



Liquid chromatography of polyethylene glycol mono- and diesters: functional macromolecules or block copolymers?

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Abstract

The chromatographic behavior of polyoxyethylene-based polymers with adsorbing hydrophobic end-fragments was studied under two types of interaction conditions for the ethylene oxide (EO) component of such heteropolymers (which are either critical or of the size-exclusion type). In a theoretical part we assume a wide-pore situation, where the molecules are smaller than pores, and consider models of two-component diblock and triblock copolymers having quite strongly adsorbing blocks. A new step is made to extend a theory from difunctional macromolecules with point-type end-groups to triblock copolymers. It is shown that A–B–A triblocks with adsorbing A-blocks and with a “critical” B-block behave in chromatography like difunctionals with effective end-group interactions. Another important finding is the existence of a second critical region, which was observed on most of the studied columns. This region can be used to separate di- and triblocks from each other. Additionally, the individual oligomers of triblocks can be separated to the baseline. Complete separations of both diblocks and triblocks in one single chromatogram can be achieved by using a step gradient between two types of studied conditions

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1. Introduction

The analysis of nonionic surfactants requires sometimes quite subtle chromatographic methods because of the various kinds of structures which can be present in such samples. Depending on the starting material and ethoxylation conditions, chains with 0, 1 and 2 hydrophobic end groups may be

formed. It must be mentioned, that a chain containing only a few ethylene oxide (EO) units and hydrophobic end fragments of a comparable size (12–18 carbon atoms) should rather be considered as a block copolymer than as a functional polymer (with point-like end groups).

In the case of some typical examples—such as fatty alcohol ethoxylates (FAE), fatty acid methyl ester ethoxylates (FAMEE) and fatty acid polyglycol esters—one has to distinguish between the following basic structures:

1. *Non-functional structures:* polyethylene glycol

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(PEG), which is often formed by a chain transfer reaction

2. *Monofunctional structures (diblocks)*: FAE and fatty acid PEG esters represent a typical diblock (A–B). The distribution of the polyoxyethylene chain (block B) depends on the conditions applied in the ethoxylation reaction (catalyst, temperature, etc.). Additionally, there can be a distribution of functionality (the chain length of the hydrophobic group, block A), if the starting material was technical grade.
3. *Difunctional structures (triblocks)*: Within this category one has to distinguish between symmetrical and asymmetrical species: A–B–A or A_1 –B– A_2 where A_1 and A_2 are the hydrophobic blocks with different length. FAMEEs can be considered as A_1 –B– A_2 structures with a very short A_2 block. In technical samples of FAE and fatty acid polyglycol esters—which should be A–B diblocks—one can always find A–B–A triblocks and additional A_1 –B– A_2 triblocks, if the starting material contained more than one fatty acid. For instance, if a sample containing C_{12} - and C_{14} -fatty acid is ethoxylated, the following diesters will be observed: C_{12} –(EO) $_n$ – C_{12} , C_{14} –(EO) $_n$ – C_{14} (symmetrical structures) and C_{12} –(EO) $_n$ – C_{14} (asymmetrical structure); a sample based on three fatty acids will contain three symmetrical and three asymmetrical diesters, and so on.

Functionality and architecture of these complex polymers are very important for the physical and chemical properties. Therefore many efforts have been made to achieve an full characterization of these molecules [1–11].

Using *only* liquid adsorption chromatography (LAC) or size exclusion chromatography (SEC) a complete characterization of polydisperse samples is not possible. In SEC mode one can only determine the overall molecular mass distribution. In LAC mode a separation according to the polar unit is possible but requires gradient elution and therefore an evaporative light scattering detector (ELSD) instead of a refractive index (RI) detector. This causes problems in the detection respectively quantitation of the lower degrees of ethoxylation because they are underestimated or even not detected at all by the ELSD [12–16].

More than 20 years ago, Russian groups postulated a critical interaction point (CIP) or critical point of adsorption [17–19]. Tennikov et al. [18] and Belenkii et al. [17] were the first to report the transition from SEC mode to LAC mode by changing the mobile phase composition (or temperature). The critical interaction point is a borderline case between SEC and LAC. In comparison to SEC with entropy controlled and LAC with enthalpy controlled separation mechanism, the entropy and enthalpy contributions compensate each other at critical conditions and the retention volume of a linear homopolymer becomes independent of molar mass. Therefore at the CIP polymer chains of all lengths elute at the same volume and can be referred to as being “chromatographically invisible”. In the meantime critical conditions have been found for many polymer–adsorbent–solvent systems.

More complex polymers such as monofunctional macromolecules or diblock copolymers have structural units of two different types. If the critical condition is realized for one type of a structural unit, only a part of the molecule becomes “invisible” and a separation according to the units of the other type can be achieved [20,21]. This separation mode is called liquid chromatography at critical conditions (LCCC) [1–5,22].

When applied to diblock structures, LCCC allows separation of these polymers according to the length of one block (at the CIP for the other one). In three-block structures (ABA), there may be two different situations: at the CIP for A they behave like two-blocks, while at the CIP for B also a separation according to the radius of gyration of B is observed. A similar type of separation was observed in [23] for difunctional macromolecules. This effect was explained in [23] by using a model of an ideal difunctional (a chain with point-type end-groups that are different from the chain units in the aspect of interaction with adsorbent). Although it is intuitively clear that triblocks A–B–A with adsorbing terminal blocks A (Fig. 1b) should behave at the CIP for B similarly to corresponding ideal difunctionals with strongly adsorbing ends (Fig. 1a), there are still no simple equations relating molecular and interaction parameters of terminal A blocks to the effective interaction parameters of ideal difunctionals. In a theoretical part we shall give a proof to the analogy

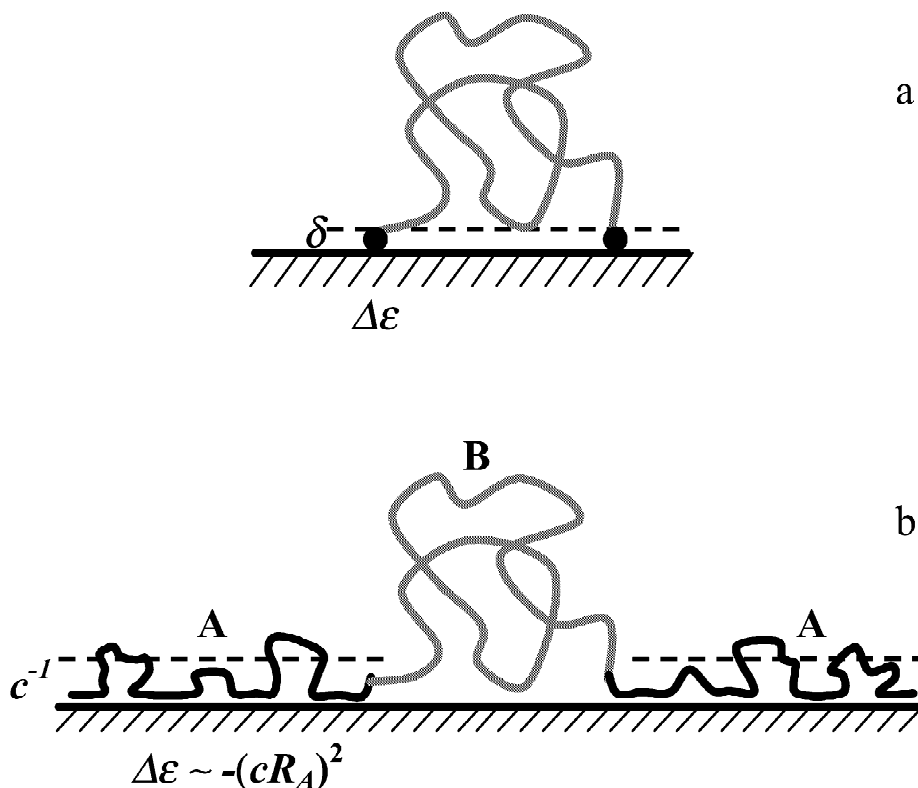


Fig. 1. Typical conformations of a difunctional macromolecule (a) and a triblock copolymer A–B–A (b) absorbed by the terminal groups (blocks) on the adsorbent surface. In the case (a) the end-group interaction parameters $\Delta\epsilon$ and δ are specified, in the case (b) their renormalized analogues are shown (see explanation in the text).

in the LCCC behavior of difunctionals and triblocks and shall present simple approximate equations describing LCCC of triblocks with adsorbing terminal blocks.

In the case of nonionic surfactants—such as fatty alcohol ethoxylates (FAE)—one can choose critical conditions for the polyoxyethylene chain and separate according to the hydrophobic end fragment. Separations at the CIP for the poly(oxyethylene) unit have been performed mostly in typical reversed-phase systems: Nucleosil RP₁₈ packings in acetonitrile–water show a critical point at about 46 wt.% of acetonitrile [2], while in methanol–water phases on most C₁₈ packings there is a wide range from 80 wt.% to 100 wt.% of methanol with almost critical conditions [8]. The advantage of this “critical region” is, that the separation can be optimized by variation of the mobile phase composition.

Sometimes it is not possible to achieve an exact compensation between the entropic and enthalpic

effects: in these systems no critical point can be observed [24]. This is the case in methanol–water phases for a Jordi Gel 500 RP column and even for some C₁₈ packings no critical point can be found.

In acetone–water C₁₈ packings typically have a sharp CIP for the poly(oxyethylene) unit between 25 and 35 wt.% of acetone [8]. Increasing the amount of acetone causes a transition to the exclusion regime for PEO, while the interaction of hydrophobic chain fragments remains of a strong adsorption type. As we have reported in previous papers [8,10,11,25], this region (up to 80 wt.% of acetone) can be used for liquid exclusion-adsorption chromatography (LEAC), which allows a separation of diblocks, such as FAE. The main problem is the poor solubility or too high retention of diblocks with longer hydrophobic chains.

Based on the retention behaviour of FAE in the region from 40 to 80 wt.% acetone, one can assume that the methylene selectivity should further decrease

with increasing acetone content. This would allow not only a separation of more hydrophobic diblocks using the LEAC mechanism, but also a separation of triblocks according to a similar mechanism. Therefore we have studied the elution behaviour of PEG and their derivatives in mobile phases with higher acetone content (far above the critical interaction point) in order to find out whether an improved separation of amphiphilic polymers could be achieved.

Another aim of these investigations was to determine whether the separation mechanism stays the same (LEAC), or if the nature of the interaction between polymer and adsorbent might change at such high acetone contents of the mobile phase.

The paper is organized as follows:

- In the theoretical part we compare the LCCC behavior of AB and ABA block-copolymers with adsorbing block(s) A and of end-functionalized polymers with adsorbing end-groups. The known theoretical results for homopolymers and functional macromolecules serve as the corner stones of our analysis. We will also show that by using the theory it is possible to predict the chromatographic behavior of diblock and triblock copolymers not only for LCCC but as well for LEAC conditions.
- In the experimental part we will first analyze the exclusion, adsorption and critical conditions for polyethylene glycol in acetone-water on different columns. Based on this analysis and on the theory we discuss possible regimes for separation of polyethylene glycol mono- and diesters. We will present the results of separation of these PEG-based diblock and triblock copolymers obtained under the LCCC and LEAC conditions as well as in the step-gradient mode.

2. Theoretical considerations

The elution volume V_e of a species in liquid chromatography is given by:

$$V_e = V_i + KV_p \quad (1)$$

where V_i stands for the interstitial volume (i.e. the volume of the solvent outside the particles of the

packing) and V_p for the entire pore volume; the void volume (V_0) of the column is given by: $V_0 = V_i + V_p$. K is the distribution coefficient relevant to the change in Gibbs free energy, which results from the changes in entropy and enthalpy. A molecular-statistical theory of chromatography is, in fact, a theory of the distribution coefficient for various types of polymers and porous adsorbents.

2.1. Homopolymers

In ideal size-exclusion chromatography there are no adsorption interactions (i.e. $\Delta H=0$), and, according to the Casassa theory [26,27], the distribution coefficients depend only on the ratio of average dimensions of molecules and pores. A more general theory describing all modes of interactive chromatography of homopolymers has been developed by Gorbunov and Skvortsov [28,29]. The general theory is based on the same model of an ideal linear polymer and a slit like pore as in the Casassa theory of SEC, but additionally takes into account adsorption interactions between macromolecules and pore walls. The theory of interactive chromatography has been verified experimentally in Refs. [30,31].

According to said theory [28,29], K is a function of three parameters: R , $2d$ and c , where c denotes the parameter of adsorption interaction, R is the radius of gyration of a macromolecule, and $2d$ is the pore width. Since in chromatographic practice R usually is less than d , we shall present here the theoretical results obtained mostly for the wide-pore situation $R < d$.

The adsorption interaction parameter c can be understood as an inverse correlation length of adsorption, characterizing the structure of a macromolecule near the surface of an adsorbent [29,32].

Negative values of c correspond to the regime of SEC, where adsorption interactions are small. Under ideal SEC conditions there are no adsorption interactions, c approaches very large negative values ($c \rightarrow -\infty$) and the unified theory is reduced to Casassa's equation:

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

In the regime of LAC c values are positive and in the case of rather strong attraction of the chain unit

to the pore wall, $cR \gg 1$, the general theory can be approximated by:

$$K \approx K_{\text{SEC}} + \frac{2}{cd} \exp(c^2 R^2) \quad (3)$$

One special case of interaction, $c=0$, is known as the critical interaction point (CIP). In this point entropy and enthalpy phenomena exactly compensate each other, and the distribution coefficient of a homopolymer takes an especially simple form:

$$K = 1 \quad (4)$$

Under experimental conditions corresponding to the CIP all linear homopolymers, regardless of their molar mass, are eluted as one narrow peak at the void volume ($V_e = V_0$). Since molecules of different length elute like very small non-adsorbing particles, non-functional polymer chains under the CIP condition may be referred to as “invisibles” [22].

2.2. Polymers with functional end-groups

In recent papers the general theory has been extended to mono- and difunctional polymers [23]. In the latter case an effective interaction parameter, $q_a = \frac{\delta}{d} [\exp(-\Delta\varepsilon) - 1]$ is introduced to account for the interaction of (a point-type) end-groups (q_{a1} and q_{a2} in the case of asymmetrical difunctionals).

The parameter $\Delta\varepsilon$ denotes the difference in the interaction free energy (expressed in the units of kT) between the terminal chain unit and the repeating one, δ being the effective radius of interaction (Fig. 1a).

Positive values of q_a correspond to more attractive interaction of an end-group compared to a repeat unit; negative values of q_a derive from a stronger attraction of the repeat unit; if the interactions of the end-group and of the repeat are the same, $q_a=0$ (in this special case the “new theory” reduces to Eqs. (2–4)). While in the general case, the equations describing the distribution coefficient of end-functional polymers are rather complicated, they reduce to a much simpler form at the critical point of interaction.

Under critical conditions for the EO-chain ($c=0$) the distribution coefficient of monofunctional macromolecules is given by:

$$K^{(a)} = 1 + q_a \quad (5)$$

In the case of difunctionals one has to distinguish between symmetrical and asymmetrical structures. According to the theory the distribution coefficient $K^{(a,a)}$ for symmetrical difunctionals is equal to:

$$K^{(a,a)} \approx 1 + 2q_a + q_a^2 \frac{d}{\sqrt{\pi} \cdot R} \quad (6)$$

while asymmetrical difunctionals—with different end groups—give:

$$K^{(a1,a2)} \approx 1 + q_{a1} + q_{a2} + q_{a1} \cdot q_{a2} \frac{d}{\sqrt{\pi} \cdot R} \quad (7)$$

These theoretical predictions for the chromatographic behaviour of mono- and difunctional polymers have been verified in previous papers [10,23].

Consequently, in a plot of K vs. $1/R$ straight horizontal lines should be obtained for nonfunctionals and monofunctionals, while for difunctionals a straight line with a positive slope is to be expected if both q_{a1} and q_{a2} have the same sign (positive or negative). It must be mentioned that even at the critical point symmetrical difunctionals will elute in the order of SEC; that means, the smallest molecule will appear at the highest elution volume.

2.3. Diblock and triblock copolymers

In the case of diblock or triblock copolymers the theory becomes more complicated [33–36], because different values for the radius R and for the interaction parameter c can be assigned to each block (we shall use index A for the alkyl group and B for the EO unit). In particular, an exact theory exists for a flexible linear diblock copolymer A–B [33]. According to this theory, the distribution coefficient is a quite complicated function of five parameters: $K_{AB} = f(d, R_A, R_B, c_A, c_B)$. Exact, but even more complicated equations are also available for multiblock copolymers [34,36]. One can use the exact theory to predict the chromatographic behavior and to simulate separation patterns for real diblock and triblock copolymers, as has been done in our previous papers [8,10]. Here we will also present an example of a theoretical simulation based on the exact theory. But first let us consider another approach, which is more simple albeit in general not exact.

It is intuitively clear that in the situation of strong adsorption of the A block(s) the chromatographic behavior of di- and triblocks (A–B and A–B–A) must be similar to that of the corresponding mono- and difunctionals. (Fig. 1a,b may better illustrate the analogous conformations of difunctionals and triblocks). Therefore one can expect that the rather simple equations of the theory [23] for macromolecules with adsorbing end-groups could be also applied to describe approximately di- and triblocks.

Under critical conditions for block B of a diblock copolymer A–B or for the terminal blocks of a triblock copolymer B–A–B, the distribution coefficients of such copolymers are known to be equal [22,33]:

$$K_{A-B} = K_{B-A-B} = K_A \quad (8)$$

where K_A denotes the distribution coefficient of a homopolymer that is identical to the block A of the reference copolymer. Evidently, the Eq. (8) for K_{A-B} is exactly equivalent to the Eq. (5) for a monofunctional polymer. If the interaction condition for the block A is of a strong attractive type ($c_A > 1/R_A$), K_A is given by the Eq. (3), and the effective q_a parameter for a copolymer can be expressed as a function of both interactive and molecular parameters of the block A:

$$q_a = K_A - 1 \approx \frac{2}{c_A d} \left[\exp(c_A^2 R_A^2) - \frac{c_A R_A}{\sqrt{\pi}} \right] \approx \frac{2 \exp(c_A^2 R_A^2)}{c_A d} \quad (9)$$

Eq. (9) has a form similar to the definition of q_a for the model with the point-type end-group. However now the conformational free energy of an adsorbing block, which is equal to $-c_A^2 R_A^2$ [29], replaces $\Delta\varepsilon$, and the former $\delta/2$ is substituted by the average thickness of the adsorbed chain A, c_A^{-1} . For the situation where critical conditions are created for the central block B of a triblock copolymer A–B–A, while the adsorption interaction of the A-component is strong enough ($c_A \gg 1/R_A$, and $c_A \gg 1/R_B$), the exact theoretical results for symmetrical and asymmetrical triblocks K_{A-B-A} and $K_{A1-B-A2}$ also can be reasonably approximated by the corresponding Eqs. (6) and (7) for symmetrical and asymmetrical difunctionals; the parameter q_a (or

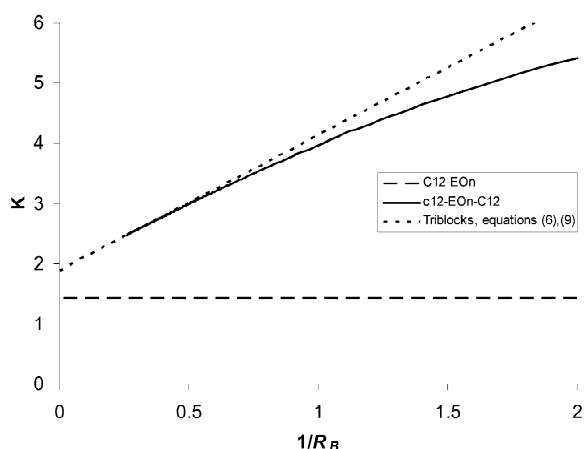


Fig. 2. Theoretical plot of K vs. $1/R_B$ for diblocks A–B and symmetrical triblocks A–B–A ($C_{12}\text{-EO}_n$ and $C_{12}\text{-EO}_n\text{-C}_{12}$) at critical conditions for the EO-component. Parameters taken for calculation: $d=20$ nm, $c_A=3$ nm $^{-1}$, $c_B=0$, $R_A=0.55$ nm. Diblocks: dashed line corresponds to the exact Eq. (8) (and also to Eqs. (5) and (9)); triblocks: solid line is calculated by using the exact theory, dotted line—Eqs. (6) and (9).

similar parameters q_{a1} , q_{a2} for asymmetrical triblocks) is again given by Eq. (9).

Fig. 2 shows an example of exact theoretical results for block copolymers in the form of a plot K vs. $1/R_B$ for A–B and A–B–A copolymers ($C_{12}\text{-EO}_n$ and $C_{12}\text{-EO}_n\text{-C}_{12}$) at critical interaction conditions for the EO-chain and strong adsorption conditions for the alkyl groups. The dashed line corresponds to diblocks as described by Eqs. (6,8 and 9). The solid and dotted lines represent triblocks A–B–A: the solid line corresponds to the exact theory; the dotted straight line corresponds to Eq. (6) with the parameter q_A calculated by using Eq. (9).

As can be seen in Fig. 2, Eqs. (6) and (9) describe A–B–A triblocks quite well, if $1/R_B \ll c_A$. The analogy between end-functional macromolecules and block copolymers is useful to understand features of LCCC of triblocks A–B–A: since in the case of difunctionals the linear dependence of K on $1/R$ takes its origin in the specific behavior of polymer loops at the CIP [23,37], it is clear that the analogous dependence of K_{ABA} on $1/R_B$ (which is almost linear at $1/R_B \ll c_A$) is of the same origin.

Thus, for analysis of diblocks one can apply LCCC by choosing critical conditions for the EO block ($c_B=0$) and adsorption conditions for the alkyl

groups ($c_A > 0$): the EO-chain gets invisible and a separation according to the length of the hydrophobic block can be achieved. The same is valid for triblocks B–A–B with a central hydrophobic block. Oligomeric A–B–A copolymers will be additionally separated according to the radius of the block B (i.e. the number of EO-groups) due to the above discussed $K(1/R_B)$ dependence.

Changing the mobile phase composition in a way which would lead to exclusion conditions for the block B, one can separate diblocks according to the LEAC mechanism [8,10,11,25]. In this case the interaction parameter for block B (c_B) is negative and c_A for the hydrophobic chain positive, which allows a separation of the individual oligomers in SEC order, but far beyond V_0 . This method has been applied to many diblock structures as it has been reported in previous papers [8,10,11,25].

As follows from both the simplified analysis based on the analogy between triblocks and difunctionals and the exact theory, for triblock structures under LEAC conditions one can expect an increased resolution of the individual oligomers.

Fig. 3 shows a theoretical simulation of a LEAC separation for a mixture of oligomeric C_{12} -EO $_n$ diblocks and C_{12} -EO $_n$ - C_{12} triblocks, which are polydisperse with respect to the EO-component. For such samples the theory predicts a SEC-order separation of individual oligomers in both series;

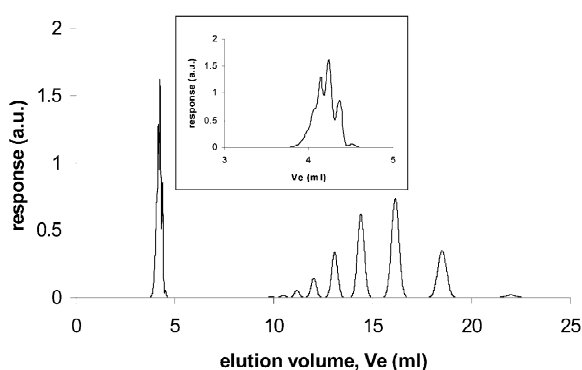


Fig. 3. Simulation of a LEAC separation of a mixture of C_{12} -EO $_n$ diblocks and C_{12} -EO $_n$ - C_{12} triblocks. Parameters used in the simulation: column: $2d=7.6$ nm, $V_0=2.74$ ml, $V_p=1.01$ ml; EO-block: $M_w=200$; $M_w/M_n=1.08$, $c=-2$; alkyl blocks: $M_w=168$, $M_w/M_n=1$, $c=3$. An insert zooms the diblock peak series. Detector response is given in arbitrary units (a.u.).

diblocks are expected to be partially separated, while for triblocks one can expect even a baseline separation.

The theory also gives the prediction about the possibility of separation of block-copolymers with respect to their architecture. It is evident from Eq. (8) that no separation between A–B and B–A–B copolymers with the same molar mass and content of component B can be achieved at the CIP condition for the component B. However, as follows from the Eqs. (5)–(9), one may expect that under this condition diblocks A–B and triblocks A–B–A (having the same total amount of the component A) or symmetrical and very asymmetrical triblocks may be separated with respect to architecture.

3. Experimental

3.1. Apparatus and materials

These investigations were performed using the density detection system DDS70 (CHROMTECH, Graz, Austria). Each system was connected to a MS-DOS computer via the serial port. Data acquisition and processing was performed using the software package CHROMA, which has been developed for the DDS70. The columns and density cells were placed in a thermostatted box, in which a temperature of 25.0 °C was maintained for all measurements on both systems (A and B).

In System A, the mobile phase was delivered by a JASCO 880 PU pump (from Japan Spectroscopic Company, Tokyo, Japan) at a flow-rate of 0.5 ml/min. Samples were injected manually using a Rheodyne 7125 injection valve (from Rheodyne, Cotati, CA, USA) equipped with a 50 μ l loop. A Bischoff 8110 refractive index (RI) detector (Bischoff, Leonberg, Germany) was connected to the DDS 70. Columns were connected to two column selection valves (Rheodyne 7060, from Rheodyne, Cotati, CA, USA):

In system B, the mobile phase was delivered by an ISCO 2350 HPLC pump and an ISCO 2360 gradient programmer (from ISCO, Lincoln, NE, USA). The flow-rate was 0.5 ml/min in all measurements. Samples were injected using a Vici injector equipped with an 50 μ l sample loop. A SEDEX 45 ELSD

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

The following columns were used in both systems:

- Jordi Gel DVB 500 RP (from Jordi, Bellingham, MA, USA): Divinylbenzene; 250×4.6 mm; particle diameter=5 µm; nominal pore size=500 Å; ser. no. 10289710
- Spherclone C₆ (from Phenomenex, Torrance, CA, USA): silica-based hexyl phase; 250×4.6 mm; particle diameter=5 µm; nominal pore size=80 Å; ser. no. 185969
- Spherisorb S5X C6 (from Phase Separations, Deeside, Clywd, UK): silica-based hexyl phase; 250×4.6 mm; particle diameter=5 µm; nominal pore size=300 Å;
- Prodigy ODS(3) column (from, Phenomenex, Torrance, CA, USA): silica-based octadecyl phase; 250×4.6 mm; particle diameter=5 µm; nominal pore size=100 Å; ser. no. 185970
- Novapak C₁₈ (from Waters, Milford, MA, USA): silica-based octadecyl phase; 300×3.9 mm; particle diameter=4 µm; nominal pore size=60 Å; serial no. W12621F_037

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

The HPLC solvents (acetone, methanol and water) were purchased from Roth (Karlsruhe, Germany) and Merck (Darmstadt, Germany). Polyethylene glycols were purchased from different companies: Aldrich (Hexaethylene glycol, PEG 900), Merck (PEG 400, 1500, 2000) and FLUKA (PEG 4000). Fatty acid polyglycol esters and fatty acid methyl ester ethoxylates were provided by Dr Wieslaw Hreczuch, “Błachownia” Institute of Heavy Organic Synthesis (ICSO), Kędzierzyn-Koźle, Poland.

4. Results and discussion

4.1. Adsorption, exclusion and critical conditions for PEG

As a first step it was important to determine the

critical interaction point for the columns used in our investigations. Therefore the elution volumes of PEGs with different molar masses were measured in various mobile phase compositions. According to the theory, under critical conditions PEGs of different molar mass should elute at the same volume. In Fig. 4, the elution volumes of different PEGs on the Prodigy ODS(3) column are plotted vs. the mobile phase composition (methanol–water). As can be seen, there is no sharp intersection point but rather a plateau with almost critical conditions in a region between 80 and 100 wt.% of methanol. This is in a good agreement with previous experiments in methanol–water on different columns [38,39].

A more pronounced adsorption-exclusion transition is observed in acetone–water instead of methanol–water as a mobile phase: as can be seen from Fig. 5, a sharp critical interaction point is found for the Prodigy ODS3 column at 28 wt.% acetone. When the acetone content was increased up to 95 wt.% of acetone, surprisingly a second “intersection point” was found around 92 wt.% acetone, as can be seen in Fig. 5.

When using acetone as the organic modifier all the other silica based columns tested in this study showed a similar behaviour with two critical points, which contrasts the situation observed with methanol. Another example is given in Fig. 6, which shows the results obtained with the Spherclone C₆ column. Table 1 summarizes the results on critical conditions for all tested columns. A possible explanation could be as follows: As the interaction of the EO unit obviously becomes stronger with decreasing polarity of the mobile phase, which is characteristic for normal-phase chromatography, one may speculate, that retention is now caused by residual silanol groups on the stationary phase. This effect should not occur on a polymer based packing, which cannot contain any silanol groups.

Fig. 7 shows the results obtained with the polymer-based Jordi Gel DVB column, where no second critical region can be found at all. In this case, one can see, that the elution volumes increase with higher acetone contents of the mobile phase but there is definitely no intersection point and the PEGs still elute in a SEC order. This is not a definite proof, but a very strong hint, that the existence of a second critical region has something to do with the residual free silanol groups, which have not been removed by

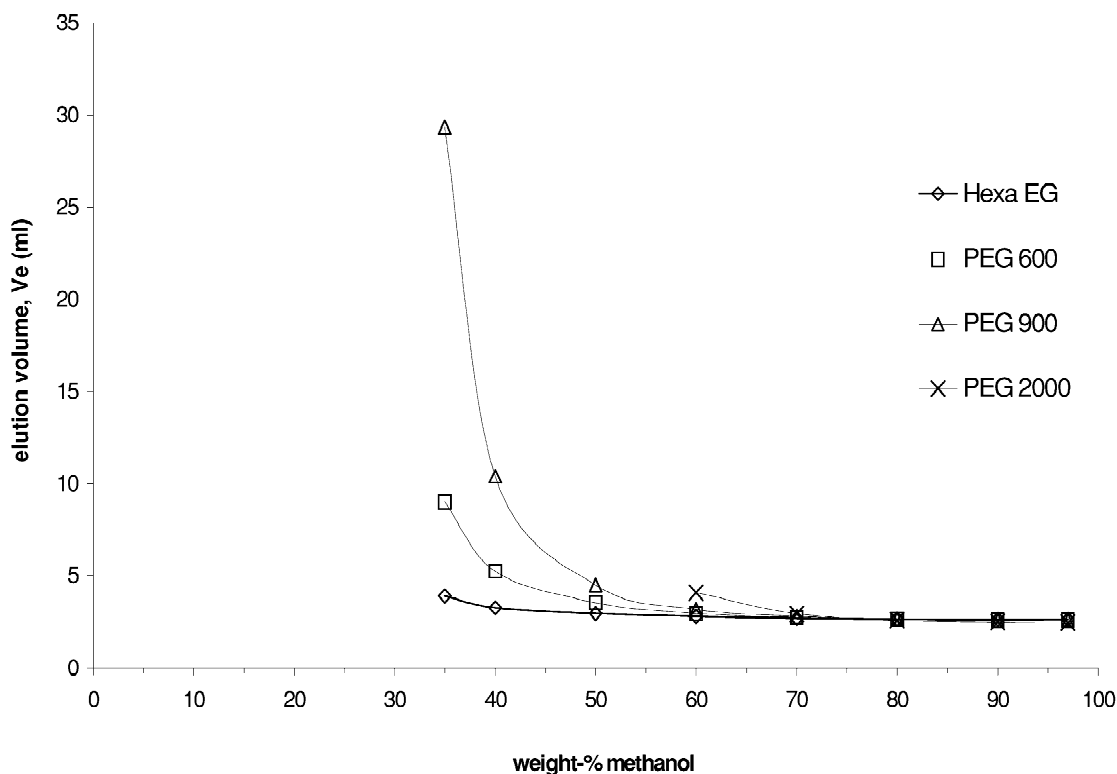


Fig. 4. Elution volumes of PEGs on a Prodigy ODS (3) column in methanol–water of different compositions (System A).

the endcapping procedure. With decreasing polarity of the mobile phase, the interaction of the EO unit with the silanol groups grows predominant, and the elution order changes again from exclusion to adsorption.

In order to determine the exact CIPs (which correspond to the intersection points in Figs. 4–7), the mobile phase composition was varied in 5% steps. One can see that in some cases there is no sharp second intersection point but a critical region where the lines for the different PEGs cross within a small range of mobile phase composition. One more interesting detail: in most cases the elution volume of PEGs at the second intersection “point” is definitely larger than at the first critical point—this is especially noticeable in Fig. 6.

These facts can be explained as follows: While in the vicinity of the first critical point (in a quite polar mobile phase) the interaction of the stationary phase with the terminal hydroxy groups of PEG can be neglected, this is no more the case at higher acetone contents. Therefore one has to consider the PEGs as

symmetrical difunctionals and not as nonfunctionals. Consequently, their elution behaviour will be described by Eq. (6), hence one may expect both an increase in K and an intersection region rather than a sharp intersection point.

Now, knowing the adsorption, exclusion and critical conditions for PEG, it turns possible to realize both the LCCC and LEAC modes of separation of PEG-based diblock and triblock copolymers, and to compare the separation results with what was expected from the theory. Of course, such separations are interesting not only from the theoretical point of view, but may also have important practical implications.

4.2. Separations under critical conditions (LCCC)

In Fig. 8 one can see a chromatogram of a C_{12} fatty acid polyglycolester ($n=3$) (this sample contains a mixture of A–B and A–B–A type block-copolymers). The chromatogram is obtained in 92 wt.% acetone as mobile phase which represents

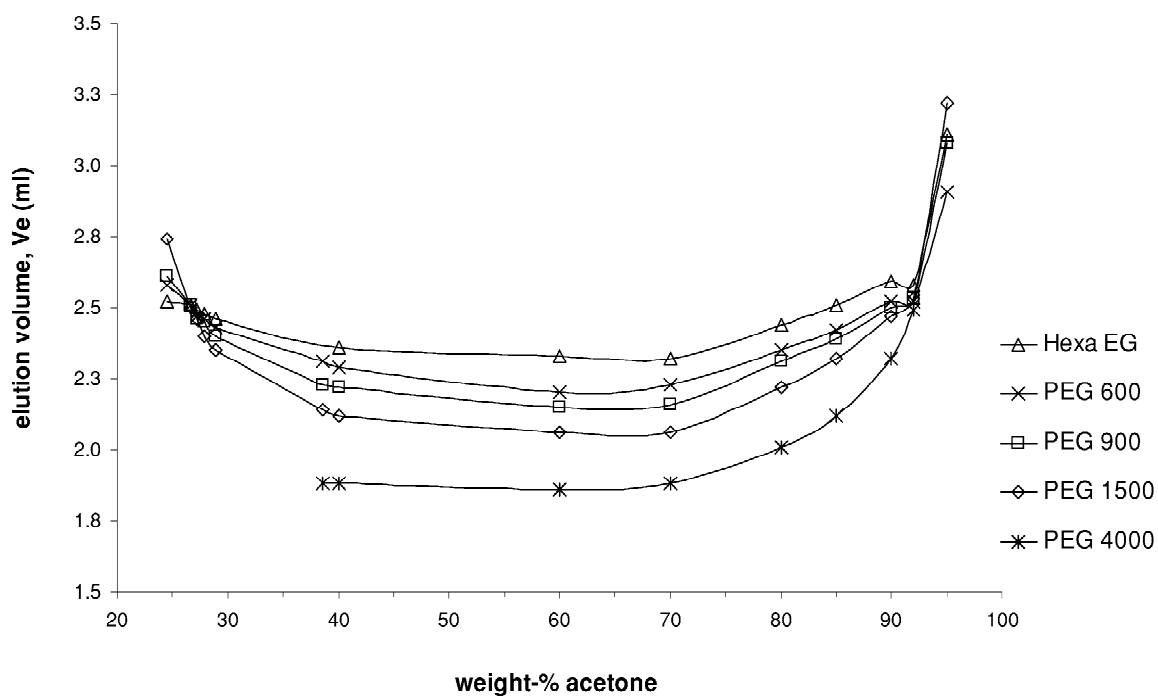


Fig. 5. Elution volumes of PEGs on a Prodigy ODS (3) column in acetone–water of different compositions (System A).

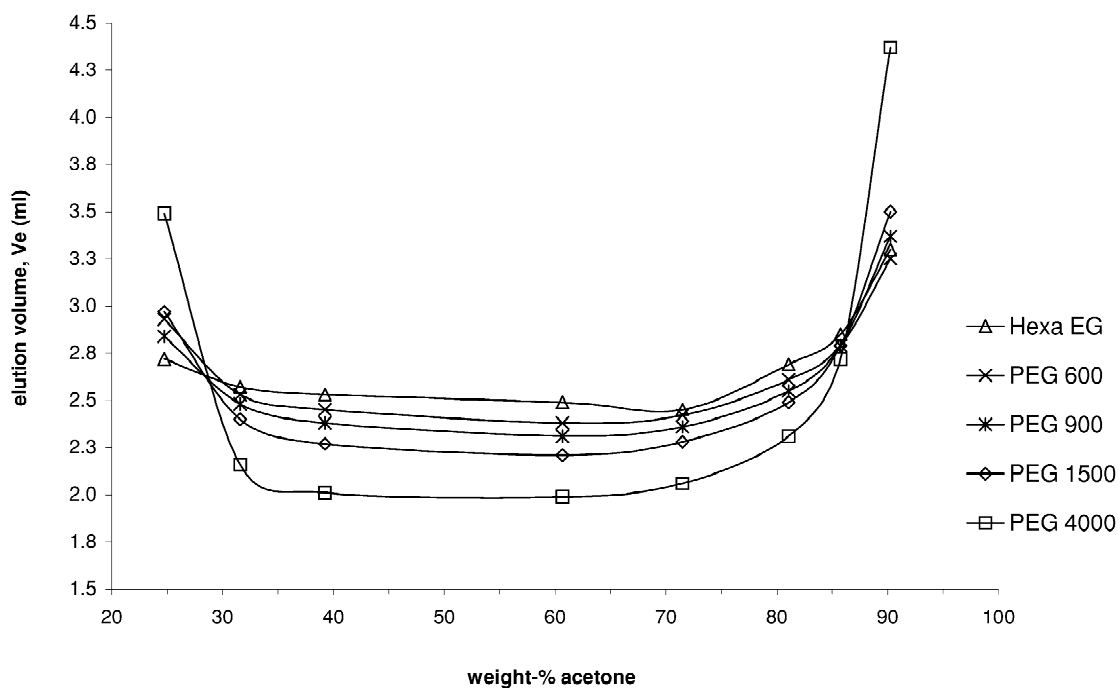


Fig. 6. Elution volumes of PEGs on a Sphreclone C₆ column in acetone–water of different compositions (System A).

Table 1
Critical conditions in acetone–water for different columns

| Columns | First critical point | | Second critical point |
|-------------|----------------------|------------|-----------------------|
| | % Acetone | V_e (ml) | % Acetone |
| Prodigy | 28 | 2.49 | 90 |
| Spherisorb | 30 | 2.49 | 85 |
| Sphereclone | 28.5 | 2.6 | 86 |
| Novapak | 28.5 | 2.05 | 90 |
| Jordi | 30 | 2.7 | – |

exactly the second critical point for EO-chain (block B) and the Prodigy ODS(3) column. As is characteristic for critical conditions, the PEG (peak 1) elute as a narrow peak just as the diblock fraction (peak 2), while the triblocks of different length of EO-chain are baseline-resolved. Even for highly asymmetrical triblocks a similar separation of the oligomers is observed, but the resolution is of course not as good as with the dilaurates. Fig. 9 shows a chromatogram of a FAMEE $N=1$ sample (with a the C_{12} -chain on one end and a methyl group on the other end), which is a good example for a very asymmetrical triblock structure.

4.3. Separations under LEAC conditions

When the mobile phase composition is changed to an acetone content, at which the EO unit is eluted in the exclusion mode, the diblocks start to split up a little bit and the retention of the triblocks becomes markedly stronger as can be seen in Fig. 10. The separation pattern in the chromatogram of Fig. 10 is very similar to what is expected from the theory, as depicted in Fig. 3. This behaviour is caused by the transition to the LEAC regime and can be used for separation of fatty acid polyglycolesters with higher degrees of ethoxylation: Fig. 11 shows a chromatogram of a C_{12} acid (9 EO's) sample in 86 wt.% of acetone where the diblocks still elute within a quite narrow peak and the triblocks are very well baseline-separated according to the increased retention.

4.4. Complete separation of both diblocks and triblocks in the step-gradient mode

For the analysis of mono- and difunctionals (diblocks and triblocks) a two-dimensional separation using the full adsorption–desorption (FAD) tech-

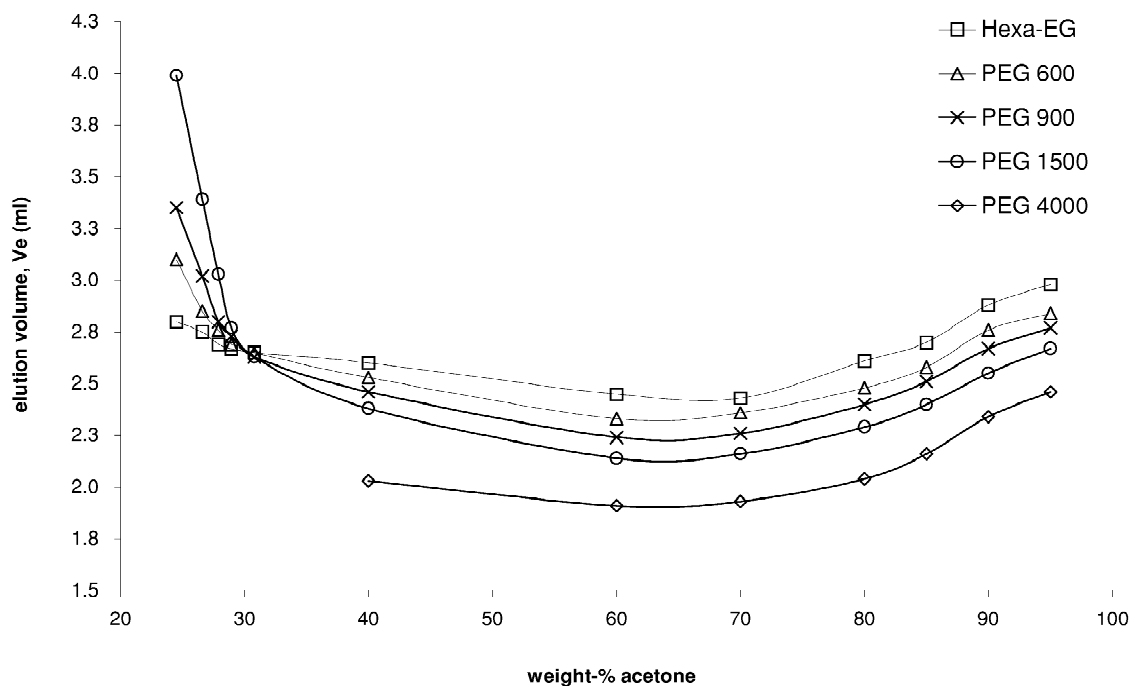


Fig. 7. Elution volumes of PEGs on a Jordi Gel DVB column in acetone–water of different compositions (System A).

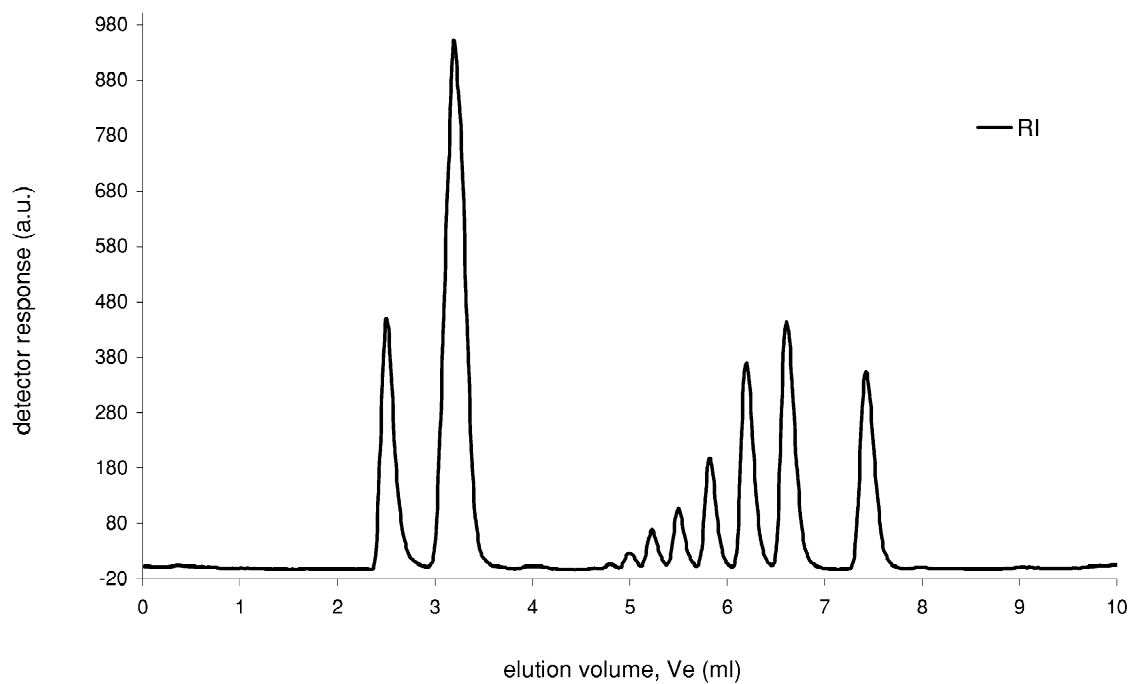


Fig. 8. LCCC of a C_{12} fatty acid polyglycolester ($n=3$) in 92 wt.% acetone on a Prodigy ODS(3) column (System A). The sample contains C_{12} - EO_n diblocks as well as C_{12} - EO_n - C_{12} triblock structures. Detector response is given in arbitrary units (a.u.).

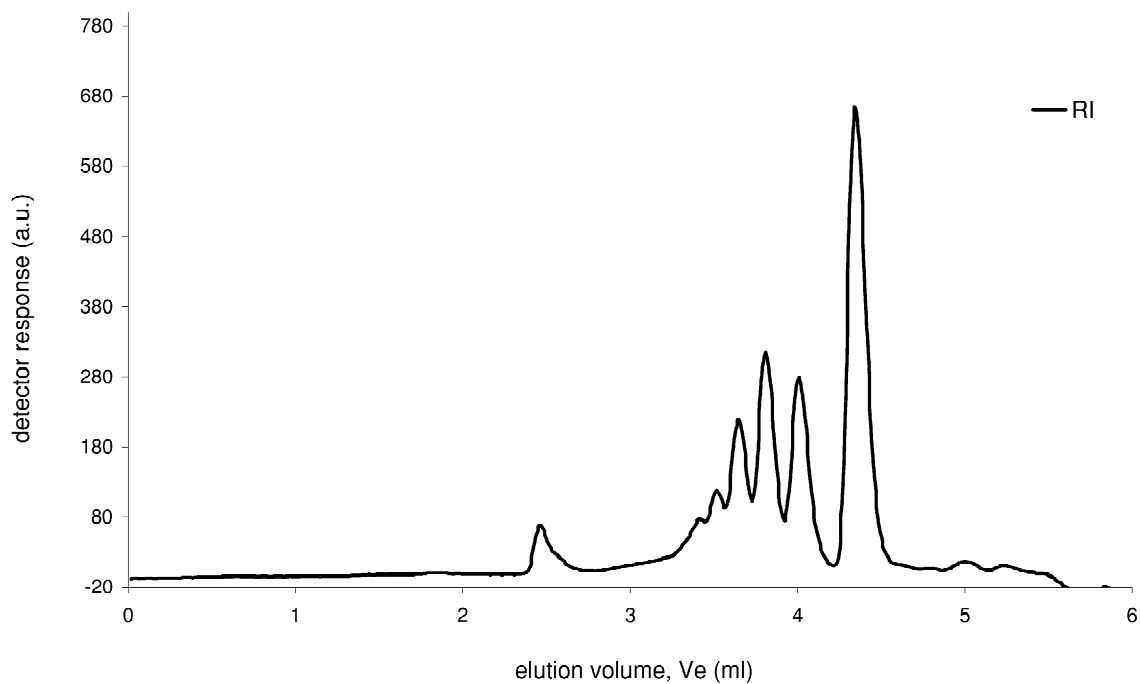


Fig. 9. LCCC of a C_{12} fatty acid methyl ester ethoxylate ($n=1$) in 92 wt.% acetone on a Prodigy ODS(3) column (System A).

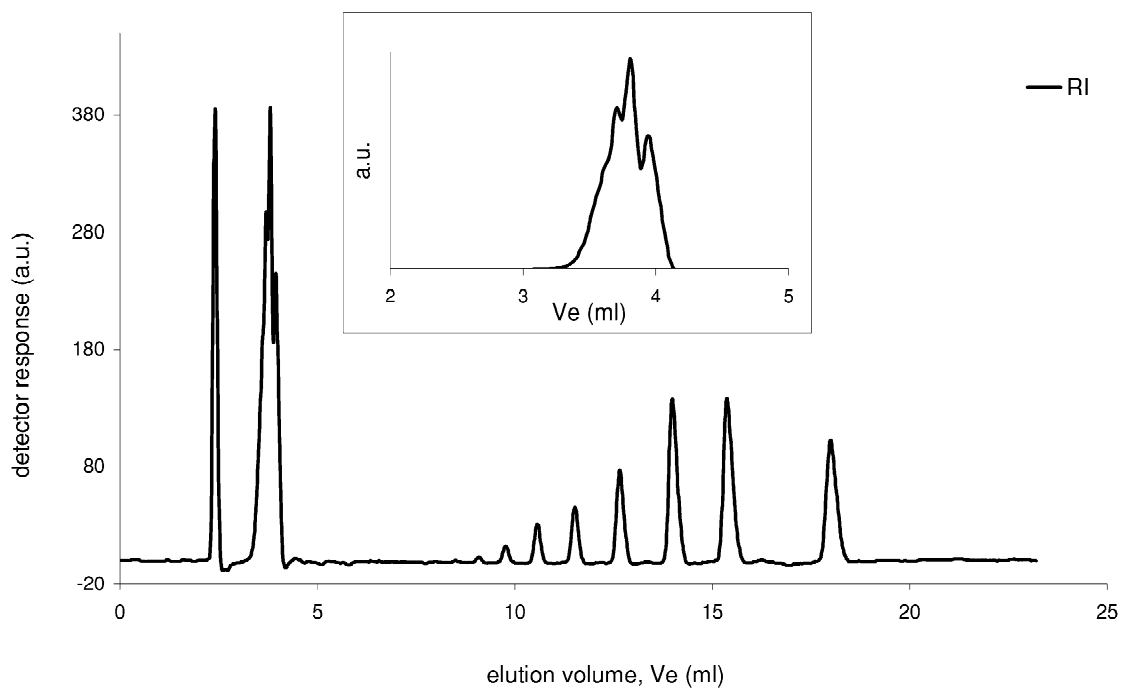


Fig. 10. LEAC of a C_{12} fatty acid polyglycolester ($n=3$) in 86 wt.% acetone on a Prodigy ODS(3) column (System A) containing a zoomed plot for the diblock peak series.

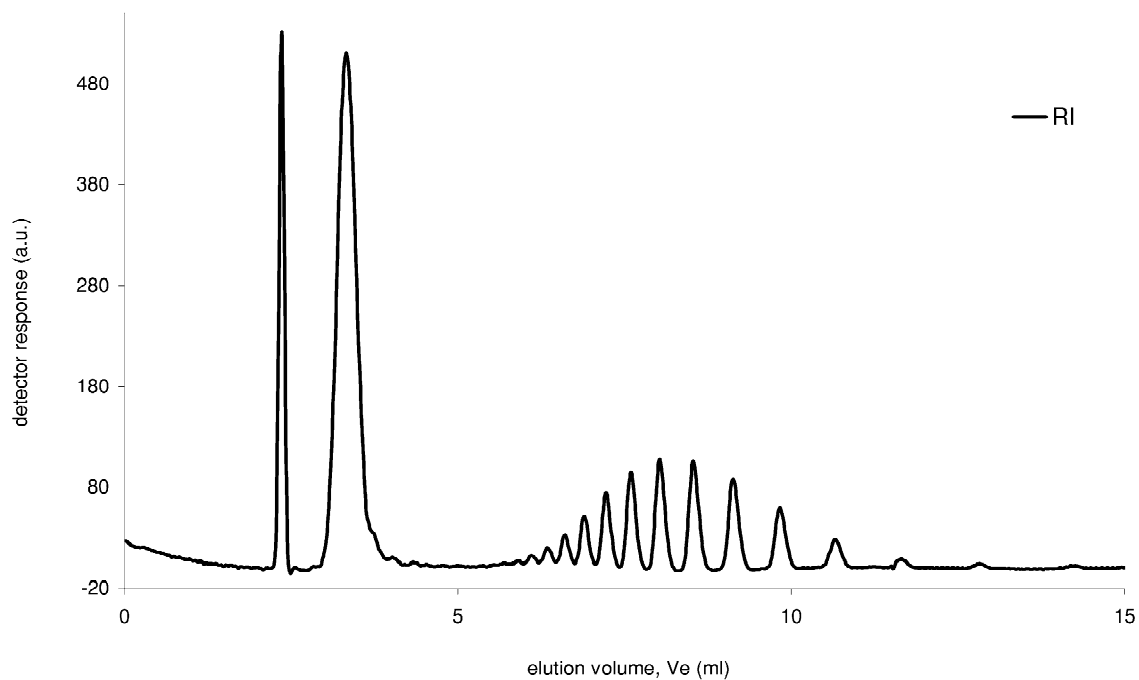


Fig. 11. LEAC of a C_{12} fatty acid polyglycolester ($n=9$) in 86 wt.% acetone on a Prodigy ODS(3) column (System A).

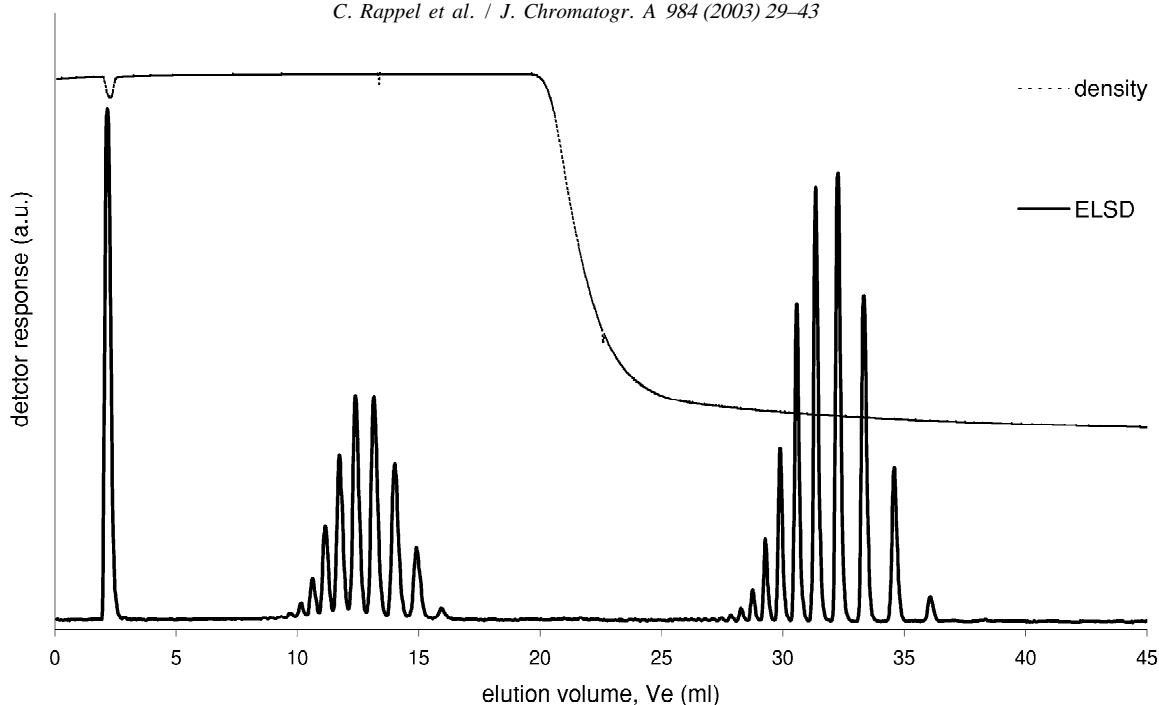


Fig. 12. LEAC of a C_{12} fatty acid polyglycolester ($n=9$) on a Prodigy ODS(3) column (System B). A step gradient from 60 to 85 wt.% acetone was applied after the diblock peak series.

nique reported in previous papers [10,25] could be applied, but this requires quite sophisticated equipment. There is, however, a much easier approach: Since diblocks can be separated very well in an acetone content of about 65% (LEAC conditions) and triblocks will be separated under conditions close to the second critical region (85–90% acetone), a step gradient (from 60 to 90 wt.% acetone) can be applied in order to achieve a separation of both (diblocks and triblocks) in one single chromatogram. As can be seen in Fig. 12, all oligomers of both homologous series are perfectly resolved.

5. Conclusions

In many cases heteropolymers may be considered either as functional macromolecules or as block-copolymers. We compared theoretically the LCCC behavior of functional macromolecules with adsorbing end-groups and of block-copolymers of A–B and A–B–A type with adsorbing A-blocks, and found it quite similar. The chromatographic behaviour of these two types of polymers can be described and

predicted using the theory with properly taken adsorption interaction parameters. We could predict the separation pattern for diblock and triblock copolymers not only for LCCC but as well for LEAC conditions, which allow an increased resolution of the individual oligomers.

Critical conditions for polyoxyethylene in acetone–water system are found experimentally for various reversed-phase columns. For many of the studied columns we observed an unusual adsorption-exclusion behavior of PEGs with two critical points and two adsorption-exclusion transitions. It looks very likely that in some cases the same PEG macromolecules behave a bit different at the first and second critical point: while exhibiting non-functional critical behavior at the first CIP, they behave rather as difunctionals at the second critical point.

Using LCCC and LEAC we have studied the chromatographic separation in copolymeric samples consisting of fatty acid polyglycol esters and fatty acid methyl ester ethoxylates. The observed chromatographic behavior is found to be quite similar to the predictions of the theory. Close to the second CIP di- and triblocks are separated from each other.

While the diblocks elute in a narrow peak, a baseline separation of the individual oligomers is achieved within the triblocks.

Complete separations of both diblocks and triblocks in one single chromatogram can be achieved by using a step gradient, which varies the mobile phase composition from LEAC conditions (to separate the diblocks) up to conditions which are very close to the second CIP (for triblock separation)

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