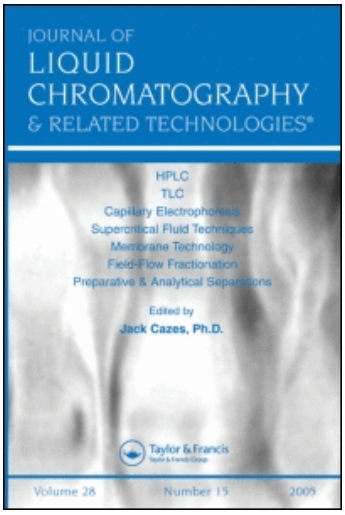

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Temperature effects in Microcolumn Size Exclusion Chromatography

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TEMPERATURE EFFECTS IN MICROCOLUMN SIZE EXCLUSION CHROMATOGRAPHY

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ABSTRACT

Effects of column temperature on the column efficiency, retention time and stability of analytes were studied in microcolumn size-exclusion chromatography. Larger theoretical plates were achieved at column temperatures betwen 70 and 100 ℃. In the constant-flow mode the retention time of analytes decreased with increasing column temperature, which was due mainly to thermal expansion of the mobile phase. When the column temperature was around or higher than the critical temperature of the mobile phase, the retention times of the analytes observed under supercritical pressure conditions still indicated dominancy of the size-exclusion mechanism, while another retention mechanism was involved under subcritical pressure conditions. In the case of the analysis of saccharides, the column temperature should be lower than 100 ℃ because oligosaccharides were decomposed at higher temperatures.

INTRODUCTION

The influence of column temperature has not been examined in great detail in size-exclusion chromatography (SEC) [1]. The column temperature affects the viscosity and density of the mobile phase, the size of solute molecules, the pore size of the gel, the internal volume of the column tubing and interactions between the solute and the gel. General-

ly, the column efficiency is better at elevated temperatures because the rise in the column temperature decreases the viscosity of the mobile phase [2-4]. It is reported that the apparent retention volume decreases with increasing column temperature in SEC, which is due to the decrease in the interactions between the solute and the gel [5,6], the expansion of the mobile phase [7], or the increase in the actual size of the solute [8]. Furthermore, the change in the column temperature leads to errors in molecular weight determinations [6,7].

The authors reported the use of microcolumns in SEC [9,10], in which the column efficiency could be improved by increasing the column length. This paper describes effecs of the column temperature on the column efficiency, the retention time, and stability of analytes in microcolumn SEC.

EXPERIMENTAL

Apparatus

The liquid chromatograph was composed of a solvent-delivery system, an oven, a micropacked separation column, a UVIDEC-100V UV spectrophotometer (JASCO, Tokyo, Japan) with a laboratory-made flow cell, an ML-425 microvalve injector with an injection volume of 20 nl (JASCO) and an outlet-pressure regulator.

In the constant-flow mode an MF-2 Microfeeder pump (Azumadenki Kogyo, Tokyo, Japan) equipped with an MS-GAN 050 gas-tight syringe (0.5 ml; Ito, Fuji, Japan) was used as the solvent-delivery system. The outlet pressure was controlled with the pressure regulator attached to a nitrogen cylinder. In the constant-pressure mode a CCPM HPLC pump (TOSOH, Tokyo, Japan) was employed, with which both inlet and outlet pressures of the column could be controlled. A pneumatic solvent-delivery system was also employed when a noise due to pulsation of the pump was observed.

The separation column was prepared from fused-silica tubing with 0.32 mm i.d. (SGE, Ringwood, Victoria, Australia). Commercially available packing materials, Shodex GPC KF-801 (6 μm; Showa Denko K.K., Tokyo, Japan) and Shodex Ionpak KS-801 (5-8 μm; Showa Denko K.K.), were employed in this work. The former was for gel permeation chromatography (GPC) with an organic mobile phase, while the latter was for SEC of water-soluble organics. Capillary Butt Connectors (Supelco, Bellefonte, PA, USA) were employed for connecting the separation column and stainless steel connecting tubes of 50 μm i.d. x 0.30 mm o.d. (Nomura Chemical, Seto, Japan), and quartz wool (1-6 μm; Wako Pure Chemical Industries, Osaka, Japan) was used as the filter. These packing materials were dispersed in the same solvent as the mobile phase and were packed into the fused-silica tubing by using an HPLC pump at 150 kgf/cm^2.

Reagents

All the reagents except for HPLC-grade distilled water (Wako Pure Chemical Industries) were of reagent-grade. Dialkyl phthalates were obtained from Tokyo Chemical Industry (Tokyo, Japan), and other reagents were obtained from Wako Pure Chemical Industries. The reagents were used without any treatment. Tetrahydrofuran and distilled water were used as the mobile phase.

RESULTS AND DISCUSSION

GPC with tetrahydrofuran as the mobile phase

It is expected that the rise in the column temperature accelerates establishment of equilibrium, leading to a higher column efficiency [2-4]. Figure 1 illustrates the relationships between the theoretical plate number, N, and the column temperature, in which a 20-cm column packed with Shodex GPC KF-801 is employed. Larger theoretical plate numbers were achieved at temperatures between 70 and 100 ℃. Since tetrahydrofuran expands at raised temperatures, the actual volumetric flow

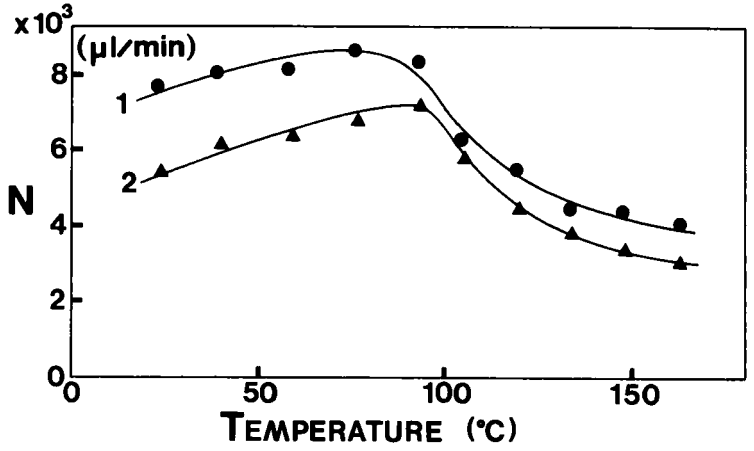

Figure 1 Temperature effect on the column efficiency in micrcolumn GPC with tetrahydrofuran mobile phase.
Column:Shodex GPC KF-801, 20 cm x 0.32 mm i.d. Mobile phase:tetrahydrofuran. Flow rates:●, 1 μl/min; ▲, 2 μl/min. Sample:DMP.

rate in the column increases with increasing column temperature. The larger the temperature difference between the column and the pump head, the larger the expansion, the larger the actual volumetric flow rate in the column. The actual volumetric flow rate in the column was corrected by assuming that no interaction between the solute and the gel was involved. The flow rates indicated in Figure 1, 1 and 2 μl/min, are corrected actual volumetric flow rates. The lower flow rate achieved larger theoretical plate numbers. Unfortunately, it is not certain why the theoretical plate number decreases with increasing column temperature in the higher column temperature region.

Figure 2 demonstrates the separations of an artificial mixture of dialkyl phthalates and alkylbenzenes at room temperature (27 °C) and 100 °C on a 1-m column packed with Shodex KF-801. In comparison with the separation at the room temperature better resolution of the analytes is achieved in a shorter time at 100 °C. Although the applied flow rate of the pump was the same for both separations, viz., 1.0 μl/min, the ob-

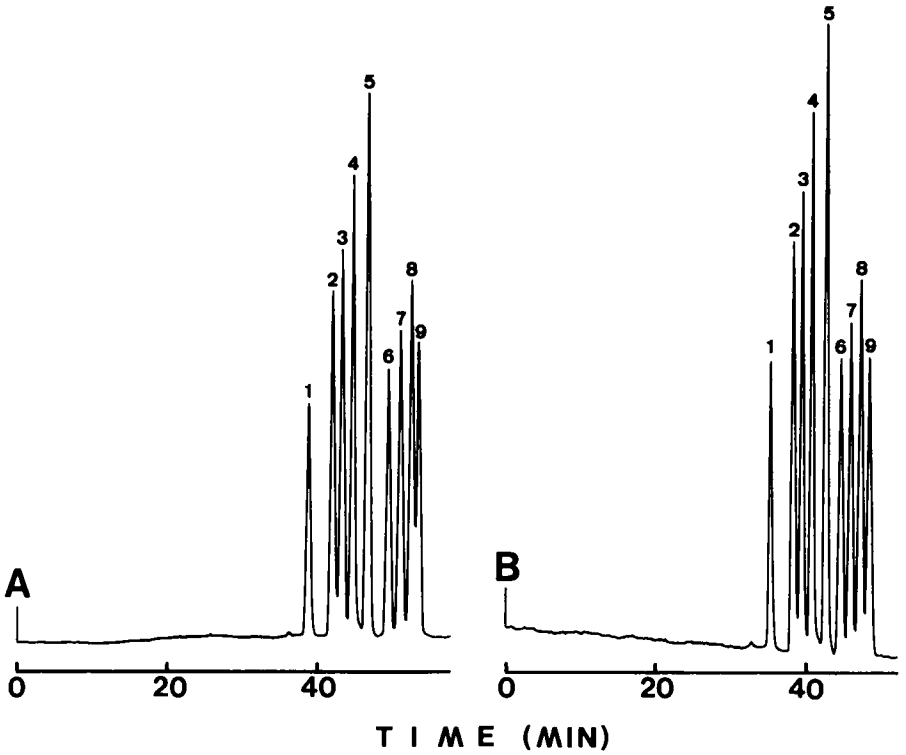

Figure 2 Separations of an artificial mixture of dialkyl phthalates and alkylbenzenes.
Column:Shodex GPC KF-801, 1 m x 0.32 mm i.d. Mobile phase:tetrahydrofuran. Flow rate:1.0 μl/min. Column temperatures:A, 27 ℃; B, 100 ℃. Samples:1=0.48 % di-2-ethylhexyl phthalate; 2=0.48 % di-n-butyl phthalate; 3=0.48 % di-n-propyl phthalate; 4=0.48 % diethyl phthalate; 5=0.48 % dimethyl phthalate; 6=1.9 % n-propylbenzene; 7= 1.9 % ethylbenzene; 8= 1.9 % toluene; 9=1.9 % benzene. Wavelength of detection:254 nm.

served retention times of the analytes for the separation at 100 ℃ were 9 % shorter than those for the separation at 27 ℃. This result may be due mainly to thermal expansion of tetrahydrofuran. In the case of the separation at 100 ℃, the outlet of the column was required to be pressurized so that the tetrahydrofuran shoud not be vapourized in the column. Otherwise, the baseline was not stable and the analytes were not eluted in a reasonable time.

The critical temperature and pressure of tetrahydrofuran are 267 °C and 52.2 kgf/cm^2, respectively. The effect of the column temperature on the retention behaviour was examined up to around 300 °C under the subcritical and supercritical pressure conditions. The inlet and outlet pressures selected as the former condition were 40 and 35 kgf/cm^2, while those selected as the latter condition were 80 and 65 (or 75) kgf/cm^2, respectively. Figure 3 shows the relative retention time of di-2-ethylhexyl phthalate (DOP) versus dimethyl phthalate (DMP) as a function of the column temperature.

Under the supercritical pressure condition the relative retention times were constant at temperatures lower than the critical temperature, denoted as CT in the figure, and slightly increased with increasing temperature in the supercritical temperature region. The results indicate that the size-exclusion mode is still dominant even under the supercritical conditions. In addition, the outlet pressure for the data at 289 and 293 °C was 75 kgf/cm^2 because the flow rate was too high to resolve DOP and DMP when the outlet pressure was 65 kgf/cm^2.

Under the subcritical pressure condition, on the other hand, the relative retention times were constant at temperatures lower than 240 °C, and the values coincided with those observed under the supercritical pressure conditions. In other words, the size-exclusion separation mode is dominant at the temperature lower than 240 °C. At temperatures higher than 240 °C the relative retention time underwent a complicated change owing to the change of the physical property of the mobile phase. In case the separation takes place by means of size exclusion, DOP should elute before DMP. However, the retention order varied with column temperature. DOP eluted after DMP at 262 °C, and DOP could not be eluted in a reasonable time at temperatures higher than 264 °C. Under the subcritical pressure condition the mobile phase vapourizes at a subcritical temperature. This means that both liquid and gaseous tetrahydrofuran exist in the column in some temperature region lower than the critical

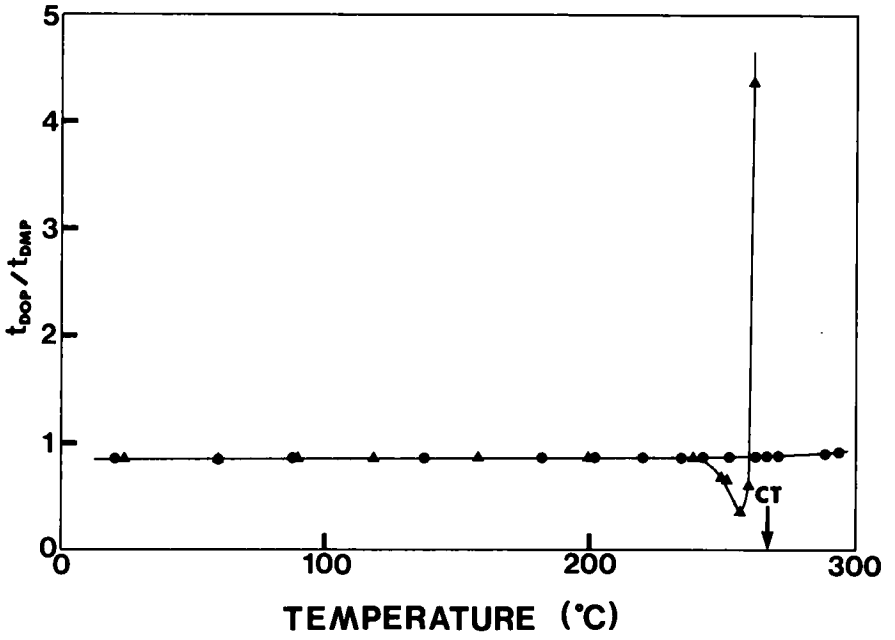

Figure 3 Relative retention time versus column temperature. Column: Shodex GPC KF-801, 20 cm x 0.32 mm i.d. Mobile phase: tetrahydrofuran. Pressures: ●=80 kgf/cm² (inlet), 65 or 75 kgf/cm² (outlet); ▲=40 kgf/cm² (inlet), 35 kgf/cm² (outlet).

temperature, where size-exclusion liquid chromatography and gas chromatography separation modes are involved.

SEC with water as the mobile phase

The effect of the column temperature on the column efficiency was also exmained in SEC of saccharides by using water as the mobile phase. Figure 4 illustrates the relationships between the theoretical plate number and the column temperature using a 21-cm column packed with Shodex KS-801. Larger theoretical plate numbers were achieved at temperatures between 70 and 100 °C. In this case the variation of the actual volumetric flow rate in the column due to thermal expansion of the mobile phase was not very significant in comparison with that in the case of the tetrahydrofuran mobile phase.

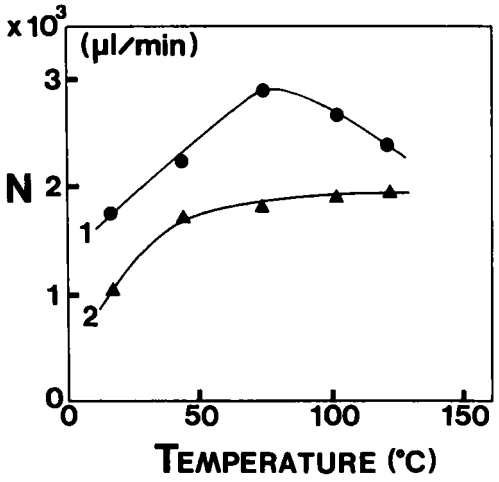

Figure 4 Temperature effect on the column efficiency in microcolumn SEC with water as the mobile phase.
Column:Shodex Ionpak KS-801, 21 cm x 0.32 mm i.d. Mobile phase:distilled water. Flow rates:●=1 μl/min; ▲=2 μl/min. Sample:glucose.

Figure 5 demonstrates the separations of raffinose and glucose at various temperatures, in which 1 μg each is injected. Raffinose and glucose are denoted as R and G in the figure, respectively. The chromatograms at 17, 44 and 74 °C were obtained by the constant-flow mode, and the applied flow rate was 1.4 μl/min. The retention volumes of the analytes slightly decreased with increasing column temperature. Under these conditions, the higher the column temperature, the narrower the peak.

At temperatures higher than 100 °C, a periodic baseline noise was observed when the Microfeeder pump was used. This was due to the pulsation of the pump. Therefore, the chromatograms at 102 and 122 °C were obtained by the constant-pressure mode. In this experiment the mobile phase was filled in PTFE tubing of 0.5 mm i.d. x ca. 1 m length, and the pressure from the nitrogen cylinder was applied to the tubing. The applied inlet pressures were 15 and 13 kgf/cm² for the former and the latter chromatogram, respectively, with the outlet pressure being ca. 3 kgf/cm².

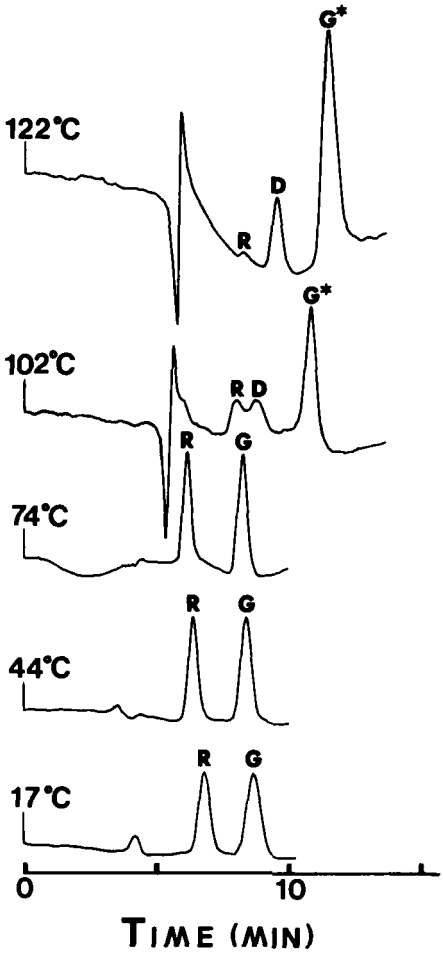

Figure 5 Chromatograms of saccharides at various temperatures. Column:Shodex Ionpak KS-801, 21 cm x 0.32 mm i.d. Mobile phase:distilled water. Flow rates and applied pressures:see text. Samples:R=raffinose; D=disaccharides; G=glucose; G*=glucose and other monosaccharides. Wavelength of detection:195 nm.

Three peaks are observed in the chromatograms of 102 and 122 °C. The second peak denoted as D is a newly appeared peak, which may be composed of disaccharides derived from raffinose. Raffinose is α-D-galactopyranosyl-(1-6)-O-α-D-glucopyranosyl-(1-2)-β-D-fructofuranoside. The

TABLE 1
Retention Time of Saccharides.
Operating conditions as in Figure 5

Temperature, ℃	Retention Time, min			Relative Retention Time	
	R	D	G(or G')	R/G	D/G
17	6.78	-	8.63	0.79	-
44	6.44	-	8.42	0.76	-
74	6.20	-	8.28	0.75	-
102	8.02	8.78	10.81	0.74	0.81
122	8.22	9.53	11.46	0.72	0.83

peak height of raffinose decreases at 102 and 122 ℃ in comparison with that observed at the lower temperatures, while the peak heights of the second and third peaks increase with increasing temperature. The third peak may involve glucose and derived monosaccharides, e.g., galactose and fructose. Therefore, the third peaks observed at 102 and 122 ℃ are denoted as G'.

Table 1 shows the retention times of these peaks and the relative retention times of the first and second peaks versus the third peak. The relative retention times are almost constant in the examined temperature region. The operation at higher temperature is not recommended for thermally unstable solutes.

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