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HIGH-SPEED HIGH-RESOLUTION GEL PERMEATION CHROMATOGRAPHY OF SMALL MOLECULES AND OLIGOMERS

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SUMMARY

High-speed high-resolution gel permeation chromatography of small molecules and oligomers was performed, using Shodex GPC KF-800 series columns packed with porous styrene–divinylbenzene copolymers of particle size 4–8 μm. The number of theoretical plates of 30-cm columns ranged from 19,800 to 23,000 when propylbenzene was used as a test sample with a mobile phase velocity of 0.09 cm/sec. The characteristics of the columns and some applications to small molecules and oligomers are reported.

INTRODUCTION

Gel permeation chromatography (GPC) employing organic solvents introduced by Moore[9] in 1964, is now applied not only to determine the molecular-weight distribution of polymers but also to separate small molecules and oligomers[1,2].

When measuring low-molecular-weight compounds, a complete separation of the individual components is often desired. It is, however, difficult with conventional long GPC columns to attain satisfactory separations in short periods of time[3]. With the development of microparticulate columns for liquid–solid and bonded-phase chromatography in the early 1970s, microparticles have also been available for GPC. However, only one report[4] has so far been published which uses packing materials of 5 μm in diameter for the separation of small molecules and oligomers. Although many other reports have been published, they refer only to the use of porous silica[5] of 10 μm in diameter and cross-linked polystyrene gel[6].

In GPC, separation is performed in the distribution constant range 0–1. The limited peak capacity n is expressed as in the following equation[7]:

$$n = (1 + 0.2N^{\frac{1}{2}}) \quad (1)$$

where N = number of theoretical plates. A column possessing a large number of theoretical plates is necessary to attain a large peak capacity in a short time. A large number of theoretical plates can be obtained by packing a column with gel of a small diameter.

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Showa Denko K.K. (Tokyo, Japan) recently made available on a commercial basis high-performance GPC columns packed with styrene–divinylbenzene copolymer gel of 4–8 μm in diameter (Shodex GPC KF-800-series high-performance columns). This paper reports their characteristics and their applications to the separation of small molecules and oligomers.

EXPERIMENTAL

Apparatus

The liquid chromatograph employed was assembled in the authors' laboratory, and consisted of a high-pressure pump (Milton-Roy), high-pressure sample valves (Rheodyne, Model 7125 with 100-μl sample loop), a UV detector (JASCO, UVIDEC-100) and a refractive index detector (Showa Denko, Shodex RI SE-11).

Columns

GPC was performed, using Shodex GPC KF-800-series columns packed with 4–8-μm particle size polystyrene gels of four different pore sizes. Technical data for the columns are given in Table I. The columns are made of seamless 316 stainless-steel tubing of 30 cm length, 10 mm O.D. and 8 mm I.D. Both ends of the column are packed with stainless steel frits of mean porosity 2 μm manufactured by Shoketsu Kinzoku, Japan, to keep the packing material firmly in place.

Reagents

Reagent-grade tetrahydrofuran (THF) (Wako, Osaka, Japan) was used as mobile phase. Standard polystyrene (Toyo Soda, Tokyo, Japan) shown in Table II was also used.

Calculations

The number of theoretical plates (N) was calculated according to the following equation:

$$N = 5.54\,(t_R/W_{\frac{1}{2}})^2 \qquad (2)$$

where t_R = retention time and $W_{\frac{1}{2}}$ = peak width at half-height.

The distribution of V_0, V_i and V_s was obtained according to the method developed by Pfannkoch *et al.*[8]: thus, the sum of the interstitial volume (V_0), pore volume (V_i) and support volume (V_s) is equal to the total internal column volume (V_c).

TABLE I

CHARACTERISTICS OF PS GEL

Column	*Particle size (μm)*	*Excluded mol. wt.*	*Linear fraction range*
Shodex GPC			
KF-801	6 ± 2	$1.5 \cdot 10^3$	50–1500
KF-802	6 ± 2	$5 \cdot 10^3$	100–5000
KF-802.5	6 ± 2	$2 \cdot 10^4$	100–20,000
KF-803	6 ± 2	$7 \cdot 10^4$	100–70,000

TABLE II

STANDARD POLYSTYRENE

Grade	*Mean molecular weight*	M_w/M_n	*Lot No.*
F-40	422,000	1.04	TS-6
F-10	107,000	1.01	TS-20
F-4	42,800	1.01	TS-19
F-2	16,700	1.02	TS-18
A-5000	6200	1.04	TS-24
A-2500	2800	1.05	TS-23
A-1000	890	*ca.* 1.1	TS-25
A-500	474	*ca.* 1.2	TS-26
A-300	370	*ca.* 1.2	TS-28

$$V_c = V_0 + V_i + V_s \tag{3}$$

The fractional pore volume (E_i), fractional void volume (E_0) and fractional support volume (E_s) were calculated according to the following equations:

$$E_i = V_i/V_c \tag{4}$$
$$E_0 = V_0/C_c \tag{5}$$
$$E_s = V_s/V_c \tag{6}$$

The following equations were used to calculate the fractional pore (E_{pv}) and fractional solid volumes (E_{sv}) of packing material.

$$E_{pv} = V_i/(V_i + V_s) \tag{7}$$
$$E_{sv} = V_s/(V_i + V_s) \tag{8}$$

RESULTS AND DISCUSSION

Characteristics of column

Fig. 1 shows the molecular weight calibration curves for standard polystyrene obtained with Shodex GPC KF-801, -802, -802.5 and -803, each packed with polystyrene gel of a different pore size. A column packed with polystyrene gel of a suitable pore size can be selected according to the molecular weight of the test sample. All curves are almost linear. Polystyrene gel is different from packing materials of the porous silica group, being is characterized by pores of a suitable sizes for separating organic compounds with lower molecular weights. It excels particularly in the separation of substances with molecular weights of 1000 or less.

Table III indicates the distribution of V_0, V_i and V_s in the four columns. V_0 is nearly constant regardless of the pore size, accounting for 35–36% of V_c. The E_0 values of columns packed with spherical silica gel[8] are reported to range from 35 to 39% and that of Shodex GPC KF-800-series columns was close to the E_0 of columns packed with rigid silica. This signifies that polystyrene gel is properly packed in the Shodex columns. The effective pore volume for separation ranged from 31 to 44% of the total internal column volume. The percentage of the pore volume ($\%E_{pv}$) was in

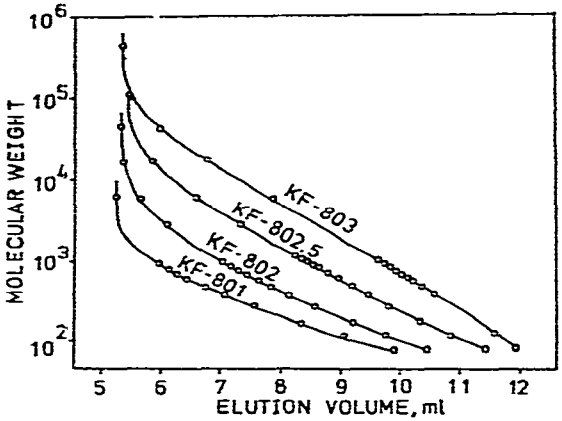


Fig. 1. Molecular weight calibration plots for Shodex GPC KF columns. Standard polystyrene; mobile phase, THF; 25°C; flow-rate, 1.0 ml/min; UV detector.

the range 48–69%. There was a tendency for the percentage to increase as the pore size becomes larger. The $\%E_{pv}$ of the Shodex columns, except for KF-803, was somewhat smaller than that of porous silica-packed columns. Although the $\%E_{pv}$ of polystyrene gel can be increased by polymerization formulation, the mechanical strength of the gel decreases with increasing $\%E_{pv}$ of polystyrene gel to such an extent that it cannot be used as the packing material for high-speed GPC.

The separation capacity of packed columns for GPC depends on the number of theoretical plates. A large number of theoretical plates can be obtained by using packing material of particle size 10 μm or less. Table IV gives the characteristics of the Shodex columns packed with polystyrene gel of 4–8 μm in particle size. The number of theoretical plates was measured at a flow-rate of 1 ml/min with propylbenzene used as a test sample and THF as the mobile phase. The guaranteed number of plates for Shodex GPC KF-800-series columns is 16,000 plates/30 cm. Kirkland and Antle[5] reported that a reduced plate height of a well made column does not exceed the range 2.0–3.5. Judging from his report, Shodex GPC KF-type columns can be recognized as being well made since their reduced plate heights are in the range 2.2–2.5.

Fig. 2 shows a plot of mobile phase velocity *vs.* the plate heights for three columns packed with Shodex GPC gels of different particle sizes. Benzene was used for measurement of the plate heights, and it was shown that the smaller the mean particle size, the lower the plate height, thereby enabling a high-performance column

TABLE III

DISTRIBUTION OF TOTAL INTERNAL COLUMN VOLUME AMONG INTERSTITIAL VOLUME (V_0), PORE VOLUME (V_i) AND SUPPORT VOLUME (V_s)

Column	V_i/V_0	V_0	V_i	V_s	E_0 (%)	E_t (%)	E_s (%)	E_{pv} (%)	E_{sv} (%)
KF-801	0.90	5.25	4.69	5.14	34.8	31.1	34.1	47.7	52.3
KF-802	0.99	5.29	5.22	4.57	35.1	34.6	30.3	53.3	46.7
KF-802.5	0.99	5.44	5.40	4.27	36.1	35.8	28.1	56.0	44.0
KF-803	1.25	5.33	6.68	3.07	35.3	44.3	20.4	68.5	31.5

TABLE IV

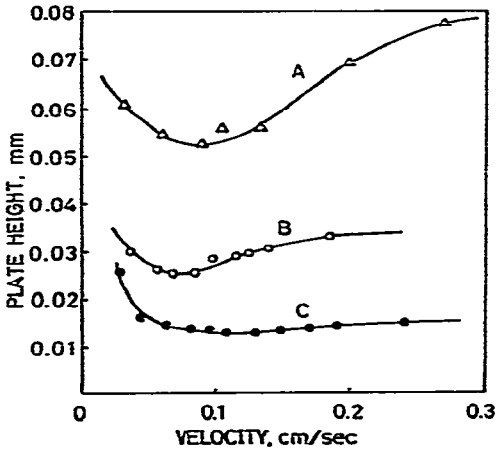
CHROMATOGRAPHIC EFFICIENCY OF COLUMN

Mobile phase, tetrahydrofuran; 24°C; 1.0 ml/min.

Column	*Pressure drop (kg/cm²)*	*Plate count (propylbenzene)*	*H (cm) (propylbenzene)*	*h* (H/d_p)
KF-801	21	23,000	0.00130	2.2
KF-802	17	21,100	0.00142	2.4
KF-802.5	15	19,800	0.00151	2.5
KF-803	15	21,100	0.00141	2.4

to be obtained. The minimum plate height can be observed for each curve and that for curve C occurs at a higher point on the mobile phase velocity axis than those for the other curves.

Fig. 3 shows the relationship of the plate heights and mobile phase velocity measured with four test samples of different molecular weights. The higher the molecular weight, the further down the mobile phase velocity axis the minimum plate height moves. Thus, when benzene (molecular weight 78) was used as a test sample, the minimum plate height was obtained at a velocity of 0.13 cm/sec; for dimethyl phthalate (DMP) (194), it was 0.065 cm/sec, with dibutyl phthalate (DBP) (278), 0.05 cm/sec and with dioctyl phthalate (DOP) (309), it was 0.04 cm/sec. Columns packed with polystyrene gel are usually used with a flow-rate of 1 ml/min. The velocity at which the minimum plate height was obtained when benzene was used as a test sample was 0.13 cm/sec, which corresponds to a flow-rate of 1.4 ml/min. Use of Shodex GPC KF-802 with a flow-rate of 1 ml/min (corresponding to a velocity of



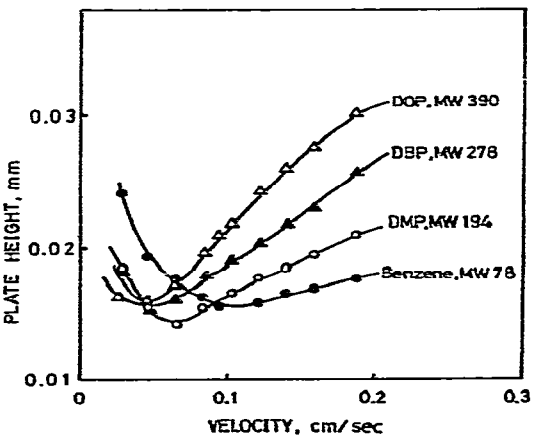


Fig. 2. Effect of particle size (d_p) of Shodex GPC gel on column efficiency. Solute, benzene; mobile phase, THF; column dimensions 30 cm × 8 mm I.D.; (A) d_p = 14–18 μm; (B) d_p = 8–12 μm; (C) d_p = 4–8 μm.

Fig. 3. Effect of velocity on plate height (H) for different molecular weight solutes. Column, Shodex GPC KF-802; mobile phase, THF; 24°C; detection UV: sample size, 10 μl.

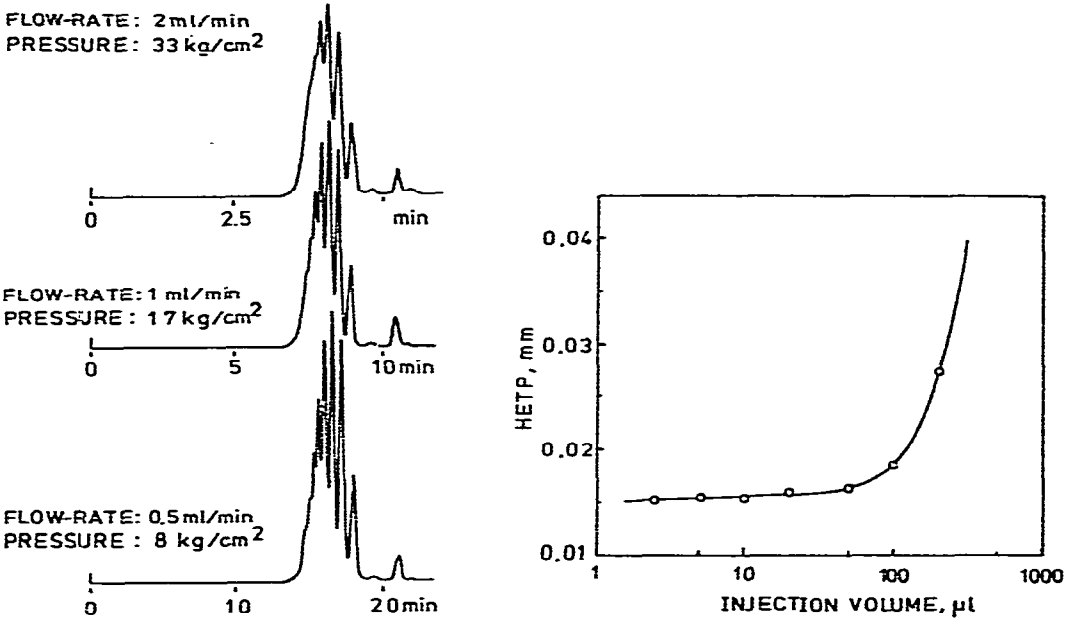


Fig. 4. Effect of flow-rate on elution patterns of polystyrene oligomers. Column: Shodex GPC KF-802, 30 cm × 8 mm I.D.; mobile phase, THF; detector, UV, 0.32 A.U.F.S. at 254 nm; sample size, 20 µl; sample, 0.5% MW 474 polystyrene; temperature, 25°C.

Fig. 5. Effect of injection volume on plate height (H). Column, Shodex GPC KF-803, 30 cm × 8 mm I.D.; mobile phase, THF; flow-rate, 1.0 ml/min; sample, benzene, 0.05 mg.

0.09 cm/sec), slightly increases the plate height for compounds of low molecular weight (such as benzene which elutes at the total permeation volume). Separation of compounds which are eluted in the vicinity of the exclusion volume, however, is more difficult than that of material which is eluted in the vicinity of the total permeation volume. A flow rate of 1 ml/min, therefore, appears appropriate for columns packed with polystyrene gel of particle size 4–8 µm in view of the relationship of the analytical time and resolution.

Fig. 4 shows chromatograms of styrene oligomers with an average molecular weight of 474. These were obtained with flow rates of 0.5, 1 and 2 ml/min.

Fig. 5 shows the effect of an injection volume of a test sample on the plate height, indicating that the volume of 50 µl maximum does not produce any effect.

APPLICATIONS

For the separation of small molecules or oligomers, GPC is advantageous in the following respects when compared with other methods of adsorption or partition chromatography. First, as long as it is soluble in the eluent, any test sample can be injected and the setting of the analytical conditions is easy. Secondly, all the components of the test sample are eluted within a certain time without adsorption onto

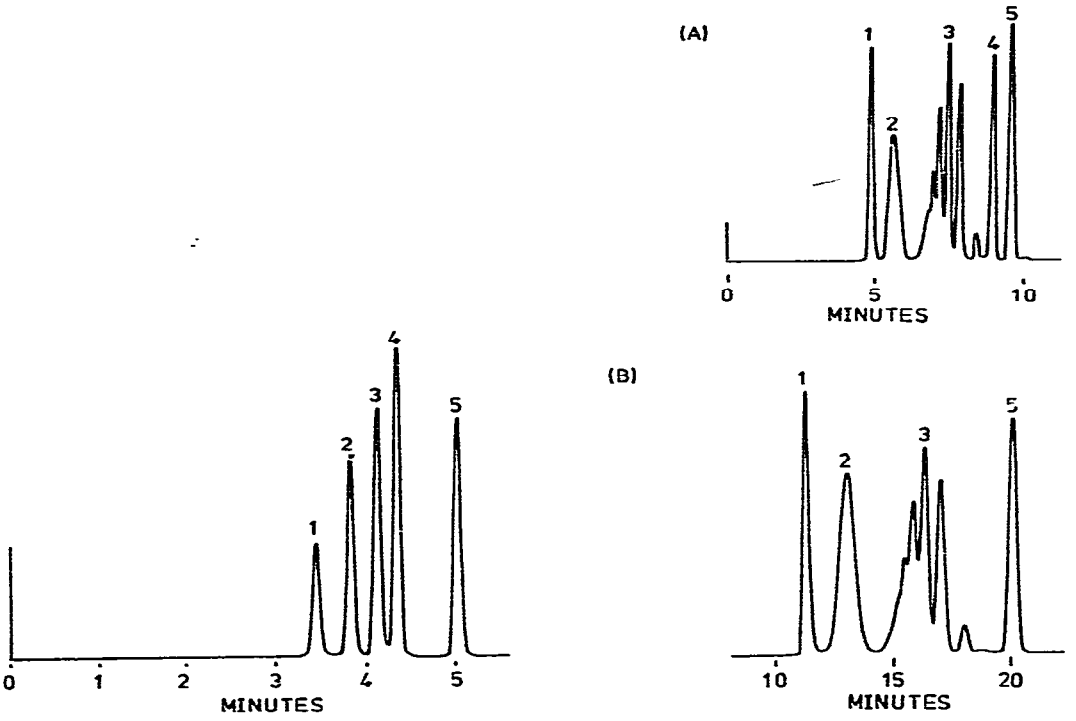


Fig. 6. Separation of mixture of DOP, DBP, DEP, DMP and benzene. Peaks: 1 = DOP, 2 = DBP, 3 = DEP, 4 = DMP, 5 = benzene; column, Shodex GPC KF-801, 30 cm × 8 mm I.D.; sample, 0.07% DOP, DBP, DEP, DMP and 0.35% benzene in THF; sample size, 10 μl; mobile phase, THF; flow-rate, 1.95 ml/min; pressure, 40 kg/cm^2.

Fig. 7. Comparison of analysis times between 4–8-μm (A) and 8–12-μm (B) PS gel-packed columns. Peaks: 1 = polystyrene MW 42,800, 2 = polystyrene MW 2,800, 3 = polystyrene MW 370, 4 = *n*-propylbenzene; 5 = benzene. (A) Column, Shodex GPC KF-802, 30 cm × 8 mm I.D.; mobile phase, THF; flow-rate, 1.1 ml/min. (B) Column, Shodex GPC A-802, 50 cm × 8 mm I.D.; mobile phase, THF; flow-rate, 1.0 ml/min.

the packing materials. The third advantage is that the molecular size of the test sample can be estimated from the elution volume.

Fig. 6 shows the separation of a mixture of DOP, DBP, diethyl phthalate (DEP), DMP and benzene. These phthalic esters are often used as plasticizers. The separation was performed at a flow rate of 2 ml/min with Shodex GPC KF-801 (30 cm × 8 mm I.D.). The esters and benzene were separated satisfactorily within 5 min.

Fig. 7 shows a comparison of the separation capacity of Shodex GPC KF-802 (30 cm × 8 mm I.D.) packed with polystyrene gel of particle size 4–8 μm with that of conventional Shodex GPC A-802 (50 cm × 8 mm I.D.) packed with polystyrene gel of particle size 8–12 μm. A mixture of standard polystyrene and benzene was used for the comparison, in which KF-802 completed separation within 10 min and A-802 within 20. KF-802 packed with polystyrene gel of particle size 4–8 μm separates twice as fast as A-802 packed with particle sizes of 10 μm yet attains the same resolution.

A satisfactory separation of oligomers over a short period of time is not easy to

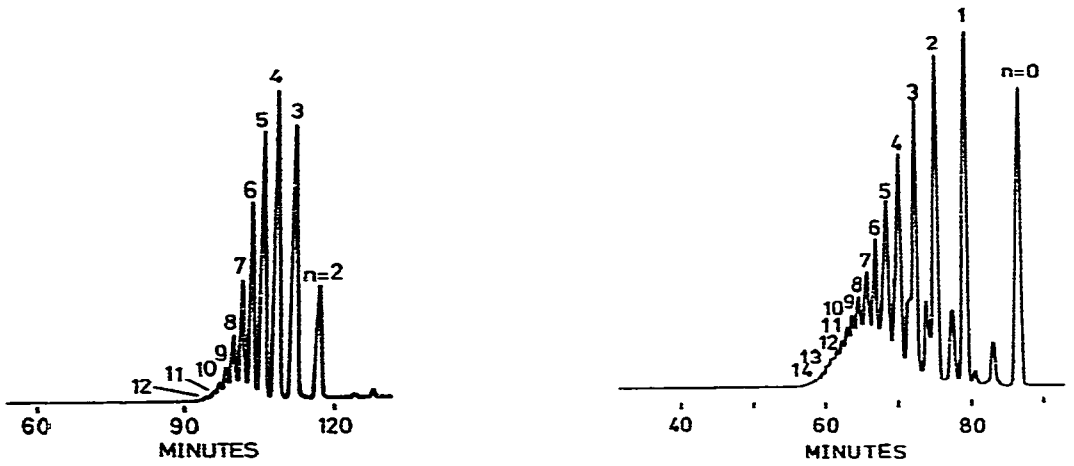


Fig. 8. Separation of polystyrene oligomers. Column, Shodex GPC KF-802.5, 4 × (30 cm × 8 mm I.D.) + KF-802, 4 × (30 cm × 8 mm I.D.); mobile phase, THF; flow-rate, 0.6 ml/min; pressure, 73 kg/cm^2; sample, 0.5% polystyrene MW 474; sample size, 100 μl.

Fig. 9. Separation of epoxy oligomers. Column, Shodex GPC KF-803, 4 × (30 cm × 8 mm I.D.) + KF-802.5, 4 × (30 cm × 8 mm I.D.); mobile phase, THF; flow-rate, 0.96 ml/min; pressure, 104 kg/cm^2; detector, UV, 254 nm; sample, 0.5% Epikote 1001; sample size, 100 μl.

achieve with conventional columns. Extremely long columns or recycle systems[2] are therefore employed for the analyses of oligomers, and these require long separation times. However, columns packed with polystyrene gel of particle size 4–8 μm have enabled short-time analyses with high resolution to be achieved.

Fig. 8 shows the separation of standard polystyrene with a molecular weight of

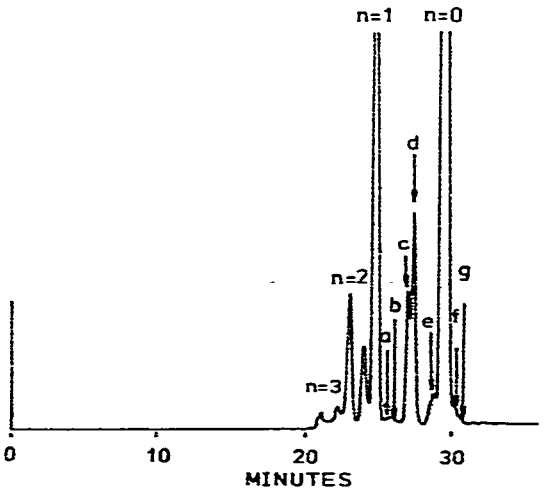


Fig. 10. Separation of epoxy oligomers. Column, Shodex GPC KF-801, 4 × (30 cm × 8 mm I.D.); sample, 0.5% Epikote 828 in THF; sample size, 15 μl; mobile phase, THF; flow-rate, 1.0 ml/min; pressure, 76 kg/cm^2.

474. The chromatogram was obtained with a flow-rate of 0.62 ml/min and four columns each of KF-802 and KF-802.5 connected in series. A low flow rate was effective in separating the test samples which were otherwise difficult to separate. The chromatogram shows good separation up to $n = 12$ within 2 h.

Fig. 9 shows a chromatogram of Epikote 1001, an epoxy oligomer. Four columns each of KF-803 and KF-802.5 were used in series and the flow rate was set to 0.96 ml/min. Peaks corresponding to the degree of polymerization ranging from $n = 0$ to $n = 14$ can be observed. Epoxy resin is used as coating material, as an electrical insulating material, as a constructional or building material and as an adhesive. When used as such, its molecular weight distribution as well as impurities formed by secondary reactions changes its performance and characteristics. Fig. 10 shows the separation of the impurities in epoxy resin. With a view to detecting traces of the impurities, the sensitivity of the dectector was amplified to such an extent that the peaks of the main components went off the recording range. Five impurities were detected between the $n = 0$ and $n = 1$ peaks. A few small peaks can also be observed at the bottom right of the $n = 0$ peak, indicating the inclusion of some other impurities. Use of high-resolution columns has made it possible to obtain peaks with narrow bases and has also improved the sensitivity of detection, thereby facilitating detection of trace impurities contained in epoxy resin.

CONCLUSION

High-speed and high-resolution GPC has become possible with the use of columns packed with polystyrene gel of particle size 4–8 μm. Small molecules or oligomers can be quickly separated with an optimum column selected from four columns each of which is packed with gel of a different pore size.

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