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Note

Effect of temperature difference between pump and column in molecular weight determination by gel permeation chromatography

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Some years ago, the effect of temperature on retention times in liquid chromatography was studied by several workers[1–6], and the importance of column thermostating in order to obtain accurate and reproducible retention times (t_R) was emphasized. In gel permeation chromatography (GPC), however, the effect of temperature on retention time is less significant than that in any other mode of chromatography, because it is based on steric exclusion and there is little contribution to retention of adsorption or partition, which is very temperature dependent. Therefore, it was considered to be more important to measure the flow-rate accurately in order to reduce errors in molecular weight calculations[7,8].

The effect of column temperature on molecular weight determinations has been reported[9], including the influence of solvent expansion and contraction. However, there has been no systematic study of the errors caused by the temperature difference between the solvent discharged from a pump and the solvent in a thermostated column.

This paper demonstrates that the independent changes in temperature of the solvent in the pump head and in the column results in significant errors in molecular weight determinations, and these errors can be compensated for simply by taking thermal expansion into account.

EXPERIMENTAL

The retention volume, V_R, is expressed by using the mass flow-rate, F_m:

$$V_R = t_R F_m[V_0(1 + \alpha T + \beta T^2 + \gamma T^3)]$$

where V_0 = volume of 1 g of the solvent at 0°C, α, β and γ are coefficients of cubic expansion of the solvent and T(°C) = temperature of the solvent. The coefficients of cubic expansion of an organic solvent are generally $\alpha \approx 10^{-3}$, $\beta \approx 10^{-6}$ and $\gamma \approx 10^{-8}$. In most instances, it can simply be taken that the volume of an organic solvent linearly expands by 0.1%/°C because the second- and third-order terms are negligibly small.

By using a volumetric displacement-type pump [any high-performance liquid chromatographic (HPLC) pump is of this type] a given volume of the solvent is simply displaced irrespective of its density or temperature. This means that the volumetric

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flow-rate metered in the pump may be constant, but the mass flow-rate from the pump varies with the temperature of the solvent. When the solvent enters a column oven at an elevated temperature from a pump at room temperature, a reduction in solvent density or a volume expansion consequently takes place. Therefore, no matter how accurately the volumetric flow-rate of the pump and the temperature of the column are controlled, the actual volumetric flow-rate or the linear velocity in the column cannot be maintained constant unless the solvent temperature in the pump head is kept constant.

Solvent, sample and column

Tetrahydrofuran (THF) was used as the mobile phase in GPC measurements with a column system consisting of two Shodex A-80M columns (50 cm × 8 mm I.D.) connected in series and packed with a mixture of polystyrene gels of nominal pore size 10^3, 10^4, 10^5 and 10^6 Å (Showa Denko, Tokyo, Japan).

Monodisperse polystyrene (PS) standards having nominal molecular weights of $2880 \cdot 10^3$, $233 \cdot 10^3$, $17.5 \cdot 10^3$ and $2.8 \cdot 10^3$ were used to measure the effect on retention times of temperature differences between the solvent and pump head and the solvent and column. These standards were obtained from Toyo Soda (Tokyo, Japan) (mol.wt. $2880 \cdot 10^3$ and $2.8 \cdot 10^3$) and Pressure Chemical (Pittsburgh, PA, U.S.A.) (mol.wt. $233 \cdot 10^3$ and $17.5 \cdot 10^3$). Ten milligrams of each PS standard and benzene were dissolved in 20 ml of THF and 100 μl of the solution were injected into the GPC system.

Apparatus

The GPC system was assembled from JASCO (Tokyo, Japan) 800 series HPLC modules. The mobile phase solvent (THF) was supplied to the delivery pump through a Model 880-50 in-line degasser (JASCO) in order to remove dissolved air, which often causes instability of the flow-rate. To control the solvent temperature in the pump head, a thermostat jacket was attached to the pump head. A water circulating bath with a built-in LC-101 refrigerator (Scinics, Tokyo, Japan) circulated water to the jacket, which contained a thermal equilibration coil (2 m × 0.8 mm I.D. × 1.6 mm O.D.) for incoming solvent. THF in a glass bottle was placed in the bath. Using this arrangement, the temperature of THF discharged from the pump was controlled to an accuracy of ± 0.1°C at specified temperatures.

The columns were thermostated to ± 0.1°C at specified temperatures in an 860-CO air circulating oven (JASCO). An 855-AS autosampler (JASCO) was used to perform automated sample injection. A heat-exchanger coil (1 m × 0.25 mm I.D.) was connected between th sampler and the column and placed in the oven for temperature equilibrium of the mobile phase solvent and sample solution before entry into the column.

An 875-UV variable-wavelength UV detector and an 805-GI data processor (JASCO) were used for monitoring and processing the GPC results. To obtain molecular weight distributions, a refractive index detector is generally used because it offers a proportional response to the mass of a sample solute. However, it is very sensitive to temperature changes of the mobile phase and room temperature, and for this reason a UV detector, which is thermally stable, was employed here for GPC measurements.

GPC measurement procedure

In order to evaluate the effect of temperature differences between the solvent and pump head and the solvent and column on errors in molecular weight determinations by GPC measurement, a calibration graph was established by injecting the polystyrene standard mixture with a solvent/pump head temperature of 20°C and a column temperature of 40°C. Retention data for each PS standard were obtained at various solvent/pump head temperatures (17, 25 and 30°C) while the temperature of the column was maintained constant at 40°C.

The molecular weight of each PS standard was calculated using the obtained

TABLE I

EFFECTS OF SOLVENT/PUMP HEAD TEMPERATURE AND RETENTION TIME COMPENSATION USING THE COEFFICIENT OF CUBIC EXPANSION ON ERRORS IN MOLECULAR WEIGHT DETERMINATION FROM THE CALIBRATION GRAPH OBTAINED AT 20°C

GPC conditions: two columns, Shodex A-80M; column temperature, 40°C; mobile phase, THF at a flow-rate of 1 ml/min.

Retention time (t_R) data used	*Calculated molecular weight ($\times 10^3$)*			
	*17.0°C**	*20.0°C*,***	*25.0°C*	*30.0°C*
Measured t_R***	3.07	2.80	2.34	2.09
	(+3.74)		(−16.43)	(−25.36)
Compensated t_R§	2.84		2.67	2.73
	(+1.43)		(−4.64)	(−2.14)
Measured t_R	18.90	17.50	14.95	13.62
	(+8.00)		(−14.57)	(−22.17)
Compensated t_R	17.67		16.76	17.67
	(+0.97)		(−4.01)	(+0.97)
Measured t_R	244.27	233.00	204.83	192.72
	(+4.84)		(−12.09)	(−17.82)
Compensated t_R	231.80		223.66	229.83
	(+0.52)		(−4.01)	(−1.36)
Measured t_R	2987.78	2880.00	2590.25	2338.10
	(+3.74)		(−10.06)	(−18.82)
Compensated t_R	2877.18		2760.67	2809.85
	(−0.10)		(−4.14)	(−2.44)

* Solvent/pump head temperature.

** The calibration graph was established by fitting the retention data at 20.0°C in the cubic equation

$$\log \text{mol.wt.} = a(t_R)^3 + b(t_R)^2 + c(t_R) + d$$

where $a = -9.3380 \cdot 10^{-6}$, $b = -2.6850 \cdot 10^{-3}$, $c = -8.1416 \cdot 10^{-2}$, $d = 9.8298$.

*** Molecular weights were calculated by entering measured retention data into the above equation. Percentage errors are shown in parentheses.

§ Retention data were compensated by using the coefficient of cubic expansion, $\alpha = 1.103 \cdot 10^{-3}$, calculated from the following equation[10], and extrapolating to 30°C:

$$\rho(T) = 0.880[1 + 0.001085(25 - T)]$$

retention data and the calibration graph. The molecular weight was also calculated using the compensated retention time by taking thermal expansion into account.

RESULTS AND DISCUSSION

Table I shows the errors in molecular weight determination without and with solvent/pump head temperature compensation. It is significant that a 10°C deviation from the temperature at which the calibration graph was established (20°C) causes 19–25% error when a wide-molecular-weight range column such as Shodex 80M is used. On the other hand, the error can be reduced to only 2.5% by applying the simple compensation for retention time with the coefficient of cubic expansion.

These results suggest that the linear velocity or volumetric flow-rate of the mobile phase solvent dominates the retention time of a sample solute in GPC, and a major contribution to the variation of retention time is made by the thermal expansion of the solvent in GPC.

In order to confirm the above conclusion, the retention time variation was evaluated by changing the temperatures of both the solvent/pump head and the solvent/column while maintaining the temperature difference constant at 15°C. With this arrangement, as the solvent/pump-head temperature increases, the mass flow-rate from the solvent delivery system decreases, but the volumetric flow-rate increases by the same factor in the column system, and *vice versa*, and accordingly the volumetric flow-rate or the linear velocity in the column remains constant. It is remarkable that even though the solvent/pump head temperature was increased from 20 to 30°C and the column temperature from 35 to 45°C, the relative changes in the measured retention times for the four standard solutes were −0.098% for PS of mol.wt. $2.8 \cdot 10^3$, −0.106% for PS of mol.wt. $17.5 \cdot 10^3$, −0.079% for PS of mol.wt. $233 \cdot 10^3$ and −0.087% for PS of mol.wt. $2880 \cdot 10^3$.

CONCLUSION

As we have demonstrated, a 10°C change in the solvent/pump head temperature results in a 19–25% error in molecular weight determination while the column temperature is kept constant. A temperature variation of a few degrees is very likely to occur in an ordinary laboratory environment, because normally only the column is thermostated and the solvent/pump head is not. Contrary to expectation, good accuracy in GPC is not always achieved when the column is thermostated. On the contrary, the smaller is the variation of the temperature difference between the solvent/pump head and the solvent/column, the smaller the variations of the retention times become, even though the overall ambient temperature varies. In other words, temperature control of only the column system may cause poorer results than when no special temperature control is applied to the room and the column system.

REFERENCES

1 G. B. Cox, *J. Chromatogr. Sci.*, 15 (1977) 385–392.
2 Cs. Horváth and W. Melander, *J. Chromatogr. Sci.*, 15 (1977) 393–404.
3 C. W. Gehrke, K. C. Kuo, G. E. Davis, R. D. Suits, T. P. Waalkes and E. Borek, *J. Chromatogr.*, 150 (1978) 455–476.

4 H. Colin, J. C. Diez-Masa, G. Guichon, T. Czajkowska and I. Miedziak, *J. Chromatogr.*, 167 (1978) 41–65.
5 W. R. Sisco and R. K. Gilpin, *J. Chromatogr. Sci.*, 18 (1980) 41–45.
6 R. K. Gilpin and W. R. Sisco, *J. Chromatogr.*, 194 (1980) 285–295.
7 W. W. Yau, H. L. Shchan and J. P. Malone, *J. Polym. Sci.*, 6 (1968) 1349–1355.
8 D. D. Bly, H. J. Stoklosa and J. J. Kirkland and W. W. Yau, *Anal. Chem.*, 47 (1975) 1810–1813.
9 S. Mori and T. Suzuki, *Anal. Chem.*, 52 (1980) 1625–1629.
10 T. E. Hogen-Esch and J. Smid, *J. Am. Chem. Soc.*, 88 (1966) 318–324.