ELSEVIER

Journal of Chromatography A, 890 (2000) 195–210

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Liquid adsorption chromatography of polyethers: experiments and simulation

Bernd Trathnigg[a,*], Alexei Gorbunov[b], Alexander Skvortsov[c]

[a]*Institute of Chemistry, Karl-Franzens Universität Graz, Heinrichstraße 28, A-8010 Graz, Austria*
[b]*Institute for Highly Pure Biopreparations, Pudozhskaya 7, 197110 St. Petersburg, Russia*
[c]*Chemical-Pharmaceutical Academy, Prof. Popova 14, 197376 St. Petersburg, Russia*

Received 15 March 2000; received in revised form 19 May 2000; accepted 19 May 2000

Abstract

The adsorption behavior of poly(ethylene glycol) (PEG) in reversed-phase chromatography is studied both experimentally and theoretically and a computer simulation of chromatograms is performed on the basis of these studies. The experimental conditions were: different reversed-phase adsorbents and a solvent methanol–water system as the mobile phase. At varying mobile phase compositions highly resolved chromatograms of PEG samples were obtained, in which all peaks could be identified, and the dependencies of the distribution coefficient on the degree of polymerization for PEG molecules were evaluated 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 stationary phase. The theory proved to describe well the experimental data in the whole range of studied molecular masses, and the thermodynamic parameters characterizing interactions of ethylene oxide repeating units in PEG molecules with the adsorbent pore walls have been determined from the comparison of the theory with the experimental data. The dispersion of chromatographic peaks corresponding to individual oligomer molecules is also estimated. In the system studied the peak width occurred to be proportional to the distribution coefficient of corresponding macromolecule. The theory is used to develop a computer-assisted procedure for simulation of chromatograms for samples of linear homopolymers. Using the obtained data on the thermodynamic parameters and the estimates of peak dispersion, chromatograms are simulated for PEG samples at two different chromatographic conditions. These simulated chromatograms were in good quantitative agreement with the real chromatograms.

Keywords: Computer simulation; Adsorption; Distribution coefficients; Peak dispersion; Poly(ethylene glycol); Polymers

1. Introduction

Reversed-phase liquid adsorption chromatography (RP-LAC) using hydrocarbon-coated stationary phases seems now to be the most feasible method to analyze the molecular mass distribution (MMD) of low-molecular-mass polyether samples, because, as demonstrated recently [1–3], this technique yields chromatograms with completely resolved peaks for all individual oligomer homologues in polyether samples of molecular mass up to several thousand. Typically, octadecylsilyl-bonded phases are used for this purpose, but also other non-polar stationary phases may be applied.

Another interesting approach is chromatography

*Corresponding author. Fax: +43-316-380-9840.

0021-9673/00/$ – see front matter
PII: S0021-9673(00)00627-0

under critical conditions, which proved to be especially useful in separations of heteropolymers [4,5].

In order to achieve good results in highly efficient separation of polymers, the experimental conditions (i.e., the combination of stationary and mobile phases as well as the operating temperature) must be selected very carefully to provide the optimal chromatographic mode.

Experimental work on finding good separation conditions could be facilitated applying theoretical approaches by which simulation of the chromatographic behavior of macromolecules seems to be the most straightforward way to the goals of optimization of analyses and chromatographic separations of polymers.

For this purpose, we have separated poly(ethylene glycol)s (PEGs) on different stationary phases in methanol–water mobile phases of different composition, and compared the results thus obtained with the simulated chromatograms.

2. Theoretical considerations

The principal features of chromatograms can be obtained even without exact accounting for all the details of the sample composition, by using, for example, simple models for MMD functions as well as for those determining other possible types of sample heterogeneity instead of the real ones.

What is generally important in the simulation of separation patterns in chromatograms, is to account in a reasonable way for the main factors determining both peak position and dispersion for all the polymeric species of which the sample consists.

For quasi-equilibrium chromatographic processes the peak position is determined by the equilibrium distribution coefficient, K, which is a function of both molecular parameters and pore structure and also of the adsorption interaction thermodynamics in the system. In its turn, the peak dispersion is also known to be dependent of molecule and pore structure and of adsorption thermodynamics, being additionally a complex function of many other operational and equipment-dependent factors like flow-rate, column geometry, adsorbent particle size and structure, together with extra-column flow contributions influencing peak-broadening in a chromatographic set [6]. In simulations of polymeric samples which are generally composed of a wide variety of macromolecular species of different molecular mass it seems to be primarily important to account for the first group of the above mentioned factors depending on the structure of macromolecules, since the latter group of factors can be considered as being about the same at given operational conditions.

The theory for the distribution coefficient of flexible homopolymers capable of being adsorbed on pore walls has been developed by Gorbunov and Skvortsov in Refs. [7,8] for a model of a Gaussian polymer chain and a slit-like pore (see also the review in Ref. [9]). It describes different features of chromatography of polymers as having an origin in different statistical properties of confined macromolecules under various conditions of adsorption.

According to the theory [7,8], the distribution coefficient, K, is a function of three parameters: polymer coil size, R, pore width, $2d$, and parameter of adsorption interaction, c. In the case where $R < d$ the formula for the distribution coefficient has the following form [9,10]:

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

where

$$g = \frac{R}{d\sqrt{6}}, \quad \Gamma = -\frac{cR}{\sqrt{6}},$$

and the function $Y(\Gamma)$ is defined as:

$$Y(\Gamma) = \exp(\Gamma^2) \cdot \mathrm{erfc}(\Gamma) \quad (2)$$

$\mathrm{erfc}(\Gamma)$ being the well-known special function (the complementary error integral), for which the tables and numerical algorithms are available [11].

The adsorption interaction parameter c has been first introduced by De Gennes in Ref. [12]; this parameter 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 Refs. [7–9], in the adsorption regime the average thickness H of a flat layer formed by a long macromolecule on a pore wall is given by $H = c^{-1}$.

Positive values of the parameter c correspond to

the regime of adsorption, $c=0$ corresponds to the 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 theory [7,8] describes both size-exclusion (SEC), 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. [13,14]. The theory [7,8] has already been successfully applied in studies of both normal- and reversed-phase liquid adsorption chromatographic behavior of polyethers and other polymers [10,15,16]. In the present paper we are using the same theory for the simulation purposes.

We have applied the theory [7,8] to RP-LAC of low-molecular mass polyethylene glycol in methanol–water mobile phase systems. This measure served to determine the adsorption interaction parameter and the mean thickness of adsorbed PEG macromolecules at varying composition of mixed methanol–water mobile phase systems. We also investigate the features of peak-broadening in the system studied and propose the simple empirical equation relating the dispersion of the peaks attributable to PEG oligomers to their distribution coefficient. For this reason, a mathematical procedure has been developed for the computer-assisted simulation of chromatograms of PEG samples, based on the molecular theory for the distribution coefficient and also accounting for peak-broadening effects. Using the experimentally determined adsorption interaction and peak-broadening parameters we can simulate chromatograms for the system studied.

3. Experimental

3.1. Equipment

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 RI detector (Bischoff, Leonberg, Germany) or an evaporative light scattering detection (ELSD) system Sedex 45 (from Sedere, Alfortville, France).

The sample loop volume was 50 μl and 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 DDS 70.

3.2. Solvents

The solvents used were HPLC grade (from Promochem, Wesel, Germany). 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).

3.3. Columns and porosimetry measurements

The following columns were used in these investigations (specifications given by the producer):

1. Spherisorb S5P (from Phase Separations, Deeside, UK) silica-based phenyl phase, 250×4.6 mm; particle diameter, 5 μm; nominal pore size, 8 nm
2. Jordi Gel 500 RP (from Jordi, Bellingham, MA, USA): 100% divinylbenzene, 250×4.6 mm; particle diameter, 5 μm; nominal pore size, 50 nm
3. Spherisorb ODS 2 (from Phase Separations) silica-based octadecyl phase, 250×4.6 mm; particle diameter, 5 μm; nominal pore size, 8 nm
4. Spherisorb ODS 2 (from Phase Separations) silica-based octadecyl phase, 100×4.6 mm; particle diameter, 3 μm; nominal pore size, 8 nm
5. Spherisorb S5X C_{18} (from Phase Separations) silica-based octadecyl phase, 250×4.6 mm; particle diameter, 5 μm; nominal pore size, 30 nm

The latter three columns were just used for comparative purposes, because they have been used in previous investigations [15].

The values of void volume V_0 and the pore volume V_p of the columns were determined with polystyrene standards (from Polymer Labs., Church Stretton, UK) in tetrahydrofuran. The measurements with the

polystyrene standards were also used to determine the pore sizes by using the SEC–porosimetry method [17].

The values of void and pore volume together with the average pore sizes obtained by porosimetry are presented in Table 1.

The average pore diameters of all columns (except one) obtained by SEC–porosimetry are in a reasonable agreement with values provided by distributor. In the case of the Jordi Gel 500 RP column, however, the pore diameter measured by SEC–porosimetry was about on tenth of that specified by manufacturer.

The porosimetry measurements revealed a certain pore-size related polydispersity for all adsorbents.

3.4. PEG samples and characterization of samples

PEGs and monodisperse ethylene oxide (EO) oligomers were purchased from Fluka (Buchs, Switzerland) and used without further purification. Oligomers used as internal standards had been prepared by preparative LAC and identified using gradient LAC, as will be described in another paper.

Before starting the simulation of chromatograms, some samples had to be characterized with respect to their MMD, which could serve as a reference.

This was done by isocratic RP-LAC in methanol–water (35:65, w/w) allowing both sufficient separation and reasonable quantitation on the ODS2 column for PEG 600 and on the S5P column for PEG 300 and 400.

Gradient elution could not be used in this case, as this would require the use of ELSD, which is rather problematic with respect to quantitation in the lower M_r range [18], whereas isocratic elution with combined density and refractive index (RI) detection can be applied, for which a simple relation between molecular mass M_r and response factor f is valid:

$$f = f_\infty + \frac{K_f}{M_r} \tag{3}$$

wherein f_∞ is the response factor for very high-molecular mass, and K_f is a constant describing the influence of the end groups [19].

Hence the first step was the determination of the response factors of the available monodisperse oligomers for density (D) and RI detection, from which f_∞ and K_f (for both detectors) were determined by linear regression. In methanol–water (35:65, w/w) as the mobile phase, the following parameters were found: $f_D = 17.46 + 1070.2/M_r$ (correlation: 0.9655); $f_{RI} = 63.514 - 807.19/M_r$ (correlation: 0.795). Once these parameters were known, chromatograms could be integrated using the correct response factors for each oligomer. The molecular mass averages thus obtained are given in Table 2.

3.5. Chromatographic measurements

We have processed a large number of chromatograms for PEG obtained under the conditions of

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

Column	Void volume V_0 (ml)	Pore volume V_p (ml)	Average pore diameter	
			Specification (nm)	Porosimetry (nm)
Jordi Gel 500 RP 250×4.6 mm	2.90	1.37	50	5.19
S5P 250×4.6 mm	3.00	1.14	8	7.03
ODS2 250×4.6 mm	2.40	0.86	8	6.80
ODS2 100×4.6 mm	1.04	0.35	8	6.80
S5W C_{18} 250×4.6 mm	3.35	1.83	30	21.5

Table 2
Molecular mass averages of poly(ethylene glycol) samples, as determined by RP-LAC [22,23] in methanol–water (35:65, w/w) on different columns[a]

Sample	Column	M_w		M_n		M_w/M_n	
		Density	RI	Density	RI	Density	RI
PEG 300	S5P	329	332	305	309	1.077	1.075
PEG 400	S5P	409	411	384	386	1.065	1.065
PEG 600	ODS2	608	607	584	583	1.040	1.041

[a] Flow-rate, 0.5 ml/min; sample loop, 50 ml; sample sizes, 600–1000 μg. Detection, density and RI.

RP-LAC in methanol–water mobile phases ranging from 30 to 100% of organic modifier.

Depending on the composition of the mobile phase, different M_r ranges can be analyzed. Using monodisperse oligomers as internal standards, peaks were identified, their number of repeating units n determined and the values of elution volume, V_e, corresponding to peak maxima positions obtained with high accuracy. In chromatograms, where some of the peaks were sufficiently resolved, the dispersions of these peaks were also calculated.

Fig. 1a shows a typical chromatogram obtained for PEG 1000 on the Jordi column at 80% (w/w) of methanol. The individual peaks were identified by adding monodisperse oligomers as internal standards, as can be seen from Fig. 1b, which shows a chromatogram of PEG 1000 on the Spherisorb S5P column in methanol–water (50:50, w/w), which was spiked with the oligomer EO12.

As can be seen the resolution is sufficient and the values of elution volume, V_e, corresponding to peak maxima positions can be obtained with high accuracy for typically 20 and more oligomers.

Thus, by processing of each chromatogram the dependence of distribution coefficient $K = (V_e - V_i)/V_p$ on the number of EO repeating units, n, was obtained. The data obtained at various chromatographic conditions will be presented and discussed together with the theory in the following section.

4. Results and discussion

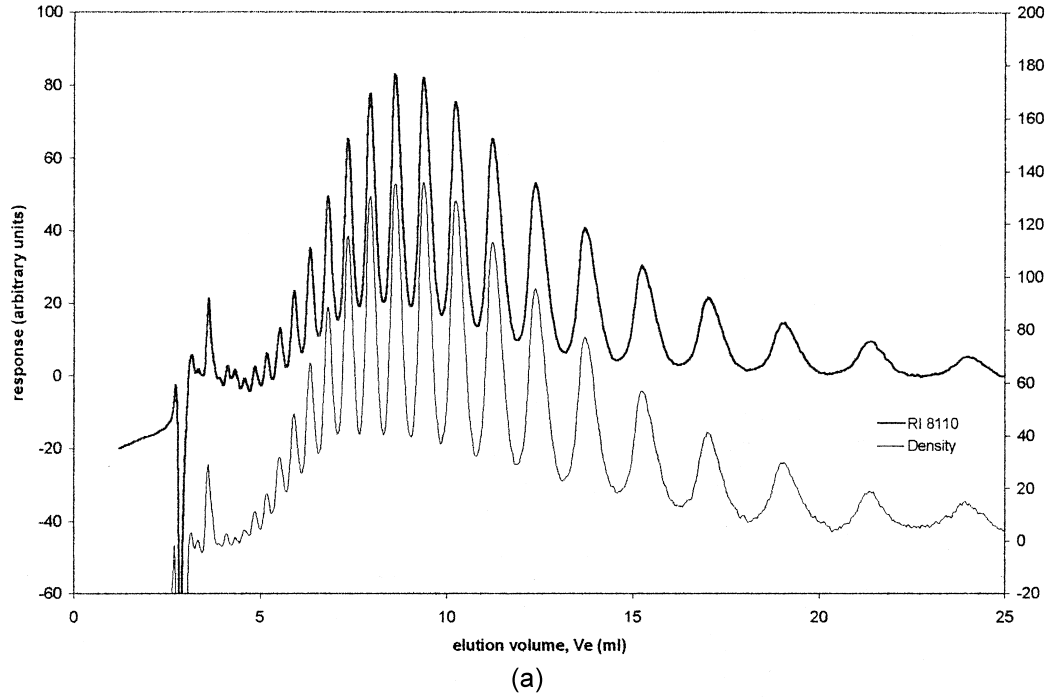
4.1. Distribution coefficient

We have studied the RP-LAC behavior of polyethylene glycols for different adsorbents in methanol–water mobile phases at organic modifier compositions ranging from 30 to 100%. In the systems studied a similar chromatographic mode was realized over a wide range of mobile phase compositions, namely that characteristic of adsorption chromatography where the retention of macromolecules increases with molecular mass.

The data obtained for Jordi Gel 500 RP and Spherisorb S5P adsorbents are presented in Figs. 2 and 3 in the form of dependencies of the distribution coefficient, K, on the number of repeating EO units.

Solid lines in Figs. 2 and 3 are drawn according to the theoretical formula (1). In the calculations the d values were used obtained by SEC–porosimetry (Table 1), and the root-mean-square sizes of PEG macromolecules were estimated according to formula $R_{PEG} = 0.079 M_r^{0.5}$ (nm) [20] 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 the adsorption interaction parameter, c, obtained in such a way are listed in the Table 3. As can be seen in Figs. 2 and 3, the theoretical formula (1) describes well the whole set of experimental data in both systems.

The dependencies of calculated parameter c upon the content of methanol in methanol–water mobile phase for S5P and Jordi Gel 500 RP columns are plotted in Fig. 4. The analogous data obtained previously in Ref. [10] for two ODS columns are also plotted in this Figure. The data for these two similar ODS columns are practically coinciding. As can be seen from Fig. 4, in the system under investigation adsorption interaction decreases with increasing the methanol content in the mixed mobile



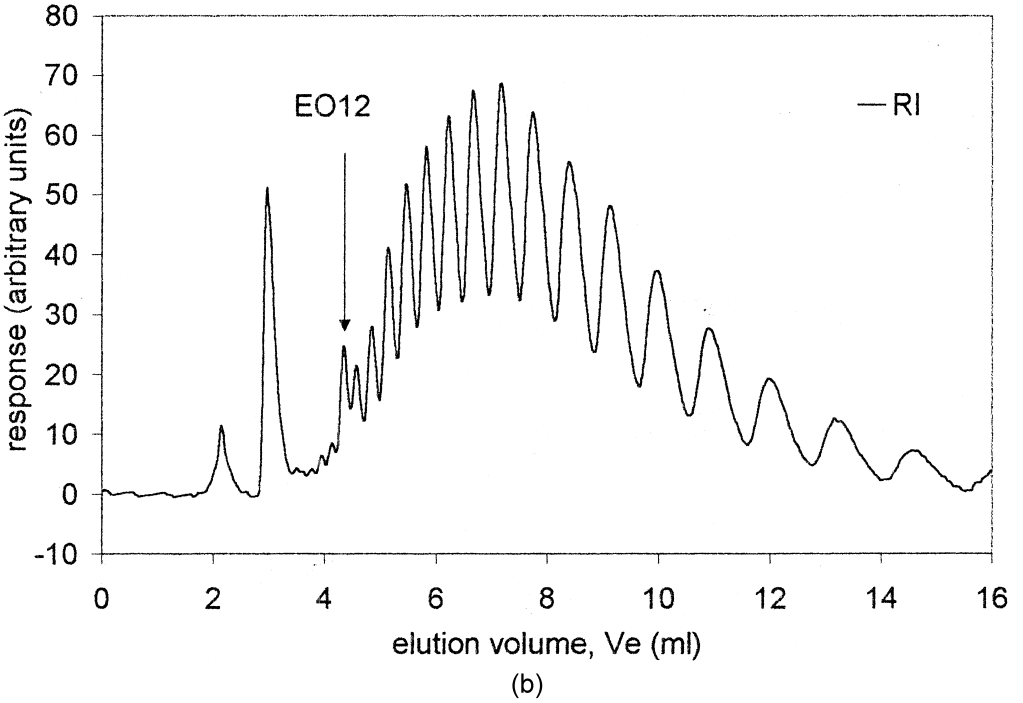


Fig. 1. (a) Chromatogram of a poly(ethylene glycol) PEG 1000 obtained with the Jordi RP 500 column in methanol–water (80:20, w/w). Flow rate, 0.5 ml/min; injected volume, 50 μl; sample size, 362.5 μg. Detection, density+RI. (b) RI trace of a chromatogram of a poly(ethylene glycol) PEG 1000 (spiked with the monodisperse oligomer EO12), as obtained with the Spherisorb S5P column in methanol–water (50:50, w/w). Flow rate, 0.5 ml/min; injected volume, 50 μl; sample size, 250 μg.

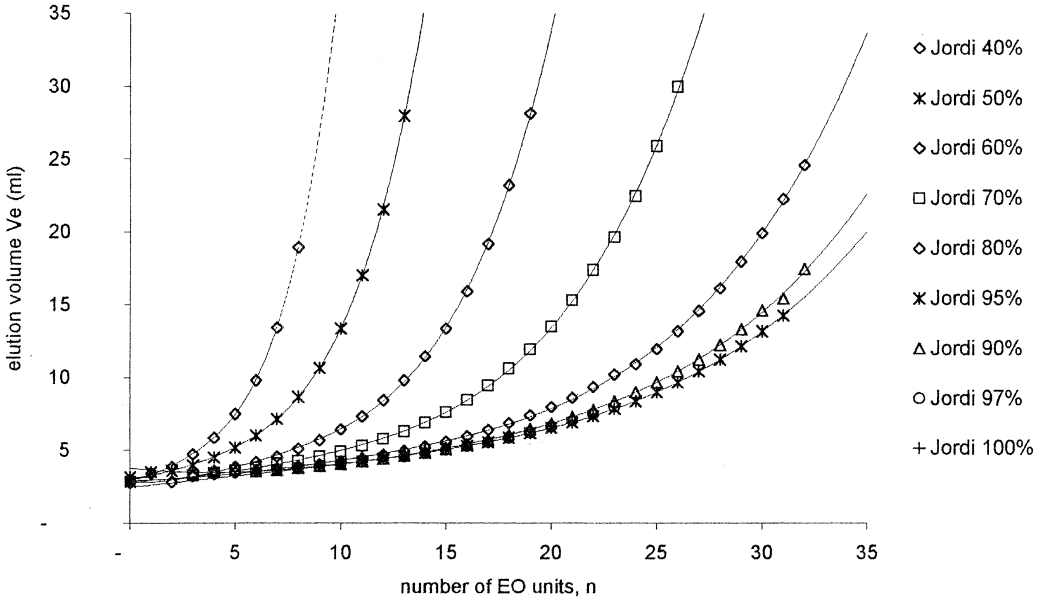


Fig. 2. Dependencies of the distribution coefficient K on the number of repeating EO units n for PEG molecules obtained on Jordi Gel 500 RP column. Experimental conditions: 30, 35, 40, 45, 50, 60, 70, 80, and 90% of methanol in mixed methanol–water solvent. Solid lines: approximation by Eq. (1).

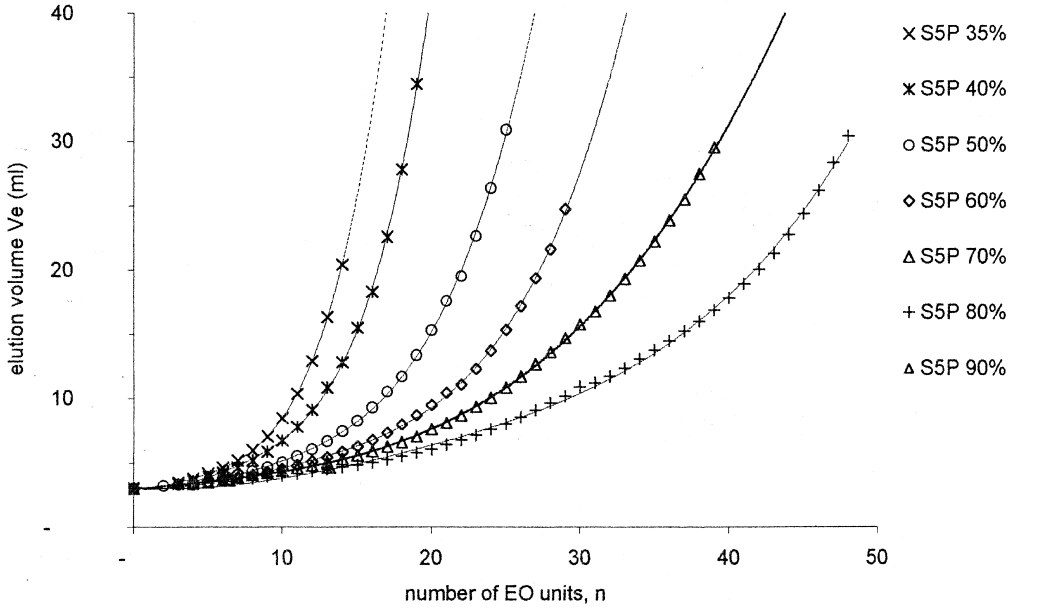


Fig. 3. Dependencies of distribution coefficient, K, upon the number of ethylene oxide repeating units, n, for PEG macromolecules at various composition of the methanol–water mobile phase. Experimental conditions: S5P column; methanol content in the mobile phase 30, 35, 40, 45, 50, 70, and 90%. Solid lines represent the approximation by Eq. (1).

Table 3
Parameter *c* describing the adsorption interaction of ethylene oxide units of PEG macromolecules in RP-LAC using Jordi Gel 500 RP and S5P as stationary phases and methanol–water mixtures as mobile phases, 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)	
	Jordi Gel 500 RP	S5P	Jordi Gel 500 RP	S5P
30	3.36	2.11	0.298	0.473
35	3.13	1.95	0.32	0.514
40	2.85	1.80	0.351	0.556
45	2.62	1.63	0.381	0.615
50	2.40	1.53	0.417	0.655
60	2.10		0.476	
70	1.75	1.34	0.571	0.745
80	1.49		0.673	
90	1.32	1.14	0.758	0.879

phase. There is, however, a considerable difference between the octadecyl bonded phases and the two other phases: while the critical point of adsorption (where *c* should equal zero) is reached at about 95–97% methanol for the ODS phases, the lines in Fig. 4 for the phenyl bonded phase (S5P) and the polydivinylbenzene phase (Jordi) do not intersect the abscissa axis, which means, that the critical conditions could not be realized in these systems at room temperature.

The average thickness of adsorbed polyether macromolecules $H = c^{-1}$ in both systems is in the order of several Ångströms and decreases with increasing of water content (see Table 3).

A comparison of chromatograms shows the difference: while PEG 900 is eluted as a narrow peak at V_0

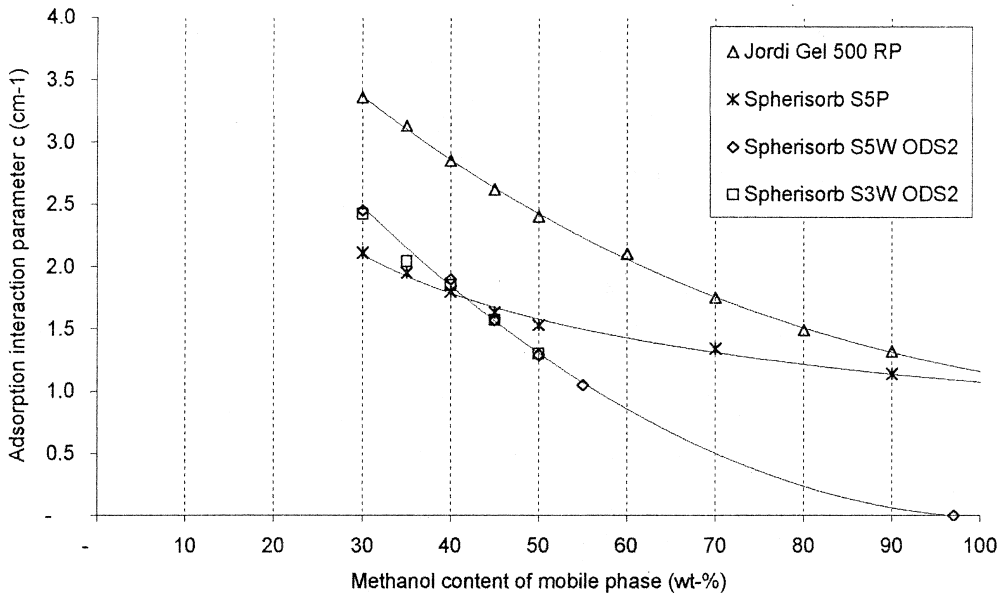


Fig. 4. Dependence of the ethylene oxide adsorption interaction parameter, *c*, upon the content of methanol in the methanol–water solvent. Data for Jordi Gel 500 RP, S5P, and two ODS columns. Lines are drawn to serve as a guide for the eye.

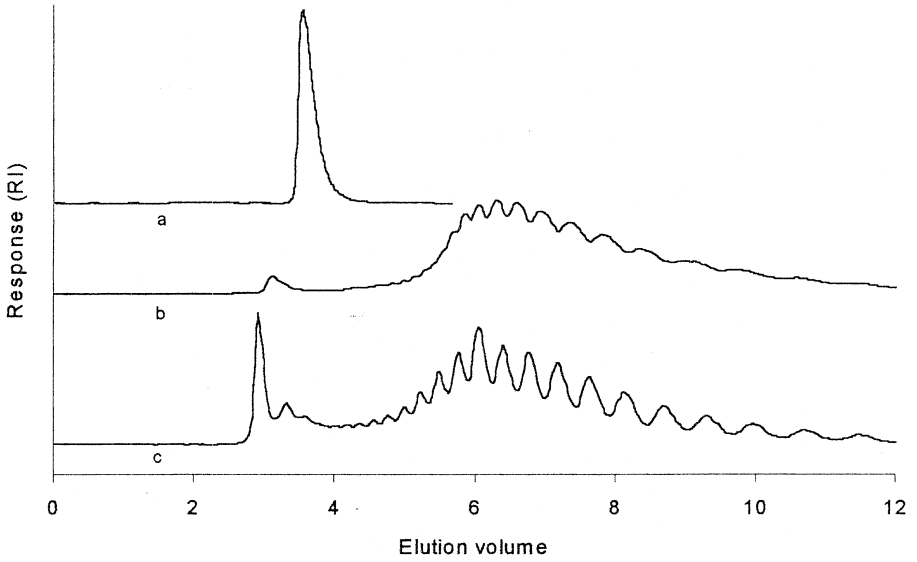


Fig. 5. RP-LAC chromatograms of poly(ethylene glycol) PEG 900, which were obtained on different 250×4.6 mm columns in methanol–water at high methanol content. Flow rate, 0.5 ml/min; injected volume, 50 μl; sample size, 350–390 μg. Detection: RI (a) S5W C_{18} column, methanol content 97%; (b) S5P column, 100% methanol; (c) Jordi Gel 500 RP column, 100% methanol.

in 97% methanol on the ODS columns (Fig. 5a) (as follows from Fig. 4, this system is close to the critical conditions), there is still a separation on the S5P (Fig. 5b) and on the Jordi column (Fig. 5c) even in 100% methanol, with about the same retention on the latter two columns, as could be expected from Fig. 4.

To summarize the results of this section it can be concluded that the retention behavior of PEG macromolecules under RP-LAC using different reversed-phase adsorbents and mixed methanol–water mobile phases in the whole range of molecular mass studied proved to be in (both qualitatively and quantitatively) good accordance with the molecular-statistic theory of chromatography of flexible homopolymers accounting for adsorption interactions. Application of this theory to the experimental data made it possible to determine the adsorption interaction parameter *c* and average adsorbed layer thickness of PEG macromolecules at different methanol–water mobile phase compositions.

Since the distribution coefficient determines the position of peaks in chromatograms, the above theory together with obtained thermodynamic parameters can serve as a basis for direct simulation of chromatograms for the systems studied.

4.2. Peak dispersion

The other problem in chromatogram simulation is to account reasonably for the peak dispersion. This is the well-known fact that under the conditions of adsorption chromatography the width of the chromatographic peaks corresponding to individual oligomeric components depends significantly on the elution time.

Unfortunately until now there is no consistent theory relating peak dispersion to the parameters of polymer molecules, mobile phase and adsorbent.

However, in this paper we show that a simple empirical rule can be successfully used in the studies of chromatographic separations of macromolecules.

Fig. 1a,b show the typical examples of chromatograms for low-molecular mass polydisperse polymeric samples obtained under the conditions of adsorption chromatography. It can be clearly seen that the width of individual chromatographic peaks in these figures increases with elution volume and therefore, largely depends on the distribution coefficient of different oligomers. To describe this dependence we propose the following empirical equation:

$$\sigma^2 \sim A + \alpha\,(V_p K)^2 \tag{4}$$

where σ^2 is the dispersion of the chromatographic peak for a polymer component with the distribution coefficient, K, while A and α are the coefficients which do not depend on K. These coefficients are believed to be constants for a given class of polymeric compounds at given chromatographic conditions.

The best way to an experimental check of the validity of Eq. (4) would be the measurement of peak dispersions and distribution coefficients under identical chromatographic conditions for a number of individual oligomers, which would require a series of monodisperse oligomeric samples, or sufficient amounts of highly monodisperse and well characterized polymeric fractions. Since such samples are commercially available only for the lowest degrees of polymerization (up to 8 in the case of PEG), we used data obtained from the processing of chromatograms of polydisperse PEG samples.

As in the case depicted in Fig. 1a, a number of peaks in some of these chromatograms were extensively baseline-resolved providing all the necessary information about both the peak positions and dispersions.

Of course, the accuracy of the dispersion measurements using chromatograms of polydisperse samples was not very good because of evident problems with partial peak overlapping and uncertain baseline position. Nevertheless this method gave us a possibility to estimate peak-broadening effects in the system investigated.

For the purpose of dispersion calculations we used two chromatograms with highly resolved peaks, which were obtained for PEG 1000 on the S5P column and for PEG 600 on Jordi Gel 500 RP column in methanol–water (70:30, w/w). The data obtained are presented in Fig. 6 in the form of σ^2 versus $(V_pK)^2$ plots.

As can be seen from Fig. 6, in both cases the data series can be approximated by straight lines, and this gives an experimental support to the empirical Eq. (4). It turned moreover that the parameters A and α

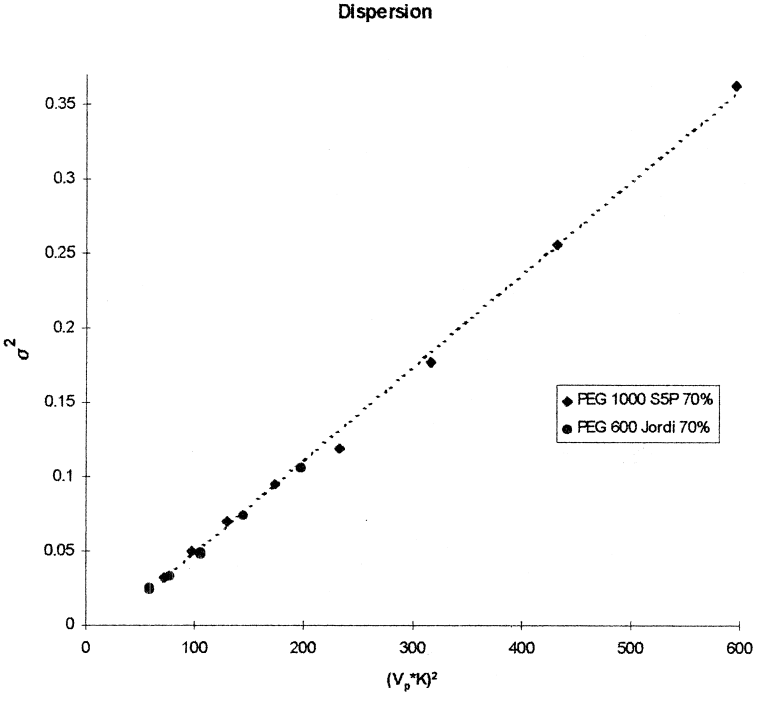


Fig. 6. Dependence of the dispersion of individual chromatographic peak, σ^2 on the parameter $(V_pK)^2$ obtained by processing the chromatograms of polydisperse PEG samples. Experimental conditions: PEG 1000, S5P column, and PEG 600, Jordi Gel 500 RP column; methanol content in the mobile phase 70%. Dots: linear approximation by Eq. (4) with $A=-0.015$, and $\alpha=0.0006$.

in the Eq. (4) have approximately the same values for both series investigated. Parameter A occurred to be of small negative value (the negative sign is of course, meaningless, most probably being the consequence of poor accuracy of dispersion measurements at low K values, where peaks are partially overlapping). The value of the α parameter in both cases occurred to be the same and was estimated as $\alpha \approx 0.0006$.

We do not think that the fact of the obviously occurring coincidence of the peak-broadening behavior for two series investigated is the general rule—this seems to be more surprising than evident. We believe however, that the expression of a dependence of the peak dispersion on the distribution coefficient in the form of Eq. (4) may be rather general. Although there is no theoretical evidence for this kind of dependence, we have other experimental

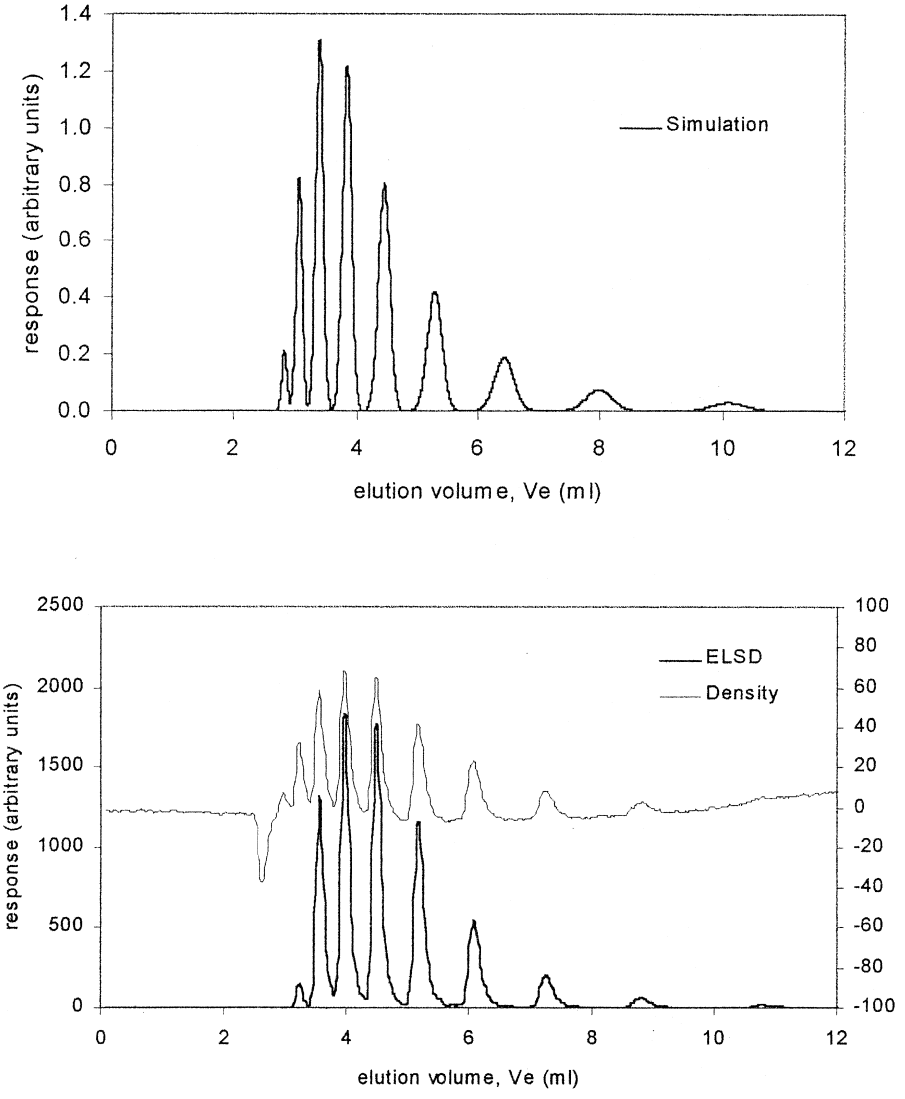


Fig. 7. (Top) Simulated chromatogram of PEG 300 on the ODS2 column in methanol–water (30:70, w/w). Parameters used: $M_w = 332$, $M_w/M_n = 1.065$, $d = 6.8$ nm, $V_0 = 2.4$ ml, $V_p = 0.86$ ml, adsorption interaction parameter $c = 2.45$ nm^{-1}, peak-broadening parameter $\alpha = 0.0006$. (Bottom) Real chromatogram obtained for PEG 300 on ODS2 in methanol–water (30:70, w/w). Detection: density and ELSD.

observations on peak-broadening phenomena under the conditions of both normal- and reversed-phase adsorption chromatography, which also seem to agree with Eq. (4).

It would be interesting to perform systematic measurements of the peak dispersion in adsorption chromatography for different polymeric homologous series using various chromatographic conditions, in order to study peak-broadening effects in more detail.

It can be concluded from the data presented in Fig. 6, that Eq. (4) holds reasonably well for the system under consideration, the main observed effect being attributable to the second term of this formula.

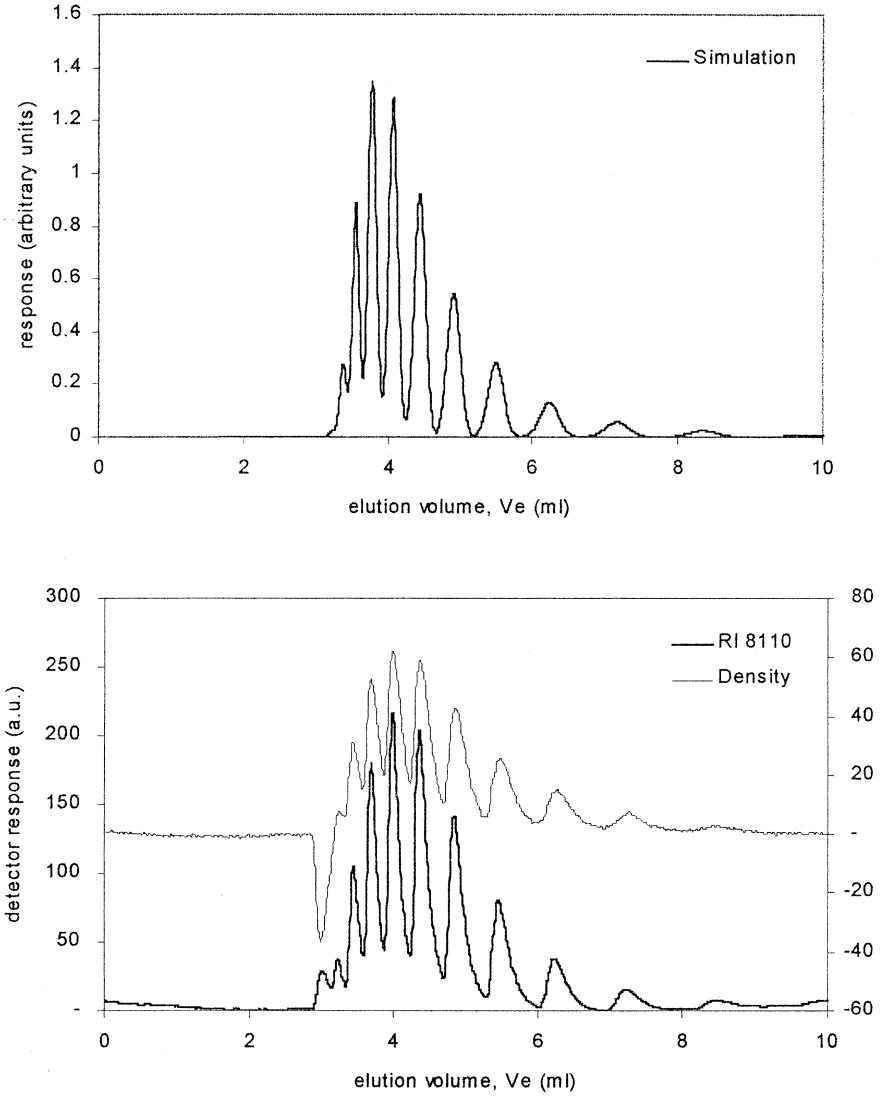


Fig. 8. (Top) Simulated chromatogram of PEG 300 on the S5P column in methanol–water (30:70, w/w). Parameters used: $M_w = 332$, $M_w/M_n = 1.065$, $d = 7.03$ nm, $V_0 = 3.0$ ml, $V_p = 1.14$ ml, adsorption interaction parameter $c = 2.11$ nm^{-1}, peak-broadening parameter $\alpha = 0.0006$. (Bottom) Real chromatogram obtained for PEG 300 on S5P in methanol–water (30:70, w/w). Detection: density and RI.

4.3. Simulation of chromatograms

After determining the adsorption interaction parameters and estimating the peak-broadening effects an attempt was made to perform a direct simulation of chromatograms at the conditions corresponding to the real ones, and to compare the simulated and experimental chromatograms.

For this purpose, we used the following computational procedure. The column parameters V_0, V_p, and pore diameter values, $2d$, were taken from Table 1 and the adsorption parameter values were chosen from Table 3 corresponding to the experimental conditions.

PEG samples were simulated by the logarithmically–normal MMD function which is fully determined by two parameters, one being the weight-average molecular mass $\bar{M}_w$, and the other one, $U = 1 + \sigma_M^2/\bar{M}^2$ being related to the dispersion of MMD function, σ_M^2 (corresponding to the parameters M_w, the number-average molecular mass M_n, and M_w/M_n from Table 2).

As in reality, model samples were assumed to be discrete mixtures of oligomeric homologues.

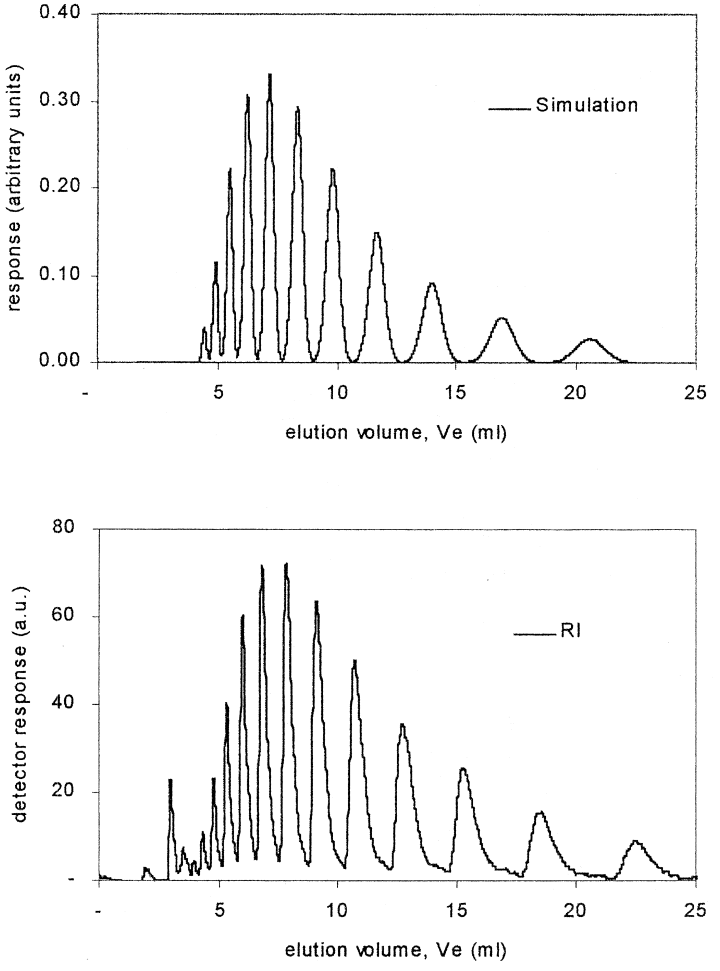


Fig. 9. (Top) Simulated chromatogram of PEG 600 on the S5P column in methanol–water (30:70, w/w). Parameters used: $M_w = 607$, $M_w/M_n = 1.041$, $d = 7.03$ nm, $V_0 = 3.0$ ml, $V_p = 1.14$ ml, adsorption interaction parameter $c = 2.11$ nm^{-1}, peak-broadening parameter $\alpha = 0.0006$. (Bottom) Real chromatogram obtained for PEG 600 on S5P in methanol–water (30:70, w/w). Detection: RI.

For every oligomeric species of molecular mass M_r (average molecule size, $R=0.079\ M_r^{0.5}$) the distribution coefficient was calculated according to Eq. (1), the peak maximum position V_{max} was determined and then the peak in the form of the Gaussian curve around this V_{max} value was simulated with the dispersion σ^2 calculated by using Eq. (4) (the coefficients in Eq. (4) were taken as $\alpha=0.0006$ and A equal to zero). Resulting chromatograms were then obtained by summation of all elementary peaks.

Calculated chromatograms together with the corresponding experimental ones are shown in Fig. 9–11.

Figs. 7 and 8 show simulated and real chromatograms of PEG 300 in the same mobile phase (methanol–water, 30:70, w/w) on different columns: Fig. 7 represents data obtained with the ODS 2

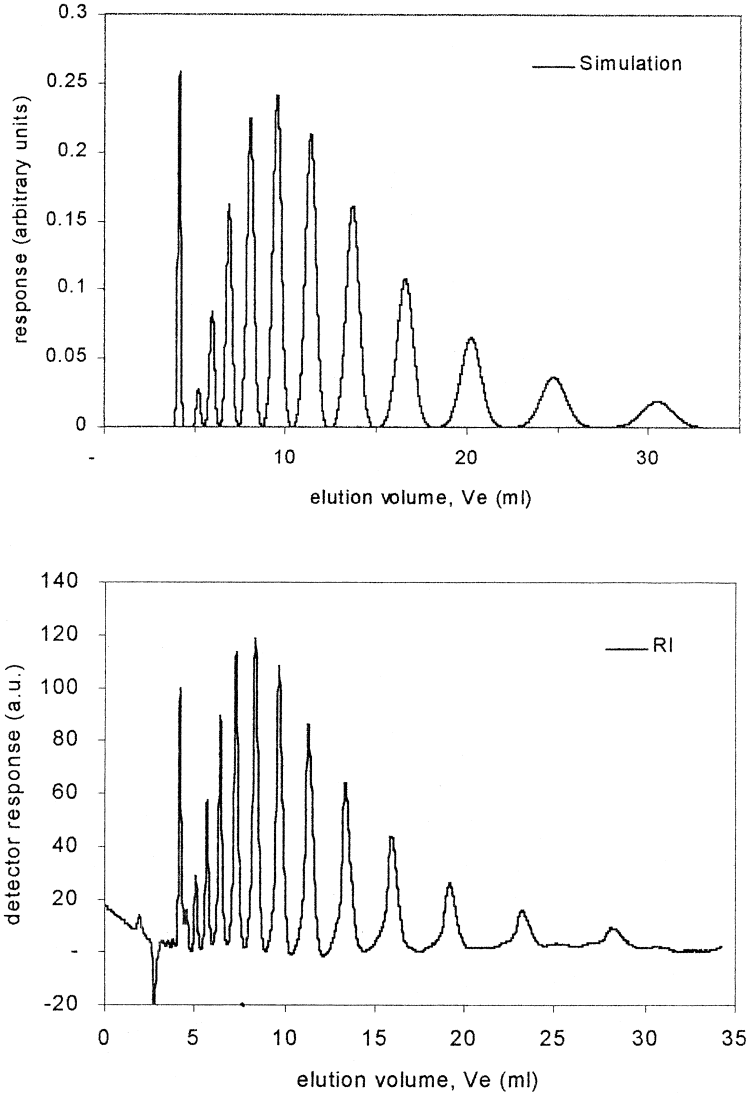


Fig. 10. (Top) Simulated chromatogram for PEG 600 on the Jordi column, spiked with hexaethylene glycol. Parameters used for the calculation: pore diameter $d=5.2$ nm, void volume $V_0=2.9$ ml, pore volume $V_p=1.37$ ml, adsorption interaction parameter $c=2.1\ nm^{-1}$, peak-broadening parameter $\alpha=0.0006$. (Bottom) Real chromatogram obtained for PEG 600 (spiked with hexaethylene glycol) on the Jordi column in methanol–water (60:40, w/w). Detection: RI.

column, Fig. 8 corresponds to those for the S5P column. As can be seen, the separation is slightly better on the ODS column, as was predicted by the simulation. It must be mentioned, that the density and RI traces agree better with the simulation, because the ELSD principle underestimates the lower oligomers (Fig. 7).

On the other hand, PEG 600 can be separated very well on the S5P column in the same mobile phase, as can be seen in Fig. 9.

On the Jordi column, retention is much higher, so the same sample will require a different mobile phase: in this case a methanol content of 60–70% proved to be appropriate. In Fig. 10, the simulation and a real chromatogram of PEG 600 on the Jordi column in methanol–water (60:40, w/w) are shown,

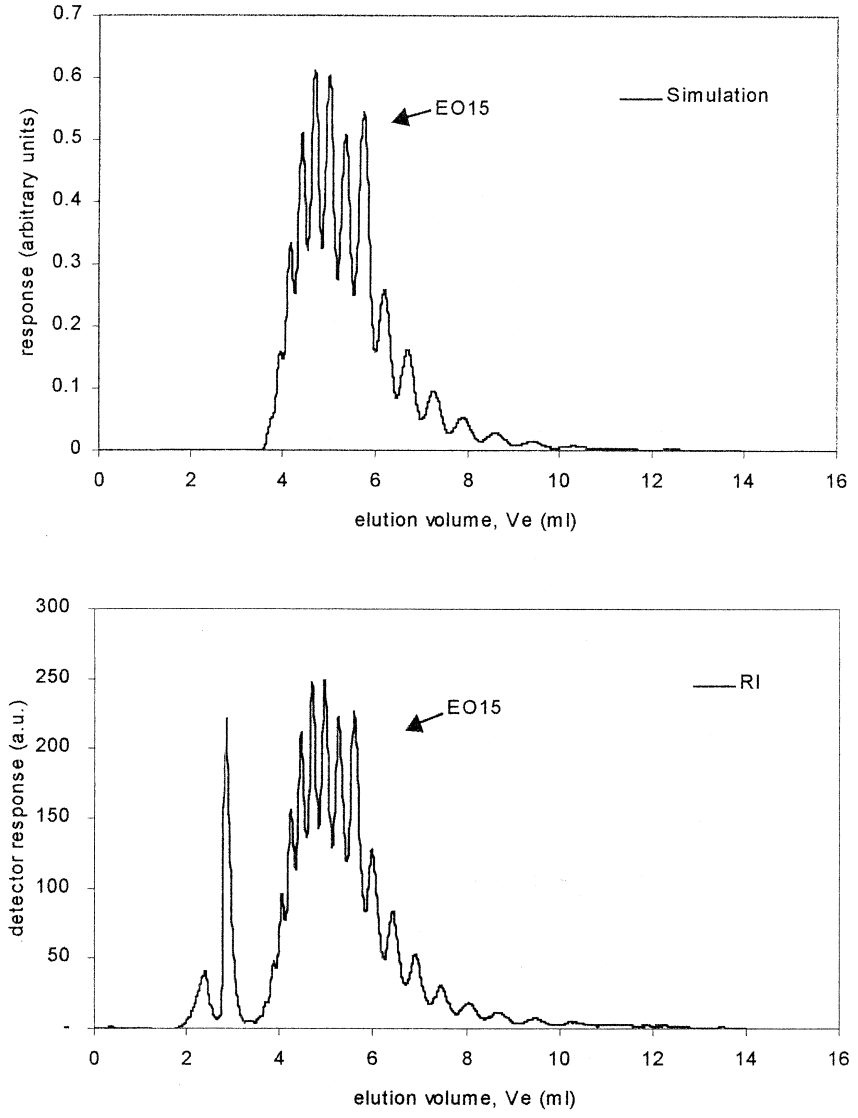


Fig. 11. (Top) Simulated chromatogram of PEG 600 on S5P, spiked with the oligomer EO15. Parameters used: $M_w = 607$, $M_w/M_n = 1.041$, $d = 7.03$ nm, $V_0 = 3.0$ ml, $V_p = 1.14$ ml, adsorption interaction parameter $c = 1.49$ nm^{-1}, peak-broadening parameter $\alpha = 0.0006$. (Bottom) Chromatogram obtained for PEG 600 on S5P column in methanol–water (80:20, w/w). Detection: RI.

to which hexaethylene glycol was added for identification purpose. The simulated chromatogram corresponds quite well to the real one, which was obtained for the mixture of PEG 600+EO6.

Furthermore, it is interesting, that using the simulation method it turns possible to obtain good estimates for MMD parameters even if chromatographic peaks are only poorly resolved: Fig. 11 shows both simulation and chromatogram of PEG 600 on the Jordi column in methanol–water (80:20, w/w), in which the pure oligomer of ethylene oxide EO15 was added as internal standard.

The comparison of real and simulated chromatograms in Figs. 7–11 shows, that in all cases not only the positions of individual peaks are in quite good accordance, but also the separation patterns and even the shape of the experimental and calculated chromatograms are very similar. It should be noted that the dependence of the response factor on the molecular mass occurring in reality was not taken into account in simulations. We suppose that after accounting for this effect the agreement between simulated and real chromatograms would become even more perfect.

In advance, we did not expect to obtain a good accordance in the shape of chromatograms, since we used the model MMD function instead of the real one. The obtained similarity of simulated and real chromatograms indicates, however, that the MMD functions of the analyzed PEGs are close to the logarithmically normal law.

It seems possible to develop analogous simulation procedures also for more complex types of polymer molecules for which the molecular-statistic theory of the distribution coefficient is available, for example, for two-block copolymers [21], and for polymers having functional end-groups.

Since the experimental study of copolymers is known to be a much more laborious task, we think that the role of the simulation in studies of such complex polymers could be of fundamental importance.

Acknowledgements

One of the authors (A.G.) wants to express his thanks for supporting two research stays in Graz to the Dr.Heinrich-Joerg-Stiftung and the Office of Foreign Relations of the Karl-Franzens-University Graz.

References

[1] B. Trathnigg, D. Thamer, X. Yan, S. Kinugasa, J. Liq. Chromatogr. 16 (1993) 2439.
[2] K. Rissler, U. Fuchslueger, H.J. Greter, J. Chromatogr. 654 (1993) 309.
[3] K. Rissler, U. Fuchslueger, J. Liq. Chromatogr. A 17 (1994) 2791.
[4] H. Pasch, B. Trathnigg, in: HPLC of Polymers, Springer, Berlin, 1997.
[5] S.G. Entelis, V.V. Evreinov, A.V. Gorshkov, Adv. Polym. Sci. 76 (1986) 129.
[6] B.G. Belenkii, L.Z. Vilenchik, in: Modern Liquid Chromatography of Macromolecules, Journal of Chromatography Library, Vol. 25, Elsevier, Amsterdam, 1983.
[7] A.A. Gorbunov, A.M. Skvortsov, Vysokomol. Soed., A 28 (1986) 2170.
[8] A.M. Skvortsov, A.A. Gorbunov, J. Chromatogr. 358 (1986) 77.
[9] A.A. Gorbunov, A.M. Skvortsov, Adv. Colloid Interface Sci. 62 (1995) 31.
[10] A.A. Gorbunov, A.M. Skvortsov, B. Trathnigg, M. Kollroser, M. Parth, J. Chromatogr. A 798 (1998) 187.
[11] E. Janke, F. Emde, F. Lösch, in: Tafeln Höherer Functionen, B.G. Teubner, Stuttgart, 1960.
[12] P.G. de Gennes, Rep. Progr. Phys. 32 (1969) 187.
[13] M.B. Tennikov, P.P. Nefyodov, M.A. Lazareva, S.Ya. Frenkel, Vysokomol. Soed., A 19 (1977) 656.
[14] B.G. Belenkii, E.S. Gankina, M.B. Tennikov, L.Z. Vilenchik, Dokl. Acad. Nauk. USSR 231 (1976) 1147.
[15] B. Trathnigg, B. Mayer, A.A. Gorbunov, A.A. Skvortsov, J. Chromatogr. A 791 (1997) 21.
[16] A.A. Gorbunov, L.Ya. Solovyova, A.M. Skvortsov, Polymer 39 (1998) 697.
[17] A.A. Gorbunov, L.Ya. Solovyova, V.A. Pasechnik, J. Chromatogr. 448 (1988) 307.
[18] B. Trathnigg, M. Kollroser, J. Chromatogr. A 768 (1997) 223–238.
[19] B. Trathnigg, X. Yan, J. Appl. Polym. Sci., Appl. Polym. Symp. 52 (1993) 193.
[20] J. Brandrup, E.H. Immergut (Eds.), Polymer Handbook, 2nd Edition, Wiley, New York, 1975, pp. IV–34.
[21] A.A. Gorbunov, A.M. Skvortsov, Vysokomol. Soed., A 30 (1988) 453.
[22] B. Trathnigg, D. Thamer, X. Yan, S. Kinugasa, J. Liq. Chromatogr. 16 (1993) 2453.
[23] B. Trathnigg, D. Thamer, X. Yan, Int. J. Polym. Anal. Char. 1 (1995) 35.