

A thermodynamic study of retention of poly(ethylene glycol)s in liquid adsorption chromatography on reversed phases

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Abstract

The retention behaviour of poly(ethylene glycol)s (PEGs) on various reversed phase columns has been studied in liquid interaction chromatography at different temperatures. Similar separations could be achieved in different mobile phases. In methanol–water practically no temperature dependence was observed, while in acetone–water retention decreased with increasing temperature. From the van't Hoff plots the thermodynamic parameters were calculated, which showed, that retention is driven by enthalpy in methanol–water, while in acetone–water the driving force is entropy.

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1. Introduction

Polyoxyethylenes (POEs) are very important products, which are used in many applications. High molecular products are usually referred to as poly(ethylene oxide)s (PEOs), POEs of less than ca. 20,000 number-average molecular mass as polyethylene glycols (PEGs).

Low molecular PEGs ($M < 800$) are usually used as intermediates for the manufacture of fatty acid ester surfactants that are used in a variety of formulated products, such as foam stabilizers, fabric softeners, and optical brighteners.

Polymers with molecular mass of ca. 1000–2000 are low-melting waxes. Their low toxicity, nonirritating nature, and smooth, creamy texture along with liquefaction at body temperatures make them particularly useful in cosmetic and pharmaceutical applications such as formulations for salves, suppositories, and ointments. The analysis of these products require a determination of their molar mass distribution (MMD) and – in the case of mono- and dialkyl ethers (or esters) – also their functionality type distribution (FTD).

In the analysis of PEGs (with terminal hydroxy groups) the following separation techniques can be applied:

- size exclusion chromatography (SEC), which separates according to molecular dimensions (hydrodynamic radius);
- interaction chromatography (or adsorption chromatography) on normal phases (NP-LAC), which separates according to the number of polar groups (O, OH) [1];
- interaction chromatography (or adsorption chromatography) on reversed phases (RP-LAC), which separates according to the number of non-polar groups (CH_2) [2,3];
- liquid chromatography under critical conditions (LCCC) makes the polyoxyethylene chain chromatographically invisible, thus allowing a separation to the end groups.

SEC is especially useful for high molecular weight samples (PEO), while it does not allow a resolution of individual oligomers (except for the lowest ones).

LAC is superior to SEC in the analysis of (lower) PEGs, where it allows a full resolution of the individual oligomers. Because of its high selectivity, isocratic elution can be applied only in the case of samples with a rather narrow MMD: with optimized conditions up to 30 oligomers can be sufficiently

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resolved. For samples with a broader MMD gradient elution is typically required [4,5]. This means in most cases a gradient from a weaker to a stronger solvent, but temperature gradient separations have also been reported [6].

These data have been used in studies of polymer retention [1,7,8] based on theoretical considerations, which allow also a prediction of retention using a special software. The agreement of experimental chromatograms with those obtained by simulation was excellent [9].

Recently, the thermodynamic background of the separation has been studied: Chang and coworkers [10] found that retention of PEG in acetonitrile–water mobile phases on normal phases (amino bonded silica) showed the normal enthalpy-driven behavior, on reversed phases (C₁₈ bonded silica), however, the separation was governed by entropy! This was explained by hydrophobic interaction [11] of the PEG molecules with the non-polar stationary phase and the aqueous mobile phase.

As other studies on the retention of amphiphilic ethoxylates on reversed phases in acetonitrile water also showed a peculiar behaviour [12–14], which was very different from that in methanol–water and acetone–water, we have studied the retention of PEG in such mobile phases on various reversed phase columns in the regime of LAC.

2. Retention of homologous series in liquid chromatography

In SEC, elution volumes are given by $V_e = V_i + KV_p$, wherein V_i is the interstitial volume (the volume between the particles of the packing), V_p is the pore volume, and K is the distribution coefficient between these volumes:

$$K = \frac{V_e - V_i}{V_p} = \frac{V_e - V_i}{V_0 - V_i} \quad (1)$$

Large molecules (with $K=0$) elute at V_i (exclusion limit!), while small molecules (having access to all pores of the stationary phase) elute at the void volume $V_0 = V_i + V_p$ (as $K=1$).

In LCCC (at the critical point for the EO chain), all oligomers having none or only one interacting terminal group elute at the same volume. In the case of non-functionals one narrow peak appears at the void volume: $V_e = V_0$, consequently $K=1$. Chains containing other groups, which are adsorbed, elute at higher V_e .

In liquid adsorption chromatography (LAC), retention is typically given in terms of the (dimensionless) retention factor k :

$$k' = \frac{V_e - V_0}{V_0} \quad (2)$$

The correct definition (and determination) of the void volume V_0 is not trivial. Basically, there are two different definitions [15]: the kinetic dead volume (corresponding to V_i), and the thermodynamic dead volume (corresponding to V_0). Differ-

ent ways to determine the void volume have been described in the literature [16–23].

A popular approach uses an empirical relation, which describes the retention of homologous series (Martin's rule) [24].

$$\ln k = A + Bn \quad (3)$$

wherein A and B are constants for each system. Extrapolation to $n=0$ should yield V_0 .

A linear relation should also be found between $\ln K$ and n :

$$\ln k = A' + B'n \quad (4)$$

Obviously, Martin's rule cannot be applied to SEC, where all peaks elute before the void volume ($V_e < V_0$, and thus $k < 0$)! As has been shown previously [25], Eq. (3) holds only in the range of strong interaction, i.e. at sufficiently high n , and there are considerable deviations at lower n . Nevertheless, most chromatographers using LAC prefer the retention factor over the distribution coefficient, as the latter requires the determination of interstitial volume and pore volume. These data can be extracted from a SEC calibration curve (inverse SEC) [26].

3. Thermodynamics of liquid chromatography

The distribution coefficient $K = \exp(-\Delta G/RT)$ is related to the change of the Gibbs energy G of the polymer chain when it transfers from the free volume V_i into the pore volume V_p .

Consequently, the distribution coefficient is related to the corresponding entropy and enthalpy changes:

$$\Delta G^0 = \Delta H^0 - T \Delta S^0 = -RT \ln K \quad (5)$$

wherein ΔG^0 , ΔH^0 , and ΔS^0 are the changes in free energy, enthalpy and entropy, R is the gas constant, and T is the absolute temperature.

The relation between distribution coefficient and thermodynamic parameters is then

$$\ln K = -\frac{\Delta G^0}{RT} = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} \quad (6)$$

From a plot of $\ln K$ versus $1/T$ (van't Hoff plot), one may calculate ΔH^0 from the slope and ΔS^0 from the intercept. Most chromatographers [27–32] use, however, the retention factor instead of the distribution coefficient, which requires the introduction of the phase ratio $\phi = (V_{st}/V_m)$:

$$\ln k = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} + \ln \phi \quad (7)$$

As has already been discussed [33], different definitions are used for the volume of the mobile phase V_m . A similar problem concerns the volume of the stationary phase V_{st} : most authors consider V_{st} as the free volume between the hydrocarbon chains of the stationary phase, the determination of which is, however, experimentally difficult [34–38].

From the definitions given in Eqs. (1) and (2) it becomes clear [25], that Eq. (7) is only an approximation, because there is no direct proportionality between k and K . Consequently, the distribution coefficient K was applied in this study.

Within (polymer) homologous series $\ln K = -\Delta G/RT$ depends on the number n of repeat units (Eq. (6)). This may be due to such a dependence of $-\Delta H/RT$ or $\Delta S/R$ (or both).

$$\ln K = A' + B'n = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} \quad (8)$$

Generally, the dependence of retention on the number of repeat units in LAC is ascribed to the contribution of enthalpy, it may, however, also originate from the entropic change [10].

The determination of the thermodynamic parameters from slope and intercept of the regression lines in the van't Hoff plots is based on a linear relation between $\ln K$ (or $\ln k$) and $1/T$, which is fulfilled in many systems [12,31,39]. In some cases, however, considerable deviations from linearity are observed, which may be due to a temperature dependence of the thermodynamic parameters or the phase ratio [40] (or both). Obviously, a temperature dependence of the void volume [18,21] will result in erroneous retention factors.

As the interstitial volume should not vary considerably with temperature, such a dependence must be expected to originate from changes in the pore volume. Consequently, the correct values for the void volume (and thus the pore volume) must be used for each temperature.

In these investigations, a constant value for V_i and a linear relation between V_p and T was assumed within the studied temperature range. The changes in entropy and enthalpy, ΔS and ΔH , were assumed to be constant within the studied temperature range. The values thus obtained were ascribed to the middle-point of the studied temperature range (25.0°).

The contributions of entropy and enthalpy changes to the free energy change were visualized by plotting the parameters $\ln K = -\Delta G/RT$, $-\Delta H/RT$, and $\Delta S/R$ as a function of the number of repeat units.

4. Experimental

These investigations were performed using the density detection system DDS70 (CHROMTECH, Graz, Austria). Data acquisition and processing was performed using the software package CHROMA, which has been developed for the DDS70.

In System A, the mobile phase was delivered by a JASCO 880 PU pump (from Japan Spectroscopic Company, Tokyo, Japan) at a flow rate of 0.5 ml/min. Samples were injected manually using a Rheodyne 7125 injection valve (from Rheodyne, Cotati, CA, USA) equipped with an 50 μ l loop.

A Bischoff 8110 refractive index detector (Bischoff, Leonberg, Germany) was connected to the DDS 70. Columns were connected to two column selection valves (Rheodyne 7060, from Rheodyne, Cotati, CA, USA).

In system B, the mobile phase was delivered by an ISCO 2350 HPLC pump (from ISCO, Lincoln, Ne, USA). The flow rate was 0.5 ml/min in all measurements. Samples were injected using a VICI injector equipped with an 50 μ l sample loop.

A SEDEX 45 ELSD (Sedere, France) was connected to the DDS 70. Nitrogen was used as carrier gas, and the pressure at the nebulizer was set to 1.0 bar. The temperature of the evaporator was 30 °C.

The columns and density cells of both systems (A and B) were placed in a thermostatted box, in which temperature (from 15.0 to 35.0 °C) was kept constant to 0.1 °C using a thermostat Lauda RM6, Lauda-Königshofen, Germany). Columns were connected to two column selection valves (Rheodyne 7060, from Rheodyne, Cotati, CA, USA).

The following columns were used in this study:

- Synergi 4 μ m MAX-RP 80A (Phenomenex, Torrance, CA, USA): silica-based dodecyl phase; 250 \times 4.6 mm; particle diameter = 4 μ m; nominal pore size = 80 Å.
- Prodigy ODS3 (Phenomenex, Torrance, CA, USA): silica-based octadecyl phase; 250 \times 4.6 mm; particle diameter = 5 μ m; nominal pore size = 100 Å.
- Symmetry C18 5 μ m (Waters, Milford, MA, USA), silica-based octadecyl phase; 150 \times 3.0 mm; particle diameter: 5 μ m.
- Chromolith Performance RP-18e 100-4.6 (Merck, Darmstadt, Germany): silica-based monolith with octadecyl groups, 100 \times 4.6 mm.

All measurements were performed with HPLC grade solvents and the mobile phases were mixed by mass and vacuum degassed. Their composition was controlled by density measurement using a DMA 60 density meter equipped with a measuring cell, DMA 602 M (A. PAAR, Graz, Austria).

HPLC solvents (acetone and water) were purchased from Roth (Karlsruhe, Germany) and Merck (Darmstadt, Germany). Polyethylene glycols were purchased from Sigma-Aldrich.

5. Results and discussion

The first step in our investigations was the determination of the pore volumes of the columns used. This was done by inverse SEC with standards of known molar mass [26,41,42].

In this study, we had to use PEG standards in an aqueous mobile phase instead of polystyrene in THF, CHCl₃, or CH₂Cl₂ (as usual). This means another limitation in the choice of the mobile phase: in pure organic phases PEG is strongly adsorbed (as most stationary phases contain residual silanol groups).

As we have shown previously [43], PEGs elute in acetone–water containing 40–70 wt.% acetone in the size exclusion mode. In other words, the interstitial volume should be the elution volume of a polymer with very high molar mass.

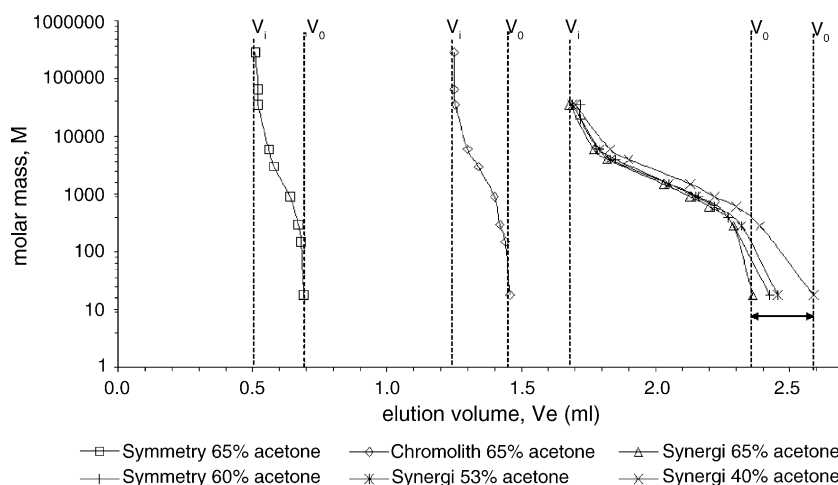


Fig. 1. SEC calibrations of different columns for PEG in acetone–water of a composition corresponding to SEC conditions.

Void volumes may vary considerably with mobile phase composition [17,21]. This is mainly due to changes in the pore volume, as variation of the interstitial volume would mean a shrinkage (or swelling) of the particles of the column packing, which is not very likely in silica-based stationary phases.

Fig. 1 shows the SEC calibration curves obtained on different columns with PEG under SEC conditions (65% acetone). The interstitial volumes V_i are obtained from the elution volume of the PEG with the highest molar mass, the void volumes V_0 from the water peak.

For the Synergi column, also the calibrations obtained in 40, 53 and 60% acetone are shown: the interstitial volume of the Synergi column is fairly constant between 40 and 65% acetone, while the void volume changes considerably in this range of mobile phase composition.

In mobile phases corresponding to LAC a direct determination of V_i is not possible. Hence, the interstitial volumes were assumed to be constant also in such mobile phases, which appears justified from data obtained with different systems [33]. The void volume was taken from the elution volume of the water peak in the corresponding mobile phase composition.

Several authors have described a temperature dependence of the void volume [18,21], which should also originate from changes in the pore volume. In the mobile phases used in this study, void volumes V_0 (Fig. 2) and pore volumes V_p (Fig. 3) showed only a very weak temperature dependence in the range of 15–35 °C. Anyway, this dependence has to be taken into account in the calculation of K .

When poly(ethylene glycol)s are separated in acetone–water of a composition corresponding to LAC conditions, retention is found to be almost independent on temperature (Fig. 4).

Very similar separations can be achieved in methanol water. In this mobile phase, however, a considerable influence of temperature is observed (Fig. 5).

In a plot of $\ln k$ as a function of the number of repeat units (Martin's rule) almost coinciding lines are obtained for the measurements in acetone–water (Fig. 6). These lines show a considerable curvature for the lower

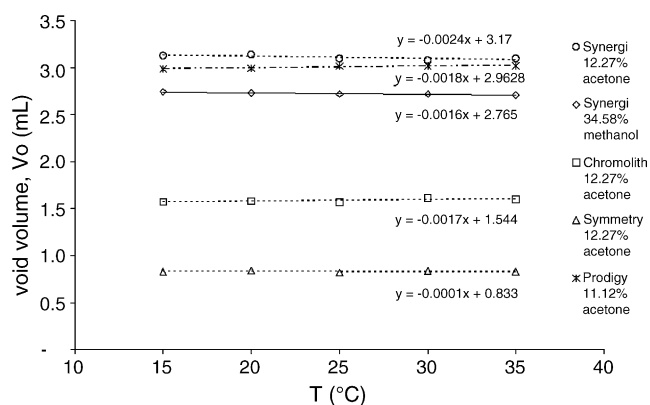


Fig. 2. Void volumes of different columns in acetone–water and methanol water mobile phases (corresponding to LAC conditions) as a function of temperature.

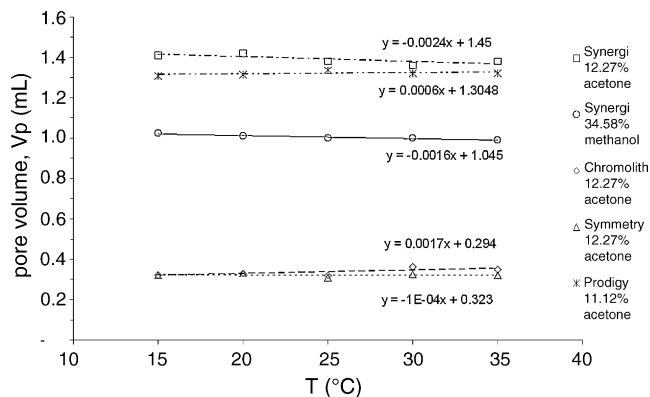


Fig. 3. Pore volumes of different columns in acetone–water and methanol water mobile phases (corresponding to LAC conditions) as a function of temperature.

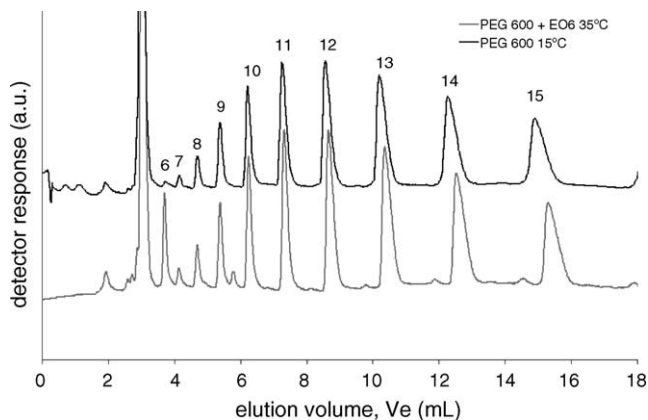


Fig. 4. Chromatograms of PEG 600 on the Prodigy column in 11.12% acetone at different temperatures (at 35 °C hexaethyleneglycol was added as internal standard).

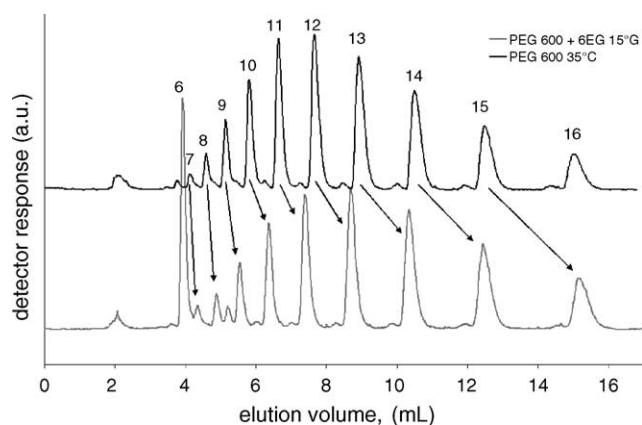


Fig. 5. Chromatograms of PEG 600 on the Synergi column in 34.58% methanol at different temperatures (at 15 °C hexaethyleneglycol was added as internal standard).

oligomers, as has been described in a previous paper [25].

When, however, $\ln K$ is plotted versus the number of repeat units, straight lines are obtained over the entire range of n , which almost perfectly coincide at all temperatures (Fig. 7).

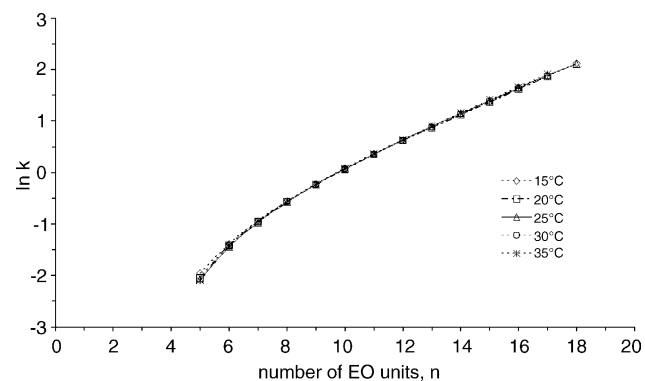


Fig. 6. $\ln k$ of PEG oligomers as a function of the number of repeat units at different temperatures. Prodigy column, 11.12% acetone.

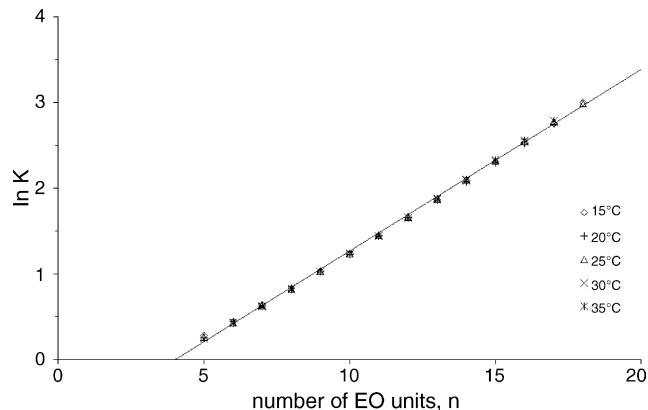


Fig. 7. $\ln K$ of PEG oligomers as a function of the number of repeat units at different temperatures. Prodigy column, 11.12% acetone.

In this mobile phase, temperature has obviously no influence on retention.

Straight lines are also found in a plot of $\ln K$ versus n for the chromatograms obtained in methanol–water. These lines have, however, a slightly different slope (Fig. 8), which decreases with increasing temperature. In the van't Hoff plots (with the distribution coefficients, Eq. (6)), straight lines are obtained in both mobile phases.

In methanol–water, these lines have a clearly positive and different slope (Fig. 9). From slope and intercept of the regression lines ΔH and ΔS were calculated using Eq. (6).

In Fig. 10 the thermodynamic parameters $\ln K = -\Delta G/RT$, $-\Delta H/RT$ obtained with the Synergi column in 34.58 wt.% methanol–water are plotted versus the number of EO units n . As can be seen, $-\Delta G/RT$ increases with n just like $-\Delta H/RT$, while $\Delta S/R$ has a very small positive value, which is almost independent on n .

This means, that adsorption in methanol–water is driven mainly by enthalpy.

A very different behaviour is, however, observed in acetone–water, where these lines appear almost parallel and horizontal (Fig. 11): as follows from Eq. (6), a vanishing slope in the van't Hoff plot indicates, that ΔH is close to zero!

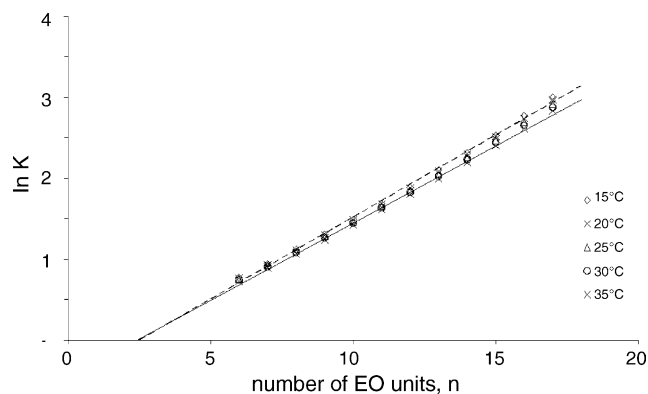


Fig. 8. $\ln K$ of PEG oligomers as a function of the number of repeat units at different temperatures. Synergi column, 34.58% methanol.

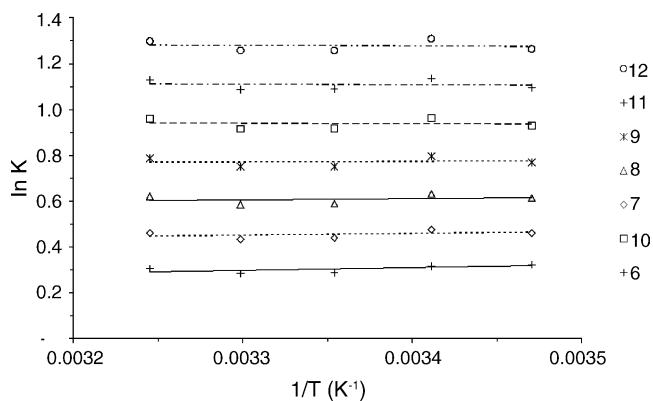


Fig. 9. van't Hoff plot of selected PEG oligomers on the Synergi column in 34.58% methanol.

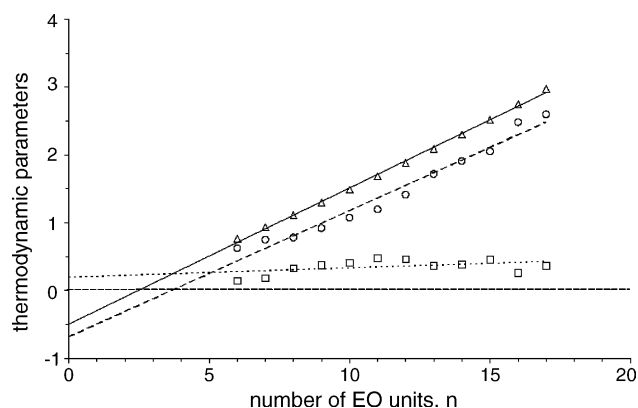


Fig. 10. Thermodynamic parameters $-\Delta G/RT$ (Δ), $-\Delta H/RT$ (\circ), and $\Delta S/R$ (\boxplus) as a function of the number EO units in PEG. Synergi column, 34.58% methanol, 25 °C.

Fig. 12 shows the results obtained with the Prodigy column in 11.12 wt.% acetone: in this case, $-\Delta G/RT$ increases with the number of EO units as $\Delta S/R$, while $-\Delta H/RT$ is quite close to zero and decreases slightly with increasing n .

Hence the driving force of adsorption in this mobile phase is mainly associated with entropy!

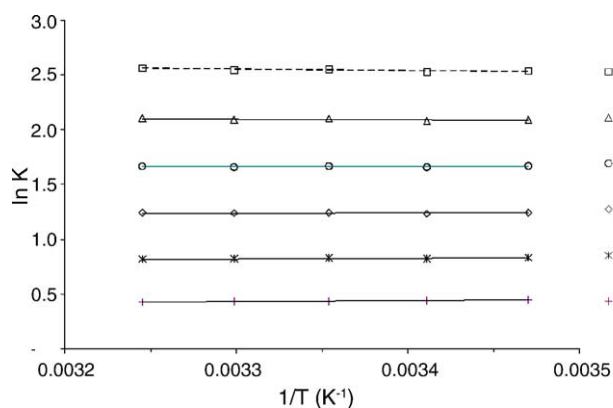


Fig. 11. van't Hoff plot of selected PEG oligomers on the Prodigy column in 11.2% acetone (w/w).

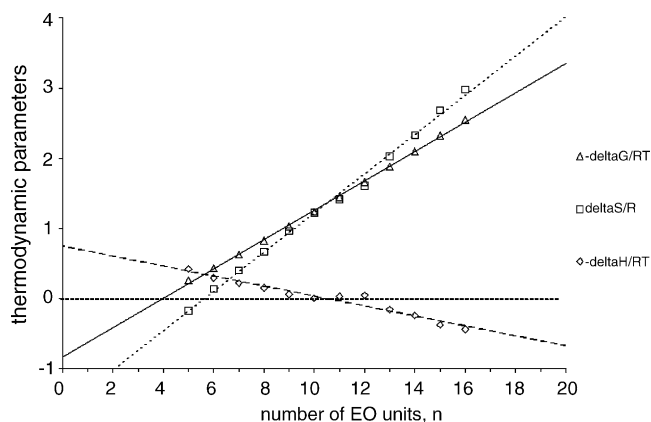


Fig. 12. Thermodynamic parameters $-\Delta G/RT$ (Δ), $-\Delta H/RT$ (\circ), and $\Delta S/R$ (\square) as a function of the number EO units in PEG. Prodigy column, 11.12% acetone, 25 °C.

When the separation was repeated on the Synergi column in 12.27 wt.% acetone–water, the same behaviour was observed as with the Prodigy column (Fig. 13). The results obtained with other columns (Symmetry and Chromolith) in acetone–water showed the same effect.

A comparison of the slopes, intercepts, and correlation coefficients in the plots of the thermodynamic parameters versus the number of EO units obtained with the different columns in different mobile phases is given in Table 1.

The slopes in the plots of $-\Delta G/RT$ versus the number of EO units, (which describe the EO selectivity) are very similar for all columns and mobile phases.

This means that in all systems there is the same adsorption mechanism of chromatography with about the same extent of adsorption interaction.

The slope of $-\Delta H/RT$ in such a plot is positive in methanol–water, while it is slightly negative in acetone–water. The opposite is true for $\Delta S/R$, the slope of which is clearly positive in acetone–water, but close to zero in methanol–water. This becomes even clearer from the graphic representation given in Fig. 14.

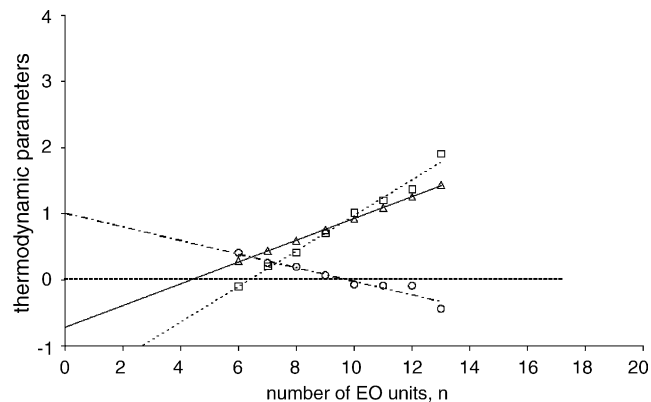


Fig. 13. Thermodynamic parameters $-\Delta G/RT$ (Δ), $-\Delta H/RT$ (\circ), and $\Delta S/R$ (\square) as a function of the number EO units in PEG. Synergi column, 12.27% acetone, 25 °C.

Table 1

Slopes, intercepts, and correlation coefficients in the plots of the thermodynamic parameters $\ln K = -\Delta G/RT$, $-\Delta H/RT$, and $\Delta S/R$ as a function of the number of EO units on different columns in methanol–water and acetone water mobile phases corresponding to LAC conditions

Thermodynamic parameters vs. number of EO units	Synergi 35% methanol	Synergi 11.57% acetone	Symmetry 11.57% acetone	Chromolith 11.57% acetone	Prodigy 11.12% acetone
Slope					
$-\Delta G/RT$	0.20	0.16	0.18	0.18	0.21
$-\Delta H/RT$	0.19	-0.10	-0.06	-0.09	-0.07
$\Delta S/R$	0.01	0.27	0.24	0.27	0.28
Intercept					
$-\Delta G/RT$	-0.49	-0.71	-0.79	-0.89	-0.83
$-\Delta H/RT$	-0.68	0.49	1.02	1.67	0.75
$\Delta S/R$	0.19	-1.20	-1.82	-2.54	-1.58
Correl					
$-\Delta G/RT$	0.9991	0.9996	0.9997	0.9999	0.9996
$-\Delta H/RT$	0.9834	-0.9614	-0.9038	-0.9731	-0.9744
$\Delta S/R$	0.4580	0.9932	0.9933	0.9976	0.9978

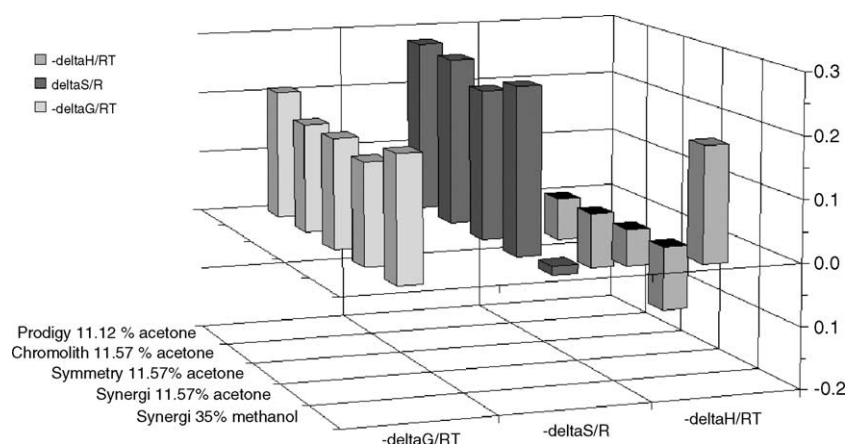


Fig. 14. Slopes in the plots of the thermodynamic parameters $\ln K = -\Delta G/RT$, $-\Delta H/RT$, and $\Delta S/R$ as a function of the number EO units in PEG.

The driving force for adsorption in the acetone–water system is the entropy change, and not the enthalpy change, as is found in methanol–water.

6. Conclusions

When PEGs are separated by LAC on reversed phase columns in methanol–water, the adsorption of oligomers is governed mostly by enthalpy changes, while entropy plays only a minor role. A completely different interplay of entropy and enthalpy is observed in acetone–water, where the driving force of adsorption is mostly entropy. These results agree with the data recently published by Chang and coworkers [10], who analyzed PEGs on a C_{18} column very similar to the Synergi or Prodigy column, but in acetonitrile–water. Although entropic and enthalpic contributions to the free energy change are very different in different systems, it is obvious that the separation mechanism in all systems is governed by adsorption; the extent of effective adsorption interactions in different systems was found to be very similar.

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