Separation Report No. 100

TSK-GEL BioAssist® Series Ion Exchange Columns

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1. Introduction

When liquid chromatography is used for purification and processing of biopolymers such as proteins, it is recommended that the purification involves a combination of orthogonal chromatography techniques in a stepwise fashion. Ion exchange chromatography is commonly used in orthogonal mode separations as a capture step due to its high protein binding capacity.

Historically, the challenge for chromatographic resin manufactures has been to introduce a relatively thick ion-exchange layer on the packing surface for high capacity binding characteristics while maintaining acceptable backpressure and separation performance. Although widely used methods of polymer chain introduction such as graft polymerization, etc. are known; these methods are limiting due to the extremely high column pressure drops that result. Consequently, the use of smaller high efficiency particles is not common in ion-exchange.

TSK-GEL BioAssist series is a group of ion-exchange columns which solve this problem by introducing ionic polymers loosely cross-linked to the porous material surface. This article describes the basic properties, applications and operating conditions for the TSK-GEL BioAssist series, which realizes high binding capacity, high retention and high resolution at a low column pressure drops equivalent to that of a conventional column.

2. Basic Properties

2-1. Ion-Exchange Capacity and Pore Characteristics

TSK-GEL BioAssist series consists of an anion-exchange column, TSKgel BioAssist Q and a cation-exchange column, TSKgel BioAssist S. TSKgel BioAssist Q is introduced with polyamine as the ion-exchange group, and the structure of the ion-exchange groups is a mixture of tertiary and quaternary ammonium. On the other hand, TSKgel BioAssist S is introduced with a polymer containing sulfopropyl groups, and the ion-exchange structure is the sulfopropyl groups. The total ion-exchange capacity of each has been prepared to approximately 0.1 meq per 1mL gel for each of the packings (**Table-1**).

Figure 1 shows the pore characteristics of TSK-GEL BioAssist series. The unfunctionalized base material of TSKgel BioAssist Q has an exclusion limit of 5 million or larger with polystyrene standards. While the unfunctionalized base material of TSKgel BioAssist S has an exclusion limit of approximately 3 million. After adding the ion-exchange groups, the exclusion limits become 1 million or larger (on a pullulan basis) for both the cation and anion exchangers. Since TSK-GEL BioAssist series contain pore diameter larger than the conventional packings, it is expected that the surface area is smaller than conventional ones. However, it possesses a high binding capacity that does not depend on the sample's molecular weight. Three-dimensional absorption is achievable for samples with small molecular weights, and the large pore diameter allows sufficient sample permeation into the pores for samples with large molecular weights.

Table-1 Features of TSK-GEL BioAssist series

Item	BioAssist Q	BioAssist S
Base material	Porous acrylate-type gel	Porous acrylate-type gel
Average particle diameter (μm)	10	7
Functional groups	Polyamine	Sulfopropyl groups
Ion-exchange capacity (eq/L)	0.1	0.1
pH range for use (long-term)	3-10	3-10
pH range for use (short-term)*	2-12	2-12
Dynamic binding capacity (g/L)	>70 (Bovine serum albumin) >70 (Thyroglobulin)	>70 (γ-globulin) >70 (Lysozyme)
Appropriate flow rate (mL/min)	1	0.8
Maximum flow rate (mL/min)	1.2	1
Maximum pressure drops(MPa)	2.5	2.5
Column member	PEEK	PEEK
Column size (mm I.D. × cm)	4.6 × 5	4.6 × 5

* 1 month or less

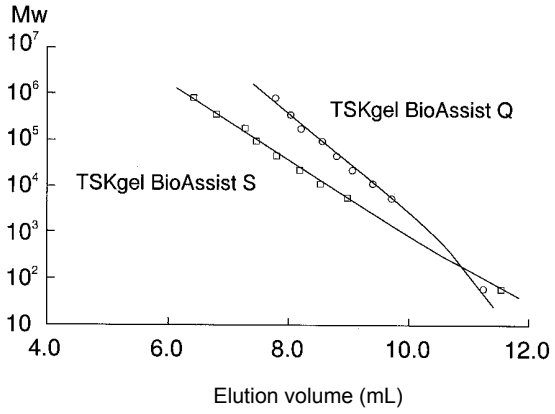

Figure-1 Calibration curves for TSK-GEL BioAssist series

Eluent: 20mmol/L Tris-HCl buffer, pH8.0 (BioAssist Q)
20mmol/L Sodium phosphate buffer, pH7.0 (BioAssist S)
Flow rate: 0.5mL/min (BioAssist Q)
0.4mL/min (BioAssist S)
Samples: pullulan
* Samples were measured by 7.8mm I.D.× 30cm column.

2-2. Separation of Standard Proteins

Figure-2 shows a comparison of standard protein separation on TSKgel BioAssist Q and conventional columns. It is clear that TSKgel BioAssist Q possesses a higher retention and resolution of the sample proteins compared to the conventional products. Likewise, as shown in **Figure-3**, comparison of separation on TSKgel BioAssist S and conventional columns also shows that TSKgel BioAssist S has a higher retention and resolution.

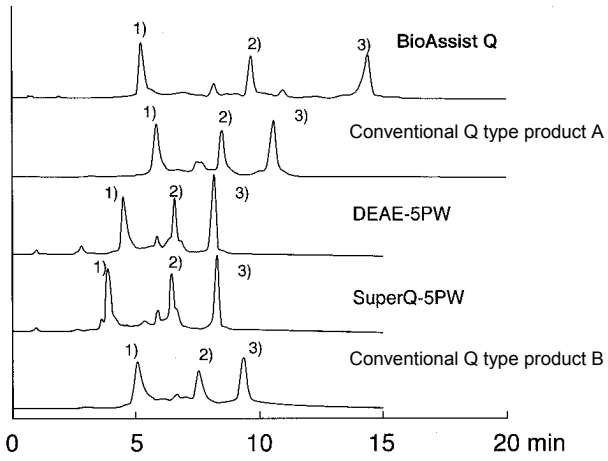

Figure-2 Comparison of standard protein separation on TSKgel BioAssist Q and conventional columns

Columns:

TSKgel BioAssist Q	4.6mm I.D. × 5cm, PEEK
Conventional Q type product A	5.0mm I.D. × 5cm, Glass
TSKgel DERE-5PW	5.0mm I.D. × 5cm, Glass
TSKgel SuperQ-5PW	5.0mm I.D. × 5cm, Glass
Conventional Q type product B	4.6mm I.D. × 5cm, PEEK

Eluent: A; 20mmol/L Tris-HCl buffer, pH8.0
B; 20mmol/L Tris-HCl buffer containing 1.0mol/L NaCl, pH8.0
Linear gradient from eluent A to B for 30 minutes
Flow rate: 1.0mL/min
Temperature: 25°C
Detection: UV (280nm)
Injection volume: 60μL
Samples:

1) Conalbumin	0.5g/L
2) Ovalbumin	1.0g/L
3) Trypsin inhibitor	1.0g/L

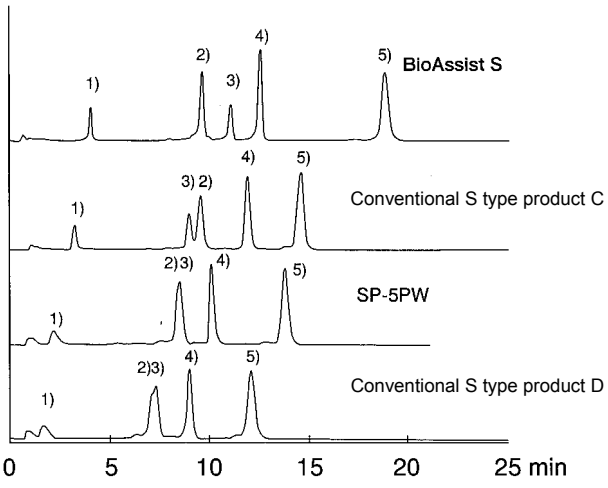

Figure-3 Comparison of standard protein separation by TSKgel BioAssist S and conventional columns

Columns:

TSKgel BioAssist S	4.6mm I.D. × 5cm, PEEK
Conventional S type product C	5.0mm I.D. × 5cm, Glass
TSKgel SP-5PW	5.0mm I.D. × 5cm, Glass
Conventional S type product D	4.6mm I.D. × 5cm, PEEK

Eluent: A; 20mmol/L sodium phosphate buffer, pH6.5
B; 20mmol/L sodium phosphate buffer containing 1.0mol/L NaCl, pH6.5
Linear gradient from eluent A to B for 32 minutes
Flow rate: 0.8mL/min
Temperature: 10°C
Detection: UV (280nm)
Injection volume: 20μL
Samples:

1) Myoglobin	1g/L
2) α-chymotrypsinogen A	2g/L
3) Ribonuclease A	4g/L
4) Cytochrome C	2g/L
5) Lysozyme	2g/L

2-3. Capacity

Figure-4 shows the results of comparing the changes in dynamic binding capacity against the molecular weight between this product and a conventional product. While the binding capacity of the conventional product decreases as the molecular weight of protein increases. TSKgel BioAssist Q maintains a high binding capacity from samples with low molecular weights to those with high molecular weights. **Tables-2 and 3** show the dynamic binding capacity of TSKgel BioAssist Q and TSKgel BioAssist S respectively, against various proteins.

Furthermore, **Table-4** shows the pH dependence of binding capacity when an antibody (mouse IgG1 pI 6.41) is used. According to **Table-3**, it is clear that TSKgel BioAssist S possesses a high binding capacity (similar to TSKgel BioAssist Q) regardless of molecular weight. In addition, **Table-4** indicates that it is capable of maintaining IgG under milder, neutral conditions compared to conventional commercial ion-exchange columns, because it has a high retention.

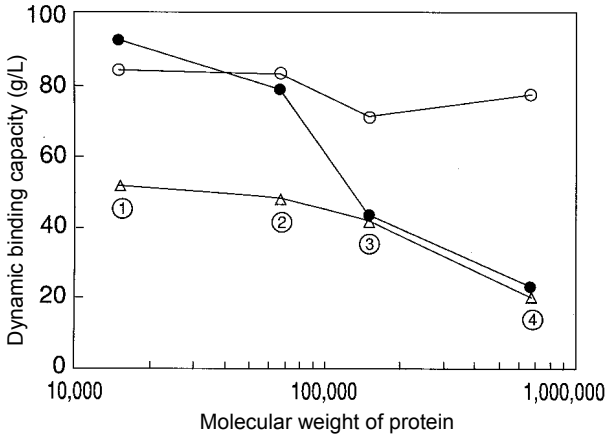

Figure-4 Effect of molecular weight of the sample on dynamic binding capacity

Columns:
- ○ TSKgel BioAssist Q 4.6mm I.D. × 1cm
- △ Conventional Q type product A 4.6mm I.D. × 1cm
- ● TSKgel SuperQ-5PW 4.6mm I.D. × 1cm

Flow rate: 0.38mL/min Temperature: 25°C
Detection: UV (280nm)
Sample solvent: 20mmol/L Tris-HCl buffer, pH8.0
Sample concentration:
- ① Trypsin inhibitor 10g/L
- ② Human serum albumin 10g/L
- ③ IgG1 2.3g/L
- ④ Thyroglobulin 5g/L

* The capacity was determined as 10% height of the breakthrough curve at UV 280nm.

Table-3 Comparison of dynamic protein binding capacity (BioAssist S)

Protein	Binding capacity (g/L)	
	BioAssist S	Conventional S type product C
γ-globulin	79	48
Lysozyme	84	63
Cytochrome C	95	43
α-chymotrypsinogen A	119	-

Columns: TSKgel BioAssist S
Conventional S type product C
Size: 4.6mm I.D. × 5mm (lysozyme, cytochrome C, α-chymotrypsinogen A)
5.0mm I.D. × 1cm (γ-globulin)
Solvent: 20mmol/L sodium phosphate buffer, pH6.5 (lysozyme, cytochrome C, α-chymotrypsinogen A)
20mmol/L sodium phosphate buffer, pH5.0 (γ-globulin)
Flow rate: 0.38mL/min
Temperature: 25°C
Detection: UV (280nm)

* The capacity was determined as 10% height of the breakthrough curve at UV 280nm.

Table-2 Comparison of dynamic binding capacity (BioAssist Q)

Protein	Binding capacity (g/L)			
	BioAssist Q	SuperQ -5PW	Conventional Q type product A	Conventional Q type product B
Thyroglobulin	**77.4**	22.9	20.2	1.8
Monoclonal IgG1	**57.8**	43.3	46.7	47.7
Human Serum Albumin	**83.1**	78.9	48.2	48.8
Trypsin Inhibitor	**84.3**	92.8	51.8	57.8

Columns:
- TSKgel BioAssist Q 4.6mm I.D. × 1cm
- TSKgel SuperQ-5PW 4.6mm I.D. × 1cm
- Conventional Q type product A 4.6mm I.D. × 1cm
- Conventional Q type product B 4.6mm I.D. × 1cm

Solvent: 20mmol/L Tris-HCl buffer, pH8.0
Flow rate: 0.38mL/min
Detection: UV (280nm)

* The capacity was determined as 10% height of the breakthrough curve at UV 280nm.

Table-4 Relationship of solvent pH to antibody binding capacity

Solvent pH	Binding capacity (g/L)	
	BioAssist S	Conventional S type product
7.0	0	0
6.5	1.5	0
6.0	67	0
5.5	62	30

Columns:
- TSKgel BioAssist S 5.0mm I.D. × 1cm
- Conventional S type product C 5.0mm I.D. × 1cm

Solvent: 20mmol/L sodium phosphate buffer, pH5.5
20mmol/L MES-HCl buffer, pH6.0
20mmol/L sodium phosphate buffer, pH6.5, 7.0
Flow rate: 0.44mL/min Temperature: 25°C
Detection: UV (280nm)
Sample: IgG1

* The capacity was determined as 10% height of the breakthrough curve at UV 280nm.

2-4. Effect of Sample Load

Figures-5 and 6 show overlaid chromatograms when proteins with different load were applied on TSKgel BioAssist Q and a commercial Q type, product A, respectively. With TSKgel BioAssist Q, little change in peak shape or separation was seen up to the load of 10mg. On the other hand, commercial Q type product A (**Figure-6**) showed an obvious change in peak shape for ovalbumin that elutes first at the load of 10mg. As you can see, TSKgel BioAssist Q is capable of being loaded with samples equivalent to commercial product or more, while maintaining separation and peak shape in spite of its smaller column size. **Figures-7 and 8** show overlaid chromatograms with sample load of 0.1mg and 2.5mg for TSKgel BioAssist S and a commercial S type product C. While peak shape and separation change at the load of 2.5mg with the commercial S type product C, TSKgel BioAssist S shows little change. Therefore, TSKgel BioAssist S is also applicable of sample loads equivalent to commercial product or more while maintaining separation and peak shape.

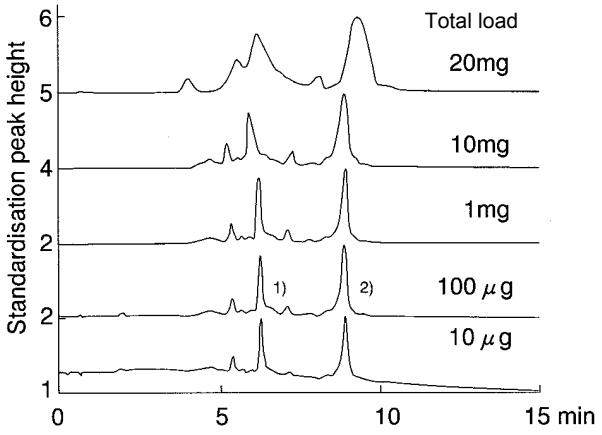

Figure-5 Effect of sample load on chromatogram (TSKgel BioAssist Q)

Column: TSKgel BioAssist Q 4.6mm I.D. × 5cm, PEEK
Eluent: A; 20mmol/L Tris-HCl buffer, pH8.0
B; 20mmol/L Tris-HCl buffer containing 1.0mol/L NaCl, pH8.0
Linear gradient from eluent A to B for 30 minutes
Flow rate: 1.0mL/min Temperature: 25°C Detection: UV (280nm)
Samples: 1) Ovalbumin 2) Trypsin inhibitor
* Chromatograms have been normalized.

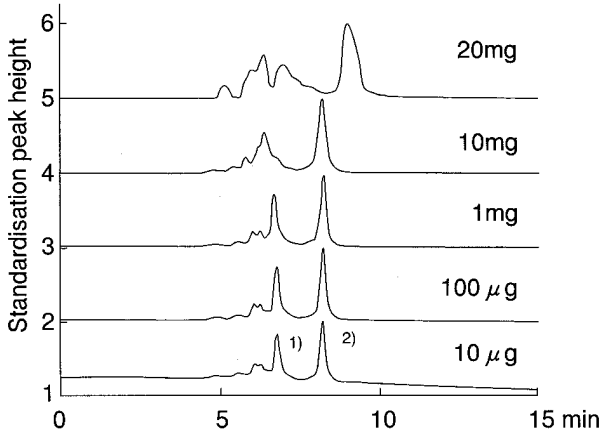

Figure-6 Effect of sample load on chromatogram (commercial Q type product A)

Column: Commercial Q type product A
5.0mm I.D. × 5cm, Glass

Other conditions are identical to Figure-5.
* Chromatograms have been normalized.

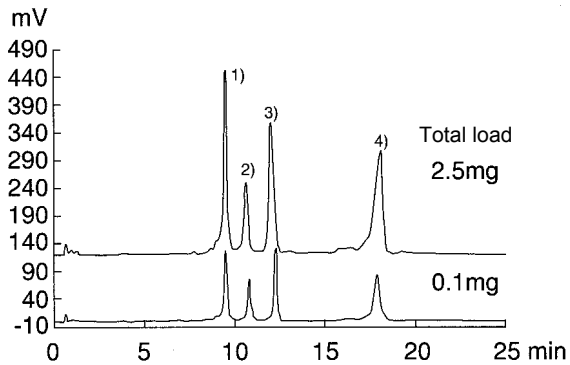

Figure-7 Effect of sample load on chromatogram (TSKgel BioAssist S)

Column: TSKgel BioAssist S 4.6mm I.D. × 5cm, PEEK
Eluent: A; 20mmol/L sodium phosphate buffer, pH6.5
B; 20mmol/L sodium phosphate buffer containing 1.0mol/L NaCl, pH6.5
Linear gradient from eluent A to B for 30 minutes
Flow rate: 0.8mL/min Temperature: 10°C
Detection: UV (280nm) (10mm cell for total load 0.1mg, 1mm cell for total load 2.5mg)
Samples: 1) α- chymotrypsinogen A 2) Ribonuclease A 3) Cytochrome C 4) Lysozyme

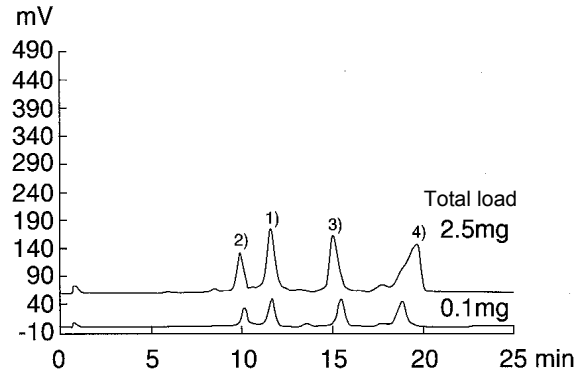

Figure-8 Effect of sample load on chromatogram (commercial S type product C)

Column: Commercial S type product C
5.0mm I.D. × 5cm, Glass

Other conditions are identical to Figure-7.

2-5. Dependence of Resolution on Flow Rate

Figure-9 shows the effect of flow rate on the peak width on TSKgel BioAssist Q. While the peak width becomes narrower as the flow rate increases, dependence of peak width on flow rate becomes small at 0.8mL/min and over. Although elution time is shortened little by little as the flow rate increases and it is possible to reduce the separation time, the flow rate of 1.0mL/min seems to be optimal, considering the fact that sample dilution by the eluent becomes larger and column's maximum pressure drops. Furthermore, similar results have been obtained on TSKgel BioAssist S, whose optimal flow rate is 0.8mL/min.

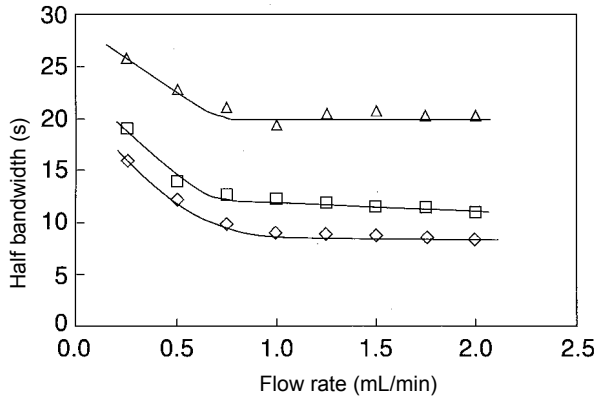

Figure-9 Dependence of peak width on the flow rate in protein separation on TSKgel BioAssist Q

Column: TSKgel BioAssist Q 4.6mm I.D. × 5cm, PEEK
Separation conditions:
same as Figure-2
(except flow rate and gradient time)
Gradient time ◇ 10 minutes
□ 15 minutes
△ 30 minutes
Sample: Ovalbumin

2-6. Dependence of Resolution on Gradient Time

The dependence of resolution on the gradient time is shown in **Figure-10** for TSKgel BioAssist Q. Although resolution is improved as the gradient time becomes longer, the slope becomes shallow when it reaches 20 minutes. Since the longer the gradient time is, the longer the analysis time becomes and the larger the sample dilution becomes, 20 to 30 minutes may be optimal for the gradient time. Similar results have been obtained on TSKgel BioAssist S.

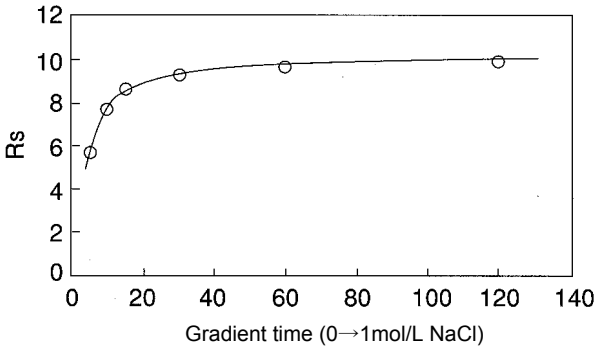

Figure-10 Dependence of resolution on gradient time in protein separation on TSKgel BioAssist Q

Column: TSKgel BioAssist Q 4.6mm I.D. × 5cm, PEEK
Separation conditions:
same as Figure-2 (except gradient time)
Samples: Ovalbumin, trypsin inhibitor

2-7. Recovery of Proteins

Tables-5 and 6 show the recovery of proteins on TSKgel BioAssist S and Q, respectively. Since TSK-GEL BioAssist series uses hydrophilic acrylate base material, it rarely causes non-specific adsorption and obtains a favorable recovery of various proteins even at a low sample load. **Figure-11** shows the antibody recovery in low sample load on TSKgel BioAssist S. While the recovery decreases when sample load decreases on a conventional styrene-type packings, the recovery is unaffected at low sample load on TSKgel BioAssist S. Sample recoveries of 90% in the range of 100ng to 20μg were observed. In addition, the recovery of angiotensin II which are a group of peptides is shown in **Figure-12**. Angiotensin II was not able to be detected in this range of loads on commercial S type product (top figure of **Figure-12**).

Table-5 Recovery of proteins on TSKgel BioAssist S

Sample name	Recovery (%)
Angiotensin II	98
Hemoglobin	93
γ-globulin	100
Lysozyme	109
Cytochrome C	105
α-chymotrypsinogen A	102

Column: TSKgel BioAssist S
4.6mm I.D. × 5cm, PEEK
Eluent: 20mmol/L sodium acetate buffer, pH5.0 (angiotensin II)
20mmol/L sodium phosphate buffer, pH6.5 (samples other than angiotensin II)
Sample load: 10μg
Measurement: Step gradient to 1.0mol/L NaCl soon after injection. Calculated from the peak area ratio against the blank test.

Table-6 Recovery of proteins on TSKgel BioAssist Q

Sample name	Recovery (%)
Angiotensin II	100
Ovalbumin	94
Trypsin inhibitor	107
Conalbumin	86
γ-globulin	93
Myoglobulin	95

Column: TSKgel BioAssist Q
4.6mm I.D. × 5cm, PEEK
Eluent: 20mmol/L Tris-HCl buffer, pH8.0
Sample load: 10μg
Measurement: Step gradient to 1.0mol/L NaCl soon after injection. Calculated from the peak area ratio against the blank test.

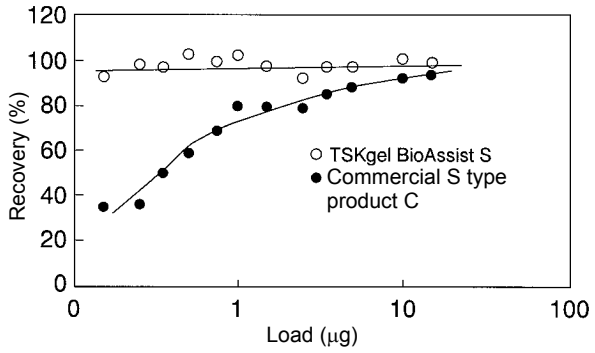

Figure-11 Comparison of γ-globulin recovery

Columns:
TSKgel BioAssist S 4.6mm I.D. × 5cm, PEEK
Commercial S type product C 5.0mm I.D. × 5cm, Glass
Measurement: Same as Table-5

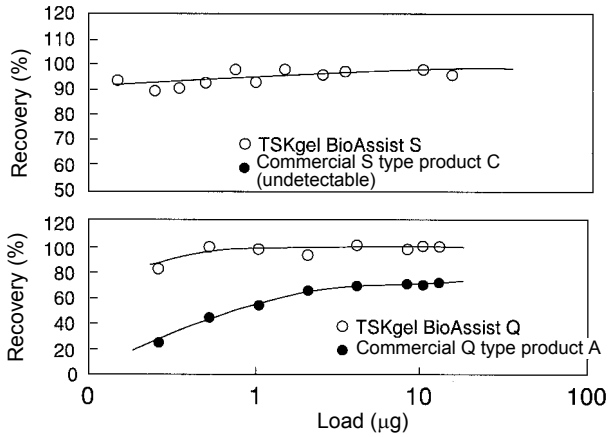

Figure-12 Comparison of recovery for Angiotensin II

Upper figure
Column:
TSKgel BioAssist S 4.6mm I.D. × 5cm, PEEK
Commercial S type product C 5.0mm I.D. × 5cm, Glass
Measurement: Same as Table-5

Bottom figure
Column:
TSKgel BioAssist Q 4.6mm I.D. × 5cm, PEEK
Commercial Q type product A 5.0mm I.D. × 5cm, Glass
Measurement: Same as Table-6

3. Operating Conditions

3-1. Pressure Drops

The maximum pressure drops of both TSKgel BioAssist Q and TSKgel BioAssist S is 2.5MPa. It is necessary to take care of pressure drop fluctuation which comes from the change of column temperature or eluent composition even though under the constant flow rate. We recommend to use at the optimal flow rate (1.0mL/min for TSKgel BioAssist Q, 0.8mL/min for TSKgel BioAssist S) in gradient analysis with increasing salt concentration that does not contain general organic solvents.

3-2. Caustic Stability

Column cleaning by alkaline solution is an effective method of column regeneration. The changes in selectivity of TSKgel BioAssist Q and S when 0.5mol/L NaOH solution is enclosed in each and left under room temperature are shown in **Figures-13 and 14**, respectively. Little change was seen in the chromatogram by either column even after 20 days. Furthermore, when a measurement method was repeated 50 times, in which 0.5mol/L NaOH solution of approximately 5 times the column volume was passed through after measuring a standard protein under the conditions similar to **Figures-2 and 3**, no change was seen in selectivity, pressure drops, etc.

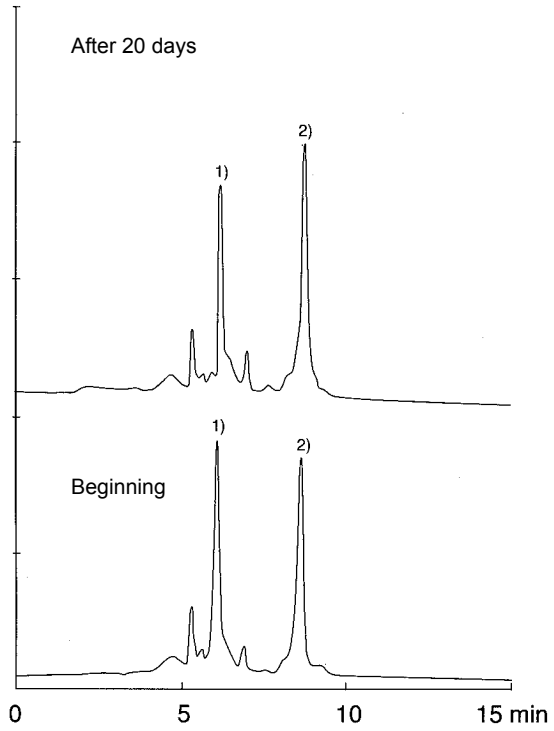

Figure-13 Caustic stability of TSKgel BioAssist Q in 0.5mol/L NaOH solution

Column:	TSKgel BioAssist Q 4.6mm I.D. × 5cm, PEEK	
Eluent:	A; 20mmol/L Tris-HCl buffer, pH8.0 B; 20mmol/L Tris-HCl buffer containing 1.0mol/L NaCl, pH8.0 Linear gradient from eluent A to B for 15 minutes	
Flow rate:	1.0mL/min	
Temperature:	25°C	
Detection:	UV (280nm)	
Injection volume:	60μL	
Samples:	1) Ovalbumin	1.0g/L
	2) Trypsin inhibitor	1.0g/L

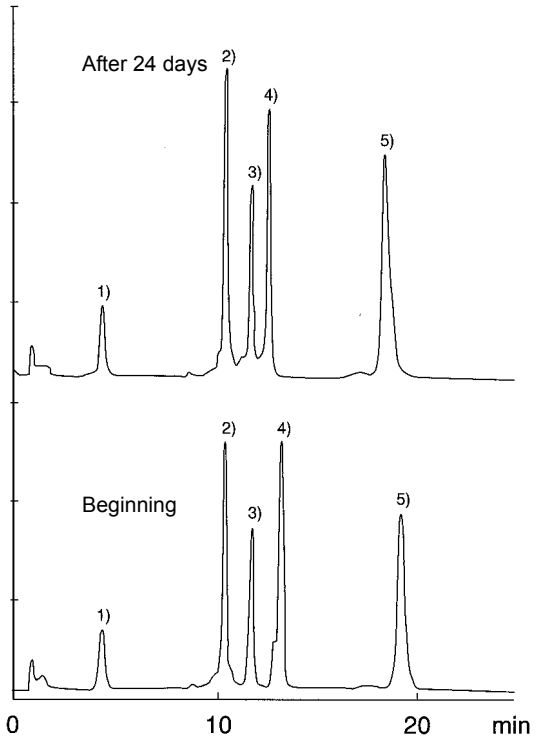

Figure-14 Caustic stability of TSKgel BioAssist S in 0.5mol/L NaOH solution

Column:	TSKgel BioAssist S 4.6mm I.D. × 5cm, PEEK	
Eluent:	A; 20mmol/L sodium phosphate buffer, pH6.5 B; 20mmol/L sodium phosphate buffer containing 1.0mol/L NaCl, pH6.5 Linear gradient from eluent A to B for 32 minutes	
Flow rate:	0.8mL/min	
Temperature:	10°C	
Detection:	UV (280nm)	
Injection volume:	20μL	
Samples:	1) Myoglobin	1.0g/L
	2) α-chymotrypsinogen A	2.0g/L
	3) Ribonuclease A	4.0g/L
	4) Cytochrome C	2.0g/L
	5) Lysozyme	2.0g/L

3-3. Buffer

Besides pollution originating from the sample, columns are also polluted by impurities included in the water or reagents used in the buffer. Therefore, make sure to use ultra pure water, distilled water for HPLC, distilled water for injection, etc. for water, and use reagents with HPLC grade or special grade. In addition, please filter the prepared buffers through a filter (0.22μm or 0.45μm).

3-4. HPLC System

In order to prevent penetration of insoluble compounds into the column in line filters are recommended between the pumping system and the injector. In addition, sample should be applied after filtration by disposable filter etc. in order to prevent penetration of insoluble component in the sample

3-5. Cleaning

Resolution may deteriorate due to impurities in the sample, clogging the end fitting or adsorbing onto the packings. In this case, resolution may be recovered by passing the eluent through the column in the backward direction or by cleaning with alkaline solution. Refer to the instruction manual attached to the column for more information.

4. Applications of Protein Separation

Figures-15 to 17 show application of protein separation on TSKgel BioAssist Q. **Figure-15** shows chromatograms of egg white. It is obvious that favorable separation is obtained on TSKgel BioAssist Q compared to commercial product. In **Figure-16**, chromatograms of mouse ascites fluid including monoclonal antibody are shown. A good separation between the antibody and albumin has been obtained. **Figure-17** shows chromatograms of commercial crude lipoxidase. It is apparent that good separation has also been achieved on this sample.
Figure-18 shows chromatograms of peptides on TSKgel BioAssist S. It is generally known that an accurate quantification is difficult to obtain when peptides, etc. are measured on a column with styrene-type base material, because sample tends to adsorb to the packings hydrophobically. However, TSKgel BioAssist S is capable of measuring peptides such as angiotensins without addition of organic solvents, etc. into the eluent since it is employed a hydrophilic acrylate as base material.

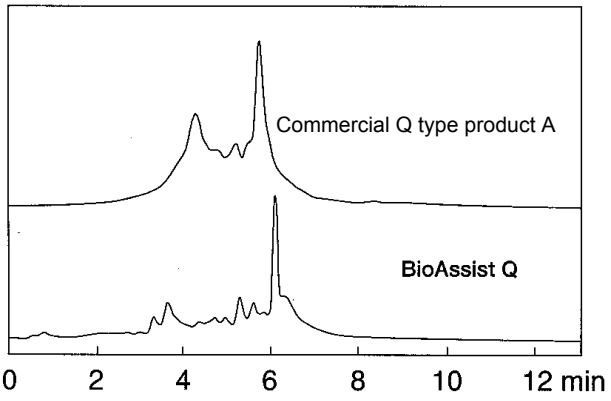

Figure-15 Comparison of egg white separation

Column:
TSKgel BioAssist Q 4.6mm I.D. × 5cm, PEEK
Commercial Q type product A 5.0mm I.D. × 5cm, Glass
Separation conditions are identical to Figure-2 except that the gradient time is changed to 15 minutes.

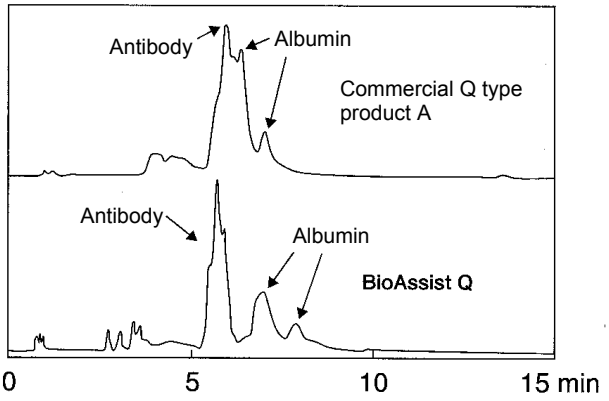

Figure-16 Comparison of mouse ascites antibody separation

Column:
TSKgel BioAssist Q 4.6mm I.D. × 5cm, PEEK
Commercial Q type product A 5.0mm I.D. × 5cm, Glass
Separation conditions are identical to Figure-2 except that the gradient time is changed to 15 minutes.

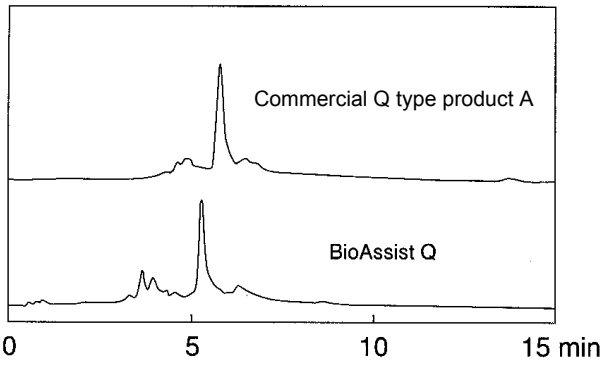

Figure-17 Comparison of lipoxidase separation

Column:
TSKgel BioAssist Q 4.6mm I.D. × 5cm, PEEK
Commercial Q type product A 5.0mm I.D. × 5cm, Glass
Separation conditions are identical to Figure-2 except that the gradient time is changed to 15 minutes.

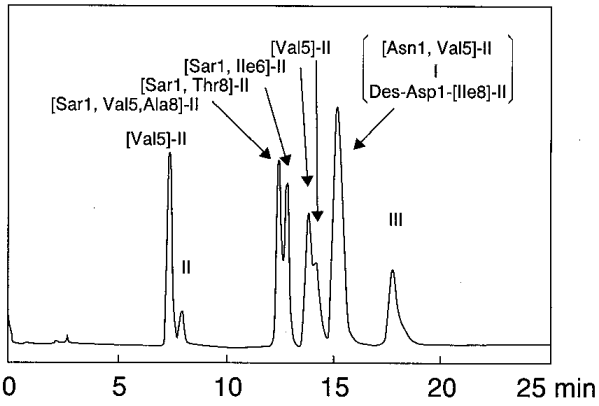

Figure-18 Separation of angiotensins

Column: TSKgel BioAssist S
4.6mm I.D. × 5cm, PEEK
Eluent: A; 20mmol/L sodium acetate buffer, pH5.0
B; 20mmol/L sodium acetate buffer containing 1.0mol/L NaCl, pH5.0
Linear gradient from eluent A to B for 20 minutes
Temperature: 25°C
Detection: UV (280nm)

5. Conclusion

This article has described the principle properties of the TSK-GEL BioAssist series including high binding capacity, high retention, and high resolution at a low column pressure drop relative to convention ion-exchangers in the marketplace. These attributes make the TSK-GEL BioAssist uniquely suited is to purification where high purity levels are required or separations of multi-component samples such as a crude extract of protein.