No.046

SEPARATION REPORT

TSKgel SW_{XL} Series

— Contents —

		Page
1.	Introduction	1
2.	Characteristics	1
3.	Basic Properties	5
	3-1 Dependence of height equivalent to a theoretical plate (HETP) on flow rate	5
	3-2 Ionic properties	5
	3-3 Sample load	6
	3-4 Protein recovery	6
4.	TSKgel SW_{XL} series applications	7
5.	Conclusion	8

1. Introduction

With all the remarkable advances that have been made in high-performance liquid chromatography (HPLC), the application of HPLC to biopolymer separation now seems natural. Until recently, the field of preparative chromatography of biopolymers had been exclusively monopolized by low-performance chromatography in which soft gels are used. However, with the development of high-speed, high-performance technology and equipment, HPLC has begun to make inroads in this area. Beginning with what now seem like classic modes of separation, gel filtration chromatography (GFC), ion exchange chromatography (IEC) and reversed-phase chromatography (RFC) have come to be established as general methods of analysis in the field of HPLC, and rapid advances have also recently been made in the fields of hydrophobic interaction chromatography (HIC) and affinity chromatography (AFC).

Tosoh has contributed to these developments in the field of HPLC by providing a variety of columns and packing materials. This is particularly the case in the area of GFC columns, as the TSKgel SW series is used worldwide, and it is no exaggeration to say that GFC using SW has now been established as the standard analytical method.

In response to demands for smaller particle sizes and increased performance in the TSKgel series, we developed and began marketing the TSKgel SW_{XL} series, the features and basic properties of which are discussed in this report. A few examples of its application are also introduced.

Table 1 TSKgel SW_{XL} Series Specifications

Column	Particle size (µm)	Guaranteed theoretical plate number (TP/column)	Column dimensions (mmI.D. × cm)
G2000SW_{XL}	5	20,000	7.8 × 30
G3000SW_{XL}	5	20,000	
G4000SW_{XL}	8	16,000	

Analytical conditions for theoretical plate number
Solvent: Distilled water
Flow rate: 1 mL/min
Sample: 1% ethylene glycol, 20 µL

Table 2 Molecular mass separation range of TSKgel SW_{XL} Series

Column	Polyethylene glycol	Dextran	Protein
G2000SW_{XL}	500～15,000	1,000～30,000	5,000～100,000
G3000SW_{XL}	1,000～35,000	2,000～70,000	10,000～500,000
G4000SW_{XL}	2,000～250,000	4,000～500,000	20,000～7,000,000

2. Characteristics

Table 1 shows the specifications of the SW_{XL} series. Table 2 shows the separation ranges for polyethylene glycol (PEG), dextran, and protein. Dimensions of all columns in the SW_{XL} series are 7.8 mmI.D.. × 30 cm. Because the packing materials used have a smaller particle size than those of the conventional SW series of columns, the guaranteed theoretical plate number is increased approximately 2-fold in comparison with the SW series, as shown in Table 1.

Figures 1 and 2 show the calibration curves created using the TSKgel SW_{XL} series when analyzing the standard samples above. Figures 3, 4 and 5 show chromatograms for standard proteins produced using the SW_{XL} series and the conventional SW series of columns. Table 3 shows the resolution (R_S) calculated from these chromatograms. From the table, it is clear that the SW_{XL} series provides separation performance that is equivalent to or better than that obtained with 60-cm columns of the conventional SW series. As a result, using the SW_{XL} series will reduce analysis times by half with no change in separation performance.

Figure 6 shows the relationship between the resolution and the molecular mass of proteins. The figure also shows the optimum separation ranges for these columns. In general, the G2000SW_{XL} is suitable for separating proteins with a molecular mass of 70,000 or less, the G3000SW_{XL} for proteins with a molecular mass between 70,000 and 300,000, and the G4000SW_{XL} for proteins with a molecular mass of 300,000 or over.

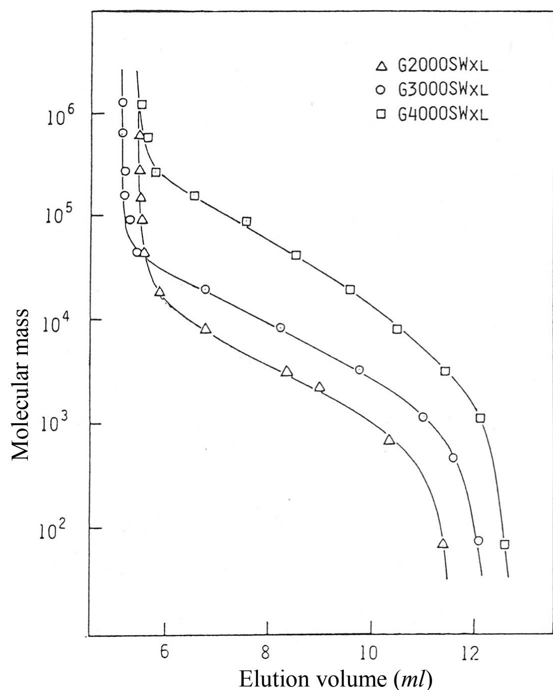


Figure 1　Calibration curves produced with PEG

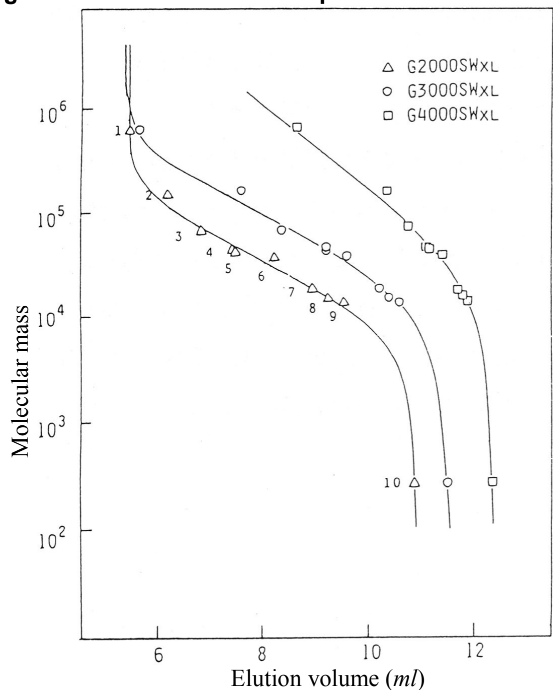
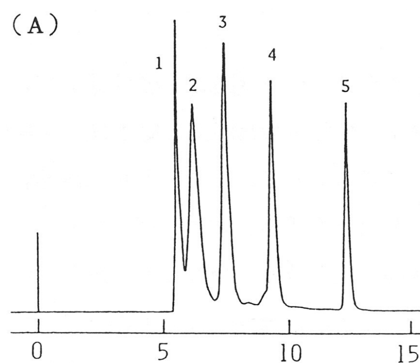
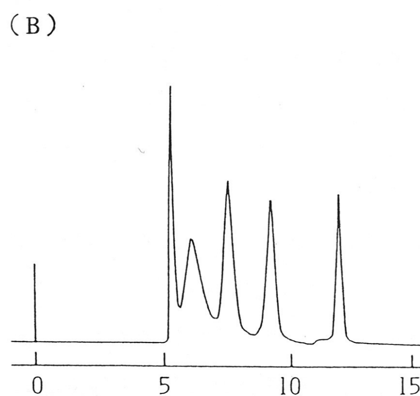


Figure 2　Calibration curves produced with proteins

Columns: TSKgel SWXL Series (7.8 mmI.D. × 30 cm)
Solvent: 0.05 mol/L phosphate buffer (pH 7) + 0.3 mol/L NaCl
Flow rate: 1 mL/min
Temperature: 25°C
Detection: UV (220 nm)
Samples:
1. Thyroglobulin
2. γ-globulin
3. Bovine serum albumin
4. Ovalbumin
5. Peroxidase
6. β-lactoglobulin
7. Myoglobin
8. Ribonuclease A
9. Cytochrome C
10. Glycine tetramer





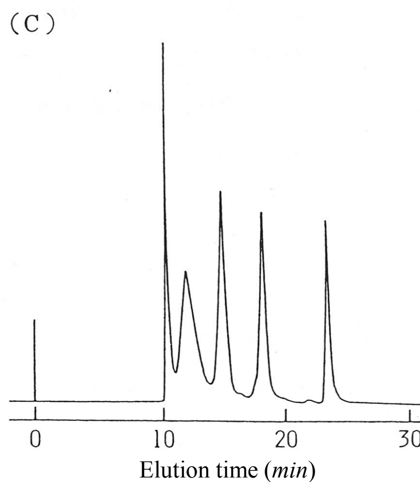
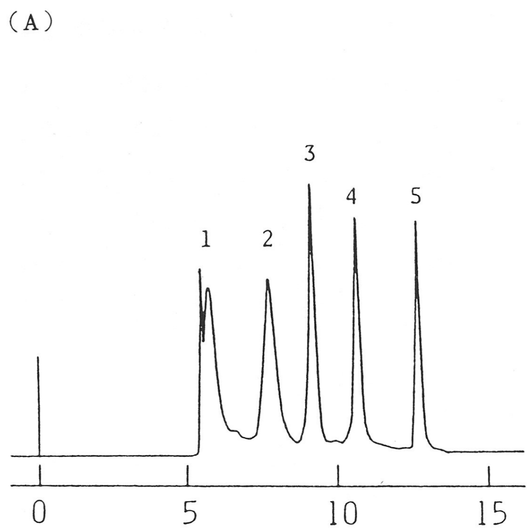
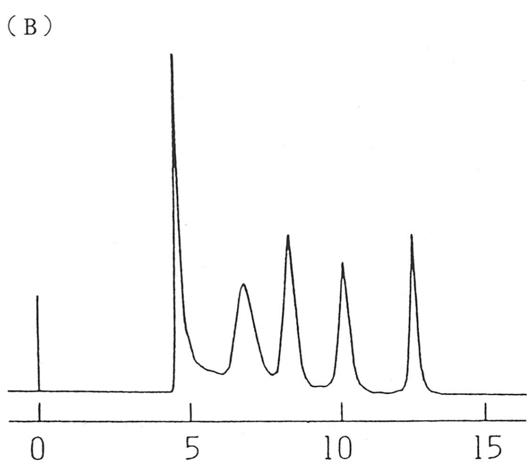


Figure 3　Comparison of SWXL and SW series (1)

Columns:
(A) TSKgel G2000SWXL 7.8 mmI.D. × 30 cm
(B) TSKgel G2000SW 7.5 mmI.D. × 30 cm
(C) TSKgel G2000SW 7.5 mmI.D. × 60 cm
Solvent: 0.05 M phosphate buffer (pH 7) + 0.03 mol/L NaCl
Flow rate: 1 mL/min
Temperature: 25°C
Detection: UV (220 nm)
Samples:
1. Thyroglobulin
2. γ-globulin
3. Ovalbumin
4. Ribonuclease A
5. p-Aminobenzoic acid





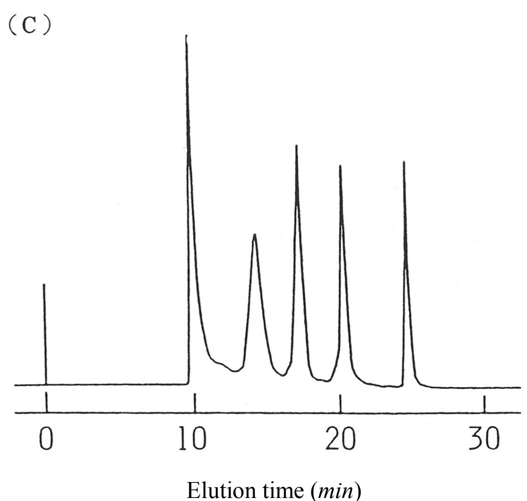
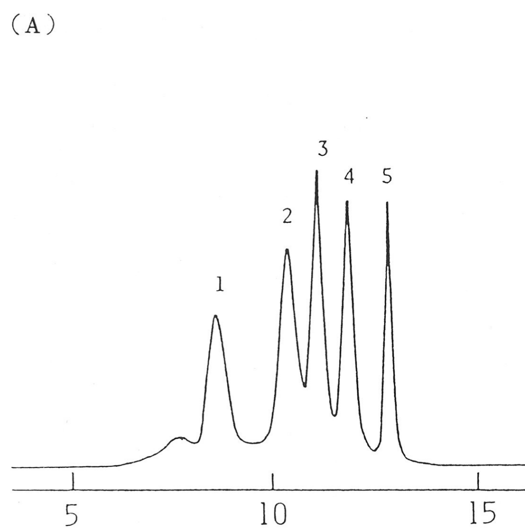
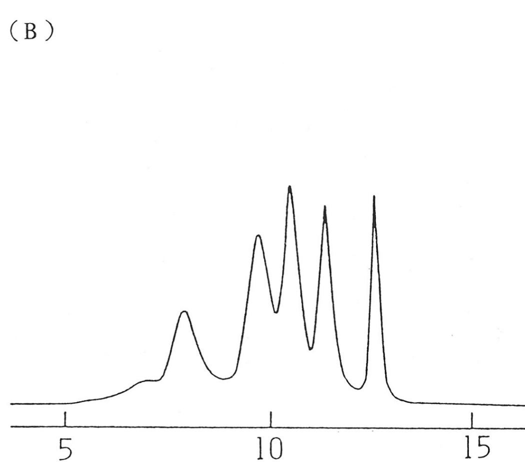


Figure 4 Comparison of SWXL and SW series (2)

Columns: (A) TSKgel G3000SWXL 7.8 mmI.D. × 30 cm
(B) TSKgel G3000SW 7.5 mmI.D. × 30 cm
(C) TSKgel G3000SW 7.5 mmI.D. × 60 cm

Same conditions as in Figure 3.





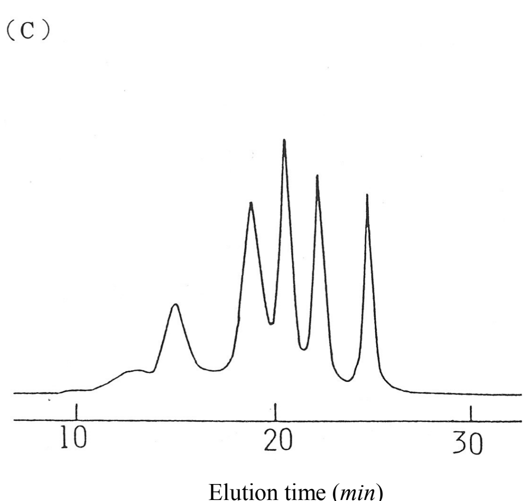


Figure 5 Comparison of SWXL and SW series (3)

Columns: (A) TSKgel G4000SWXL 7.8 mmI.D. × 30 cm
(B) TSKgel G4000SW 7.5 mmI.D. × 30 cm
(C) TSKgel G4000SW 7.5 mmI.D. × 60 cm

Same conditions as in Figure 3.

Table 3. Comparison of resolution (Rs) of SWXL and SW series

Sample	Rs		
	G2000SWXL	G2000SW 30 cm	G2000SW 60 cm
Thyroglobulin			
	2.43	1.57	2.24
γ-globulin			
	3.13	2.24	2.48
Bovine serum albumin			
	6.44	2.93	5.00
Peroxidase			
	9.07	5.76	8.03
Myoglobin			
	12.98	5.19	6.61
Cytochrome C			
	2.89	1.50	2.23
Glycine tetramer			

Sample	Rs		
	G3000SWXL	G3000SW 30 cm	G3000SW 60 cm
Thyroglobulin			
	4.13	4.35	6.33
γ-globulin			
	3.73	2.30	3.46
Bovine serum albumin			
	7.14	4.23	6.14
Peroxidase			
	8.29	5.66	9.31
Myoglobin			
	8.53	4.30	6.49
Cytochrome C			
	1.68	1.34	2.46
Glycine tetramer			

Sample	Rs		
	G4000SWXL	G4000SW 30 cm	G4000SW 60 cm
Thyroglobulin			
	3.19	2.77	3.07
γ-globulin			
	1.54	1.28	1.95
Bovine serum albumin			
	3.31	1.98	3.11
Peroxidase			
	2.99	2.77	3.69
Myoglobin			
	3.28	2.35	2.07
Cytochrome C			
	0.69	0.70	0.75
Glycine tetramer			

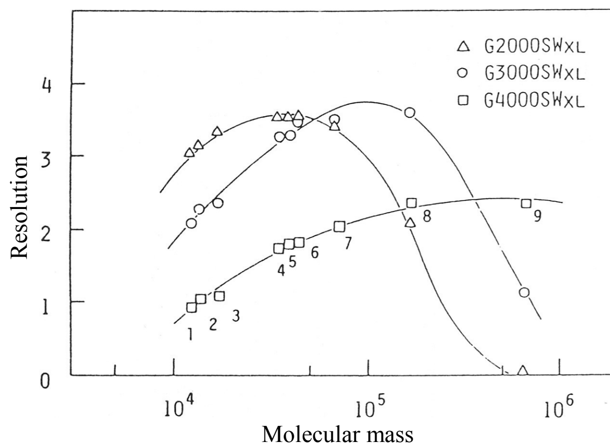


Figure 6 Relationship between molecular mass and resolution

Columns: TSKgel SWXL Series (7.8 mmI.D. × 30 cm)
Solvent: 0.05 mol/L phosphate buffer (pH 7) + 0.3 mol/L NaCl
Flow rate: 1 mL/min
Temperature: 25°C
Detection: UV (220 nm)
Samples: 1. Cytochrome C 2. Ribonuclease A 3. Myoglobin 4. β-lactoglobulin 5. Peroxidase 6. Ovalbumin 7. Bovine serum albumin 8. γ-globulin 9. Thyroglobulin

3. Basic Properties

3-1 Dependence of height equivalent to a theoretical plate (HETP) on flow rate

The effect of flow rate on HETP depends on the particle size of the packing material, the molecular size of the sample, and the viscosity of the solvent. Using bovine serum album and myoglobin as representative examples, Figure 7 shows the dependence of HETP on flow rate in the SWXL and conventional SW series of columns.

In the SWXL series, HETP changes little as the flow rate increases, while in the SW series HETP depends significantly on flow rate. This is due to the small particle size of the packing materials used in the SWXL series.

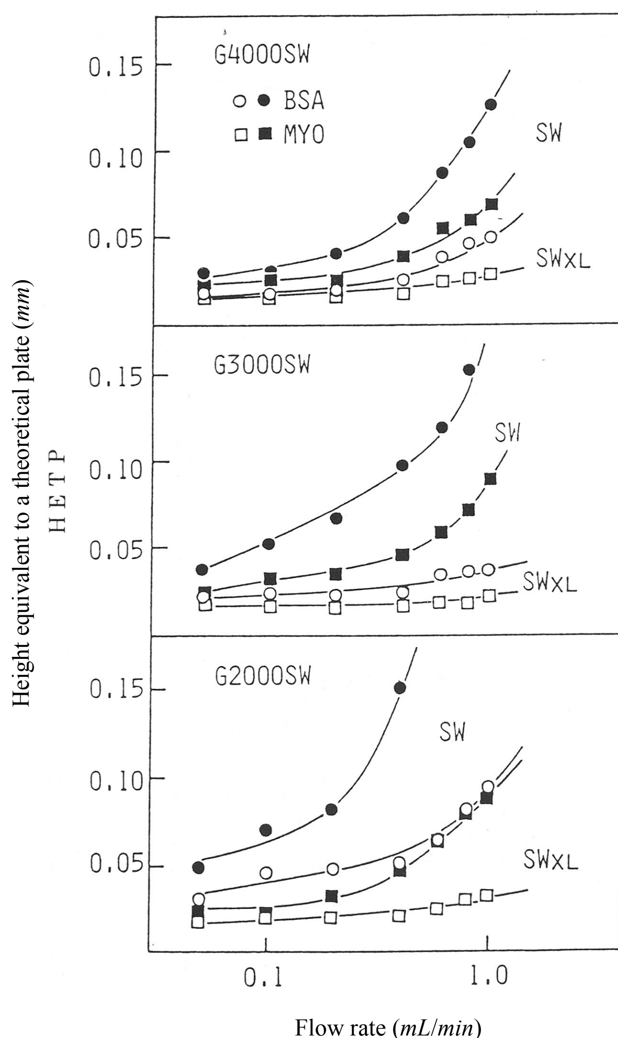


Figure 7 Dependence of height equivalent to a theoretical plate (HETP) on flow rate

Columns: ○, □SWXL series
●, ■ SW series
Solvent: 0.05 mol/L phosphate buffer (pH 7) + 0.3 mol/L NaCl
Samples: BSA: Bovine serum albumin
MYO: Myoglobin

3-2 Ionic properties

Silica gel-based packing materials contain functional groups. Silanol functional groups have a negative charge in neutral solution. On the other hand, there are 3 types of protein that are used for samples: basic proteins, which have a positive charge in solution; acidic proteins, which have a negative charge; and neutral proteins, which have no charge. As a result, ionic interactions between the packing material and samples will occur.

Figure 8 shows the dependence of the protein elution volume on salt concentration. The elution volume of cytochrome C, a basic protein, increases at ≤0.2 mol/L in both the SWXL series the conventional SW series of columns, indicating that cytochrome C readily bonds with the packing material. On the other hand, the elution volumes of bovine serum albumin and ovalbumin, which are acidic proteins, decrease as the salt concentration decreases due to ionic interaction with the packing material. Moreover, myoglobin, a neutral protein, shows no change in elution volume.

Thus, in this way, at low salt concentrations, ionic interactions occur between the packing material and biopolymers such as nucleic acids and proteins. Consequently, to negate this effect, 0.2 to 0.5 mol/L of salt should be added to the solvent.

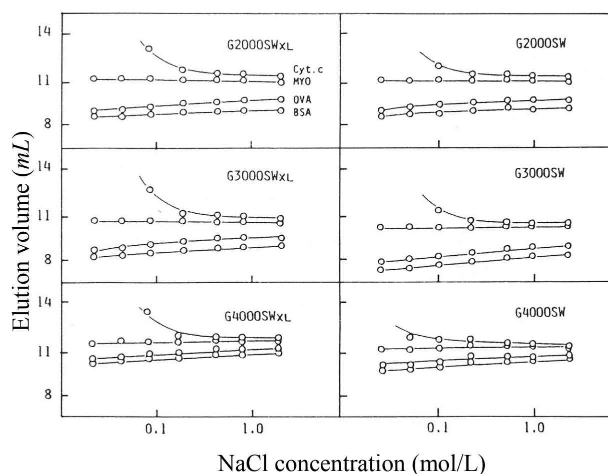


Figure 8 Dependence of elution volume on salt concentration

Solvent: 0.05 mol/L phosphate buffer (pH 7) + NaCl
Flow rate: 1 mL/min
Samples: Cyt.C: Cytochrome C
MYO: Myoglobin
OVA: Ovalbumin
BSA: Bovine serum albumin

3-3 Sample load

Figure 9 shows the dependence of HETP on sample load in the separation of bovine serum albumin. As is also shown in Figure 6, although the overall HETP is lower in the SWXL series than the conventional SW series, in both series, sample load changes very little up to 250 µg. Sample loads used in the SWXL series are similar to those used in the conventional SW series.

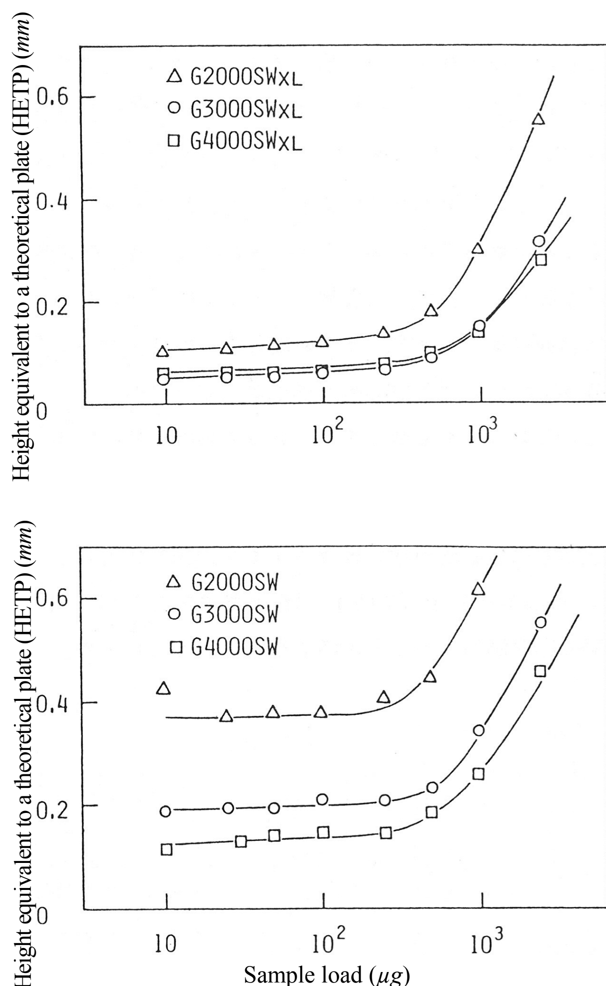


Figure 9 Effect of sample load (at a constant injection volume) on HETP

3-4 Protein recovery

Table 4 shows protein recovery at various sample loads. In the G2000SWXL and G3000SWXL, the recovery of ribonuclease, thyroglobulin, and γ-globulin was virtually quantitative, regardless of the sample load. Myoglobin, cytochrome C, chymotrypsinogen, lysozyme, and trypsin inhibitor were all recovered quantitatively. In the G4000SWXL, ribonuclease, γ-globulin and the 5 other proteins noted above were recovered quantitatively. However, for thyroglobulin, there was a decrease in recovery when the sample load was small (1 µg).

In the SWXL series, although recovery is quantitative in the case of the vast majority of proteins regardless of the sample load, the recovery does decrease at low sample loads in the case of some exceptional proteins. (Similar results occur with the conventional SW series as well).

Table 4 Protein recovery (%)

	Sample load (µg)				
	1	5	10	50	100
G2000SWXL					
Ribonuclease A	95	83	96	98	94
Thyroglobulin	107	92	101	-	-
γ-globulin	103	109	116	98	107
G3000SWXL					
Ribonuclease A	96	97	97	95	94
Thyroglobulin	92	97	101	99	91
γ-globulin	106	103	97	97	108
G4000SWXL					
Ribonuclease A	104	106	103	103	94
Thyroglobulin	78	90	91	102	101
γ-globulin	91	90	107	97	104

4. TSKgel SWXL series applications

Figures 10 and 11 show examples of the separation of a crude extract of rat liver and the separation of polypeptides using the G2000SWXL. Figures 12 and 13 show examples of the separation of a crude extract of guinea pig stomach and a crude extract of *Ricinus communis* lectin (RCA) using the G3000SWXL. Figures 14 and 15 show examples of the separation of a crude extract of spinach leaf and the separation of ø X 174 RF DNA-Hae III digest using the G4000SWXL.

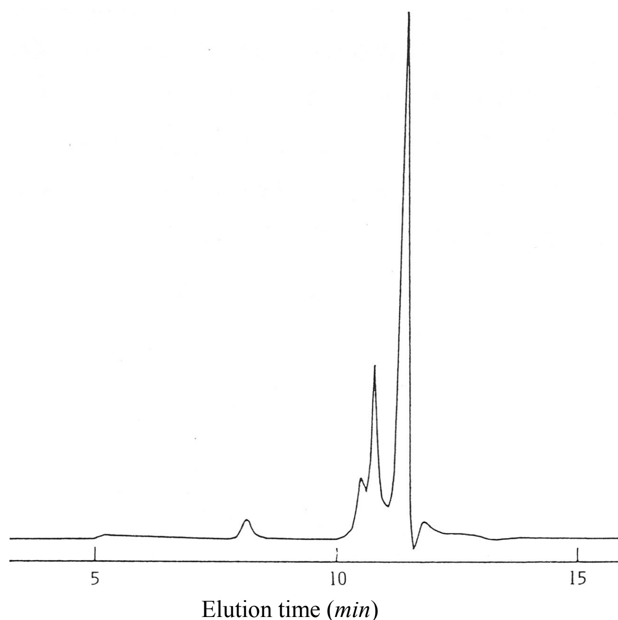


Figure 10 Separation of crude extract of rat liver (10 μL)

Column: TSKgel G2000SWXL 7.8 mmI.D. × 30 cm
Solvent: 0.05 mol/L phosphate buffer (pH 7) + 0.3 mol/L NaCl
Flow rate: 1 mL/min
Temperature: 25°C
Detection: UV (220 nm)

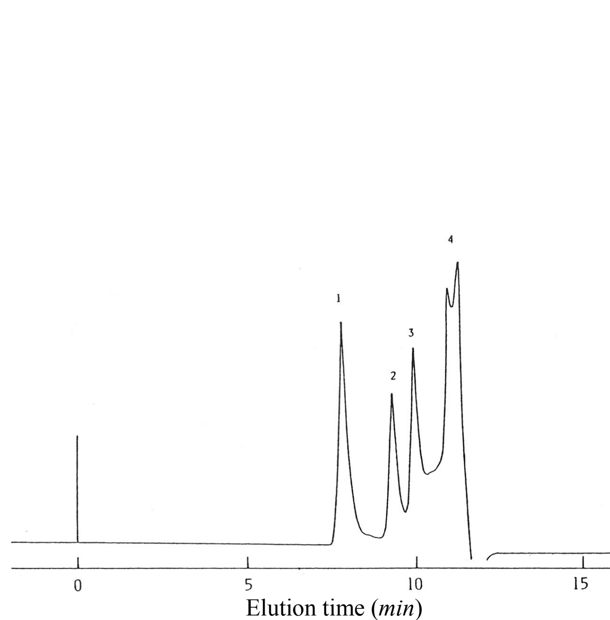


Figure 11 Separation of polypeptides

Column: TSKgel G2000SWXL 7.8 mmI.D. × 30 cm
Solvent: 40% acetonitrile + 0.05% TFA
Flow rate: 1 mL/min
Temperature: 25°C
Detection: UV (215 nm)
Samples: 1. Cytochrome C 2. Insulin 3. *α*-endorphin 4. Leu-enkephalin

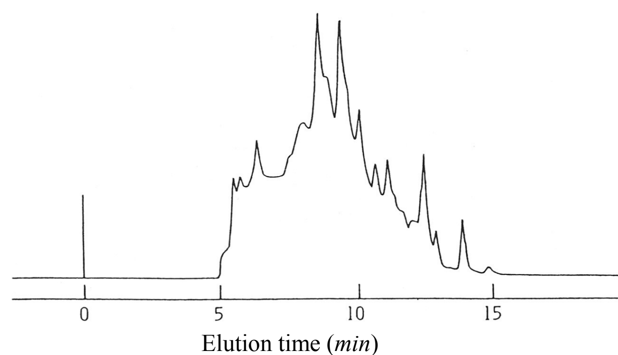


Figure 12 Separation of crude extract of guinea pig (marmot) stomach (25 μL)

Conditions are the same as in Figure 10 except for the column.
Column: TSKgel G3000SWXL 7.8 mmI.D. × 30 cm

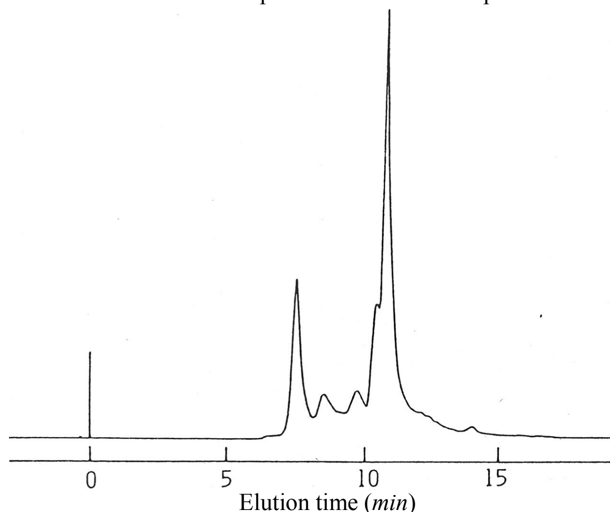


Figure 13 Separation of crude extract of *Ricinus communis* lectin (RCA) (25 μL)

Conditions are the same as in Figure 12.

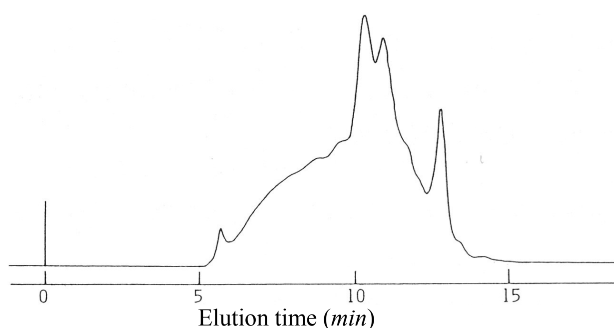


Figure 14 Separation of crude extract of spinach leaf (25 μL)

Conditions are the same as in Figure 10 except for the column.

Column: TSKgel G4000SWXL 7.8 mmI.D. × 30 cm

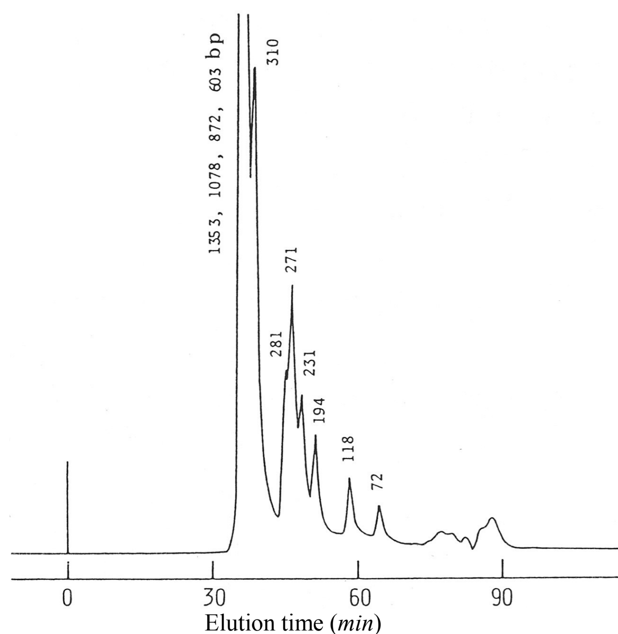


Figure 15 Separation of φ X 174 RFDNA-Hae III digest (4.5 μg/50 μL)

Column: TSKgel G4000SWXL 7.8 mmI.D. × 30 cm

Solvent: 0.05 mol/L phosphate buffer (pH 7) + 0.3 mol/L NaCl + 1 mmol/L EDTA

Flow rate: 0.15 mL/min

Temperature: 25°C

Detection: UV (260 nm)

5. Conclusion

The TSKgel SWXL series of columns has uses packing materials with smaller particle size and delivers vastly improved performance when compared to the conventional SW series. The 30-cm columns in this series provide separation performance that is equivalent to or better than the separation provided by conventional 60-cm SW columns. This results in several advantages, as both analysis time and solvent use are reduced by half, while at the same time, because there is no change in sample load, sample dilution can be kept to a minimum.

It is expected that in the future, high-performance GFC will be increasingly used to separate biopolymers by taking advantage of the increased performance of the SWXL series.