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Reversed-phase high-performance liquid chromatography of polyethers

Comparison with a theory for flexible-chain macromolecules

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Abstract

Reversed-phase adsorption chromatography retention modes of polyethylene glycol (PEG), its mono and dimethyl ethers (MME and DME) and polypropylene glycol (PPG) are studied both experimentally and theoretically. The experimental conditions were: narrow- and wide-pore Spherisorb C₁₈ adsorbents and two mixed-solvent systems (methanol–water and acetone–water) as mobile phases. At varying compositions of the mobile phase, fully-resolved chromatograms of polyether samples were obtained, in which all peaks could be identified, and the dependencies of the distribution coefficient on the degree of polymerization for PEG and PPG molecules were extracted by processing these chromatograms. The data were interpreted by using a theory of homopolymers based on a continuum Gaussian chain model of flexible macromolecules and a slit-like model of pores of the stationary phase. Two regimes of an adsorption chromatography of macromolecules are examined in relation to the well-known Martin rule, the role of pore size and the end-group effects being discussed. The theory proved to describe the experimental data on both polymers well, in the whole range of molecular masses studied, and thermodynamic parameters characterizing interactions of ethylene oxide and propylene oxide (PO) repeating units in polyether molecules with the adsorbent pore walls have been determined from a comparison of the theory with the experimental data. The conditions corresponding to the critical chromatography mode are estimated from the thermodynamic data for PEG and methanol–water system as being at about 76% of methanol, while for the PPG and acetone–water system, it is shown that such critical conditions at the given temperature are not attainable. Additionally, the mean thickness H of adsorbed PEG and PPG macromolecules was estimated as being equal to about 3–10 Å. The parameter H proved to be a decreasing function of the water content in the mixed solvent. The same estimate of thickness $H \approx 2.6$ Å is obtained for both polymers in pure water. © 1998 Elsevier Science B.V.

Keywords: Thermodynamic parameters; Adsorption; Polyethers; Poly(ethylene glycol); Poly(propylene glycol); Polymers

1. Introduction

Reversed-phase liquid adsorption chromatography (RP-LAC) using hydrocarbon-coated stationary phases now seems to be the most feasible method for

analysing the molecular mass distribution of low-molecular-mass polyether samples.

As has been demonstrated recently [1–3], this technique can yield chromatograms with completely resolved peaks for all individual oligomer homologues in polyether samples of molecular masses up to several thousands.

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Another interesting approach is chromatography under critical conditions, which proved to be especially useful in separations of heteropolymers [4,5]. It must be mentioned that good results in the separation of polyethers and of other similar compounds can only be achieved, if the experimental conditions are selected very carefully. In particular, the combination of stationary and mobile phases as well as the operating temperature have to be chosen properly to provide an optimal chromatographic mode.

To achieve a deeper understanding of the mechanisms involved in the chromatographic separations of polyethers, a theory is required which could adequately describe the main features of the reversed-phase chromatography of these macromolecules.

In our previous paper [6] we have studied the features of RP-LAC of polyethers in the low-molecular-mass range, up to several hundred. A model of a rigid-rod molecule was used in the theoretical considerations. It was shown that, in the case of polyethers which are known as macromolecules of very high flexibility, the rigid-rod model theory can only be applied to very short molecules. In order to describe the RP-LAC behaviour of polyethers in a wide range of molecular masses, a quite different theory is necessary, accounting for the flexibility of polyether macromolecules.

A theoretical description of chromatography of flexible-chain macromolecules has been first realized by Casassa [7] who developed the theory of gel-permeation (size-exclusion) chromatography (SEC) of polymers; adsorption effects have not been included into the Casassa theory. A general theory of chromatography of homopolymers accounting for the adsorption effects has been suggested by Gorbunov and Skvortsov [8,9]. This theory describes both size-exclusion, adsorption and critical modes of chromatography of polymers, and also explains a transition from the size-exclusion to the adsorption regime via the critical condition chromatography mode, which had been first observed by Tennikov et al. [10,11].

According to the theory [8,9] (see also the review in Ref. [12]), different features of chromatography of polymers have an origin in different statistical properties of confined macromolecules under various conditions of adsorption.

This theory [8,9] has recently been shown to be able to describe the chromatographic behaviour of polyether macromolecules under the conditions of normal-phase LAC [13]. There was also an attempt to apply this theory to the interpretation of the chromatographic data on PEG obtained under conditions of hydrophobic interaction chromatography [14].

In the present paper, we shall use the results of the theory in Ref. [8,9] applying this theory to the experimental data on the RP-LAC of polyethylene glycol (PEG) and polypropylene glycol (PPG) in the molecular-mass range covered in this study.

This gives us an opportunity to determine the adsorption interaction parameter and the mean thickness of adsorbed polyether macromolecules at varying compositions of mixed mobile phases, methanol–water and acetone–water.

2. Experimental

PEGs, their mono-(MME) and dimethyl ethers (DME), and monodisperse EO-oligomers were purchased from Fluka (Buchs, Switzerland) and used without further purification.

Analytical measurements were performed on a modular HPLC system consisting of a Jasco 880 PU HPLC pump (Japan Spectroscopic, Tokyo, Japan) equipped with a Rheodyne 7125 injector (Rheodyne, Cotati, CA, USA), and a density detection system DDS 70 (Chromtech, Graz, Austria) coupled with a Bischoff 8110 refractive index (RI) detector (Bischoff, Leonberg, Germany) or an evaporative light-scattering detection system (ELSD) SEDEX 45 (from SEDERE, Alfortville, France). The sample loop volume was 50 μ l, the injected concentrations were 5–15 g/l. Measurements were performed at a flow-rate of 0.5 ml/min and a temperature of 25.0°C. Data acquisition and processing was performed using the software CHROMA, which is part of the DDS70.

For these investigations, three different columns were used, which were purchased from Phase Separations (Deeside, UK):

- Spherisorb S5 ODS2 (5 μ m, 80 Å, 250×4.6 mm) Serial 831955, Batch 34/188.
- Spherisorb S3 ODS2 (3 μ m, 80 Å, 100×4.6 mm) Serial 115506, Batch 34/188.

- Spherisorb S5X C₁₈ (5 μm, 300 Å, 250×4.6 mm). Serial 836515, Batch 95/121.

The values of void volume V_0 , the interstitial volume V_i and the pore volume V_p of the columns were determined with polystyrene standards (from Polymer Labs., Church Stretton, UK) in tetrahydrofuran. These data were also used to check the pore sizes by using the SEC-porosimetry method [15]. The porosimetry measurements revealed a certain polydispersity according to pore size in all three adsorbents, and the average pore sizes obtained by porosimetry resulted to be somewhat smaller than the data provided by the distributor, as can be seen from Table 1.

The solvents used were HPLC grade (from Promochem, Wesel, Germany). For the preparative fractionation, methanol was distilled prior to use.

Mobile phases were mixed by mass and degassed in vacuum. The composition was controlled by density measurement using a density meter DMA 60 equipped with a measuring cell DMA 602 M (A. PAAR, Graz, Austria).

We have processed a large number of chromatograms for PEG and for its MME and DME obtained under the condition of the RP-LAC in methanol–water mobile phase (in the range of 30 to 55% of methanol), and also for polypropylene glycol in acetone–water mobile phase (at 20 to 90% of acetone).

Depending on the composition of the mobile phase, different ranges of molar mass can be analyzed. Using monodisperse oligomers (either commercial ones or those obtained by semipreparative HPLC [3]) as internal standards, peaks could be

identified and their number of repeating ethylene oxide (EO) groups, n , determined.

Fig. 1 shows a typical chromatogram obtained at 35% (w/w) of methanol for PEG 900, which was spiked with the oligomer EO16 (peak 6). As can be seen the resolution is good, and the values of the elution volume, V_e , corresponding to peak maxima positions can be obtained with high accuracy for 16 oligomers. Owing to this fact, the processing of each chromatogram offers an opportunity to obtain the dependence of a distribution coefficient $K=(V_e-V_i)/V_p$ on the number of EO repeating units, n . Chromatograms for MME, DME and PPG were processed in a similar way.

The data obtained at various chromatographic conditions will be presented below in order to be discussed together with the theory.

3. Theory

Polyethers are known to be polymers having high chain flexibility. The PEG molecules with a degree of polymerization of the order of ten are known to take, in solution, the chaotically rolled up and entangled coil-shaped conformations which can be described by the $R \sim M^{0.5}$ relationship (R being the characteristic average size of a macromolecule, commonly defined as the root-mean-square dimension between two ends of a polymer chain).

The theories describing the properties of such molecules are usually based on the model of an ideal polymer chain (i.e. on the Gaussian coil model).

The basic result following from the well-known

Table 1
Pore volume of columns and pore diameters of adsorbents used, as obtained from porosimetry, and data by the distributor

Column	Void volume V_0 (ml)	Pore volume V_p (ml)	Average pore diameter	
			Specification (nm)	Porosimetry (nm)
S5 ODS2 (25 cm)	2.40	0.86	8	6.8
S3 ODS2 (10 cm)	1.04	0.354	8	6.8
SX5 C ₁₈ (25 cm)	3.35	1.83	30	21.5

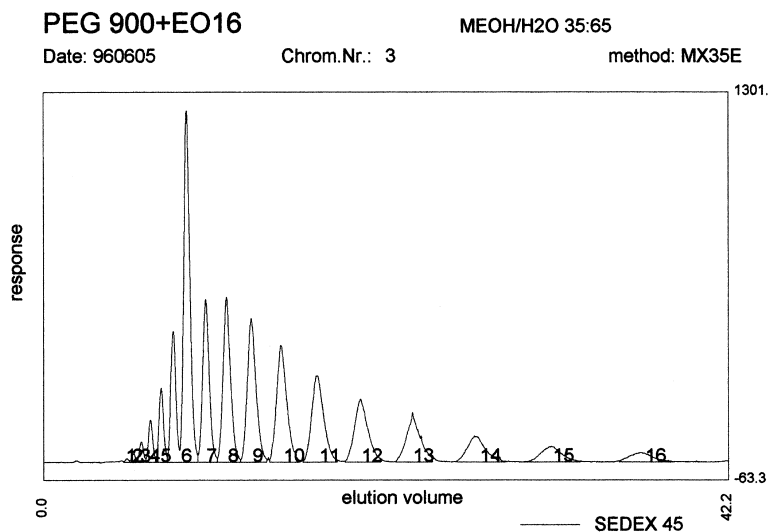


Fig. 1. Chromatogram of a poly(ethylene glycol) 900 (spiked with the oligomer EO16), which was obtained on a 250×4.6 -mm S5X C₁₈ column (particle size 5 μm , pore diameter 30 nm) in methanol–water (35:65, w/w) at a flow-rate of 0.5 ml/min. Injected volume: 50 μl , sample concentration: 5 g/l. Detection: ELSD.

Casassa theory [7] describing the features of SEC of macromolecules (for which the situation of absence of adsorption interactions is characteristic) is the conclusion that the distribution coefficient $K_{\text{SEC}} = (V_e - V_i)/V_p$ is determined by a macromolecule-to-pore size ratio.

In particular, for wide slit-like pores having the width, $2d$, which is much more than the average size of the molecule (root-mean-square end-to-end distance), R , the distribution coefficient is given by:

$$K_{\text{SEC}} \approx 1 - \frac{2}{\sqrt{6\pi}} \cdot \frac{R}{d} \quad (1)$$

The theory of chromatography of homopolymers capable of being adsorbed on pore walls has been suggested by Gorbunov and Skvortsov in Refs. [8,9] for a model of a Gaussian polymer chain and a slit-like pore. According to [8,9], the distribution coefficient, K , is a function of three parameters: polymer coil size, R , pore width, $2d$, and the parameter of adsorption interaction, c .

The adsorption interaction parameter c was first introduced by De Gennes in Ref. [16], and was used in many continuum-model-based theories [9,12,17–19] describing the behaviour of macromolecules at the interfaces and in porous media. The parameter c has a meaning of the inverse correlation length of

adsorption, which can serve as a characteristic of the structure of a macromolecule near the surface of an adsorbent. According to [8], in the adsorption regime, the value of $H = c^{-1}$ is equal to the mean thickness of a flat layer formed by a long macromolecule on a pore wall, as is shown schematically in Fig. 2.

Positive values of the c parameter correspond to the regime of adsorption, $c=0$ corresponds to the so-called critical conditions when the entropy losses of a macromolecule in a pore are precisely compensated by an energy gain due to the adsorption, and negative c values are characteristic of the situation, where adsorption interactions are small or absent.

According to [12], the parameter of adsorption interaction, c , is directly related to the adsorption energy parameter, ϵ , which was commonly in use in the lattice-model-based theories [20,21] of polymer adsorption.

In the vicinity of the critical point of adsorption, c is proportional to the deviation of ϵ from the critical value ϵ_{cr} : $c \sim (\epsilon_{\text{cr}} - \epsilon)$.

At large $-\epsilon$ values corresponding to a strong adsorption regime, $c \sim \epsilon^{1/2}$.

The general formulae expressing the distribution coefficient K at arbitrary c , R and d values, and also the approximate formulae for the cases of narrow

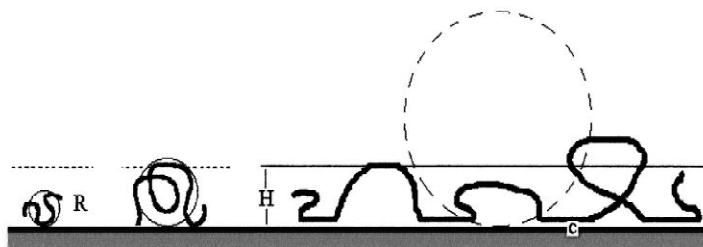


Fig. 2. The structure of short- and long-polymer molecules near an adsorbing surface. A polymer chain forms on the surface a layer of average thickness $H=c^{-1}$ if the size of a macromolecule in the solution R is greater than this H value.

and wide pore were obtained in Ref. [8,9]. In the case where $R < d$, the formula for the distribution coefficient has the following form [9,12]:

$$K \approx 1 - \frac{2}{\sqrt{\pi}} \cdot g + \frac{g}{\Gamma} [1 - Y(\Gamma)], \quad (2)$$

where $g = \frac{R}{d\sqrt{6}}$, $\Gamma = -\frac{cR}{\sqrt{6}}$, and the function $Y(\Gamma)$ is defined as:

$$Y(\Gamma) = \exp(\Gamma^2) \cdot \operatorname{erfc}(\Gamma), \quad (3)$$

and $\operatorname{erfc}(\Gamma)$ being the well-known special function (the complementary error integral), for which the tables and numerical algorithms are available [22]. Asymptotic forms of the function Y are also known:

$$Y(\Gamma) \approx \begin{cases} \frac{1}{\sqrt{\pi}} \Gamma^{-1} & \text{at } \Gamma \gg 1 \\ 1 - \frac{2}{\sqrt{\pi}} \Gamma + \Gamma^2 - \frac{4}{3\sqrt{\pi}} \Gamma^3, & \text{at } |\Gamma| \ll 1^- \\ 2\exp(\Gamma^2), & \text{at } -\Gamma \gg 1 \end{cases} \quad (4)$$

Eq. (2) was analyzed in Ref. [8,9]. It has been shown, in particular, that depending on the value and sign of a product $\Gamma = -cR/\sqrt{6}$ three main modes of the chromatography of polymers are possible.

At $\Gamma \gg 1$, Eq. (2) can be reduced to Eq. (1) describing the mode of size-exclusion chromatography of macromolecules.

At $\Gamma = 0$, the distribution coefficient becomes equal to unity, which is characteristic of the polymer chromatography under critical conditions.

The case of $-\Gamma > 0$ corresponds to the conditions of adsorption chromatography at which K increases with molecular mass. This case is of special interest

for us, since the data have been obtained under the conditions of adsorption chromatography.

At $-\Gamma \gg 1$, the function $Y(\Gamma)$ is approximately equal to $2\exp(\Gamma^2)$, hence Eq. (2) can be reduced to a more simple expression, describing the distribution coefficient of sufficiently long macromolecules in the adsorption chromatography mode:

$$K \approx K_{\text{SEC}} + \frac{2}{cd} \exp\left(\frac{R^2 c^2}{6}\right) \quad (5)$$

It should be noted that according to the theory [8,9] this formula for the adsorption chromatography mode is approximately valid not only for wide-pore adsorbents, but as well for narrow-pore adsorbents, provided the adsorption interactions are strong enough, that is, under the condition of $Rc \gg 1$.

Eq. (5) can be rewritten in a form:

$$K \approx K_{\text{SEC}} + A \exp(Bn) \quad (6)$$

where n is a degree of polymerization, and the coefficients A and B are given by:

$$A = \frac{2}{cd} \quad (7)$$

and

$$B = M_0 Q^2 c^2 \quad (8)$$

Here M_0 is the molecular mass of a repeating unit, and Q is a parameter depending upon the chain flexibility, Q being the coefficient in the relationship between the size of a flexible-chain macromolecule, R , and molecular mass, M :

$$R = QM^{0.5} \quad (9)$$

As follows from Eq. (6), the plot of $\ln(K - K_{\text{SEC}})$

versus the number of repeating units, n , at large n should give a straight line:

$$\ln(K - K_{\text{SEC}}) \approx \ln(A) + Bn \quad (10)$$

provided the condition of $Bn \gg 1$ is fulfilled which means the sufficiently strong adsorption of macromolecules.

Eq. (10) is very similar to the well-known Martin equation [23], which is widely used in the interpretation of adsorption chromatography data. The latter equation assumes a linear dependence of the logarithm of a capacity factor, k' , on the repeating group number, n . Keeping in mind a relation between the capacity factor, k' , and the distribution coefficient, K :

$$k' = \frac{V_p}{V_0} (K - 1) \quad (11)$$

one can make sure of the full equivalence of these two equations at large K values, and can also use Eqs. (7) and (8) to give meaning to an intercept and a slope in the $\ln k'$ vs. n linear approach.

At $-\Gamma = cR/\sqrt{6} < 1$ (that is at small size of a molecules or at weak adsorption energies) Eqs. (5)–(10) describing the high-molecular-mass perfect adsorption regime do not work any longer. According to Eqs. (2)–(4), at small $-\Gamma$ values, the distribution coefficient is of the order of unity and has the following asymptotic form:

$$K \approx 1 + \frac{cR^2}{6d} \left(1 + \frac{4}{3\sqrt{6}\pi} cR \right) \quad (12)$$

Obviously, at these conditions neither $\ln(K - K_{\text{SEC}})$ nor $\ln k'$ are linear functions of the number of repeating units, n .

The condition $-\Gamma = cR/\sqrt{6} \approx 1$, which is equivalent to:

$$n^* \approx \frac{6}{M_0(Qc)^2} \quad (13)$$

may serve to define a conventional point of demarcation (a limiting number n^* of repeating units), between high- and low-molecular-mass adsorption regimes. This condition simply means that a perfect adsorption layer formed by a macromolecule on an adsorbent pore wall surface disappears if the charac-

teristic size of a macromolecule $s = R/\sqrt{6}$ is less than the average layer thickness $H = c^{-1}$ (see Fig. 2).

4. Results and discussion

We have studied the RP-LAC behaviour in two polymer–solvent systems. The first system was PEG and its MME and DME in the methanol–water mobile phase (the examined interval of mobile phase composition was from 30 to 55% of methanol), the second one was PPG in the acetone–water mobile phase (the interval from 20 to 90% of acetone was covered).

Two adsorbents of approximately equal pore diameter 8 nm, were used for the first system: S5 ODS2 (particle size 5 μm) and S3 ODS2 (particle size 3 μm). The same S5 ODS2 stationary phase, and also the Spherisorb SX5 C₁₈ (particle size 5 μm , pore diameter 30 nm) were used in the studies of PPG in the acetone–water system.

In both systems, a similar chromatography mode was realized in the investigated ranges of mobile phases composition, namely the mode characteristic of the adsorption chromatography where the retention of macromolecules increases with molecular mass. The data obtained for PEG and PPG are presented in Figs. 3 and 4 in the form of dependencies of the distribution coefficient, K , on the number of repeating EO units, n_{EO} [or propylene oxide (PO) units, n_{PO}].

Solid lines in Figs. 3 and 4 are drawn according to the theoretical, Eq. (2). In the calculations, the d values from porosimetry were used (Table 1), and the root-mean-square sizes of PEG and PPG macromolecules were estimated according to the formula $R_{\text{PEG}} = 0.079M^{0.5}$ (nm) [24] corresponding to the unperturbed dimensions of long polyether chains.

For each mobile phase composition, one certain value of interaction parameter c was selected, ensuring the best fit of the theoretical curve to the experimental points corresponding to the whole molecular mass series.

The values of adsorption interaction, parameter c , obtained in such a way are listed in the Tables 2 and 3. As can be seen in Figs. 3 and 4, the theoretical Eq. (2) describes the whole set of experimental data well, in both the PEG/methanol–water and PPG/

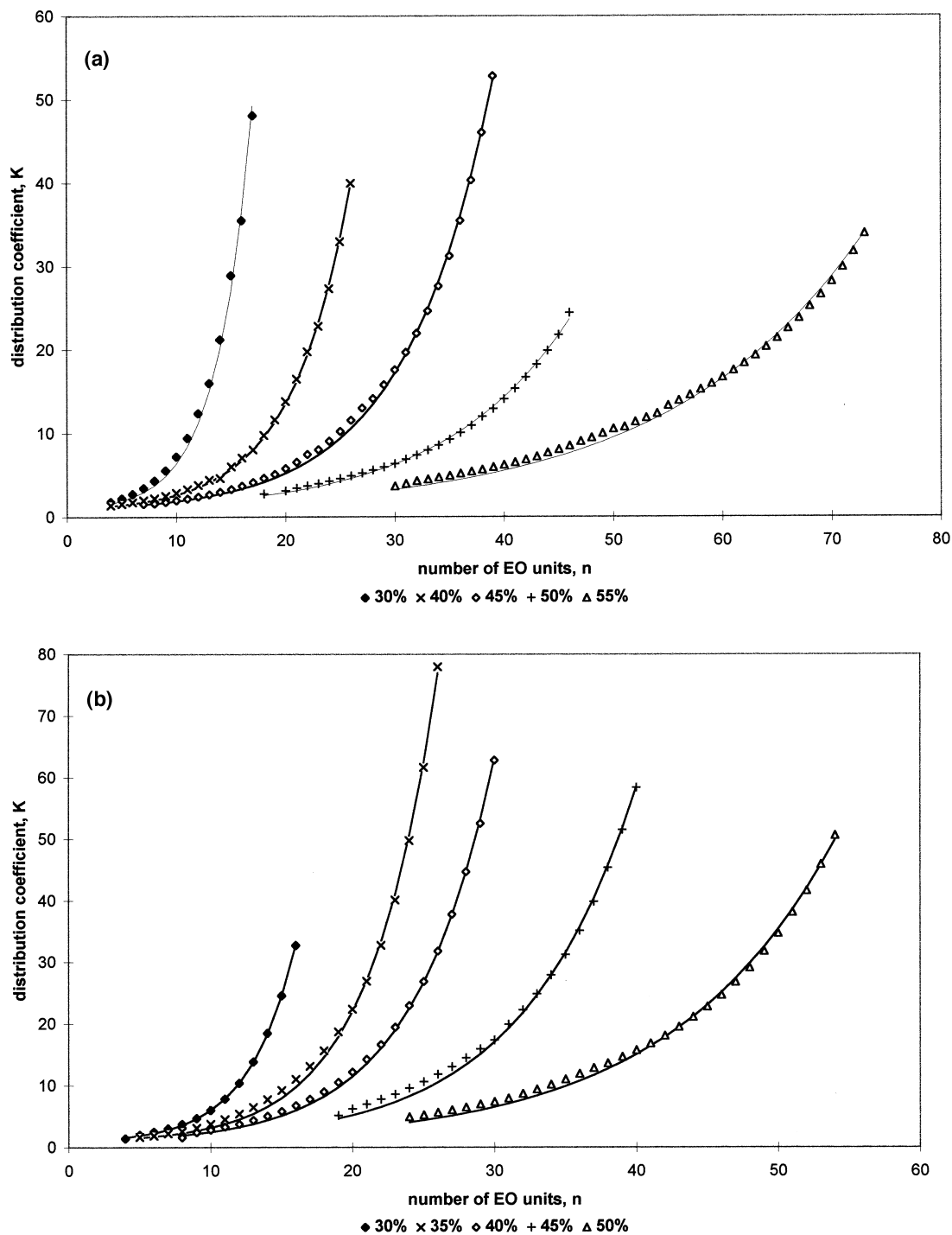


Fig. 3. Dependencies of distribution coefficient K on the number of repeating EO units n for PEG molecules obtained on S5 ODS2 (a) and on S3 ODS2 (b) columns. Experimental conditions: (a) 30%, 40%, 45%, 50% and 55%; (b) 30%, 35%, 40%, 45% and 50% of methanol in mixed methanol–water solvent. Solid lines—approximation by Eq. (2).

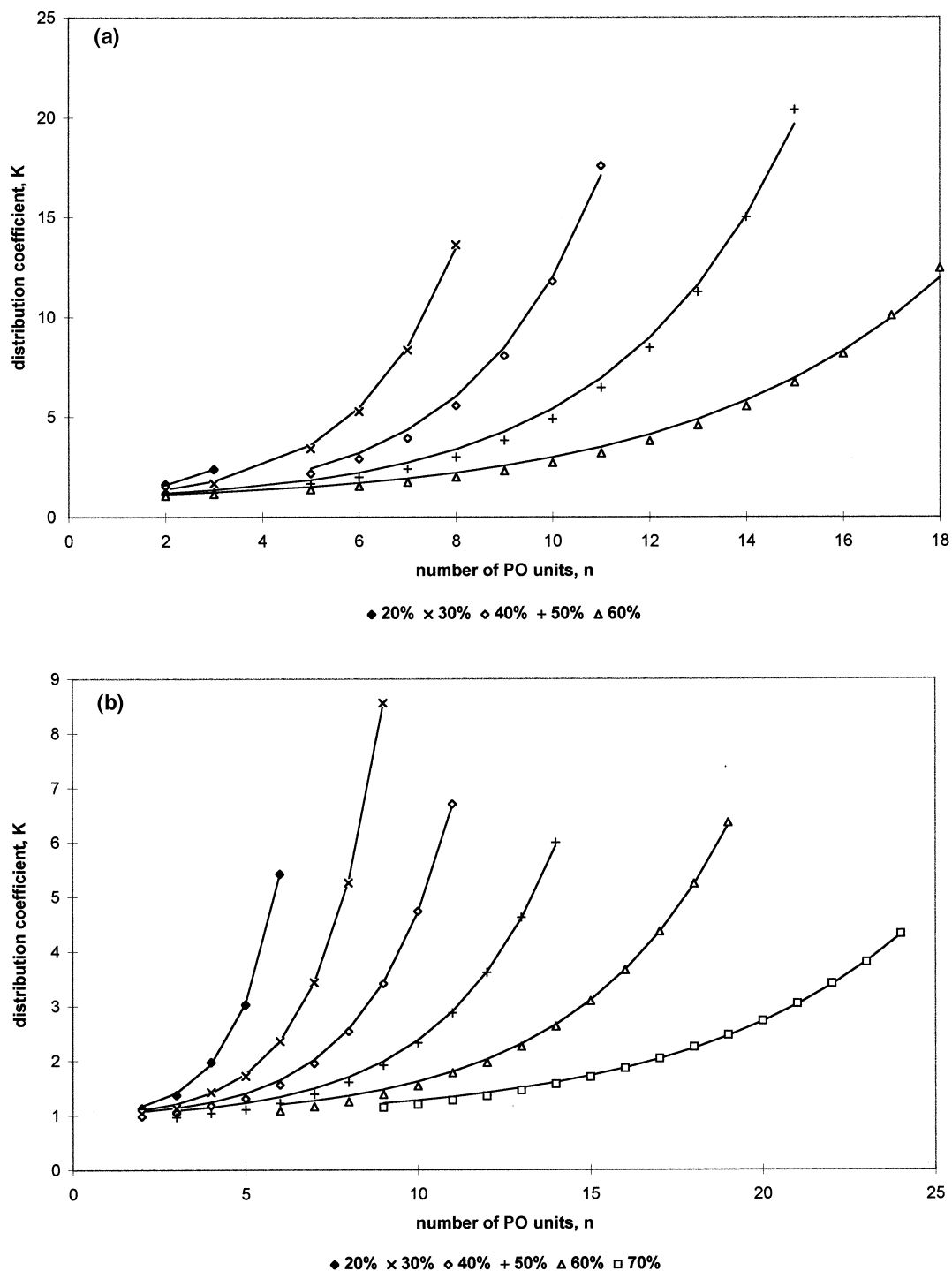


Fig. 4. Dependencies of distribution coefficient, K , upon the number of propylene oxide repeating units, n_{PO} , for polypropylene glycol macromolecules at various composition of the acetone–water mobile phase. Experimental conditions: (a) S5 ODS2 and (b) SX5 C₁₈ columns. Acetone content in the mobile phase is specified in the legend. Solid lines are the approximation by Eq. (2).

Table 2

Parameter c describing the adsorption interaction of ethylene oxide units of PEG, MME, and DME macromolecules in RP-LAC using S5 and S3 C₁₈ as stationary phases and the methanol–water as mobile phase, and the average thickness of adsorbed polyethylene oxide macromolecules, H

Methanol (%, w/w)	Adsorption interaction parameter, c (nm ⁻¹)				Average adsorbed layer thickness H (nm)
	PEG		MME		
	S3W ODS2	S5W ODS2	S5W ODS2	S5W ODS2	
30	2.42	2.45	2.37	2.32	0.41
35	2.04				0.49
40	1.85	1.90	1.78	1.80	0.53
45	1.57	1.57			0.64
50	1.30	1.29	1.34	1.39	0.77
55		1.05	1.08	1.08	0.95

acetone–water systems. Furthermore, we shall discuss in more detail, the features of a chromatographic behaviour of these macromolecules.

4.1. High- and low-molecular-mass regimes

Fig. 5a demonstrates the typical dependence of PEG distribution coefficient on the number of repeating EO groups n , while Fig. 5b shows the same experimental data in the $\ln(K - K_{SEC})$ vs. n coordinates, the lines corresponding to the theory are also shown in these figures. The solid lines in Fig. 5a and b correspond to the general Eq. (2) at $c = 1.29 \text{ nm}^{-1}$, and the dashed straight line corresponds to Eq. (10) with the slope and intercept values which are calculated using Eqs. (7) and (8) with the given d , M_0 and

Table 3

Adsorption interaction parameter c and average thickness H of adsorbed PPG macromolecules measured in RP-LAC using S5 ODS2 and SX5 C₁₈ as stationary phases and acetone–water as mobile phase

Acetone (%)	c (nm ⁻¹)		H (nm)
	S5 ODS2 (8 nm)	SX5 C ₁₈ (30 nm)	
20	3.14	3.34	0.31
30	2.7	2.86	0.36
40	2.35	2.48	0.41
50	2.02	2.14	0.48
60	1.85	1.82	0.55
70		1.5	0.67

Q , and with this $c = 1.29 \text{ nm}^{-1}$ value. It can be seen in Fig. 5a and b that the general Eq. (2) reasonably describes the whole range of n .

In Fig. 5b one can see two regimes: beginning from about $n^* \approx 15$ the straight line approximation of the data by Eq. (10) works well (this is the high-molecular-mass adsorption regime), but considerable deviation of experimental points from this straight line is seen at $n < n^*$ (in the low-molecular-mass regime). Eq. (13) gives a value of $n^* \approx 12$ for this example, and this estimate agrees well with what is seen in Fig. 5b.

There are several consequences to be drawn from Eq. (13): the stronger the adsorption, the lower is n^* and the less pronounced is the nonlinear region in the $\ln(K - K_{SEC})$ vs. n plots, and therefore the better is the accuracy of determination of adsorption interaction parameter c from such linear plots.

If the interval of n is large enough (as in the example of Fig. 5), then both methods (by using Eq. (2) directly, and from the slope of the linear plot according to Eq. (10)) give fairly close values of the c parameter.

Further, we shall discuss only the features of the high-molecular-mass adsorption regime because in most cases n^* values were small enough (of the order of $n = 10$ and less) and there were only a few examples where the low-molecular-mass regime was evidently noticeable.

4.2. Influence of pore size

Fig. 6a shows the data obtained for PEG at 40% of methanol on S3 ODS2 and S5 ODS 2 stationary phases having approximately the same average pore diameters. Lines are the straight line approximations by Eq. (10), with A and B calculated by Eqs. (7) and (8) using the average pore size values $2d = 6.8 \text{ nm}$ taken from the porosimetric measurements and c values from Table 2. So, the observed intercepts are in a reasonable quantitative agreement with Eqs. (7) and (10).

Fig. 6b demonstrates the influence of the pore size on the $\ln(K - K_{SEC})$ vs. n plots. Two dependencies in Fig. 6b describe the chromatographic behaviour of PPG molecules at the same acetone content in the acetone–water mobile phase, but on two adsorbents S5 ODS2 and Spherisorb SX5 C₁₈,

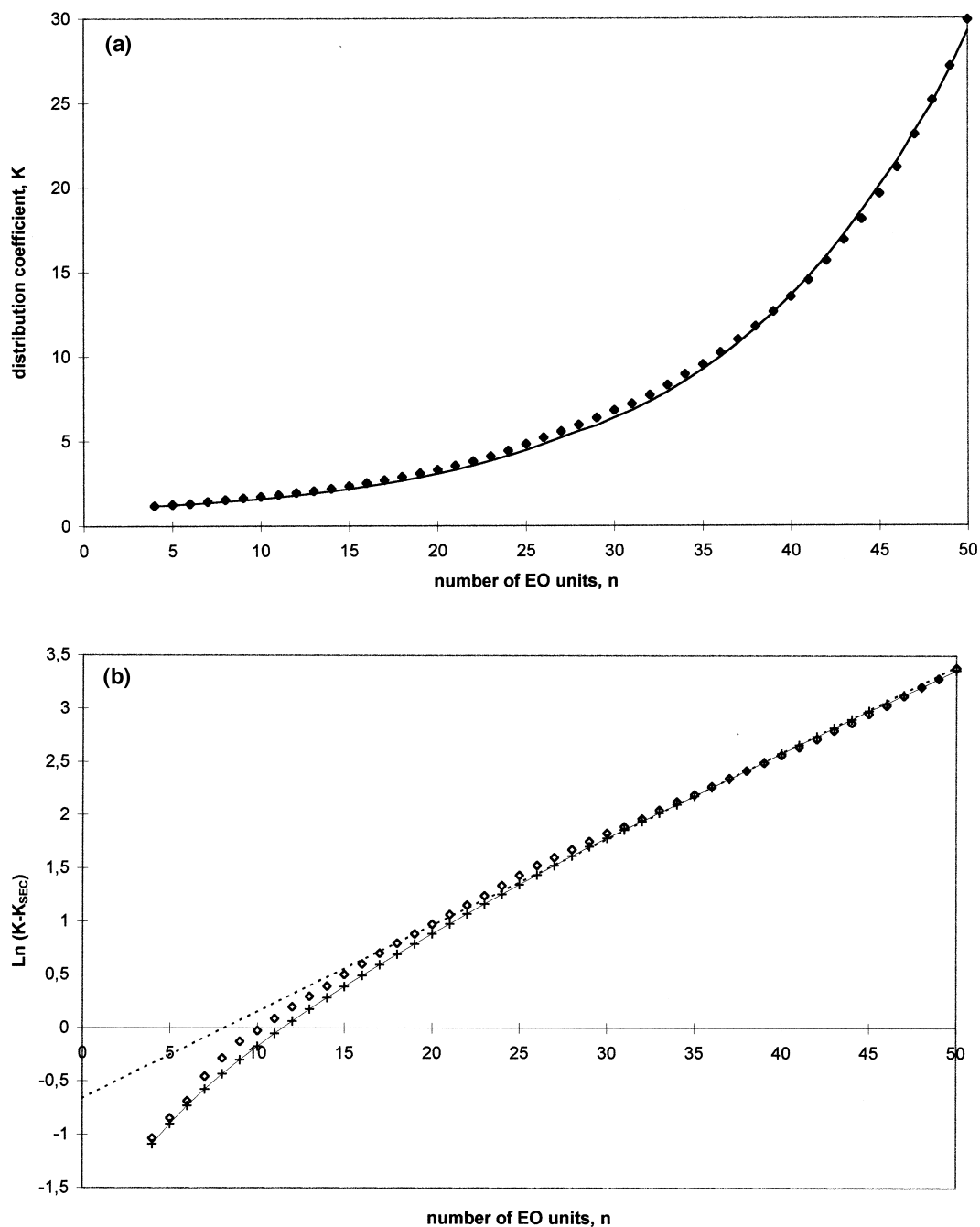


Fig. 5. (a) Distribution coefficient of polyethylene glycol molecules, K , and (b) $\ln(K - K_{\text{sec}})$ as a function of the number of repeating ethyleneoxide units, n , obtained from the chromatograms for several different PEG samples. S5 ODS2 column, 50% of methanol in the methanol–water mobile phase. Solid lines are calculated using Eq. (2), dashed line— Eq. (10) with A and B according to Eqs. (7) and (8).

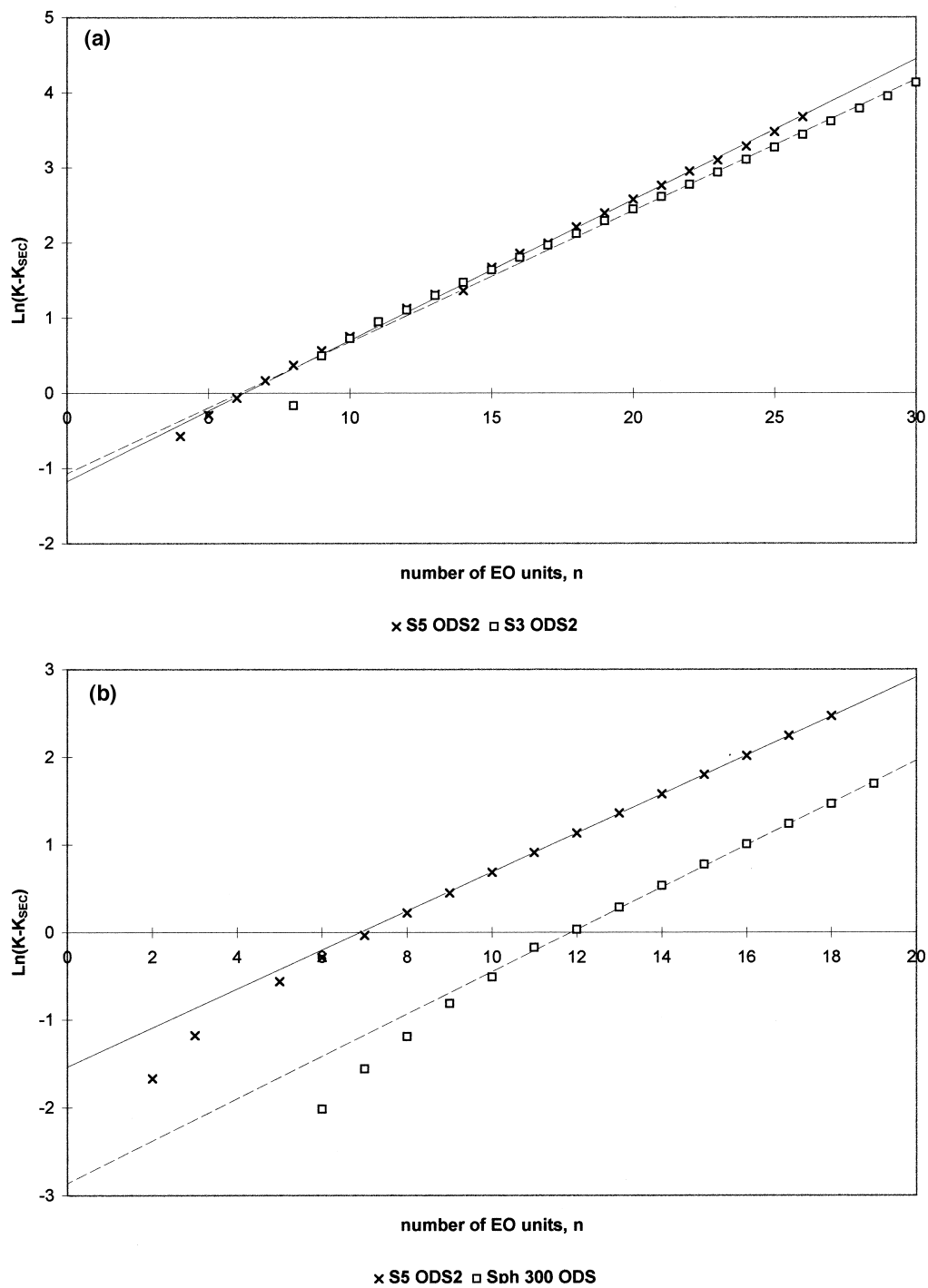


Fig. 6. Dependences of $\ln(K - K_{\text{sec}})$ on the number of repeating units (n) for (a) polyethylene glycols on S3 ODS2 and S5 ODS2 stationary phases at 40% of methanol in mixed methanol–water solvent, and (b) polypropylene glycol on S5W ODS2 and SX5 C₁₈ stationary phases at 60% of acetone in the acetone–water solvent. Lines are linear approximation by Eq. (10) at high n .

differing in pore sizes (nominal pore diameters of these adsorbents are 8 and 30 nm correspondingly). The approximating lines are almost parallel, but the line for the more wide-pore stationary phase is shifted down just to the same extent, as follows from Eqs. (7) and (10).

4.3. End-group effects

A plot of $\ln(K - K_{SEC})$ vs. n (which is shown in Fig. 7) demonstrates the influence of the end groups in PEG, MME and DME molecules on the chromatographic behaviour of these macromolecules in the S5 ODS2/methanol–water system. It must be mentioned, that Eqs. (2) and (5) are derived for a homopolymer model, therefore they cannot describe, in all details, the features of LAC of macromolecules with terminal units which are different in their adsorption ability from the repeating-chain units. In fact, Fig. 7 shows the considerable effect of the end groups, and that the values of intercept obtained for MME and DME macromolecules do not agree with

Eq. (7). However, one can see in Fig. 7, that all three series at large n give practically parallel straight lines, this means that the slope of these lines should have the same physical meaning. Therefore the adsorption interaction parameter for repeating EO units of MME and DME molecules can also be determined by using Eq. (8) from the slope of straight lines.

4.4. Dependence of adsorption interaction parameter and of adsorption layer thickness on mobile phase composition

The dependence of calculated parameter c upon the content of methanol in methanol–water mobile phase becomes clear from Fig. 8a. This figure shows a plot of all data on PEG, MME and DME, which were obtained on the S5 ODS2 adsorbent (and also the data for PEG, which were obtained on the S3 ODS2 packing).

Obviously the data from all the series are about the same, and can be approximated by a common

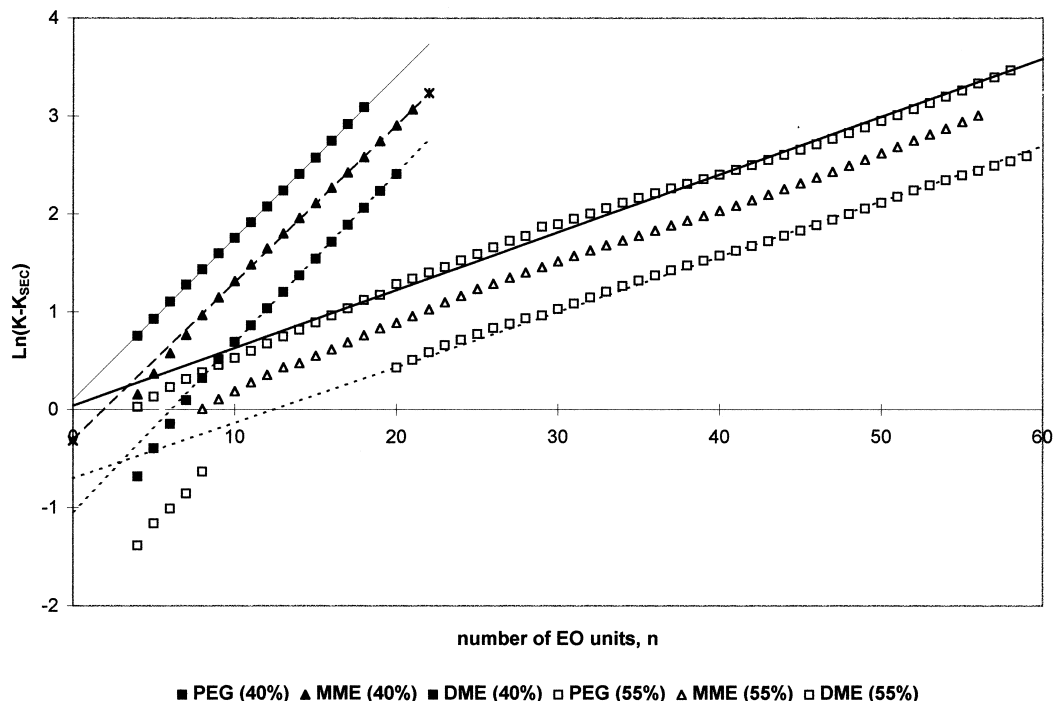


Fig. 7. $\ln(K - K_{SEC})$ vs. n plot for PEG, MME and DME macromolecules at (a) 40%, and (b) 55% of methanol. Column: S5W ODS2. Lines represent straight linear approximation at $n > 10$.

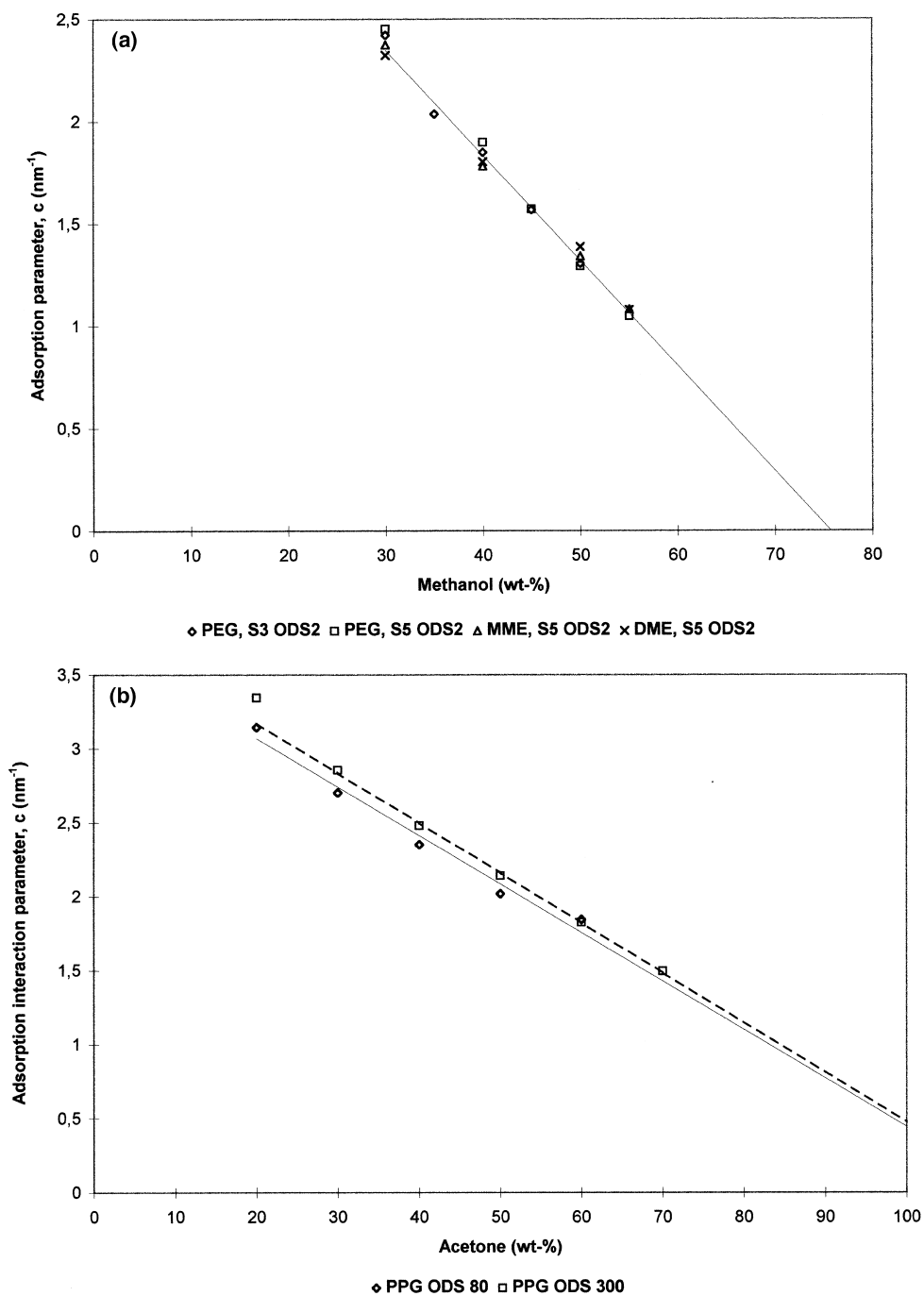


Fig. 8. (a) Dependence of the ethylene oxide adsorption interaction parameter, c , upon the content of methanol in the methanol–water solvent. Data obtained for PEG, MME and DME on S5 ODS2 column, and for PEG on S3 ODS2 columns. The common straight line approximation over all points is shown by a solid line. (b) Parameter of adsorption interaction c for PPG macromolecules as a function of composition of acetone–water mobile phase. Data for S5 ODS2 and SX5 C₁₈ columns. The straight-line approximations for each series are shown.

straight line dependence: as can be seen from Fig. 8, in the system under investigation adsorption interaction decreases with increasing methanol content in the mixed mobile phase. According to the theory, the zero value of the c parameter corresponds to the critical condition, at which the dependence of the distribution coefficient on polymer molecular mass disappears. By suggesting the linear tendency in Fig. 8a and by extrapolating this tendency to $c=0$, the value for the critical methanol content 76%, is obtained for this system.

Fig. 8b presents the analogous data for PPG in the acetone–water mobile phase. Again, the data obtained for different C_{18} -stationary phases are close enough.

Fig. 8b shows that, as in the case of PEG and methanol–water, the less water that is in the mobile phase, the less is the adsorption interaction. However in the PPG and acetone–water system it seems impossible to reach the critical conditions at normal temperature: lines in Fig. 8b do not intersect the abscissa axis. Possibly the critical conditions could be realized in this system at a different temperature.

The average thickness of adsorbed polyether macromolecules $H=c^{-1}$ in both systems has an order of several Ångström and decreases with increasing of water content—see Tables 2 and 3.

The linear extrapolation of the data of Fig. 8a and b to pure water gives for both PEG and PPG, the same estimate of $H\approx 2.6$ Å for thickness of adsorbed layers formed by these polymers in pure water.

5. Conclusions

The following main conclusions can be drawn from these studies:

(1) The retention behaviour of PEG macromolecules in RP–LAC using Spherisorb C_{18} adsorbents and mixed methanol–water mobile phase in the whole range of molecular mass studied proved to be (both qualitatively and quantitatively) in good accordance with the molecular-statistic theory of chromatography of flexible homopolymers accounting for adsorption interactions. The same also proved to be true for the PPG and acetone–water system. The theory expresses the distribution coefficient K as a function of three parameters: the molecule dimension

in the solution, R , the adsorbent pore size, d , and adsorption interaction parameter, c . Application of this theory to the experimental data made it possible to determine the adsorption interaction parameter c of PEG and PPG macromolecules at varying mobile phase composition.

(2) It is shown that dependent on the value of the Rc product, two regimes of adsorption chromatography of polyether macromolecules can be realized. At high values of Rc , the high-molecular-mass regime takes place, at which adsorbing macromolecules form pancake-type structures on the pore walls, characterized by an average thickness $H=c^{-1}$. At low Rc , most macromolecules inside adsorbent pores retain their three-dimensional structure, and only a moderate chromatographic retention is characteristic for this low-molecular-mass regime.

(3) In the high-molecular-mass regime, the value of $\ln(K - K_{SEC})$ is a linear function of the number of repeating-chain units, n . The formulae are obtained by expressing the slope and intercept of this linear dependence as functions of the adsorption interaction parameter c and pore size, d . This linear dependence is equivalent to the well-known Martin rule relating $\ln(k')$ to n , the present theory giving meaning to the slope and intercept of linear $\ln(k')$ vs. n plots. In the low-molecular-mass regime, both linear approaches are not applicable. The general theory reasonably describes both high- and low-molecular mass regimes.

(4) The studies on chromatography of PEG, MME and DME macromolecules revealed that even though the theory for homopolymers cannot correctly describe the chromatographic behaviour of macromolecules with terminal units differing in their adsorption ability from nonterminal ones, the high-molecular-mass linear approach can also be used for such polymers to determine the correct values of adsorption interaction parameter for repeating chain units.

(5) The dependencies of adsorption interaction parameter, c , and of the adsorption layer thickness, H , on the composition of the mobile phase for both experimental systems are obtained: the lower the water content in mobile phase, the weaker is the adsorption, and the higher the layer thickness. By extrapolation of parameter c to the $c=0$ level, the critical conditions for the PEG and methanol–water

system are estimated as being at about 76% of methanol. At the same time, in the PPG and acetone–water system, the critical conditions are shown not to be attainable at the temperature studied.

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