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Theory of liquid adsorption chromatography of macromolecules applied to polyethylene glycol and fatty alcohol ethoxylates

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Abstract

Liquid adsorption chromatography retention modes of poly(ethylene glycol) (PEG), its mono- and dimethyl ethers (MMEs and DMEs) and also of fatty alcohol ethoxylates (FAEs) are studied both experimentally and theoretically. The experimental system under investigation was a Spherisorb S5W 80 Å column and isopropanol–water as the mobile phase. At various compositions of the mobile phase (in the range of 75 to 87% of isopropanol) chromatograms exhibiting good peak resolution were obtained and the dependencies of the elution volume on the number of repeating EO units for both PEG and FAE samples evaluated on the basis of a closer inspection of the chromatographic data. The experiments did not reveal any differences between the chromatographic behavior of PEGs, MMEs and DMEs, while the FAEs gave substantially smaller values of elution volume at all mobile phase compositions. These data were interpreted by using a molecular-statistic theory of homopolymers (to describe both PEG, MME and DME) and two-block copolymers (for FAEs) based on a continuum Gaussian chain model of macromolecules and a slit-like model of pores of stationary phase, wide pore approximation and the adsorption chromatography mode for PEG molecules were assumed in the development of this theory. This theory described very well the experimental data obtained, and two thermodynamic parameters characterizing interactions of EO units of PEG and both EO and $(\text{CH}_2)_n$ chains of FAE molecules with the adsorbent pore walls have been determined from the comparison of the theory and the experiments. Although the mean thickness of adsorbed oxyethylene chain, H , was estimated as being equal to about 3.5–4.5 Å, H proved to be slightly decreasing with increasing isopropanol content in mobile phase. Chromatograms visualizing the adsorption of PEG and FAE molecules are presented, and the correspondence between the theoretical approach and the experimental situation under investigation is discussed. © 1997 Elsevier Science B.V.

Keywords: Thermodynamic parameters; Adsorption; Poly(ethylene glycol); Fatty alcohol ethoxylates; Ethoxylates; Polymers; Polyethers; Block copolymers

1. Introduction

Polyethylene glycol (PEG) and its derivatives are extensively used in pharmaceutical and biomedical technologies, and also serve as important ingredients

in the food and perfumery industries. Water soluble ethoxylated fatty alcohols (FAEs) typically containing polymer homologous series of polyoxyethylene with hydrophobic fatty alcohol end groups are also of a great importance from both the fundamental and industrial point of view. FAEs are used as rheology modifiers in paint additives, oil recovery, drug

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delivery systems, etc., as steric stabilizers in ink and pigment vehicle and are also in widespread use as non-ionic surfactants. Fatty alcohol derivatives of PEG are used as wetting and antistatic agents in textile industry, as detergent components, and also in the production of urethane fibers.

With respect to their chemical structure, ethoxylated fatty alcohol molecules are block-copolymers. To give a full characterization to FAEs as block-copolymers, i.e., to obtain the link between the distributions of molecular mass and chemical composition, it is necessary to use two-dimensional analytical methods, of which the chromatographic methods are the main ones [1–3]. In analyses of PEGs and of PEG derivatives and in separations of these polymers according to the number of EO groups, liquid adsorption chromatography (LAC) using silica based porous materials as stationary phases is widely employed [4].

As has been demonstrated recently in studies of PEG separation [5–7] this technique can yield chromatograms with completely resolved peaks for all individual oligomer homologues for samples of average degree of polymerization up to 40 repeating EO units. Another interesting approach is a chromatography under critical conditions, which proved to be especially useful in separations of heteropolymers [2,8].

To achieve a better understanding of the mechanisms involved in the chromatographic separations of PEG and PEG derivatives, a theory is required which could adequately describe main features of chromatography of homopolymers and copolymers.

A theoretical description of chromatography was first realized by Casassa [9–11] who developed the theory of gel-permeation (size-exclusion) chromatography of polymers, but adsorption effects have not been taken into account in the Casassa theory.

A general theory of chromatography of homopolymers accounting for adsorption effects has been proposed by Gorbunov and Skvortsov [12,13]. This theory proved capable of describing both size-exclusion, adsorption and critical modes of chromatography of polymers, and also to explain a transition from the size-exclusion to the adsorption mode via the critical condition chromatography approach, which had been first observed by Tennikov and

coworkers [14,15]. According to the theory [12,13] (see also the review in Ref. [16]), different features of chromatography of polymers have an origin in different statistical properties of confined macromolecules under various adsorption conditions.

In the present paper we make use of the results of the theory [12,13] applying them to the experimental data obtained from the chromatography of polyethylene glycol. This will give an opportunity to determine the adsorption interaction parameter and the mean thickness of adsorbed PEG or FAE molecules at varying compositions of the isopropanol–water mobile phase.

It is reasonable to consider FAE macromolecules as two-block copolymers. It is a quite complicated task to develop a molecular-statistic theory of chromatography of copolymers, since this requires not only to simulate a conformational structure of macromolecules and a structure of porous stationary phase, but also to account for the chemical structure of copolymer molecules (their composition, architecture and compositional heterogeneity), as well as for possible modes of interaction of copolymer chain units with a stationary phase. The principles of the theory of chromatography of block-copolymers have been developed [18,19], but till now for many important modes of chromatography of block-copolymers there is a lack of theoretical results suitable for experimental data treatment. In this paper we present the results of a theory of chromatography of two-block copolymers, applicable for systems including wide-pore adsorbents and copolymers containing only one component capable of strong adsorption. This theory will then be applied to describe the chromatographic behavior of FAE macromolecules, and thus provide an opportunity to estimate the parameters of adsorption interaction at various values of mobile phase composition.

2. Experimental

PEGs, their mono- and dimethyl ethers, monodisperse EO-oligomers, fatty alcohols and the corresponding ethoxylates (Brij) were purchased from Fluka (Buchs, Switzerland) and used without further purification. Several ethoxylates based on pure fatty

alcohols were provided by H.-R. Holzbauer, Institute of Applied Chemistry Adlershof (Berlin, Germany).

Brij 30 (Fluka), a commercial fatty alcohol ethoxylate with an average degree of ethoxylation of 2, which contains mainly dodecyl, but also tetradecyl and hexadecyl end groups, was fractionated by semi-preparative liquid chromatography under critical conditions (LCCC) on a 250×10 mm I.D. column packed with Spherisorb ODS2 (5 μm particles, average pore size 8 nm) from Phase Separations (Deeside, Clywd, UK) with methanol–water (90:10, w/w) at a flow-rate of 2.0 ml/min, as described in previous papers [3,7].

Analytical measurements were performed on a modular high-performance liquid chromatography (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 RI detector (Bischoff, Leonberg, Germany). Data acquisition and processing was performed using the software CHROMA, which is part of the DDS 70.

All measurements were performed on a 250×4.6 mm I.D. column packed with Spherisorb S5W (bare silica, average particle size 5 μm, pore diameter 8 nm) from Phase Separations at a flow-rate of 0.50 ml/min and a column temperature of 25.0°C.

The void volume and the pore volume of the column were determined with polystyrene standards obtained from Polymer Laboratories (Church Stretton, UK) in tetrahydrofuran (THF), presuming that no interaction with the stationary phase takes place under these conditions:

$$V_0 = 3.32 \text{ ml}; \quad V_p = 1.37 \text{ ml}$$

The sample loop volume was 50 μl and the injected concentrations were 5–15 g/l. The solvents obtained from Promochem (Wesel, Germany) were HPLC grade. For the preparative fractionation, methanol was distilled prior to use. Mobile phases were mixed by weight and vacuum degassed. The composition was controlled by density measurement using a type DMA 60 density measuring device (mechanical oscillator principle) equipped with the

measuring cell DMA 602 M, both from A. Paar (Graz, Austria).

3. Chromatographic data and reproducibility

We investigated a large number of chromatograms for PEG and for its mono- and dimethyl ethers (MME and DME, correspondingly), as well as for several samples of FAEs. These data were obtained under the condition of the normal-phase LAC at different compositions of isopropanol–water mobile phase (in the range of 75 to 87% of isopropanol). It seems tedious to present an extensive set of quite similar original data and, therefore, we shall demonstrate only a few typical examples.

As has been shown previously [3,4,6,7], PEGs and their derivatives can be separated very well according to molecular mass by isocratic normal-phase LAC using isopropanol–water as the mobile phase.

Fig. 1a shows a chromatogram obtained at 85% (w/w) of isopropanol for PEG monomethyl ether 350, and Fig. 1b a section of a chromatogram obtained for an ethoxylate of 1-dodecanol containing an average number of EO groups of 20 [in the following text, abbreviations like $C_{12}(EO)_{20}$ will be used for such samples]. As can be seen, in both cases the resolution is good, and peak maxima positions can be obtained with high accuracy for 15–20 oligomers. Depending on the composition of the mobile phase, different ranges of molar mass can be analyzed. Using monodisperse oligomers (either commercial ones or such obtained by semipreparative HPLC [3]) as internal standards, peaks can be identified and their number of repeating EO groups, n , determined. Owing to this fact, the processing of each chromatogram offers an opportunity to obtain the dependence of elution volume, V_e , on the number of EO groups, n . Such dependencies, obtained for different polydisperse PEG samples at 80% of isopropanol in the mobile phase are shown in Fig. 2.

Sufficient reproducibility was obtained and, in order to achieve more precise results, the data shown in Fig. 2 can be averaged. Further we shall use only the results, averaged over a number of data sets (from two up to five sets have been used as a rule for averaging).

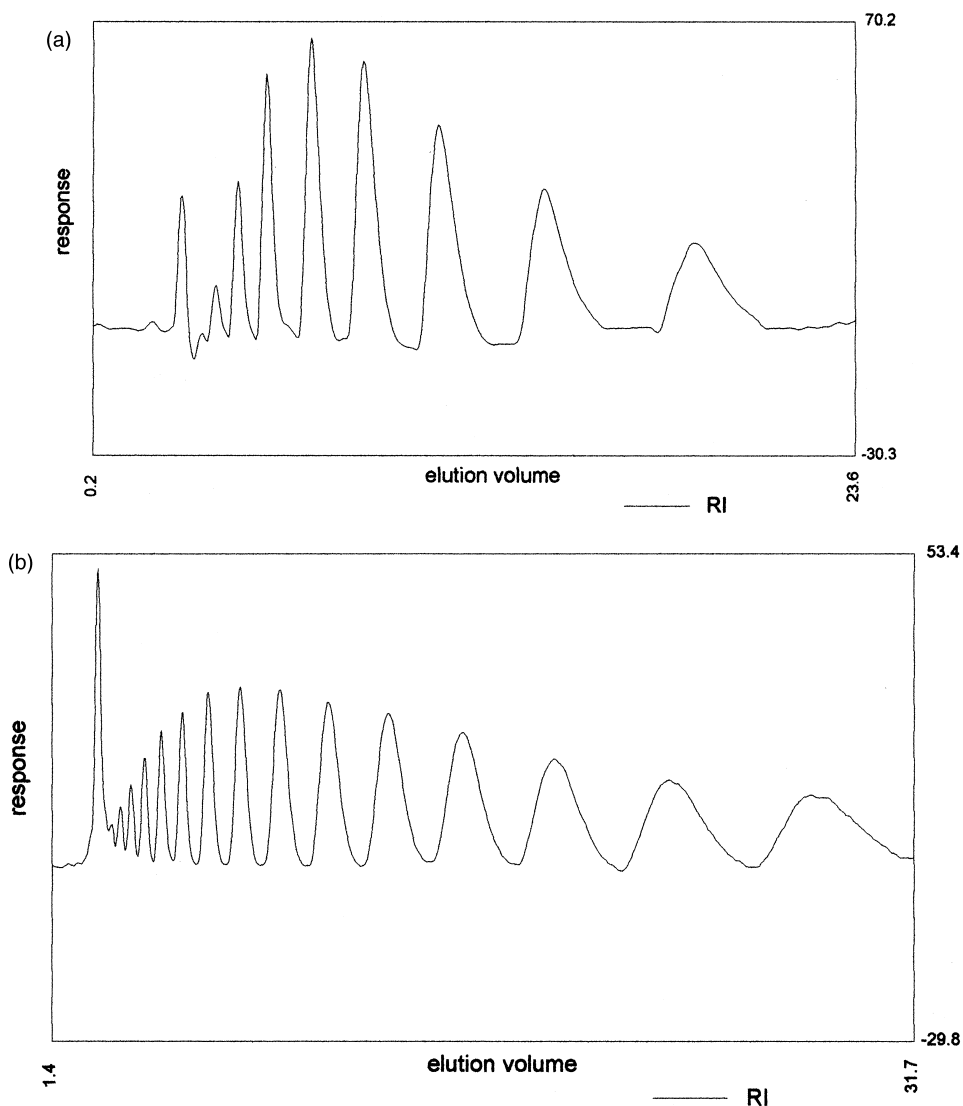


Fig. 1. (a) Chromatogram of a PEG monomethyl ether, MME 350 (Fluka), which was obtained on a 250×4.6 mm column packed with Spherisorb S5W (particle size $5 \mu\text{m}$, pore diameter 80 nm) in isopropanol–water (85:15, w/w) at a flow-rate of 0.5 ml/min . Injected volume: $50 \mu\text{l}$, sample concentration: 6.49 g/l . Detection: RI. (b) Chromatogram of a FAE based on 1-dodecanol, average degree of ethoxylation of 20, which was provided by H.-R. Holzbauer, Institute of Applied Chemistry Adlershof, Berlin, Germany. Column: 250×4.6 mm, Spherisorb S5W (particle size $5 \mu\text{m}$, pore diameter 80 nm) in isopropanol–water (82:18, w/w) at a flow-rate of 0.5 ml/min . Injected volume: $50 \mu\text{l}$, sample concentration: 12.06 g/l . Detection: RI.

Fig. 3a and Fig. 3b show a comparison of data on PEG and its mono- and dimethyl ethers. As can be seen, the results for PEG, MME and DME are very close to each other, the differences being of the order of the reproducibility errors. Similar results were obtained for PEG, MME and DME at all other

mobile phase compositions investigated. Thus, in the present system end-group effects proved to play a negligibly small role in the chromatographic behavior of these three types of polymers. Therefore one can suppose that under the conditions of LAC in an isopropanol–water system PEG, MME and DME

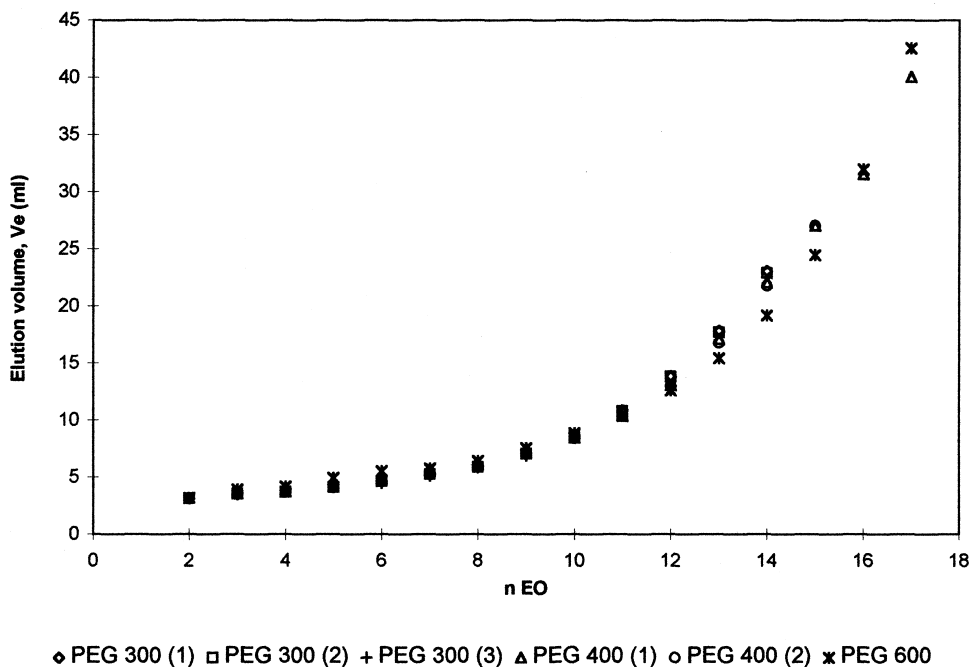


Fig. 2. Elution volume of PEG, V_e , as a function of the number of repeating ethylene oxide units, n , obtained from the chromatograms for repeated injections of different samples (PEG 300, PEG 400 and PEG 600, all from Fluka). S5W column, isopropanol–water (80:20).

macromolecules behave as homopolymers, all of the repeating units of these molecules contributing to the same extent to the adsorption onto the stationary phase.

A comparison of the chromatographic data for PEG and FAE macromolecules shows, however, a quite different picture: in Fig. 4, the elution volumes measured for PEG and for several FAE samples at 87% isopropanol are plotted versus the number of EO groups, n . As can be seen, the elution volumes obtained for FAE molecules are substantially lower than those measured for PEG at the same n values. Obviously, there is no substantial dependence of the chromatographic data upon the average number of CH_2 groups in FAE samples (at least for those with 8 to 14 carbon atoms in the alkyl group).

Very similar results for PEG and FAE were obtained at all other mobile phase compositions studied. It can be concluded from these data that the hydrocarbon block of the FAE molecule is either not adsorbing at all, or has an only weak adsorption ability, which indicates, that the net effect resulting from entropy and enthalpy contributions into the

interaction between this block and an adsorbent has features characteristic of repulsive interactions.

These qualitative conclusions were the basis for the further development of the theory which will be then used for the quantitative data treatment.

4. Theoretical

The main goal of a molecular statistic theory of chromatography is to obtain a distribution coefficient for molecules of a given type – this parameter being in the close relation with the directly measurable chromatographic peak position values.

The distribution coefficient is an equilibrium thermodynamic parameter which is known to be equal to the ratio of partition functions of a molecule in the stationary and mobile chromatographic phases (i.e., inside the adsorbent pores and in an unrestricted space of the same volume). Thus, the partition functions for a mathematical model simulating the given specific “molecule–mobile phase–stationary phase” real system have to be calculated. The factors

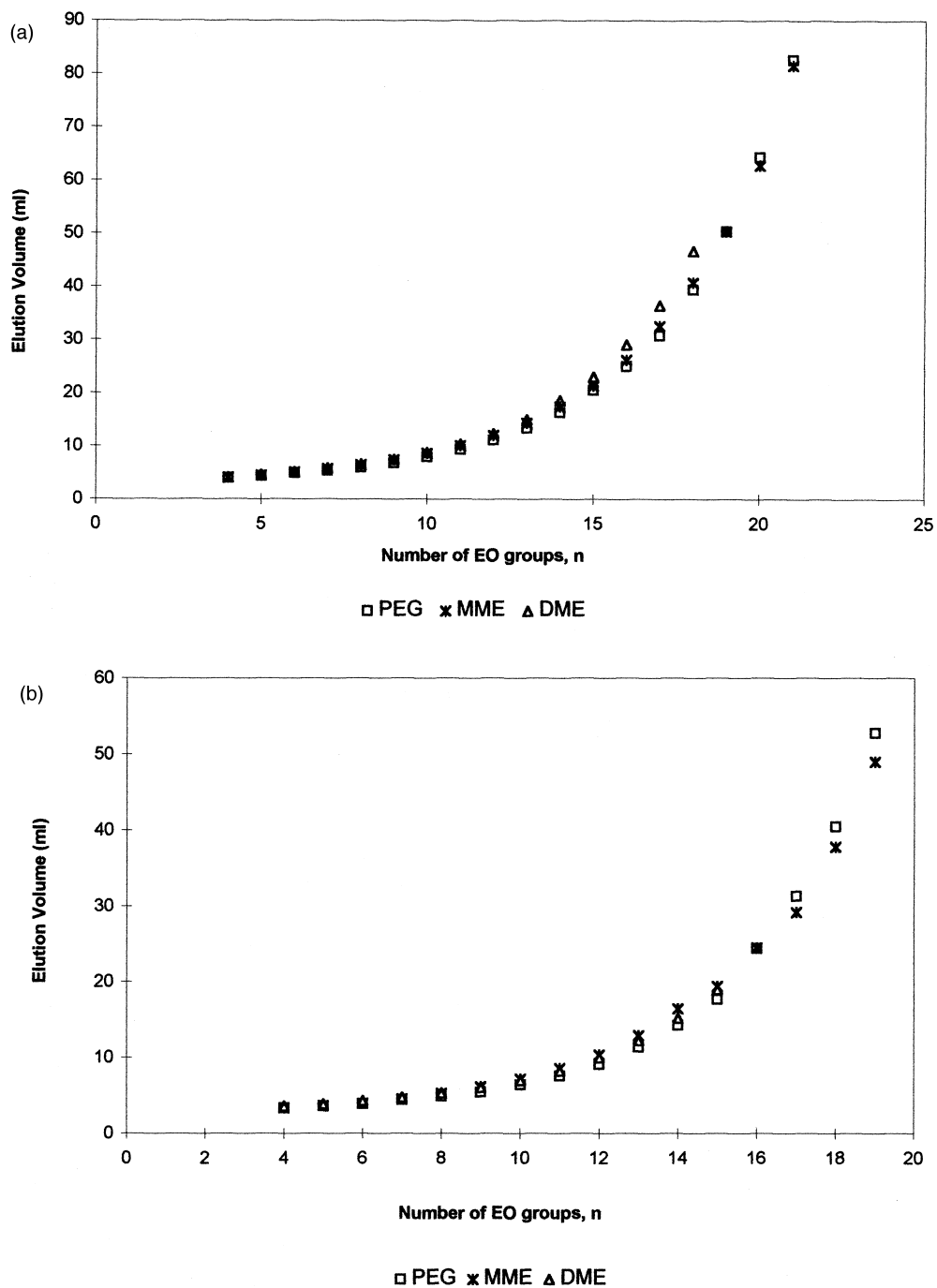


Fig. 3. Dependences of elution volume V_e on the number of repeating EO units n for molecules of PEG, MME and DME obtained at 75% (a), and at 82% (b) of isopropanol.

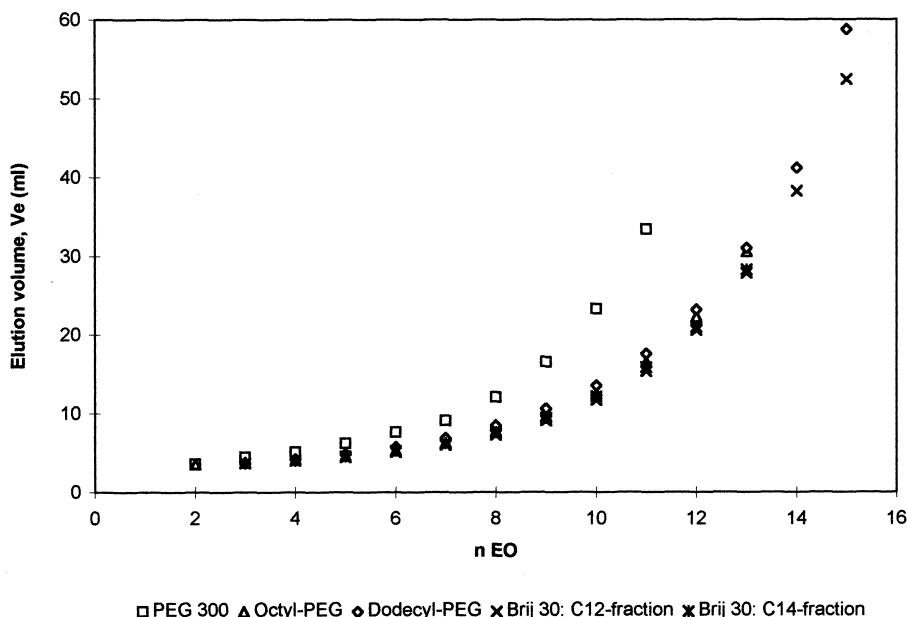


Fig. 4. Plot of the elution volume V_e vs. the number of EO units plots for PEGs and several samples of FAEs. Experimental conditions: S5W column, isopropanol–water (87:13).

usually taken into account in such calculations are the chemical and conformational structure of molecules, the pore size and shape, and also the possible interactions between molecules and pore walls.

4.1. Theory of chromatography of flexible-chain homopolymers

PEG and its derivatives, the chromatographic behavior of whom is studied in the present paper, are polymers having a high chain flexibility. The PEG molecules with a degree of polymerization of the order of ten are known to adapt the chaotically rolled up and entangled coil-shaped conformations in solution, 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 usually are based on the model of an ideal polymer chain (i.e., on the Gaussian chain model). It is a well-known fact, that every Gaussian chain conformation corresponds to a certain trajectory of a Brownian particle random walk, and this fact

produces direct analogies between polymer and diffusion-type problems. In a continuum approach the use of this model very often gives an opportunity to obtain the strict formulae for statistical and thermodynamic parameters of macromolecules by solving the diffusion equation together with appropriate boundary conditions.

The first molecular-statistical theory of this kind describing the features of gel permeation (size-exclusion) chromatography of macromolecules has been developed by Casassa [9–11]. The Casassa theory has been verified in numerous experiments and has gained universal recognition. Casassa used the pore models of a simple shape (namely the slit-like, cylindrical and spherical ones) and considered the situation for which the absence of adsorption interactions between a molecule and pore walls was characteristic. The basic result following from the theory [9–11] is the conclusion that the distribution coefficient in the exclusion chromatography 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

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

Further progress in the theory of chromatography of polymers was made by considering appropriately the effect of adsorption of macromolecules on pore walls in the theory.

The problem of polymer adsorption on a planar surface has been intensively studied over the last 4 decades, and several theoretical approaches were elaborated to describe the polymer adsorption phenomena. The well known early theoretical papers on polymer adsorption [19–23] were based on lattice models of an infinitely long polymer chain, where the energy parameter, ε , served to account for the adsorption of chain units. In continuum model based theories [12,13,24–28] the adsorption effects were simulated by applying appropriate boundary conditions at the adsorbing surface, and by solving the correspondent diffusion equation boundary problem.

The boundary conditions describing the adsorption of macromolecule in an adequate way was proposed by De Gennes in Ref. [29], and the corresponding parameter of adsorption interaction, c , was considered in this fundamental paper.

The parameter c defines the inverse correlation length of adsorption, which can serve as a characteristic of the structure of a macromolecule inside a pore. According to [30], in the adsorption regime the value of $H=c^{-1}$ is equal to the mean thickness of a layer formed by a macromolecule on a pore wall – as is shown schematically in Fig. 5b.

Positive values of parameter c 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. The parameter of adsorption interaction in a continuum model, c , was found to be directly related to the adsorption energy parameter in a lattice model, ε . According to Ref. [31], this relation has the following form:

$$ac = \sqrt{\frac{6}{1 - \exp(\varepsilon_{cr})}} [\exp(\varepsilon_{cr}) - \exp(\varepsilon)],$$

at $-\varepsilon < -\varepsilon_{cr}$

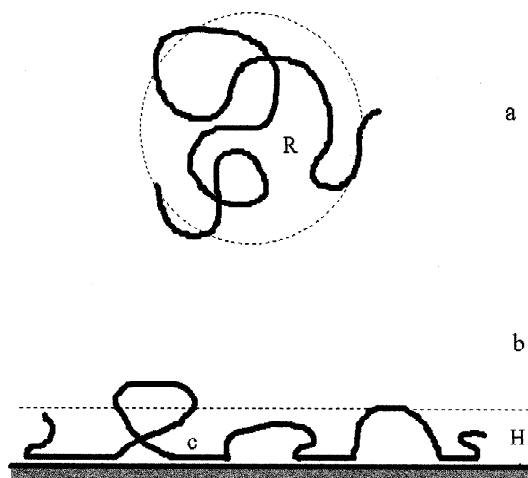


Fig. 5. Typical conformations of a flexible-chain macromolecule in the solution (a), and near the adsorbing surface (b). Model parameters are indicated: R : root-mean-square end-to-end distance for a macromolecule in the solution, c : parameter of adsorption interaction, and $H=c^{-1}$: average thickness of an adsorbed polymer molecule.

$$ac = \sqrt{6\{\ln[2\exp(\varepsilon_{cr}) - 1] - \varepsilon\}},$$

at $-\varepsilon > -\varepsilon_{cr}$ (2)

where a is the chain unit size (mean random walk step length). In the vicinity of the critical point c is proportional to the deviation of ε from the critical value, ε_{cr} : $c \sim (\varepsilon_{cr} - \varepsilon)$. Large $-\varepsilon$ values are in accordance with strong adsorption interaction expressed by the term $c \sim \varepsilon^{1/2}$.

The theory of chromatography of homopolymers being adsorbed on the pore walls has been built both for the lattice [32] and for the continuum [12,13] models of a Gaussian chain and a slit-like pore. According to Refs. [12,13], the distribution coefficient, K , is a function of three parameters: polymer coil size, R , pore width, d , and parameter of adsorption interaction, c . The rigorous formulae expressing the distribution coefficient K at arbitrary c , R and d values, obtained in Refs. [12,13], have a complicated form, which is not convenient for the treatment of experimental data. Herein we are primarily interested in the case where $R < 2d$, since it is this situation that is characteristic of the experimental system under investigation.

At $R < 2d$ it becomes possible to derive an approximate formula for the distribution coefficient making

use of the known solution of the diffusion equation with adsorption boundary conditions for a macromolecule at a single flat surface, which has been obtained in Refs. [24,25]. We consider it useful to give here the basic steps of the derivation of the formula for distribution coefficient, because this similar approach will be used in developing the theory of chromatography for a two-block copolymer.

In Ref. [24] the formula has been obtained for a partition function $P(z,R,c)$ of a macromolecule, having one of its ends at a point on a distance z from an adsorbing plane, $z=0$, the other end being at a different position within the molecule.

This formula has the following form:

$$P(z,R,c) = 1 + \exp(-\eta^2) \cdot [Y(\eta + \Gamma) - Y(\eta)] \quad (3)$$

where $\eta = z\sqrt{6}/2R$, $\Gamma = -cR/\sqrt{6}$, and the function $Y(t)$ is defined as:

$$Y(t) = \exp(t^2) \cdot \operatorname{erfc}(t) \quad (4)$$

where $\operatorname{erfc}(t)$ is the well-known special function (the complementary error integral), for which the tables and numerical algorithms are available [33].

Asymptotic forms of the function Y are also known:

$$\begin{aligned} & \frac{1}{\sqrt{\pi}} t^{-1}, \quad \text{at } t \gg 1 \\ Y(t) & \approx 1 - \frac{2}{\sqrt{\pi}} t, \quad \text{at } |t| < 1 \\ & 2\exp(t^2), \quad \text{at } -t \gg 1 \end{aligned} \quad (5)$$

The full partition function of a macromolecule inside a pore can be calculated by integrating Eq. (3) over the whole space within the slit of the width of $2d$. In this way, one can obtain the following expression for the distribution coefficient of a homopolymer at $R < 2d$

$$K = \frac{1}{d} \int_0^d P(z) dz \approx 1 - \frac{2}{\sqrt{\pi}} \cdot g - \frac{g}{\Gamma} [1 - Y(\Gamma)] \quad (6)$$

where $g = R/d\sqrt{6}$, $\Gamma = -cR/\sqrt{6}$ and the Y function being defined by Eq. (4).

Eq. (6) was analyzed in Refs. [12,13]. It has been shown, in particular, that depending on the value and

sign of a product $\Gamma = -cR/\sqrt{6}$ three main regimes of the chromatography of polymers are possible.

At $\Gamma \gg 1$, Eq. (6) can be reduced to Eq. (1) describing the regime of size-exclusion chromatography of macromolecules. At $\Gamma = 0$ the distribution coefficient becomes equal to unity, what is characteristic of the polymer chromatography under critical conditions. The case of $-\Gamma > 1$ corresponds to the conditions of adsorption chromatography at which K increases with molecular mass in accordance with an approximately exponential law. Obviously, it is this latter regime that has been realized in the experimental system under consideration.

4.2. Theory of chromatography of two-block copolymers

The fundamentals of the theory of chromatography of two-block copolymers are described in Refs. [17,18], and the general formulae for the distribution coefficient of two-block copolymer are available. These formulae, which contain infinitely long power series, are, however, too complicated and inconvenient for use in the treatment of experimental data.

In this section we shall derive more simple approximate formulae, which are expected to be applicable in the case where the size of a macromolecule is less than the pore size. We shall also assume that one of the blocks in a copolymer molecule is able to adsorb much more strongly than the other one.

In Fig. 6, the adsorption of a two-block copolymer of the $A_m B_n$ type is visualized. As in Section 4.1 we shall use the continuum model of a Gaussian coil in a slit-like pore. Let us introduce for block A the root-mean-square end-to-end distance, R_A , characterizing the mean size of this block in the solution, and let R_B be introduced analogously. Adsorption interactions of A and B components are assumed to be different, and therefore c_A and c_B are introduced, which describe the A and B adsorption interactions. Block B will be assumed as the more strongly adsorbing one.

An expression for the distribution coefficient of a two-block copolymer, K_{AB} , may be derived in a following way. Let us consider a two-block macromolecule AB, having the point of AB-junction at the

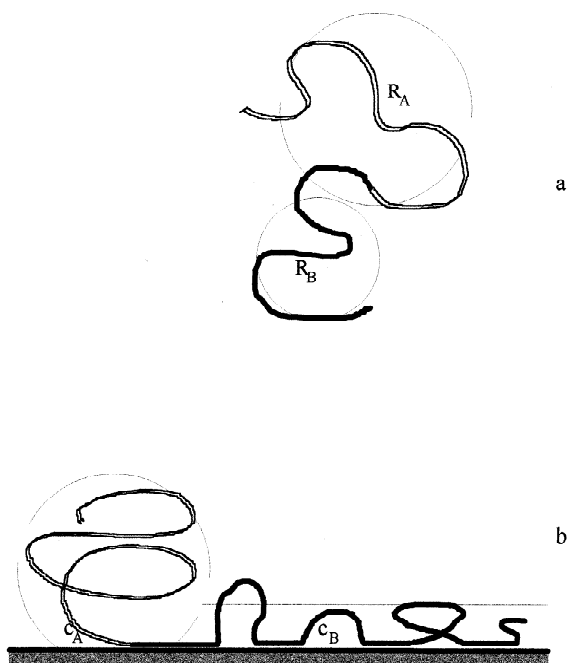


Fig. 6. Gaussian chain continuum model of a macromolecule of two-block copolymer in solution (a), and near the pore wall (b). A situation is shown, where block B is adsorbed, and the interactions of the type A block with the pore wall bear repulsive features.

distance z from the adsorbent surface. We may then arrange K_{AB} in the form:

$$K_{AB} = \frac{1}{d} \int_0^d P_A(z, R_A, c_A) \cdot P_B(z, R_B, c_B) dz \quad (7)$$

where P_A and P_B are the partition functions of the form of Eq. (2) for the blocks A and B.

After substitution of the corresponding expressions for P_A and P_B into this integral, and making the assumption that $-c_B R_B \gg 1$, which means that block B is adsorbed more strongly, the integration of Eq. (7) can be carried out. The final result will then have the following form:

$$K_{AB} \approx K_B - qY\left(-\frac{c_B R_B}{\sqrt{6}}\right) \quad (8)$$

where K_B is the distribution coefficient of an adsorbing macromolecule identical to the block B having the form of Eq. (6), and q is a parameter independent

on the length of the block B, q being the function of the size of the other block A, of pore width, and also of both interaction parameters, c_A and c_B :

$$q = \frac{1}{c_B d} \cdot \frac{c_A \left[1 - Y\left(\frac{c_B R_A}{\sqrt{6}}\right) \right] + c_B \left[1 - Y\left(-\frac{c_A R_A}{\sqrt{6}}\right) \right]}{c_A + c_B} \quad (9)$$

It follows from Eq. (8), that the dependence of a distribution coefficient of two-block copolymer on molecular mass of the adsorbing block should be always of approximately exponential type, provided the pore size is sufficiently large in comparison with the molecule size. This conclusion is also valid for the situation where the second block A is not adsorbing at all. In this special case the coefficient q acquires a more simple form

$$\begin{aligned} q &= \frac{1}{c_B d} \cdot \left[1 - Y\left(\frac{c_B R_A}{\sqrt{6}}\right) \right] \\ &\approx \frac{1}{c_B d}, \quad \text{at } R_A \gg c_B^{-1} \\ &\approx \sqrt{\frac{\pi}{6}} \cdot \frac{R_A}{2d}, \quad \text{at } R_A < c_B^{-1} \end{aligned} \quad (10)$$

If the conditions for block A are critical ones ($c_A = 0$), then the well-known interesting result [17,18,34] can be derived from Eqs. (8) and (9):

$$K_{AB} = K_B \quad (11)$$

which means that the chromatography of block-copolymers under the conditions critical for block A proceeds in such a way as if this block was not present at all.

5. Comparison of theory and experiment

For the purpose of comparison with the theory, experimental values of elution volume, V_e were transformed into the values of distribution coefficient, K , by using the formula:

$$K = (V_e - V_i) / V_p \quad (12)$$

where V_i is the interstitial volume, and V_p is the pore volume.

Fig. 7 demonstrates the dependencies of PEG

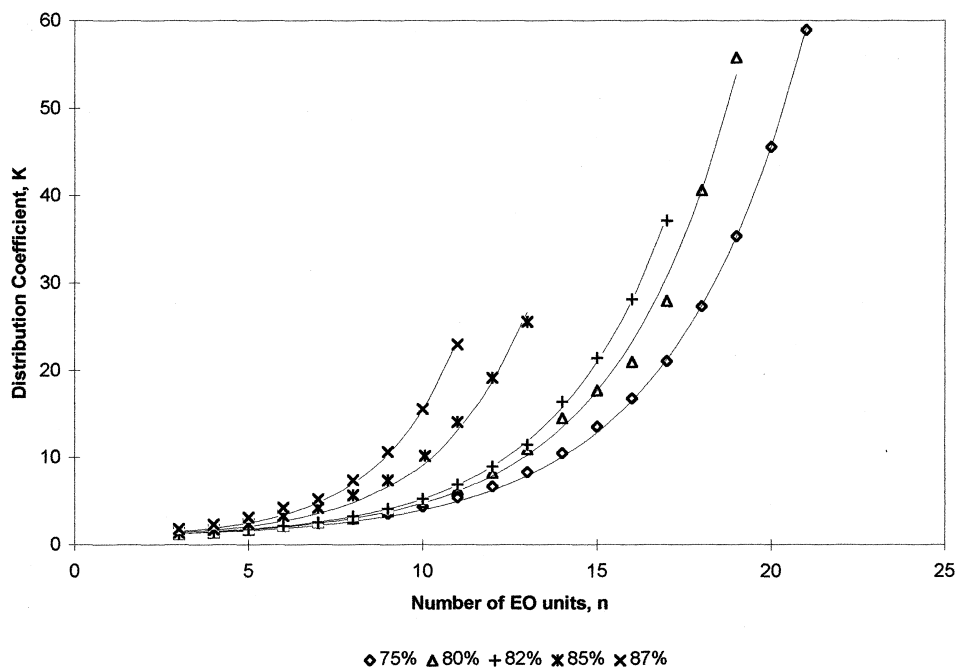


Fig. 7. Dependences of the distribution coefficient, K , upon the number of EO units of PEG molecules. Experimental conditions: S5W column, various percentages of isopropanol in the mobile phase (75%, 80%, 82%, 85% and 87% w/w). Lines are plotted according to Eq. (6) at values of parameter c listed in Table 1.

distribution coefficients on the number of repeating EO groups n at several compositions of isopropanol–water mobile phases. The lines in this figure are drawn according to Eq. (6). In the calculations the d value was taken equal to 4 nm, and the root-mean-square size of PEG macromolecules was estimated according to formula $R_{\text{PEG}} = 0.079M^{0.5}$ (nm) [35] corresponding to the unperturbed dimension of a long polyoxyethylene chain. At every mobile phase composition the appropriate value of the interaction parameter c was selected ensuring the best fit of the theoretical curve to data points. The values of c obtained in such a way are presented in Table 1.

As can be seen in Fig. 7, Eq. (6) describes well the whole set of the experimental data obtained for PEG at different isopropanol–water mobile phase compositions.

As can be deduced from Table 1, there is a systematic trend in c values, when the mobile phase composition is changed: the adsorption interaction parameter c decreases, when the isopropanol percentage in the mixed mobile phase is decreased. It

can be seen, however, that this decrease is not substantial, and there is no evidence that c approximates zero at any isopropanol percentage. This means that it is impossible to realize the critical chromatography mode for the PEG–S5W–isopropanol–water system.

As mentioned above, the value of the parameter $H = c^{-1}$ in the adsorption regime is equal to the mean thickness of a layer formed by an adsorbing macromolecule on a pore wall. For the system under consideration the calculated thickness of adsorbed PEG macromolecules was in the order of a few Å,

Table 1

Adsorption interaction parameter, c , and average thickness of adsorbed PEG macromolecules, H , at varying isopropanol percentages in the isopropanol–water mobile phase

Isopropanol (%)	c (nm ⁻¹)	H (nm)
75	2.26	0.442
80	2.36	0.423
82	2.42	0.413
85	2.7	0.371
87	2.9	0.345

which slightly decreases with an increase of isopropanol percentage – see Table 1.

Now we pass to the treatment of experimental data on the chromatography of FAE molecules. For this purpose we use Eq. (8) from Section 4.2, substituting as c_B into this formula the values of c determined from the data of the chromatography of PEGs, and selecting the coefficient q in such a way, that the best fit between the theory and the experimental data was achieved.

As can be deduced from Eq. (8), the difference $K_B - K_{AB}$ (between the distribution coefficients of a homopolymer identical to the copolymer block B and a two-block copolymer AB) must be proportional to $Y(-c_B R_B / \sqrt{6})$, the slope of this $K_B - K_{AB}$ vs. $Y(-c_B R_B / \sqrt{6})$ straight line being equal to the value of the coefficient q .

Such plots for two isopropanol–water compositions are shown in Fig. 8. As can be seen, the experimental data plotted in the above specified coordinates indeed give the expected straight lines, and the q values for FAE molecules can be easily calculated.

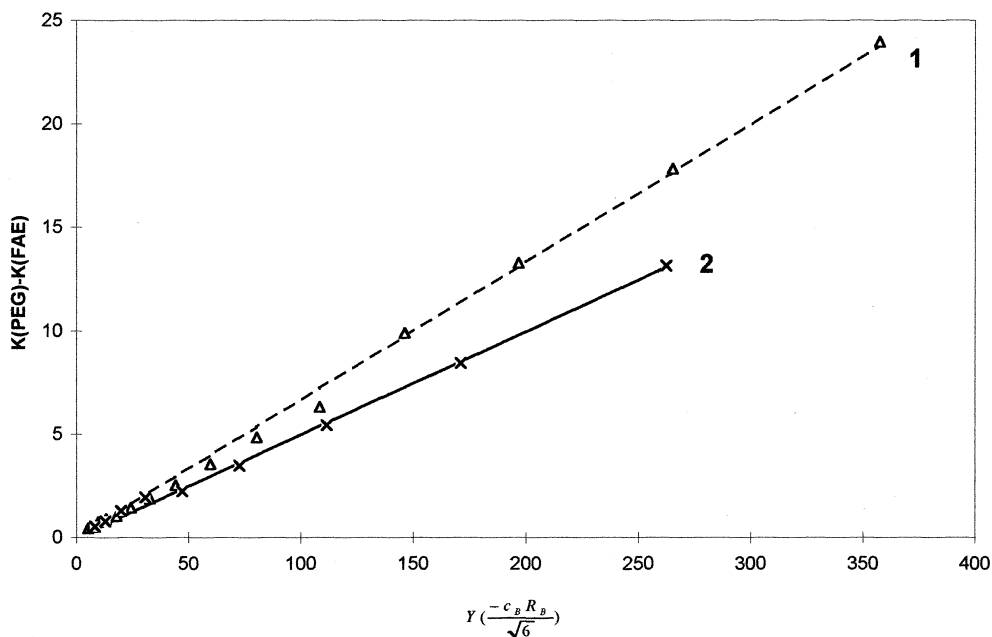


Fig. 8. Difference between the distribution coefficients of PEG and FAE macromolecules plotted versus the function $Y(-c_B R_B / \sqrt{6})$ which was calculated for the ethylene oxide chain using Eq. (4), and the values of $c_B = c$ from Table 1. Experimental conditions: S5W column, 1: $C_{12}(EO)_{10}$ at 82% of isopropanol, 2: Brij 30, C_{12} -Fraction at 87% of isopropanol. Straight lines are drawn according to the linear least-square approximation method.

Fig. 9 shows the dependencies of distribution coefficients, K_{FAE} and K_{PEG} on the number of repeating EO groups, measured under identical conditions (82% isopropanol). The solid line for PEG is calculated according to Eq. (6) at $c = 2.42 \text{ nm}^{-1}$, the line for FAE corresponds to Eq. (8) at the same value of $c_B = c = 2.42 \text{ nm}^{-1}$, and at $q = 0.067$.

It can be seen that by proper selection of only two adjustable parameters, c and q , Eqs. (6) and (8) describe very well the chromatographic behavior of both homopolymer series (PEG) and block-copolymer series (FAE).

All data obtained for other samples of FAE differing in the average number of CH_2 -groups in the alkyl chain as well as varying isopropanol percentages have been processed in the same way and the obtained q values are listed in Table 2.

According to Eq. (9), which is applicable to a block-copolymer of the $A_m B_n$ type with the adsorbing block B, the factor q is a function of four parameters: pore size, d , block A size, R_A , and of two polymer–adsorbent interaction parameters, c_A and c_B . Therefore it is possible to calculate the value

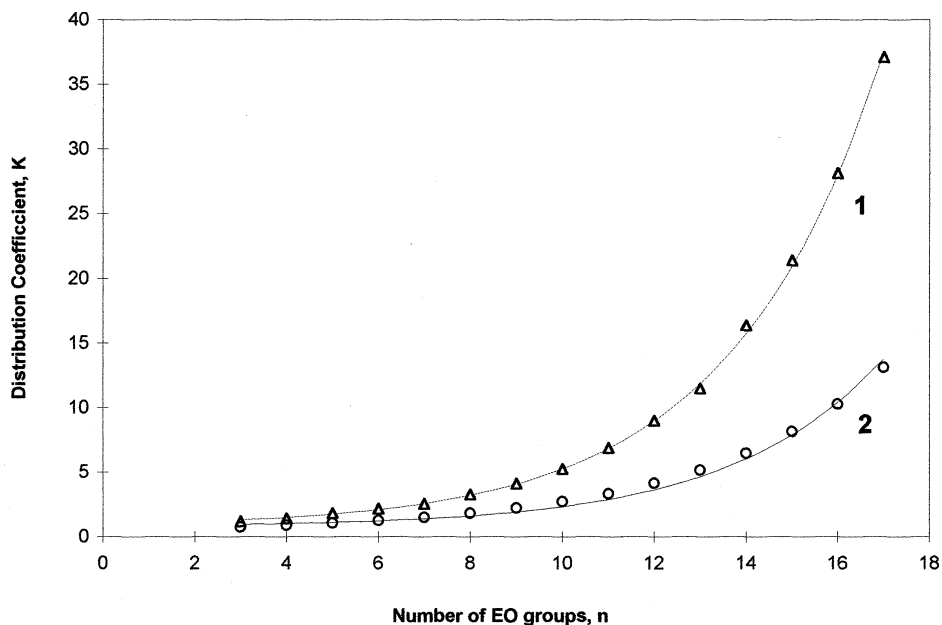


Fig. 9. Experimental (points) and theoretical (lines) dependencies of the distribution coefficient, K , upon the number of EO groups, n , for PEG (1) and FAE $C_{12}(EO)_{10}$ (2) at 82% of isopropanol. Lines (1) and (2) are drawn according to Eqs. (6) and (8), respectively, using the following parameter values: $c_B = c = 2.42 \text{ nm}^{-1}$ and $q = 0.067$.

of c_A by solving Eq. (9) numerically, provided that the q value is determined and the parameters d , R_A and c_B are also known. We have performed these calculations for the FAE copolymers at various solvent compositions, substituting into Eq. (9) the values of c_B and q obtained as stated above and

listed in Table 2. The value of d was taken equal to 4 nm, and R_A values were estimated by using the formula for the unperturbed dimension of a long $(-\text{CH}_2-)_m$ chain $R_A = 0.106M^{0.5}$ (nm) [35]. The calculated c_A values are also listed in Table 2. All calculated values of the interaction parameter for the

Table 2

Factor q in Eq. (8), and interaction parameters of the oxyethylene block, c_B , and of the alcohol block, c_A , obtained for various samples of FAEs at various isopropanol–water mobile phase compositions

Copolymer	Isopropanol (%)	q	c_B (nm^{-1})	c_A (nm^{-1})
$C_{12}(EO)_n$	80	0.055	2.36	-1.39
$C_{16}(EO)_n$	80	0.059	2.36	-1.2
$C_{12}(EO)_{10}$	82	0.067	2.42	-1.59
$C_{12}(EO)_{20}$	82	0.067	2.42	-1.61
$C_{12}(EO)_n$	85	0.05	2.7	-1.35
$C_{14}(EO)_n$	85	0.05	2.7	-1.22
$C_{16}(EO)_n$	85	0.053	2.7	-1.2
$C_8(EO)_n$	87	0.047	2.9	-1.75
$C_{12}(EO)_n$	87	0.042	2.9	-1.22
Brij 30 (C_{12} -fraction)	87	0.05	2.9	-1.43
Brij 30 (C_{14} -fraction)	87	0.048	2.9	-1.25

alkyl moiety of FAE copolymer, c_A , proved to be negative.

This means that the entropic effects prevail in the interactions of alkyl blocks with the S5W porous stationary phase in an isopropanol–water system, resulting in repulsive interactions. At the same time, the absolute values of c_A occurred were not of the order of infinity but rather in the order of one, which implies that enthalpy effects were also involved, but these enthalpy effects were too weak to affect repulsive interactions.

Unfortunately, the method of estimation of c_A does not allow a determination of c_A with high accuracy, as can be seen from the scatter of the values in Table 2. Nevertheless, it is obvious that there is no substantial dependence of c_A parameter on mobile phase composition in the studied range.

6. Discussion

Now let us discuss to what extent the present theoretical approach does correspond to the experimental situation under investigation.

The theoretical formulae (Eqs. (6)–(9)) have been derived under the assumption that the size of macromolecules is substantially lower than the pore size. The average pore size of the porous stationary phase, $2d$, used in the present experiments was equal to 8 nm, whereas the maximum average sizes of PEG and FAE molecules did not exceed the value of 3 nm and, for this reason, the above assumption was fulfilled. The approximate formulae (Eqs. (6)–(9)) for two-block copolymers contain also the assumptions that one of the block is adsorbing, while the other one is not adsorbed or only able to weak adsorption. The estimated values of c_B and c_A listed in Table 2 are in accordance with these assumptions.

During the development of the theory we made use of the Gaussian-coil model which is known to describe correctly the properties of sufficiently long macromolecules. Usually such models work successfully enough, when a polymer chain contains at least three to five and more statistical chain segments (i.e., Kuhn segments). The investigated PEG and FAE samples contain polyethylene oxide molecular chains having up to approximately four statistical segments, and therefore, the real system was only partially

consistent with the theoretical model. However, surprisingly Eqs. (6) and (8) have adequately described the chromatographic behavior of both PEG and FAE macromolecules in the whole range of their molecular mass. With respect to our calculation of the interaction parameter for the non-adsorbing block, c_A , by solving Eq. (9), one should of course consider the calculated values of c_A as an only rough estimate. The reason for this consists in the fact that the investigated FAE copolymers contain alkyl blocks of only 8 to 16 repeating CH_2 -groups, (i.e., corresponding to an equivalent of 1–2 Kuhn segments), but also in the low sensitivity of Eq. (9) when this equation is used for the determination of c_A in the range of negative c_A values. Nevertheless we consider the approach based on Eqs. (8) and (9) being quite useful and informative, especially if this approach is applied to the chromatographic behavior of higher molecular mass copolymer samples.

In the present paper we have compared the experimental data on LAC of PEGs and of their FAEs with the results of the molecular-statistic theory of chromatography of homopolymers and two-block copolymers, and succeeded in the calculation of two adsorption interaction parameters. Thus, this theory describes the chromatographic behaviour in the system under consideration in an appropriate way.

Apart from the evident advantages in understanding the mechanism of chromatography and resulting in good agreement between theory and experiment, such calculations may be of additional interest due to their relation not only to chromatography but also to the other phenomena including polymer adsorption. The theories describing the structure of macromolecules near the surface of the stationary phase have been published [16]). In the present paper we use only one of the results of the structural theory, namely, the conclusion that the value of $H=c^{-1}$ defines the mean adsorption layer thickness. At the same time, provided that the interaction parameter is determined from the chromatographic measurements, it is possible in principle to obtain the other characteristics of the structure of macromolecules (the fraction of adsorbed units, the chain unit density profiles near the adsorbing interface and inside the pores, etc.).

In the case of block copolymers the knowledge

about the interaction parameters allows to predict the chromatographic behavior and structures for molecules which are very different with respect to their molecular mass and chemical composition.

In our opinion, it would be very interesting to obtain data on the structure of macromolecules inside porous stationary phases using the independent method for structural elucidation (for example, those described in Ref. [36]), and to compare these data with the results calculated from the chromatographic measurements.

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