Shodex™

HPLC Columns

Shodex™ KW400 series columns

High performance and downsized columns for protein analysis

Technical notebook
No. 5

SHOWA DENKO EUROPE

Contents

1. Introduction

Size exclusion chromatographic (SEC) columns are suitable to identify molecular weight of biopolymers, such as proteins. Shodex has developed a brand-new KW400 series of high-performance SEC semi-micro columns. This series is a downsized and higher performance version of PROTEIN KW-800, specialized for protein analyses. Both series are filled with silica gel. This notebook introduces the features of KW-400 series.

2. Specification

Table 2-1. Specification of KW400 Series Columns

Product Code	Product Name	Exclusion Limit		Plate	Particle Size (μm)	ID x Length (mm)
		(Pullulan)	(Protein)			
F6989201	KW402.5-4F	60,000	150,000	≥ 35,000	3	4.6 x 300
F6989202	KW403-4F	150,000	600,000	≥ 35,000	3	4.6 x 300
F6989203	KW404-4F	500,000	1,000,000	≥ 25,000	5	4.6 x 300
F6989204	KW405-4F	1,300,000	20,000,000	≥ 25,000	5	4.6 x 300
F6700132	KW400G-4A	Guard column		—	5	4.6 x 10

For all Columns

Packing Material : Silica Gel with Hydrophilic Polymer Coating
Housings : Stainless Steel
Recommended Flow Rate : ≤ 0.35mL/min
Maximum Flow Rate : 0.5mL/min
Maximum Pressure : 10 MPa (KW402.5-4F, KW403-4F, KW404-4F), 7MPa (KW405-4F)
Temperature : 5 - 45℃
pH : 3.0 - 7.5
Organic Solvent : Up to 100% of Methanol, Ethanol or Acetonitrile

(Attention) The KW400 series is a semi-micro type of a SEC column.
It is recommended to use it with a semi-micro type HPLC system.

2-1. Eluent Condition of KW400 Series

(1) Use of Salt

Buffers, including phosphate, TRIS-hydrochloric acid, and acetate buffers, are normally used along with a salt, such as sodium chloride, sodium sulfate, potassium sulfate, or ammonium sulfate. An appropriate salt content is between 0.1 M and 0.3 M.

Note 1. The pH range of the eluent should be between 3 and 7.5.

Note 2. Chloride ions erode columns and tubings in a HPLC system. In case of adding a chloride containing salt, it is recommended to replace the solvent in tubings with a chloride-free solvent after analysis.

(2) Use of Urea or Guanidine Hydrochloride

Aqueous solutions of urea or 6M guanidine hydrochloride as a protein denaturant can also be used as an eluent. These denaturants are so viscous that the recommended flow rate is 0.15 mL/min. Preparing a column exclusively for each of these solvents is recommended, because solvent replacement takes time.

3) Use of Surfactants

Aqueous solutions containing a surfactant, such as 1% SDS or Brij, can be used as an eluent.

4) Use of Polar Organic Solvents

Polar organic solvents, including acetonitrile, methanol, and ethanol, can be used as an eluent, whether it is pure or an aqueous solution.

2-2. Calibration Curves

Figures 2-1, 2-2 and 2-3 describe calibration curves of the KW400 series.

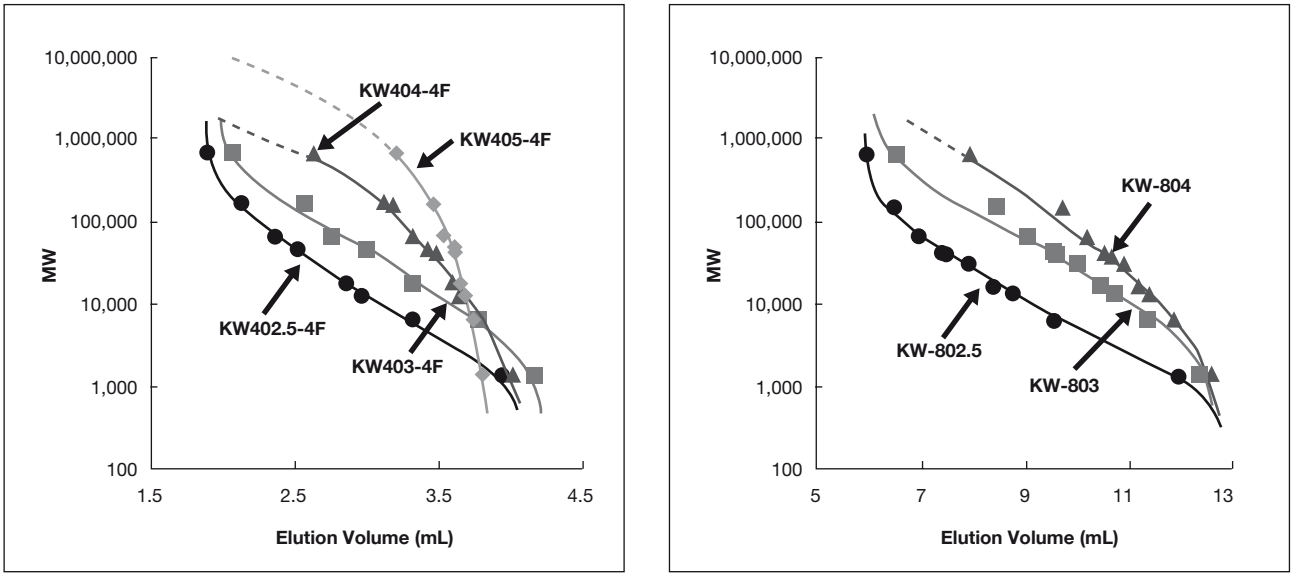


Fig. 2-1 Calibration Curves of KW400 and KW-800 Series with Proteins

Columns : Shodex KW400-4F Series (4.6mmID x 300mm each)
Eluent : 50mM Sodium Phosphate Buffer + 0.3M NaCl (pH7.0)
Flow Rate : 0.33mL/min
Detector : UV (280nm)
Column Temp. : 25℃

Columns : Shodex PROTEIN KW-800 Series (8.0mmID x 300mm each)
Eluent : 50mM Sodium Phosphate Buffer + 0.3M NaCl (pH7.0)
Flow Rate : 1.0mL/min
Detector : UV (280nm)
Column Temp. : 25℃

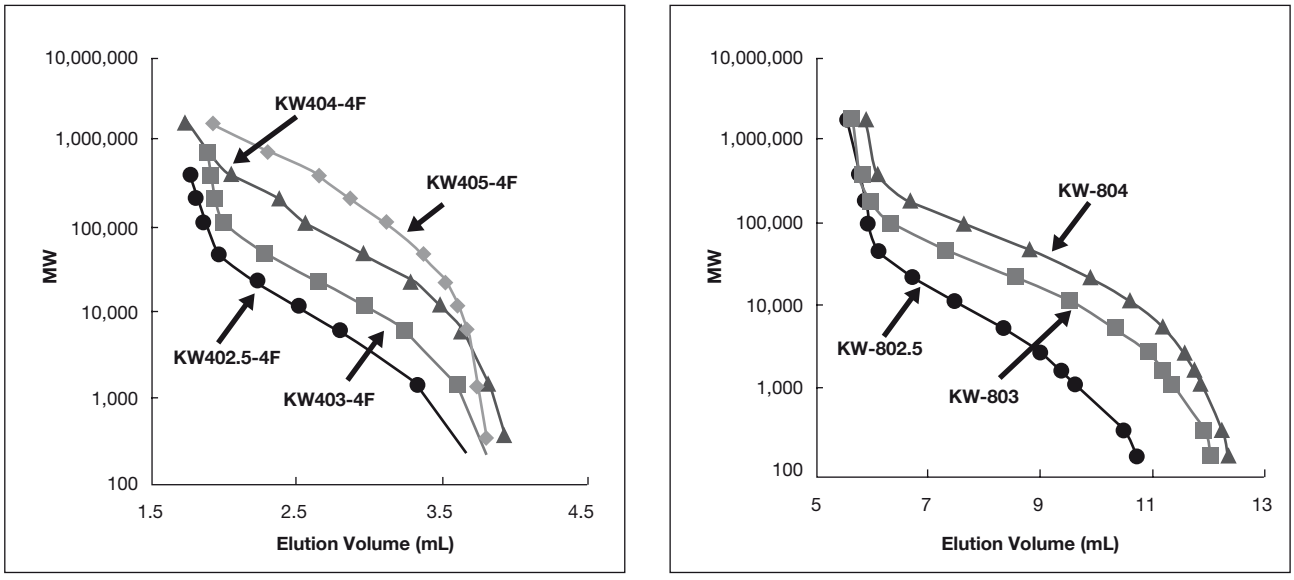


Fig. 2-2 Calibration Curves of KW400 and KW-800 Series with Pullulan

Columns : Shodex KW400-4F Series (4.6mmID x 300mm each)
Eluent : H_2O
Flow Rate : 0.33mL/min
Detector : Shodex RI
Column Temp. : 25℃

Columns : Shodex PROTEIN KW-800 Series (8.0mmID x 300mm each)
Eluent : H_2O
Flow Rate : 1.0mL/min
Detector : Shodex RI
Column Temp. : 25℃

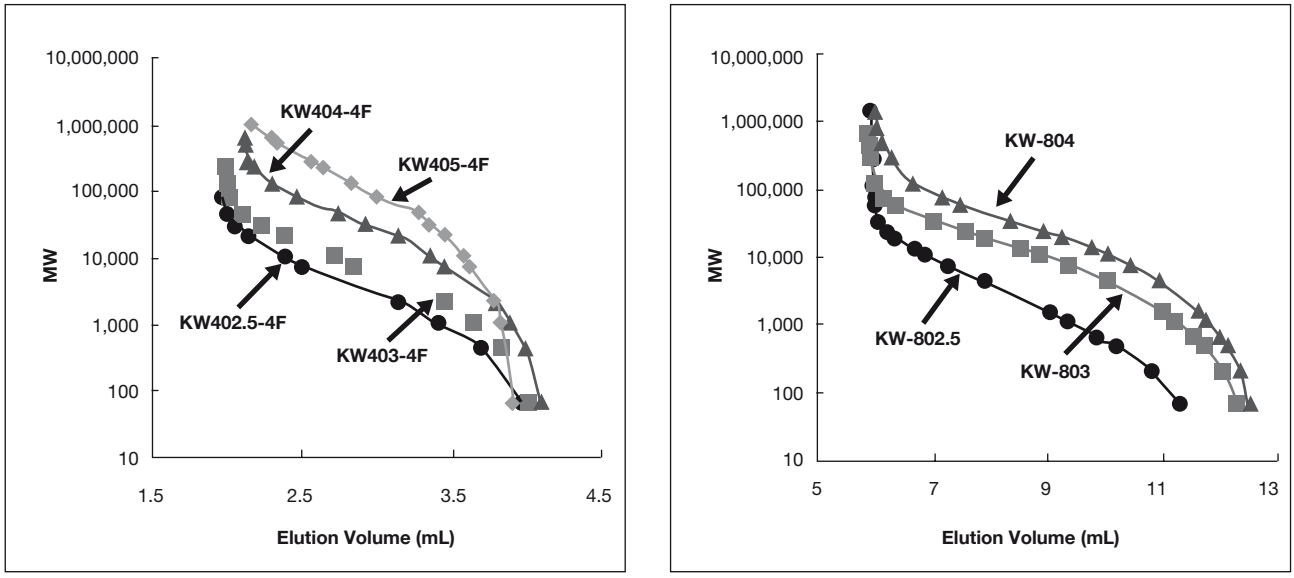


Fig. 2-3 Calibration curves of KW400 and KW-800 series with PEG/PEO

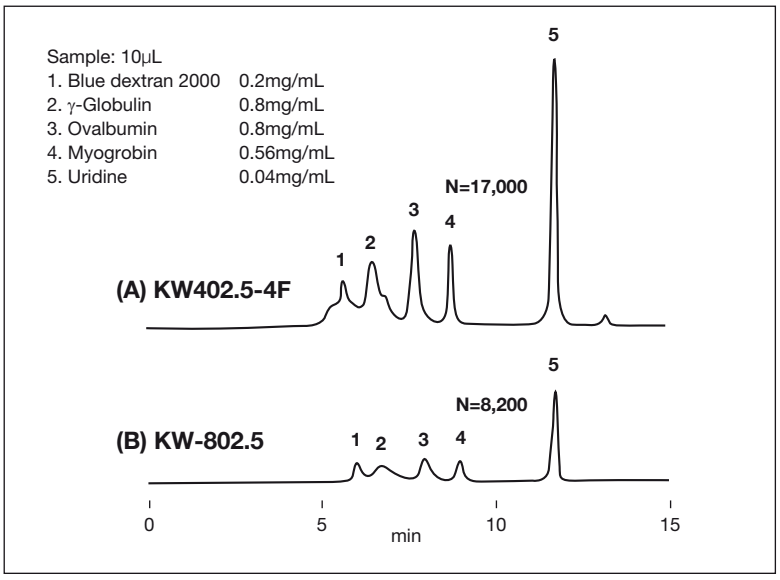
Columns : Shodex KW400-4F Series (4.6mmID x 300mm each)
Eluent : H_2O
Flow Rate : 0.33mL/min
Detector : Shodex RI
Column Temp. : 25℃

Columns : Shodex PROTEIN KW-800 Series (8.0mmID x 300mm each)
Eluent : H_2O
Flow Rate : 1.0mL/min
Detector : Shodex RI
Column Temp. : 25℃

3. Advantages of KW400 Series

3-1. Separation Performance

The KW400 series is a high performance version of the conventional PROTEIN KW-800 series. Its finer packing material enabled a downsized column. Figure 3-1 shows chromatograms using the KW400 series and KW-800 columns for a protein mixture. A semi-micro type HPLC was used in this datum. The theoretical plate number of KW402.5-4F is 1.5 times better than that of KW-802.5.



Columns : (A) Shodex KW402.5-4F (4.6mmID x 300mm)
(B) Shodex PROTEIN KW-802.5 (8.0mmID x 300mm)
Eluent : 50mM Sodium Phosphate Buffer + 0.3M NaCl (pH7.0)
Flow Rate : (A) 0.33mL/min, (B) 1.0mL/min
Detector : UV (280nm) (Semi-micro Type)
Column Temp. : 25℃
Injector : Rheodyne 8125
Tube : 0.1mmID sus

Fig. 3-1 Comparison of KW402.5-4F and KW-802.5

3-2. Detection Sensitivity

Figure 3-1 describes a better detection sensitivity of KW400 series, compared with KW-800 columns. KW402.5-4F has an approximately 3 to 4 times better detection sensitivity of KW402.5-4F as compared with that of KW-802.5.

3-3. Recovery of Proteins

Recovery data of seven kinds of proteins are shown in Table 3-1. Both of KW402.5-4F and KW403-4F columns achieve a high recovery by the very low level adsorption of proteins onto the packing materials.

Table 3-1. Recovery of proteins

Protein	Recovery (%)	
	KW402.5-4F	KW403-4F
γ-Globulin	98	96
Bovine serum albumin	89	96
Ovalbumin	89	97
Myoglobin	90	89
Cytochrome c	92	92
Lysozyme	87	98
α-Chymotrypsinogen A	95	94

Column : Shodex KW402.5-4F, KW403-4F (4.6mmID x 300mm each)
Eluent : 50mM Sodium phosphate buffer + 0.3M NaCl (pH7.0)
Flow rate : 0.33mL/min
Detector : UV (280nm)
Column temp. : 25℃

4. Features of KW400 Series

4-1. Influence of Flow Rate

Figure 4-1 shows the relationship between the theoretical plate number and the flow rate. Figure 4-2 describes chromatograms of proteins at different flow rates. Table 4-1 shows the relationship between the flow rate and the peak separation. As shown in Figure 4-1 and Table 4-1, a lower flow rate contributes to a higher theoretical plate number and a better peak separation. Figure 4-2 indicates that a slower flow rate leads to a higher peak height. The analysis is normally performed around 0.3 mL/min. For a better resolution and a higher sensitivity, the flow rate is recommended to be 0.2 mL/min or less. Please note that longer analysis time is needed in this case.

* The height equivalent of a theoretical plate (HETP) is the column length divided by the theoretical plate number. The smaller the HETP, the better the separation efficiency.

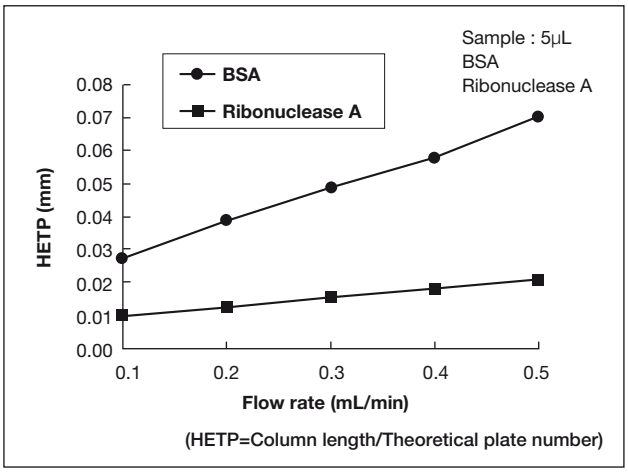


Fig. 4-1 Relationship of TPN and flow rate of KW402.5-4F

Column : Shodex KW402.5-4F (4.6mmID x 300mm)
Eluent : 50mM Sodium phosphate buffer + 0.3M NaCl (pH7.0)
Detector : UV (280nm)
Column temp. : 25℃

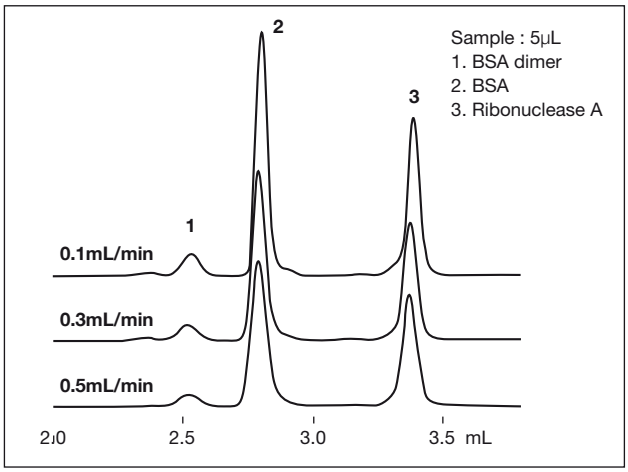


Fig. 4-2 Chromatograms of proteins at different flow rates

Column : Shodex KW402.5-4F (4.6mmID x 300mm)
Eluent : 50mM Sodium phosphate buffer + 0.3M NaCl (pH7.0)
Detector : UV (280nm)
Column temp. : 25℃

Table 4-1. Relationship of Flow Rate and Resolution

KW402.5-4F		Flow Rate (mL/min)		
		0.5	0.3	0.1
Resolution	BSA Dimer / BSA	1.49	1.68	2.04
	BSA / Ribonuclease A	5.28	6.17	7.88

4-2. Influence of Injection Volume of Sample

Figure 4-3 shows the relationship between the injection volume and the height equivalent of a theoretical plate (HETP) using KW402.5-4F and KW403-4F with bovine serum albumin (BSA) as a sample. Less than 10 µL of injection volume is suitable for both columns.

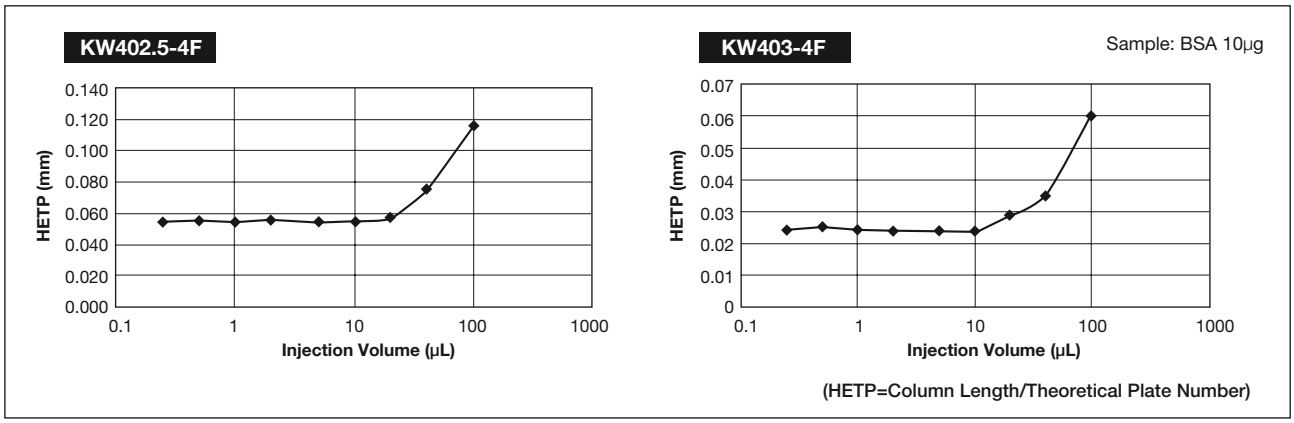


Fig. 4-3 Relationship of Injection Volume and HETP using KW402.5-4F and KW403-4F

Column : Shodex KW402.5-4F (4.6mmID x 300mm)
Eluent : 50mM Sodium Phosphate Buffer + 0.3M NaCl (pH7.0)
Flow Rate : 0.35mL/min
Detector : UV (280nm)
Column Temp. : 25℃

Column : Shodex KW403-4F (4.6mmID x 300mm)
Eluent : 50mM Sodium Phosphate Buffer + 0.3M NaCl (pH7.0)
Flow Rate : 0.35mL/min
Detector : UV (280nm)
Column Temp. : 25℃

4-3. Influence of Sample Loads

Figure 4-4 shows the relationship of sample loads and the HETP using KW402.5-4F and KW403-4F with bovine serum albumin (BSA) as a sample. Less than 100µg of sample load is suitable for both columns.

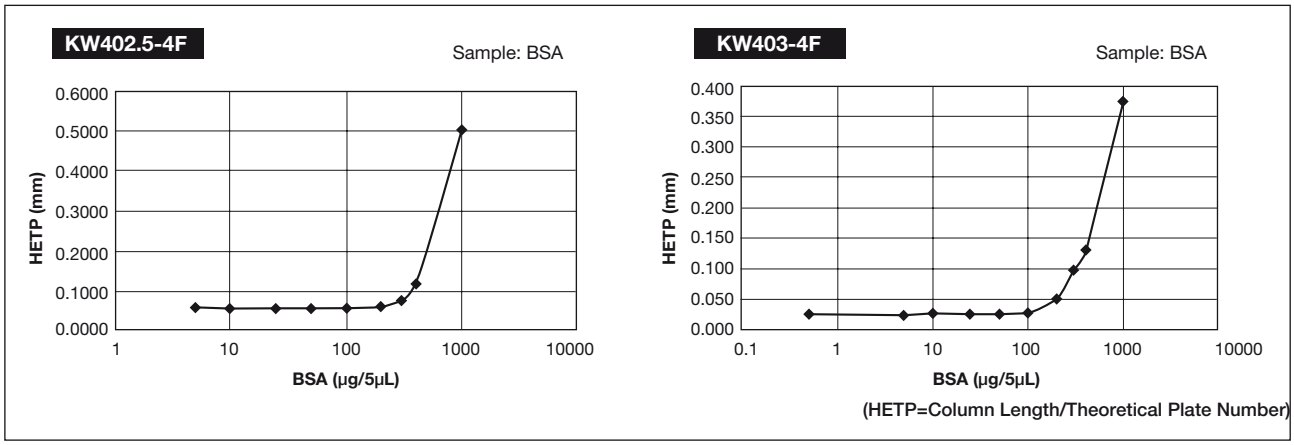


Fig. 4-4 Relationship of Sample Loads and the HETP using KW402.5-4F and KW403-4F

Column : Shodex KW402.5-4F (4.6mmID x 300mm)
Eluent : 50mM Sodium Phosphate Buffer + 0.3M NaCl (pH7.0)
Flow Rate : 0.35mL/min
Detector : UV (280nm)
Column Temp. : 25℃

Column : Shodex KW403-4F (4.6mmID x 300mm)
Eluent : 50mM Sodium Phosphate Buffer + 0.3M NaCl (pH7.0)
Flow Rate : 0.35mL/min
Detector : UV (280nm)
Column Temp. : 25℃

5. Optimization of HPLC system

Semi-micro columns are designed to minimize sample diffusion inside, compared to conventional columns. Therefore, it is recommended to use a semi-micro type HPLC system to obtain the optimal performance, because a conventional HPLC system could cause sample diffusion except for columns where it is connected with a semi-micro columns.

Table 5-1 shows the theoretical plate numbers for a uridine analysis with a KW402.5-4F column in combination with a conventional HPLC system and a semi-micro type HPLC system, respectively. KW402.5-4F with a conventional HPLC system has a lower theoretical plate number, about 60% of that of the same column with a semi-micro HPLC system. The following data illustrate how each HPLC system component for a semi-micro type improves separation compared to the conventional HPLC system.

Table 5-1. Comparison of Resolution with a Conventional and a Semi-micro Type of HPLC System

	Injector [1]	Inner Diameter of Tubings [2]	Cell Volume of UV [3]	Theoretical Plate Number [4]
Conventional Type	Rheodyne 7725i	0.25mm	17.7μL	26,000
Semi-micro Type	Rheodyne 8125	0.13mm	2.4μL	43,700

Column : Shodex KW402.5-4F (4.6mmID x 300mm)
Eluent : 50mM Sodium Phosphate Buffer + 0.3M NaCl (pH7.0)
Flow Rate : 0.35mL/min
Detector : UV (280nm)
Column Temp. : 25℃

1) Rheodyne 7125i: Conventional Type, Rheodyne 8125: Low Dead Volume Type.
2) The length of tubing between injector and column inlet: 100mm.
Tubing between the column outlet and the UV detector: accessory of the cell.
Conventional type cell: approx. 0.2mmID x 600mmL, semi-micro type cell: approx. 0.1mmID x 500mmL
3) Optical path length of a UV cell: conventional type 10mm, semi-micro type: 3mm
4) The theoretical plate numbers are measured by 0.1% Uridine with 1μL injection.

5-1. Injector

The relationship between injector types and theoretical plate numbers is shown in Table 5-2. Only changing an injector to a lower dead-volume type instead of a conventional type doesn't improve theoretical plate numbers as well as changing the other components to lower dead-volume ones, too.

Table 5-2. Relationship of Injector Types and Theoretical Plate Numbers

Injector [1]	Inner Diameter of Tubings [2]	Cell Volume of UV [3]	Theoretical Plate Number [4]
Rheodyne 7725i	0.25mm	17.7μL	26,000
Rheodyne 8125	0.25mm	17.7μL	26,100

Column : Shodex KW402.5-4F (4.6mmID x 300mm)
Eluent : 50mM Sodium Phosphate Buffer + 0.3M NaCl (pH7.0)
Flow Rate : 0.35mL/min
Detector : UV (280nm)
Column Temp. : 25℃

Please refer to the captions in Table 5-1.

5-2. Tubing

Table 5-3 shows the relationship between inner diameters of tubings and theoretical plate numbers. A better theoretical plate number can be achieved by using a narrower tubing.

Table 5-3. Relationship of Inner Diameters of Tubings and Theoretical Plate Numbers

Injector [1]	Inner Diameter of Tubings [2]	Cell Volume of UV [3]	Theoretical Plate Number [4]
Rheodyne 7725i	0.25mm	17.7μL	26,000
Rheodyne 8125	0.13mm	17.7μL	27,000

Column : Shodex KW402.5-4F (4.6mmID x 300mm)
Eluent : 50mM Sodium Phosphate Buffer + 0.3M NaCl (pH7.0)
Flow Rate : 0.35mL/min
Detector : UV (280nm)
Column Temp. : 25℃

Please refer to the captions in Table 5-1.

5-3. UV Cells

Table 5-4 describes theoretical plate numbers in a uridine analysis with a UV detector for a semi-micro cell and a conventional cell. Here the conventional tubings and injectors are used. The larger conventional cell, which has over seven times the volume of the semi-micro cell, showed greater sample diffusion and the theoretical plate number was also extremely affected.

Table 5-4. Relationship of cell volume and theoretical plate number

Injector [1)]	Inner Diameter of Tubings [2)]	Cell Volume of UV [3)]	Theoretical Plate Number [4)]
Rheodyne 7725i	0.25mm	17.7μL	26,000
Rheodyne 7725i	0.25mm	2.4μL	43,500

Column : Shodex KW402.5-4F (4.6mmID x 300mm)
Eluent : 50mM Sodium Phosphate Buffer + 0.3M NaCl (pH7.0)
Flow Rate : 0.35mL/min
Detector : UV (280nm)
Column Temp. : 25°C

Please refer to the captions in Table 5-1.

As shown in Tables 5-1, 5-2, 5-3 and 5-4, it is recommended to use a semi-micro HPLC system to obtain the optimal performance of KW400 series columns. It is also possible to use the KW400 series with a conventional HPLC system, but the sample diffusion at a UV cell is especially large, therefore, it is recommended to at least use a semi-micro type UV cell.

[Information]
The sensitivity of a UV detector (or peak height) is proportional to the cell volume (or optical path length), therefore a conventional cell might be more sensitive than a semi-micro cell, as shown in Figure 5-1.

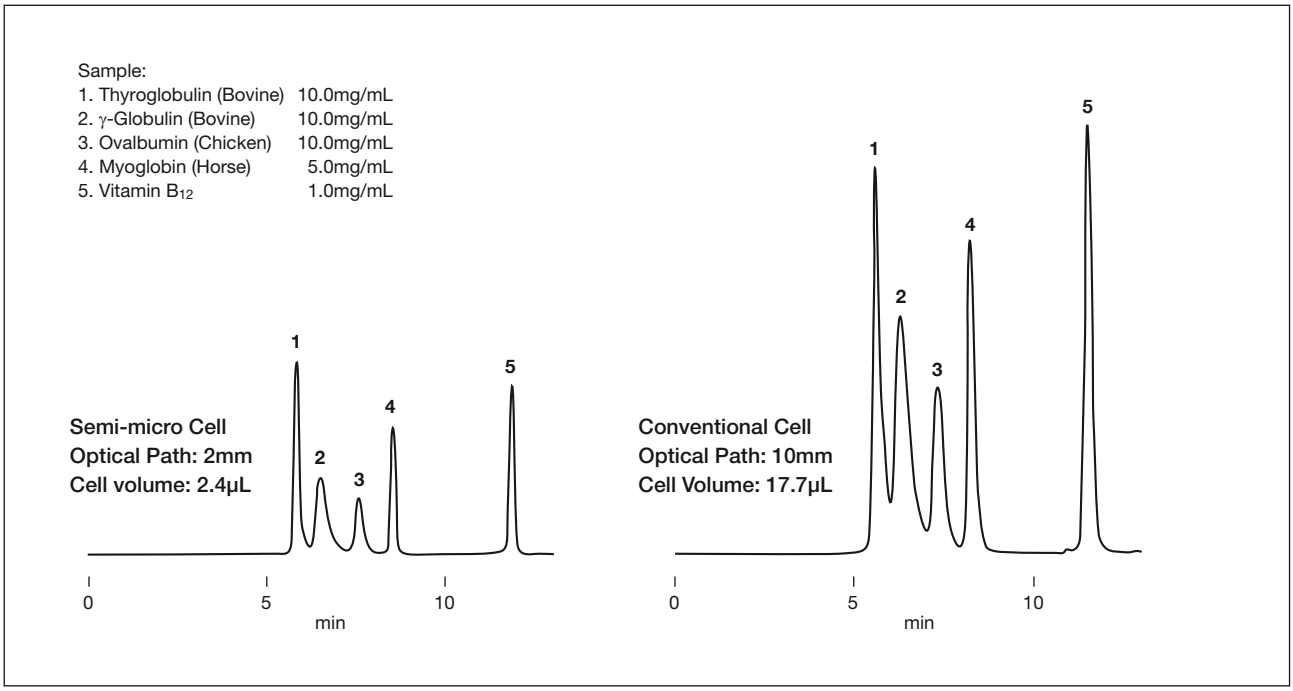


Fig. 5-1 Difference of Peak Height with UV Cell Types

Column : Shodex KW402.5-4F (4.6mmID x 300mm)
Eluent : 50mM Sodium Phosphate Buffer + 0.3M NaCl (pH7.0)
Flow Rate : 0.35mL/min
Detector : UV (280nm)
Column Temp. : 25°C

6. Applications

6-1. Control Serum

Figure 6-1 shows chromatograms using KW403-4F and KW405-4F for a control serum. Since the pore size of the packing material of KW404-4F is larger than that of KW403-4F, KW404-4F can analyze large substances whose molecular weight is above the exclusion limit of KW403-4F.

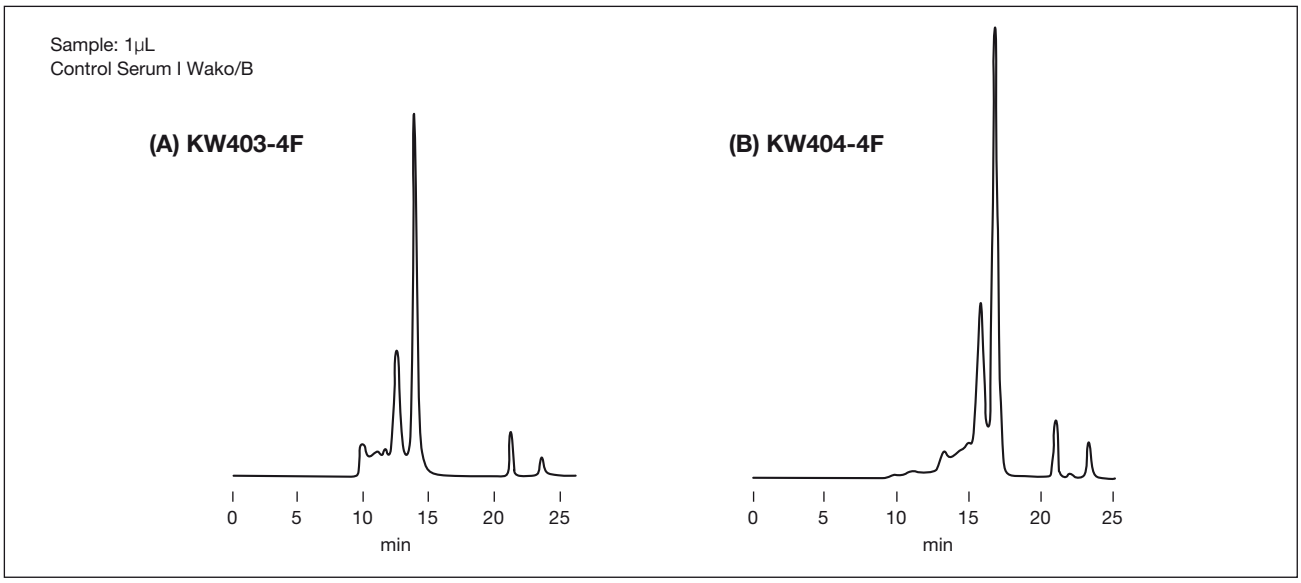


Fig. 6-1 Control Serum

Columns : (A) Shodex KW403-4F (4.6mmID x 300mm), (B) Shodex KW404-4F (4.6mmID x 300mm)
Eluent : 50mM Sodium Phosphate Buffer + 0.3M NaCl (pH7.0)
Flow Rate : 0.20mL/min
Detector : UV (280nm)
Column Temp. : 25℃

6-2. Whey

Figure 6-2 shows a chromatogram of whey in yoghurt.

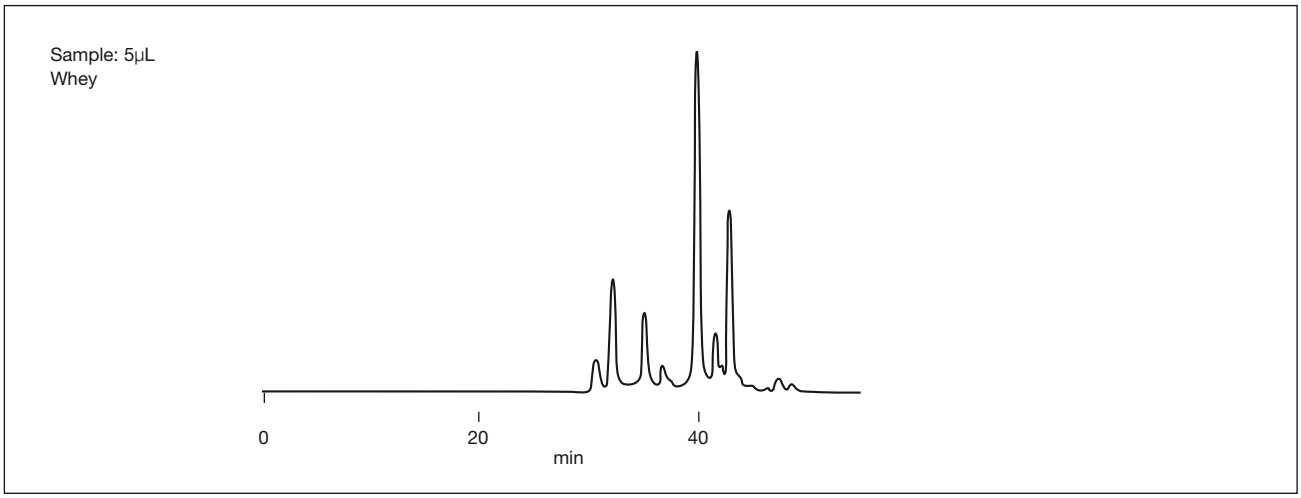


Fig. 6-2 Whey in Yoghurt

Columns : Shodex KW402.5-4F + KW403-4F (4.6mmID x 300mm each)
Eluent : 50mM Sodium Phosphate Buffer + 0.3M NaCl (pH7.0)
Flow Rate : 0.20mL/min
Detector : UV (280nm)
Column Temp. : 30℃

6-3. Lectins

Figure 6-3 shows chromatograms of lectins. Lectins are proteins with a special affinity to specific kinds of sugar and their origins are diverse, such as glycoproteins and metal-containing.

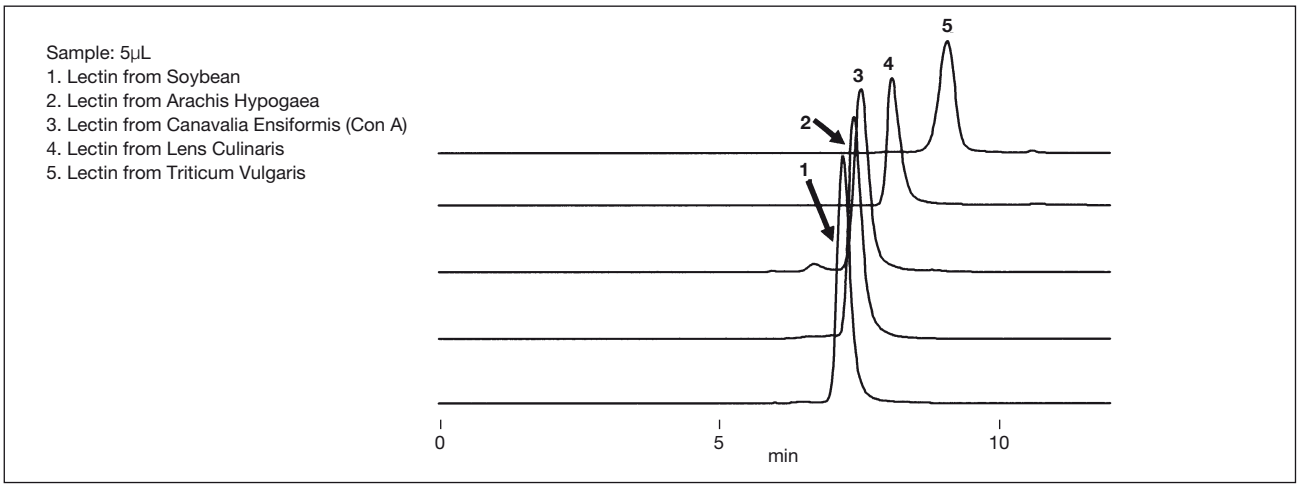


Fig. 6-3 Lectins

Column : Shodex KW402.5-4F (4.6mmID x 300mm)
Eluent : 50mM Sodium Phosphate Buffer + 0.3M NaCl (pH7.0)
Flow Rate : 0.33mL/min
Detector : UV (220nm)
Column Temp. : 30˚C

6-4. Peptides

Figure 6-4 indicates chromatograms for peptides with a molecular weight of 269 to 1734. Because characteristics of side chains of amino acids influence the separation in peptide analyses, not like protein analyses, peptides containing lots of hydrophobic amino or basic acids might cause interactions besides size exclusion.

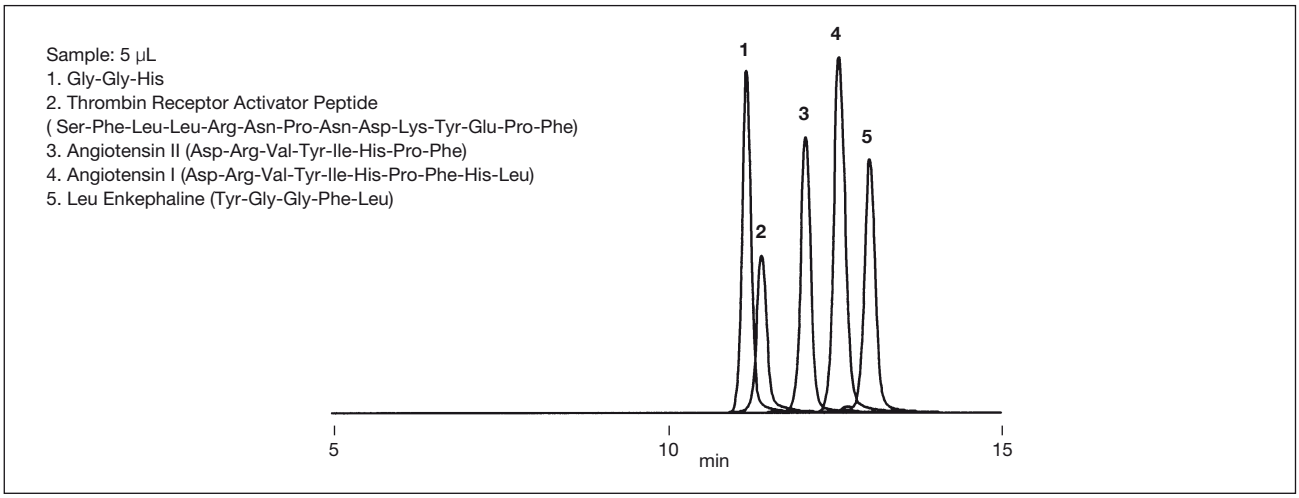


Fig. 6-4 Peptides with a Molecular Weight of 269 to 1734

Column : Shodex KW402.5-4F (4.6mmID x 300mm)
Eluent : 50mM Sodium Phosphate Buffer + 0.3M NaCl (pH7.0)
Flow Rate : 0.33mL/min
Detector : UV (220nm)
Column Temp. : 30˚C

7. Column Selection for Proteins and Peptides

To analyze proteins and peptides, a wide variety of Shodex columns is available using various separation modes, including SEC, reversed phase, ion exchange, hydrophobic interaction chromatography, multimode and affinity chromatography.

GFC columns are suitable for the first screening of unknown samples, whereby substances elute in a decreasing order of molecular weight. Reversed phase columns, which are most widely used, separate substances by using the different grades of hydrophobicity.

In addition to standard columns with ID 4.6mm, we provide micro columns with ID 300micron-800micron, semi-micro columns with ID 1.0mm-2.0mm and preparative columns with ID 10mm and above. Please select the suitable size according to your purpose.