Separation Report No. 024

Applications for High-Performance Gel Filtration Columns (TSKgel SW and TSKgel PW columns): Focus on Biopolymers

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TOSOH BIOSCIENCE

3604 Horizon Drive, Suite 100, King of Prussia, PA 19406
Phone: (484) 805-1219 FAX: (610) 272-3028
Orders and Technical Service: (800) 366-4875
Member of the TOSOH Group

1. Introduction

Among the dramatic developments in high performance liquid chromatography (HPLC) that occurred during the early 1980's, advances in the application of HPLC for the analysis of biopolymers have played a major role. In earlier years there was a sense that biopolymer analysis was the sanctuary of low speed chromatography using soft gels such as cross-linked dextran gels and their derivatives, and was therefore outside the scope of HPLC. However, this myth broke down in the face of the rapid developments that took place during the early '80s in terms of producing LC packing materials with smaller particle sizes. For example, from the perspective of separation mode, there was a sense that while gel filtration chromatography (GFC) was still the premier mode of protein isolation, that high performance applications for ion exchange chromatography (IEC) and reversed phase chromatography (RPC) were making rapid inroads.

As shown in Table 1, Tosoh contributed to these developments by supplying a variety of outstanding columns and packing materials for this field. In particular, the high performance TSKgel SW and TSKgel PW columns established Tosoh's reputation as pioneers in the field. During the 1980's various uses for these columns were developed and published by researchers in laboratories all over the world.

This report reviews the important role played by high performance TSKgel GFC columns as tools in the analysis and purification of biopolymers.

For basic information on TSKgel SW columns (theoretical background, comparison with competitive products, selection of solvents and precautions on use, etc.) see the review paper by Horio et al.[1], which discuss these issues in detail.

To learn about more recent developments in the field of high performance gel filtration chromatography, see references 33-36, which are available for download on the Tosoh Bioscience website.

Table 1 TSKgel columns used in biopolymer separation

□ Used very often (or can be used)
A Used often
B Used to a certain extent

			Sample					
Separation mode	TSKgel Columns		Proteins	Peptides	Polynucleotides	Oligonucleotides	Polysacharides	Oligosaccharides
Gel filtration	SW	TSKgel G2000SW	A	A	B	A	B	B
		TSKgel G3000SW	□	B	A	B	A	D
		TSKgel G4000SW	A	D	A	D	A	D
	PW	TSKgel G2000PW	D	C	D		C	□
		TSKgel G3000PW	C	C	D	A	□	B
		TSKgel G4000PW	C	D	C	D	A	D
		TSKgel G5000PW	B	D	A	D	□	D
		TSKgel G6000PW	C	D		D	A	D
Ion exchange	Cation exchange	TSKgel SP-2SW	B	A				
		TSKgel CM-2SW	B	A				
		TSKgel CM-3SW	A	B				
		TSKgel SP-5PW	□	B				
	Anion exchange	TSKgel QAE-2SW	B	A	B	A		
		TSKgel DEAE-2SW	B	A	B	A		
		TSKgel DEAE-3SW	A	B	A	B		
		TSKgel DEAE-5PW	□	B	A	B		

2. Applications for TSKgel SW columns

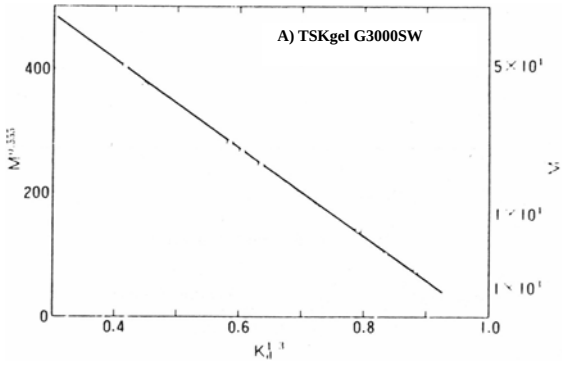
2-1. Methods for analyzing molecular mass of proteins and peptides

TSKgel SW columns have many merits, including excellent speed, operability, and reproducibility. Moreover, methods for analyzing molecular mass using the TSKgel SW columns now tend to be used for more diverse applications, replacing gel filtration methods using conventional soft gels and polyacrylamide-SDS electrophoresis. These columns may be used with both ordinary buffer systems and denatured solvent systems. Table 2 shows the characteristics of the main solvent systems.

Table 2 Characteristics of representative solvent systems used to analyze the molecular mass of proteins and peptides

Characteristic Ordi	nary buffer	SDS	Guanidine-HCl	Urea
1. Native or denaturated	□ native	denaturated	denaturated	denaturated
2. Separation range	○ wide	narrow	medium	medium
3. Linearity I) MW > 10,000	good	○ very good	□ excellent	good
2) MW < 10,000	not good	× bad	○ very good	good
4. Sensitivity to ionic strength	sensitive	× very sensitive	○ not sensitive	sensitive
5. Corrosion concern	not severe	not severe	× severe	not severe
6. Operating cost	○ low	○ low	× high	low
Typical condition	0.25mol/L sodium phosphate (SP)	0.2% SDS 0.2mol/L SP	6mol/L Guanidine-HCl	8mol/L urea

Guanidine hydrochloride systems, which have been investigated in detail by Ui[2], produce plots with outstanding linearity, as shown in Figure 1. As these systems contain a high concentration of chlorine ions, careful system maintenance is a necessity. SDS (sodium dodecyl sulfate) systems represent a popular method that has been investigated in detail by Takagi et al.[3] and Konishi et al.[4] However, as shown in Figure 2, this method has drawbacks in the limited range of linearity, as well as the fact that results depend heavily on the concentration of the salt in the solvent. Figure 3 shows a representative chromatogram produced by Konishi et al., using an SDS system. Ordinary buffer systems can handle proteins without causing denaturation, representing a major advantage, and characteristically show broad separation range. However, care must be exercised with this method, which depends heavily on the elution conditions (pH, salt concentration, etc.). Ui[5] investigated the applicability of guanidine hydrochloride systems for analyzing glycoproteins and found that results showed good linearity (Figure 4).



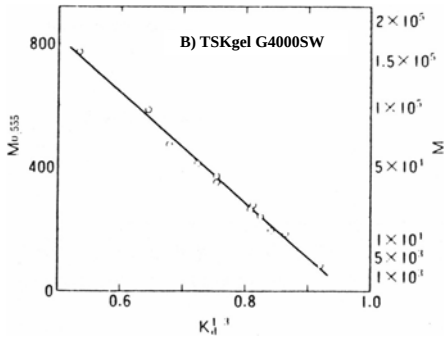


Figure 1 Protein calibration curves for TSKgel G3000SW and G4000SW columns in a 6mol/L guanidine hydrochloride system

Columns: TSKgel G3000SW and TSKgel G4000SW

Solvent: 6mol/L guanidine hydrochloride + 10mmol/L phosphate + 1mmol/L EDTA

Flow rate: 0.5mL/min

Detection: UV@280 nm

Samples*: polypeptides obtained by reduction and alkylation of various proteins

*Courtesy of Professor Nobuo Ui of Gunma University

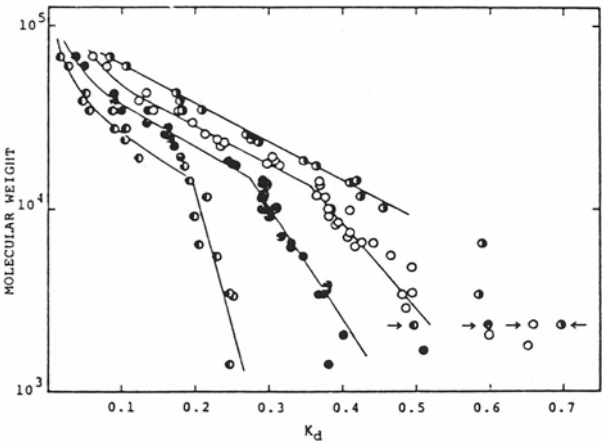


Figure 2 Calibration curves for polypeptides using a TSKgel 3000SW column in an SDS system (salt concentration dependence)

Column: TSKgel G3000SW

Solvent: 0.05mol/L sodium phosphate buffer (pH 7.0) + 0.1% SDS + NaCl

- ◐ 0.025mol/L NaCl
- ● 0.05mol/L NaCl
- ○ 0.10mol/L NaCl
- ◑ 0.20mol/L NaCl

Flow rate: 1.0mL/min

Detection: UV@280 nm

Samples*: various proteins and polypeptides produced by breaking down these proteins with CNBr

*Courtesy of Professor Katsutoshi Konishi of Dokkyo Medical University

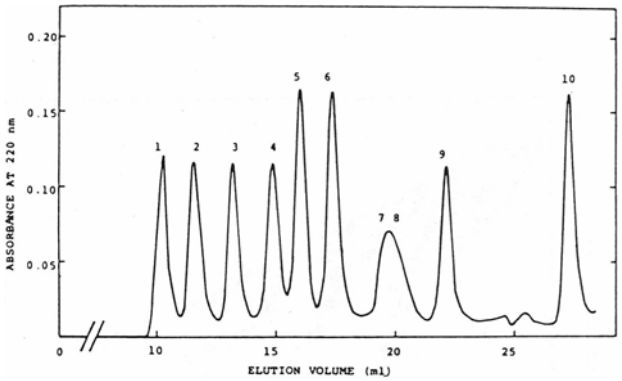


Figure 3 Separation of mixture of peptides in SDS system

Column: TSKgel G3000SW, 7.5mm ID x 60cm

Solvent: 0.2mol/L sodium phosphate buffer (pH 7.0) + 0.2% SDS + 0.2mol/L NaCl

Flow rate: 0.5mL/min

Detection: UV@280 nm

Samples*:
1. blue dextran
2. serum albumin
3. pepsin
4. tripsinogen
5. myoglobin
6. cytochrome C
7. aprotinin
8. insulin B chain
9. insulin A chain
10. 2-mercaptoethanol

*Courtesy of Professor Katsutoshi Konishi of Dokkyo Medical University

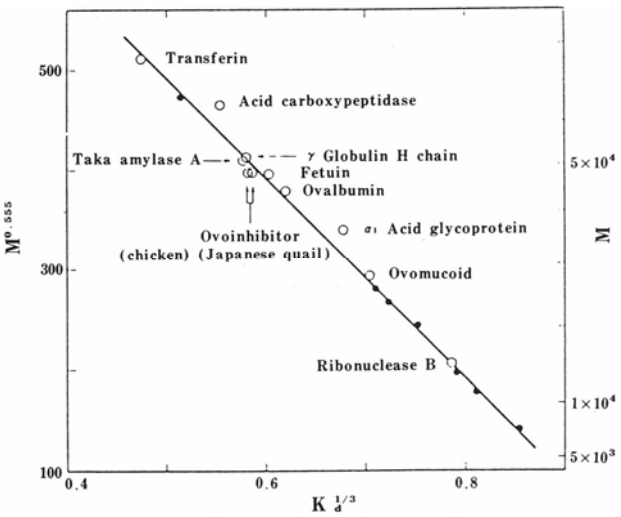


Figure 4 Calibration curve for polypeptides and glycopolypeptides in a 6mol/L guanidine hydrochloride system

Column: TSKgel G3000SW

Solvent: 6mol/L guanidine hydrochloride + 10mmol/L sodium phosphate + 1mmol/L EDTA (pH 6.5)

Flow rate: 0.5mL/min

Detection: UV@280 nm

Samples*: polypeptides and glycopolypeptides obtained by reduction and alkylation of proteins and glycoproteins

*Courtesy of Professor Nobuo Ui of Gunma University

Takagi et al.[6] and Takamatsu et al.[7] conducted detailed studies using HPLC-LALLS, which uses low-angle laser light scattering (LALLS) photometry for detection in combination with a differential refractometer. This powerful technique, which does not require calibration curves from standard samples, is considered to be the ultimate solution for analyzing molecular mass, and holds great promise for future development. Figure 5 shows changes in elution patterns produced by Takagi et al. by heating a solubilized biomembrane protein (porin).

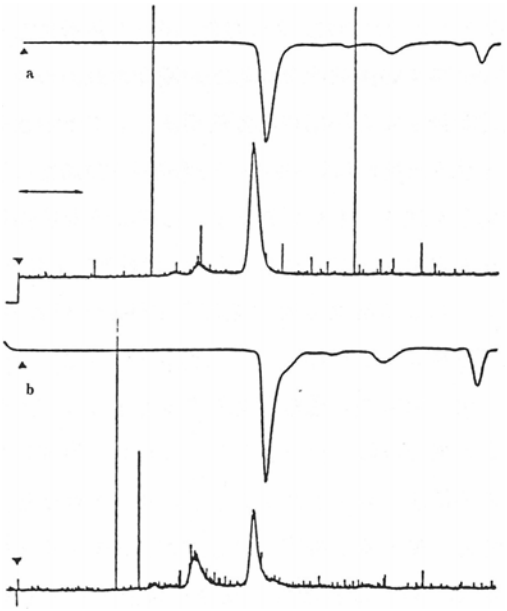


Figure 5 Changes in elution pattern of porin due to heat (HPLC-LALLS application example)

Column: TSKgel G3000SW, 7.5mm ID x 60cm

Solvent: 0.1mol/L sodium phosphate buffer (pH 6.9) + 2mmol/L SDS + 2mmol/L NaN_3

Flow rate: 0.16mL/min

Detection: low-angle laser light scattering photometer + differential refractometer

Samples*: a: porin solution before heating
b: porin solution after heating at 100°C for 5min

*Courtesy of Professor Toshio Takagi of the Institute for Protein Research, Osaka University

2-2. Analysis of Proteins and Peptides

Numerous ground breaking methods of analysis continue to be developed based on the outstanding properties of TSKgel SW columns, such as their speed, high performance and excellent reproducibility. High speed is most often employed in methods used to analyze the course of chemical reactions associated with the chemical alteration of proteins (denaturation, condensation, degradation, etc.). Table 3 shows representative application examples of this application.

Tomono et al.[8] tracked the course of enzyme digestion of commercial IgG by pepsin in a TSKgel G3000SW column (Figure 6). Ingham et al.[10] has tracked the course of thermal denaturation of antithrombin III while studying the inhibitory effect of stabilizers on denaturation.

Table 3 Examples of experiments tracking changes over time in protein reactions using TSKgel SW columns

No.	Content	Author
1	Analysis of peptic fragmentation of human immunoglobulin G	T. Tomono et al.[8]
2	Proteolytic conversion of active to inactive ubiquitin	K. D. Wilkinson et al.[9]
3	Thermal denaturation of antithrombin III	K. C. Ingham et al.[10]
4	Thermal stability of human chorionic gonadotropin	K. C. Ingham et al.[11]
5	Renin-RBP complex equilibrium	K. Murakami et al.[12]

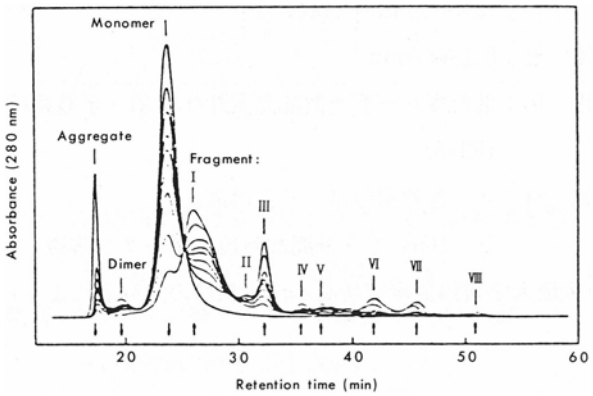


Figure 6 Tracking changes over time in degradation products of commercial IgG by pepsin

Column: TSKgel G3000SW, 7.5mm ID x 60cm x 2

Solvent: 0.05 mol/L acetate buffer (pH 5.0) + 0.1 mol/L sodium sulfate

Flow rate: 1.0mL/min

Samples*: 100µL solutions produced by digestion of IgG (20g/L) by pepsin after 0, 2, 4, 6, 8, 10, 15, 30, and 60min

*Courtesy of Tsugikazu Tomono, Director of Japanese Red Cross Central Blood Center

Hara et al.[11] have conducted a detailed study of the methods of analyzing lipoproteins in serum and established a method for measuring the molecular mass of lipoproteins and lipids. Speed, peak sharpness, and the high degree of sensitivity resulting from post-labeling techniques are major advantages of this method for practical applications. Serum samples of around 10µL are sufficient, and can be loaded without pretreatment. Figure 7 shows some representative chromatograms.

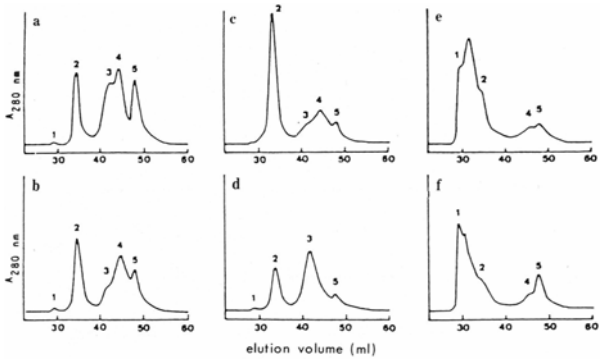


Figure 7 Degradation patterns of various serum lipoproteins

Columns: TSKgel G5000PW, 7.5mm ID x 60cm
+ TSKgel G3000SW, 7.5mm ID x 60cm x 2

Solvent: 0.1mol/L tris-HCl buffer (pH 7.4)

Flow rate: 1.0mL/min

Detection: UV@280nm

Samples*: human serum
(a) young woman
(b) elderly man
(c) myocardial infarction patient
(d) hepatic cirrhosis patient
(e), (f) hyperlipidemia

Peak 1	VLDL
Peak 1	VLDL
Peak 2	LDL
Peak 3	HDL2
Peak 4	HDL3
Peak 5	Albumin

*Courtesy of Professor Ichiro Hara, Tokyo Medical and Dental University

Suzuki et al.[15] have conducted detailed studies involving the quantitative analysis of metallothionein. In these studies, the liver and kidney of cadmium-administered rats were used as samples, and the columns were directly coupled to an atomic absorption detector. The advantage of this technique is that metallothionein can be separated into two isozymes for analysis. Presumably the cation exchange capacity of the TSKgel SW gel due to its silanol group plays a role in this isozyme separation. Representative chromatograms are shown in Figure 8.

Tomono et al.[15] has conducted a detailed study of the analysis of plasma proteins. The results are widely used for quality control purposes in the manufacture of blood preparations. Figure 9 shows a chromatogram of serum analyzed by Tomono et al. Table 4 shows the results of analysis of immunoprecipitation of the five fractions shown in the figure.

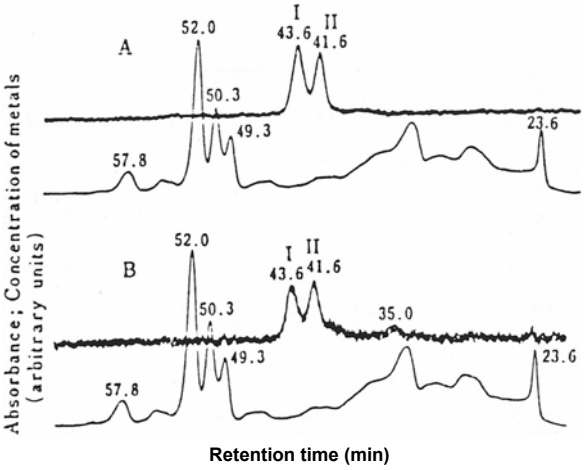


Figure 8 Chromatogram of liver supernatant of Cd-administered rat

Column: TKgel G3000SW, 21.5mm ID x 60cm

Solvent: 50mmol/L Tris-HCl buffer

Flow rate: atomic absorption (Cd, Zn) + UV@280 nm
A: Cd
B: Zn

Samples*: rat liver supernatant

Peak I	Metallothionein I
Peak II	Metallothionein II

*Courtesy of Professor Kazuo Suzuki of the National Institute for Environmental Studies

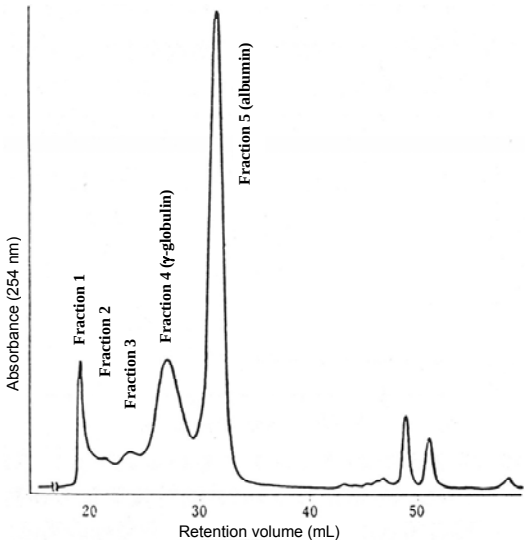


Figure 9 Chromatogram of human serum proteins produced using TSKgel G3000SW

Column: TSKgel G3000SW, 7.5mm ID x 60cm x 2

Solvent: 0.05mol/L sodium acetate buffer (pH 5.0) + 0.1mol/L sodium sulfate

Flow rate: 1.0mL/min

Samples*: human serum, 30μL

*Courtesy of Tsugikazu Tomono, Director of Japanese Red Cross Central Blood Center)

Table 4 Results of analysis of immunoprecipitation of human serum fractions using TSKgel G3000SW

Antiserum	Serum	Fr-1	Fr-2	Fr-3	Fr-4	Fr-5	Molecular mass	Quantity in serum (mg/dl)
Prealbumin	+	–	–	–	–	+	61,000	20
Albumin	++	–	–	–	–	++	67,000	4,000
α_1-Lipoprotein	+	–	±	+	+	–	195,000～435,000	360
α_1-Acidglycoprotein	+	–	–	–	–	+	44,000	60
α_1-Antitrypsin	+	–	–	–	–	+	54,000	300
Ceruloplasmin	+	–	–	–	+	–	160,000	35
Haptoglobin	+	–	+	+	+	–	100,000～400,000	100
α_2 HS-glycoprotein	+	–	–	–	+	+	49,000	50
α_2-Macroglobulin	+	±	±	±	–	–	820,000	250
Transferrin	+	–	–	–	±	+	90,000	250
β-Lipoprotein	+	+	±	–	–	–	3,200,000	360
C3-Component	+	–	–	–	+	–	240,000	100
Immunoglobulin G	++	–	–	±	++	±	150,000	1,300
Immunoglobulin A	+	–	±	+	±	–	160,000	210
Immunoglobulin M	+	+	–	–	–	–	900,000	140

2-3 Protein and peptide purification

TSKgel SW columns allow for fast purification and produce a high level of purity, and are thus now frequently used in preparative chromatography. Table 5 shows important examples of this usage.

Even with standard analytical columns (internal diameter, 7.5mm), samples on the order of tens of milligrams can be processed daily if several milligrams are processed each run or if processes are run repeatedly.

If a preparative column is used (internal diameter, 21.5mm), samples can be purified in quantities ranging from tens of milligrams up to about 100mg per run, and if used repeatedly, quantities ranging from hundreds of milligrams to several grams per day can be processed. Kato et al.[23] has investigated sample loads for preparative columns using bovine serum albumin. This study showed that ß-galactosidase and urease could be purified efficiently and with excellent recovery of activity (Figures 10, 11). Wehr et al. conducted detailed studies on sample load in the purification of apolipoproteins in a guanidine hydrochloride system. Although the sample load appeared to be smaller than that found by Kato et al., this is presumably due to the high viscosity of the solvent.

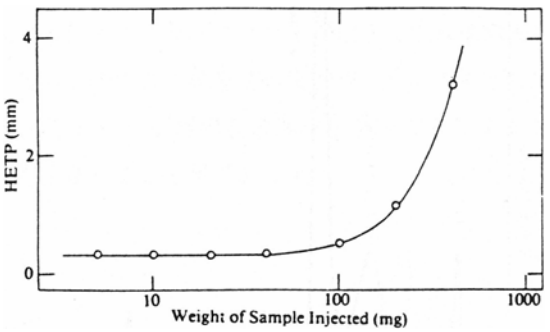


Figure 10 Investigation of sample loads for TSKgel G3000SW (preparative column) using bovine serum albumin *[Relationship between height equivalent to theoretical plates (HETP) and sample load (mg)]*

Column: TSKgel G3000SW, 21.5mm ID x 60cm

Solvent: 0.1mol/L phosphate buffer (pH 7) + 0.3mol/L sodium chloride

Flow rate: 8mL/min

Detection: differential refractometer (RI)

Samples: bovine serum albumin solution, 4mL

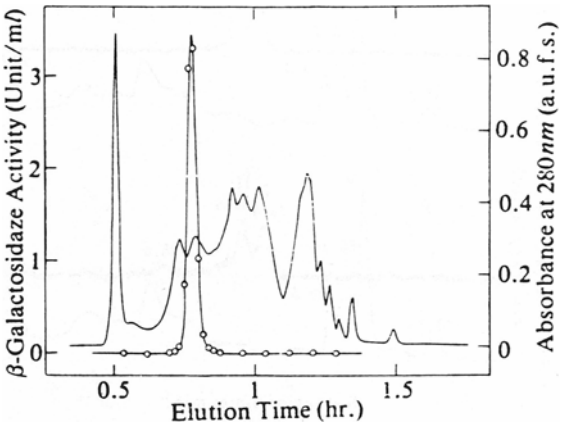


Figure 11 Purification of crude ß-galactosidase using TSKgel G3000SW

Column: TSKgel G3000SW, 21.5mm ID x 60cm

Solvent: 0.2mol/L phosphate buffer (pH 6.7)

Flow rate: 5mL/min

Detection: UV@280 nm + enzymatic activity

Sample: crude ß-galactosidase, 2.5% solution, 3mL

Table 5 Protein purification examples using TSKgel SW columns

Author	Sample	Column size ID (mm)	Eluent
D. Polacek[16]	Apolipoprotein	7.5	6mol/L Urea
K. K. Kohli[17]	Cytochrome P-450	7.5	Phosphate buffer (PB) + detergent
J. H. Collins[18]	Triptic digest of acanthamoeba myosin II	7.5	6mol/L Guanidine-HCl
M. R. Maurizi[19]	Glutamine synthetase from E.Coli	7.5	PB or Trisacetate
L. E. Walker[20]	HLA-DR1 antigen	7.5	PB + 0.1% SDS
J. E. Hurley[21]	Cyclic CMP phosphodiesterase	7.5	Morpholinopropane sulfonic acid + Na_2SO_4
E. Furuya[22]	Fructose-6-phosphate-2-kinase	7.5	PB
Y. Kato[23]	β-Galactosidase	21.5	PB
Y. Kato[24]	Human serum	21.5	PB
T. Tomono[25]	Plasma protein	7.5, 21.5	PB
T. Wehr[26]	HDL apolipoprotein	21.5	6mol/L Guanidine-HCl
R. Asada	γ-Globulin	55	PB

Very large scale columns (internal diameter, 55-108mm) are capable of purification in quantities ranging from hundreds of milligrams to several grams per run, and from several grams to tens of grams per day. With scaling up to this level, these columns are expected to be sufficient for use in purification processes on an industrial scale for high value-added proteins, enzymes, and peptides. Purification by high performance GFC has a number of advantages, including a) speed (high throughput), b) high purity, c) ease of automation, d) solvents recovered in high concentrations, and e) fast method development. However, perhaps most important is the fact that with HP-GFC, the time and effort required to scale up can be dramatically reduced. As a result, a major advantage is the absence of any decline in separation performance when scaling up from the analytical level to large scale columns. Figures 12 and 13 show that TSKgel SW large scale columns are designed to provide the same level of separation as analytical level columns.

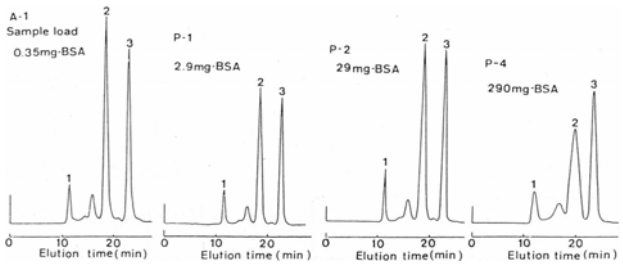


Figure 12 Comparison of TSKgel G3000SW analytical and preparative columns

Columns: TSKgel G3000SW (A: 7.5mm ID x 60cm)
TSKgel G3000SW (P: 21.5mm ID x 60cm)

Solvent: 0.1mol/L phosphate buffer (pH 6.8) + 0.1mol/L sodium chloride

Flow rate: 120mL/hr·cm^2 (A=0.89mL/min, P=7.3mL/min)

Detection: UV@280nm

Samples: 1. blue dextran 2. bovine serum albumin
3. myoglogin

2-4 Separation of nucleic acids

Until recently, applications of HP-GFC technology to nucleic acid have lagged far behind those for proteins, but the development of applications is expected to advance rapidly in the future. High performance GFC is inferior to electrophoresis in terms of separation performance, but continues to receive high marks due to many advantages, including superior operability and speed, as well as good yields and the ease with which high volume fractionation can be accommodated.

Figure 14 shows an RNA separation exampled from Konishi et al.[26] Wilkinson et al.[9] reported on a very effective method for purifying rabbit 9S-globin mRNA by high performance GFC using a TSKgel SW column. In that study, better results were obtained with a 6mol/L urea solution system than with a normal buffer solution system. In the future, TSKgel SW columns are expected to see frequent use in DNA fragment analysis and crude fractionation.

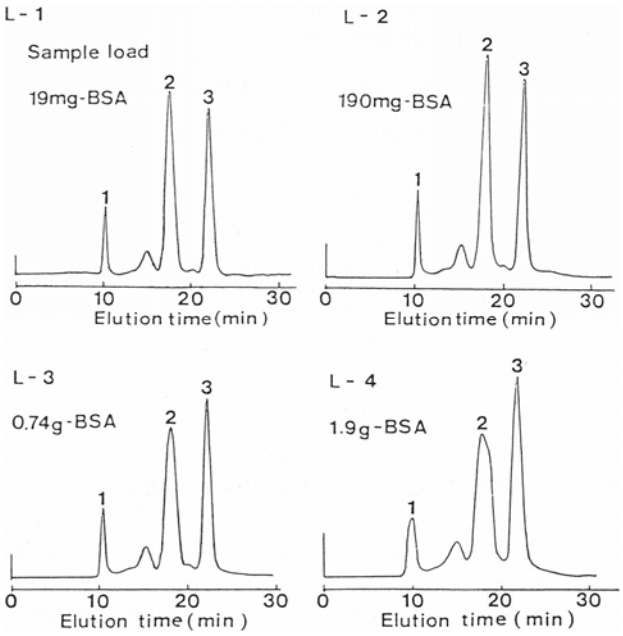


Figure 13 Effect of sample load on TSKgel G3000SW large scale preparative column

Columns: TSKgel G3000SW, 5.5mm ID x 60cm

Solvent: 0.1mol/L phosphate buffer (pH 6.8) + 0.1mol/L sodium chloride

Flow rate: 48mL/min

Detection: UV@280nm

Samples: 1. blue dextran 2. bovine serum albumin
3. myoglogin

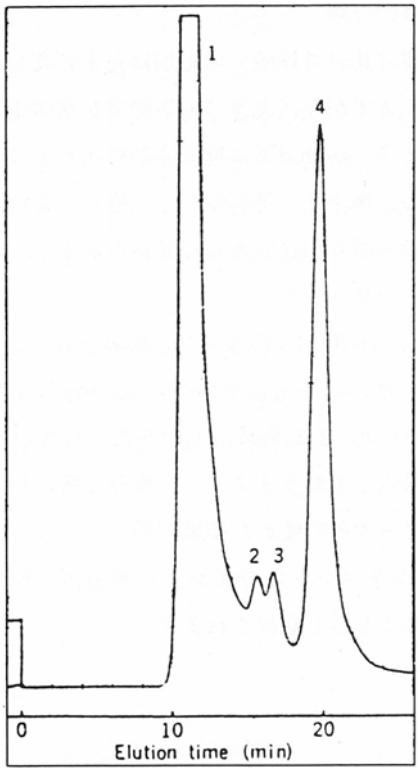


Figure 14 Elution pattern of rat liver cytoplasmin RNA produced by TSKgel G3000SW

Column: TSKgel G3000SW, 7.5mm ID x 60cm

Solvent: 0.2mol/L phosphate buffer (pH 7.0) + 0.1% SDS

Flow rate: 0.90mL/min

Detection: UV@260nm

Samples: rat liver cytoplasmin extracted at 65°C
Peak 1 28 S + 18 S
Peak 2 5.8 S
Peak 3 5 S
Peak 4 4 S RNA

3. Application of TSKgel PW-type columns

Table 6 shows representative application examples for the PW-type column. PW-type columns are mainly used for the analysis of water-soluble synthetic polymers, but under some circumstances can also be used for biopolymers to supplement SW-type columns. TSKgel PW-type columns offer the following advantages: a) several grades with large exclusion limit (TSKgel G5000PW and TSKgel G6000PW); b) good resolution in the oligomer range (TSKgel G2000PW); c) good linearity of the calibration curve; and d) excellent stability under alkaline conditions.

3-1 Po lysaccharide analysis

A coupled column system in which the TSKgel G5000PW (or TSKgel G6000PW) is coupled to a TSKgel G3000PW column is often used in the analysis of polysaccharides. Under these circumstances, combining the features described under a) and c) above, good linearity of the calibration curve is obtained over a wide molecular mass range (from 10million to several hundred dalton). In manufacturing clinical grade dextran, a TSKgel G5000PW column coupled to a TSKgel G3000PW column, Alsop et al.[28] reported very good reproducibility over a long period of time in quality control analyses in which molecular mass distribution was measured. Kato et al.[29] used a TSKgel PW-type column for the purpose of characterizing commercial dextran and pullulan.

Table 6 Representative application examples for TSKgel PW columns

Classification	Examples
1. Synthetic polymers • nonionic • cationic • anionic	• PEG, polyglycerin, polyacrylamide • polyethyleneimine, polyvinylpyrolidone • Poly (sodium acrylate), Poly (sodium styrene sulfonate)
2. Polysaccharides and derivatives	• standard dextran, clinical dextran, pullulan inulin, heparin, chitosan • carboxymethylcellulose
3. Very large biopolymers • polynucleotides • viruses • proteins	• DNA fragments • TMV, SBMV, TBSV • lipoprotein (VLDL,LDL), apoferritin, gelatin, sea worm chlorocruorin
4. Small molecules • oligomers • others	• oligossaccharides (dextran hydrolysate, cyclodoxtrin hydrolysate), cyclodextrins • oligopeptides • oligonucleotides

3-2 Oligosacchari de analysis

The TSKgel G2000PW column exhibits noticeably better separation performance than the TSKgel G2000SW column in a molecular mass range up to 1,000, and is frequently used to analyze oligosaccharides. Hiromi et al.[30] obtained good results using the TSKgel G2000PW column to analyze degradation products of amylose produced by amylase. Hayashi et al.[31] has used the TSKgel G2000PW column to analyze chito-oligosaccharides.

3-3 Pro tein analysis

From the perspective of separation range, either the TSKgel G3000PW or TSKgel G4000PW column can be used for normal size proteins. However, a TSKgel PW column is not often used, as its resolution is inferior (because the theoretical plate numbers are lower and the slope of the calibration curve is steeper) to that of the TSKgel SW column. However, a TSKgel PW column should be considered when alkaline mobile phase conditions are required for protein stability. TSKgel PW-type columns can also be cleaned using high pH solutions, and they are more robust than TSKgel SW columns when using samples that contain strongly adsorbing components. The larger pore size TSKgel G5000PW and G6000PW columns are also more appropriate when analyzing proteins that exceed the separation range of the TSKgel G4000SW column. Hara et al.[13] often use a coupled column system consisting of TSKgel G5000PW combined with the TSKgel G3000SW column for the analysis of lipoproteins. With a coupled column system, in which the TSKgel G4000SW and TSKgel G3000SW columns are coupled together, both VLDL and LDL elute in the void volume. In contrast, with a coupled column system that employs the TSKgel G5000PW column, both of these substances can be separated, and a pattern that can be seen over a wide area ranging from VLDL to HDL is generated (Figure 7). Himmel et al.[32] studied the elution behavior of viruses and giant proteins using the TSKgel G5000PW preparative column (internal diameter 21.5mm). They reported that sea worm chlorocruorin (molecular mass: 2.9million) and apoferritin dimer (molecular mass: 960,000) were within the separation range. Figure 15 shows a chromatogram obtained by Himmel et al. for proteins and viruses.

TSKgel SW columns can also be used to analyze gelatin. However, in general, because gelatin has a broad molecular mass distribution and the polymer domain is often partially excluded even with the TSKgel G4000SW column, column sets centering on the TSKgel G5000PW or TSKgel G6000PW column are frequently used.

3-4 Applications of very large biopolymers (nucleic acid and viruses, etc.)

The TSKgel G4000SW column has the ability to separate DNA fragments up to about 700 base pairs, while the TSKgel G5000PW column is useful for analyzing larger fragments.

As discussed above, Himmel et al.[32] investigated the analysis of several types of viruses using a TSKgel G5000PW preparative column.

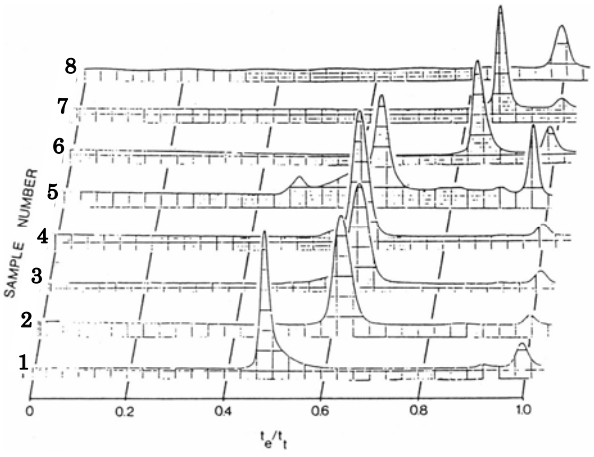


Figure 15 Virus and protein elution patterns produced by TSKgel G5000PW

Column: TSKgel G5000PW, 21.5mm ID x 60cm

Solvent: 0.01mol/L phosphate buffer (pH 7.0) + 0.1mol/L potassium chloride

Flow rate: 0.96mL/min

Detection: UV@280 nm

Samples:
1. TMV (tobacco mosaic virus)
2. TBSV (tomato bushy stunt virus)
3. SBMV (southern bean mosaic virus)
4. TYMV (turnip yellow mosaic virus)
5. apoferritin dimer
6. hemoglobin
7. myoglobin
8. cytochrome C

4. Conclusio ns

In this Separation Report we have highlighted important studies that were performed by researchers in Japan and elsewhere in the early 1980's following the introduction of TSKgel SW- and PW-type columns. These studies laid the groundwork for many breakthroughs in biochemical analysis that have taken place since that time. And while academic and industrial scientists advanced our understanding of the natural world, Tosoh researchers have continued the development of GFC columns to keep up with the pace of innovation. For details of recent developments in Gel Filtration columns, we recommend that you consult the Separation Reports listed in references 33-36 that can be downloaded from our web site.

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TOSOH BIOSCIENCE

TOSOH Bioscience LLC
3604 Horizon Drive, Suite 100
King of Prussia, PA 19406
Orders & Service: (800) 366-4875
Fax: (610) 272-3028
www.separations.us.tosohbioscience.com
email: info.tbl@tosoh.com

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