Separation Report No. 028

Principles and Applications of High Performance GPC Columns: TSKgel H_{XL} Type Columns for Oligomer Analysis

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1. Intr oduction

High performance liquid chromatography can be classified into four categories based on the separation mode: gel permeation* (GPC, often referred to as Size Exclusion Chromatography or SEC)), adsorption (liquid-solid chromatography: LSC), partition (liquid-liquid chromatography: LLC), and ion exchange (IEC) chromatography. Among these separation modes, GPC is the only one that does not use a physical interaction between the packing material and the sample. Instead, GPC is characterized by separation based on the molecular size of the sample. Consequently, most of the data obtained from GPC relates to the size (molecular weight) of the sample, and thus it is an extremely useful procedure for analyzing unknown molecules.

An additional advantage of GPC is that the analysis process is simple, as there is no need to evaluate the composition of the mobile phase, as is required with other modes.

Currently not only is GPC being used in analysis and process control to analyze low molecular weight substances, oligomers and polymers, it is also being employed in pretreatment procedures and preparative separation, etc., of complex samples.

In this report the use of GPC is introduced, focusing on the characteristics of the TSKgel H_{XL} Series high-performance columns, which reduce the analysis time of more conventional GPC columns by half.

* In the broad sense of the term, GPC may refer to procedures in which an organic solvent or an aqueous solution is used as the mobile phase. Recently, the use of an aqueous mobile phase has been categorized separately as gel filtration chromatography (GFC).

2. Principles of GPC Separation

The separation performance of a column used in GPC is evaluated by the calibration curve. Figure 1 shows a calibration curve and graphical representation of how a sample is separated in a column. The packing material used in GPC contains countless pores in sizes ranging from dozens to thousands of Angstroms and it is believed that the sample molecules can freely enter the pores.

In the example illustrated in Figure 1.1, three compounds of different molecular weight are injected into the column. The substances which are larger than the pore size of the packing material (A) elute through the column in the mobile phase outside of the packing material (total exclusion volume: Vo). Those molecules smaller than the pore size of the packing material (C) will elute more slowly as they will diffuse into the pores of the packing material (Vi).

The percentage of pores that can be permeated by the sample varies depending on the size of the sample. Shown in Figure 1.1 is sample (B) with a size between (A) and (C). Sample B will elute from the column at a time between (A) and (B). By monitoring the eluate from the column with an appropriate detector, a chromatogram is obtained, such as the one shown in Figure 1.2. The elution time, or elution volume, is shown on the horizontal axis. In GPC, the largest samples always are eluted first, and consequently the molecular weight can be estimated by comparing elution volumes. A calibration curve is constructed by plotting the sample elution volume (time) against the logarithm of the molecular weight of the sample, as is shown Figure 1.3. The calibration curve includes two components: a part normally composed of two lines with a comparatively steep slope, and one line with a shallower slope.

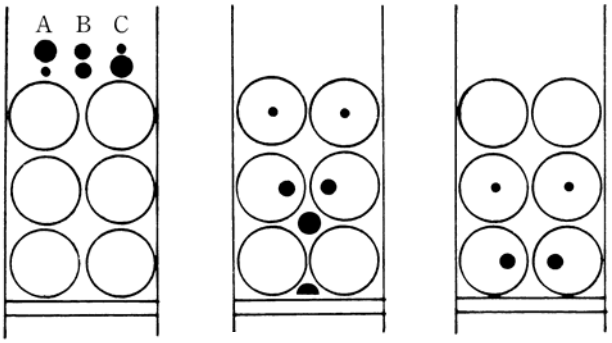
The quality of the separation of compounds can be determined from the slope of the calibration curve; the more gradual the slope, the better the separation.

Section (a) of the calibration curve reveals that the compound is being eluted at Vo, the total exclusion volume. This occurs because the component is larger than the largest pores of the packing material. The highest molecular weight that can permeate the pores is referred to as the exclusion limit.

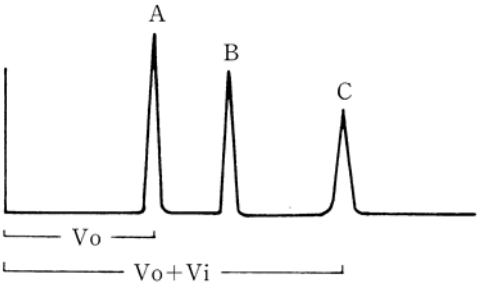
In section (c), also called the total inclusion volume, the smallest molecule has completely entered the pores. In reality, effective separation occurs only in section (b), and it is imperative to select a packing material (column) with a pore size or exclusion limit that is compatable with the size of the sample components.

It is important to note that the vertical axis of the calibration curve is the logarithm of the molecular weight, thus separation is not based on the difference between the molecular weights, but on the ratio between the molecular weights ($\log M_2 - \log M_1 = \log M_2/M_1$). Moreover, the calibration curve will vary depending on the chemical composition of the calibration standards used in analysis. Therfore, in order to accurately measure the molecular weight of the sample, standards must be prepared that have the same or similar characteristics as the sample component being analyzed.

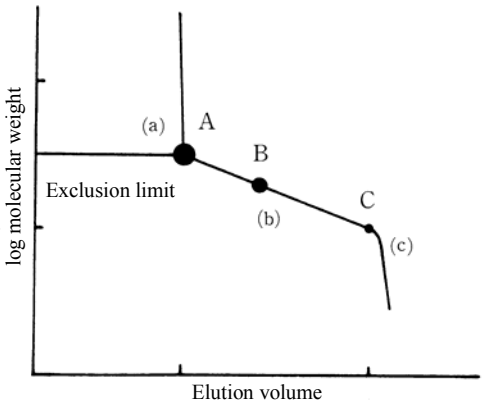
Figure 2 shows an example of a calibration curve constructed by analyzing n-paraffins, aliphatic acids and alcohols using the TSKgel G2000H_{XL} column. Aliphatic acids and n-paraffins elute along the same curve, but with alcohols the elution tends to occur slightly delayed as molecular weight decreases.



1) Separation of samples



2) Chromatogram



3) Calibration curve

Fig. 1 Principles of GPC separation

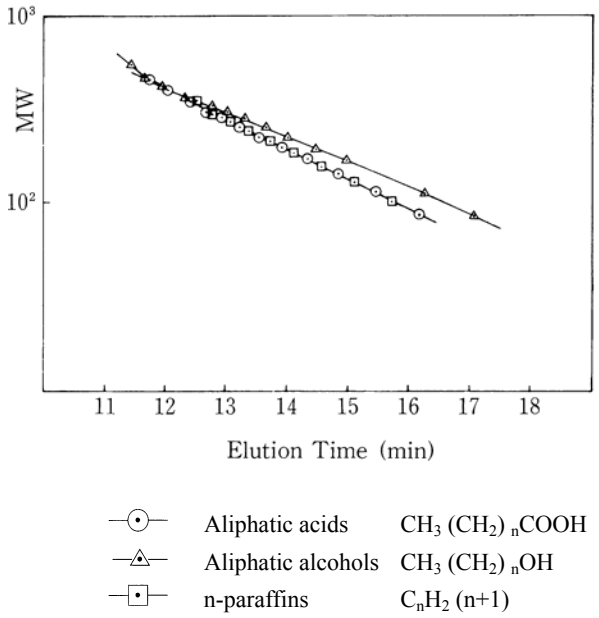


Fig. 2 Calibration curves for n-paraffin, aliphatic acid, and alcohol

Column: TSKgel G2000H_{XL}
(7.8mm ID x 30cm) x 2
Solvent: THF
Flow rate: 1.0 mL/min

3. TSK gel H_{XL} type columns

The matrix in the TSKgel H_{XL} type columns is a styrene-divinylbenzene copolymer, similar to what is used in TSKgel H-type columns. Table 1 shows the 9 grades of TSKgel H_{XL}-type columns. The number of theoretical plates is greatly increased for H_{XL} columns compared to conventional H-type columns. In the low molecular weight grades, where high performance is particularly in demand, there is a two-fold increase in the number of theoretical plates. As a consequence, column length has been reduced to 30cm, half the length of 60cm conventional columns, which reduces analysis time and solvent consumption. These columns have the further advantage of having a greater number of theoretical plates, resulting in less broadening of the peaks and in an increase in peak height, which improves detection sensitivity.

Commonly, polystyrene standards are used to calibrate TSKgel H_{XL}-type columns, not just for analyzing polystyrene polymer samples, but also for many other polymers for which standards with narrow molecular weight distribution are not readily available.

Tetrahydrofuran (THF) is most often used as a mobile phase, but when the objective is preparative separation, chloroform is more appropriate, as THF can form explosive polymers when heated. If a sample to be analyzed cannot be dissolved in either of these chemicals, a solvent such as DMF or m-cresol/chloroform is used.

Table 1 TSKgel H_{XL} Columns

Name	Exclusion limit	Number of theoretical plates
TSKgel G1000H_{XL}	1 x 10^3	16,000TP/30cm
TSKgel G2000H_{XL}	1 x 10^4	16,000
TSKgel G2500H_{XL}	2 x 10^4	16,000
TSKgel G3000H_{XL}	6 x 10^4	16,000
TSKgel G4000H_{XL}	4 x 10^5	16,000
TSKgel G5000H_{XL}	4 x 10^6	14,000
TSKgel G6000H_{XL}	4 x 10^7 (estimate)	14,000
TSKgel G7000H_{XL}	4 x 10^8 (estimate)	14,000
TSKgel GMH_{XL}	4 x 10^8 (estimate)	14,000
TSKgel GMH_{XL}-HT	4 x 10^8 (estimate)	5,500

4. Column Selection

The column grade should be selected based on the calibration curve. Figure 3 shows calibration curves for each grade.

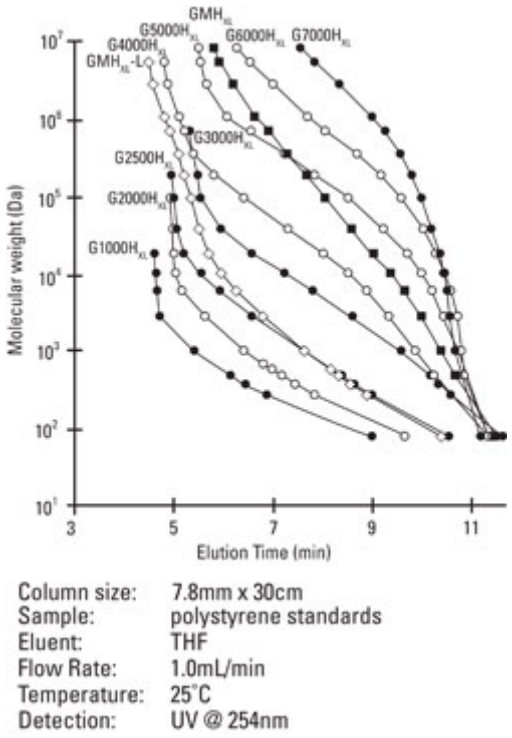


Fig. 3 TSKgel H_{XL} calibration curves

The purpose of the mixed-bed TSKgel GMH_{XL} column is to analyze the molecular weight distribution of polymers. Because mixed-bed columns provide a linear relationship over a wide range of molecular weights, the slope of the calibration curve is steeper than that for the individual pore size columns. Mixed-bed columns are not the preferred option when the objective is to obtain the best possible separation.

If the molecular weight of the sample being analyzed can be estimated, the pore size grade can be chosen directly based on the calibration curve. If the molecular weight of the sample is known to be in the in the range of 100 to 100,000 dalton, the sample can be analyzed using a system in which TSKgel $G4000H_{XL}$ and TSKgel $G2000H_{XL}$ columns are placed in series. Select the mixed-bed TSKgel GMH_{XL} column if the molecular weight of the sample is completely unknown.

In order to demonstrate changes in separation that occur when analysis is conducted with the various column grades, Figure 4 depicts chromatograms produced when a typical epoxy resin (Epikote 1004) was analyzed using grades ranging from TSKgel $G1000H_{XL}$ through TSKgel $G4000H_{XL}$. In the chromatogram produced with the TSKgel $G1000H_{XL}$ column, most of the components are eluted at the exclusion limit, and although this grade does not show the molecular weight distribution accurately, this column can be used in preparative separations and when analyzing oligomers that have a polystyrene-converted molecular weight of 800 or less (elution time $\geq$ 10 minutes). Analysing oligomer content in the presence of polymer is convenient for bulk determination in the high molecular weight region and reduces analysis time.

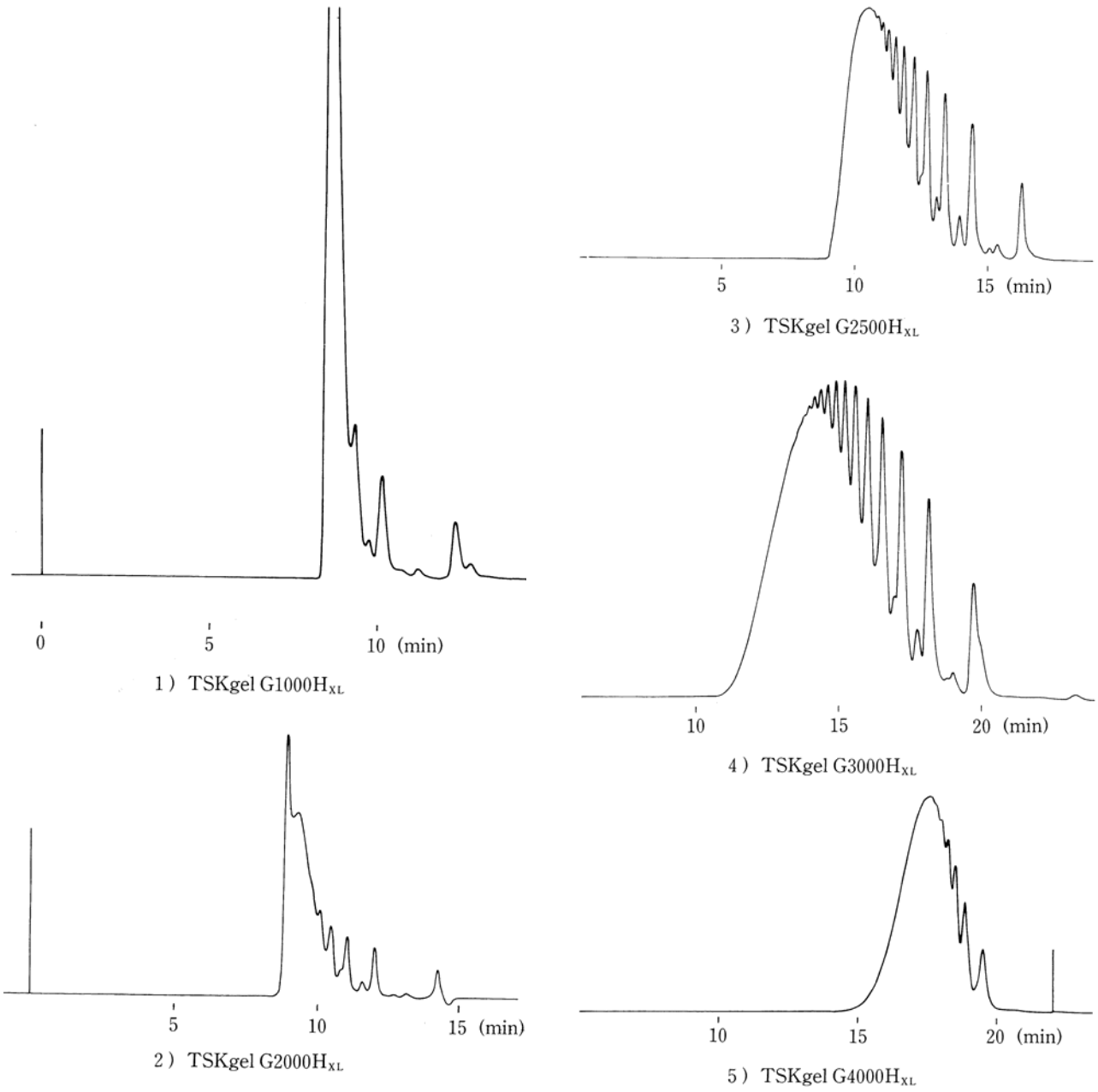


Fig. 4 Separation of Epikote 1004 by TSKgel G1000H$_{XL}$ through TSKgel G4000H$_{XL}$ columns

Although the TSKgel G2000H_{XL} produces more peaks than the TSKgel G1000H_{XL}, some components are still excluded, but separation is improved in the polystyrene-converted molecular weight range of 800 to 1,000.

Virtually the entire molecular weight distribution is provided by the TSKgel G2500H_{XL} column. However, the shape of the peaks at the higher molecular weight range of the distribution suggests the existence of a component with a molecular weight above the exclusion limit of the column. Separation in the polystyrene-converted molecular weight range of 2,000 to 10,000 has improved; however, resolution of the last eluting components from the TSKgel G2500H_{XL} column has deteriorated (MW ≈ 250).

The number of peaks is greatest with the TSKgel G3000H_{XL} column, which is the best column to use to obtain the full molecular weight distribution. Moreover, the peak from an excluded polymer component (anticipated from the TSKgel G2500H_{XL} chromatogram) is not observed.

Components with a polystyrene-converted molecular weight of 1,000 or less elute in or close to the inclusion volume with the TSKgel G4000H_{XL}column. It is apparent that the pores in this grade are too large for the sample.

As is indicated from the above discussion, even with the same sample, the chromatogram will differ greatly depending on the column grade, and accurate data will not be obtained if the wrong column is selected.

The focal point of the data provided by each grade can be estimated from the calibration curves. Calibration curves of the TSKgel G1000H_{XL} through TSKgel G4000H_{XL} grade columns are shown in Figure 3. With the TSKgel G1000H_{XL}, the slope becomes shallow after elution of 10.5mL and components with a molecular weight of 600 or below are well separated. The TSKgel G2000H_{XL} is suitable for separation of compounds with a molecular weight of 1,000 or less. In the low molecular weight range, the slope of the calibration curve is not as steep with the TSKgel G1000H_{XL} as the TSKgel G2000H_{XL}, thus separation of low molecular weight components is best with the TSKgel G1000H_{XL}. With the TSKgel G2500H_{XL}, the slope is shallow up to a molecular weight of 3,000, but slightly steeper than with the TSKgel G2000H_{XL}. The chromatogram of the TSKgel G2500H_{XL} is separated on the high molecular weight side, but low molecular weight separation is inferior to that of the TSKgel G2000H_{XL}. The calibration curve of the TSKgel G3000H_{XL} has a gradual slope in the molecular weight range of 1,500 to 10,500. On the chromatogram, separation on the high molecular weight side is better than that of the TSKgel G2500H_{XL}, but low molecular weight separation is not as good. The optimal molecular weight range for the TSKgel G4000H_{XL} is 10,000 to 200,000. There is a noticeable difference in comparison to samples in a molecular weight range of 200 to 30,000, as the separation between polymers in this range is inadequate.

Thus as discussed above, in GPC, calibration curves provide crucial information and should always be checked when conducting sample analyses.

5. Connecting Columns in Series

Multiple columns are often connected in series in GPC analyses. The reasons for this are as follows:

(1) To improve separation or

(2) to expand the molecular weight range in which separation can be achieved

Separation of the sample components in liquid chromatography is expressed by Equation (1) below.

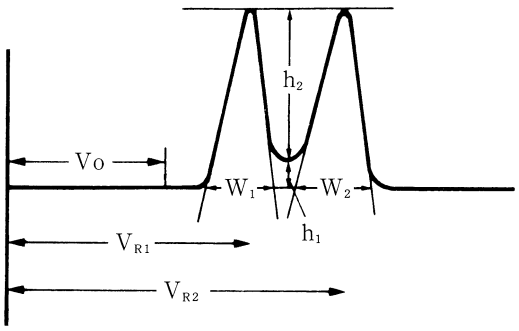
$$R=\frac{1}{4}\sqrt{N}\cdot\left(\frac{\alpha-1}{\alpha}\right)\cdot\left(\frac{k'}{k'+1}\right) \quad \cdots\cdots (1)$$

In Equation 1, R expresses the resolution between two components. As R increases, separation improves. N is the number of theoretical plates, α is the selectivity, and k′ the retention factor, which are computed by Equations (2) and (3), respectively.

$$\alpha=\frac{V_{R2}-V_O}{V_{R1}-V_O} \quad \cdots\cdots (2)$$

$$k'=\frac{V_R-V_O}{V_O} \quad \cdots\cdots (3)$$

(See Figure 5 for V_R and V_O)



$$R=\frac{2\,(V_{R2}-V_{R1})}{W_1+W_2} \quad \cdots\cdots (4)$$

or

$$\frac{h_2}{h_1+h_2}\times 100 \quad \cdots\cdots (5)$$

In Equation (4) the value increases as separation improves and in Equation (5), complete separation is achieved at 100%.

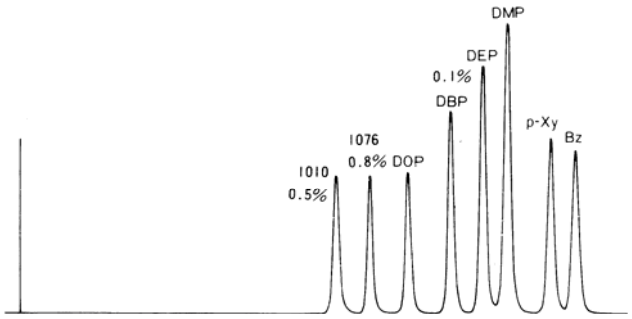
Fig. 5 Calculation method for resolution

As discussed in the section on the "Principles of GPC Separation", because the sample components do not interact with the packing material in ideal GPC, resolution (R) is governed by the number of theoretical plates and the slope of the calibration curve.

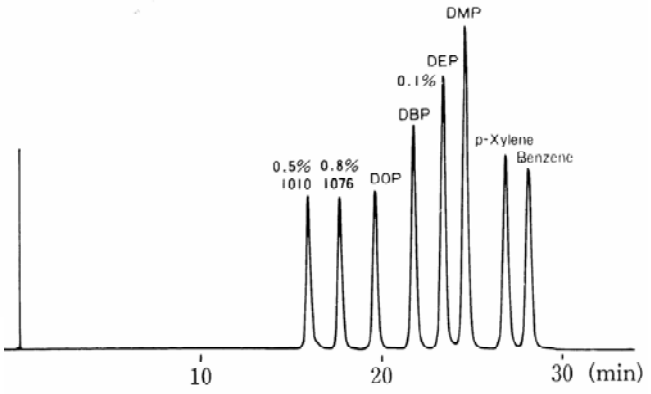
When sample components S_1 and S_2 are separated by GPC, α and k′ are constants, with no correlation to the length of the column. If the length of the column is doubled, V_R and V_O also double. Consequently, Equation (1) becomes

$$R = K\sqrt{N} \quad \cdots\cdots\cdots\cdots\cdots\cdots \quad (6) \quad K: \text{constant}$$

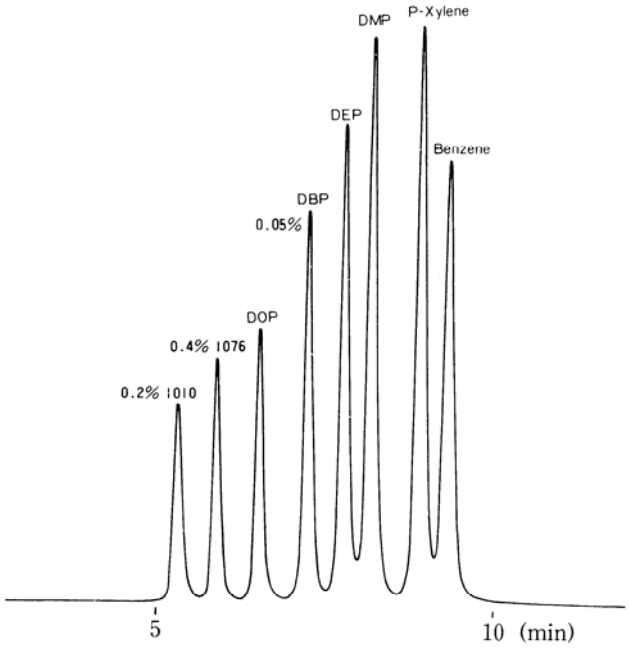
The easiest way to increase the number of theoretical plates is to increase the length of the column. Figure 6 shows chromatograms produced by analyzing phthalic acid esters, etc. using from one to four TSKgel G2000H_{XL} columns. As the length of the column increases, the number of theoretical plates increases proportionally (as measured with benzene) and separation improves.



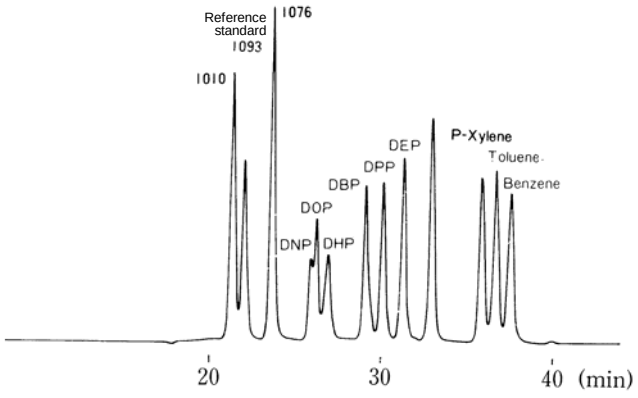
2 columns, N = 34,000



3 columns, N = 51,000



1 column, N = 17,000



4 columns, N = 68 000

Fig. 6 Separation of phthalic acid esters by TSKgel G2000H_{XL}

Figure 7 shows the results of plotting the resolution of DEP and DMP* (R) against the square root of the number of theoretical plates ($\sqrt{N}$). That there is a linear relationship between R and the square root of the number of theoretical plates $\sqrt{N}$ is evident from Figure 7, in that R increases proportionately with the square root of $\sqrt{N}$. In general, in GPC, the number of theoretical plates is expressed as the sum of the theoretical plates in each column. As a result, when the separation of sample components needs to be improved, the required number of columns can be calculated from the square root of $\sqrt{N}$ based on the targeted resolution.

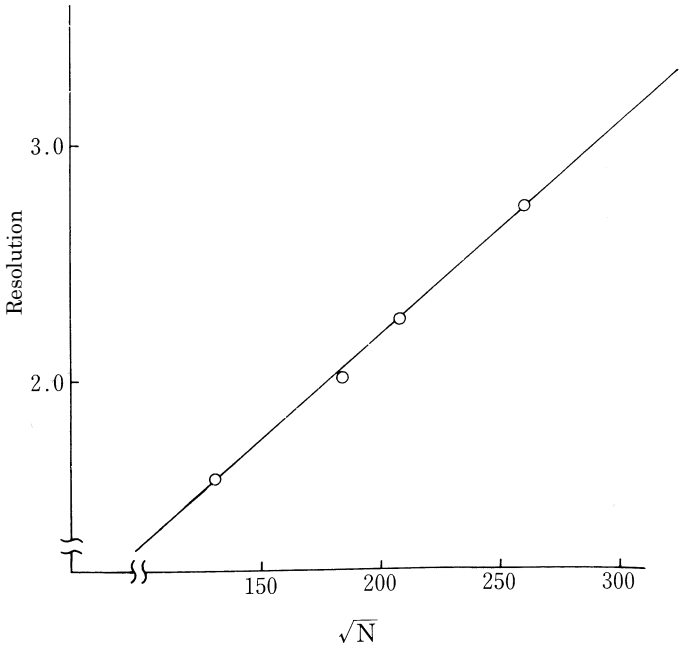


Fig. 7 Effect of number of theoretical plates on DEP-DMP resolution

Next, if separation is impossible or can only be achieved for a narrow range of molecular weights when using a single grade (pore size) of columns, it is recommended to connect multiple columns of different grades in series. The calibration curve produced by a system composed of multiple columns of different grades in tandem can be calculated from the calibration curves of each individual column. Table 2 shows an example of this, using two TSKgel G2000H_{XL} columns and two TSKgel G3000H_{XL} columns. Under the column headings in the table representing TSKgel G2000H_{XL} and TSKgel G3000H_{XL}, the elution volumes determined separately for each column are shown. The values indicated for G2H + G3H_{XL} (cal) correspond to the calculated elution volume when two TSKgel G2000H_{XL} columns are coupled with two TSKgel G3000H_{XL} columns. The G2H + G3H_{XL} (obs) column shows the actual values that were measured when two TSKgel G2000H_{XL} columns were coupled to two TSKgel G3000H_{XL} columns. The resulting calibration curve is displayed in Figure 8. The solid line represents the values generated by calculation; the points on the graph are actual measured values. The computed values and measured values are in excellent agreement. When it is necessary to combine columns of various grades, if the calibration curve of each individual column is known, the calibration curve of any system of columns can be derived through calculation.

Table 2 Method for calculating calibration curves

Molecular weight	G2H_{XL}	G3H_{XL}	G2H+3H_{XL} (cal)	G2H+3H_{XL} (obs)
6200	9.0 (mL)	13.9 (mL)	22.9 (mL)	22.9 (mL)
2800	9.8	15.6	25.4	25.2
682	12.1	18.3	30.4	30.4
578	12.5	18.6	31.1	31.0
474	12.9	18.9	31.8	31.8
370	13.5	19.3	32.8	32.8
266	14.4	19.9	34.3	34.3
162	15.8	20.7	36.5	36.4

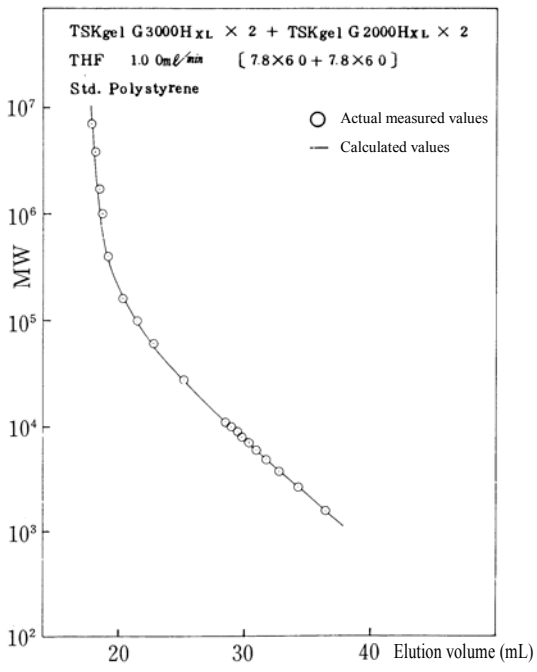


Fig. 8 Calibration curve of TSKgel G2000H_{XL} + TSKgel G3000H_{XL}

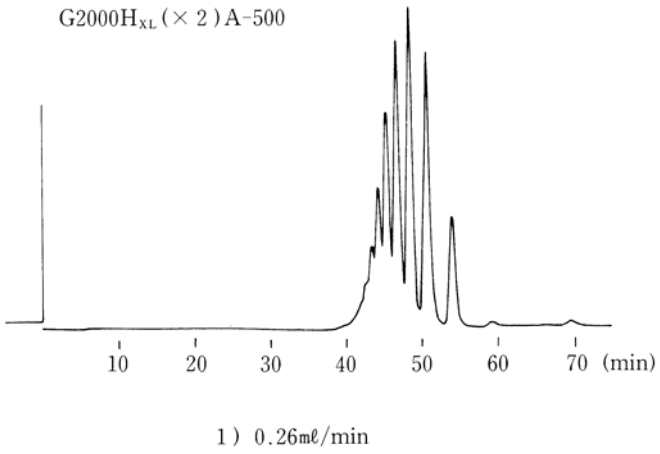
6. Effect of Flow Rate

It is well known that in high performance liquid chromatography, column efficiency and sample resolution vary depending on the flow rate of the mobile phase. Figure 9 shows the results of analyses of polystyrene oligomer A-500 using TSKgel 2000H_{XL} at various flow rates of THF. Table 3 shows resolution* calculated for components with a degree of polymerization (n) 5-4 and 4-3.

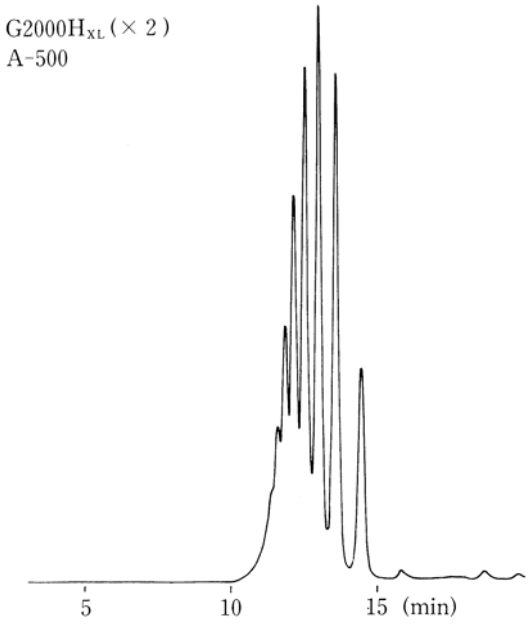
Table 3 Changes in resolution due to flow rate

Flow rate	0.26 (mL/min)	0.50	1.00
R_{5-4}	86 (%)	83	81
R_{4-3}	94 (%)	92	90

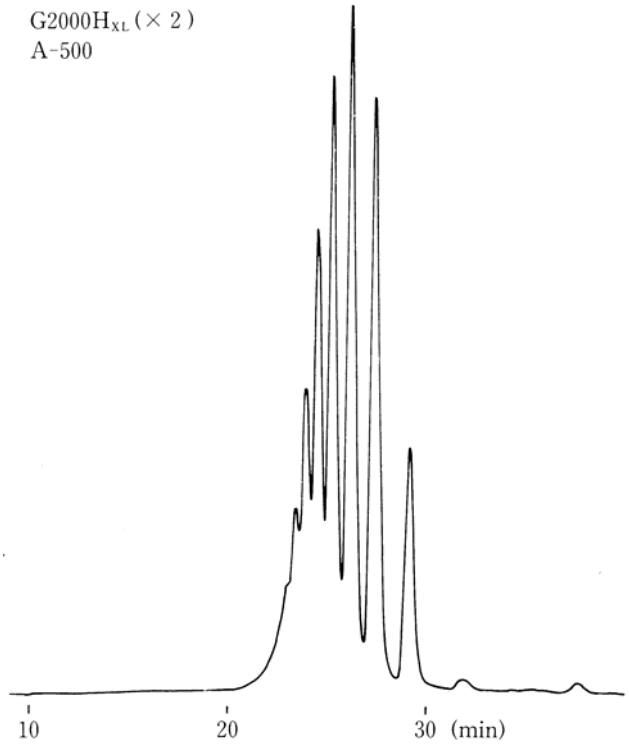
*Calculated using method shown in Fig. 5 (1)



1) 0.26mℓ/min



3) 1.0mℓ/min



2) 0.5mℓ/min

Fig. 9 Changes in separation due to flow rate

Even at a flow rate of 0.26mL/min, the separation is not substantially better in comparison to 1.0mL/min. Due to the small particle size of the packing material in TSKgel H_{XL} type columns, the flow rate has less impact than it has in conventional columns, particularly for low molecular weight compounds. In contrast, as flow rate decreases, analysis time increases. Figure 10 demonstrates the results when A-500 is analyzed using four TSKgel G2000H_{XL} columns. The analysis time in Figure 10 is about the same as it is in Figure 9. As is evident from the chromatograms, if the measurement time is identical, using additional columns significantly improves resolution (R_{5-4} and R_{4-3} are 95% and 98%, respectively.)

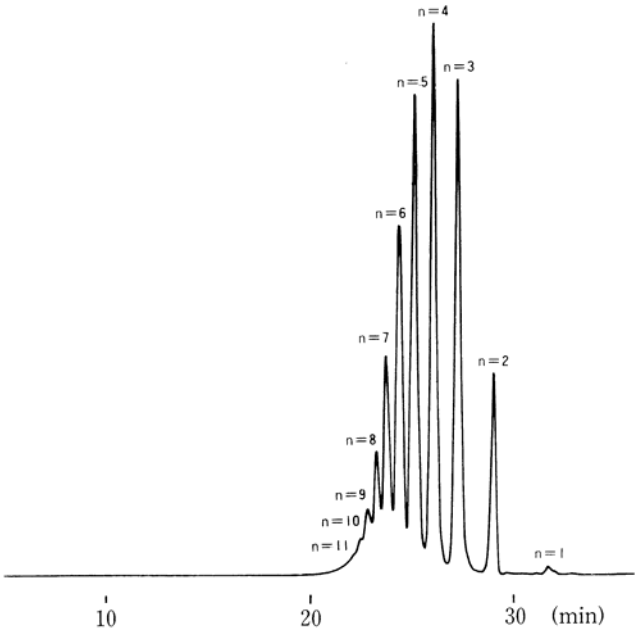


Fig. 10 Separation of A-500 using four G2000H_{XL} columns

Mobile phase: THF
Flow rate: 1.0mL/min

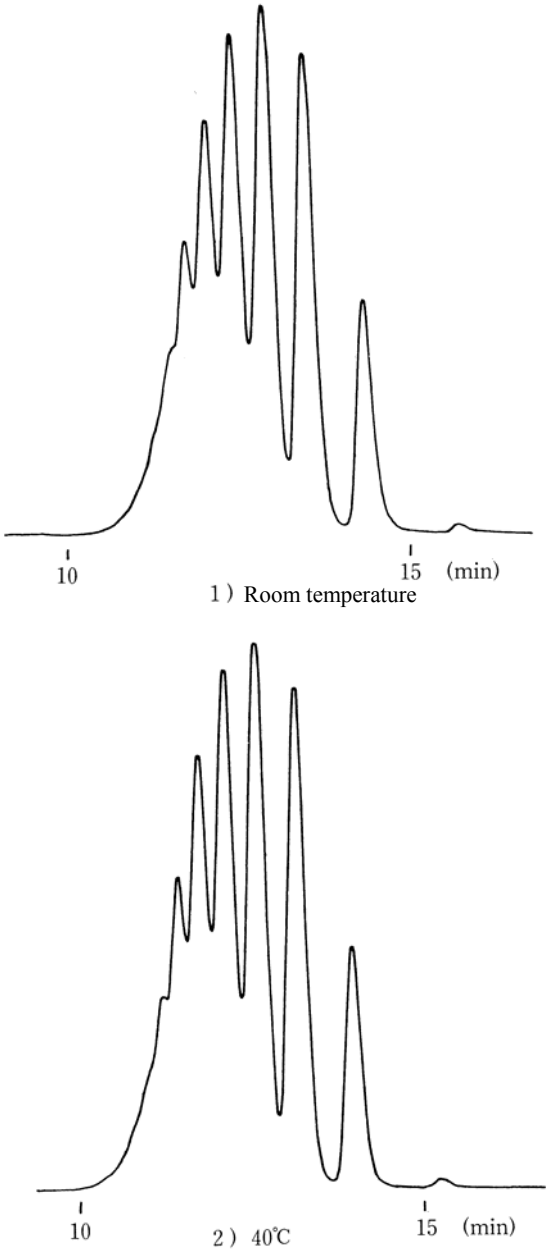


Fig. 11 Effects of column temperature on separation

Column: G2000H_{XL}
Solvent: THF
Flow rate: 1.0mL/min
Sample: polystyrene A500

7. Effect of Temperature

In general in GPC, peaks become sharper and separation improves when the column temperature is elevated. This is because the viscosity of the mobile phase decreases as temperature is increased, which accelerates diffusion of the sample. Figure 11 shows chromatograms obtained when polystyrene 500 was analyzed at room temperature versus 40ºC. Table 4 indicates the results of calculations of the resolution for each peak. As is shown from Figure 11 and Table 4, separation of low molecular weight components was little affected by temperature, but the resolution improved slightly for high molecular weight components at 40ºC. When analyzing substances of higher molecular weight, better results can be obtained by increasing the column temperature.

Table 4 Effects of column temperature on resolution

Column temp.	R_{3-2}	R_{4-3}	R_{5-4}	R_{5-6}	R_{6-7}
Room temp.	98 (%)	86	64	44	28
40ºC	98 (%)	87	65	46	32

8. Effect of Sample Size

In addition to analysis, preparative scale separations are another common GPC application. When a large quantity of a sample component is required for further studies, a large scale preparative column is selected, but a standard analytical column is suitable when only a small quantity of sample is required.

In preparative separations it is important to select the appropriate volume of injection and also sample mass. Typically, increasing the sample injection volume can lead to peak distortion due to overloading. Overloading tends to be more pronounced with higher performance columns as peak volumes are narrower. Mass overloading is another concern that one needs to be aware of. Figure 12 depicts the relationship between the height equivalent to a theoretical plate (H or HETP) and sample mass of benzene and Irganox 1010, using TSKgel G2000H_{XL}. Variations in H, resulting from changes in sample mass (often referred to as sample load), are dependent on the molecular weight of the sample. The higher the molecular weight, the more readily H is affected by increased sample mass. The quantity of a sample that can be injected depends on the elution position of the components to be separated, and although generally not expressed in numerical values, it is safe to state that preparative separations can be performed with TSKgel G2000H_{XL} for sample quantities ranging from one to several mg.

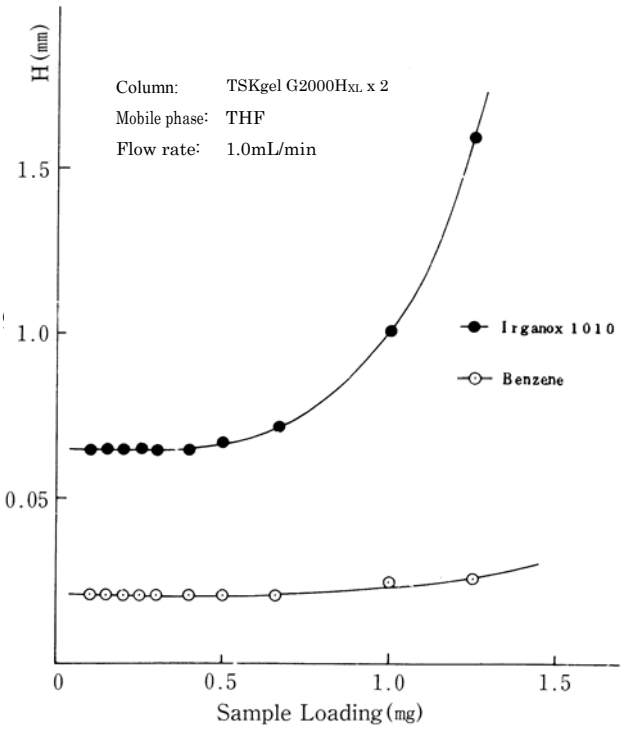
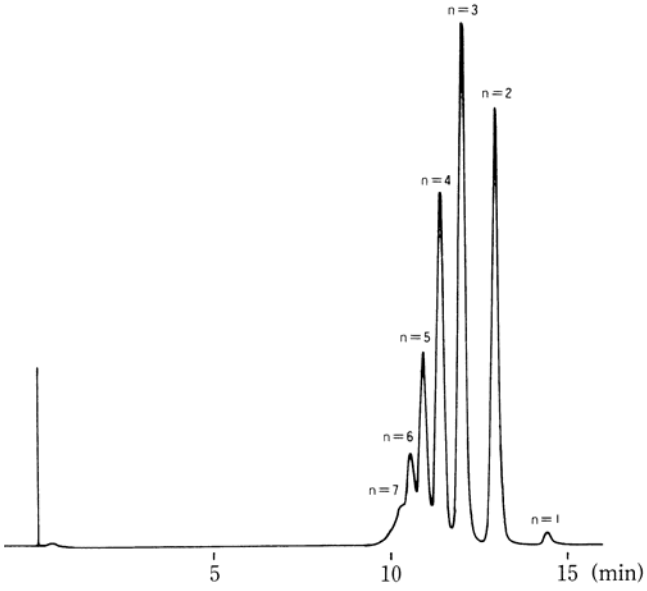
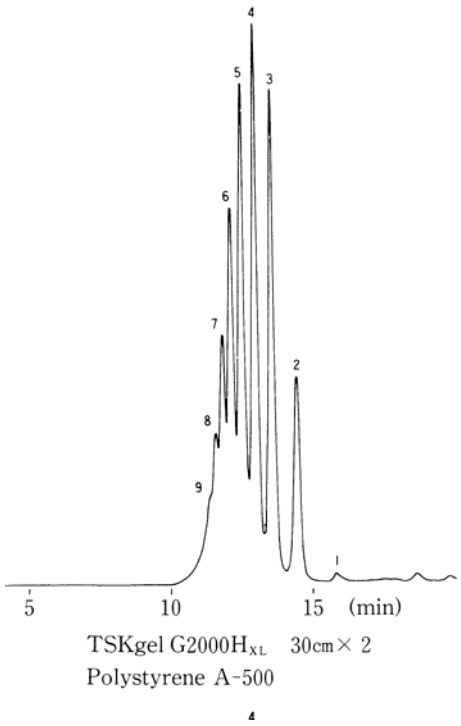


Fig. 12 Relationship between number of theoretical plates and sample mass

9. Application Examples

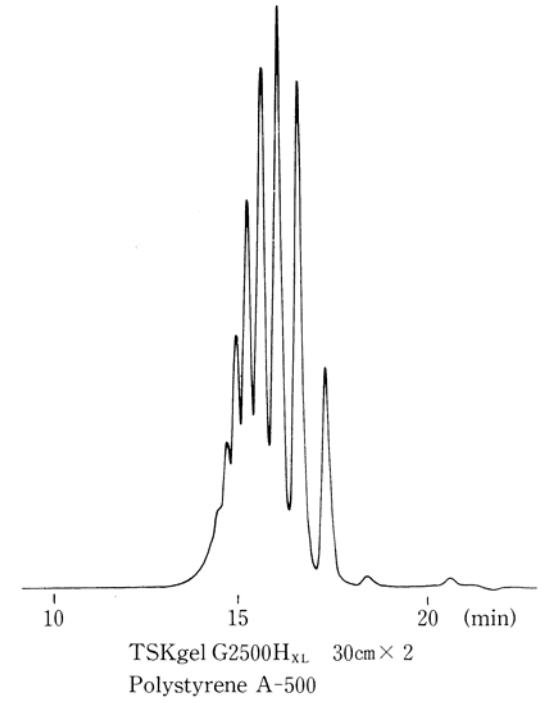


1) TSKgel G1000H_{XL} 30cm× 2
Polystyrene A-300



TSKgel G2000H_{XL} 30cm× 2
Polystyrene A-500

Fig. 13 Separation of polystyrene oligomer by TSKgel H_{XL} type columns



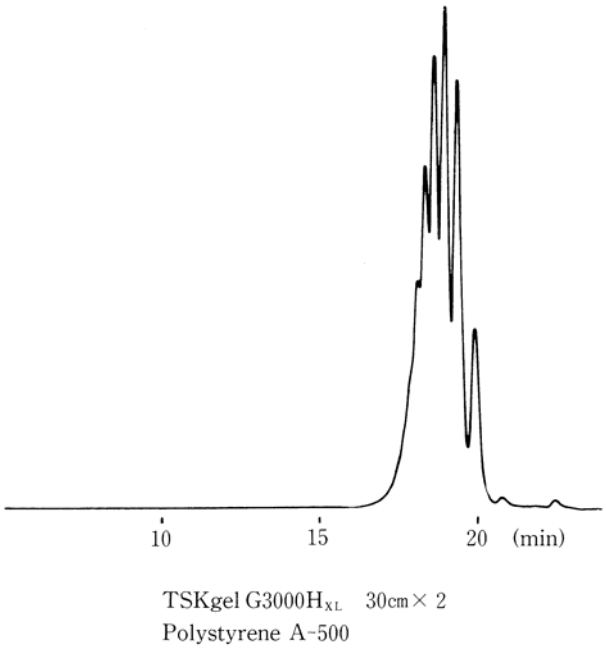


Fig. 13 Continued Separation of polystyrene oligomer by TSKgel H_{XL} type columns

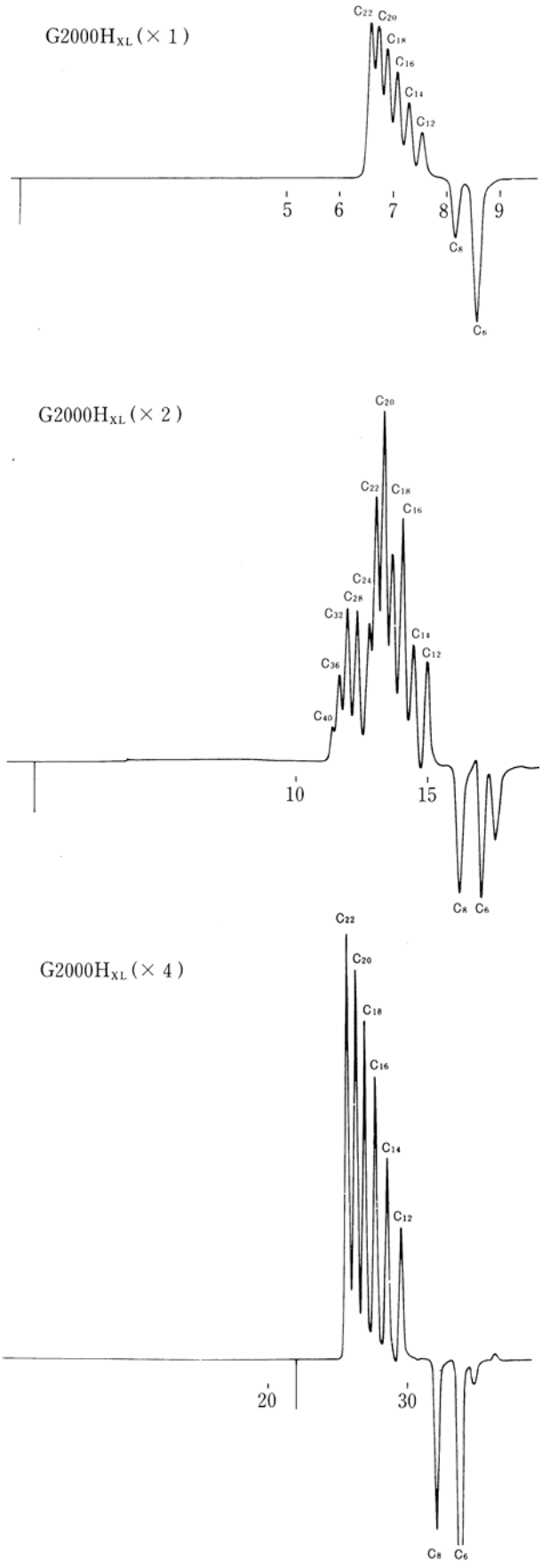
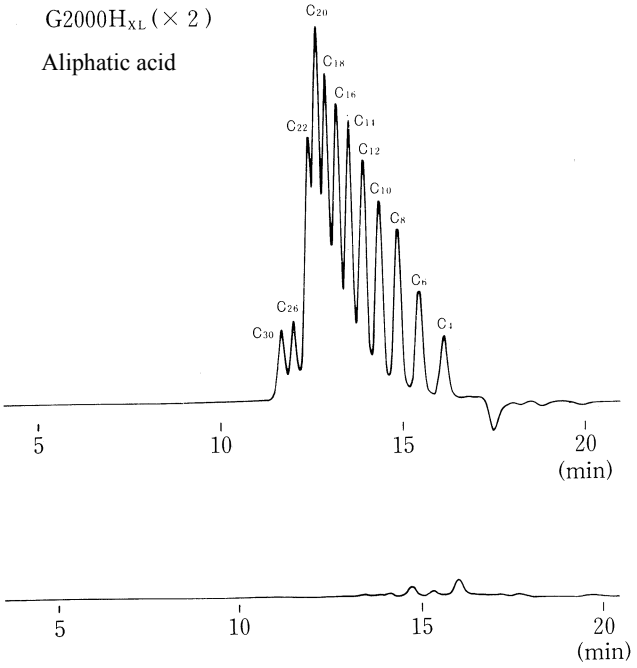
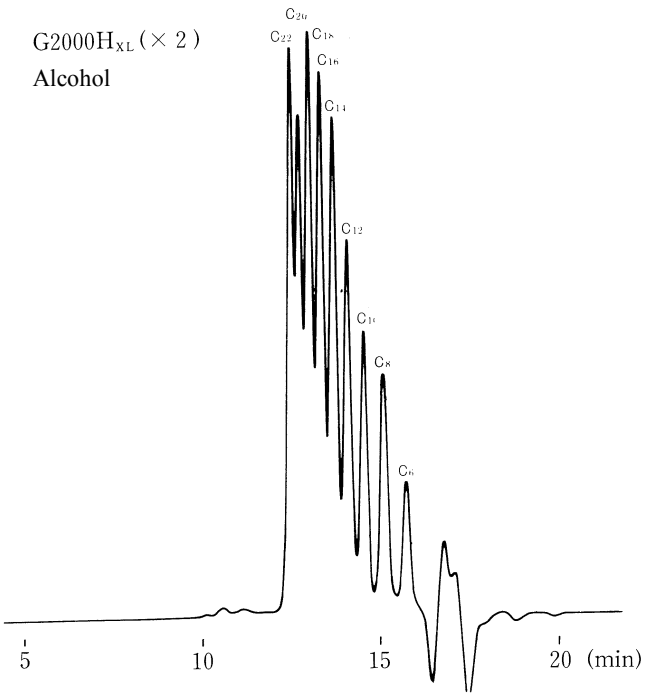
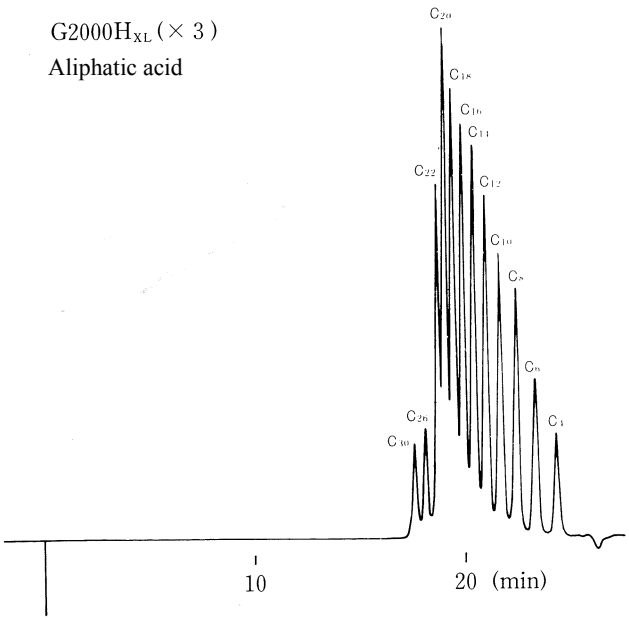


Fig. 14 Separation of n-paraffin

Column: G2000H$_{XL}$
Mobile Phase: THF
Flow rate: 10mL/min







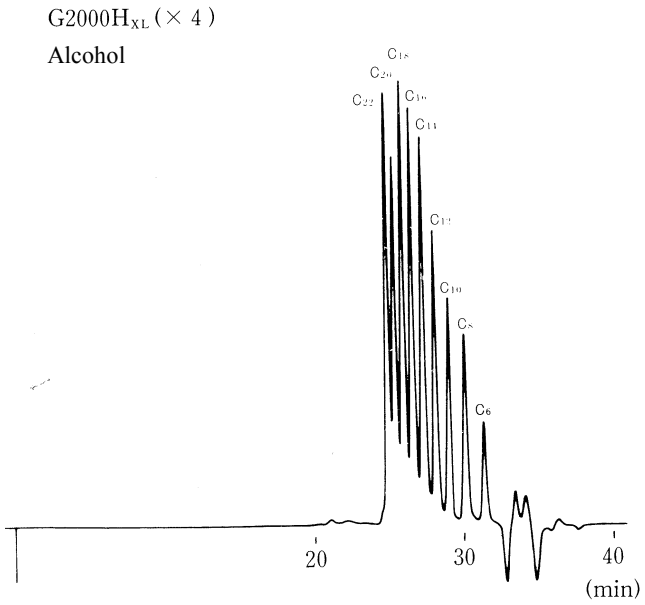
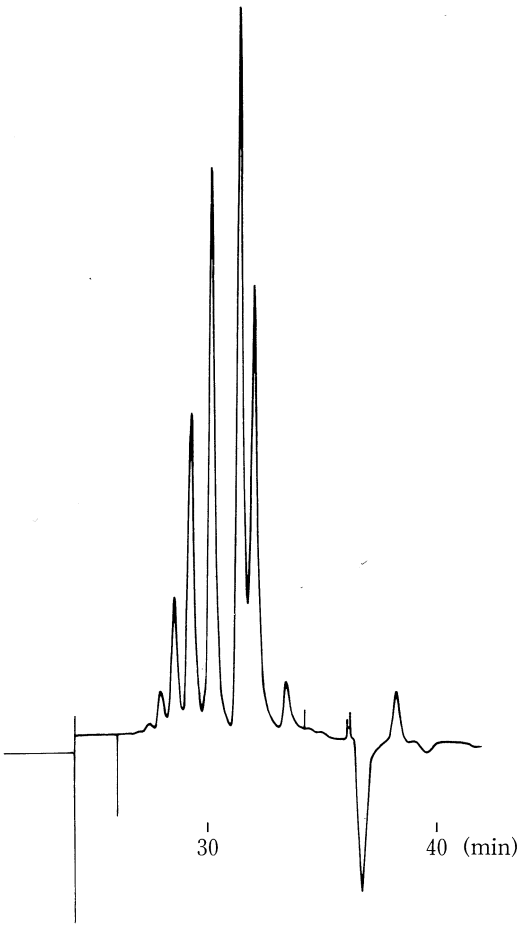
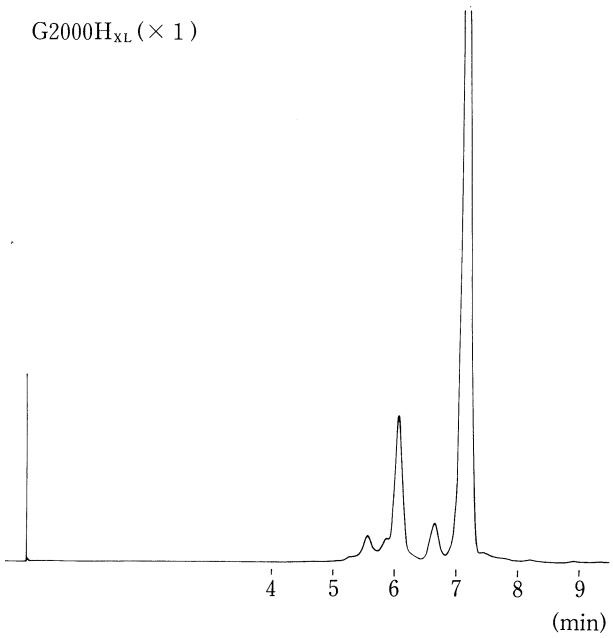
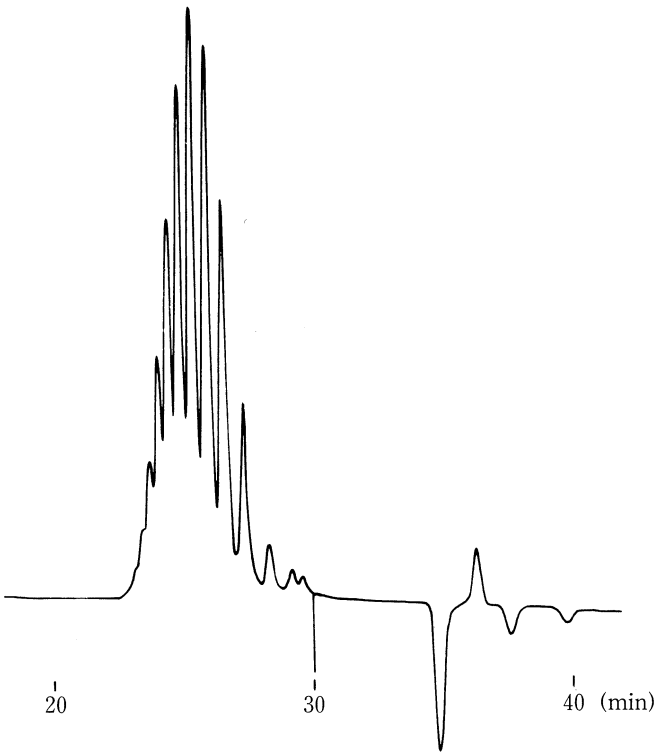


Fig. 15 Separation of aliphatic acid

Fig. 16 Separation of aliphatic alcohol







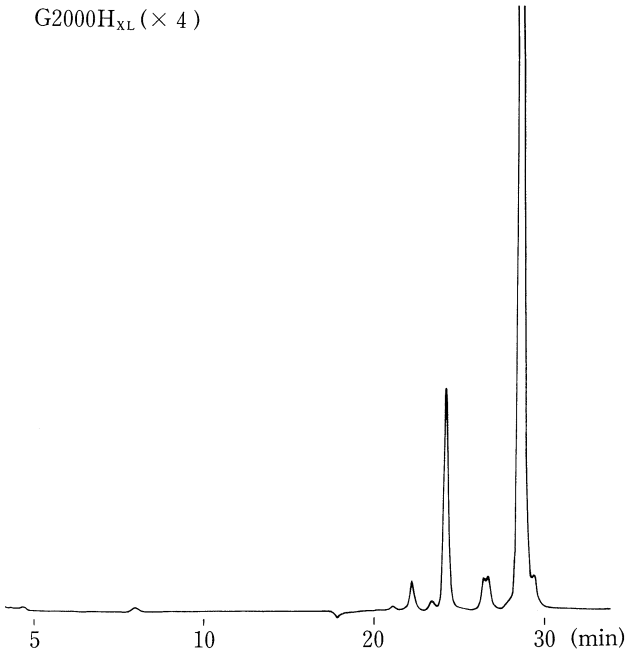
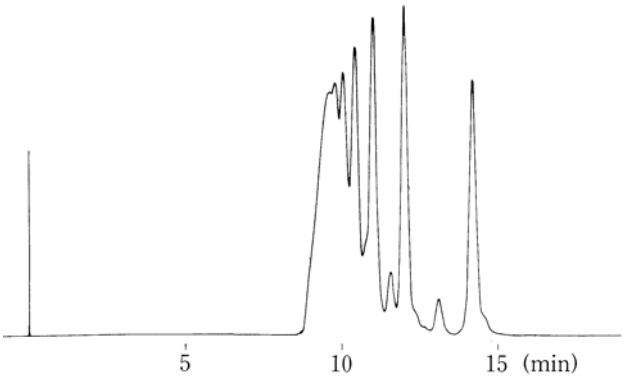
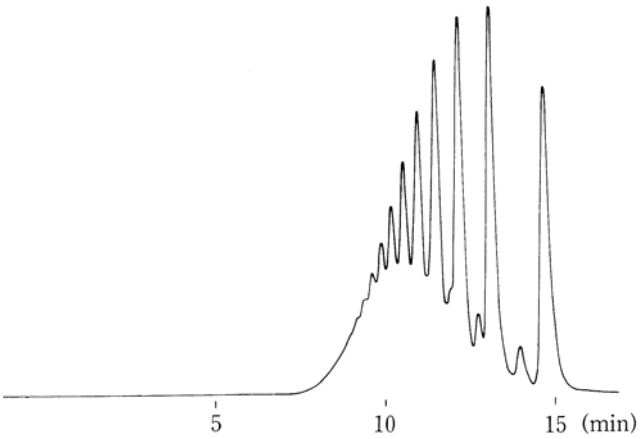


Fig. 17 Separation of polyethylene glycol

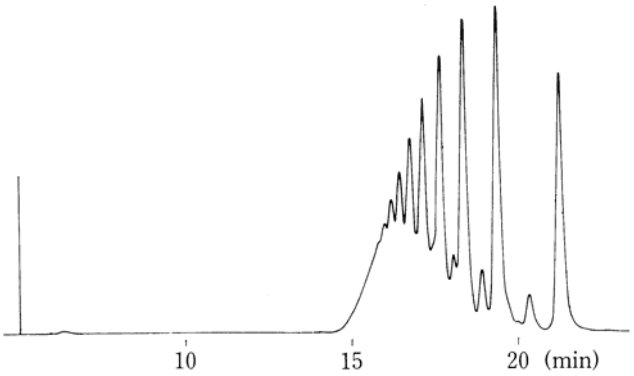
Fig. 18 Separation of Epikote 828



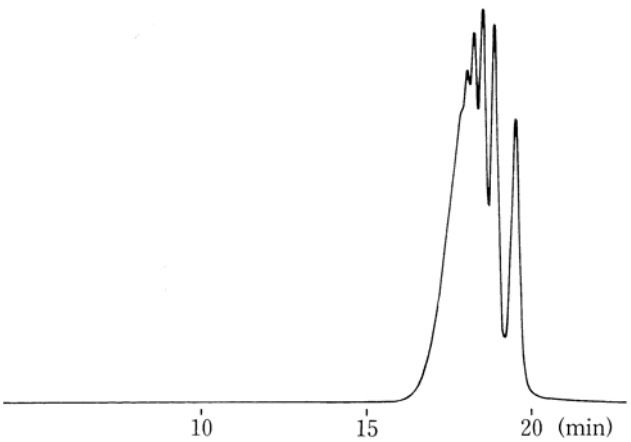
(1) G2000H_{XL}: 2 columns



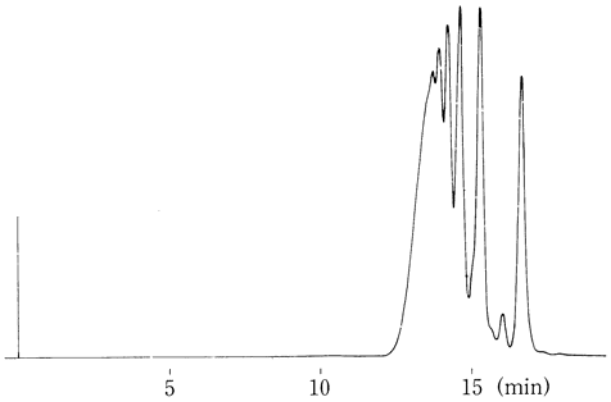
(2) G2500H_{XL}: 2 columns



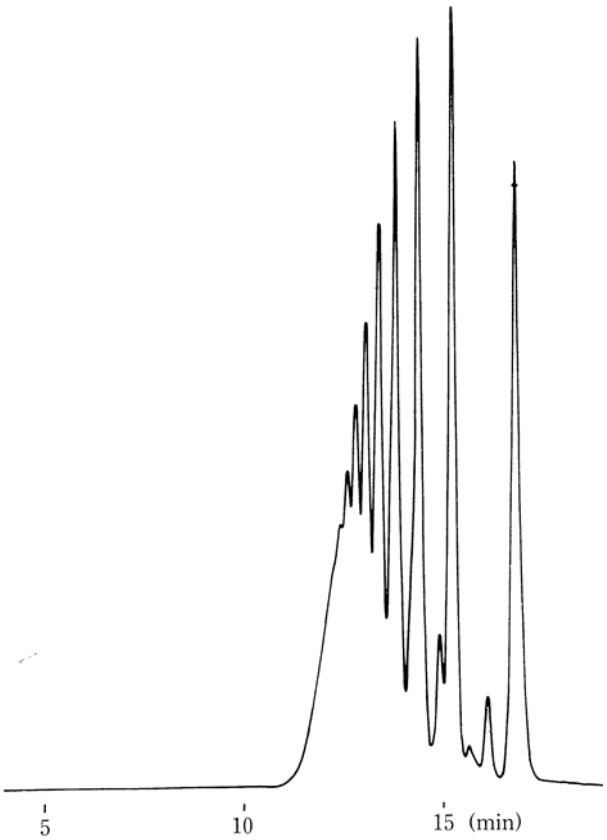
(3) G3000H_{XL}: 2 columns



(4) G4000H_{XL}: 2 columns

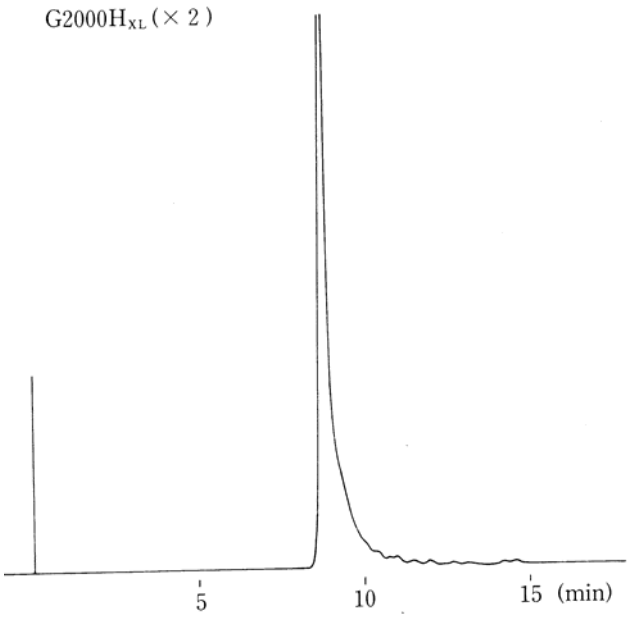


(5) TSKgel G4000H_{XL} + TSKgel G2000H_{XL}

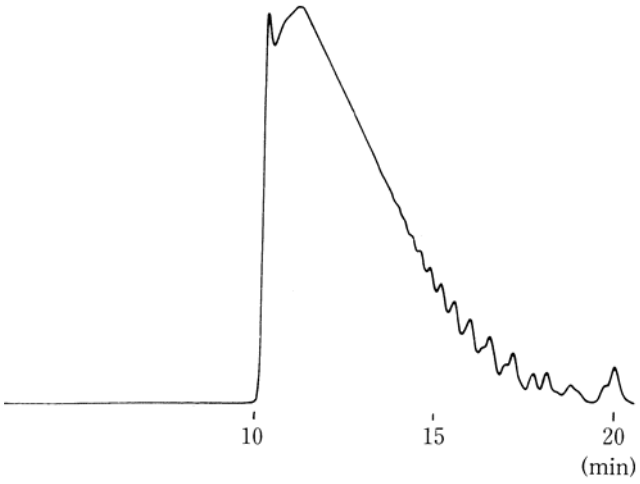


(6) TSKgel G4000H_{XL} + TSKgel G3000H_{XL} + TSKgel G2500H_{XL} + TSKgel G3000H_{XL}

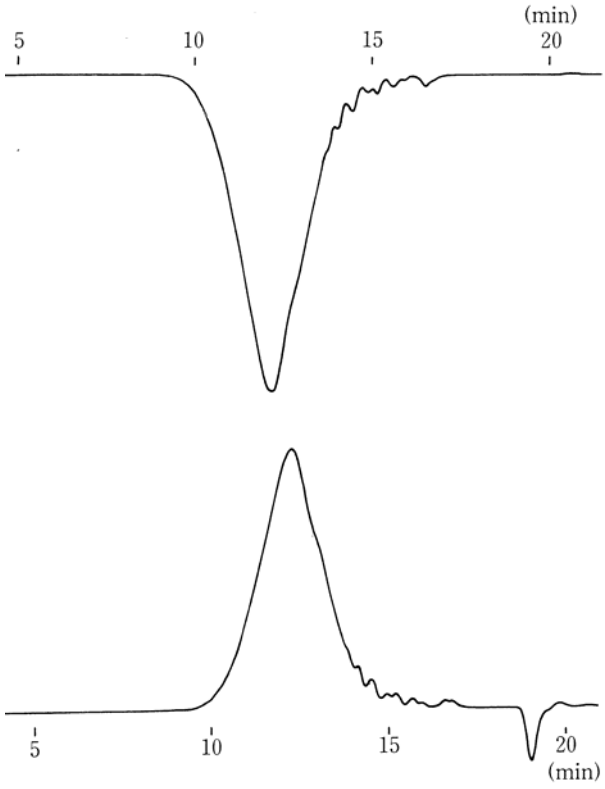
Fig. 19 Separation of Epikote 1001



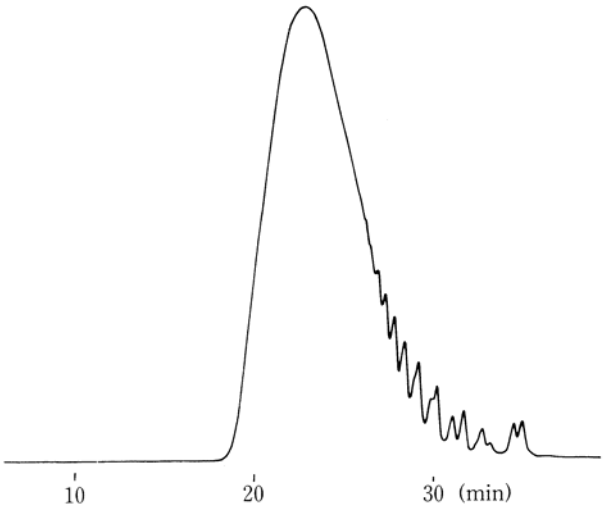
(1) G2000H$_{XL}$: 2 columns



(2) G3000H$_{XL}$: 2 columns



(3) TSKgel G4000H$_{XL}$ + TSKgel G2000H$_{XL}$



(4) TSKgel G4000H$_{XL}$ + TSKgel G3000H$_{XL}$ + TSKgel G3000H$_{XL}$ + TSKgel G2000H$_{XL}$

Fig. 20 Separation of Epikote 1009

10. Conclusion

In general, analysis by conventional GPC is more time consuming than separation by reversed phase or normal phase HPLC. The TSKgel H_{XL} type columns provide a significant improvement in this regard, by reducing analysis time over more conventional GPC column types. Using TSKgel H_{XL} type columns yields a number of major advantages, including reducing analysis time by half, increasing analysis performance, and decreased solvent consumption and disposal costs. High performance GPC with TSKgel H_{XL} type columns is expected to be widely applicable, as this column technology better addresses three key demands of analysis: ease of use, speed and sensitivity.