

Temperature Effect on Retention and Separation of Poly(ethylene glycol)s in Reversed-Phase Liquid Chromatography

C.H. Lochmüller*, Martin A. Moebus, Qicai Liu, and Chun Jiang

Paul M. Gross Chemical Laboratory, Department of Chemistry, Duke University, Durham, North Carolina 27708

Matti Elomaa

Laboratory of Polymer Chemistry, P.O. Box 55, FIN-00014 University of Helsinki, Finland

Abstract

The isocratic retention of poly(ethylene glycol)s (PEGs) is studied on an octadecyl (C_{18}) bonded-phase column at different temperatures. Linear relationships between the natural logarithm of the capacity factor ($\ln k'$) and the inverse of temperature ($1/T$) are found. Some thermodynamic parameters, such as the apparent change of enthalpy (ΔH°) and the apparent change of entropy (ΔS°), are obtained using van't Hoff plots. Principal component factor analysis (PCFA) and target transformation factor analysis (TTFA) are used to analyze the retention data. The PEG molar mass is found to be a real factor in the solute space, and unknown molar mass can be calculated via the "free-floating" method. The information obtained from the thermodynamic study is discussed in developing strategies for separation of homopolymers and copolymers according to the end-group functionality.

Introduction

Since the early 1980s, there has been significant interest in using reversed-phase high-performance liquid chromatography (HPLC) in the separation of synthetic polymers (1–21). A few successful separations of homopolymers and copolymers have been reported by gradient elution methods, although the retention mechanism has been a subject of much debate (4–11,13–15). One of the concerns in retention mechanism discussions is if isocratic elution can be observed. Fundamental retention parameters, such as the apparent equilibrium constant (K), can only be obtained through isocratic retention measurement, and therefore, isocratic elution is central to the understanding of the fundamental retention mechanism components such as the thermodynamics of polymer retention in reversed-phase HPLC.

In many cases, for small molecules, retention process thermodynamics and the fundamental retention mechanism have been studied through temperature effects on retention (22–32). Usually, van't Hoff plots are used to obtain thermodynamic

parameters such as the apparent change of standard enthalpy. In contrast, polymers, regarded as large homologous compounds, have been separated by imposing a temperature gradient on columns of inert packing materials such as glass beads (33,34). One classical example is the Baker–Williams-type separation that was developed in the 1950s (33). The mechanism of Baker–Williams-type separations describes the dependence of polymer solubility on temperature. Although successfully applied in many cases, this method has a limitation in that the difference in chemical nature for different polymers is neglected.

The thermodynamics of polymer retention in reversed-phase HPLC can shed light on the retention mechanism. Furthermore, temperature could be a controllable factor in polymer separations. Resolution and efficiency of separation might be improved through effective thermal control without the need of a solvent gradient. Therefore, the study of the thermodynamics of polymer retention becomes indispensable.

Earlier investigations demonstrated the feasibility of obtaining isocratic retention data for polymers (14,35). In this study, isocratic retention data of poly(ethylene glycol)s (PEGs) and poly(ethylene oxide)s (PEOs) of seven different molar masses were obtained at different temperatures. Hereafter, both PEG and PEO will be referred to as PEG for the purpose of simplification. These data are used in making van't Hoff plots and obtaining thermodynamic parameters, in other words, changes in enthalpy (ΔH°) and entropy (ΔS°).

Factor analysis is a useful technique to analyze multivariate data without the need of knowing the explicit form of the functions or equations to describe the data, in other words, a soft model approach (36–38). It has been shown that a factor analytical model can be applied to precise retention prediction without knowing the form of any functions (38). In this study, factor analysis is used to correlate the retention data with the molecular properties of polymers, such as average molar mass, so that such properties can be obtained or predicted from retention data. Because the natural logarithm of the capacity factor ($\ln k'$) is directly related to the energies of the chromatographic process, the $\ln k'$ data at different temperatures is factor analyzable. In this study, the retention data set ($\ln k'$)

* Author to whom correspondence should be addressed.

of seven different PEG samples (row designee) under different temperatures is used in principal component factor analysis (PCFA) and target transformation factor analysis (TTFA). The background to the use of factor analysis in chromatography has been described previously (37,38) and will not be reviewed in detail here. The information obtained in this thermodynamic study suggests new strategies for separation of homopolymers and copolymers according to the end-group functionality.

Experimental

Instrumentation and procedures

A Perkin-Elmer Series 4 liquid chromatograph and 420B autosampler (Perkin-Elmer; Norwalk, CT) were used. The detector used was a Perkin-Elmer LC-85B detector with LC autocontrol operated at a wavelength of 195 nm with a 1.4- μ L flow cell. The flow rate was 1 mL/min. A sample loop of 5 μ L was used. Retention data were collected using a Nelson Analytical Chromatography package (Nelson Analytical; Cupertino, CA). Samples were dissolved right before injection in the mobile phase. The concentrations of all samples were 2 mg/mL. Before each injection, the samples were shaken for 10–20 min. The temperature of the column was controlled by immersing the column in a circulating water bath with both heating and cooling units (Forma Scientific; Marietta, OH). The temperature was measured with a Hewlett-Packard digital thermometer (Model 2042A) (Böblingen, Germany). The precision of temperature control was $\pm 0.01^\circ\text{C}$. Alternatively, three aluminum blocks were fabricated in-house to hold the column at three locations: the column inlet, the middle of the column, and the column outlet. These aluminum blocks were individually heated or cooled with three separate temperature controls. Thus, a thermal gradient could be generated along the column.

Materials

The Hypersil C_{18} bonded-phase columns (Hewlett-Packard) that were used had dimensions of $200 \times 2\text{-mm}$ i.d. or $100 \times 4.6\text{-mm}$ i.d., an average pore size of 120 Å, and an average particle size of 5 μm . HPLC-grade acetonitrile (ACN) and water were used as received. The following PEG standards (in Daltons) were purchased from Polysciences, Inc. (Warrington, PA): 400 ($M_w/M_n = 1.10$); 1500 ($M_w/M_n = 1.10$); 9000 ($M_w/M_n = 1.20$); and 15,000 ($M_w/M_n = 1.10$). The following PEO standards (in Daltons) were purchased from Millipore (Amherst, MA): 26,000 ($M_w/M_n = 1.20$); 46,000 ($M_w/M_n = 1.10$); 95,000 ($M_w/M_n = 1.04$); 170,000 ($M_w/M_n = 1.04$); 250,000 ($M_w/M_n = 1.04$); 510,000 ($M_w/M_n = 1.05$); and 913,000 ($M_w/M_n = 1.08$).

Retention data

The capacity factor was calculated by the following equation: $k' = (t_R - t_0)/t_0$ where t_R is the retention time of the polymer peak maximum, and t_0 is the void time. The void times of the PEG samples were measured with mobile phases of the highest volume fraction of acetonitrile at the lowest temperatures. The standard deviation of k' calculated from three measure-

ments was always within 2% error overall. The mobile phases ranged from 40% acetonitrile to 50% acetonitrile. The range of temperature in this study was from 20°C to 45°C . The computer program used in matrix calculations of PCFA and TTFA was MATLAB (Mathworks Inc.). The correlation coefficients in linear regression were all above 0.995.

Results and Discussion

In a chromatographic process, the quantitative contributions to retention from molecular interactions, such as London forces and hydrogen bonding, are still unknown after years of study. However, because the chromatographic process is an equilibrium distribution of molecules between a stationary phase and a mobile phase, the apparent changes of state functions, such as standard enthalpy (ΔH°) and standard free energy (ΔG°), can be calculated from the equilibrium constants (K) via the following relationships (Equations 1, 2, and 3):

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad \text{Eq 1}$$

$$\Delta G^\circ = -RT - \ln K \quad \text{Eq 2}$$

$$\ln k' = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} - \ln\left(\frac{V_S}{V_M}\right) \quad \text{Eq 3}$$

where T is the temperature (in Kelvin), k' is the capacity factor, R is the gas constant, and V_S and V_M are the volumes of stationary phase and mobile phase, respectively.

A fundamental question that remains to be answered in chromatography is whether the phase ratio V_S/V_M is constant over a range of temperatures because it would be impossible to draw a plane to divide the volume of the stationary phase and the mobile phase. The volume of the mobile phase, which is the same as the void volume of the column for a specific solute, has been studied (39–44). However, it is assumed in this study

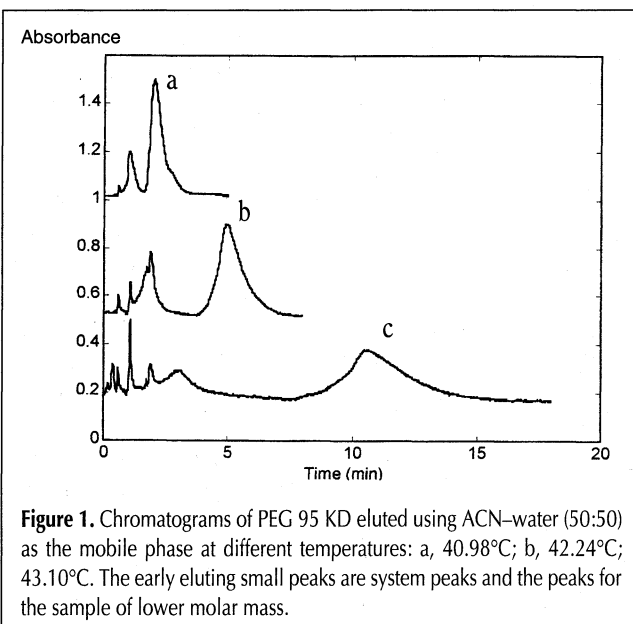


Figure 1. Chromatograms of PEG 95 KD eluted using ACN–water (50:50) as the mobile phase at different temperatures: a, 40.98°C ; b, 42.24°C ; c, 43.10°C . The early eluting small peaks are system peaks and the peaks for the sample of lower molar mass.

that the phase ratio is constant within a temperature range of 25°C. This situation results in a linear plot of $\ln k'$ versus $1/T$ for a constant mobile phase, where $1000/T$ is used for scaling purposes. This linear relationship has been widely observed in the study of small molecules (22–32). From the plot of $\ln k'$ versus $1/T$, the slope is the measure of $-\Delta H^\circ/R$, and the intercept is $\Delta S^\circ/R$ plus the term of $-\ln(V_S/V_M)$. Because $-\ln(V_S/V_M)$ is assumed to be a constant, the intercept used is $\Delta S^*/R$ hereafter. Because the phase ratio is usually between 0.3 and 0.9 (45), ΔS^* is a good approximation of ΔS° .

Figure 1 shows sample chromatograms of PEG 95-KD at different temperatures. It was found that an increase in temperature results in an increase in retention. Such a retention behavior correlates with the inverse solubility-temperature relationship of PEG molecules; in other words, the solubility of PEGs in aqueous solution decreases as temperature increases

(46). In addition, the temperature coefficient of the unperturbed dimension of PEGs in athermal aqueous solvents was reported as $d(\ln \langle r^2 \rangle)/dT \sim 2.3 \times 10^{-4} \text{C}^{-1}$ (47). Therefore, an increase in temperature will result in an increase in the size of PEG molecules. The enlarged random coils of PEGs under higher temperature may experience stronger interactions with the stationary phase due to the increased surface area.

Figures 2A–2E show the van't Hoff plots of $\ln k'$ versus $(1000/T)$ of PEG samples at five different mobile phase compositions. Over the temperature range in our experiments, the van't Hoff plot was linear for all of the PEG samples. The slope and intercept values were converted to ΔH° and ΔS^* and are listed in Table I. As seen in Table I, ΔH° and ΔS^* increase rapidly as the molar mass of PEG increases. Note that the sign of ΔH° is positive, indicating the retention process for PEGs is endothermic. The values of ΔS^* are also much larger than

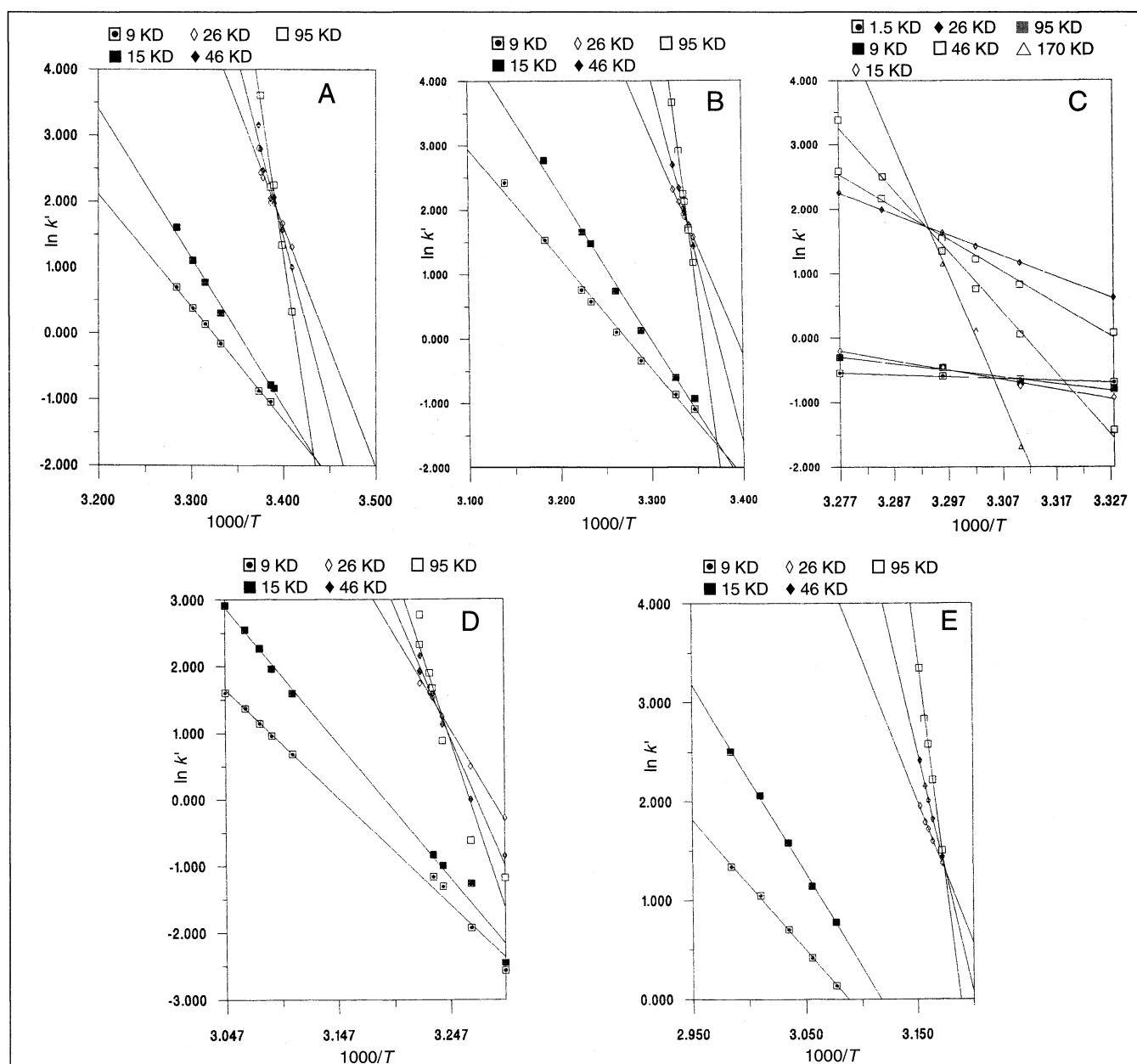


Figure 2. van't Hoff plot of different ratios of ACN-water: A, 40.0:60.0; B, 42.1:57.9; C, 44.2:55.8; D, 47.1:52.9; and E, 50.0:50.0.

those of small molecules, indicating that the retention of polymers onto the stationary phase results in large changes in the conformations and configurations of polymers and surface structures of the bonded phase (45). Both ΔH° and ΔS^* values in Table I reach apparent maxima at 42.1% ACN. The causes for such maximum values of ΔH° and ΔS^* currently are under investigation.

The plots of ΔH° values of PEG samples versus their molar masses show linear relationships (Figure 3). Similarly, the linear relationships in plots of the ΔS^* values versus PEG molar mass are observed (Figure 4). From Figures 3 and 4,

$$\Delta H^\circ = n\Delta H_m^\circ + \Delta H_{\text{end}}^\circ \quad \text{Eq 4a}$$

$$\Delta S^\circ = n\Delta S_m^\circ + \Delta S_{\text{end}}^\circ \quad \text{Eq 4b}$$

where ΔH_m° and ΔS_m° are the standard enthalpy change and standard entropy change, respectively, for a repeating unit, $-\text{CH}_2\text{CH}_2\text{O}-$. Constant $\Delta H_{\text{end}}^\circ$ and constant $\Delta S_{\text{end}}^\circ$ can be regarded as ΔH° and ΔS° contributed by end groups. From Equations 4a and 4b, the total free energy of the retention process can be expressed by Equation 4c:

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ = n\Delta G_m^\circ + \Delta G_{\text{end}}^\circ \quad \text{Eq 4c}$$

where ΔG_m° and $\Delta G_{\text{end}}^\circ$ are the changes of the standard free energy for a repeating unit and end groups, respectively. Thus from Equation 4c, there is a linear relationship between ΔG° and the molar mass, which correlates well with the conformational studies on polymers in the adsorbed state. The conformation of a globular polymer at the adsorbed state is either a cylindrical disc (train type) or globular one (7,48). Each of the conformations has a contact area with the stationary phase proportional to the molar mass of the polymer. Therefore, the adsorption energy should be proportional to the molar mass.

These linear relationships can be used as the calibration curve for the determination of PEG molar masses. The ΔH° value for the sample of an unknown molar mass can be calculated from its retention time at two different temperatures (Equation 3). The molar mass of the unknown sample can be

Table I. ΔH° and ΔS^* Values for Five PEG Samples					
PEG (KD)	Volume percent acetonitrile				
	50	47.1	44.2	42.1	40
ΔH° (kJ/mol K)					
9	109.5	137.2	148.1	147.5	148.2
15	158.0	184.9	190.5	196.9	187.9
26	238.1	279.9	272.4	306.5	270.5
46	405.7	430.5	429.8	480.2	430.4
95	763.3	794.9	820.8	851.4	802.9
ΔS^* (J/mol K)					
9	338.0	431.8	976.9	482.2	492.6
15	492.7	587.7	618.3	648.2	625.5
26	771.9	917.4	910.1	1039.1	933.6
46	1299.1	1404.5	1428.2	1619.3	1476.2
95	2433.8	2583.2	2717.1	2859.6	2740.3

found from an existing plot of ΔH° versus molar mass constructed from standards. This strategy was tested with PEG standards. It was found that the calculated molar masses closely track the actual reported values. These results further support Martin's assumption (49) that the total free energy for transfer of a molecule from the stationary phase to the mobile phase, ΔG° , can be fragmented according to the compound structure. That is, ΔG° is a sum of the free energies of "transferring" each fragment of the compound from the stationary phase to the mobile phase.

$$\Delta G^\circ = a\Delta G_A^\circ + b\Delta G_B^\circ + c\Delta G_C^\circ + d\Delta G_D^\circ + \dots \quad \text{Eq 5}$$

where a, b, c, d, \dots are the coefficients and $\Delta G_A^\circ, \Delta G_B^\circ, \Delta G_C^\circ, \Delta G_D^\circ, \dots$ are the free energies of the fragments. From Equations 2 and 5, $\ln k'$ can be expressed as

$$\ln k' = \sum_i \ln k_i' + \text{constant} \quad \text{Eq 6}$$

where $\ln k_i'$ is the logarithm of the capacity factor for fragment i of the molecule. Therefore, for a polymer, Martin's assumption is expressed as

$$\ln k' = f(n) \ln k_i + \text{constant} \quad \text{Eq 7}$$

where $\ln k_i$ is the logarithm of the capacity factor for the repeating unit i of the polymer, $f(n)$ is a function related to the number of repeating units (n), in other words, the molar mass of the polymer.

The retention data set ($\ln k'$) of seven different PEG samples (row designee) under different temperatures is used in PCFA. As indicated in Table II by the columns labeled *Eigenvalues*,

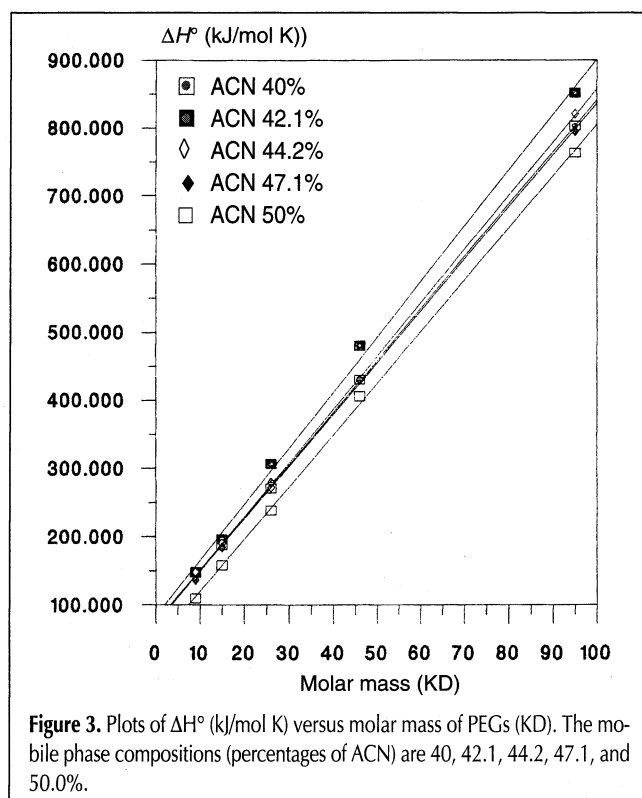


Figure 3. Plots of ΔH° (kJ/mol K) versus molar mass of PEGs (KD). The mobile phase compositions (percentages of ACN) are 40, 42.1, 44.2, 47.1, and 50.0%.

Variance explained, and *Probability test*, there are two principal factors. The data set is then reduced by using two factors. After the solute submatrix was regenerated, TTFA was performed using the reported molar masses of the PEG standards as a test target vector for the row vector matrix because the molar mass is one of the primary molecular properties. There is a good correlation between the test vector (column 1, Table III) and the predicted vector (column 2, Table III), and the vector of molar mass was found to be the real vector. The molar mass of one PEG sample was deliberately left out as a "free-floating" point, and, as shown in Table III, the molar mass of this unknown can be predicted. Because the number of data points is small, target testing provides a good estimation and a reasonable check of the PEG molar masses.

The thermodynamic study of PEG retention can be applied to optimize the separation of polymers. Figure 5 shows the chromatogram of the separation of a mixture of seven PEG polymers using a solvent gradient. To improve the separation without a solvent gradient, two types of temperature gradients were employed: gradient *T* raises or lowers the temperature across the whole column linearly with respect to time and gradient *L* generates a thermal gradient along the column. To examine the effects from both temperature gradients, three PEG polymer mixtures of molar mass 26 KD, 46 KD, and 95 KD were used. Figures 6A and 6B show the chromatograms of the mixture of three PEG samples. Both elution modes were isocratic, and one had gradient *T* and the other had gradient *L*. Under isothermal isocratic conditions, PEG samples were hardly separated, whereas imposing a thermal gradient *T* or *L* can facilitate separation. It should be noted that these results are preliminary. Studies on the precise control of the thermal

gradients of both *T* and *L* to obtain high resolutions are in progress.

The results from this study suggest that the temperature effect may be used to separate PEG polymers and other polymers that have similar temperature-retention relationships according to the end-group functionality. It is assumed that there are two PEG polymers, A and B, A with an average degree of polymerization, *m*, and B with an average degree of polymerization, *n*. Then Equations 4a and 4b can be used for A and B:

$$\Delta H_A^\circ = m\Delta H_0^\circ + \Delta H_A^\circ \quad \text{Eq 8a}$$

$$\Delta S_A^\circ = m\Delta S_0^\circ + \Delta S_A^\circ \quad \text{Eq 8b}$$

$$\Delta H_B^\circ = n\Delta H_0^\circ + \Delta H_B^\circ \quad \text{Eq 8c}$$

$$\Delta S_B^\circ = n\Delta S_0^\circ + \Delta S_B^\circ \quad \text{Eq 8d}$$

where ΔH_0° and ΔS_0° are the changes of standard enthalpy and entropy for the repeating unit of $-\text{CH}_2\text{CH}_2\text{O}-$. From the linear regression of ΔH and ΔS versus the molar mass (Figures 3 and 4), ΔH_0 and ΔS_0 can be calculated. Therefore,

$$\begin{aligned} \ln(k'_A / k'_B) &= \ln \alpha \\ &= -\frac{(n-m)\Delta H_0^\circ}{RT} + \frac{(n-m)\Delta S_0^\circ}{R} \\ &\quad - \frac{(\Delta H_A^\circ - \Delta H_B^\circ)}{RT} + \frac{(\Delta S_A^\circ - \Delta S_B^\circ)}{R} \end{aligned} \quad \text{Eq 9}$$

Table II. Principal Component Factor Analysis of Retention Data of Seven PEG Samples

No. of Factors	Eigenvalue	Variance explained	Probability test*
1	1.89e+03	93.00	0.001
2	1.42e+02	7.00	0.000
3	7.60e-27	0.00	0.076
4	1.66e-27	0.00	0.141
5	3.69e-28	0.00	0.293
6	1.30e-28	0.00	0.387
7	2.75e-29	0.00	1.000

* From reference 49.

Table III. Target Transformation Factor Analysis of PEG Solute Retention Data Space With Molar Mass as Target Factors

Test vector	Predicted vector	Free floating calculation
1.5	3.47	
9	12.86	11.47
15	18.06	16.08
26	34.03	31.13
46	51.46	45.76
95	94.68	
170	166.17	

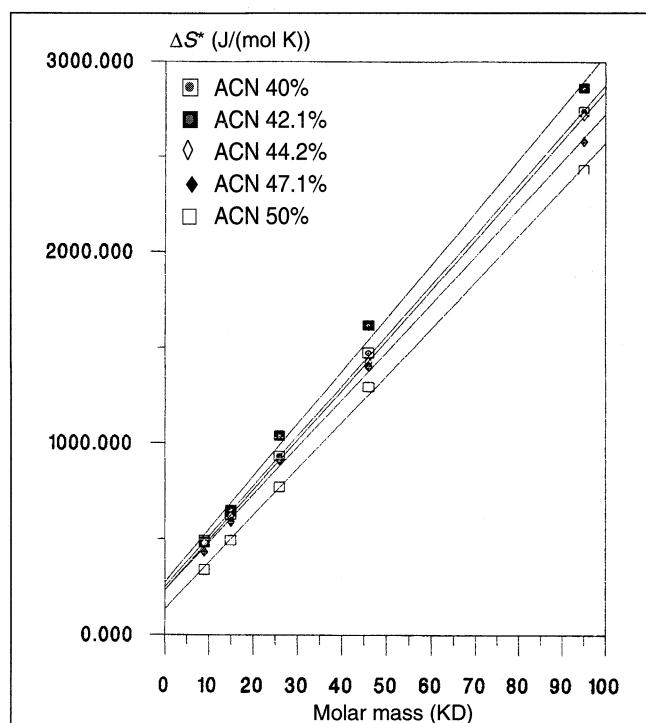


Figure 4. Plots of ΔS^* (J/mol K) versus molar mass of PEGs (KD). The mobile phase compositions (percentage of ACN) are: 40, 42.1, 44.2, 47.1, and 50.0%.

By selecting a temperature (T) so that

$$-\frac{(n-m)\Delta H^{\circ}_0}{RT} + \frac{(n-m)\Delta S^{\circ}_0}{R} = 0$$

$\ln(k'_A/k'_B)$ can become independent of degree of polymerization. Such a temperature, which is $\Delta H^{\circ}_0/\Delta S^{\circ}_0$, can be termed the enthalpy-entropy compensation temperature (EECT). At EECT, Equation 9 can be simplified as

$$\ln(k'_A/k'_B) = \ln \alpha = -\frac{(\Delta H^{\circ}_A - \Delta H^{\circ}_B)}{RT} + \frac{(\Delta S^{\circ}_A - \Delta S^{\circ}_B)}{R} \quad \text{Eq 10}$$

In Equation 10, the selectivity factor is independent of the molar mass and depends only on the functionality of the end groups. The EECT was observed in this study. Almost all of the lines intersect on a same point in Figures 2A–2E. At this particular temperature, all PEG samples have the same retention regardless of their molar mass. Table IV shows the values of EECT obtained from the linear regression results of Figures 3 and 4. These equations can also provide an explanation for the fact that there are two groups of lines in Figures 2A–2E. The top group are PEGs of higher molar mass from Millipore, and their exact name should be poly(ethylene oxide) because their terminal groups may be the same but not their hydroxyl groups. The bottom group are PEGs of lower molar mass from Polysciences, and they terminate in hydroxyl groups. One possible reason that might lead to such a difference in bottom and top groups of PEGs could be the dependence of PEG conformation on molar mass. PEG in the solid crystalline state has a helical structure. After dissolution in aqueous solution, partial helical structure persists in the crystalline PEG. However, for high molar mass PEG ($M_n > 4,000$ Daltons) in very dilute solution, the structure can be treated as a typical random coil (50). Melander and co-workers (51) reported that for PEG oligomers and their derivatives there exist two conformations. For oligo-PEGs of low molar mass, the conformation is usually a zig-zag shape or a helical structure. As the number of degree of polymerization reaches 9, which corresponds to a molar mass of about 500 Daltons, the oligo-PEG has a meandering

structure. They also observed a conformation change of oligo-PEGs from zig-zag to meandering structure within the temperature range of 20°C to 80°C. The equilibrium of such an isomerization process was used to explain the nonlinearity in

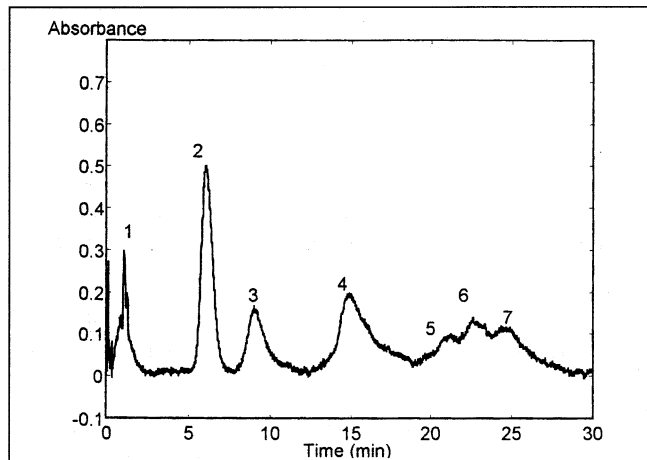
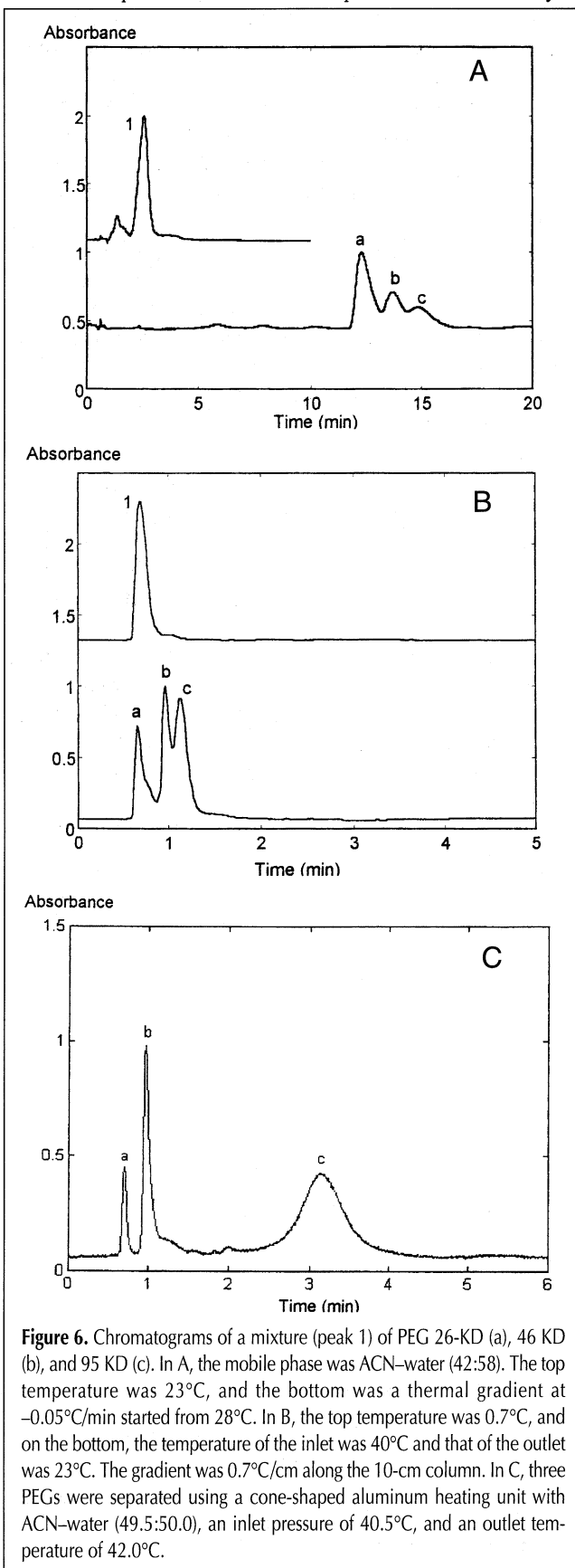


Figure 5. Gradient elution of a mixture of seven PEG standards with ACN–water from 41:59 to 43:57 in 45 min. Peaks 1–7 are PEG (in KD): 26, 46, 95, 170, 250, 510, and 913, among which the sample sizes of 2 and 4 were spiked.



van't Hoff plots over the wide temperature range. In this study, the molar mass of PEGs are well above 1-KD and there is excellent linearity in the van't Hoff plots, so it can be concluded that the conformations of PEGs in this study are random coils or meandering structures. Therefore, the substantial difference in retention between PEGs of low and high masses near the EECT can be attributed to their end-group differences. The advantage of using the temperature effect in separation by end-group functionality is that one does not need to know the molar mass of the polymer sample or need to prefractionate a polymer. This study suggests that choosing a proper solvent as the mobile phase (mobile phases of different kind yield different ΔH° and ΔS° values (Table IV and reference 51) and suitable temperature at the EECT, PEG polymer mixtures can be separated according to their terminal groups regardless of their molecular masses. Experiments are currently underway to examine this hypothesis.

In contrast to this method, there are reports that attempted to use critical composition to separate polymers by the end groups (16). This technique is based on the fact that for polymers of very high molar mass, the range of mobile phase composition to obtain isocratic retention is so narrow that polymers behave the same except for those that differ in end groups. However, good separation was only achieved when the molar mass was below 2-KD (16).

Conclusion

Linear van't Hoff plots were observed in this study, and the apparent change of enthalpy and the apparent change of entropy were obtained. It was found that both ΔH° and ΔS° values of the PEG polymers were much larger than those of small molecules. Principal component factor analysis revealed that two factors can be used to span the retention data space. With the molar mass of PEG polymers as the test vector, TTFA confirmed that molar mass was the real vector in the solute space. The molar mass of unknown PEG samples can be obtained either through the linear calibration plot of ΔH versus the molar mass or the free-floating method of factor analysis. The temperature effect on retention showed that the separation of PEG polymers can be realized by using thermal gradients, and PEG polymers can be separated according to the end groups.

Table IV. Enthalpy-Entropy Compensation Temperature (EECT) for PEG with Different Mobile Phases

	Percent ACN				
	40	42.1	44.2	47.1	50
ΔH°_0 (kJ/mol K)	7.660	8.182	7.853	7.624	7.614
ΔS°_0 (J/mol K)	26.296	27.608	26.125	24.912	24.375
EECT (K)	291.42	296.36	300.59	306.53	312.37

References

1. *Modern Methods of Polymer Characterization*. H.G. Barth and J.W. Mays, Eds. Wiley and Sons, New York, NY, 1991.
2. *Polymer Analysis and Characterization*. H.G. Barth and J. Janca, Eds. Journal of Polymer Science: Applied Polymer Symposium 51, Wiley and Sons, New York, NY, 1991.
3. G. Glöckner. *Polymer Characterization by Liquid Chromatography*. Elsevier, Amsterdam, The Netherlands, 1987.
4. D.W. Armstrong and K.H. Bui. Non-aqueous reversed-phase liquid chromatographic fractionation of polymers. *Anal. Chem.* **54**: 706-708 (1982).
5. L.R. Snyder, M.A. Stadalius, and M.A. Quarry. Gradient elution in reversed-phase HPLC separation of macromolecules. *Anal. Chem.* **55**: 1412-30A (1983).
6. D.W. Armstrong and R.E. Bohem. Gradient liquid chromatographic separation of macromolecules: theory and mechanism. *J. Chromatogr. Sci.* **22**: 378-85 (1984).
7. R.E. Bohem, D.E. Matire, D.W. Armstrong, and K.H. Bui. Theory of homopolymer retention in semidilute solution using liquid chromatography. *Macromolecules* **17**: 400-407 (1984).
8. G. Glöckner and J.H.M. VanDen Berg. Gradient elution chromatography of polymers on reversed-phase columns with tetrahydrofuran as an eluent component. *Chromatographia* **19**: 55-61 (1984).
9. G. Glöckner and J.H.M. VanDen Berg. Two-dimensional analysis of copolymers by size-exclusion chromatography and gradient elution reversed-phase precipitation chromatography. *J. Chromatogr.* **317**: 615-24 (1984).
10. M.A. Stadalius, M.A. Quarry, T.H. Mourey, and L.R. Snyder. General model for the separation of large molecules by gradient elution: sorption versus precipitation. *J. Chromatogr.* **358**: 1-16 (1986).
11. M.A. Stadalius, M.A. Quarry, T.H. Mourey, and L.R. Snyder. Conventional chromatographic theory versus critical solution behavior in the separation of large molecules. *J. Chromatogr.* **358**: 17-38 (1986).
12. P. Jandera and J. Rozkosna. Isocratic and gradient liquid chromatographic elution of styrene oligomers on silica gel. *J. Chromatogr.* **362**: 325-43 (1985).
13. G. Glöckner and J.H.M. VanDen Berg. Precipitation and adsorption phenomena in polymer chromatography. *J. Chromatogr.* **352**: 511-22 (1986).
14. C.H. Lochmüller and M.B. McGranaghan. Isocratic elution of high molecular weight monodisperse polystyrenes. *Anal. Chem.* **61**: 2449-55 (1989).
15. A. Alhedai, R.E. Rohem, and D.E. Matire. Reversed-phase liquid chromatographic retention behavior of polystyrene homopolymers. *Chromatographia* **29**: 313-21 (1990).
16. A.V. Gorshkov, H. Much, H. Becker, H. Pasch, V.V. Evreinov, and S.G. Entelis. Chromatographic investigation of macromolecules in the "critical range" of liquid chromatography: I. Functionality type and composition distribution in polyethylene oxide and polypropylene oxide copolymers. *J. Chromatogr.* **523**: 91-102 (1990).
17. H. Sato, K. Ogino, S. Maruo, and M. Sasaki. Separation of styrene-methacrylate copolymer by composition using normal and reversed phase high performance liquid chromatography. *J. Polymer Sci.* **29**: 1073-80 (1991).
18. D.M. Northop, D.E. Matire, and R.P.W. Scott. Liquid chromatographic retention behavior of polystyrene homopolymers on a C_4 bimodal pore diameter reversed-phase column. *Anal. Chem.* **64**: 16-21 (1992).
19. R.A. Shalliker, P.E. Kavanagh, I.M. Russell, and D.G. Hawthorne. The measurement of molecular weight distribution of some polydisperse polystyrene by reversed-phase and size-exclusion chromatography. *Chromatographia* **33**: 427-33 (1992).
20. G. Glöckner and D. Wolf. Reversed-phase liquid chromatography.

- graphic retention behavior of polystyrene homopolymers. *Chromatographia* **34**: 363–68 (1992).
21. J.J. Kirkland. Superfacially porous silica microspheres for the fast high performance liquid chromatography of macromolecules. *Anal. Chem.* **64**: 1239–45 (1992).
 22. G. Guiochon and F. Dondi. *Theoretical Advancement in Chromatography and Other Related Separation Techniques*. NATO ASI series, 1992.
 23. W.R. Melander and C. Horvath. Enthalpy–entropy compensation in reversed-phase chromatography. *J. Chromatogr.* **158**: 215–25 (1978).
 24. W.R. Melander, B.K. Chen, and C. Horvath. Mobile phase effects in reversed-phase chromatography: I. Concomitant dependence of retention on column temperature and eluent composition. *J. Chromatogr.* **185**: 99–109 (1979).
 25. W.R. Melander, C.A. Mannan, and C. Horvath. Mobile phase effects in reversed-phase chromatography: IV. Retention by *n*-alkylbenzenes as a function of column temperature and the nature and the concentration of the organic coeluent. *Chromatographia* **15**: 611–15 (1982).
 26. W.R. Melander, B. K. Chen, and C. Horvath. Mobile phase effects in reversed-phase chromatography: VII. Dependence of retention on mobile phase composition and column temperature. *J. Chromatogr.* **318**: 1–10 (1985).
 27. M. Kuchar and H. Tomkova. Gas–liquid chromatography and lipophilicity of esters of benzoic acids: Enthalpy–entropy compensation. *J. Chromatogr.* **398**: 43–51 (1987).
 28. M. Kuchar and E. Kraus. Enthalpy–entropy compensation in reversed-phase liquid chromatography of series of aryloxoalkanoic and arylhydroxyalkanoic acids. *J. Chromatogr.* **449**: 391–401 (1988).
 29. J. Li, A.J. Dallas, and P. Carr. Empirical scheme for the classification of gas chromatography stationary phase based on solvatochromic linear energy relationships. *J. Chromatogr.* **517**: 103–21 (1990).
 30. J. Li, Y. Zhang, A.J. Dallas, A.J. Carr, and P. Carr. Measurement of solute dipolarity and polarizability and hydrogen bond acidity by inverse gas chromatography. *J. Chromatogr.* **550**: 101–34 (1991).
 31. K.S. Reddy and E. Kovats. Pair-wise interaction by gas chromatography: I Interaction free enthalpies of solutes with non-associated primary alcohol groups. *J. Chromatogr.* **609**: 229–59 (1992).
 32. J. Li and P. Carr. Gas chromatographic study of solvation enthalpies by solvatochromically based linear free solvation energy relationships. *J. Chromatogr.* **659**: 367–80 (1994).
 33. C.A. Baker and R.J.P. Williams. A new chromatography procedure and its application to high polymers. *J. Chem. Soc.* 2352–62 (1956).
 34. *Fractionation of Synthetic Polymers—Principles and Practices*. L.H. Tung, Ed. Marcel Dekker, New York, NY, 1977.
 35. C. Jiang, Ph.D. Dissertation, Duke University, 1994.
 36. E.R. Malinowski and D.G. Howery. *Factor Analysis in Chemistry*. Wiley, New York, NY, 1980.
 37. C.H. Lochmüller, S.J. Breiner, C.E. Reese, and M.N. Koel. Characterization and prediction of retention behavior in reversed-phase chromatography using factor analytical modeling. *Anal. Chem.* **61**: 367–78 (1989).
 38. C.H. Lochmüller, C.E. Reese, and S. Hsu. Cross-column retention prediction in reversed-phase liquid chromatography using factor analytical modeling. *Anal. Chem.* **66**: 3806–13 (1994).
 39. B.L. Karger, J.R. Grant, A. Hartkopf, and P.H. Weiner. Hydrophobic effects in reversed-phase liquid chromatography. *J. Chromatogr.* **128**: 65–78 (1976).
 40. H. Colin, J.C. Diez-Masa, G. Guiochon, and T. Gzajkivska, and I. Miedziak. The role of HPLC using pyrocarbon containing adsorbents. *J. Chromatogr.* **167**: 41–65 (1978).
 41. J.R. Gant, J.W. Dolan, and L.R. Snyder. Systematic approach to optimize resolution in reversed-phase chromatography with emphasis on the role of temperature. *J. Chromatogr.* **185**: 153–77 (1979).
 42. C.S. Horvath and H. Lin. Band spreading in liquid chromatography: general plate height equation and a method for evaluation the individual plate height contributions. *J. Chromatogr.* **149**: 43–70 (1978).
 43. V. Kazakevich and H.M. McNair. Thermodynamic definition of HPLC dead volume. *J. Chromatogr. Sci.* **31**: 317–22 (1993).
 44. F. Rideo and E. Kovats. Adsorption from liquid mixtures and liquid chromatography. *J. Chromatogr.* **239**: 1–28 (1982).
 45. F.M. Yamamoto, S. Rokushika, and H. Hatano. Comparison of thermodynamic retention behaviors on various C18 columns different in their hydrophobicity. *J. Chromatogr. Sci.* **27**: 704–709 (1989).
 46. F.E. Bailey and J.V. Koleske. *Poly(ethylene oxide)*. Academic Press, New York, NY, 1976.
 47. P.J. Flory. *Statistical Mechanics of Chain Molecules*. Wiley, New York, NY, 1969.
 48. A. Takahashi and M. Kawaguchi. The structure of macromolecules adsorbed on interfaces. In *Behavior of Macromolecules. Advances in Polymer Science*. Speinger-Verlag, New York, NY, 1982.
 49. A.J.P. Martin. Some theoretical aspects of partition chromatography. *Biochem. Soc. Symp.* **3**: 4–20 (1949).
 50. N. Abbott, D. Blankschtein, and T.A. Hatton. Protein partitioning in two-phase aqueous polymer systems I. Novel physical pictures and a scaling thermodynamic formulation. *Macromolecules* **24**: 4334 (1991).
 51. W.R. Melander, A. Nahum, and C. Horvath. Mobile phase effects in reversed-phase chromatography: III. Changes in conformation and retention of oligo(ethylene glycol) derivatives with temperature and eluent composition. *J. Chromatogr.* **185**: 129 (1979).

Manuscript accepted June 9, 1995.