



Retention mechanism of poly(ethylene oxide) in reversed-phase and normal-phase liquid chromatography

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Abstract

The retention behavior of low- and high-molecular-mass poly(ethylene oxide) (PEO) in reversed-phase (RP) and normal-phase (NP) liquid chromatography was investigated. In RPLC using a C_{18} bonded silica stationary phase and an acetonitrile–water mixture mobile phase, the sorption process of PEO to the stationary phase showed $\Delta H^\circ > 0$ and $\Delta S^\circ > 0$. Therefore, PEO retention in RPLC separation is an energetically unfavorable, entropy-driven process, which results in an increase of PEO retention as the temperature increases. In addition, at the enthalpy–entropy compensation point the elution volume of PEO was very different from the column void volume. These observations are quite different from the RPLC retention behavior of many organic polymers. The peculiar retention behavior of PEO in RPLC separation can be understood in terms of the hydrophobic interaction of this class of typical amphiphilic compounds with the non-polar stationary phase, on the one hand, and with the aqueous mobile phase, on the other. The entropy gain due to the release of the solvated water molecules from the PEO chain and the stationary phase is believed to be responsible for the entropy-driven separation process. On the other hand, in NPLC using an amino-bonded silica stationary phase and an acetonitrile–water mixture mobile phase, PEO showed normal enthalpy-driven retention behavior: $\Delta H^\circ < 0$ and $\Delta S^\circ < 0$, with the retention decreasing with increasing temperature and PEO eluting near the column void volume at the enthalpy–entropy compensation point. Therefore, high-resolution temperature gradient NPLC separation of high-molecular-mass PEO samples can be achieved with relative ease. The molecular mass distribution of high-molecular-mass PEO was found to be much narrower than that measured by size-exclusion chromatography.

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1. Introduction

Poly(ethylene oxide) (PEO) and derivatives are widely used in various scientific and industrial fields. For the characterization of PEO and derivatives,

HPLC techniques, including normal-phase (NP) and reversed-phase (RP) liquid chromatography, have been widely applied [1,2]. Good RPLC resolution of low-molecular-mass PEO has been reported by a number of authors [3–10]. There are also a few reports of good oligomer resolution of low-molecular-mass PEO on bare silica gel and an amino-bonded silica column with mobile phases generally used in RP systems [7,11,12]. There have also been

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several theoretical efforts to explain the RPLC retention of low-molecular-mass PEO [8,10,13]. Nevertheless, the HPLC retention mechanism of PEO is not fully understood. Their RPLC retention shows a rather unusual temperature dependence as well as a molecular mass dependence. Also, there are few successful reports of the high-resolution separation of high-molecular-mass PEO samples [14].

The chromatographic process is a consecutive distribution process of solutes between the mobile and stationary phases when they move through a separation column. The equilibrium distribution constant (K) of solute molecules between a stationary phase and a mobile phase is related to changes of the standard free energy (ΔG°), enthalpy (ΔH°) and entropy (ΔS°) associated with the transfer of one mole of analyte from the mobile to the stationary phase:

$$\Delta G^\circ = \Delta H^\circ - T \Delta S^\circ = -RT \ln K \quad (1)$$

$$k' \equiv \frac{(V_R - V_0)}{V_0} = K\phi \quad (2)$$

$$\ln k' = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} + \ln \phi \quad (3)$$

where k' is the retention factor, ϕ is the volume ratio of stationary phase to mobile phase, and V_R and V_0 are the retention volume of the solute and the total void volume of the column, respectively. The thermodynamic variables can be obtained from the temperature-dependent retention data via the van't Hoff plot (Eq. (3)).

Under typical interaction chromatographic separation conditions, the interaction strength of polymeric solutes with the stationary phase increases with molecular mass. Martin [15] predicted that the interaction strength of a polymeric solute with a stationary phase was proportional to the degree of polymerization and a linear relationship has been confirmed in many polymer systems [16,17]. Then the retention of polymers would increase exponentially with the degree of polymerization. Therefore, in the interaction chromatographic separation of polymeric materials, either solvent gradient or temperature gradient elution is employed to control the retention of polymeric solutes. Gradient elution is performed by reducing the interaction strength of

polymeric solutes, and the polymers elute in increasing order of molecular mass. In solvent gradient elution, the solvent power is increased during elution, while the temperature is increased in temperature gradient elution [17]. This general scheme is based on the concept that the sorption process of a polymeric solute to the stationary phase is energetically favorable, i.e. an exothermic process ($\Delta H^\circ < 0$), and that ΔS° does not play a key role in the interaction chromatographic (IC) separation process. Also, it is common to observe a negative ΔS° since the sorption of a large polymer chain to a restricted stationary phase (or pores) should be entropically unfavorable as in the size-exclusion chromatographic (SEC) separation process.

PEO shows unique RPLC retention behavior quite distinct from many other organic polymers. Lochmüller et al. reported the temperature effect on the retention of PEO in RPLC, C_{18} bonded silica and mixtures of acetonitrile and water as stationary and mobile phase, respectively [14]. The retention of PEO increased as the temperature increased. Kamiyusuki et al. reported similar results for low-molecular-mass PEO with a branched polyfluoroalkylsilane-coated silica stationary phase [18]. The thermodynamic parameters associated with the separation process showed that the partition process of PEO from the mobile to stationary phase is endothermic ($\Delta H^\circ > 0$), while the entropy change is positive ($\Delta S^\circ > 0$) [14]. This means that the sorption process of PEO in RPLC is an energetically unfavorable, entropy-driven process, which is rather unique since most IC separations of organic polymers involve an enthalpy-driven, exothermic sorption process.

Therefore, in order to achieve a good resolution in the temperature gradient RPLC separation of PEO (to reduce retention during elution), it was necessary to reduce the temperature during the elution [14,19] while the opposite temperature program was used for other polymer systems such as polystyrene, poly(methyl methacrylate) and polyisoprene [20–24]. Lochmüller et al. explained that the peculiar retention behavior is correlated with the inverse solubility–temperature relationship, viz. the solubility of PEOs in aqueous solution decreases as temperature increases. In addition, they conjectured that the increase in temperature would result in an increase in

the size of the PEO molecules and the enlarged random coils of PEOs would experience a stronger interaction with the stationary phase due to the increased surface area. However, it is not straightforward to explain the retention behavior by considering the endothermic nature of the sorption process since the increasing contact of the enlarged PEO chain with the stationary phase would result in a stronger endothermic enthalpic interaction. In this study, we investigated the temperature dependence of the RPLC and NPLC retention of PEO in detail to shed light on their separation mechanisms.

2. Experimental

The low-molecular-mass poly(ethylene oxide) (PEO 2300) was purchased from Aldrich, and the high-molecular-mass PEO samples (weight-average molecular mass, M_w : 21, 45, 85, and 270 kg/mol) were acquired from Tosoh. The PEO samples were prepared by anionic polymerization of ethylene oxide and have one end hydroxyl group. The HPLC apparatus consists of a solvent delivery pump (LDC, CM 3200), a six-port sample injector (Rheodyne, 7125), and a refractive index detector (Shodex, RI-71). For the RPLC analysis, a C_{18} bonded silica column (Luna C_{18} , Phenomenex, 100 Å pore, 250 × 4.6 mm) was used. For the NPLC system, an amino-bonded silica column (Nucleosil-NH₂, Macherey-Nagel, 100 Å pore, 250 × 2.1 mm) was used. The eluents for both RPLC and NPLC were mixtures of acetonitrile (Duksan, HPLC grade) and water (deionized and filtered through a 0.45 μm pore membrane filter) of different compositions. The injection samples were prepared by dissolving the polymers in a small volume of the eluent. The temperature of the column was controlled by circulating fluid from a programmable bath/circulator (Neslab, RTE-111) through a laboratory-made column jacket.

3. Results and discussion

3.1. RPLC retention behavior

Fig. 1 shows RPLC chromatograms of a PEO 2300 sample obtained at different column tempera-

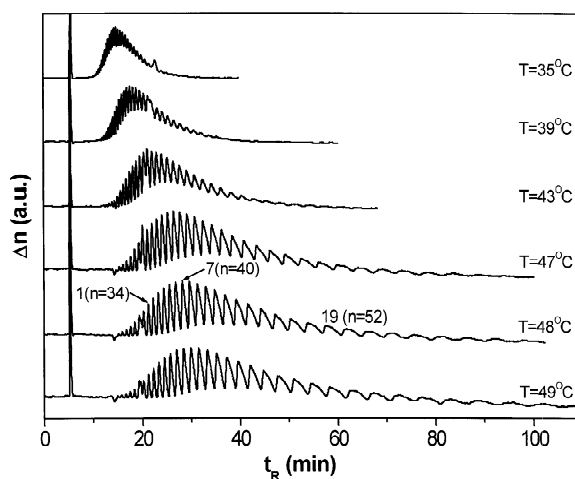


Fig. 1. RPLC chromatograms of PEO 2300 recorded at different temperatures. Each oligomer peak of PEO 2300 at 48 °C was identified by MALDI-TOF-MS. The numbers in parentheses are the degree of polymerization, n . The retention of PEO increases with increasing temperature, indicative of positive ΔH° . Column, Luna C_{18} , 100 Å pore, 250 × 4.6 mm; eluent, CH₃CN–water (35:65, v/v) at a flow-rate of 0.45 mL/min.

tures. A mixture of acetonitrile–water (35:65, v/v) was used as mobile phase. The degree of polymerization for the oligomer peaks was determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and labeled in the chromatogram recorded at 48 °C. The sharp peak appearing near $t_R = 5$ min is the injection solvent peak. The chromatograms clearly show an increase in retention as the temperature increases.

Fig. 2 shows the van't Hoff plots of PEO 2300 for the odd-numbered oligomer peaks (from 1 to 19). The void volume of the column was measured from the injection solvent peak and it showed a slight variation with temperature, likely due to thermal expansion, but the effect on the calculation of retention factors was negligible. All the oligomers show a good linear relationship. From the slope and intercept of the van't Hoff plots in Fig. 2, ΔH° and ΔS^* ($\Delta S^* = \Delta S^\circ/R + \ln \phi$) were obtained according to Eq. (3) and are plotted in Fig. 3. The volume ratio of the stationary phase to the mobile phase, ϕ , is usually between 0.06 and 0.47 in common reversed-phase columns and ΔS^* is used in place of ΔS° assuming that ϕ does not change significantly with temperature [25]. They show an excellent linear

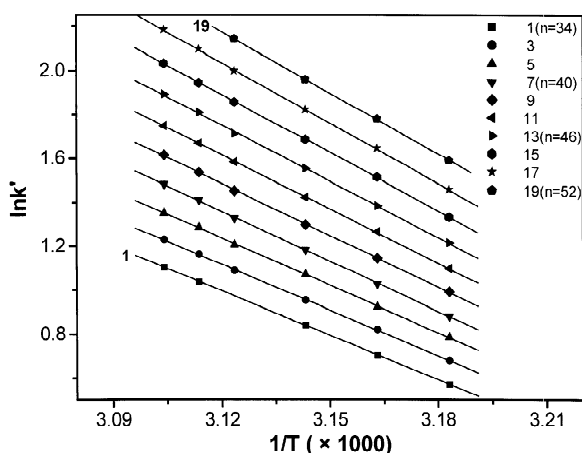


Fig. 2. van't Hoff plots of PEO 2300 prepared from the RPLC chromatograms shown in Fig. 1. Peak numbers and corresponding degree of polymerization are also shown in the plot. Only the odd-numbered peaks are shown. They show a good linear relationship.

relationship with the degree of polymerization following Martin's rule. It is clear that both ΔH° and ΔS^* are positive. From these plots, we obtained values of ΔH° and ΔS^* per oxyethylene unit of 1.23 kJ/mol and $4.39 \text{ J K}^{-1} \text{ mol}^{-1}$, respectively.

Fig. 4 shows the van't Hoff plots for three high-molecular-mass PEO samples (21, 45 and 85 kg/mol) in RPLC. The chromatographic separation conditions are the same as for PEO 2300 except for the mobile phase composition (acetonitrile–water, 45:55, v/v). It was necessary to use a different composition with a greater acetonitrile content to elute the high-molecular-mass samples in a moderate temperature range. In this RPLC system, acetonitrile reduces the retention of PEO by acting as a desorption-promoting solvent. For the high-molecular-mass PEO samples, we found the same trend as for PEO 2300, i.e. both ΔH° and ΔS^* were positive. From the slope and intercept of the van't Hoff plots, the standard enthalpy and entropy changes for the oxyethylene unit were determined to be 0.52 kJ/mol and $1.68 \text{ J K}^{-1} \text{ mol}^{-1}$, respectively. These values can be compared with 1.23 kJ/mol and $4.39 \text{ J K}^{-1} \text{ mol}^{-1}$, respectively, for the separation of PEO 2300 (acetonitrile–water, 35:65, v/v). It is apparent that the high content of acetonitrile reduces both ΔH° and ΔS^* .

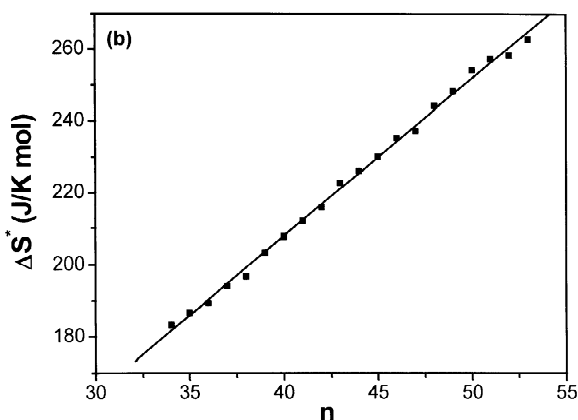
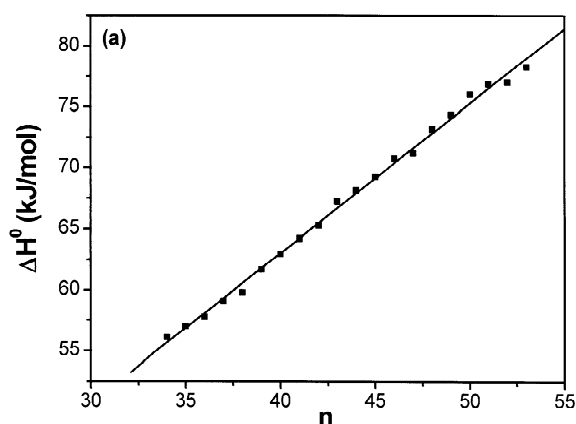


Fig. 3. Molecular mass dependence of the thermodynamic parameters for PEO 2300 obtained from the slope and intercept of the van't Hoff plots in Fig. 2: (a) ΔH° vs. degree of polymerization, (b) $\Delta S^* = \Delta S^\circ/R + \ln \phi$ vs. degree of polymerization. They show a good linearity following Martin's rule. From the slope of the plots, ΔH° and ΔS^* per oxyethylene unit were determined as 1.23 kJ/mol and $4.39 \text{ J K}^{-1} \text{ mol}^{-1}$, respectively.

At 34 °C, all PEO samples elute at the same retention time independent of the molecular mass. Across this transition temperature the elution order of the three PEO samples is reversed. Above the crossover temperature, lower-molecular-mass PEO elute first as observed for PEO 2300 in Fig. 1. Below the crossover temperature, high-molecular-mass PEO elute first. As already reported by Lochmüller et al. [14] this is the enthalpy–entropy compensation point found in various polymer systems. However, such a compensation point in the HPLC separation of

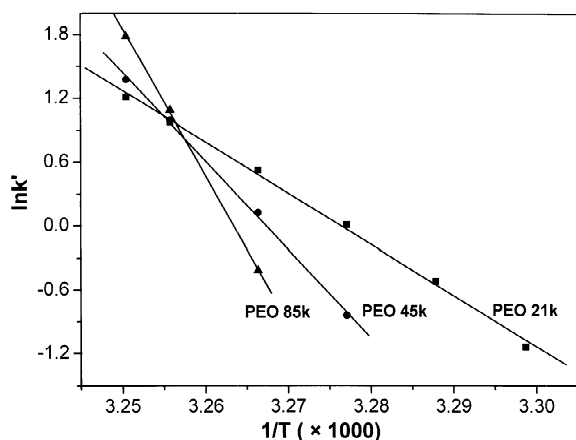


Fig. 4. van't Hoff plot of three high-molecular-mass PEO samples (21, 45 and 85 kg/mol) in the RPLC system. The slope and intercept of the van't Hoff plot indicate that both ΔH° and ΔS^* are positive. Column, Luna C₁₈, 100 Å pore, 250×4.6 mm; eluent, CH₃CN–water (45:55, v/v). The enthalpy and entropy compensation point occurs at 34 °C, at which the molecular mass dependence of PEO retention disappears.

polymers is usually a result of the compensation of the entropic size-exclusion effect and the enthalpic interaction effect. Therefore, at the compensation point the polymers elute near the total column void volume ($k' \approx 0$) independent of their molecular masses [26,27]. In the case of PEO, however, the transition point appears far from the usual position, which indicates that the nature of the enthalpy and entropy compensation in the PEO system is different from many other polymers.

3.2. NPLC retention behavior

On the other hand, in the NPLC separation using an amino-bonded silica column, the elution behavior of PEO shows quite different behavior from the RPLC system. Fig. 5 shows NPLC chromatograms of PEO 2300 obtained at four different column temperatures. A mixture of acetonitrile–water (90:10, v/v) was used as mobile phase. The resolution of NPLC is somewhat poorer than RPLC, but individual peaks are resolved at low temperatures. As the temperature increases the NPLC retention decreases and the elution peaks of oligomers of different degrees of polymerization merge into a single sharp peak. This is the retention behavior

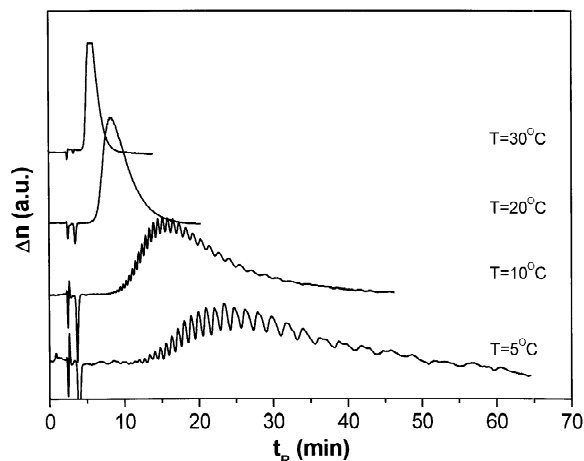


Fig. 5. NPLC chromatograms of PEO 2300 recorded at different temperatures. Column, Nucleosil-NH₂, 100 Å pore, 250×2.1 mm; eluent, CH₃CN–water (90:10, v/v) at a flow-rate of 0.2 mL/min. The retention of PEO decreases with increasing temperature, indicative of negative ΔH° .

commonly found in the IC separation of many organic polymers involving an exothermic sorption process.

Analogous to PEO 2300, high-molecular-mass PEO show NPLC elution behavior much different from RPLC, as shown in Fig. 6. In the NPLC separation of high-molecular-mass PEO, the chromatographic separation conditions are the same as

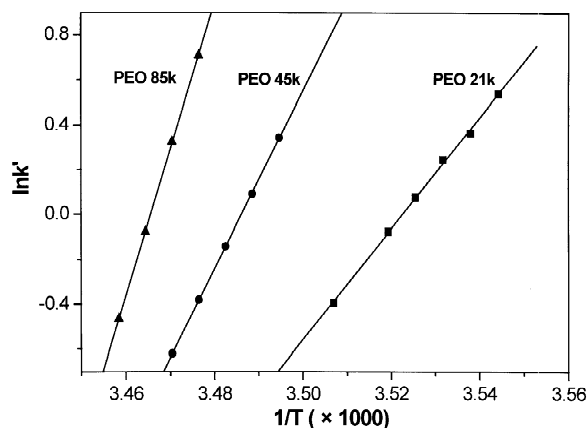


Fig. 6. van't Hoff plot for the NPLC separation of three high-molecular-mass PEO (21, 45 and 85 kg/mol). The slope and intercept of the van't Hoff plot indicates that both ΔH° and ΔS^* are negative. Column, Nucleosil-NH₂, 100 Å pore, 250×2.1 mm; eluent, CH₃CN–water (70:30, v/v).

for PEO 2300 except for the mobile phase composition (acetonitrile–water, 70:30, v/v). Contrary to RPLC separation, in NPLC separation, water behaves as a desorption-promoting liquid and increasing the water content of the eluent decreases the retention of PEO. Retention behavior typically observed in many other polymer systems was found, which is represented as $\Delta H^\circ < 0$ and $\Delta S^\circ < 0$. The standard enthalpy and entropy changes per monomer segment are -0.23 kJ/mol and -0.80 J K⁻¹ mol⁻¹, respectively. This indicates that the sorption of PEO chains to the stationary phase is dictated by favorable energetic interaction with the polar stationary phase. The enthalpy and entropy compensation point can be estimated as $T_c = \Delta H^\circ / \Delta S^\circ \approx 288$ K. At this temperature, $\ln k'$ is extrapolated to a large negative value ($k' \approx 0$, $V_R \approx V_0$), which is the typical behavior observed for many organic polymers.

NPLC retention increases as the molecular mass increases or as the temperature decreases over the whole molecular mass range. Therefore, we can easily separate a wide range of molecular masses of PEO samples by NPLC with an increasing temperature gradient. Fig. 7 shows the temperature gradient

interaction chromatography chromatogram of four high-molecular-mass PEO samples (21, 45, 85, and 270 kg/mol). All PEO samples were separated to the baseline and the average molecular mass and the molecular mass distribution were calculated with respect to the calibration curve prepared from the four PEO samples. The number- and weight-average molecular masses are in reasonable agreement with the value provided by the manufacturer, but the molecular mass distribution is much narrower than the manufacturer's value ($M_w/M_n = 1.07$, where M_n is the number-average molecular mass). This is an expected result since the manufacturer's values were obtained by SEC analysis and it is well established that polymers prepared by anionic polymerization often have narrower molecular mass distributions than those measured by SEC [20–23,28]. SEC analysis cannot provide accurate molecular mass distributions of narrowly distributed polymer samples due to the band-broadening problem [29].

3.3. Hydrophobic interaction

What would cause the peculiar RPLC retention behavior of PEO? We believe that it is the hydrophobic interaction. The hydrophobic interaction is an entropy-driven phenomenon frequently found in aqueous solutions of amphiphilic molecules due to the unfavorable interaction of nonpolar moieties of the molecules with water molecules. If nonpolar compounds are placed in water they tend to spontaneously associate due to the hydrophobic interaction. The best-known example is the micelle formation of surfactants, which induces an entropy increase resulting from a displacement of the water molecules ordered around the non-associated hydrophobic groups to the more unstructured bulk water. The hydrophobic interaction is also known to play an important role in protein folding and structure stabilization [30–32].

Another well-known example is the low critical solution behavior of amphiphilic organic polymers such as PEO [33,34]. PEO dissolves in water due to the strong hydrogen bonding interaction between water molecules and the backbone oxygen [35–37], but water molecules do not favor contact with the hydrophobic ethylene group in the backbone. In order to reduce the unfavorable contact, the water

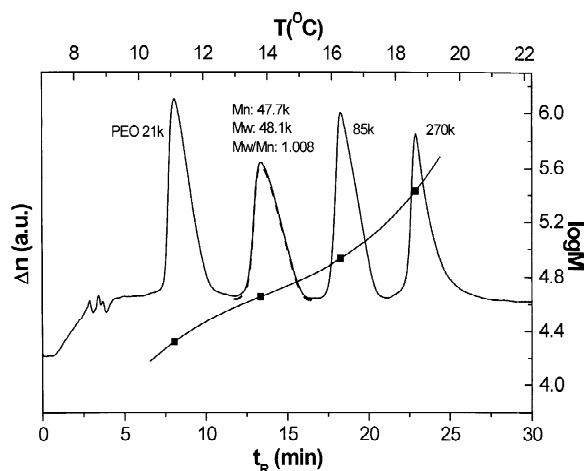


Fig. 7. Solid line: Temperature gradient interaction chromatography chromatogram of four high-molecular-mass PEO samples (21, 45, 85 and 270 kg/mol). Dashed line: Separate injection of a PEO sample (45 kg/mol). M_n and M_w values were calculated from the calibration curve (shown in the plot) constructed from the peak positions of the four PEO samples. Column, Nucleosil-NH₂, 100 Å pore, 250×2.1 mm; eluent, CH₃CN–water (70:30, v/v) at a flow-rate of 0.2 mL/min.

molecules surrounding the amphiphiles adopt a more organized structure that reduces the system entropy. If the PEO chains precipitate, the surface area for unfavorable contact decreases and the surrounding water molecules are relieved from the low entropic state. This hydrophobic interaction causes a solubility decrease of polymers such as PEO as the temperature increases since the enthalpic stability of the mixed state due to the hydrogen bonding of the PEO chain between water molecules is reduced relative to the entropic penalty at elevated temperatures.

In RPLC separation, water molecules adjacent to the PEO chains and the octadecyl stationary phase are in a low entropy state due to the unfavorable contact. When PEO chains are adsorbed to the stationary phase, the water molecules are released from the low entropy state, increasing the entropy of the whole system. The entropy gain associated with the hydrophobic interaction is larger than the conformational entropy loss of the polymer chains in the sorption process and there is a net entropy gain from the sorption process. The net entropy gain in the sorption process has to be large enough to overcome the enthalpy penalty of the endothermic sorption process and PEO is retained in the RPLC separation.

Therefore, it is the hydrophobic entropy effect that is responsible for the increase of RPLC retention, in particular for this compound class, while the unfavorable enthalpy effect reduces retention. The relative entropic ($\Delta S^* = \Delta S^\circ/R + \ln \phi > 0$) and enthalpic ($-\Delta H^\circ/RT < 0$) contribution to the retention ($\ln k'$) varies with temperature according to Eq. (3). As the temperature increases the enthalpic contribution decreases relative to the entropy effect due to the RT factor in the denominator. This explains the inverse temperature dependence of the RPLC retention of the PEO samples: the enthalpic contribution decreases and the retention increases as the temperature increases. This also explains the molecular mass dependence of RPLC retention. At 34 °C, the enthalpy and the entropy contributions per oxyethylene unit compensate each other and the retention of high-molecular-mass PEO becomes independent of molecular mass as shown in Fig. 4. At temperatures higher than the compensation point, the entropic contribution dominates and higher-molecular-mass PEO is retained longer (since ΔH° and ΔS^*

are proportional to the degree of polymerization, as shown in Fig. 3). For the same reason, at temperatures lower than the compensation point, the enthalpic contribution dominates and the retention of higher-molecular-mass PEO becomes shorter. We can easily infer from Fig. 2 that the separation of PEO 2300 was performed above the compensation temperature. The compensation point can be estimated from the ΔH° and ΔS^* values per oxyethylene unit, 1.23 kJ/mol and 4.39 J K⁻¹ mol⁻¹: $T_c = \Delta H^\circ/\Delta S^* \approx 280$ K. Therefore, in this case, the low-molecular-mass PEO elutes first since the RPLC separation was performed above the transition temperature.

In NPLC separation, the sorption of PEO molecules to the amino-bonded stationary phase is an exothermic process. Also, the entropy gain from the hydrophobic interaction is smaller than with RPLC due to the smaller content of water in the mobile phase and the favorable interaction of water molecules with the amino group of the stationary phase. Therefore, the entropy change associated with the sorption process is negative and normal elution behavior is observed.

4. Conclusion

For the HPLC separation of polymers, it is generally accepted that SEC separation is an entropy-driven process, while interaction chromatographic separation is enthalpy driven. It is also commonly assumed that interaction chromatographic separation requires an exothermic solute sorption process and high-molecular-mass polymers are retained longer in the separation column. However, the example of the RPLC separation of PEO clearly shows that this is not necessarily the case and an entropically driven non-size-exclusion separation process is possible. In fact, RPLC with an aqueous mobile phase is widely used in the separation of biomolecules under the name of hydrophobic interaction chromatography [38,39] and the thermodynamics involved in the separation principle is very similar to the present case [40]. We have found for the first time, to our knowledge, a hydrophobic interaction chromatography separation mechanism with a synthetic polymer system.

Acknowledgements

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