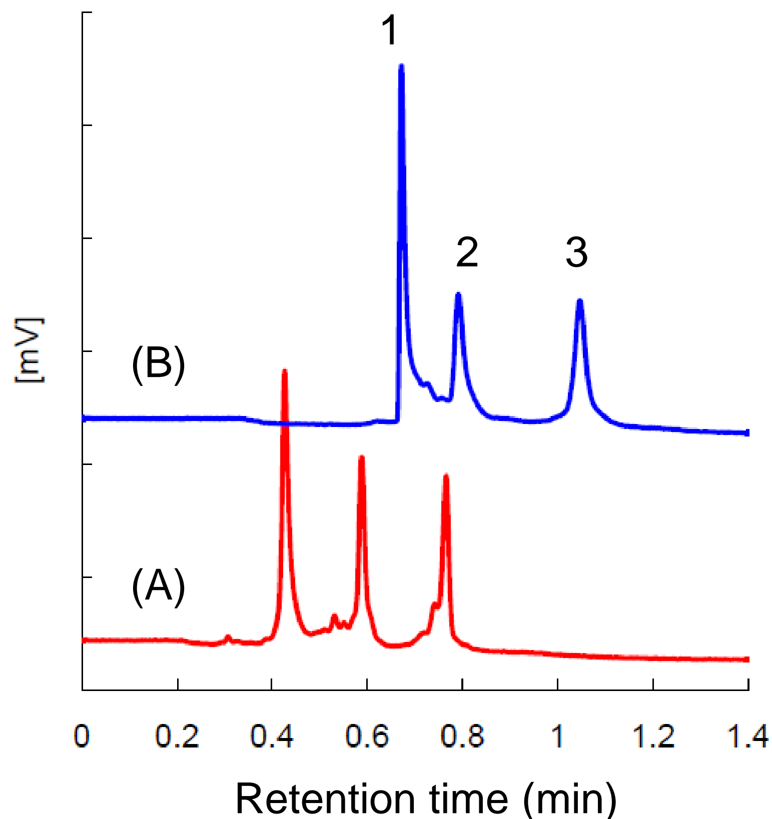
Flow rate: 1.0mL/min

Detection: UV@280nm

Samples: 1. conalbumin
2. ovalbumin
3. trypsin Inhibitor

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

Fast Protein Separation on Monolithic and Non-Porous Anion Exchange Columns



Columns: A: TSKgel Q-STAT, 10μm, 3.0mm ID x 3.5cm
B: Brand B, Monolithic WAX-type, 4mm ID x 5cm

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

Gradient: 0% B (0min), 100% B (1min)

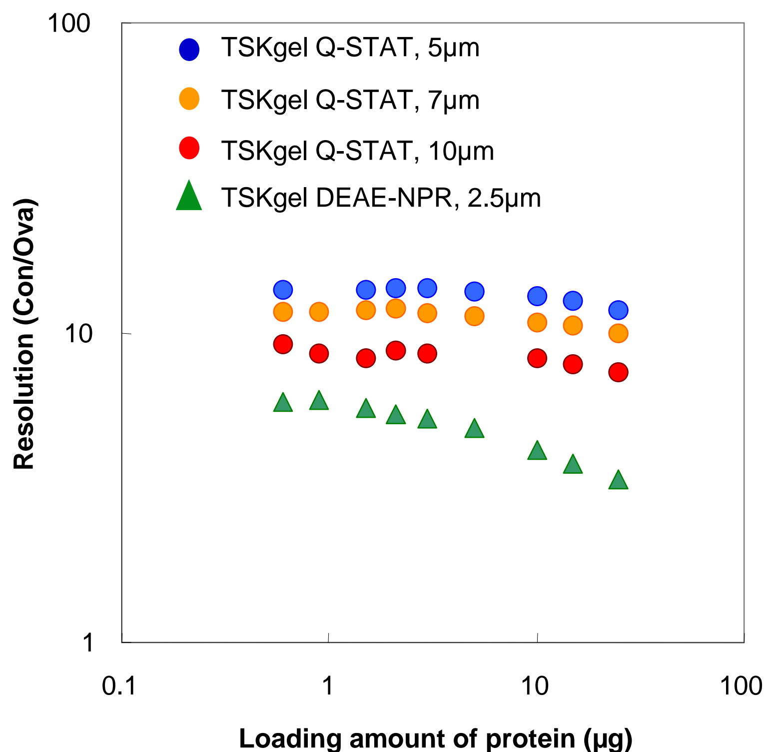
Flow rate: 2.0mL/min

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.

Loading Capacities versus Resolution on Non-Porous TSK-GEL Anion Exchange Columns



Columns: TSK-GEL Q-STAT, 5µm, 7µm, 10µm
TSKgel DEAE-NPR, 2.5µm
Dimensions: 4.6mm ID x 3.5cm

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

The effect of sample mass on resolution was investigated on the TSK-GEL Q-STAT series columns and a TSKgel DEAE-NPR column of the same column dimensions. TSK-GEL Q-STAT series columns show higher resolution over a wide range of sample mass compared with the TSKgel DEAE-NPR column.

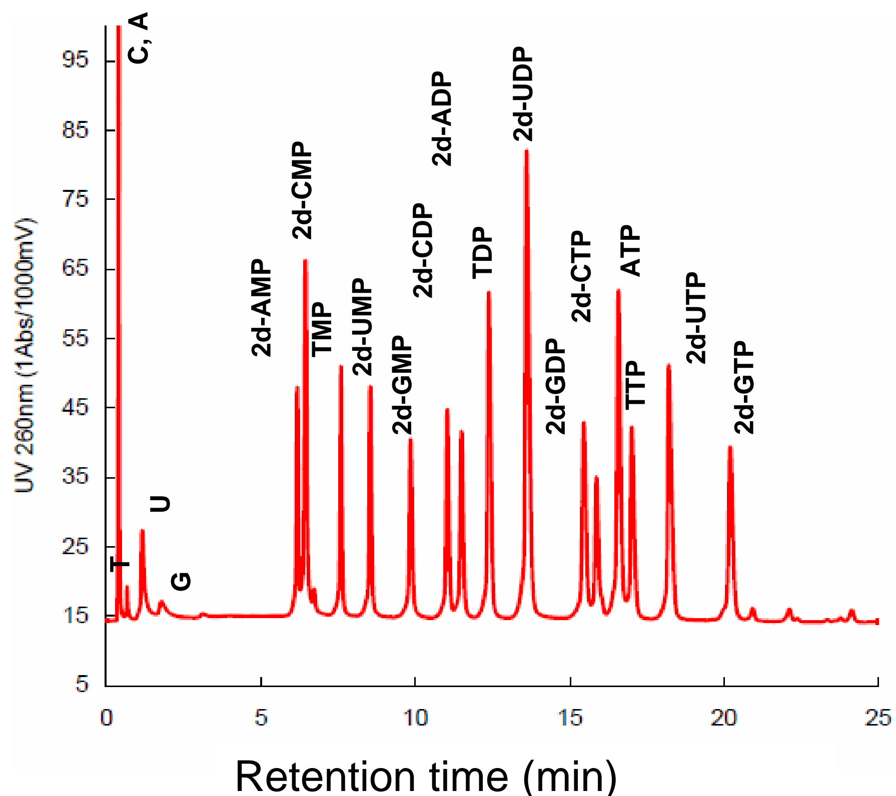
Static Binding Capacity (SBC) of BSA on Non-Porous TSK-GEL Anion Exchange Resins

Property	TSK-GEL DEAE-NPR	TSK-GEL Q-STAT		
Particle size	2.5µm	5µm	7µm	10µm
Static binding capacity (mg BSA/mg dry gel)	9.1	38.6	27.0	20.9

Despite the fact that surface area decreases with increasing particle size, the larger Q-STAT particles have higher binding capacities than the smaller DEAE-NPR particles. The novel bonding chemistry used in the preparation of 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.

Separation of DNA on TSK-GEL DNA-STAT Columns

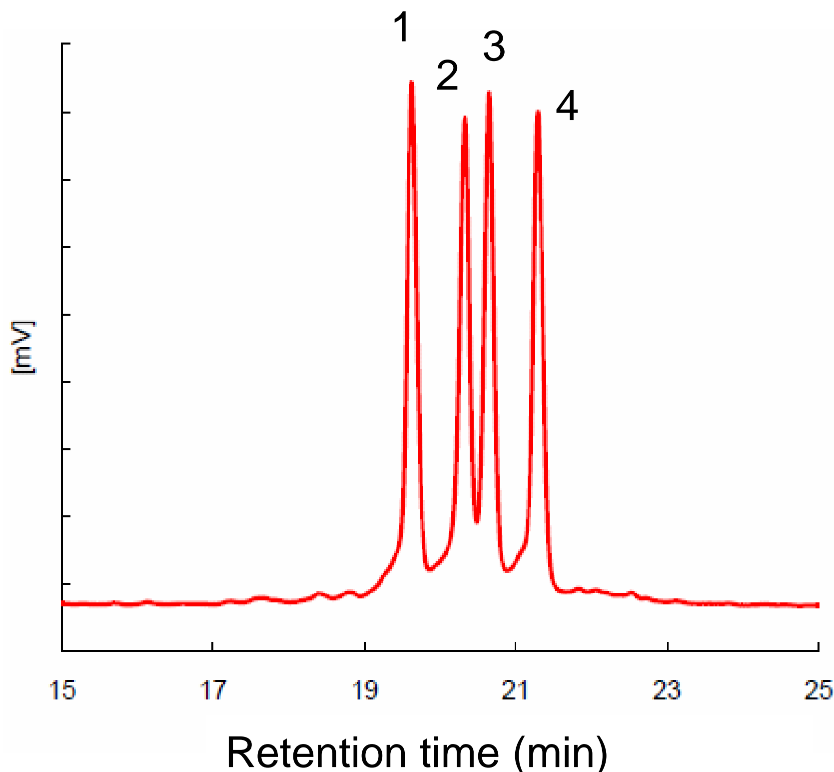
Separation of Nucleotides on TSK-GEL DNA-STAT Column



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

Low molecular weight nucleotides were separated with excellent peak shape, demonstrating the absence of micro-pores on the TSKgel DNA-STAT column.

Synthetic Oligonucleotides on TSKgel DNA-STAT



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'-TCTTTACTTTAGTCACAAAGCGATAA-3'-NH2
3. 5'-GACTCCGTTCTTCTATATTTTCGAGG-3'-NH2
4. 5'-GGACGTGCTGGGTGTCTTCTCCGTCG-3'-NH2

Four 18-mers of different composition were separated successfully on a TSKgel DNA-STAT column.

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

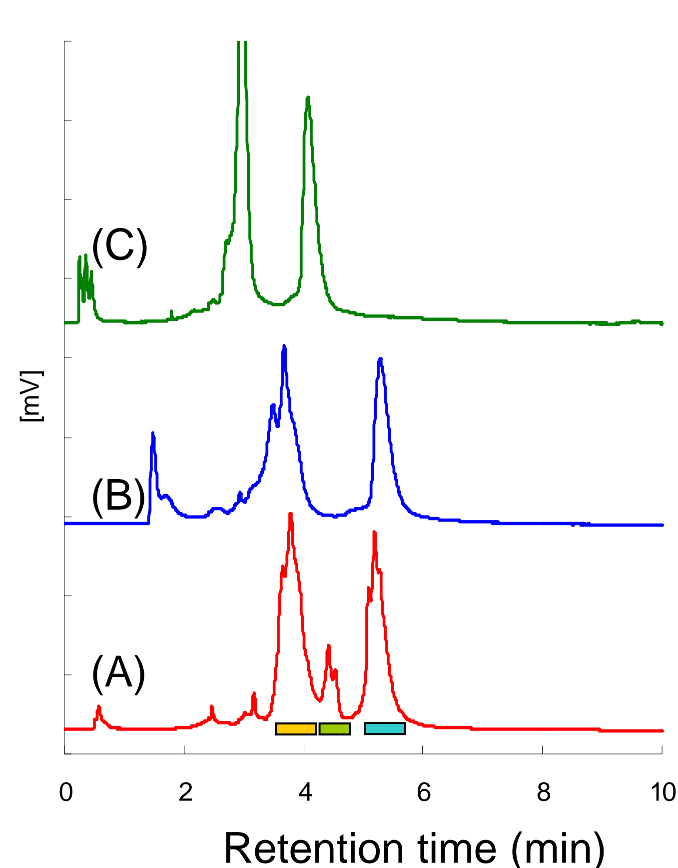
Sample: 1kb ladder

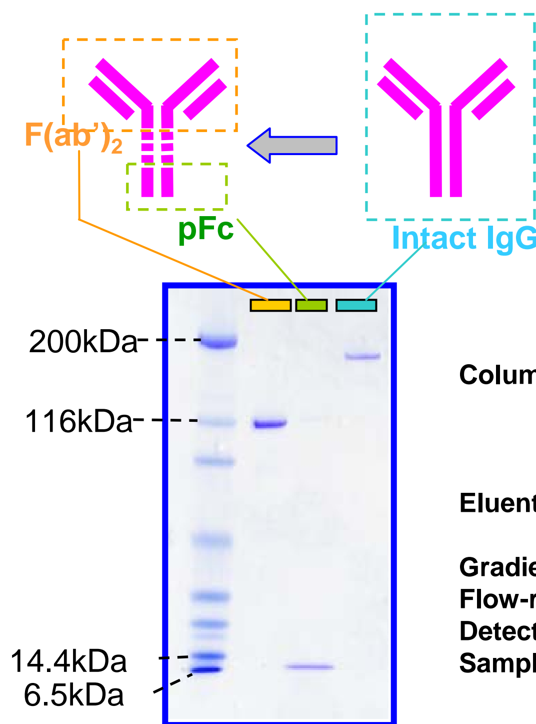
Large DNA fragments containing more than 10kb were successfully separated on a TSKgel DNA-STAT column packed with 5µm particles.

Applications of TSK-GEL Q-STAT and TSK-GEL DEAE-NPR Columns

Chromatographic Profiles of IgG Pepsin Digest on Non-Porous Anion Exchange Columns

TOSOH



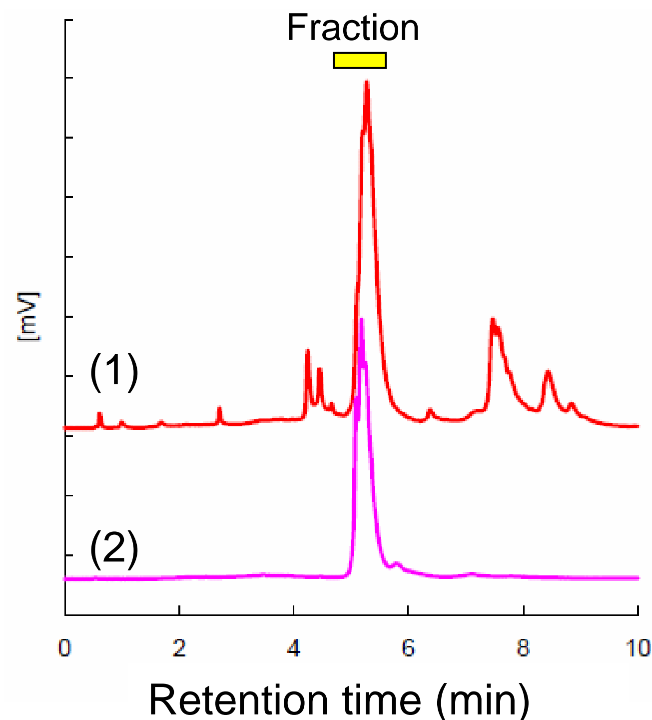


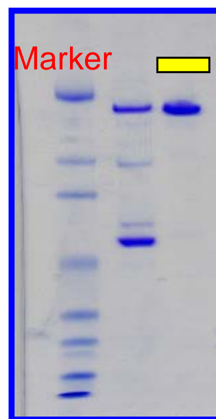
Non-reduced SDS-PAGE

Columns:	**A: TSKgel Q-STAT, 7µm, 4.6mm ID x 10cm**
	B: Brand A: Non-porous WAX, 4mm ID x 25cm
	C: TSKgel DEAE-NPR, 2.5µm, 4.6mm ID x 3.5cm
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**
Sample:	**Pepsin digested mAb**

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



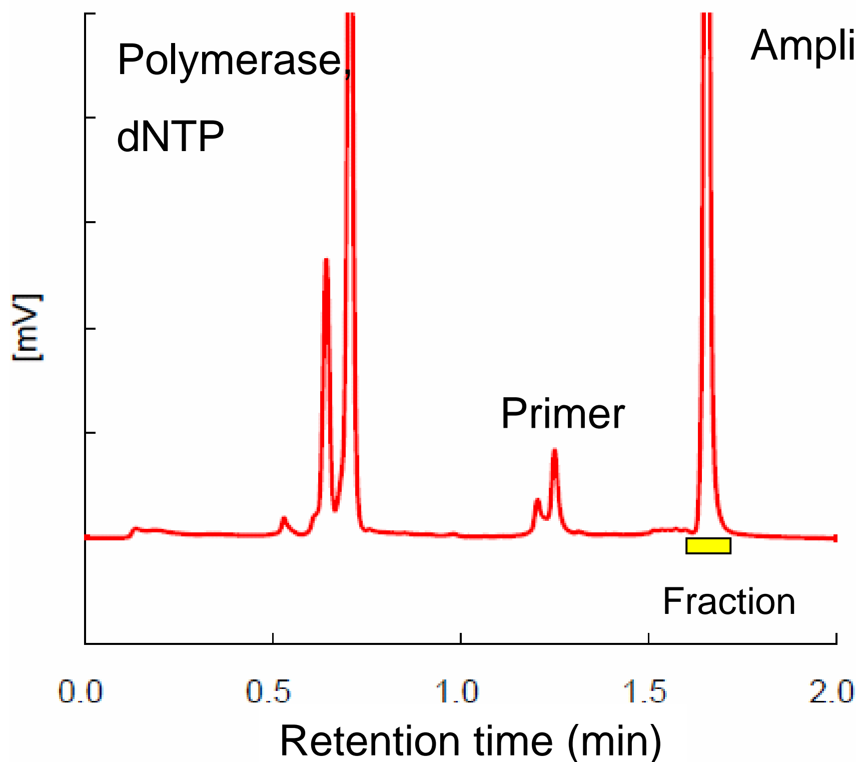


Non-reduced SDS-PAGE

Column:	**A: TSKgel Q-STAT, 7µm, 4.6mm ID x 10cm**
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**
Sample:	**Mouse ascites containing mAb2**
Chromatograms:	**1: Crude mouse ascites**
	2: Analysis of isolated fraction

Analysis results from the isolated fraction (yellow band in the upper chromatogram) suggests a single component.

High Throughput Analysis of Amplified DNA on a TSKgel Q-STAT Column



Agarose gel electrophoresis

Column:	A: TSKgel Q-STAT, 10µm, 3.0mm ID x 3.5cm
Eluent:	A: 20mmol/L Tris-HCl (pH8.5) B: 1.0mol/L NaCl in buffer A
Gradient:	0% B (0min), 100% B (1.5min)
Flow-rate:	2.0mL/min
Detection:	UV@260nm
Sample:	Amplified by PCR

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 new anion exchange columns were evaluated for the analysis of biological samples. The short (3.5cm) TSKgel Q-STAT column packed with 10μm particles was very useful for 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.
- 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.