



# SEPARATION REPORT

## Principles and Applications of High Performance GPC Columns: TSKgel H<sub>XL</sub> Type (Oligomer Edition)

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## 1. Introduction

High performance liquid chromatography can be classified into 4 categories based on the separation mode: gel permeation\* (GPC), adsorption (liquid-solid chromatography: LSC), partition (liquid-liquid chromatography: LLC), and ion exchange (IxC) 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 substances.

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<sub>XL</sub> high-performance columns, which reduce the analysis time of conventional GPC 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).

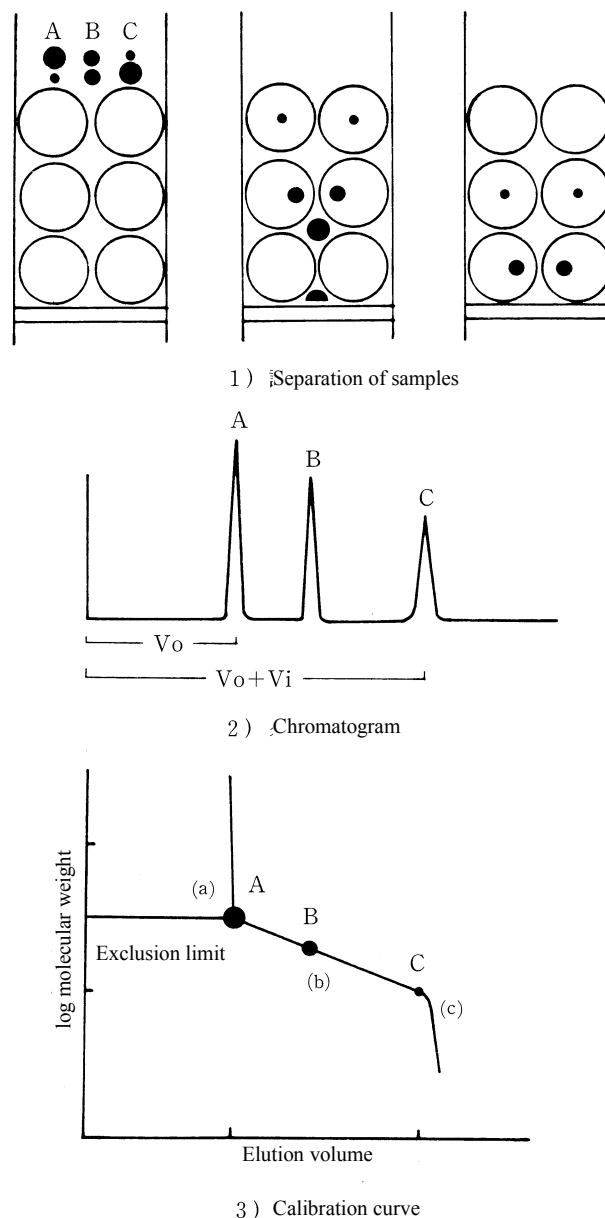
## 2. Principles of GPC Separation

The separation performance of a column used in GPC is evaluated by the calibration curve. Fig. 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 permeate the pores.

When three types of samples with different molecular weights are injected into the column (Fig. 1.1), substances that are larger than the pore size of the packing material (A) are eluted through the column on the outside of the packing material (column dead volume:  $V_0$ ), following the flow of the mobile phase.

On the other hand, substances that are smaller than the pore size of the packing material (C) will be eluted more slowly as they will permeate the pores of the packing material ( $V_i$ ).

The percentage of pores that can be permeated by the sample varies depending on the size of the sample, and a sample (B) with a size between (A) and (C) will be eluted 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 Fig. 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



**Fig.1 Principles of GPC separation**

molecular weight can be estimated by comparing elution volumes. In Fig. 1.3, the sample elution volume (time) is plotted against a logarithm of the molecular weight of the sample, on what is called a calibration curve. The calibration curve is composed of two parts: a part normally composed of two lines with a comparatively steep slope, and one line with a shallower slope.

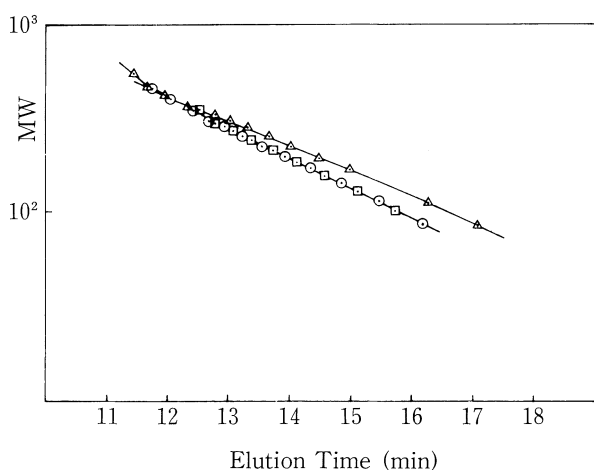
The separation of samples can be judged from the slope of the calibration curve: the more gradual the slope, the better the separation.

Section (a) of the calibration curve shows that the sample is being eluted in  $V_0$ , because it is larger than the pores of the packing material. The largest molecular weight that can permeate the pores is called the exclusion limit.

In section (c), the sample has completely permeated 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 that corresponds to the size of the sample.

It is important to note that the vertical axis of the calibration curve is the log of the molecular weight, thus separation is determined 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 sample used in analysis, thus in order to accurately measure the molecular weight of the sample, a standard sample of the same substance as the sample being analyzed must be prepared.

Figure 2 shows an example of a calibration curve calculated by analyzing n-paraffin, aliphatic acid, and alcohol with the TSKgel G2000H<sub>XL</sub>. Aliphatic acid and n-paraffin are eluted along the same curve, but with alcohol, the elution tends to occur later as the molecular weight decreases.



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

Column: TSKgel G2000H<sub>XL</sub> x 2 (7.8 mm I.D. x 30 cm) x 2

Solvent: THF

Flow rate: 1.0 mL/min

- Aliphatic acid       $\text{CH}_3(\text{CH}_2)_n\text{COOH}$
- △ Aliphatic alcohol    $\text{CH}_3(\text{CH}_2)_n\text{OH}$
- n-paraffin           $\text{C}_n\text{H}_{2(n+1)}$

### 3. TSKgel H<sub>XL</sub> type

The matrix in the TSKgel H<sub>XL</sub> type column is a styrene-divinylbenzene copolymer, similar to what is used in TSKgel H type columns. Table 1 shows the 9 grades of TSKgel H<sub>XL</sub> type columns. The number of theoretical plates is greatly improved compared to conventional GPC 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. In association with this, the column length has been reduced to 30 cm, half the length of conventional columns, which reduces analysis time and can reduce solvent consumption. These columns have the further advantage in that, because the number of theoretical peaks is increased, there is little spreading of elution peaks, and because the peak height is increased, relative sensitivity is improved.

The mobile phase selected for use in TSKgel H<sub>XL</sub> type columns must be a solvent that dissolves polystyrene.

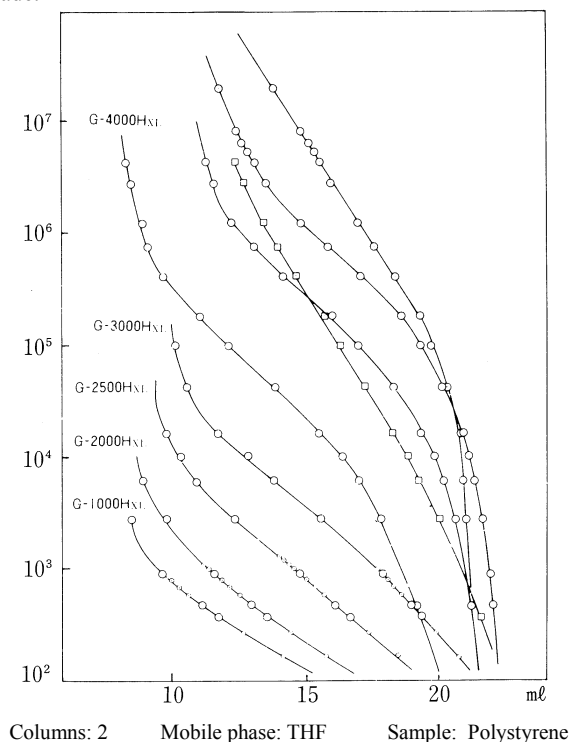
**Table 1 TSKgel H<sub>XL</sub>**

Name	Exclusion limit*	Number of theoretical plates
TSKgel G1000H <sub>XL</sub>	$1 \times 10^3$	16,000TP/30cm
TSKgel G2000H <sub>XL</sub>	$1 \times 10^4$	16,000
TSKgel G2500H <sub>XL</sub>	$2 \times 10^4$	16,000
TSKgel G3000H <sub>XL</sub>	$6 \times 10^4$	16,000
TSKgel G4000H <sub>XL</sub>	$4 \times 10^5$	16,000
TSKgel G5000H <sub>XL</sub>	$4 \times 10^6$	14,000
TSKgel G6000H <sub>XL</sub>	$4 \times 10^7$ (estimate)	14,000
TSKgel G7000H <sub>XL</sub>	$4 \times 10^8$ (estimate)	14,000
TSKgel GMH <sub>XL</sub>	$4 \times 10^8$ (estimate)	14,000
TSKgel GMH <sub>XL</sub> -HT	$4 \times 10^8$ (estimate)	5,500

Generally, tetrahydrofuran (THF) is most often used, but when the objective is preparative separation, chloroform is used, as THF produces explosive polymers when heated. Moreover, when a sample is analyzed that cannot be dissolved in either of these solvents, a solvent such as DMF or m-cresol/chloroform is used.

### 4. Column Selection

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

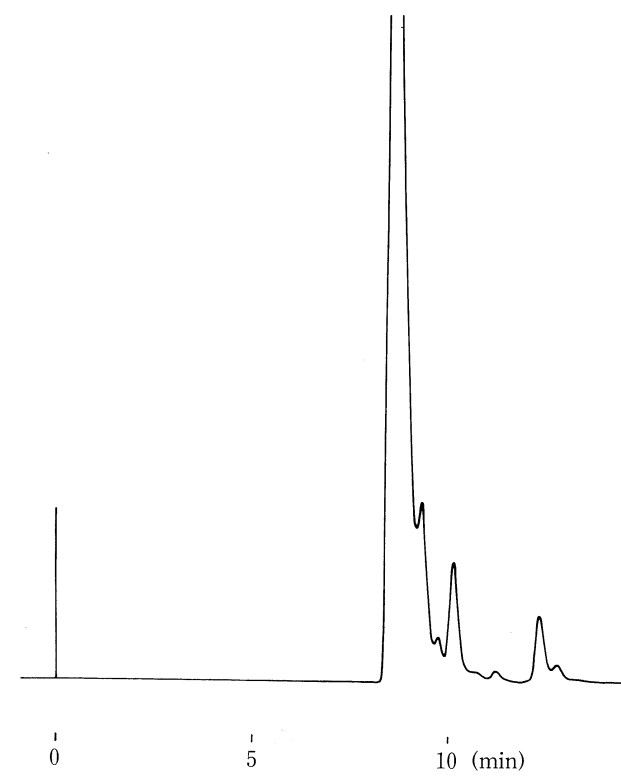


**Fig. 3 TSKgel G-H<sub>XL</sub> calibration curves**

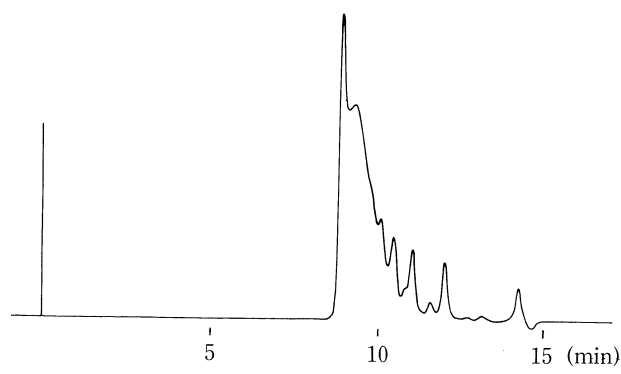
The purpose of TSKgel GMH<sub>XL</sub> columns is to analyze the molecular weight distribution of polymers. Because these columns provide a linear relationship over a wide range of molecular weights, the gradients are relatively steep and they are not suitable for the purpose of separation.

If the molecular weight of the sample being analyzed can be estimated, the grade can be chosen directly based on the calibration curve. If the molecular weight of the sample is unknown, the sample can be analyzed using a system in which the TSKgel G4000H<sub>XL</sub> and TSKgel G2000H<sub>XL</sub> are combined, and the molecular weights of samples ranging from 10's to 100,000's can be estimated.

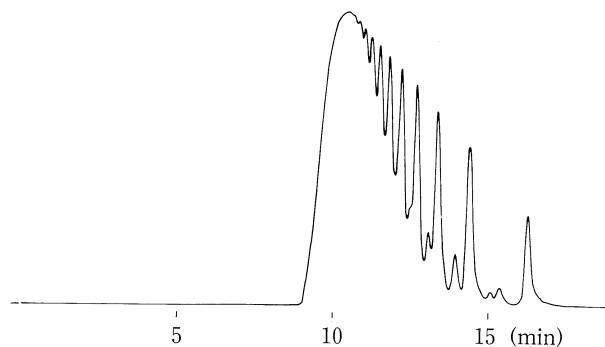
In order to show changes in separation that occur when analysis is conducted with the various column grades, Figure 4 shows chromatograms produced when an epoxy resin, a typical sample, was analyzed using grades ranging from the TSKgel G1000H<sub>XL</sub> through the TSKgel G4000H<sub>XL</sub>. In the chromatogram produced with the TSKgel G1000H<sub>XL</sub>, most of the components are eluted at the exclusion limit, and although



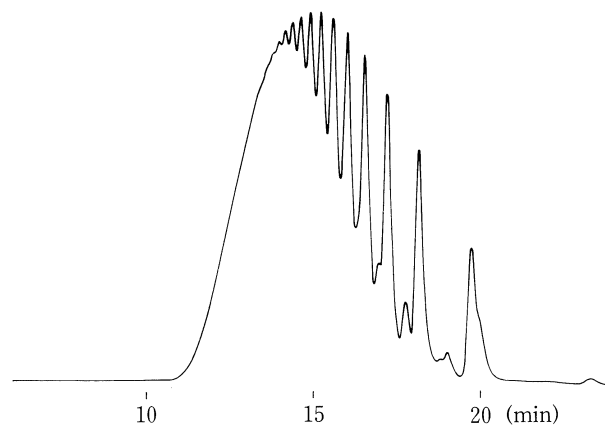
1) TSKgel G1000H<sub>XL</sub>



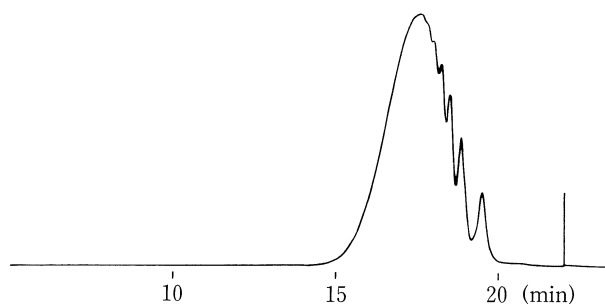
2) TSKgel G2000H<sub>XL</sub>



3) TSKgel G2500H<sub>XL</sub>



4) TSKgel G3000H<sub>XL</sub>



5) TSKgel G4000H<sub>XL</sub>

Columns: 2    Mobile phase: THF    Flow rate: 1.0 mL/min

**Fig. 4 Separation of Epikote 1004 by TSKgel G1000H<sub>XL</sub> through TSKgel G4000H<sub>XL</sub> columns**

this grade does not show distribution accurately, it can be used in preparative separations and in the determination of components that have a polystyrene-converted molecular weight of 800 or less (elution time  $\geq 10$  minutes). This is convenient for bulk determination in the high molecular weight region and reduces analysis time.

Although the TSKgel G2000H<sub>XL</sub> produces more peaks than the TSKgel G1000H<sub>XL</sub>, some components are still excluded, but separation is improved in the polystyrene-converted molecular weight range of 800 to 1,000.

Virtually the entire distribution is provided by the TSKgel G2500H<sub>XL</sub>, however, the shape of the peaks on the higher part of polymer suggests the existence of a component with a molecular weight above the exclusion limit. Separation in the polystyrene-converted molecular weight range of 2,000 to

10,000 is improved, however, separation of the last component eluted in the TSKgel G2000H<sub>XL</sub> is poor (MW ≈ 250).

The number of peaks is greatest with the TSKgel G3000H<sub>XL</sub>, which is the best column to use to obtain the full distribution. Moreover, effects of a polymer component thought to be excluded in the TSKgel G2500H<sub>XL</sub> are not observed. Separation of components with a polystyrene-converted molecular weight of 1,000 or less are in the dead volume with the TSKgel G4000H<sub>XL</sub>, and elution is late. It is clear that the pores in this grade are too large for the sample.

As is clear from the above, even with the same sample, the chromatogram will differ greatly depending on the column grade, and correct 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<sub>XL</sub> through TSKgel G4000H<sub>XL</sub> grade columns are shown in Fig. 3. With the TSKgel G1000H<sub>XL</sub>, the slope becomes shallow after elution of 10.5 mL, and components with a molecular weight of 600 or below are well separated. The TSKgel G2000H<sub>XL</sub> is suitable for separation of components with a molecular weight of 1,000 or less. In the low molecular weight range, the gradient is not as steep with the TSKgel G1000H<sub>XL</sub> as the TSKgel G2000H<sub>XL</sub>, thus separation of low molecular weight components is best with the TSKgel G1000H<sub>XL</sub>. With the TSKgel G2500H<sub>XL</sub>, the slope is shallow up to a molecular weight of 3,000, but slightly steeper than with the TSKgel G2000H<sub>XL</sub>. The chromatogram of the TSKgel G2500H<sub>XL</sub> is separated on the high molecular weight side, but low molecular weight separation is inferior to that of the TSKgel G2000H<sub>XL</sub>. The calibration curve of the TSKgel G3000H<sub>XL</sub> 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<sub>XL</sub>, but low molecular weight separation is not as good. The optimal molecular weight range for the TSKgel G4000H<sub>XL</sub> 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 components 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. Combining Columns

Multiple columns are often linked together for use in GPC analysis. The reasons for this can roughly be categorized as follows:

- (1) To improve sample separation
- (2) To expand the molecular weight range in which separation can be achieved

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

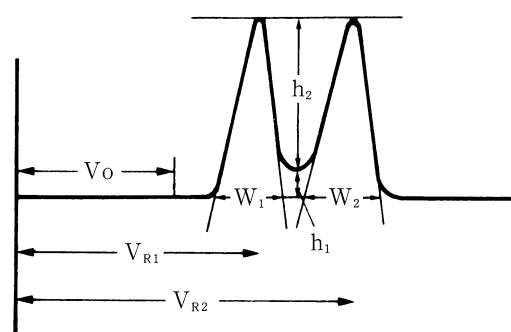
$$R = \frac{1}{4} \sqrt{N} \cdot \left( \frac{\alpha - 1}{\alpha} \right) \cdot \left( \frac{k'}{k' + 1} \right) \quad \dots\dots\dots (1)$$

where R expresses the resolution of a sample. As R increases, separation improves. N expresses the number of theoretical plates; α the separation coefficient of a sample, and k' the capacity ratio, which are calculated by Equations (2) and (3), respectively.

$$\alpha = \frac{V_{R2} - V_0}{V_{R1} - V_0} \quad \dots\dots\dots (2)$$

$$k' = \frac{V_R - V_0}{V_0} \quad \dots\dots\dots (3)$$

(See Fig. 5 for V<sub>R</sub> and V<sub>0</sub>)



$$R = \frac{2(V_{R2} - V_{R1})}{W_1 + W_2} \quad \dots\dots\dots (a)$$

or

$$\frac{h_2}{h_1 + h_2} \times 100 \quad \dots\dots\dots (b)$$

In (a) the value increases as separation improves, and in (b) complete separation is achieved at 100%.

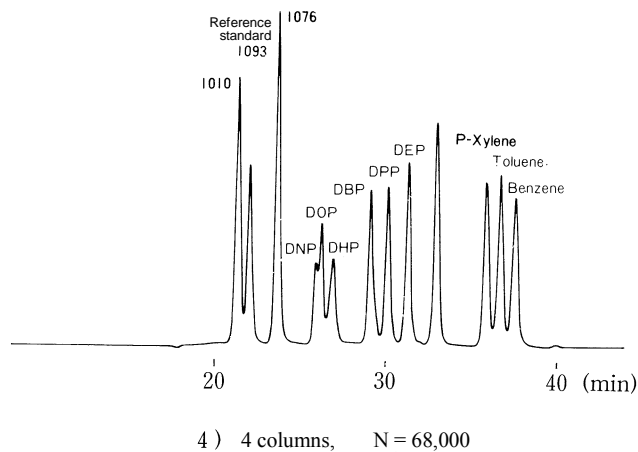
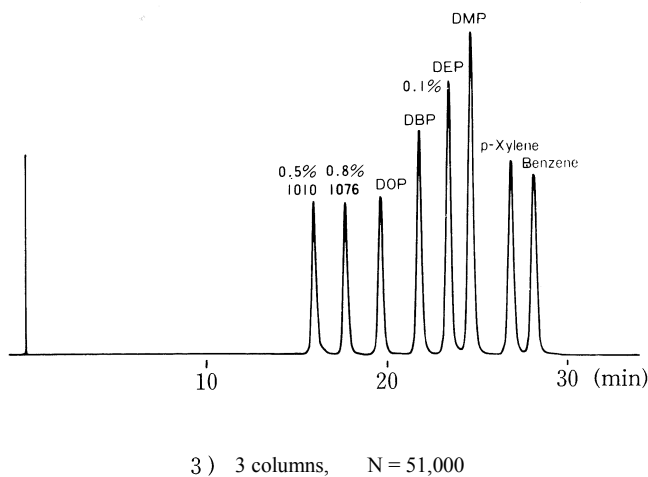
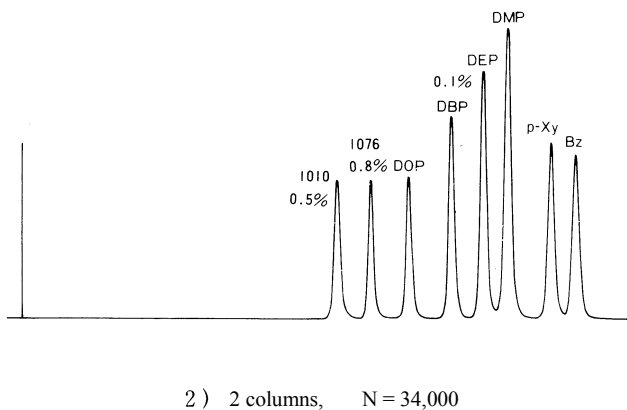
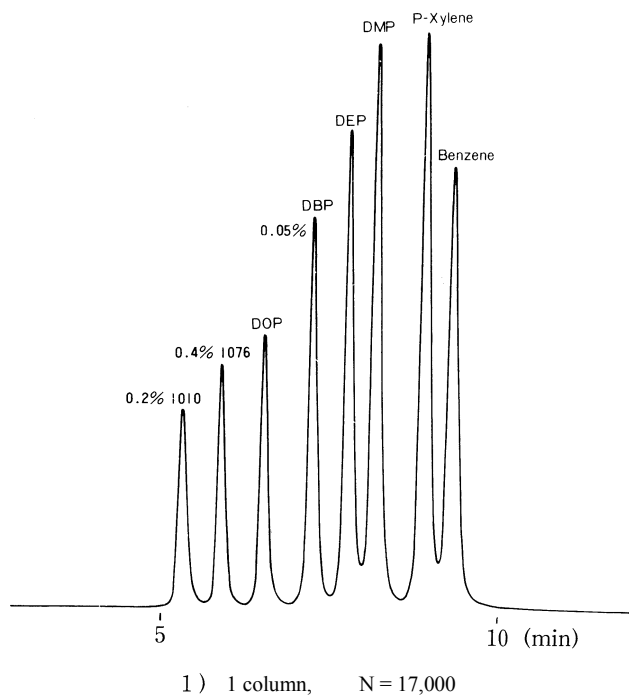
**Fig. 5 Calculation method for resolution**

As discussed in the section on the “Principles of Separation,” because GPC does not utilize the interaction between the sample and the packing material, separation of samples (R) is governed by the number of theoretical plates and the slope of the calibration curve.

When samples S<sub>1</sub> and S<sub>2</sub> 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<sub>R</sub> and V<sub>0</sub> also double. Consequently, Equation (1) becomes

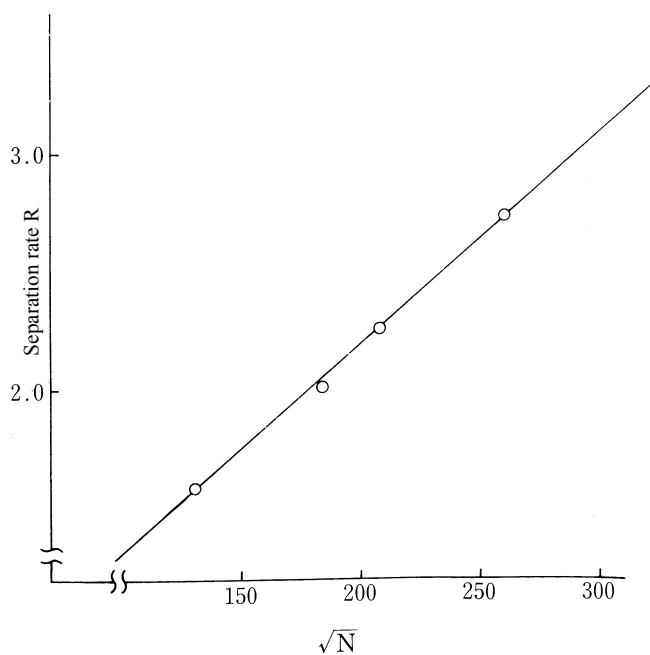
$$R = K \sqrt{N} \quad \dots\dots\dots (1)' \quad K: \text{constant}$$

The easiest way to increase the number of theoretical plates is to increase the length of the column. Fig. 6 shows chromatograms produced by analyzing phthalic acid esters, etc. using from one to four TSKgel G2000H<sub>XL</sub> columns. As the length of the column increases, the number of theoretical plates increases (measured with benzene) and separation improves.



**Fig. 6 Separation of phthalic acid esters by TSKgel G2000H<sub>XL</sub>**

Fig. 7 shows the results of plotting the resolution of DEP and DMP\* (R) against the square root of the number of theoretical plates ( $\sqrt{N}$ ). There is a linear relationship between R and the square root of the number of theoretical plates  $\sqrt{N}$ , as it is clear that separation of the samples improves 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 samples was improved, the required number of columns can be calculated by calculating the square root of  $\sqrt{N}$  based on the targeted resolution.



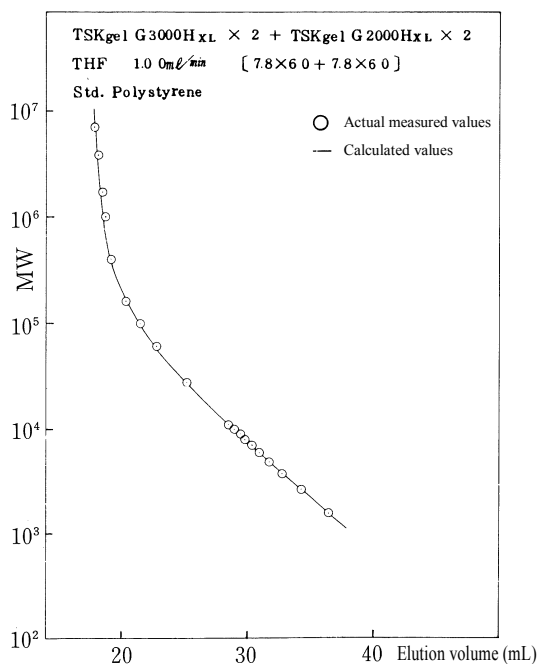
**Fig. 7 Effect of number of theoretical plates on DEP-DMP resolution**

Next, if separation is impossible or only possible within a narrow range of molecular weights when only one grade of column is used, multiple columns of different grades are linked together and used. The calibration curve produced by a system composed of multiple columns of different grades linked together can be calculated from the calibration curves of each individual column. Table 2 shows an example using the TSKgel G2000H<sub>XL</sub> and the TSKgel G3000H<sub>XL</sub>. Under the column headings in the table representing TSKgel G2000H<sub>XL</sub> and TSKgel G3000H<sub>XL</sub>, the elution volumes calculated separately for each column are shown. The values shown for TSKgel G2000H + TSKgel G3000H<sub>XL</sub> (cal) corresponds to

**Table 2 Method for calculating calibration curves**

Molecular weight	G2H <sub>XL</sub>	G3H <sub>XL</sub>	G2H+3H <sub>XL</sub> (cal)	G2H+3H <sub>XL</sub> (obs) <sup>1)</sup>
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

the elution volume when two TSKgel G2000H<sub>XL</sub> columns and two TSKgel G3000H<sub>XL</sub> columns are linked together. The TSKgel G2000H + TSKgel 3000H<sub>XL</sub> (obs) column shows the actual values measured when two TSKgel G2000H<sub>XL</sub> columns and two TSKgel G3000H<sub>XL</sub> columns were linked together. The calibration curve is displayed in Fig. 8. The solid line represents the values obtained by calculation; the points on the graph are actual measured values. The calculated values and

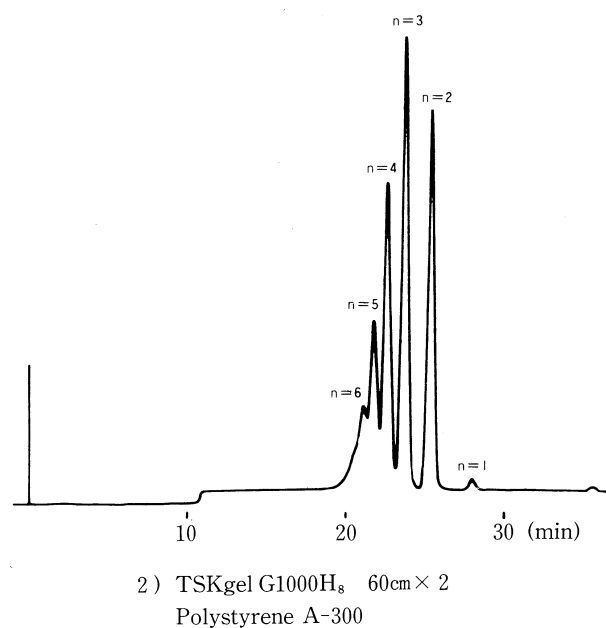
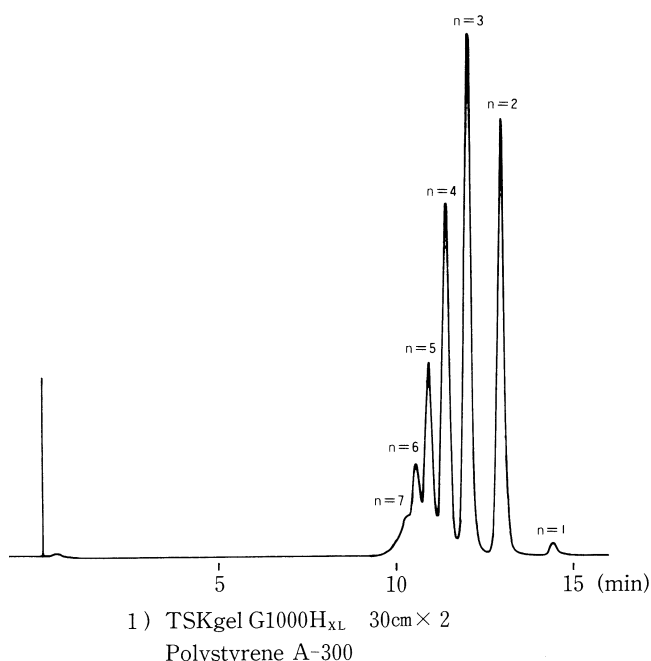


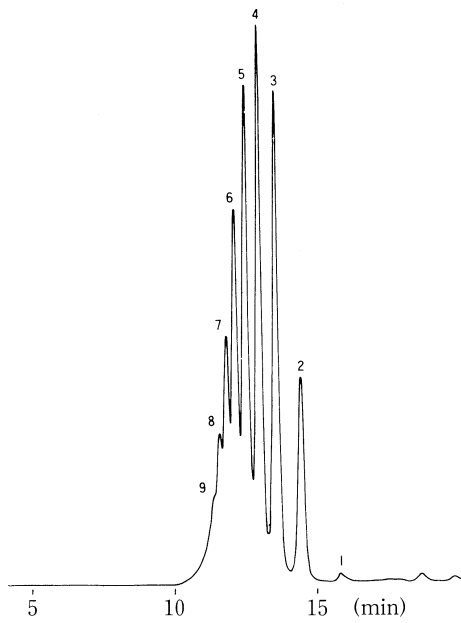
**Fig. 8 Calibration curve of TSKgel G2000H<sub>XL</sub> + TSKgel G3000H<sub>XL</sub>**

measured values align perfectly. 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.

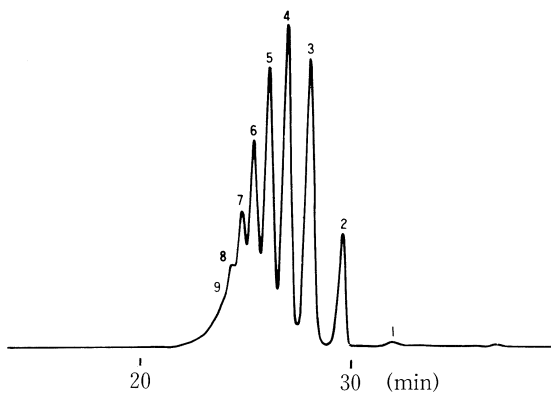
## 6. Comparison of TSKgel H<sub>XL</sub> and H-type Columns

Fig. 9 shows examples of analyses of a polystyrene oligomer produced using grades TSKgel G1000H<sub>XL</sub> through TSKgel G3000H<sub>XL</sub> versus the TSKgel G1000H through TSKgel G3000H.

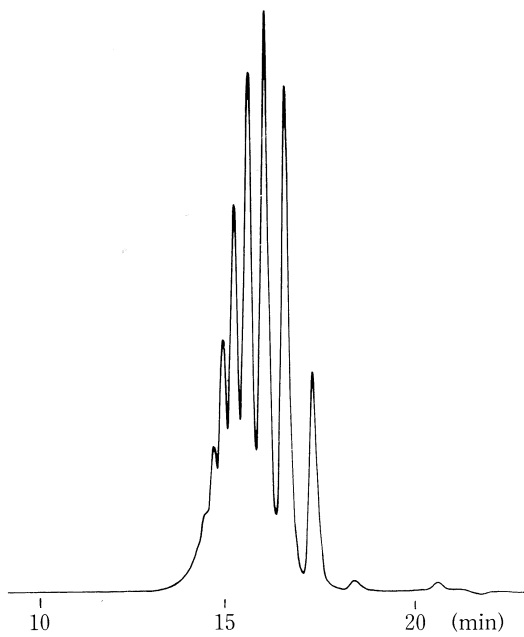




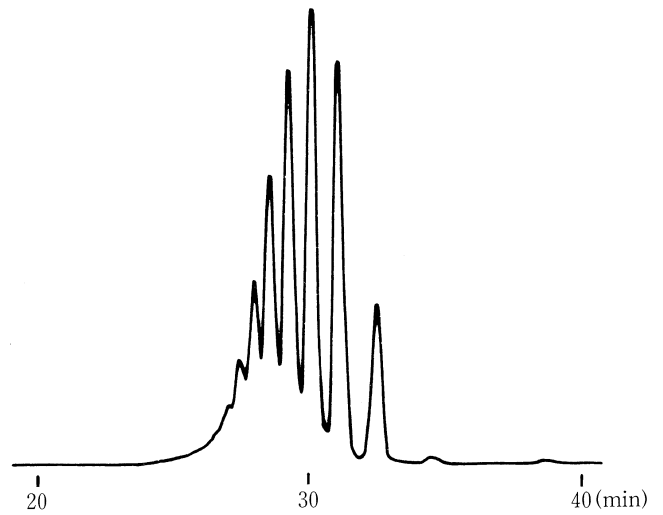
3) TSKgel G2000H<sub>XL</sub> 30cm × 2  
Polystyrene A-500



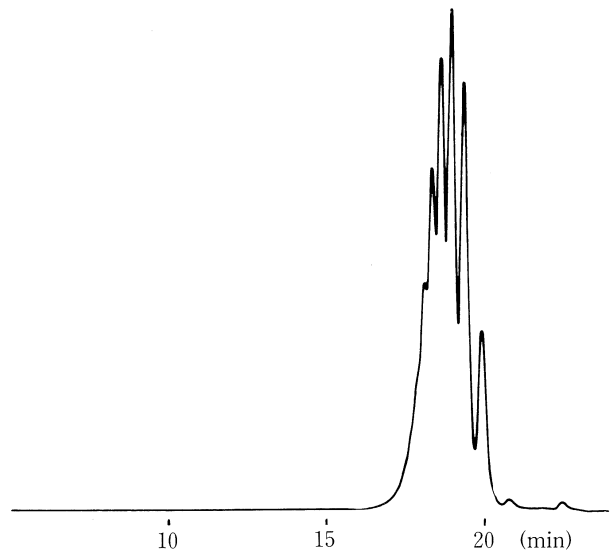
4) TSKgel G2000H<sub>8</sub> 60cm × 2  
Polystyrene A-500



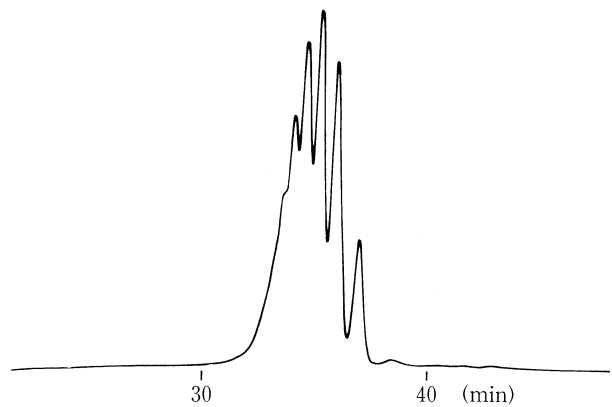
5) TSKgel G2500H<sub>XL</sub> 30cm × 2  
Polystyrene A-500



6) TSKgel G2500H<sub>8</sub> 60cm × 2  
Polystyrene A-500



7) TSKgel G3000H<sub>XL</sub> 30cm × 2  
Polystyrene A-500



8) TSKgel G3000H<sub>8</sub> 60cm × 2  
Polystyrene A-500

**Fig. 9 Separation of polystyrene oligomer by TSKgel G-H<sub>XL</sub> type and TSKgel G-H columns**



Because flammable organic solvents are used in GPC, to conduct unmanned continuous operations at night, special facilities and considerations are required. Improving sample processing capacity is considered an extremely significant issue, both from this perspective, as well as from an economic perspective.

TSKgel H<sub>XL</sub> columns are half the length of TSKgel H-type columns, and consequently, under the same analysis conditions, analysis time can be reduced by half. Separation is at least equivalent to what is obtained with conventional columns. As a result, by replacing TSKgel H type columns with the TSKgel H<sub>XL</sub> type, analysis time can be greatly reduced, and sample processing capacity can be doubled.

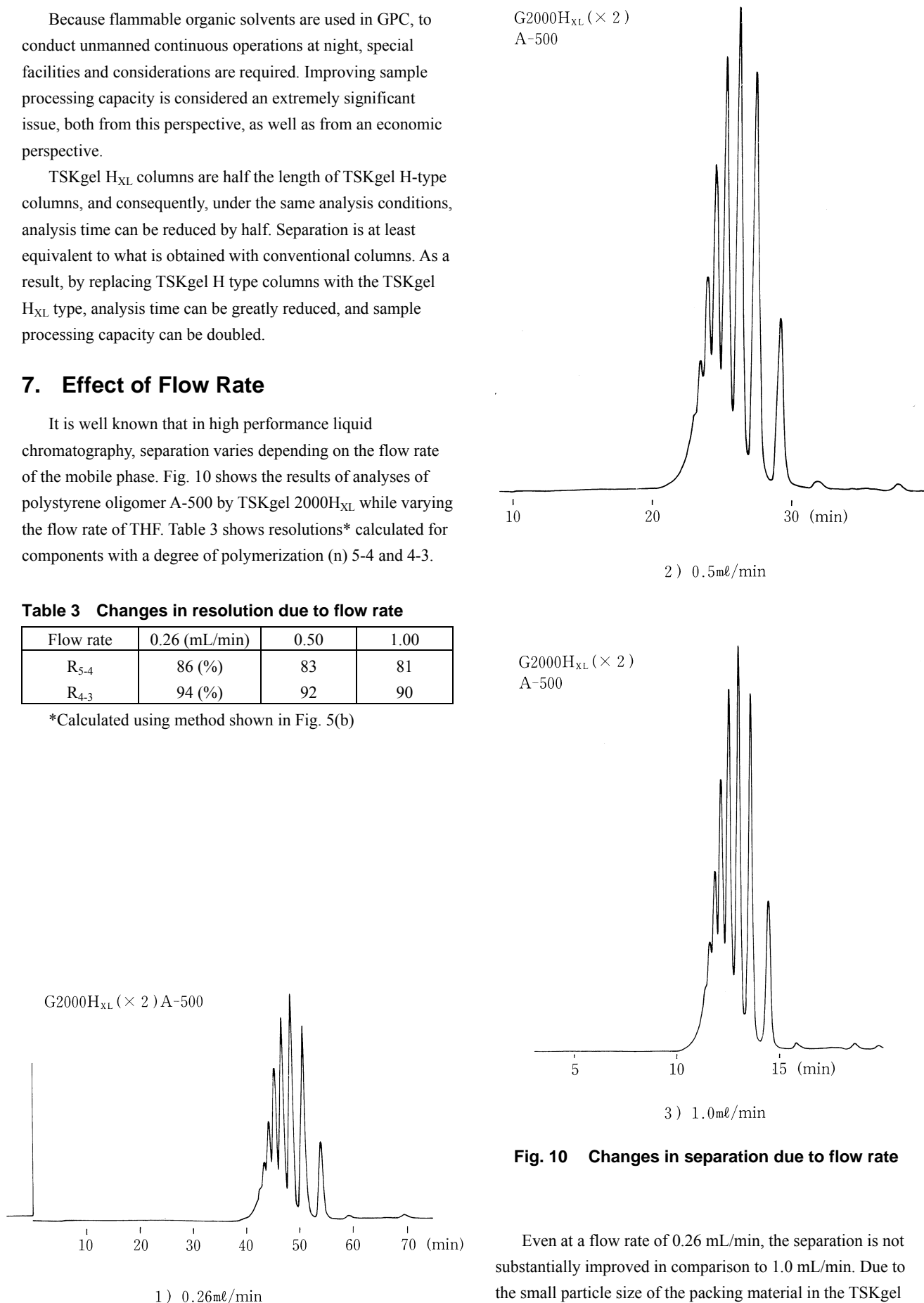
## 7. Effect of Flow Rate

It is well known that in high performance liquid chromatography, separation varies depending on the flow rate of the mobile phase. Fig. 10 shows the results of analyses of polystyrene oligomer A-500 by TSKgel 2000H<sub>XL</sub> while varying the flow rate of THF. Table 3 shows resolutions\* 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 <sub>5-4</sub>	86 (%)	83	81
R <sub>4-3</sub>	94 (%)	92	90

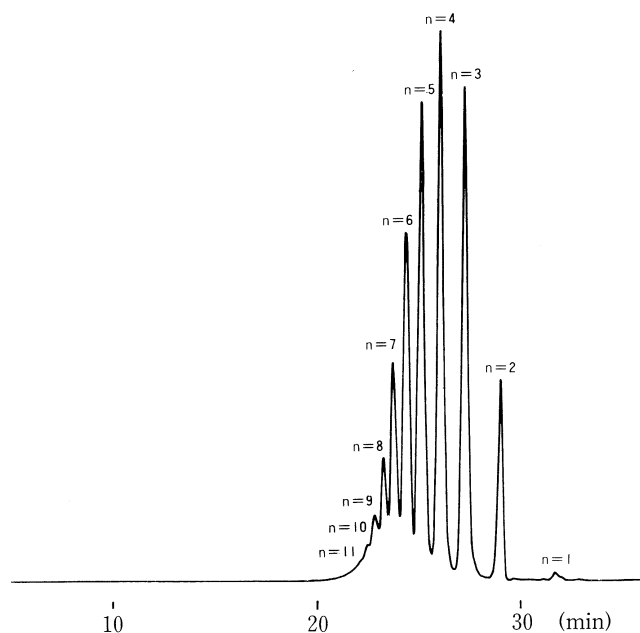
\*Calculated using method shown in Fig. 5(b)



**Fig. 10 Changes in separation due to flow rate**

Even at a flow rate of 0.26 mL/min, the separation is not substantially improved in comparison to 1.0 mL/min. Due to the small particle size of the packing material in the TSKgel H<sub>XL</sub> type, flow rate has less effect than it has in conventional columns. On the other hand, as flow rate decreases, analysis time increases. Fig. 11 shows the results when A-500 is

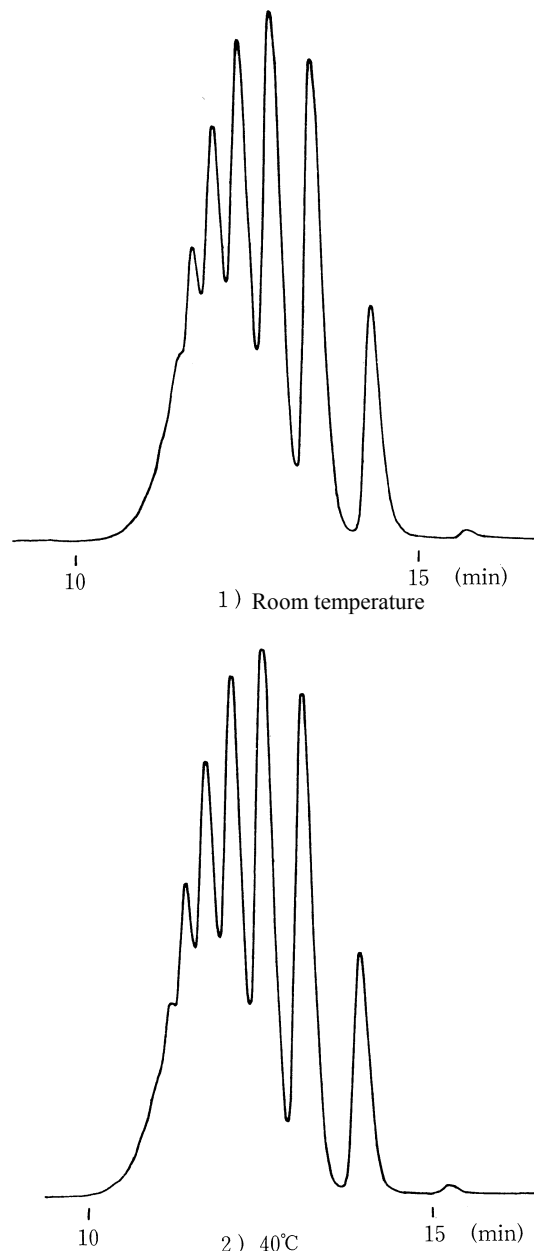
analyzed using four TSKgel G2000H<sub>XL</sub> columns. The measuring time in Fig. 11 is about the same as it is in Fig. 10. As is clearly evident from the chromatograms, if the measurement time is the same, increasing the number of columns significantly improves separation ( $R_{5-4}$  and  $R_{4-3}$  are 95% and 98%, respectively.)



**Fig. 11 Separation of A-500 using four G2000H<sub>XL</sub> columns**  
**Mobile phase: THF Flow rate: 1 mL/min**

## 8. Effect of Temperature

In general in GPC, peaks become sharper and separation improves when the column temperature is increased. This is believed to be because the viscosity of the mobile phase decreases when the temperature is increased, which speeds up diffusion of the sample. Fig. 12 shows chromatograms produced when polystyrene 500 was analyzed at room temperature versus 40 °C. Table 4 shows the results of calculations of the resolution for each peak. As is clear from Fig. 12 and Table 4, separation of low molecular weight components was little affected by temperature, but the resolution improved slightly with high molecular weight components when the temperature was 40 °C. The effect of column temperature is believed to vary depending on the sample, and when analyzing substances with a high molecular weight, a better effect can be obtained by increasing the column temperature.



**Fig. 12 Effects of column temperature on separation**

Column: G2000H<sub>XL</sub>  
 Solvent: THF Flow rate: 1.0 mL/min  
 Sample: Polystyrene A500

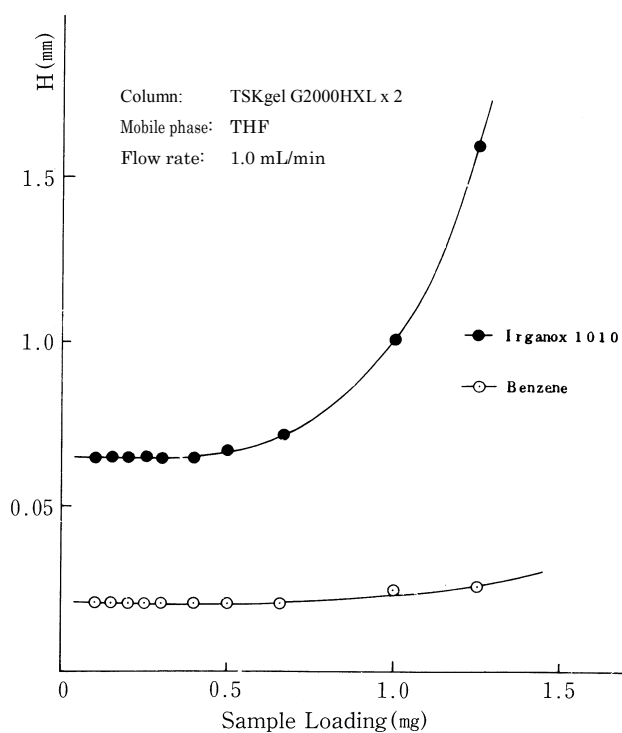
**Table 4 Effects of column temperature on resolution**

Column temperature	$R_{3-2}$	$R_{4-3}$	$R_{5-4}$	$R_{5-6}$	$R_{6-7}$
Room temperature	98 (%)	86	64	44	28
40 °C	98 (%)	87	65	46	32

## 9. Effect of Sample Size

In addition to analysis, preparation is another application for which GPC is frequently used. When a large quantity of a sample is needed for preparation, a large scale preparative column is used, but when preparing a small quantity of a sample, an ordinary analysis column can be used. In preparative separation of a sample, the relationship between separation and the injection volume is crucial. In general,

increasing the sample injection volume can lead to distortion of the peaks due to overloading. Moreover this tends to be more noticeable with higher performance columns as the particle size of the packing material decreases. Fig. 13 shows the relationship between height equivalent to theoretical plate (H) and sample injection of benzene and Irganox 1010, using the TSKgel G2000H<sub>XL</sub>. Variations in H resulting from changes in sample load are based on the molecular weight of the sample, as the larger the molecular weight, the more readily H is affected by sample loading. The quantity of a sample that can be injected is limited by the elution position of the components to be separated, and though generally not indicated in numerical values, it is believed that preparative separation can be performed with the TSKgel G2000H<sub>XL</sub> for sample quantities ranging from one to several mg.

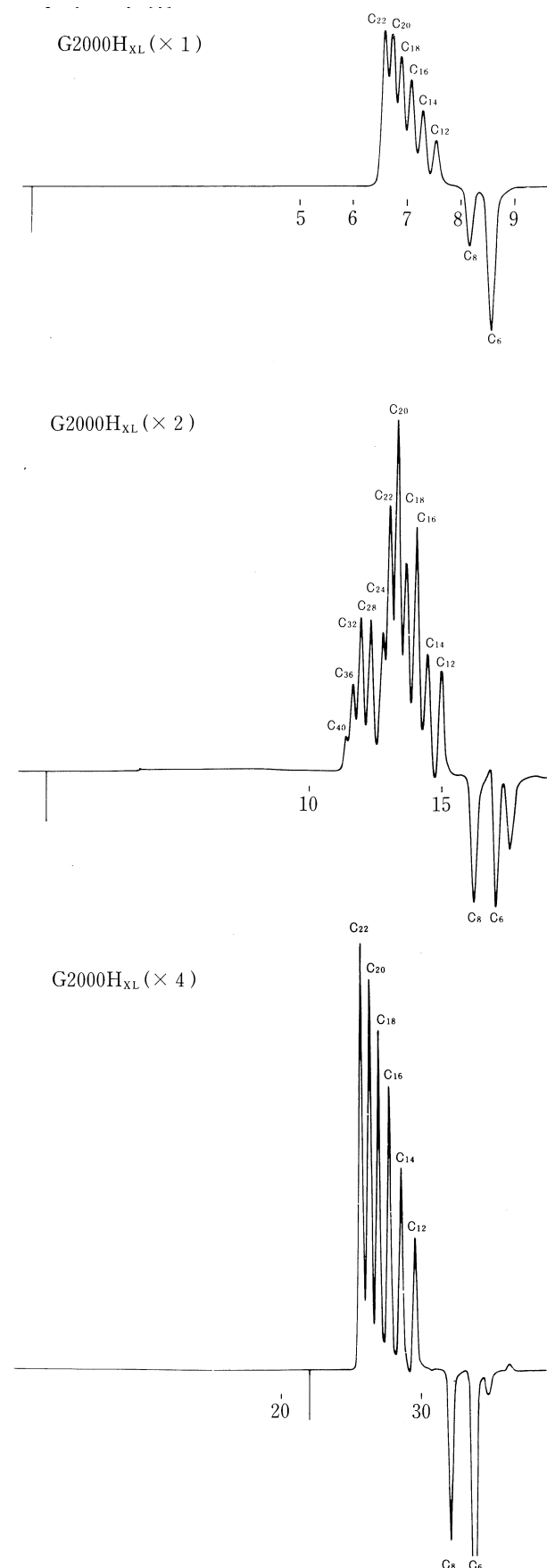


**Fig. 13 Relationship between number of theoretical plates and sample loading**

## 10. Conclusion

The TSKgel H<sub>XL</sub> type columns have much better performance than conventional GPC columns. Using this column yields a number of major advantages including reducing analysis time by half, increasing analysis performance, and providing economic benefits. In general, one problem with conventional GPC is that analysis requires more time than LLC and LSC, etc. But the introduction of the TSKgel H<sub>XL</sub> type of column results in significant improvement in this area. High-speed, high-performance GPC is expected to have many applications in the future, as this technology better satisfies three key areas demanded in analysis: ease of use, speed, and sensitivity.

## 11. Data



**Fig. 14 Separation of n-paraffin**

Column: G2000H<sub>XL</sub>  
Mobile phase: THF  
Flow rate: 10 mL/min

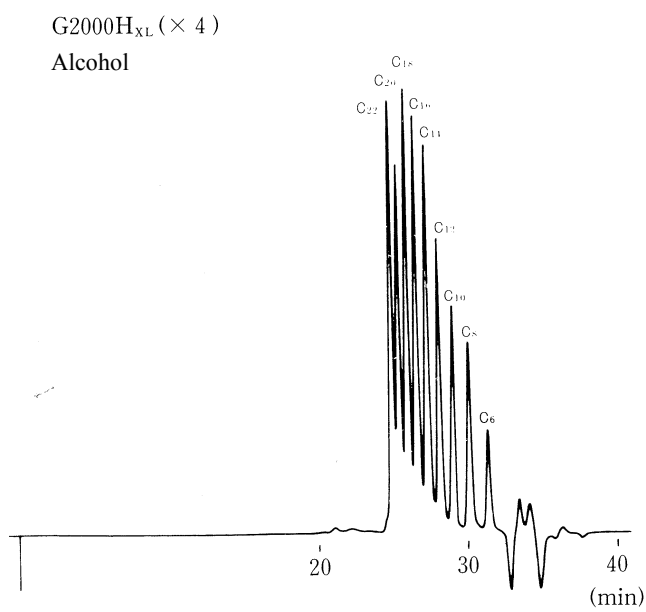
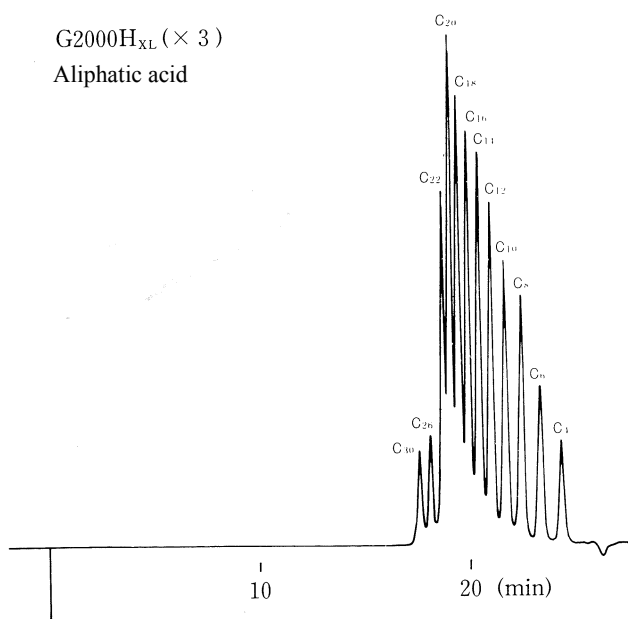
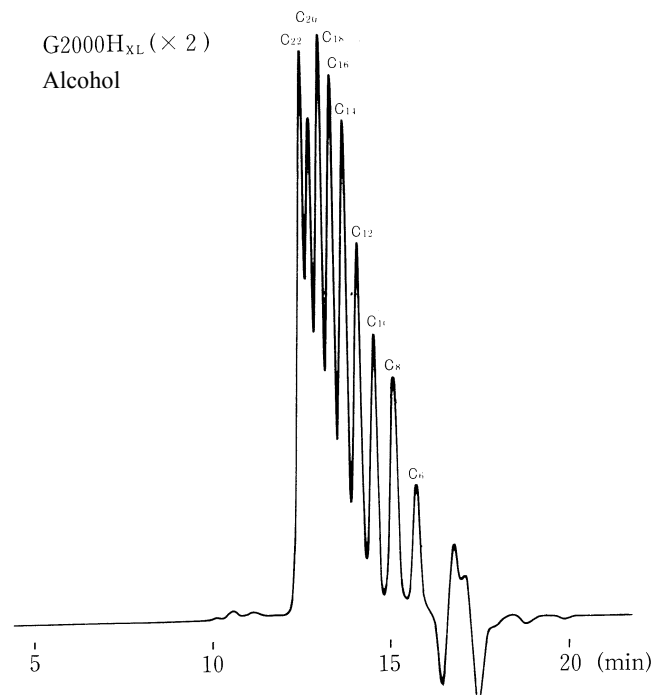
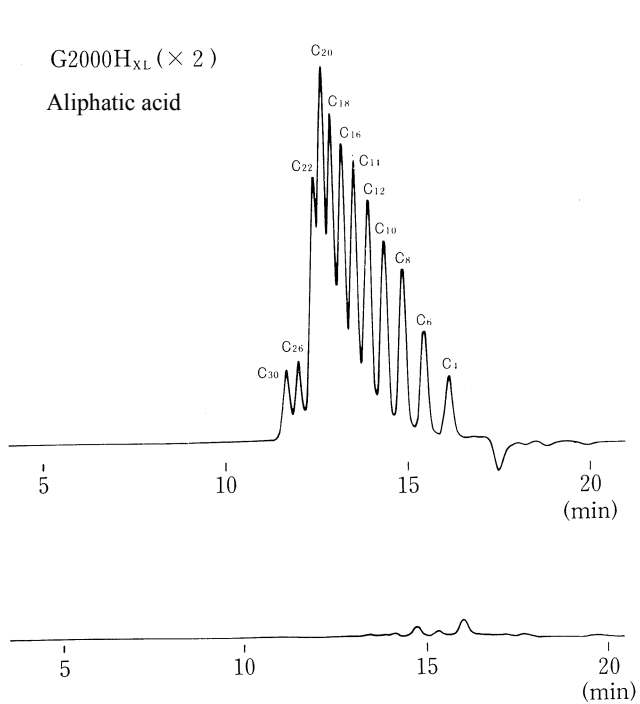
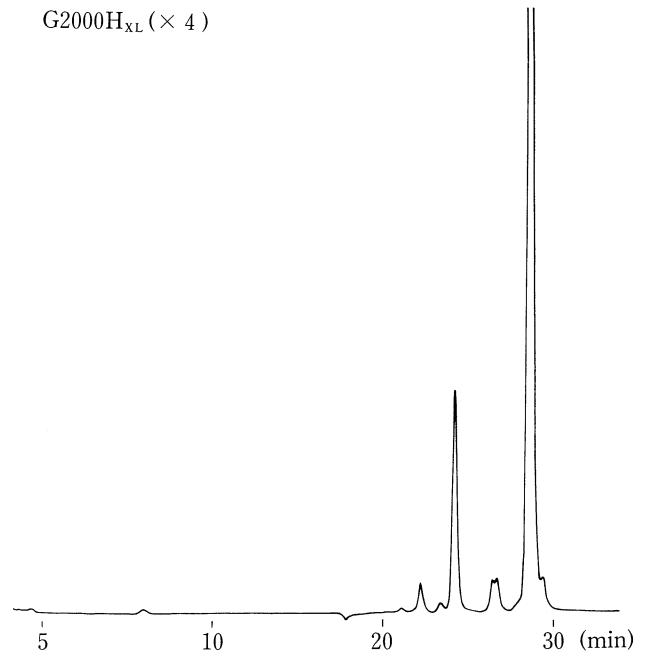
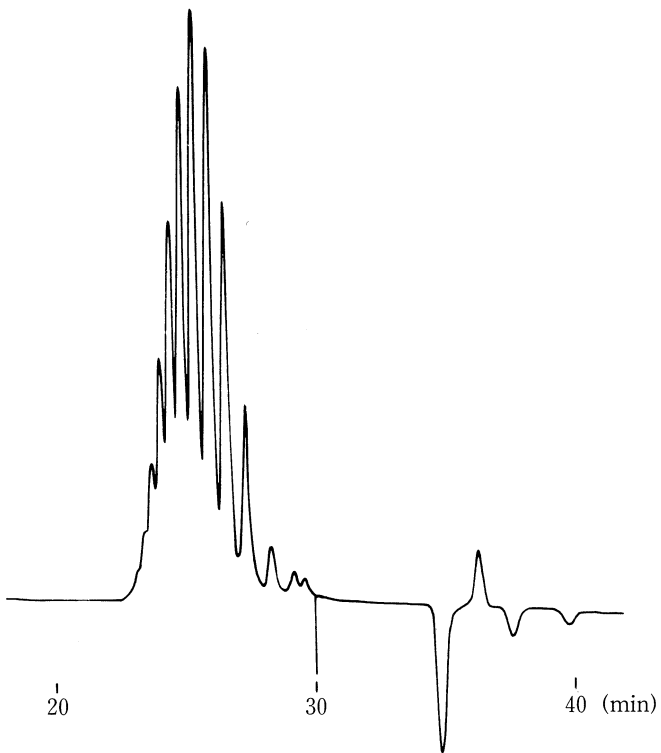
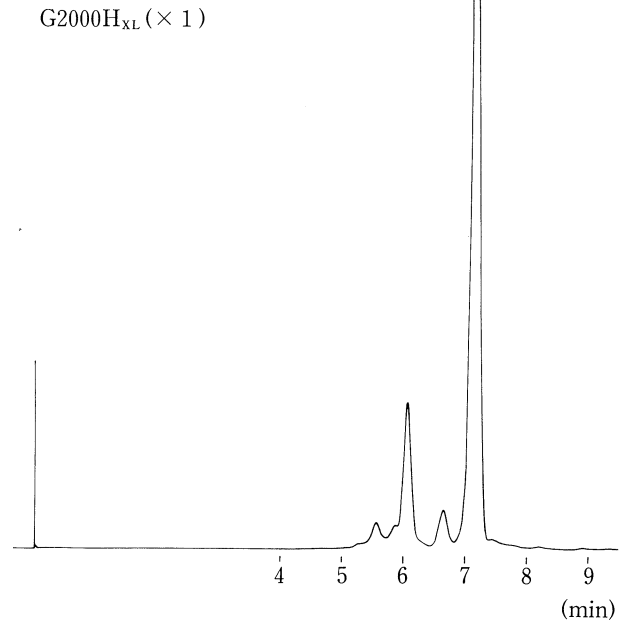
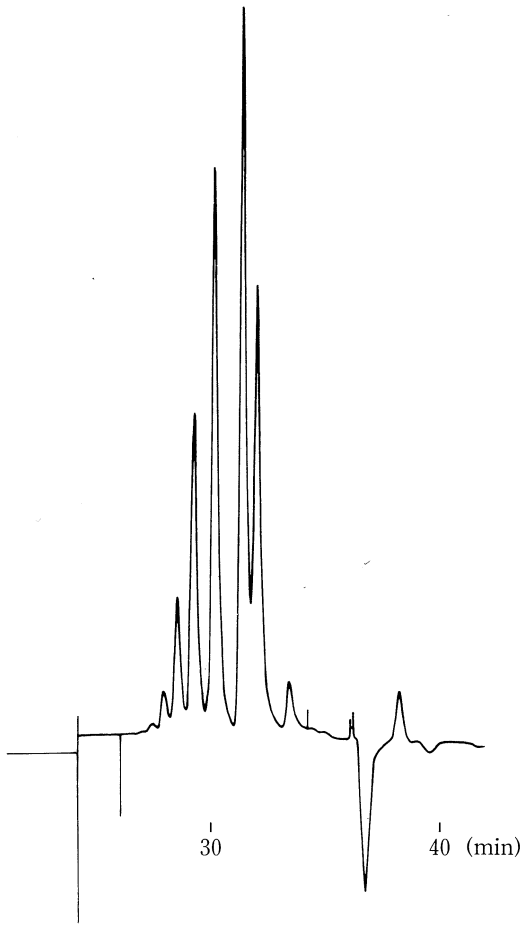


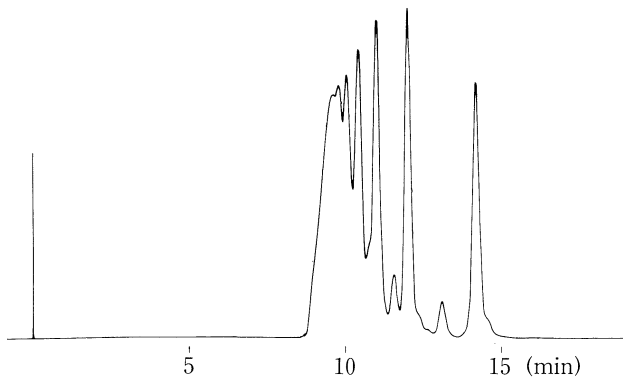
Fig. 15 Separation of aliphatic acid

Fig. 16 Separation of aliphatic alcohol

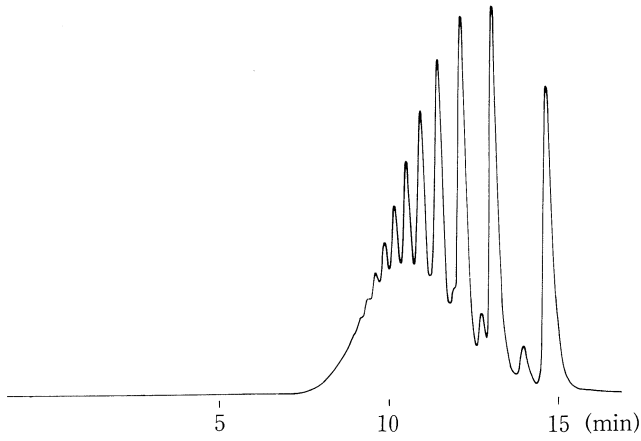


**Fig. 17** Separation of polyethylene glycol

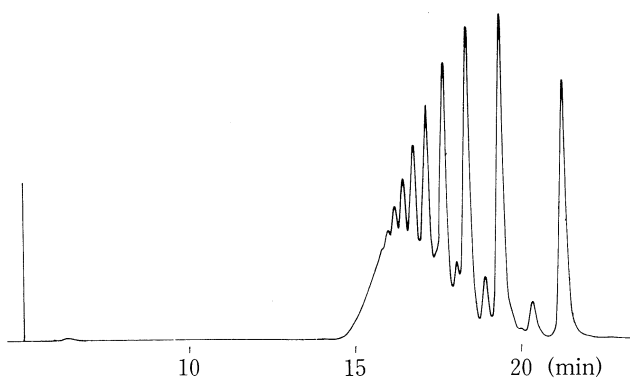
**Fig. 18** Separation of Epikote 828



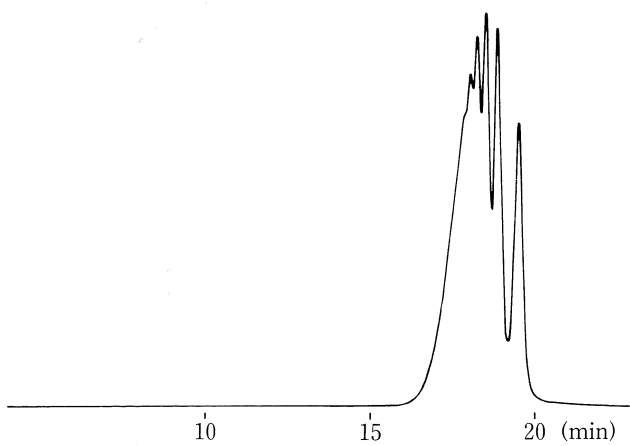
(1) G2000H<sub>XL</sub>: 2 columns



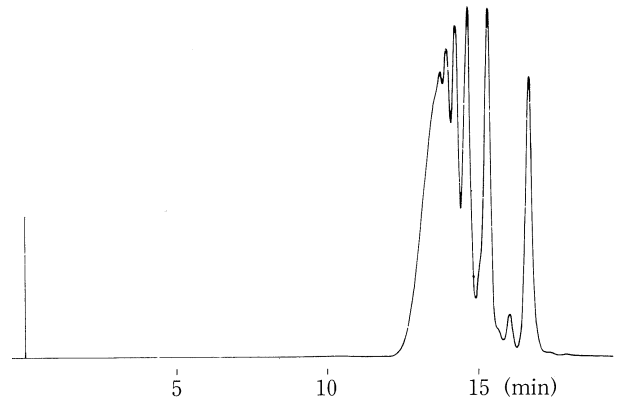
(2) G2500H<sub>XL</sub>: 2 columns



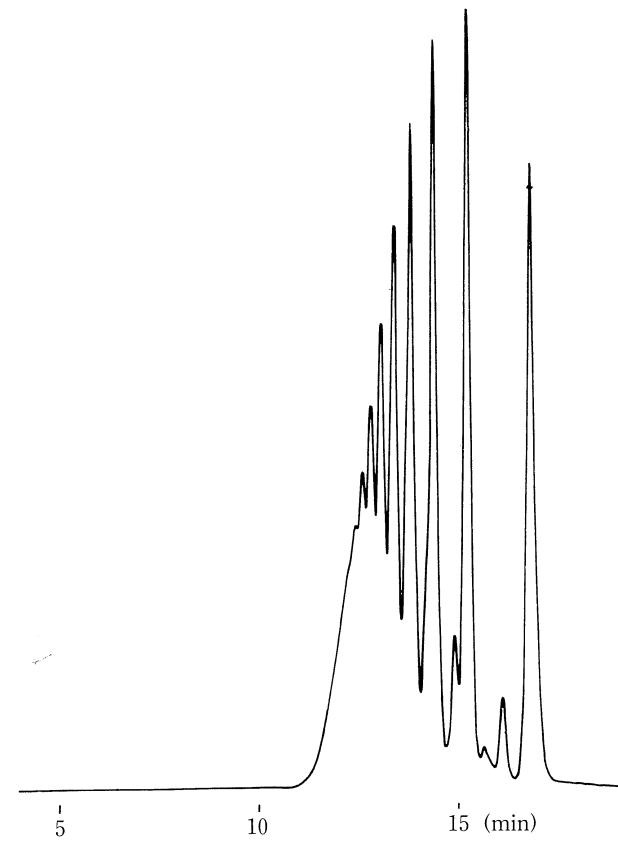
(3) G3000H<sub>XL</sub>: 2 columns



(4) G4000H<sub>XL</sub>: 2 columns



(5) TSKgel G4000H<sub>XL</sub> + TSKgel G2000H<sub>XL</sub>



(6) TSKgel G4000H<sub>XL</sub> + TSKgel G3000H<sub>XL</sub> +  
TSKgel G2500H<sub>XL</sub> + TSKgel G3000H<sub>XL</sub>

**Fig. 19 Separation of Epikote 1001**

