TOSOH

SEPARATION REPORT NO. 46

TSKgel SWXL SERIES COLUMNS

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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 the early 1980's, the field of preparative chromatography of biopolymers had been exclusively monopolized by low performance chromatography using soft gels. 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 (RPC) have become general methods of analysis in HPLC. 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-type columns are used worldwide, and it is no exaggeration to say that GFC using TSKgel SW columns is now a standard analytical method.

In response to demands for smaller particle sizes and increased performance in TSKgel GFC columns, Tosoh, in 1987, introduced the TSKgel SW_{XL} series columns, the features and basic properties of which are discussed in this report. Several application examples are included in this report.

2. Characteristics

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

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

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

Table 1 TSKgel SW_{XL} Series Specifications

TSKgel Column	Particle size (μm)	Guaranteed theoretical plate number (TP/30cm)	Column dimensions
$G2000SW_{XL}$	5	20,000	7.8mm ID × 30cm
$G3000SW_{XL}$	5	20,000	
$G4000SW_{XL}$	8	16,000	

Analytical conditions for theoretical plate number:
Solvent: Distilled H_2O
Flow rate: 1mL/min
Sample: 1% ethylene glycol, 20μL

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

TSKgel Column	PEG/PEOs	Dextrans	Proteins
$G2000SW_{XL}$	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

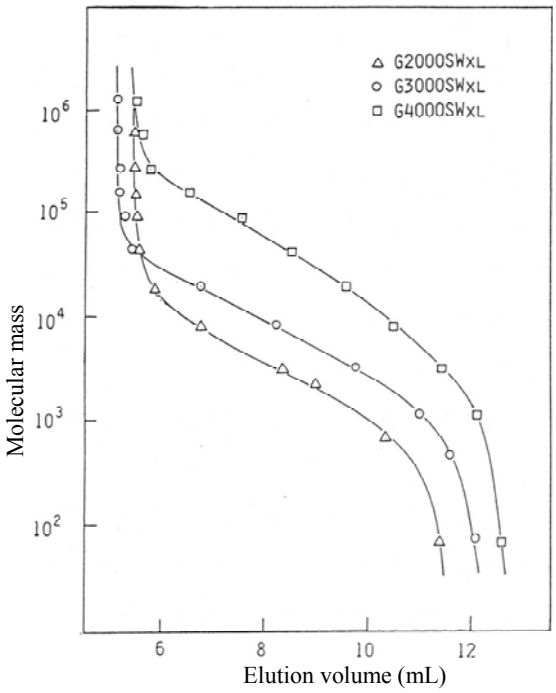

Figure 1 Calibration curves produced with PEG

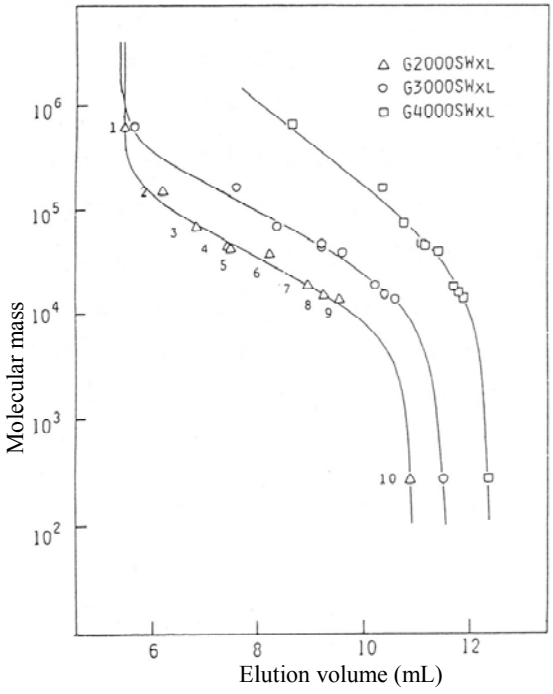

Figure 2 Calibration curves produced with proteins

Columns: TSKgel SW_{XL} Series, 7.8mm ID x 30cm
Solvent: 0.05mol/L phosphate buffer (pH 7) + 0.3mol/L NaCl
Flow rate: 1mL/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
9. cytochrome C
10. glycine tetramer

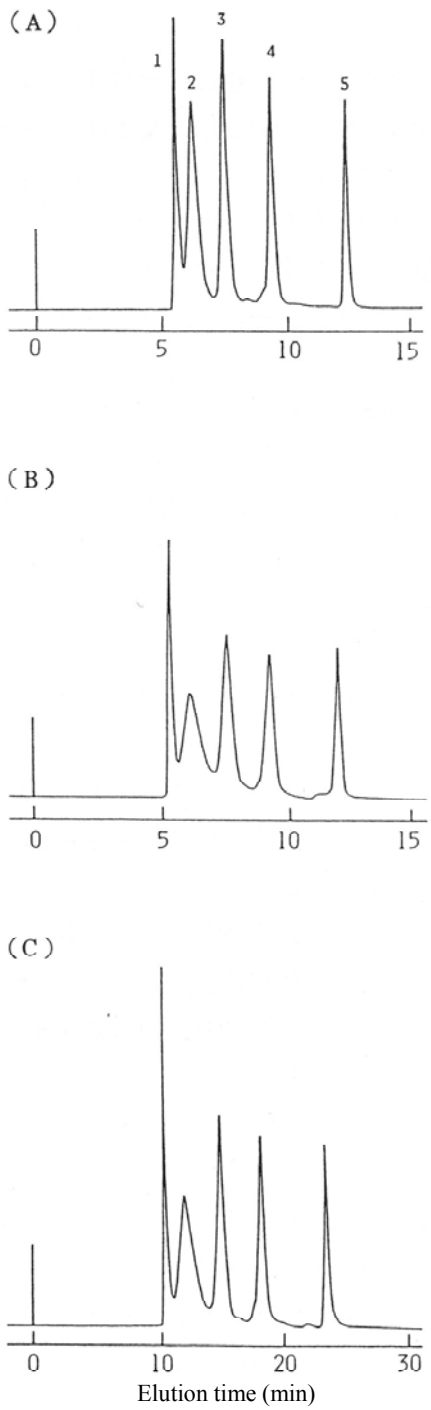

Figure 3 Comparison of SW_{XL} and SW columns (1)

Columns: A: TSKgel G2000SW_{XL}, 7.8mm ID x 30cm
B: TSKgel G2000SW, 7.5mm ID x 30cm
C: TSKgel G2000SW, 7.5mm ID x 60cm
Solvent: 0.05mol/L phosphate buffer (pH 7) + 0.03mol/L NaCl
Flow rate: 1mL/min
Temperature: 25°C
Detection: UV@220 nm
Samples:
1. thyroglobulin
2. γ-globulin
3. ovalbumin
4. ribonuclease A
5. p-aminobenzoic acid

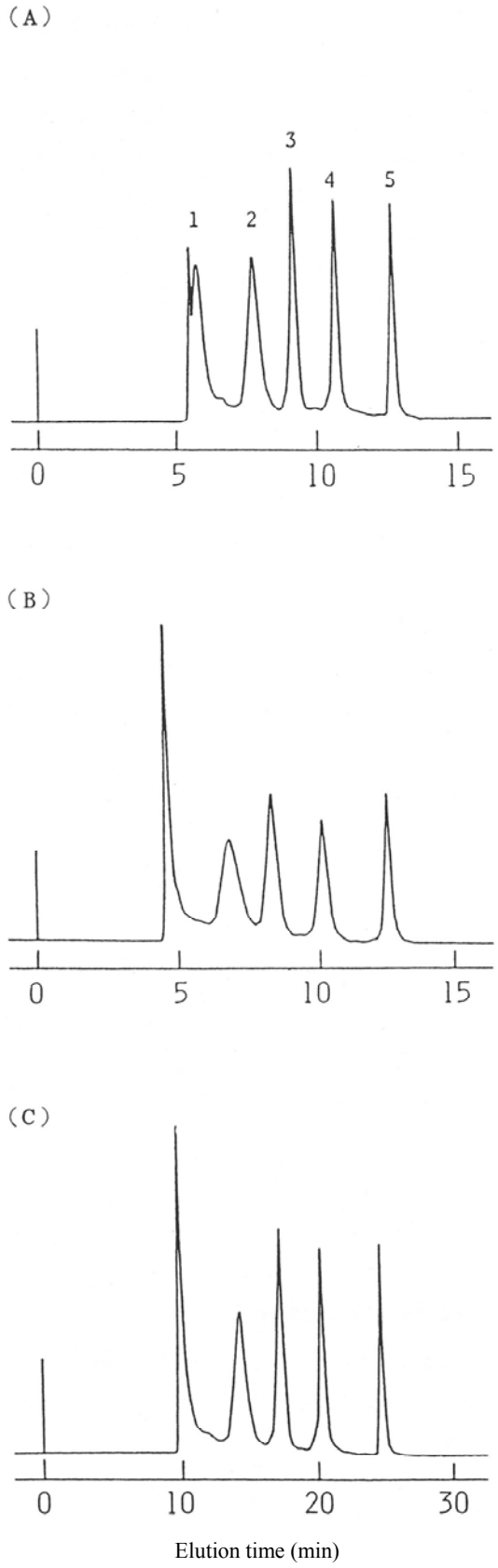

Figure 4 Comparison of SW_{XL} and SW columns (2)

Columns: A: TSKgel G3000SW_{XL}, 7.8mm ID x 30cm
B: TSKgel G3000SW, 7.5mm ID x 30cm
C: TSKgel G3000SW, 7.5mm ID x 60cm

Same conditions as in Figure 3.

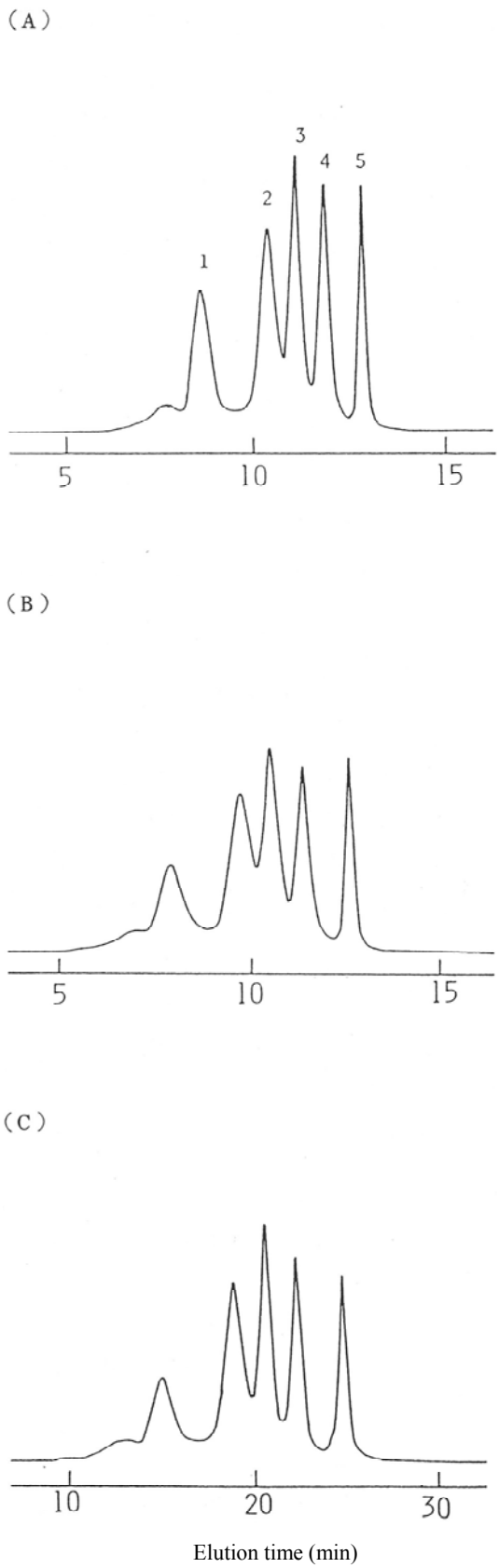

Figure 5 Comparison of SW_{XL} and SW columns (3)

Columns: A: TSKgel G4000SW_{XL}, 7.8mm ID x 30cm
B: TSKgel G4000SW, 7.5mm ID x 30cm
C: TSKgel G4000SW, 7.5mm ID x 60cm

Same conditions as in Figure 3.

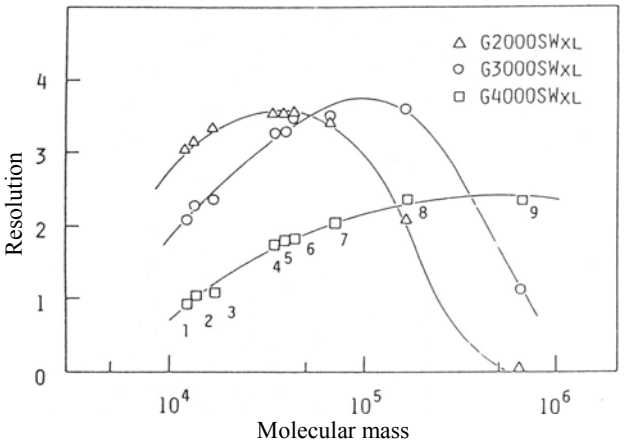

Figure 6 Relationship between molecular weight and resolution

Columns: TSKgel SW_{XL} Series, 7.8mm ID x 30cm
Solvent: 0.05mol/L phosphate buffer (pH 7) + 0.3mol/L NaCl
Flow rate: 1mL/min
Temperature: 25°C
Detection: UV@220nm
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

Table 3. Comparison of resolution (Rs) of SW_{XL} and SW columns

Sample	Rs		
	TSKgel $G2000SW_{XL}$	TSKgel G2000SW 30cm	TSKgel G2000SW 60cm
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		
	TSKgel $G2000SW_{XL}$	TSKgel G2000SW 30cm	TSKgel G2000SW 60cm
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		
	TSKgel $G2000SW_{XL}$	TSKgel G2000SW 30cm	TSKgel G2000SW 60cm
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			

3. Basic Properties

3-1 Dependence of HETP on flow rate

The effect of flow rate on height equivalent to a theoretical plate (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 for the TSKgel SW_{XL} and conventional SW columns.

For the TSKgel SW_{XL} columns, HETP changes little as the flow rate increases, while for the TSKgel SW columns HETP decreases significantly with increasing flow rate. This is due to the smaller particle size of the packing materials used in the TSKgel SW_{XL} columns, which reduces dispersion from convection (Eddy dispersion) and from mass transfer in the mobile phase.

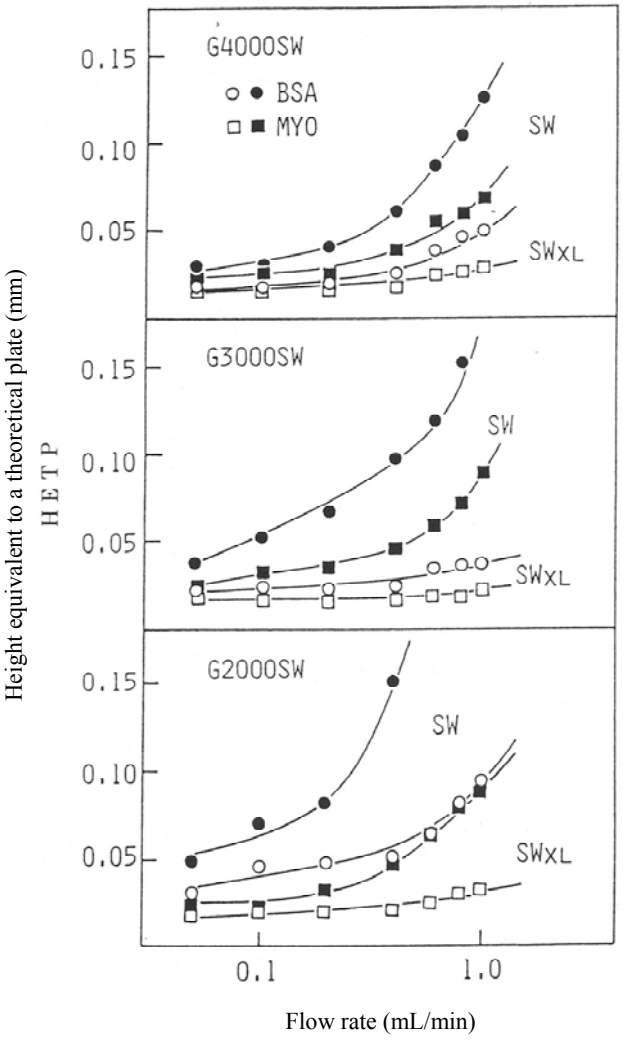

Figure 7 Dependence of height equivalent to a theoretical plate (HETP) on flow rate

Columns: ○, □ TSKgel SW_{XL} Series
●, ■ TSKgel SW series
Solvent: 0.05mol/L phosphate buffer (pH 7) + 0.3mol/L NaCl
Samples: BSA: bovine serum albumin
MYO: myoglobin

3-2 Ionic properties

Silica gel-based packing materials contain silanol functional groups. Silanol groups are weakly acidic and thus have a net negative charge in neutral solution. From a sample perspective, basic proteins have a positive charge in solution; acidic proteins have a negative charge, while neutral proteins have no charge. As a result, ionic interactions between the packing material and samples will occur.

Figure 8 shows the dependence of protein elution volume on salt concentration. The elution volume (retention) of cytochrome C, a basic protein, increases at a salt concentration of ≤0.2mol/L on both the TSKgel SW_{XL} columns and the conventional TSKgel SW columns, indicating that cytochrome C readily interacts 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 repulsion with the negatively charged silanol groups on the surface of the packing material. As expected, myoglobin, which is a neutral protein, shows no change in elution volume with varying salt concentration.

Thus, 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 0.5mol/L of salt should be added to the solvent to balance these interactions.

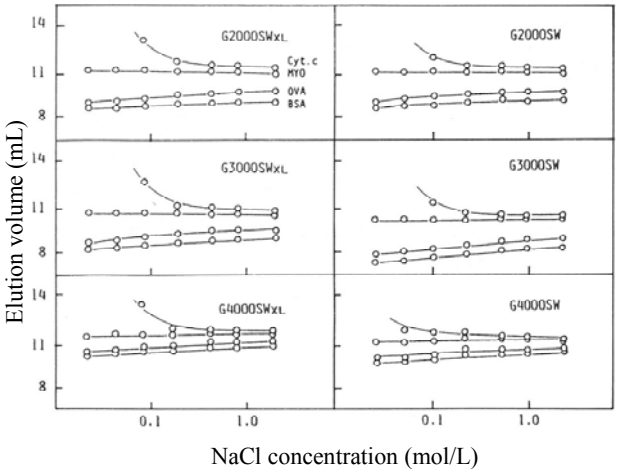

Figure 8 Dependence of elution volume on salt concentration

Solvent: 0.05mol/L phosphate buffer (pH 7) + NaCl
Flow rate: 1mL/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 was shown earlier in Figure 6, although the overall HETP is lower for the TSKgel SW_{XL} columns than the conventional TSKgel SW columns, for both column types, sample load changes very little up to about 250µg injected on-column. Sample loads used on the TSKgel SW_{XL} columns are similar to those used on the conventional TSKgel SW columns.

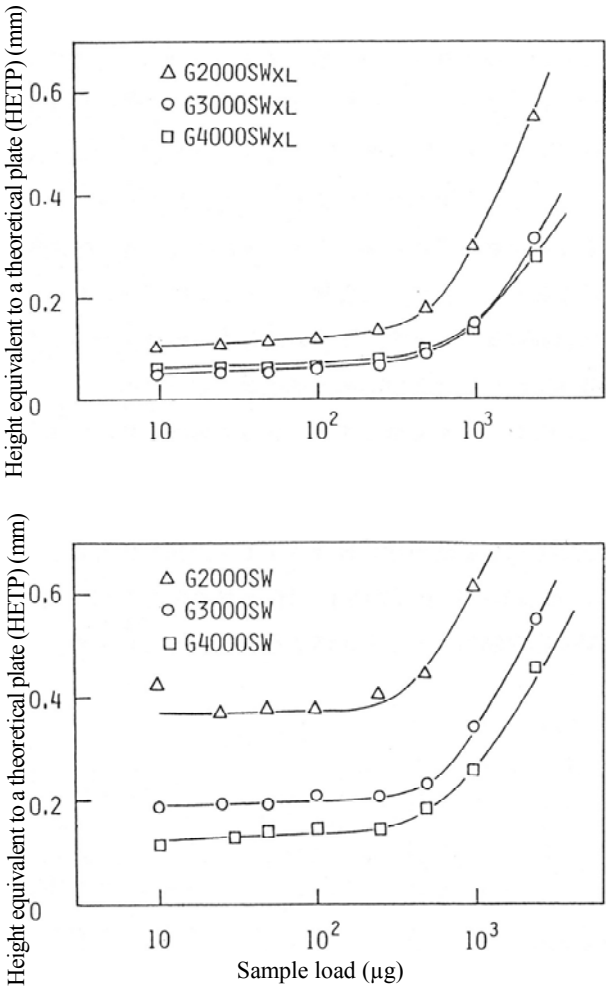

Figure 9 Effect of sample load (at a constant injection volume) on HETP

3-4 Protein recovery

Table 4 shows protein recovery at various sample loads. For the TSKgel $G2000SW_{XL}$ and $G3000SW_{XL}$ columns, the recovery of ribonuclease, thyroglobulin, and *γ*-globulin was virtually quantitative, regardless of the sample mass injected (sample load). Myoglobin, cytochrome C, chymotrypsinogen, lysozyme, and trypsin inhibitor were all recovered quantitatively. In the TSKgel $G4000SW_{XL}$ column, 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*µ*g).

On the TSKgel SW_{XL} columns, although recovery is quantitative for the vast majority of proteins regardless of sample load, recovery does decrease at low sample loads in the case of some exceptional proteins. (Similar results occur with the conventional TSKgel SW columns as well).

Table 4 Protein recovery (%)

	Sample load (µg)				
	1	5	10	50	100
TSKgel $G2000SW_{XL}$					
ribonuclease A	95	83	96	98	94
thyroglobulin	107	92	101	-	-
γ-globulin	103	109	116	98	107
TSKgel $G3000SW_{XL}$					
ribonuclease A	96	97	97	95	94
thyroglobulin	92	97	101	99	91
γ-globulin	106	103	97	97	108
TSKgel $G4000SW_{XL}$					
ribonuclease A	104	106	103	103	94
thyroglobulin	78	90	91	102	101
γ-globulin	91	90	107	97	104

Columns: TSKgel SW_{XL} Series, 7.8mm ID x 30cm

Same conditions as in Figure 2.

4. Applications

Figures 10 and 11 show examples of the separation of a crude extract of rat liver and the separation of polypeptides using a TSKgel G2000SW$_{XL}$ column. 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 a TSKgel G3000SW$_{XL}$ column. Figures 14 and 15 show examples of the separation of a crude extract of spinach leaf and the separation of øX174 RF DNA-Hae III digest using a TSKgel G4000SW$_{XL}$ column.

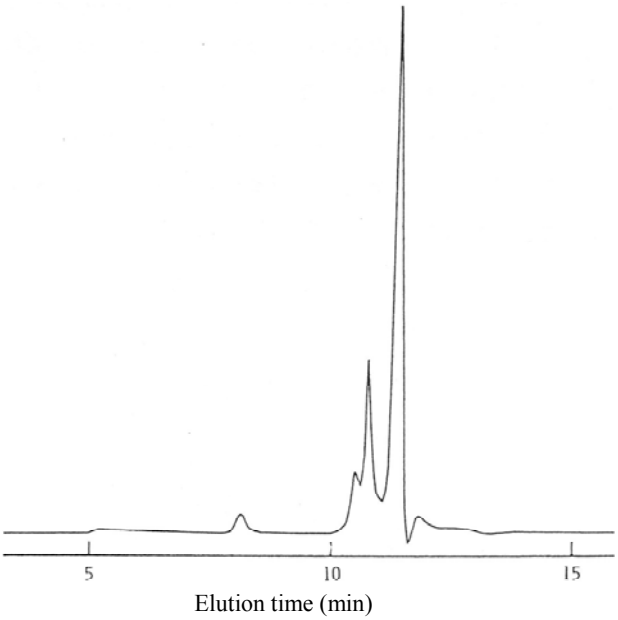

Figure 10 Separation of crude extract of rat liver (10µL)

Column: TSKgel G2000SW$_{XL}$, 7.8mm ID x 30cm
Solvent: 0.05mol/L phosphate buffer (pH 7) + 0.3mol/L NaCl
Flow rate: 1mL/min
Temperature: 25°C
Detection: UV@220 nm

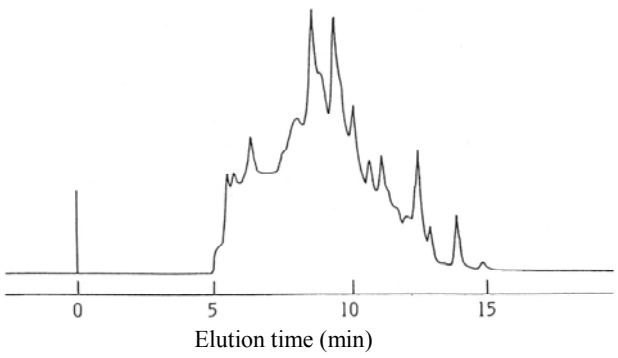

Figure 12 Separation of crude extract of guinea pig (marmot) stomach (25µL)

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

Column: TSKgel G3000SW$_{XL}$, 7.8mm ID x 30cm

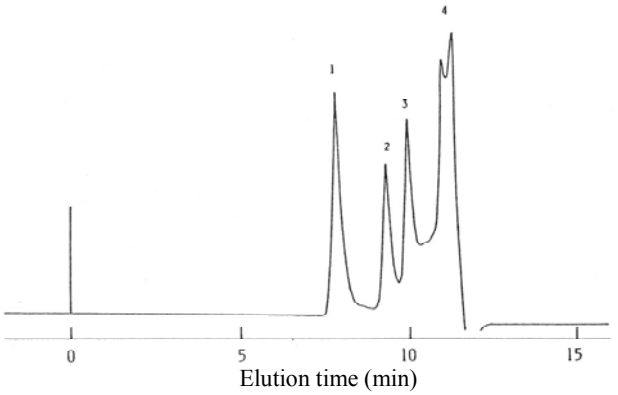

Figure 11 Separation of polypeptides

Column: TSKgel G2000SW$_{XL}$, 7.8mm ID x 30cm
Solvent: 40% ACN + 0.05% TFA
Flow rate: 1mL/min
Temperature: 25°C
Detection: UV@215 nm
Samples: 1. cytochrome C
2. insulin
3. α-endorphin
4. leu-enkephalin

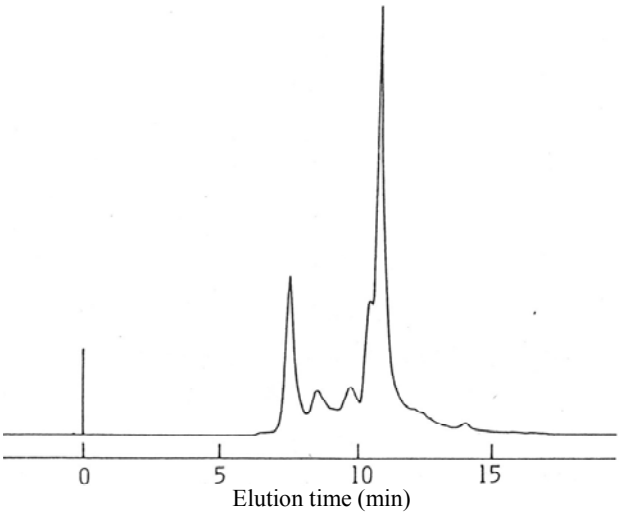

Figure 13 Separation of crude extract of *Ricinus communis* lectin (RCA) (25µL)

Conditions are the same as in Figure 12.

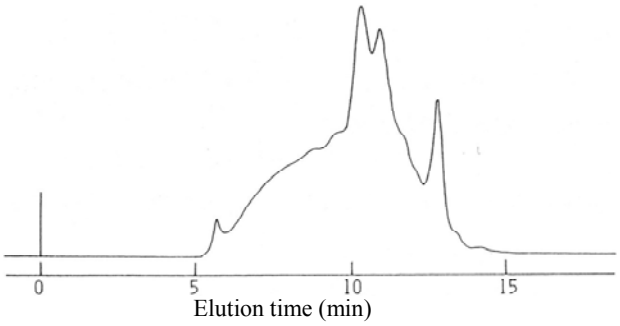

Figure 14 Separation of crude extract of spinach leaf (25µL)

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

Column: TSKgel G4000SW$_{XL}$, 7.8mm ID x 30cm

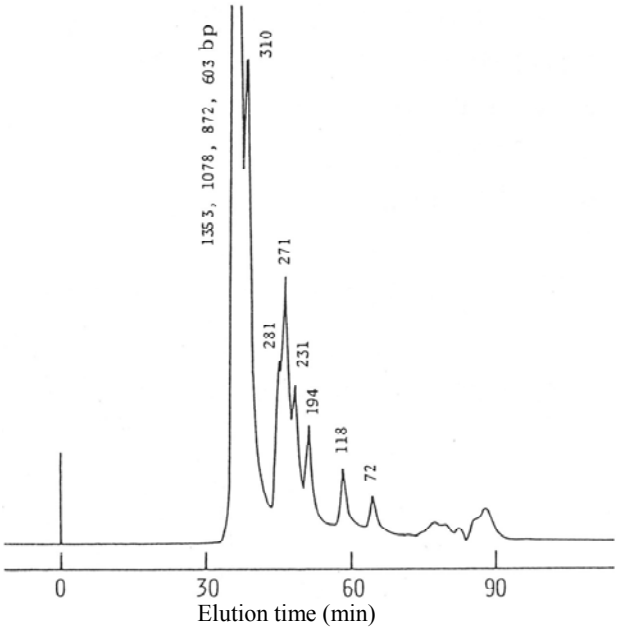

Figure 15 Separation of φX174 RF DNA-Hae III digest (4.5µg/50µL)

Column: TSKgel G4000SW$_{XL}$, 7.8mm ID x 30cm
Solvent: 0.05mol/L phosphate buffer (pH 7) + 0.3mol/L NaCl + 1mmol/L EDTA
Flow rate: 0.15mL/min
Temperature: 25°C
Detection: UV@260 nm

5. Conclusions

TSKgel SW$_{XL}$ columns use packing materials with smaller particle size and deliver vastly improved performance when compared to the conventional TSKgel SW columns. The 30cm columns in this series provide separation performance that is equivalent to *(at double the analysis time)* or better *(at the same analysis time)* than the separation provided by conventional 60cm TSKgel 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.

Looking back to the time when the TSKgel SW$_{XL}$ columns were introduced, high performance GFC columns have become the norm for analyzing biopolymers by taking advantage of the increased performance of the TSKgel SW$_{XL}$ columns.

For further information about the TSKgel SW$_{XL}$ and 4 micron SuperSW columns for higher performance GFC, we recommend that you study the following Separation Reports available at www.tosohbioscience.com:

SR062: Separation of IgG and Albumin by High Performance Gel Filtration Chromatography Using TSKgel G3000SW$_{XL}$

SR095: TSKgel SuperSW Series