Retention mechanism, isocratic and gradient-elution separation and characterization of (co)polymers in normal-phase and reversed-phase high-performance liquid chromatography

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Abstract

Synthetic (co)polymers or (co)oligomers with two (or more) repeating groups show not only molar mass distribution, but also composition and sequence distribution of the individual repeat units. To characterize such two- (or more-) dimensional distribution, liquid chromatography under ''critical conditions'' has been suggested, where the separation according to one type of repeating units is suppressed by balancing the adsorption and the size-exclusion effects. In present work it is shown that by combination of adequately selected separation conditions in normal-phase and in reversed-phase systems, the two-dimensional distribution mode can be adjusted to result in the separation following the distribution of any of the two repeat units in ethylene oxide–propylene oxide block (co)oligomers. Based on the retention mechanism suggested, prediction and optimization of the conditions for isocratic and gradient-elution separations of (co)oligomers is possible. HPLC–MS with atmospheric-pressure chemical ionization is a valuable tool for unambiguous identification of the individual (co)oligomers and their tracking in course of method development. Gradient elution can be used for the separation and characterization of block (co)oligomers of ethylene oxide (EO) and propylene oxide (PO) according to the number of the units in one block, while the separation according to the distribution of the units in the other block is suppressed. The effects of the arrangement of the individual EO and PO blocks in the block (co)oligomers (the sequence distribution) affects significantly the retention behavior and the selection of the optimum separation conditions. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Gradient elution; Retention mechanism; Polymers; Ethylene oxide; Propylene oxide

1. Introduction

With widespread use of synthetic polymer- or oligomer-based materials in industry, agriculture and households, the demands increase on the properties of technical products with respect to mechanical and chemical resistance, biodegradability, etc. Technical polymers and oligomers contain varying types and numbers of structural units, the distribution of which controls their quality and suitability for specific applications. For the evaluation of the quality of technical products and for further development of polymerization techniques leading to new tailor-made materials with improved properties, characterization methods are necessary to provide information on the polymer structure. In addition to classical wet analytical and spectroscopic methods, separation techniques and especially size-exclusion (SEC) and high-performance liquid chromatography (HPLC) can provide most detailed information on the composition of the polymer products.

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The basic information on the character of synthetic polymers and oligomers is their molecular mass. However, technical products never are a pure single species, but are more or less complex mixtures of different compounds containing one or more structural repeat units. The composition of these mixed products can be characterized by one or more types of distribution.

(1) Oligomers or polymer products composed of a single repeat monomeric unit, U, are always mixtures containing individual species with various numbers of repeat units, n. The distribution of the species with different numbers of the repeat units represents the molecular mass distribution in the product, which can be schematically represented as:

$-U-, -U-U-, -U-U-U-, -U-U-U-U-,$
$-U-U-U-U-U-, \ldots$
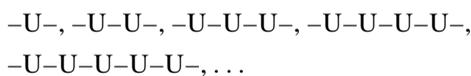
(2) Some polymers may contain various end groups, E_1, E_2, ... so that the functionality type distribution can be attributed to such products, e.g.,:

$E_1-U-U-U-U-E_1, E_2-U-U-U-U-E_2,$
$E_1-U-U-U-U-E_2$
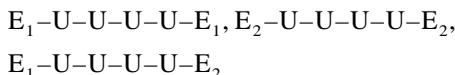
(3) (Co)polymers and (co)oligomers contain two or more different monomeric repeat units, U_1, U_2, ... in various ratios, $n_1:n_2 \ldots$, controlling the chemical composition distribution of the product, schematically represented as:

$-U_1-U_2-U_1-U_1-U_1-, -U_1-U_2-U_1-U_2-U_1-,$
$-U_1-U_2-U_2-U_1-U_2-, -U_1-U_2-U_2-U_2-U_2-$
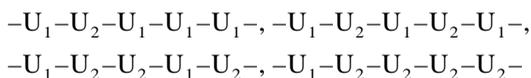
(4) Finally, different monomeric units can be present in different sequences of microblocks with different numbers of the individual repeat units along the polymer chain in random or block (co)polymers. This polymer heterogeneity is characterized by the sequence distribution, such as:

$-U_1-U_2-U_1-U_2-U_1-U_2-,$
$-U_1-U_1-U_1-U_2-U_2-U_2-,$
$-U_1-U_1-U_2-U_2-U_2-U_1-$
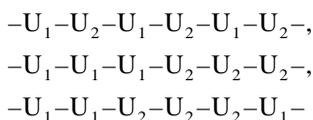
The molecular mass distribution can be determined by size-exclusion chromatography, but vari-

ous distribution types can be characterized using HPLC in normal-phase and in reversed-phase modes (interaction chromatography).

Ethylene oxide–propylene oxide (EO–PO) block copolymers are nonionic surfactants frequently used in washing machines. Because of their thickening capacity and low skin sensitivity they found also applications as the emulsifiers and solubilizers of flavors and fragrances in cosmetic products.

The oxypropylene chain $-[CH_2-CH(CH_3)-O]_m-$ $=(PO)_m$ in the EO–PO block copolymers is hydrophobic, but the oxyethylene chain $-[CH_2-CH_2-O]_n-=(EO)_n$ is hydrophilic. Two types of block (co)polymers with different sequence distribution can be prepared using different technologies: The $(EO)_n-(PO)_m-(EO)_n$ type is prepared by ethoxylation of polypropylene glycol. The reversed reaction sequence, reaction of polyethylene glycol with propylene oxide is used to prepare the second type of block (co)polymers, $(PO)_m-(EO)_n-(PO)_m$. All three types of distribution – the molecular mass distribution, the chemical composition distribution and the sequence distribution – affect the properties of the technical products. With increasing average molecular mass the gelation becomes easier, whereas increasing number of PO units improves the wetting properties of the products and increasing number of EO units improves the solubility in water, but impairs the wetting properties. The (co)polymers with the $(PO)_m-(EO)_n-(PO)_m$ structure are characterized by reduced foam formation and gelation with respect to the (co)polymers of the $(EO)_n-(PO)_m-(EO)_n$ type [1].

Efficient separations of ethoxylated non-ionic surfactants can be achieved by HPLC both in normal-phase systems on columns packed with unmodified silica gel or with amino, nitrile or diol chemically bonded phases [2–16] and in reversed-phase systems [17–26]. Few reports have been published so far on the systematic investigation of their retention behavior [27,28].

Individual ethoxylated oligomers are eluted in the order of increasing number of oligomeric oxyethylene units in normal-phase systems. In reversed-phase systems, the order of elution of the oligomers depends on the oligomeric series and on the mobile phase. For example, oligoethylene glycol phenyl ethers are eluted in the order of increasing number of

oxyethylene units on an octadecylsilica column [29] and so are oligoethylene glycol octylphenyl ethers on a trimethylsilica column in mobile phases comprised of water and methanol [24], whereas oligoethylene glycol nonylphenyl ethers are almost unseparated on a C_{18} column in these mobile phases (Fig. 7 in Ref. [30]) and their order of elution is reversed in aqueous acetonitrile [26,31,32], propanol or dioxane [30], like in chromatography on a mixed-mode reversed-phase/ion-exchange column [33]. We have studied earlier simultaneous effects of the degree of polymerization, n, and of the concentration of the stronger solvent in binary mobile phases on the retention in nonionic oligomeric series, both in reversed-phase [30,34], and in normal-phase [35] systems. Recently, we have extended this investigation to the anionic oligomeric surfactants in reversed-phase [36] and normal-phase ion-pair systems [37] and to ethoxylated alcohol surfactants with different numbers of oxyethylene units and alkyl chain lengths [38].

In the present work, we investigated possibilities of using HPLC in normal-phase and in reversed-phase modes for the determination of the molecular mass distribution, chemical composition distribution and block sequence distribution in block copolymers, with special attention to the EO–PO block copolymers.

2. Theoretical

SEC has been widely used for the characterization of the molecular mass distribution. In the separations controlled solely by size-exclusion mechanism, polymeric compounds, dependent on their size, partly penetrate into the pores of the inert column packing. As the volume of the pores accessible to the molecules decreases as the size of the molecules increases, the elution volumes, V_e, decrease with increasing molecular masses of the individual species and V_e of all sample components are in the range between the column interparticle volume, V_i, and the column hold-up volume, V_M, so that the volume of the eluate available for the separation is rather limited.

In reversed-phase (RP) HPLC, in normal-phase (NP) HPLC and in ion-exchange HPLC (HPIEC), all

sample compounds are retained because of the interactions with the stationary and (or) with the mobile phase. If the three HPLC modes are applied to polymer separations, they can be classified as interaction chromatography to be distinguished from SEC. If size- or ionic-exclusion effects are absent in interaction chromatography, all analytes are eluted with elution (retention) volumes $V_e = V_R$ greater than V_M and are limited only by the acceptable time of analysis. Hence, the separation selectivity for the species with different numbers of oligomeric units (the oligomeric selectivity) is usually greater than in SEC. The retention of polymers and oligomers in interaction chromatography depends on the number of repeat units, n, and on the concentration of the strong eluent, φ, in the mobile phase. The retention characterized by the retention factors, $k = (V_R - V_M)/V_M$, usually increases so that the logarithms of the retention factors change in a linear manner as the number of repeat units, n, i.e., the molecular mass, M_r increases [30].

In reversed-phase systems, the logarithms of the retention factors usually decrease in a linear manner with increasing concentration of the organic solvent (stronger eluent, B) in a binary aqueous–organic mobile phase, φ

$$\log k = a - m\varphi \tag{1}$$

The dependence of k on the number of repeat units, φ, and on n can often be described by Eq. (2) [30,34]:

$$\log k = a_0 + a_1 n - (m_0 + m_1 n)\varphi \tag{2}$$

In normal-phase systems with binary organic mobile phases containing two solvents, one less polar (A) and the other more polar (B), the dependence of the retention factors on the concentration φ of the solvent B can be often described by a simple Eq. (3) [35]:

$$\log k = a - m \log \varphi \tag{3}$$

(this equation formally applies also in HPIEC, where φ is the concentration of a salt, acid or base in an aqueous or aqueous–organic mobile phase).

In some normal-phase systems, Eq. (3) fails to fit the retention data and a three-parameter Eq. (4)

should be used to describe adequately the retention behavior [39]:

$$k = \frac{1}{(a + b\varphi)^m} \qquad (4)$$

For NP systems where Eq. (3) applies, the dependence of k on n and on the concentration of a more polar solvent B, φ, in a mobile phase comprised of two organic solvents with different polarities can be described by Eq. (5), formally similar to Eq. (2) [27,35]:

$$\log k = a_0 + a_1 n - (m_0 + m_1 n) \log \varphi \qquad (5)$$

where a_0, a_1, m_0, m_1 in Eqs. (2) and (5) are constants depending on the character of the repeat units and of the end groups in the oligomer or polymer, on the chemistry of the stationary phase or of the adsorbent in the column and on the type of the mobile phase components, but are independent of the concentrations of the mobile phase components. For some chromatographic systems and oligomeric series, additional terms, quadratic with respect to n or φ, should be added to the right-hand side of Eqs. (2) or (5).

In liquid chromatography of polymers on a porous active column packing, size-exclusion effects and interactions with the stationary (and with the mobile) phases can affect simultaneously the chromatographic behavior. Sometimes conditions can be found at which the size-exclusion effects exactly compensate the interactions of the polymers with the components of the chromatographic system [40–42], so that individual polymers or oligomers with different number of repeat units are co-eluted in a single peak at the same elution volume, V_e independent of n. These conditions are known as ''liquid chromatography at the critical point of adsorption'' or as the ''point of exclusion–adsorption transition'' and have been recently reviewed [43]. Recently, Thrathnigg et al. have presented theoretical explanation of the behavior of lower [44] and of higher [45] polyethylene glycol ethers in reversed-phase systems by combined effects of adsorption and size exclusion.

The elution volume, V_e, contributed to by both size-exclusion effect and interactions causing the retention in the stationary phase can be expressed as:

$$V_e = V_i + K_{sec} V_p + K_i V_s \qquad (6)$$

where V_i is the interstitial volume (volume outside the particles in the column), V_p is the pore volume in the column and V_s is the volume of the stationary phase, K_{sec} and K_i are the distribution constants for size exclusion and for the retention equilibrium between the stationary and the mobile phases.

Exact compensation of the size-exclusion effects and of the interactions causing the retention in the stationary phase for oligomers with different numbers of repeat units should result in the elution of all oligomers close to the column hold-up volume (no exclusion and no retention). The size-exclusion effects may influence also the behavior of retained compounds, but the interaction effects predominate. The size-exclusion effects may either influence the values of the constants a_0, a_1, m_0, m_1 of Eqs. (2) and (5) or – possibly – may contribute to non-linearity of the $\log k$ versus n plots.

From Eqs. (2) and (5) it follows that co-elution can occur also in pure retentive interaction-controlled separations at a certain concentration of the component B, φ_i in the mobile phase if:

either: $\varphi_i = a_1/m_1$ (RP systems)   or

$\quad\quad \varphi_i = 10^{a_1/m_1}$ (NP systems).

If the contribution of each repeat unit [such as PO and EO in EO–PO (co)oligomers] to the retention energy of copolymers is constant, the dependence of the retention of the individual species on the number of the two units, n_{EO} and n_{PO}, can by described Eq. (7):

$$\log k = \log \beta + n_{EO} \log \alpha_{EO} + n_{EO}^2 \log \gamma_{EO}$$
$$+ n_{PO} \log \alpha_{PO} + n_{PO}^2 \log \gamma_{PO} \qquad (7)$$

Here, α_{EO} and α_{PO} express the oxyethylene and the oxypropylene separation selectivities, respectively, i.e., the elution ratio of the oligomers differing by one oxyethylene or oxypropylene oligomeric repeat unit, $\log \beta$ represents the contribution to the retention by the end group(s) and $\log \gamma$ the deviations of all kinds from the linear $\log k$ versus n plots, i.e., from the Martin rule (for more details see Refs. [34] and [46]). The dependence of the retention and separation selectivity of the individual block units

can be described by equations similar to Eqs. (2) and (5) in reversed-phase and in normal-phase systems, respectively.

The conditions for co-elution of oligomers with different numbers of a certain type of repeat unit have been employed for the separation of various polymers according to their functionalities. The ''critical conditions'' are especially useful for characterization of block copolymers which represent very complex mixtures of individual species. To facilitate the analysis, the separation of one block is intentionally suppressed to make possible the analysis of the other block with respect to its molar mass distribution [47]. This method has been applied to various polymers and oligomers, including polyethylene glycol ethers. Reversed-phase chromatography with acetonitrile–water [48] or with methanol–water [49] mobile phases was employed for this purpose.

If Eq. (2) in RP systems or Eq. (5) in NP systems control the retention of the individual blocks in the block copolymers, the separation solely according to the distribution of one type, i, of repeat units can be achieved if the deviations from the Martin rule are not very significant, i.e., if $\gamma_i \approx 1$ and if:

$\varphi_i = a_i / \boldsymbol{m_i}$ (for Eq. 2 in the RP systems) or

$\varphi_i = 10^{a_i / m_i}$ (for Eq. 5 in the NP systems)

In chromatography of polymers, isocratic elution allows separation of a limited number of compounds and gradient elution is necessary for the separation of more complex samples. To accomplish gradient-elution separation of block copolymers according to a single distribution mode for one type of oligomer units and close to ''critical conditions'' for the second block, the oligomer selectivity for the second type of oligomer units, α_2, should not depend significantly on the composition of the mobile phase, i.e., $m_1 \approx 0$.

The identification of the individual compounds in the chromatograms of products containing (co)oligomers with two different distribution modes can be difficult if each mode contributes to the retention. In normal-phase systems, polar repeat units contribute more strongly to the retention than the less polar oligomer units and the effect is opposite in reversed-phase systems. Hence, two-dimensional

chromatographic separations of complex mixtures of (co)oligomers with bimodal distribution should be theoretically possible – the separation into groups with the same number of polar units and with different less-polar units in reversed-phase systems, or into groups with the same number of non-polar units, but with different numbers of more polar units in normal-phase systems.

LC–MS techniques are particularly useful for the analysis of (co)polymers and (co)oligomers, as they allow mass spectral identification of individual oligomers in the groups separated by HPLC under conditions close to ''critical'' [38,50,51], so that two-dimensional separations are not necessary. Off-line coupling with fast atom bombardment (FAB) or desorption chemical ionization was originally used for LC–MS of surfactants [52]. Later, direct on-line coupling was possible using new interfaces based on different ionization techniques such as continuous flow FAB [53,54], thermospray or electrospray [55,56]. In an earlier work [38], we applied LC–MS with atmospheric pressure chemical ionization (APCI) for the investigation of retention mechanism of oligomers with bimodal distribution of alkyls and of EO groups in samples of ethoxylated alcohols in normal- and reversed-phase systems.

3. Experimental

3.1. Materials

n-Hexane and dichloromethane were obtained from Baker (Deventer, The Netherlands), acetonitrile and 2-propanol from Labscan (Dublin, Ireland). All solvents were of HPLC grade. Water was doubly distilled in glass (with addition of potassium permanganate and sodium hydrogencarbonate). Glass cartridge columns (150×3 mm I.D.), packed with Separon SGX C_{18}, particle size 7 μm (octadecyl silica) and Separon SGX NH_2, 7 μm (aminopropyl silica), both average pore size 8 nm, were purchased from Tessek, Prague, Czech Republic.

Technical samples of EO–PO block copolymers were obtained from Sloveca (Nováky, Slovak Republic): Slovank 310, $(EO)_n$–$(PO)_m$–$(EO)_n$ type,

Novanik 600/20, 600/40 and 600/50, $(PO)_m$–$(EO)_n$–$(PO)_m$ type, with different average numbers of EO and PO units.

3.2. Chromatographic and mass spectrometric instrumentation

The liquid chromatograph used consisted of a Model 616 pump, a Model 717+ autosampler, a four-channel solvent delivery system (low-pressure gradient system), a thermostatted column compartment, a Model 996 photodiode-array detector and a Millenium chromatography manager (all from Waters, Milford, MA, USA). As the sample compounds do not absorb significantly in the UV region, the outlet from the column was connected either to a Model PL-EMD 950 evaporative light scattering detection (ELSD) system (Polymer Labs., Shropshire, UK) or directly to a VG Platform quadrupole mass analyzer (Micromass, Manchester, UK) with atmospheric pressure chemical ionization in the positive mode, molecular mass range up to 3000. The ELSD signal was processed using a Vectra V12 personal computer (Hewlett-Packard, Avondale, CA, USA) with a CSW 1.6 chromatographic data station (Data Apex, Prague, Czech Republic).

3.3. Procedures

Mobile phases for isocratic experiments were prepared by pre-mixing appropriate volumes of solvents, filtered through a 0.45-μm Millipore filter prior use and degassed by continuous stripping with helium during the analysis. The samples were prepared by dissolving in the mobile phase in concentrations ca. 1 mg/ml. The column temperature was kept at 40°C and the flow-rate at 1 ml/min in all experiments. A 10- or 20-μl sample volume was injected into the liquid chromatograph.

Mass spectrometric data were acquired in the molecular mass range from 35 to 1500 at the scan duration of 1.9 s in the positive-ion APCI mode. A potential of 3.05 kV was applied on the discharge needle. The temperature was held at 500°C in the APCI probe and at 90°C in the ion source. Nitrogen was used as the drying, sheath and nebulizing gas.

Mild ionization conditions with cone voltage of 10 V were selected to suppress the fragmentation.

Each experiment was repeated at least twice. From the retention volumes, V_R, the retention factors, $k = (V_R/V_M - 1)$ were determined. The column hold-up volumes, V_M, were determined as the elution volumes of non-retained compounds (methanol and acetonitrile for a Separon SGX C_{18} column, $V_M = 0.74$ ml and n-hexane for a Separon SGX Amine column in normal-phase systems, $V_M = 0.90$ ml) and both V_R and V_M were corrected for the volumes of the connecting tubing. Each chromatographic peak was attributed to the individual oligomer species on the basis of the mass of its $[M+H]^+$ ion in the APCI mass spectrum. Peaks of the $[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$ ions were identified as the most significant peaks in the mass spectra. From their m/z values, corresponding combinations of the numbers of the oxypropylene ($-CH_2-CH(CH_3)-O-$, PO) and oxyethylene ($-CH_2-CH_2-O-$, EO) groups in the individual oligomers were determined. With the cone voltage of 10 V, the fragmentation is negligible. From the total ion current (TIC) chromatograms, the chromatograms corresponding to the species with a constant number of oxyethylene but different numbers of oxypropylene units or to the species with a constant number of oxypropylene but different numbers of oxyethylene units were reconstructed selecting only the ion currents of the corresponding $[M+H]^+$ ions.

Multiparameter nonlinear regression analysis using the ADSTAT software (TRILOBYTE, Prague, Czech Republic) was used to calculate the constants of the dependencies of log k on the numbers of oxyethylene and oxypropylene units (Eq. (7)). The constants of this equation are given in Tables 1, 3 and 5 separately for the $(EO)_n-(PO)_m-(EO)_n$ and $(PO)_m-(EO)_n-(PO)_m$ block copolymer types in the three chromatographic systems studied. Linear or nonlinear regression was used to determine the parameters of the equations describing the dependence of the retention factors on the composition of the mobile phase, which are listed in Table 2 for the Separon SGX C_{18}/acetonitrile–water RP system (Eq. (1)), in Table 4 for the Separon SGX Amine/2-propanol–n-hexane and in Table 6 for the Separon SGX Amine/acetonitrile–water–dichloromethane NP systems (Eq. (4)).

Table 1

Constants of the dependences of the retention factors, k, on the number of oxyethylene, n_{EO} and oxypropylene, n_{PO}, repeat units of $(PO)_m-(EO)_n-(PO)_m$ (Novanik) and $(EO)_n-(PO)_m-(EO)_n$ (Slovanik) block copolymers on a Separon SGX C_{18} column in acetonitrile–water mobile phases: $\log k = \log \beta + n_{EO} \log \alpha_{EO} + n_{EO}^2 \log \gamma_{EO} + n_{PO} \log \alpha_{PO} + n_{PO}^2 \log \gamma_{PO}$

	50% CH_3CN	60% CH_3CN	70% CH_3CN	80% CH_3CN	90% CH_3CN
Novanik					
Log β	-1.26 ± 0.02	-1.72 ± 0.08	-0.40 ± 0.05	-0.24 ± 0.02	0.5 ± 0.1
Log α_{EO}	-0.0011 ± 0.0004	0.0005 ± 0.0003	0.0003 ± 0.0001	0.00009 ± 0.00006	-0.0006 ± 0.0005
Log α_{PO}	0.187 ± 0.005	0.22 ± 0.02	0.01 ± 0.01	-0.021 ± 0.005	-0.04 ± 0.04
Log γ_{PO}	-0.0012 ± 0.0003	-0.004 ± 0.001	0.0044 ± 0.0008	0.0044 ± 0.0003	0.002 ± 0.003
C.D.[a]	0.9995	0.9914	0.9868	0.9945	0.8723
Slovanik					
Log β	-1.193 ± 0.008	-1.05 ± 0.02	-0.839 ± 0.005	-0.764 ± 0.004	-0.570 ± 0.006
Log α_{EO}	-0.025 ± 0.004	-0.02 ± 0.01	-0.026 ± 0.002	-0.019 ± 0.002	0.001 ± 0.003
Log γ_{EO}	0.0039 ± 0.0009	0.001 ± 0.002	0.0018 ± 0.0006	0.0030 ± 0.0005	0.0040 ± 0.0007
Log α_{PO}	0.1771 ± 0.0006	0.147 ± 0.001	0.1112 ± 0.0003	0.0826 ± 0.0003	0.063 ± 0.003
C.D.[a]	0.9996	0.9967	0.9996	0.9995	0.9985

[a] C.D. = Coefficient of determination.

4. Results and discussion

4.1. HPLC–APCI-MS analysis of the EO–PO block (co)oligomers

Mass spectrometric detection with APCI ionization in the positive ion mode allows sensitive detection of the samples of EO–PO (co)oligomers, which do not absorb radiation in the UV region. Further, unambiguous identification of the individual species is possible even in the chromatograms with overlapping peaks, as the ionization conditions (sample cone voltage) were adjusted to yield only $[M+H]^+$ ions. Fig. 1 shows examples of mass spectra of overlapped peaks of the $(EO)_n-(PO)_m-(EO)_n$ (Slovanik, Fig. 1A) and the $(PO)_m-(EO)_n-(PO)_m$ (Novanik, Fig. 1B) (co)oligomers, in which series of peaks with different EO or PO numbers are apparent at characteristic m/z values.

By selecting the ions corresponding to a series with a single EO unit and with different numbers of PO units, or the ions corresponding to a series with a single PO unit and with different numbers of EO units, chromatograms corresponding to the individual oligomer units distribution modes were extracted, so that overlapping peaks could be deconvolved. In the chromatograms reconstructed in this way, retention volumes could be determined for the investigation of

Table 2

Constants of the dependences of the retention factors, k, of $(EO)_n-(PO)_m-(EO)_n$ (Slovanik) block copolymers on the concentration of acetonitrile, φ (%, v/v, $\cdot 10^{-2}$), on a Separon SGX C_{18} column in acetonitrile–water mobile phases (50–80% acetonitrile): $\log k = a - m\varphi$

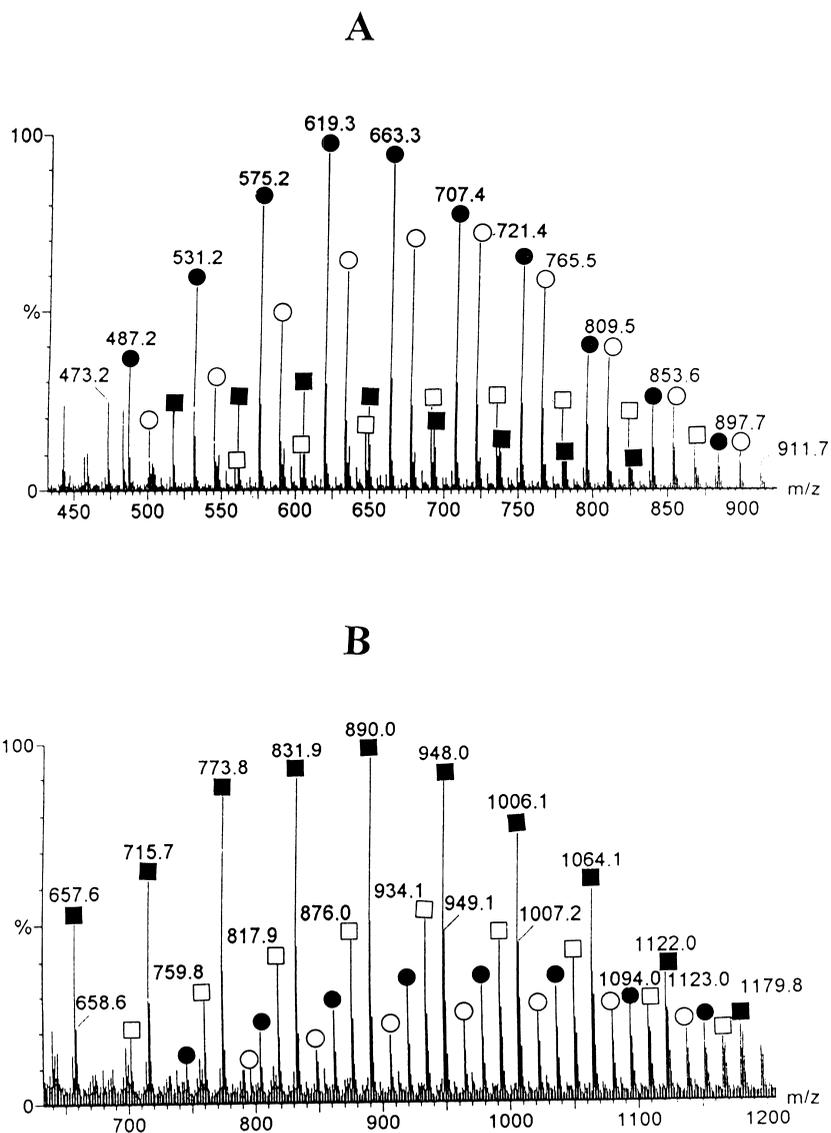| $(n_{EO}=2)$	$n_{PO}=11$	$n_{PO}=12$	$n_{PO}=13$	$n_{PO}=14$	$n_{PO}=15$
a	1.79 ± 0.06	2.11 ± 0.05	2.42 ± 0.05	2.74 ± 0.02	3.06 ± 0.01
m	2.07 ± 0.09	2.39 ± 0.07	2.66 ± 0.08	2.97 ± 0.04	3.27 ± 0.02
k[a]	0.9960	0.9983	0.9981	0.9997	0.9999
$(n_{PO}=13)$	$n_{EO}=0$	$n_{EO}=1$	$n_{EO}=2$	$n_{EO}=3$	$n_{EO}=4$
a	2.45 ± 0.05	2.43 ± 0.04	2.42 ± 0.05	2.42 ± 0.02	2.40 ± 0.04
m	2.66 ± 0.08	2.65 ± 0.05	2.66 ± 0.08	2.69 ± 0.03	2.67 ± 0.06
k[a]	0.9983	0.9992	0.9981	0.9997	0.9990

[a] k = Correlation coefficient.

A

B

Fig. 1. APCI(+) mass spectra of coeluted peaks of EO–PO block copolymers; Column: Separon SGX C_{18}, gradient 15–100% acetonitrile in water in 25 min (0.5 ml/min, 40°C). Peaks are marked for series of [M+H]+ ions with various numbers of EO and PO units: (A) $(PO)_m$–$(EO)_n$–$(PO)_m$ (Novanik); $n_{PO}=1$, $n_{EO}=10$–17 (■); $n_{PO}=2$, $n_{EO}=8$–17 (●); $n_{PO}=3$, $n_{EO}=7$–16 (○) and $n_{PO}=4$, $n_{EO}=7$–14 (□). (B) $(EO)_n$–$(PO)_m$–$(EO)_n$ (Slovanik); $n_{EO}=0$, $n_{PO}=11$–20 (■); $n_{EO}=1$, $n_{PO}=11$–19 (□); $n_{EO}=2$, $n_{PO}=11$–18 (●) and 4 $n_{EO}=3$, $n_{PO}=11$–17 (○).

the effects of the chromatographic conditions on the separation. Further, peak areas in the deconvolved chromatograms can be integrated and the distribution of the EO and PO units among the individual species can be determined.

4.2. Effect of the molecular mass and chemical composition distribution on the retention behavior of the EO–PO block (co)oligomers

In the reversed-phase systems studied, i.e., on a

Separon SGX C_{18} column with acetonitrile–water mobile phases, the retention of both the $(EO)_n$–$(PO)_m$–$(EO)_n$ (Slovanik) and the $(PO)_m$–$(EO)_n$–$(PO)_m$ (Novanik) (co)oligomers can be described by Eq. (7). The constants of this equation are given in Table 1. The quadratic terms with log γ_{EO} are negligibly small for the Novanik samples and the quadratic terms with log γ_{PO} for the Slovanik samples. The quadratic terms with log γ_{EO} for the Slovanik samples and with log γ_{PO} for the Novanik samples are more significant, but are one- to two-orders of magnitude smaller than the linear terms and can be neglected to first approximation, as it is demonstrated by the log k versus n_{EO} and n_{PO} plots in Figs. 2 and 3, which are close to straight lines.

The oligomer selectivity for rather polar EO units is low in the reversed-phase systems studied (the term log α_{EO} is approximately two-orders of magnitude lower than the oligomer selectivity for less polar PO units, log α_{PO}). For reversed-phase systems the retention (log k) increased in linear manner with increasing number of the repeat PO oligomer units, n_{PO}, so that these systems are principally well suited for the separations of the EO–PO (co)oligomers according to the number of PO units. The $(EO)_n$–$(PO)_m$–$(EO)_n$ (Slovanik) (co)oligomers could be separated according to the number of PO units in all mobile phases studied, but the $(PO)_m$–$(EO)_n$–$(PO)_m$ (Novanik) (co)oligomers only in mobile phases with 60% or less acetonitrile. The oligomer selectivities for PO units in the Slovanik and Novanik samples are approximately equal to each other in 50% acetonitrile as the mobile phase. With the Slovanik samples, log α_{PO} regularly decreases with decreasing concentration of acetonitrile in the mobile phase from 50 to 90%. (Table 1). On the other hand, the dependence of the oligomer selectivity for PO units in the Novanik samples is more complex. There is little difference between the log α_{PO} values in 50 and 60% acetonitrile, but the oligomer selectivity for the PO units is much lower in mobile phases with 70 and 80% acetonitrile and reversed elution order is observed for the oligomers with different numbers of PO units in 90% acetonitrile (line 5 in Fig. 3A). We have observed earlier similar retention behavior as with the Novanik samples for ethoxylated fatty alcohol surfactants with two blocks of different polarities – $(EO)_n$ and $(CH_2)_m$ [38]. In the Novanik

Fig. 2. Dependencies of the retention factors, k, on the number of PO units, n_{PO}, for the oligomers with two EO units (A) and on the number of EO units, n_{EO}, for the oligomers with 13 PO units (B) in a Slovanik sample, type $(EO)_n$–$(PO)_m$–$(EO)_n$. Column: Separon SGX C_{18}, mobile phase: acetonitrile–water (50:50) (1), (60:40) (2), (70:30) (3), (80:20) (4), (90:10) (5), temperature: 40°C.

(co)oligomers, the inner block is formed by the EO units and the end blocks by the PO units, whereas the EO and the PO blocks in the Slovanik samples are arranged in the opposite order. The sequence distribution in the EO–PO (co)oligomers affects
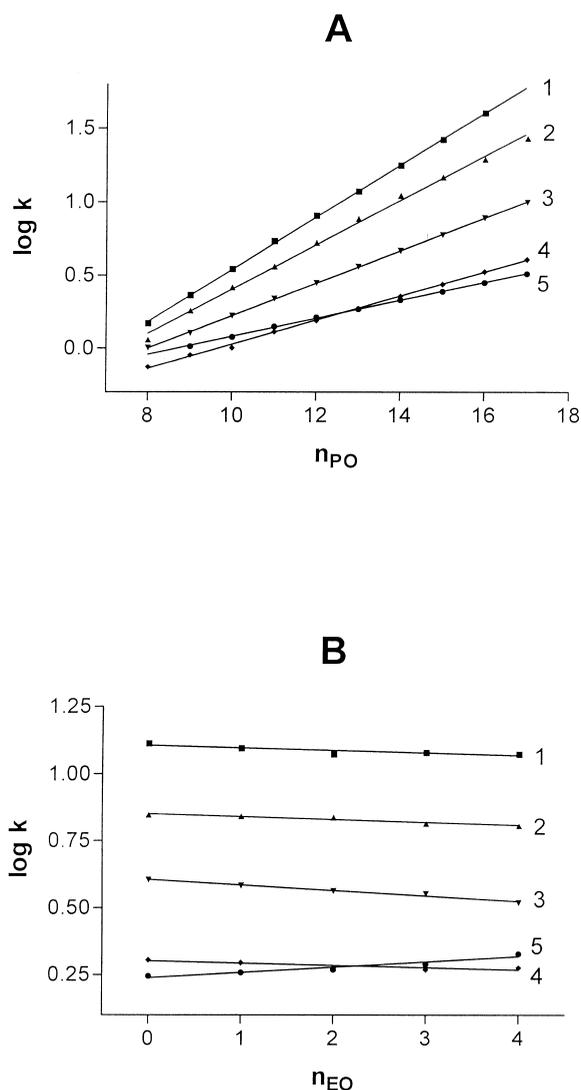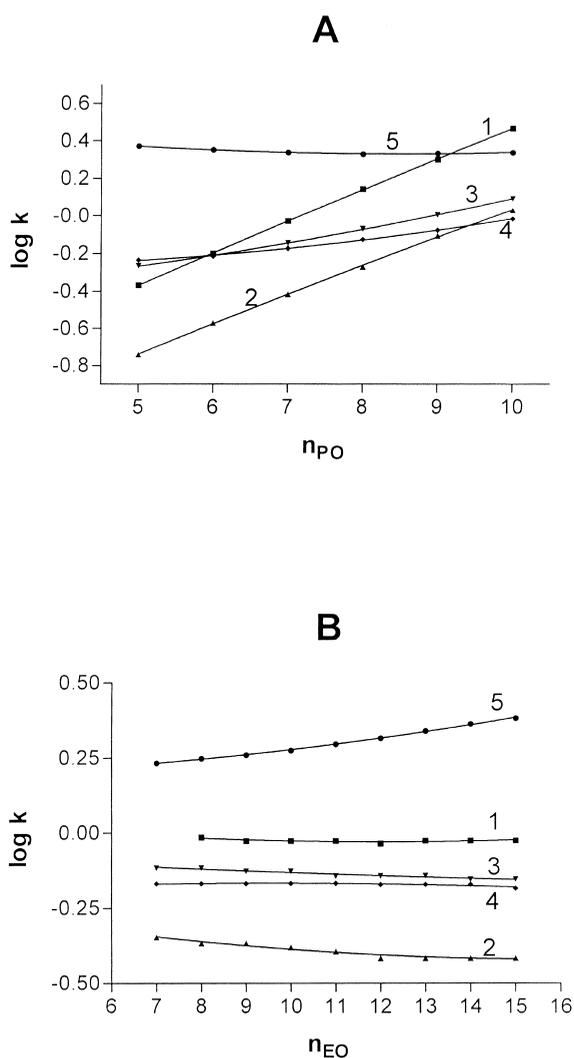
A

B

Fig. 3. Dependencies of the retention factors, k, on the number of PO units, n_{PO}, for the oligomers with 13 EO units (A) and on the number of EO units, n_{EO}, for the oligomers with seven PO units (B) in a Novanik sample, type $(PO)_m$–$(EO)_n$–$(PO)_m$. column: Separon SGX C_{18}, mobile phase: acetonitrile–water (50:50) (1), (60:40) (2), (70:30) (3), (80:20) (4), (90:10) (5), temperature: 40°C.

significantly the retention behavior, as is discussed below in more detail.

The retention either decreases or increases with increasing number of EO units, n_{EO}, with both Slovanik and Novanik (co)oligomers, but the effect of the number of EO units on the retention is much lower than with PO units and the oligomer selectivity

for the EO units is close to zero in the whole range of the mobile phase composition tested (Figs. 2B and 3B). However, the oligomer selectivity for the EO units is one- to two-orders of magnitude lower for the Novanik samples than for the Slovanik samples. This means that the separation according to the number of PO units under "critical conditions" for the EO block is possible in reversed-phase systems for Novanik sample types in mobile phases containing 70% or less acetonitrile, but is difficult to achieve for the Slovanik samples.

Eq. (7) also describes well the retention of the EO–PO block (co)oligomers in the normal-phase systems on a Separon SGX Amine column in 2-propanol–n-hexane (Table 3) and in acetonitrile–water–dichloromethane (Table 5) mobile phases. In 2-propanol–hexane mobile phases the retention (log k) of the two types of block (co)oligomers exhibits a linear increase with increasing number of polar EO units (Fig. 4), the quadratic term with γ_{EO} in Eq. (7) is negligible. On the other hand, the retention of (co)oligomers decreases with increasing number of relatively non-polar PO units, but this decrease is much less significant than the increase of retention with increasing number of EO units (Fig. 5). The values of the oligomer selectivity for PO units, log α_{PO}, are negative and an order of magnitude lower than the positive values of the oligomer selectivity for EO units, log α_{EO} (Table 3). Hence, the separation according to the number of EO units under close-to "critical elution conditions" for the PO block is principally possible over a wide range of concentrations of 2-propanol in the mobile phase, where the species with different numbers of PO units are co-eluted in a common peak. However, the oligomer selectivity for the PO units (negative) in Novanik is approximately seven-times larger than the selectivity for the PO units in Slovanik, so that in this chromatographic system "critical elution conditions" and separation according to the number of EO units can be achieved for Slovanik type (co)oligomers only.

The quadratic term with γ_{PO} can be neglected only for the $(EO)_n$–$(PO)_m$–$(EO)_n$ (Slovanik) (co)oligomers, but it is approximately four-times larger for the $(PO)_m$–$(EO)_n$–$(PO)_m$ (Novanik) (co)oligomers whose log k versus n_{PO} plots are significantly curved (Table 3, Fig. 5A and B).

Table 3
Constants of the dependences of the retention factors, k, on the number of oxyethylene, n_{EO} and oxypropylene, n_{PO}, repeat units of $(PO)_m–(EO)_n–(PO)_m$ (Novanik) and $(EO)_n–(PO)_m–(EO)_n$ (Slovanik) block copolymers on a Separon SGX Amine column in 2-propanol–hexane mobile phases: $\log k = \log \beta + n_{EO} \log \alpha_{EO} + n_{EO}^2 \log \gamma_{EO} + n_{PO} \log \alpha_{PO} + n_{PO}^2 \log \gamma_{PO}$

Novanik	30% 2-Propanol	Slovanik	30% 2-Propanol	10% 2-Propanol
Log β	0.39±0.03	Log β	−0.200±0.005	0.253±0.004
Log α_{EO}	0.0821± 0.0004	Log α_{EO}	0.127±0.003	0.223±0.002
Log α_{PO}	−0.154±0.009	Log α_{PO}	−0.0222±0.0003	−0.0119±0.0003
Log γ_{PO}	0.0059±0.0006	Log γ_{PO}	0.0015±0.0007	−0.0024±0.0005
C.D.[a]	0.9983	C.D.	0.9985	0.9996

[a] C.D.=Coefficient of determination.

The retention of the EO–PO (co)oligomers on a Separon SGX Amine column in acetonitrile–water–dichloromethane mobile phases increases with increasing number of EO units (Fig. 6) and with decreasing number of PO units (Fig. 7) and can be also adequately described by Eq. (7), whose parameters are given in Table 5. In contrast to the other two chromatographic systems studied, the oligomer selectivities for both EO and PO units are significant so that ''critical conditions'' neither for PO nor for EO blocks are observed and peaks corresponding to the two types of distribution are seriously overlapped at any mobile phase composition. This chromatographic system shows some features found with normal-phase systems and other typical for reversed-phase retention behavior. The identification of the individual species was possible only using APCI-MS detection for the deconvolution of overlapped peaks (Fig. 8).

The log k versus n_{EO} plots for the $(EO)_n–(PO)_m–(EO)_n$ (Slovanik) (co)oligomers and the log k versus n_{PO} plots for the $(PO)_m–(EO)_n–(PO)_m$ (Novanik) (co)oligomers are significantly curved as demonstrated by the relatively high values of the quadratic terms with γ_{EO} for Slovanik and with γ_{PO} for Novanik samples (Table 5). In this chromatographic system, preferential adsorption of water on the bonded amino column occurs. Consequently, oligomeric units in different distances from the other block can be solvated to different extent by the mobile phase components and their contributions to the retention may differ. It can be assumed that the adsorbed individual species have the EO block(s) oriented towards the surface of the amino phase and the PO block(s) towards the less polar bulk mobile phase. The EO units close to the surface of the stationary phase are better solvated by the mobile phase more rich in water while the PO units more distant from the surface are better solvated by the bulk mobile phase with a lower concentration of water.

In the Novanik samples, the inner polar EO block contributes to the retention, but the PO end blocks of low polarities diminish the retention. The contribution of the EO units in the center of the inner block to the retention is higher than that of the EO units close to and shielded by the PO end blocks (Fig. 6B). The contribution of the PO units in the end blocks close to the inner block to the decrease of the retention is probably more significant than the effect of more distant PO units, which are better solvated by the bulk mobile phase (Fig. 7A). In the Slovanik samples, the EO units in the end blocks have better access to the surface of the adsorbent and are possibly not affected by the inner PO block, oriented towards the bulk mobile phase so that their retention is affected less significantly by the PO block than with the Novanik samples and show more regular contribution to the retention (Fig. 6A). The PO units in the inner non-polar block oriented into the bulk liquid phase also show more regular contribution to decreasing retention than the PO units in the end blocks of the Novanik samples (Fig. 7B).

Like in RP system with aqueous acetonitrile, the block sequence in the (co)oligomers affects significantly the retention behavior in the NP systems studied.

4.3. Effect of the mobile phase on the retention of the EO–PO block copolymers
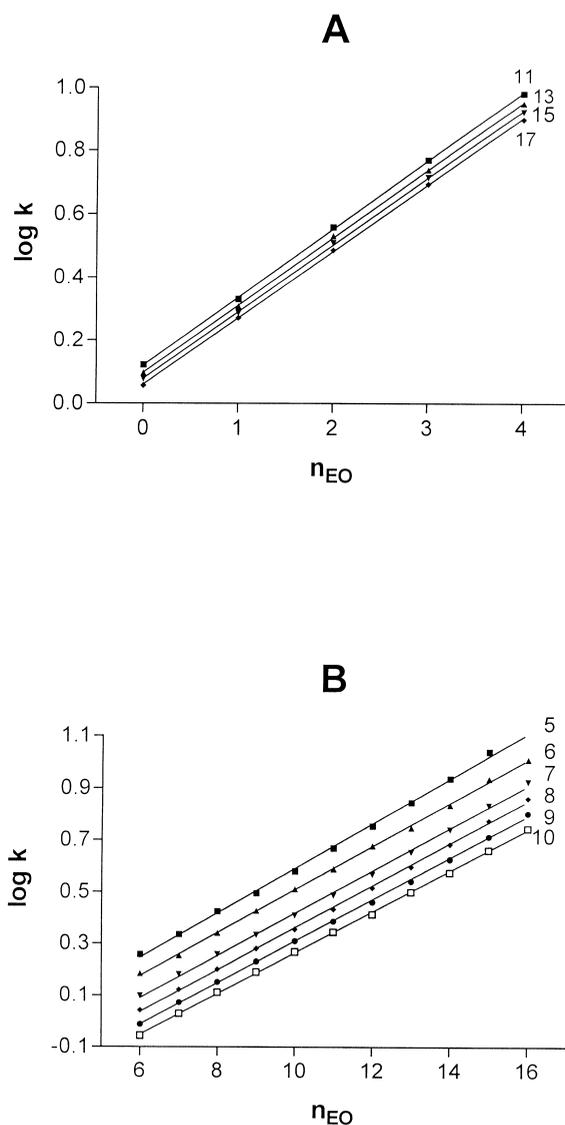
In reversed-phase systems, the effect of the con-

A

A

B

Fig. 4. Dependencies of the retention factors, k, on the number of EO units, n_{EO}, for the oligomers with 11–17 PO units in a Slovanik sample, type $(EO)_n–(PO)_m–(EO)_n$, in 2-propanol–n-hexane (10:90), mobile phase A and for the oligomers with 5–10 PO units in a Novanik sample, type $(PO)_m–(EO)_n–(PO)_m$, in 2-propanol–n-hexane (30:70), mobile phase B. Normal-phase chromatography on a Separon SGX Amine column, temperature: 40°C.
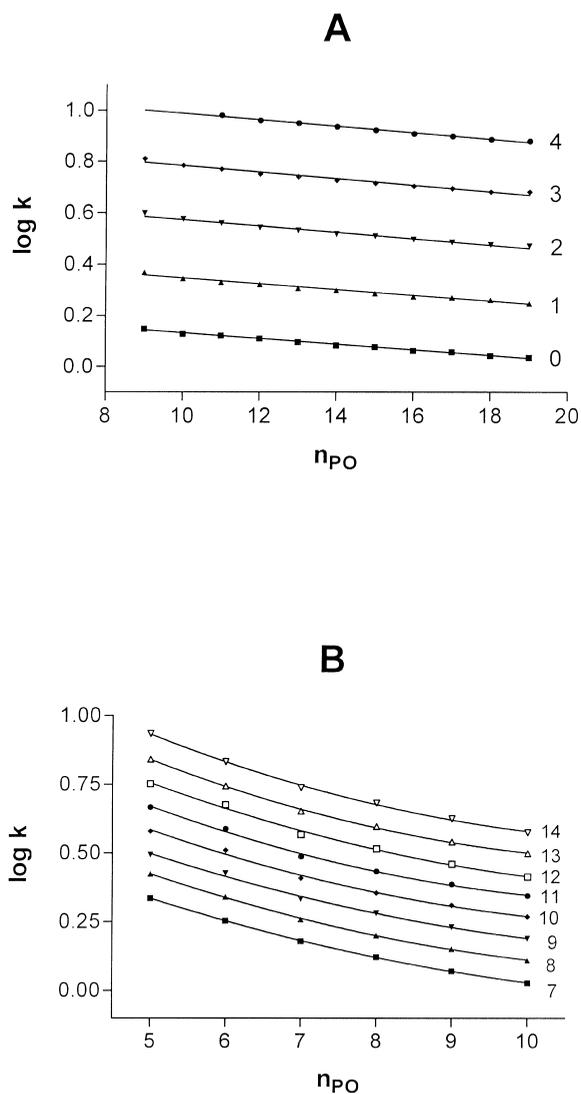
B

Fig. 5. Dependencies of the retention factors, k, on the number of PO units, n_{PO}, for the oligomers with 0–4 units in a Slovanik sample, type $(EO)_n–(PO)_m–(EO)_n$, in 2-propanol–n-hexane (10:90), mobile phase A for the oligomers with 7–14 EO units in a Novanik sample, type $(PO)_m–(EO)_n–(PO)_m$, in 2-propanol–n-hexane (30:70), mobile phase B. Normal-phase chromatography on a Separon SGX Amine column, temperature: 40°C.

centration of acetonitrile in the mobile phases on the retention is adequately described by Eq. (1) only for the $(EO)_n–(PO)_m–(EO)_n$ (Slovanik) (co)oligomers (Table 2). The retention of $(PO)_m–(EO)_n–(PO)_m$

(Novanik) (co)oligomers exhibits minimum retention in 60% acetonitrile as the mobile phase.

In normal-phase systems with a Separon SGX Amine column and 2-propanol–n-hexane mobile phases, the effect of the concentration of 2-propanol in the mobile phase should be described by the
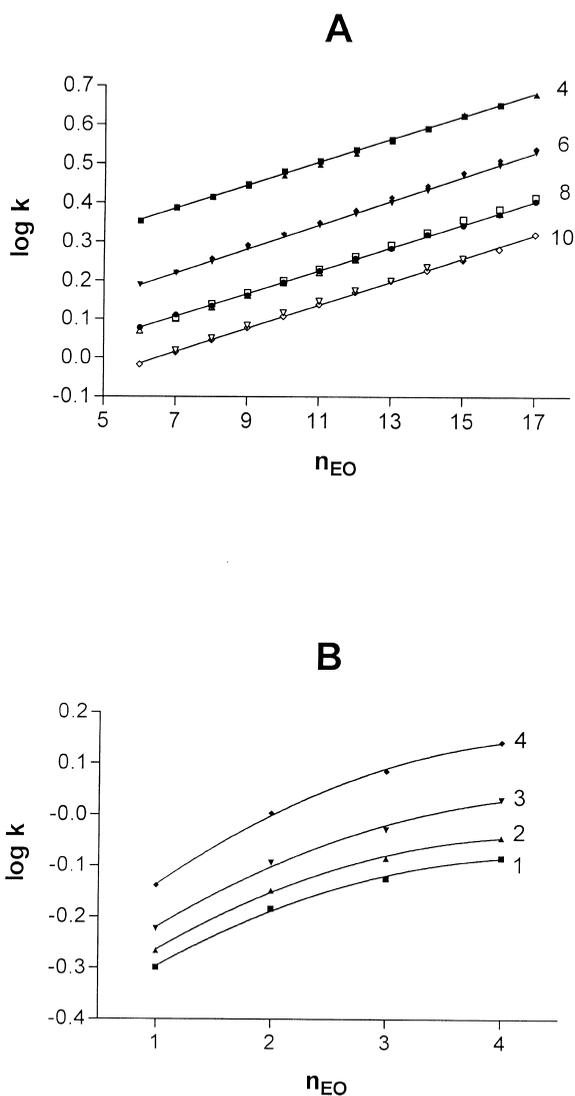
A

A

B

B

Fig. 6. Dependencies of the retention factors, k, on the number of EO units, n_{EO}, for the oligomers with 4, 6, 8 and 10 PO units in a Slovanik sample, type $(EO)_n–(PO)_m–(EO)_n$, in acetonitrile–water–dichloromethane (39.6:0.4:60), mobile phase A and for the oligomers with 14 PO units in a Novanik sample, type $(PO)_m–(EO)_n–(PO)_m$, in acetonitrile–water–dichloromethane (69.3:0.7:30) (1), (59.4:0.6:40) (2), (49.5:0.5:50) (3) and (39.6:0.4:60) (4), mobile phases B. Normal-phase chromatography on a Separon SGX Amine column, temperature: 40°C.

Fig. 7. Dependencies of the retention factors, k, on the number of PO units, n_{PO}, for the oligomers with 8, 12 and 15 EO units in a Novanik sample, type $(PO)_m–(EO)_n–(PO)_m$, in acetonitrile–water–dichloromethane (59.4:0.6:40), mobile phase A and for the oligomers with three EO units in a Slovanik sample, type $(EO)_n–(PO)_m–(EO)_n$, in acetonitrile–water–dichloromethane (69.3:0.7:30) (1), (59.4:0.6:40) (2), (49.5:0.5:50) (3) and (39.6:0.4:60) (4), mobile phases B. Normal-phase chromatography on a Separon SGX Amine column, temperature: 40°C.

three-parameter Eq. (4), the parameters of which are given in Table 4 for the $(EO)_n–(PO)_m–(EO)_n$ (Slovanik) (co)oligomers.

In the NP systems with Separon SGX Amine and

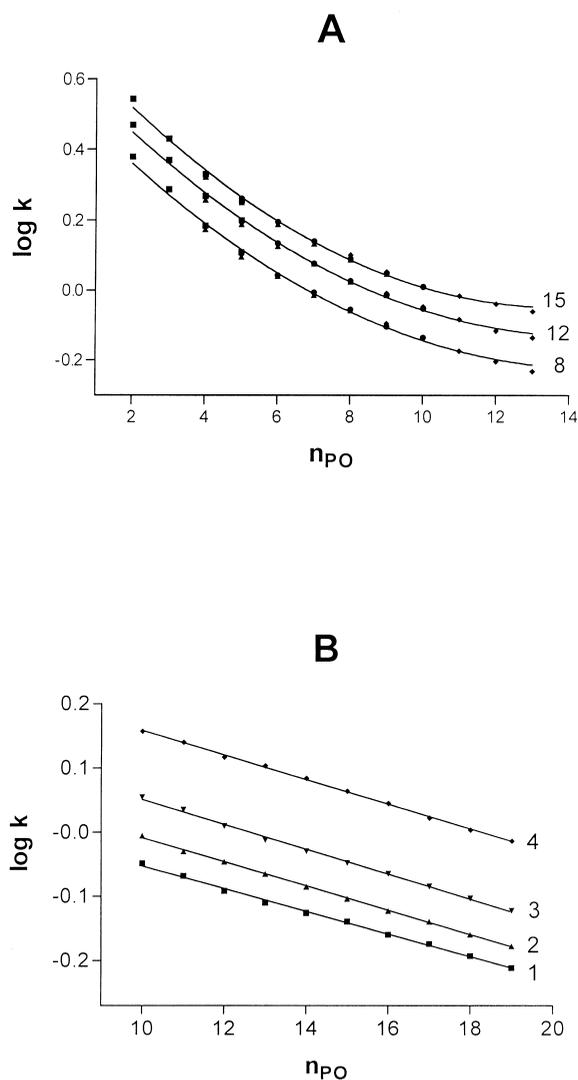acetonitrile–water–dichloromethane mobile phases (Table 5), the plots of log k versus the concentration of aqueous acetonitrile, φ, are linear in agreement with Eq. (3) only for the $(PO)_m–(EO)_n–(PO)_m$ (Novanik) (co)oligomers (Fig. 9). The dependence of

A

B
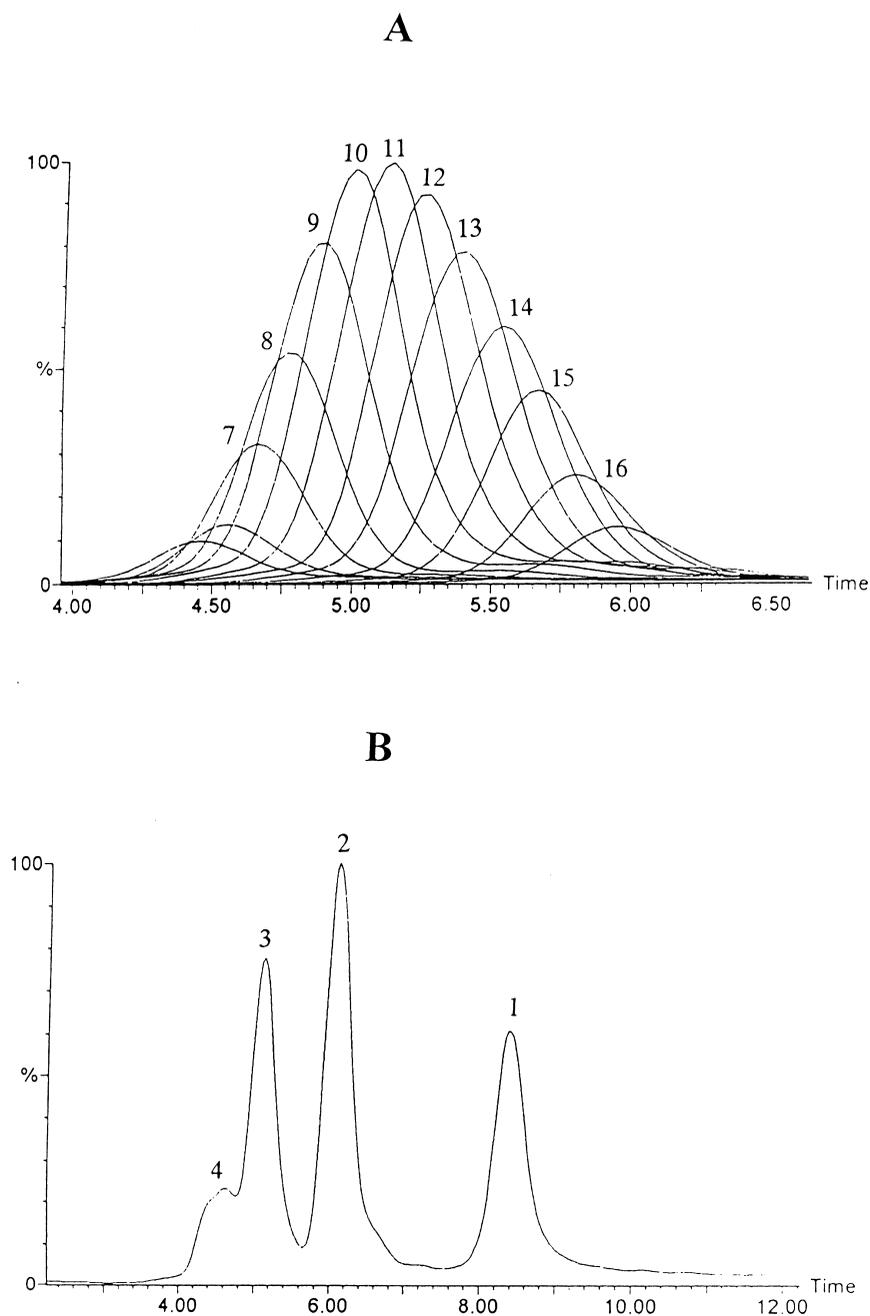
Fig. 8. Normal-phase separation of a $(PO)_m$–$(EO)_n$–$(PO)_m$ type sample (Novanik) on a Separon SGX Amine (5 μm) column (150×3.3 mm I.D.) in acetonitrile–water–dichloromethane (69.3:0.7:30). Flow-rate: 0.5 ml/min; temperature: 40°C; detection: APCI(+)-MS. (A) Chromatographic peaks extracted for $[M+H]^+$ ions with three PO units; numbers $= n_{EO}$. (B) Chromatographic peaks extracted for $[M+H]^+$ ions with 11 EO units; numbers $= n_{PO}$.

Table 4

Constants of the dependences of the retention factors, k, of $(EO)_n$–$(PO)_m$–$(EO)_n$ (Slovanik) block copolymers on the concentration of 2-propanol, φ (%, v/v, $\cdot 10^{-2}$) on a Separon Amine column in 2-propanol–hexane mobile phases (10–25% 2-propanol): $k = (a + b\varphi)^{-m}$

	$n_{EO} = 0$	$n_{EO} = 1$	$n_{EO} = 2$	$n_{EO} = 3$
a	-1.22 ± 0.01	-0.43 ± 0.03	-0.432 ± 0.007	-0.3280 ± 0.0001
b	14.0 ± 0.1	7.0 ± 0.2	5.59 ± 0.06	4.413 ± 0.002
m	0.699 ± 0.008	1.24 ± 0.07	1.22 ± 0.04	1.413 ± 0.001
C.D.[a]	1.00	1.00	1.00	1.00

[a] C.D. = Coefficient of determination.

the retention of the $(EO)_n$–$(PO)_m$–$(EO)_n$ (Slovanik) (co)oligomers should be described by the three-parameter Eq. (4) and the plots of log k versus the concentration of aqueous acetonitrile, φ, are significantly curved (Fig. 10). The constants of Eq. (4) for this NP system are listed in Table 6, together with the coefficients of determination demonstrating a good fit to the experimental data.

4.4. Effect of the sequence distribution on the separation of the EO–PO block (co)oligomers

The $(PO)_m$–$(EO)_n$–$(PO)_m$ (Novanik) (co)oligomers with inner EO block and PO end blocks can be separated according to the number of PO units in reversed-phase systems on a Separon SGX C_{18} column in acetonitrile–water mobile phases only. Gradient elution is necessary to separate more than

four peak groups. Gradient-elution separation of a Novanik sample according to the number of PO units is shown in Fig. 11. The oligomer selectivity for the EO units for the samples with inner EO block is low enough over a wide composition range of the mobile phase, so that it allows reversed-phase gradient-elution separation at close-to ''critical elution conditions''. However, the oligomer selectivity for the PO units in the $(EO)_n$–$(PO)_m$–$(EO)_n$ (Slovanik) (co)oligomers is high enough to preclude reversed-phase separations of these products under ''critical elution conditions''for the PO block.

The $(EO)_n$–$(PO)_m$–$(EO)_n$ (Slovanik) (co)oligomers can be separated according to the number of EO units in normal-phase systems on a Separon SGX Amine column in 2-propanol–hexane mobile phases. Like in reversed-phase chromatography of Novanik samples, the oligomer selectivity for the PO units for

Table 5

Constants of the dependences of the retention factors, k, on the number of oxyethylene, n_{EO} and oxypropylene, n_{PO}, repeat units of $(PO)_m$–$(EO)_n$–$(PO)_m$ (Novanik) and $(EO)_n$–$(PO)_m$–$(EO)_n$ (Slovanik) block copolymers on a Separon SGX Amine column in acetonitrile–water–dichloromethane mobile phases: $\log k = \log \beta + n_{EO} \log \alpha_{EO} + n_{EO}^2 \log \gamma_{EO} + n_{PO} \log \alpha_{PO} + n_{PO}^2 \log \gamma_{PO}$

|	Mobile phase: acetonitrile–water–dichloromethane			
	69.3:0.7:30	59.4:0.6:40	49.5:0.5:50	39.6:0.4:60
Novanik				
Log β	0.236 ± 0.008	0.338 ± 0.007	0.437 ± 0.008	0.58 ± 0.01
Log α_{EO}	0.0188 ± 0.0001	0.0217 ± 0.0001	0.0247 ± 0.0001	0.0312 ± 0.0001
Log α_{PO}	-0.082 ± 0.002	-0.097 ± 0.002	-0.107 ± 0.002	-0.121 ± 0.003
Log γ_{PO}	0.0025 ± 0.0002	0.0032 ± 0.0001	0.0036 ± 0.0001	0.0043 ± 0.0002
C.D.[a]	0.9973	0.9982	0.9985	0.9992
Slovanik				
Log β	-0.15 ± 0.02	-0.11 ± 0.01	-0.06 ± 0.01	0.01 ± 0.01
Log α_{EO}	0.15 ± 0.01	0.153 ± 0.008	0.158 ± 0.008	0.182 ± 0.009
Log γ_{EO}	-0.017 ± 0.002	-0.016 ± 0.002	-0.015 ± 0.002	-0.020 ± 0.002
Log α_{PO}	-0.0197 ± 0.0007	-0.0203 ± 0.0005	-0.0211 ± 0.0005	-0.0206 ± 0.0006
C.D.[a]	0.9816	0.9899	0.9925	0.9891

[a] C.D. = Coefficient of determination.
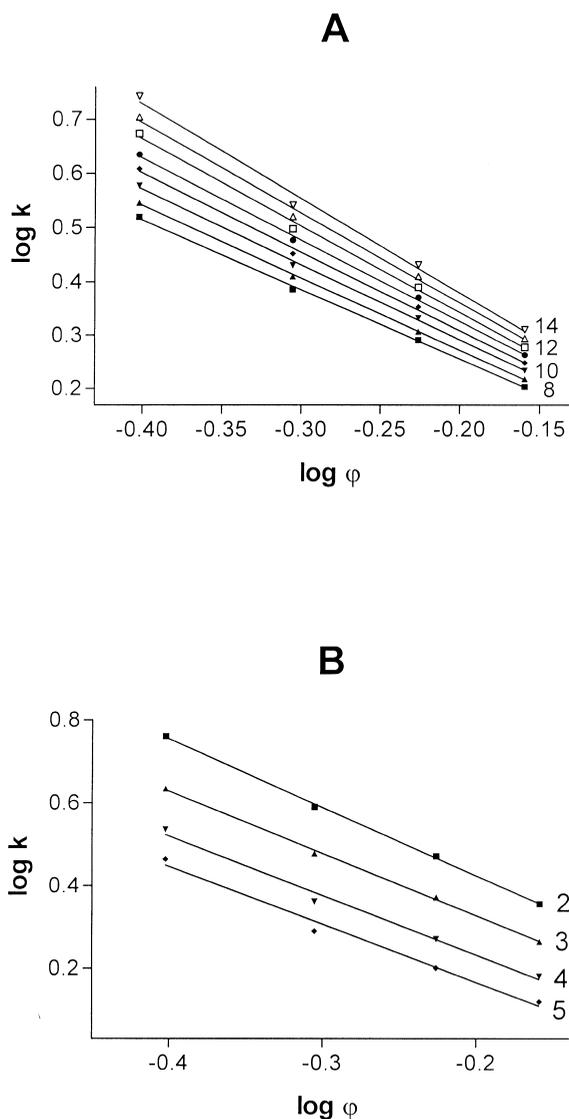
Fig. 9. Dependencies of the retention factors, k, of the oligomers with three PO units and 8, 10, 12 and 14 EO units (A) and with 12 EO units and 2–5 PO units (B) in a $(PO)_m–(EO)_n–(PO)_m$ type (Novanik) block copolymer sample, on the concentration, φ (%, v/v, $\cdot 10^{-2}$) of aqueous acetonitrile (99% acetonitrile, 1% water) in acetonitrile–water–dichloromethane mobile phases on a Separon SGX Amine column; temperature$=40°C$.
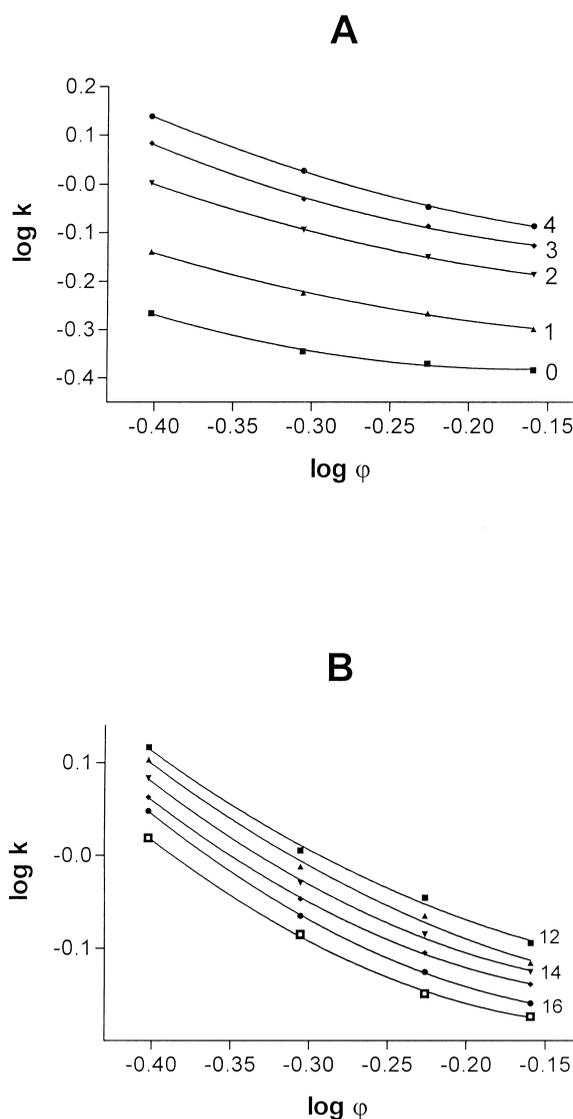
Fig. 10. Dependencies of the retention factors, k, of the oligomers with 14 PO units and 0–4 EO units (A) and with three EO units and 12–17 PO units (B) in a $(EO)_n–(PO)_m–(EO)_n$ type (Slovanik) block copolymer sample, on the concentration, φ (%, v/v, $\cdot 10^{-2}$) of aqueous acetonitrile (99% acetonitrile, 1% water) in acetonitrile–water–dichloromethane mobile phases on a Separon SGX Amine column; temperature$=40°C$.

the samples with inner PO block is very low and allows baseline resolution of oligomers with different numbers of EO units using gradient elution under virtually "critical elution conditions". The oligomers

are eluted in the order of increasing number of EO units and successful separation into seven peaks was obtained for Slovanik samples with a linear gradient from 10% to 25% 2-propanol in hexane in 25 min

Table 6

Constants of the dependences of the retention factors, k, $(PO)_m-(EO)_n-(PO)_m$ (Novanik) and $(EO)_n-(PO)_m-(EO)_n$ (Slovanik) block copolymers with different numbers of oxyethylene, n_{EO} and oxypropylene, n_{PO}, repeat units on the concentration of aqueous acetonitrile (99% acetonitrile: 1% water) in mobile phases acetonitrile–water–dichloromethane on a Separon SGX Amine column: $k = (a + b\varphi)^{-m}$

Novanik $n_{PO} = 7$	$n_{EO} = 9$	$n_{EO} = 10$	$n_{EO} = 11$	$n_{EO} = 12$	$n_{EO} = 13$	$n_{EO} = 14$
a	-1.0 ± 0.1	-0.97 ± 0.05	-0.80 ± 0.02	-0.71 ± 0.03	-0.717 ± 0.008	-0.65 ± 0.01
b	3.4 ± 0.2	3.02 ± 0.08	2.62 ± 0.03	2.38 ± 0.04	2.24 ± 0.01	2.09 ± 0.01
m	0.41 ± 0.04	0.40 ± 0.03	0.47 ± 0.02	0.50 ± 0.03	0.46 ± 0.01	0.51 ± 0.02
C.D.[a]	0.9998	0.9999	1.0000	0.9999	1.0000	1.0000
$n_{EO} = 12$	$n_{PO} = 5$	$n_{PO} = 6$	$n_{PO} = 7$	$n_{PO} = 8$	$n_{PO} = 9$	$n_{PO} = 10$
a	-0.44 ± 0.03	-0.55 ± 0.03	-0.71 ± 0.03	-1.01 ± 0.02	-1.22 ± 0.05	-1.75 ± 0.06
b	1.55 ± 0.04	1.89 ± 0.03	2.38 ± 0.04	3.16 ± 0.04	3.83 ± 0.08	5.3 ± 0.1
c	0.60 ± 0.06	0.55 ± 0.04	0.50 ± 0.03	0.42 ± 0.01	0.42 ± 0.02	0.37 ± 0.01
C.D.[a]	0.9998	0.9999	0.9999	1.0000	1.0000	1.0000

Slovanik $n_{PO} = 14$	$n_{EO} = 1$	$n_{EO} = 2$	$n_{EO} = 3$	$n_{EO} = 4$		
a	-18 ± 4	-4.0 ± 0.4	-2.7 ± 0.1	-1.3 ± 0.3		
b	56 ± 11	12 ± 1	8.0 ± 0.3	4.3 ± 0.7		
m	0.23 ± 0.01	0.28 ± 0.01	0.273 ± 0.009	0.38 ± 0.07		
C.D.[a]	0.9999	0.9999	1.0000	0.9990		
$n_{EO} = 3$	$n_{PO} = 12$	$n_{PO} = 13$	$n_{PO} = 14$	$n_{PO} = 15$	$n_{PO} = 16$	$n_{PO} = 17$
a	-1.6 ± 0.2	-2.3 ± 04	-27 ± 0.1	-3.41 ± 0.08	-3.5 ± 0.02	-4.7 ± 0.01
b	5.0 ± 0.4	6.8 ± 0.8	8.0 ± 0.3	10.0 ± 0.2	10.6 ± 0.5	13.9 ± 0.3
m	0.32 ± 0.03	0.28 ± 0.03	0.273 ± 0.009	0.253 ± 0.003	0.274 ± 0.008	0.250 ± 0.003
C.D.[a]	1.0000	0.9996	0.9999	1.0000	1.0000	1.0000

[a] C.D. = Coefficient of determination.
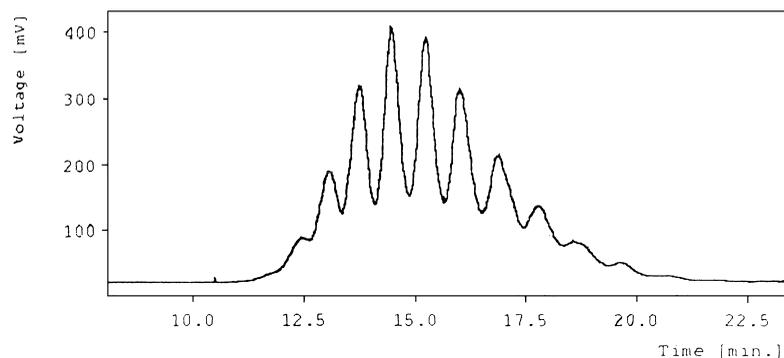

Fig. 11. Gradient-elution reversed-phase separation of a $(PO)_m-(EO)_n-(PO)_m$ type (co)oligomer sample (Novanik) on a Separon SGX C_{18} (7 μm) column (150×3.3 mm I.D.) with evaporative light scattering detection. Gradient 15–100% acetonitrile in water in 25 min. Flow-rate: 0.5 ml/min, temperature: 40°C. Peaks correspond to the oligomers with 5–14 PO units.

(Fig. 12A). Each peak contains species with the same number of EO units, but various numbers of PO units.

The oligomer selectivity for the PO units in the products with the end PO blocks is significantly higher than for the samples with the inner PO block, so that the individual oligomers in the $(PO)_m$–$(EO)_n$–$(PO)_m$ (Novanik) products are co-eluted in normal-phase systems on the bonded Amine column in 2-propanol–hexane mobile phases (Fig. 12B).

In the HPLC of (co)oligomers, the "critical elution

conditions" are usually observed at a very narrow mobile phase composition range. The reversed-phase system with a C_{18} column and acetonitrile–water mobile phases and the normal-phase system with a bonded amine column and propanol–hexane mobile phases in this work differ from the earlier systems in that the composition range of the mobile phases yielding "critical elution conditions" for EO or for PO units in the block EO–PO (co)oligomers is very broad, which makes possible the application of gradient elution at the "critical elution conditions".
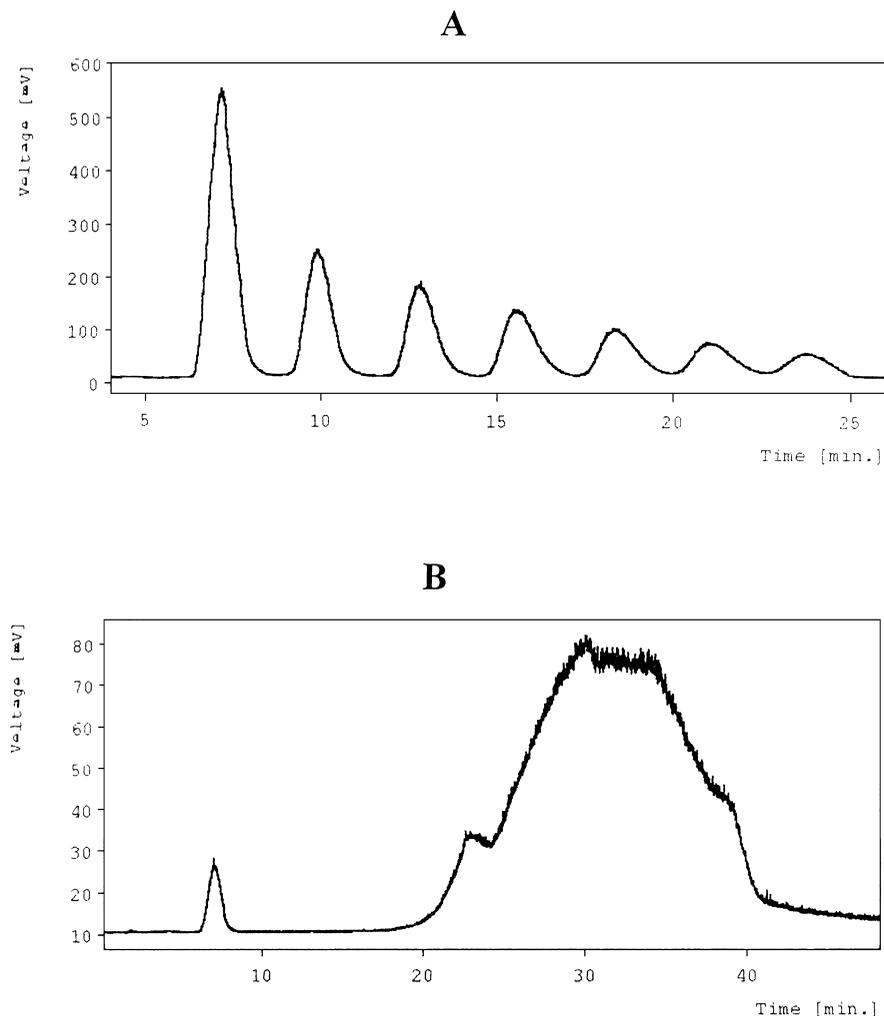
A

B

Fig. 12. Gradient-elution normal-phase separation of samples of block (co)oligomers on a Separon SGX Amine (5 μm) column (150×3.3 mm I.D.) with evaporative light scattering detection. Gradient 10–30% 2-propanol in *n*-hexane in 30 min. Flow-rate: 0.5 ml/min, temperature: 40°C. (A) $(EO)_n$–$(PO)_m$–$(EO)_n$ type sample (Slovanik); peaks correspond to the oligomers with 0–6 EO units; (B) $(PO)_m$–$(EO)_n$–$(PO)_m$ type sample (Novanik); oligomers with 6–18 EO units and 5–10 PO units.

However, the sequence distribution of the individual blocks in the (co)oligomers controls whether the ''critical elution conditions'' can be achieved or not. The present results suggest that the separation according to the number of repeat units in the end blocks at the ''critical elution conditions'' for the inner block units can be achieved, but such separation is not feasible for the products with the opposite block sequence. This can be possibly explained by the conformation of the (co)oligomers, in which the end blocks are better exposed to the interactions with the surface of the stationary phase, whereas direct interactions of the units in the inner block are partially sterically hindered by the outer blocks.

The oligomers in both $(EO)_n$–$(PO)_m$–$(EO)_n$ (Slovanik) and $(PO)_m$–$(EO)_n$–$(PO)_m$ (Novanik) products are co-eluted in normal-phase systems on a Separon SGX Amine column in acetonitrile water–CH_2Cl_2 mobile phases. The individual species can be separated according to the number of EO or PO units in the reconstructed chromatograms using HPLC–MS, as illustrated by the example in Fig. 8.

5. Conclusions

Reversed-phase or normal-phase HPLC can be successfully used for the separations of EO–PO (co)oligomers. The separation selectivity for low polarity repeat PO units is better in reversed-phase systems, but the selectivity for more polar EO repeat units is superior in normal-phase systems on a bonded amine column with propanol–hexane mobile phases.

In both RP or NP separation systems, the separation of the EO–PO (co)oligomers according to one distribution mode can be suppressed and the separation according to the other distribution mode enhanced over a wide composition range of the mobile phases, so that the ''critical elution conditions'' can be achieved using gradient elution. A surprising aspect of these separations is strong dependence of the separation on the sequence of the EO and PO blocks in the (co)oligomer products, making feasible only the separation according to the number of the repeat units in the end blocks at the ''critical elution conditions'' for the inner block

units. For the separation according to the number of the repeat units in the inner block, peak deconvolution and reconstruction of the chromatograms using HPLC–MS with APCI can be used. As an alternative approach, two-dimensional HPLC techniques can be used for the separation according to the bimodal repeat units distribution, which is being presently investigated.

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