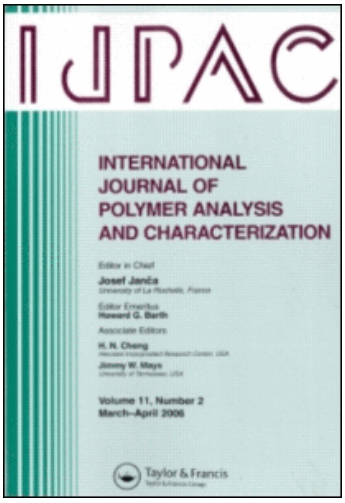

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Retention Behavior of Oligomers and Cooligomers in Reversed-phase and in Normal-phase Interactive Liquid Chromatographic Systems*

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Models describing the retention of small molecules combined with the additivity rules for the contributions to the retention can be used for prediction of the retention and resolution of oligomers, small synthetic polymers and copolymers in interactive liquid chromatography with dependency on the molar mass distribution and on the composition of the mobile phase, both in reversed-phase and in normal-phase chromatographic systems, for isocratic or gradient elution. Synthetic copolymers or cooligomers with two (or more) repeat monomer units show not only molar mass distribution, but also composition and sequence distribution of the individual repeat units. By combination of adequately selected separation conditions in normal-phase and in reversed-phase systems, the separation conditions can be adjusted to enhance or to suppress the chromatographic resolution according to each of the two distribution modes and to achieve the separation following the distribution of any of the two repeating groups under "critical conditions" of interactive HPLC. The sequence distribution, *i.e.*, the distribution of the position of the blocks was found to affect significantly the retention behavior of the individual species in the block cooligomers. Based on the retention mechanism suggested, optimization of the conditions for isocratic and gradient-elution separations of cooligomers is possible.

Keywords: Copolymers; Cooligomers; Interactive liquid chromatography; Oligoethylene glycol derivatives

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INTRODUCTION

Technical products based on synthetic polymers are more or less complex mixtures which contain various structural units, the type, number and distribution of which controls the product quality and suitability for specific applications.

(1) Polymer products composed of species with various numbers of a single repeat monomer unit, U, are characterized by the molar mass distribution, which can be schematically represented as:

$$\text{-U-, -U-U-, -U-U-U-, -U-U-U-U-, -U-U-U-U-U-, } \ldots$$

(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.*,

$$E_1\text{-U-U-U-U-}E_1, E_2\text{-U-U-U-U-}E_2, E_1\text{-U-U-U-U-}E_2$$

(3) Copolymers contain two or more different repeat monomer units, U_1, U_2, ... in various ratios, $n_1 : n_2 \ldots$, controlling their chemical composition distribution, schematically represented as:

$$\text{-}U_1\text{-}U_2\text{-}U_1\text{-}U_1\text{-}U_1\text{-, -}U_1\text{-}U_2\text{-}U_1\text{-}U_2\text{-}U_1\text{-, -}U_1\text{-}U_2\text{-}U_2\text{-}U_1\text{-}U_2\text{-,}$$
$$\text{-}U_1\text{-}U_2\text{-}U_2\text{-}U_2\text{-}U_2\text{-}$$

(4) Finally, different monomer units can be present in different sequences of microblocks with different numbers of the individual repeat monomer units along the polymer chain. This polymer heterogeneity is characterized by the sequence distribution, such as:

$$\text{-}U_1\text{-}U_2\text{-}U_1\text{-}U_2\text{-}U_1\text{-}U_2\text{-, -}U_1\text{-}U_1\text{-}U_1\text{-}U_2\text{-}U_2\text{-}U_2\text{-,}$$
$$\text{-}U_1\text{-}U_1\text{-}U_2\text{-}U_2\text{-}U_2\text{-}U_1\text{-}$$

For the evaluation of the quality of technical products, adequate characterization methods are necessary. Size-exclusion chromatography (SEC) has been widely used for characterization of the molar mass distribution. In the separations controlled solely by the size-exclusion

mechanism, polymers of different sizes are eluted in the order of decreasing molar masses, before the column hold-up volume V_0, so that the volume of the eluate available for the separation is limited. Recently, high-performance liquid chromatography (HPLC) in normal-phase (NP) and in reversed-phase (RP) modes has attracted considerable attention for the separation of lower polymers and oligomers. These chromatographic modes, sometimes classified as "interactive chromatography" (IC), can be used for characterization of various types of distribution of the individual samples in complex polymer mixtures, most of which are impossible to achieve using SEC.[1] The objective of this work is to present an overview of the results obtained by application of a simple general retention model to the retention behavior of various oligomers, lower polymers and cooligomers in reversed-phase and in normal-phase interactive HPLC under isocratic and gradient–elution conditions.

EXPERIMENTAL

Materials

n-Hexane and dichloromethane were obtained from Baker (Deventer, Netherlands), methanol, dioxane, tetrahydrofuran, acetonitrile and 2-propanol from Labscan Ltd. (Dublin, Ireland). All solvents were of HPLC grade. Water was doubly distilled in glass (with addition of potassium permanganate and sodium bicarbonate). Glass cartridge columns (150 × 3 mm I.D.), packed with Separon SGX (silica), Separon SGXC18, (octadecyl silica) and Separon SGXNH$_2$, all 7 μm particle size and average pore size 8 nm, were purchased from Tessek Ltd. (Prague, Czech Republic). Silasorb Diol, 5 μm column (250 × 4 mm I.D.), was obtained from Lachema (Brno, Czech Republic).

Technical samples of EO–PO block cooligomers were obtained from Sloveca (Nováky, Slovak Republic): Slovanik 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. Samples of oligoethylene glycol nonylphenyl and hexadecyl ethers were obtained from Servo (Delden, Netherlands) and Bohemiachem (Děčín, Czech Republic), respectively.

Chromatographic and Mass Spectrometric Instrumentation

The liquid chromatograph used was either a HP 1090 M instrument (Hewlett-Packard, Palo Alto, CA, USA), or consisted of a Model 616 pump, a Model 717+autosampler, a four-channel solvent delivery system (low-pressure gradient system), a thermostated column compartment, a model 996 photodiode-array detector and a Millennium chromatography manager (all from Waters, Milford, MA, USA), either connected to a Model PL-EMD 950 evaporative light scattering detector (ELSD, Polymer Laboratories, Shropshire, UK) or to a VG Platform quadrupole mass analyser (Micromass, Manchester, UK) with the probe for atmospheric pressure chemical ionization (APCI), mass range up to 3000 Da.

Procedures

Mobile phases for isocratic experiments were prepared by premixing 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 approximately 1 mg/mL. The column temperature was kept at 40°C and the flow rate at 1 mL/min in all experiments. 10 or 20 μL sample volumes were injected into the liquid chromatograph.

Mass spectrometric data were acquired in the range from 35 to 1500 Da 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 nebulising 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 times t_R, the retention factors $k=(t_R/t_0-1)$ were determined. The column hold-up times t_0 were determined as the elution times of nonretained compounds (methanol and acetonitrile in reversed-phase and *n*-hexane in normal-phase systems) and both t_R and t_0 were corrected for the volumes of the connecting tubing. Chromatographic

peaks were attributed to the individual oligomer on the basis of the mass of its $[M+H]^+$ ion in the APCI mass spectrum. Peaks of $[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$ ions were identified as the most significant peaks in the mass spectra, from which the molecular masses and the numbers of the methylene, oxypropylene (—CH_2—CH(CH_3)—O—, PO) and oxyethylene (—CH_2—CH_2—O—, EO) groups in the individual oligomers were determined.

MOLAR MASS DISTRIBUTION

In the "interactive chromatography" (IC) of polymers and oligomers, all species are retained because of the interactions with the stationary and (or) with the mobile phase and are eluted with elution (retention) volumes V_R greater than the column hold-up volume V_0. Unlike SEC, the volume of the eluate available for the separation is limited only by the time of separation and, possibly, by the decreased detectability of species that elute at high retention volumes. This often results in a greater repeat unit separation selectivity in an oligomer or polymer series and in better characterization of the molar mass distribution than with SEC techniques. The retention of polymers and oligomers in dependence on the number of repeat monomer units, n, can be described by a second-order polynomial equation.[2,3]

$$\log k = \log \beta + n \log \alpha + n^2 \log \gamma \qquad (1)$$

Here, the term β is the contribution of the end groups to the retention factor, $k = V_R/V_0 - 1$, the term α is the repeat unit selectivity, which characterizes the quality of the chromatographic separation of adjacent peaks of oligomers differing by a single monomer unit, *i.e.*, the quality of separation according to the molar mass distribution. The first two terms on the right-hand side Eq. (1) reflect the Martin rule of additivity of structural contributions to the retention.[4] The term γ is a measure of possible deviations from this rule occasionally observed experimentally[5,6] and often is small enough to be neglected.

Equation (1) generally applies to all interactive HPLC systems (reversed-phase, normal-phase, ion-exchange), In these systems, the quadratic term can be neglected if a repeat monomer unit contributes

by a regular increment to the retention. The first assumption for such behavior is that the interaction effects predominate over the size-exclusion effects in a given chromatographic system. This is usually the case for lower oligomers or polymers and packing materials with sufficiently wide pores. Size-exclusion effects are relatively unimportant if the compounds are significantly retained. For larger species with limited access to the adsorbent surface inside the pores, the size of the molecules may affect the retention in two different ways. First, the size-exclusion effect can limit to different extent the pore volume accessible to the polymer species differing in size. Second, the surface of the adsorbent available for interactions diminishes and consequently the phase ratio, *i.e.*, the ratio of the volumes of the stationary and of the mobile phases in the column may change, too. The retention factor is directly proportional to the distribution constant in the system with the phase ratio being the proportionality constant. The distribution constant should be independent of possible size-exclusion effects, but this is not necessarily the case with the phase ratio and consequently the retention factor may depend on the size-exclusion effects even if the column hold-up volume is corrected for the limited pore volume accessibility. These two effects could be theoretically accounted for in Eq. (1), by introducing the dependence of the hold-up volume (and of the phase ratio in the column) on the number of the repeat monomer units, but the parameters characterizing such dependencies are difficult to determine. If size-exclusion effects are not accounted for, they can result in possible significant contribution of the nonlinear term in Eq. (1).

Further, all repeat monomer unit in any polymer or oligomer species should have the same access to the stationary phase. Not only size-exclusion effects, but also possible conformation changes, especially of the larger species, may limit the access of a part of the molecule to the adsorbent. The conformation effect can result in a nonlinear form of the dependence of log k on the number of repeat units n. In such a case, the contribution of the quadratic term in Eq. (1) may become important and if a sudden conformation change occurs at a certain length of the oligomer chain, breaks on the log k versus n plots may be observed.

Nevertheless, Eq. (1) provides useful tool for the characterization of retention in IC of oligomers and lower polymers and, in spite of its

inherent limitations, can be helpful to explain many aspects of their retention behavior, sometimes surprising at first glance.

In IC, the retention factor and the repeat unit selectivity depend strongly not only on the character of the column packing (stationary phase), but also on the composition of the mobile phase. In various IC modes, binary mobile phases are most often used, comprised of an eluent with a low elution strength (weak solvent A) and an eluent with a greater elution strength (strong solvent B), even though ternary or more complex mobile phases are occasionally used to optimize the retention and the repeat unit selectivity. Adjusting the concentration of the strong solvent B, φ, in a binary mobile phase is a very efficient tool for optimizing the retention and the repeat unit selectivity, α. For this purpose, simple equations can be derived describing simultaneous dependencies of the retention factors and of α on the number of repeat monomer units n, and on φ, in a binary mobile phase. However, different retention mechanisms occurring in reversed-phase and in normal-phase modes, which are most suitable for IC separations of uncharged polymers and oligomers, should be taken into account.

Reversed-Phase Liquid Chromatography of Polymers and Oligomers

In reversed-phase liquid chromatography systems, columns packed with nonpolar alkyl-bonded stationary phases and aqueous–organic mobile phases are usually employed. As the surface of the column packing material is covered by relatively long alkyl chains with certain degree of flexibility, the retention of solute molecules occurs on the surface of or in between the bonded alkyl chains rather than on specific adsorption sites. To describe such retention behavior, various models were suggested. The interaction indices approach is especially suitable to describe the retention of oligomers with various repeat monomer units. This model is based on the assumption that the retention in reversed-phase systems is controlled essentially by solvophobic mechanism. The main driving force of retention can be understood as the difference in the free energy of polar interactions between the molecules of the mobile phase ΔG_{M-M}, and the free energy of polar interactions between the molecules of the solute and the molecules of the mobile phase ΔG_{M-X}. The energy of polar

interactions between two molecules is characterized here as the product of the contributions from each molecule. These contributions are assumed to be directly proportional to the index of interaction I, as a measure of the polarity of the molecule. As every solute is characterized by a unique value of I, a proportionality constant, $c_i > 0$, accounts for the specific character of the polar interactions connected with the type of the organic solvent used in the mobile phase. Then $\Delta G_{M-X} = c_M \cdot I_M \cdot c_X \cdot I_X \cdot V_X$ and $\Delta G_{M-M} = c_M \cdot I_M \cdot c_M \cdot I_M \cdot V_X$, where I_X is the interaction index of the solute and I_M that of the mobile phase and $c_X > 0$, $c_M > 0$ are c_i of the solute and of the mobile phase, respectively. The logarithm of the retention factor is proportional to the free energy of retention, $-\Delta G$, resulting from the difference between the energies of interactions ΔG_{M-M} and ΔG_{M-X}.[7]

Both the size (molar volume, V_X), and the polarity (interaction index, I_X) of oligomers either increase or decrease regularly with the number of repeat units, n.[3, 8]

$$V_X = V_{0X} + \Delta V_X n \tag{2}$$

$$I_X = I_{0X} + \Delta I_X n \tag{3}$$

V_{0X} and I_{0X} are the molar volume and the interaction index, respectively, of the end group(s) in the series and ΔV_X, ΔI_X are the increments of the molar volume and of the interaction index caused by the contribution of a repeat monomer unit. The validity of Eq. (2) is straightforward and has been verified experimentally.[8] The validity of Eq. (3) has also been verified experimentally,[8] *e.g.*, the ΔV_X of the repeat methylene unit in various series is approximately $16\,\mathrm{cm^3/mol^{-1}}$.

Combining Eqs. (2) and (3) with the basic relationship correlating the contribution of I_X and V_X to retention, the following quadratic expression for the dependence of the logarithms of k on the number of the repeat monomer units n was derived.[2]

$$\begin{aligned} \log k = \log \Phi &+ \frac{V_{0x} c_M}{2.31RT}(c_M I_M^2 - c_X I_M I_{0X}) \\ &+ \frac{c_M}{2.31RT}[\Delta V_X (c_M I_M^2 - c_M I_M I_{0X}) - V_{0X} c_X I_M \Delta I_X] n \\ &+ \frac{\Delta V_X \Delta I_X c_M c_X}{2.31RT} n^2 \end{aligned} \tag{4}$$

Here, Φ is the phase ratio, *i.e.*, the ratio of the volumes of the stationary and of the mobile phases in the column, respectively.

In most reversed-phase systems, where the solvent B is an organic solvent in an aqueous–organic mobile phase, the interaction index of the mobile phase, I_M in Eq. (4) can be expressed as a linear function of the concentration of the organic solvent φ (volume fraction, % vol/vol 10^{-2}), much like the Snyder's polarity indices P'.[12]

$$I_M = (1 - \varphi)I_{H_2O} + \varphi I_{org} \tag{5}$$

I_{H_2O} and I_{org} are the interaction indices of water and of the organic solvent, respectively.

Neglecting the quadratic terms and introducing I_M from Eq. (5), Eq. (4) can be rewritten to describe the dependence of the retention factor of a solute in an oligomer series k on the concentration of the stronger solvent in the mobile phase φ and on the number of repeat monomer units, n.[2,3,9]

$$\log k = a_0 + a_1 \cdot n - (m_0 + m_1 \cdot n)\varphi = \log k_0 - m\varphi \tag{6}$$

$$\alpha = 10^{a_1}/10^{m_1\varphi} \tag{7}$$

α is the repeat group selectivity and:

$$a_0 = \log \Phi + c_1 V_{0X} I_{H_2O}(c_M I_{H_2O} - c_X I_{0X}) \tag{8}$$

$$m_0 = c_1 V_{0X}(2c_M I_{H_2O} - c_X I_{0X})(I_{H_2O} - I_{org}) \tag{9}$$

$$a_1 = c_1 I_{H_2O}[(c_M I_{H_2O} - c_X I_{0X})\Delta V_X - c_X \Delta I_X V_{0X}] \tag{10}$$

$$m_1 = c_1 (I_{H_2O} - I_{org})[(2c_M I_{H_2O} - c_X I_{0X})\Delta V_X - c_X \Delta I_X V_{0X}] \tag{11}$$

with $c_1 = c_M/2.31\ RT$.

The coefficients a_0, a_1, m_0, m_1 in Eqs. (6) and (7) depend on the character of the repeat monomer units and of the end groups in the oligomer or polymer, on the chemistry of the stationary phase and on the type of the mobile phase components. Equations (8)–(11) correlate the coefficients of Eqs. (6) and (7) with the characteristics of the oligomers or polymers and of the chromatographic system. For example, these equations predict that the repeat unit selectivity

generally should increase with increasing volume ΔV_X and decreasing polarity ΔI_X of the repeat monomer unit and with increasing polarity of the organic solvent I_{org} in the mobile phase, which is in agreement with many experimental observations. Further conclusion that can be drawn from this model is that the retention is expected to increase with increasing degree of polymerization for oligomers with nonpolar repeat monomer units, but, in relatively rare cases when the polarity of the repeat monomer unit is large and its volume is relatively small, the individual oligomer species can be eluted in order of decreasing molar masses, depending on the polarities and concentrations of the solvents in the mobile phase. As the retention is contributed to also by the end group(s), their size and polarity can affect the useful range of mobile phase composition necessary for the elution to be accomplished in reasonable time and consequently, the elution order may differ for series with the same repeat monomer units, but with different end groups, as it is illustrated by some examples in Table I.

The four-parameter Eq. (6) has been first introduced to characterize the retention behavior of various homologous series in dependence on the number of repeat methylene units and on the concentration of the organic modifier in binary aqueous–organic mobile phases.[2] This equation with a suitable homologous series was used for the calibration of retention of various small nonhomologous series in mobile phases with different composition, to predict the retention and optimize the separation conditions.[13,14] Similar approach was

TABLE I Examples of the coefficients of Eq. (6) for various oligomers (O) in RP systems

O	*Repeat unit*	*S*	a_0	a_1	m_0	m_1	φ *range*	*Retention*
PS	C_6H_5—CH—CH_2—	D	2.49	0.77	3.12	0.83	< 93%	↑
OEG	—CH_2—CH_2—O—	M	−1.10	0.36	0.61	0.60	< 61%	↑
OEG	—CH_2—CH_2—O—	P	−0.90	0.34	−1.40	3.26	< 10%	↑
OEP	——CH_2—CH_2—O	P	2.69	0.00	3.89	0.00	< 100%	↓
OEA	—CH_2—CH_2—O—	M	7.37	0.00	7.49	0.00	< 95%	↑
OEA	—CH_2—CH_2—O—	A	4.10	0.00	3.90	−0.10	< 78%	↓

O–oligomer series: PS–polystyrenes, OEG–oligoethylene glycols, OEP–oligoethylene glycol nonylphenyl ethers, OEA–oligoethylene glycol hexadecyl ethers; column: Separon SGX C18, binary mobile phases containing various organic solvents, S: dioxane–D, methanol–M, 2-propanol–P, acetonitrile–A in water; retention either increases (↑) or decreases (↓) in the order of increasing number of repeat monomer units.

later suggested to characterize the properties of the stationary phase–mobile phase systems in reversed-phase chromatography.[15]

Table I illustrates by a few examples the validity of the present model for different oligomer and polymer classes with different repeat monomer units and different end groups. Earlier results achieved for polystyrenes,[11] oligoethylene glycols, oligoethylene glycol nonylphenyl ethers [9] and oligoethylene glycol hexadecyl ethers[16] in reversed-phase chromatography on a Separon SGX C18 column in binary mobile phases containing dioxane, methanol, 2-propanol and acetonitrile in water are included in the table, for the sake of better comparison possibilities. For all the oligomer series studied, good validity of Eq. (1) was found even when the quadratic term was neglected. Good linearity of the plots of log k versus the concentration of methanol and regular spacing of the plots obtained for oligomers with increasing numbers of PO repeat units in Figure 1 illustrates the validity of the four-parameter Eq. (6) for ethylene oxide–propylene

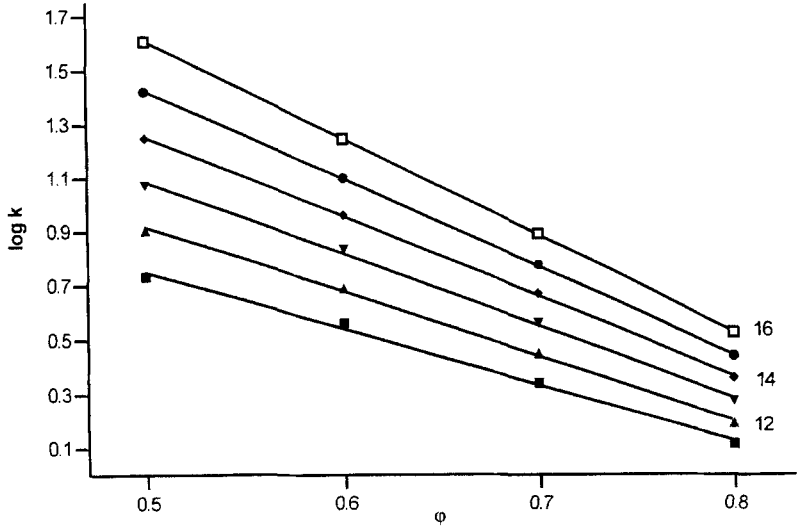

FIGURE 1 Dependency of retention factors k of the EO–PO oligomers with two oxyethylene units in a Slovanik 310 sample on the concentration, φ (%vol. 10^{-2}), of methanol in methanol–water mobile phases on a Separon SGX C18 column, 10 µm, (150 × 3.2 mm I.D.). The numbers of the plots correspond to the numbers of oxypropylene units in the individual species.

oxide block cooligomers on a C18 column in acetonitrile–water mobile phases.[17] Similar results were obtained for all other oligomer series studied. The coefficients of Eq. (6) applying to the individual oligomer series are compared in Table I.

The retention of most oligomers increases with increasing number of repeat monomer units in the useful mobile phase composition range (positive values of the coefficients a_1, m_1). Relatively high values of the coefficients a_1 demonstrate reasonably high repeat unit selectivities for polystyrenes and oligoethylene glycols, while low values of a_1 for oligoethylene glycol ethers indicate rather poor repeat unit selectivity for the EO derivatives in reversed-phase systems. The retention decreases with increasing number of oxyethylene units n for oligoethylene glycol nonylphenyl ethers in propanol–water mobile phases and for oligoethylene glycol hexadecyl ethers in acetonitrile–water mobile phases (negative values of these coefficients). The differences in the order of elution and in the repeat unit selectivity originate from different values of the a_1, m_1 coefficients for the series with different end groups but the same oxyethylene repeat units in the same binary mobile phases (OEG and OEA in methanol–water; OEG and OEP in propanol–water). The reversed order of elution may seem strange at first glance, but Eqs. (10) and (11) offer explanation of this behavior, as these coefficients depend on the size (V_{0X}) and polarity (I_{0X}) of the end groups. Figure 2 illustrates decreasing retention of oligoethylene glycol nonylphenyl ethers with increasing number of oxyethylene units in a reversed-phase system, which was verified both by on-line mass spectroscopic identification of the peaks and by comparison with the chromatographic profiles of the products with different nominal polymerization degree. This behavior cannot be explained by size-exclusion effect, as the differences between the retention volumes of the individual oligomers are in between 2–3 mL, much higher than the total pore volume in the column, which is approximately 0.4–0.5 mL. Most probable explanation based on the present model consists in a stronger contribution of increasing number n to increasing polarity (diminishing the retention) than the contribution of an EO unit to increasing molar volume (promoting the retention), resulting in lower values of the terms with ΔV_X than of the terms with ΔI_X in Eqs. (10) and (11) and negative values of the coefficients a_1, m_1.

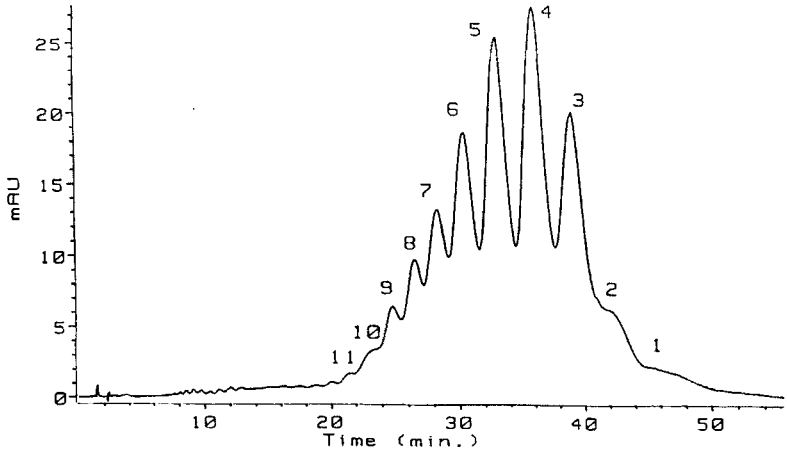

FIGURE 2 Reversed-phase separation of a Serdox NNP 4 sample containing oligoethylene glycol nonylphenyl ethers with 1–11 oxyethylene units on a Separon SGX C18 column in 40% 2-propanol in water as the mobile phase. Flow rate 1 mL/min, UV detection at 230 nm. The numbers of the peaks correspond to the numbers of oxypropylene units in the oligomers.

Normal-Phase Liquid Chromatography of Polymers and Oligomers

In normal-phase chromatography, the columns are usually packed either with inorganic adsorbent (most often, silica), either unmodified or carrying chemically bonded polar groups such as amino-, diol-, *etc.* Here, the active polar adsorption centers are localized on the adsorbent surface at more or less fixed positions and the retention is mainly controlled by the competition for these centers by molecules of solutes and the molecules of the mobile phase. The attraction forces to the adsorption centers increases with increasing polarities of the molecules. The mobile phase is usually a mixture of a nonpolar solvent A with one or more polar organic solvent(s). With binary mobile phases, which are used most often in practice, the Snyder model of adsorption [18] can be adapted to describe the dependence of the retention factor on the number of repeat monomer units n in the oligomer and on the concentration of the more polar of the two solvents, B, φ. If the oligomers are strongly retained in pure nonpolar solvent A as the mobile phase, Eqs. (11) and (12) can often be used for

this purpose.[10, 11]

$$\log k = a_0 + a_1 n - (m_0 + m_1 \cdot n) \cdot \log \varphi = \log k_0 - m \log \varphi \quad (12)$$

$$\alpha = 10^{a_1} / \varphi^{m_1} \quad (13)$$

Like in reversed-phase chromatography, α is the repeat unit selectivity, but Eqs. (12) and (13) differ from Eqs. (6) and (7) because of different mechanism controlling the retention. The constants a_0, a_1, m_0 and m_1 are related to the adsorption energy of the repeat structural unit Q_i and of the end group(s) Q_0 in pure nonpolar solvent and to the surface area of the adsorbent occupied by an adsorbed repeat monomer unit A_i and by the end group(s) A_0.

$$a_0 = K + \alpha'(Q_0 - A_0 \cdot \varepsilon_b) \quad (14)$$

$$a_1 = \alpha'(Q_i - A_i \cdot \varepsilon_b) \quad (15)$$

$$m_0 = \frac{A_0}{n_b} \quad (16)$$

$$m_1 = \frac{A_i}{n_b} \quad (17)$$

where K is an auxiliary adsorbent dependent constant, ε_b is the solvent strength (a measure of polarity) of the solvent B and n_b is the surface area of the adsorbent occupied by an adsorbed molecule of the more polar solvent B in the binary mobile phase.

This model was used to explain the behavior of oligoethylene glycol nonylphenyl ethers (OEGNPEs) nonionic surfactants on unmodified silica gel and on polar diol-, nitrile- and amino-bonded phases.[6, 10] The validity of Eq. (12) in normal-phase systems is illustrated by good linearity of the log k versus φ plots in Figure 3 for ethylene oxide–propylene oxide block cooligomers on a bonded amino column in mobile phases with various concentrations of aqueous acetonitrile in dichloromethane.

In Eqs. (14)–(17), a_0 is the contribution of the end groups and a_1 the contribution of the repeat monomer unit to the retention in the pure polar solvent B to the retention and both contributions increase with increasing energy of adsorption of the structural units in pure

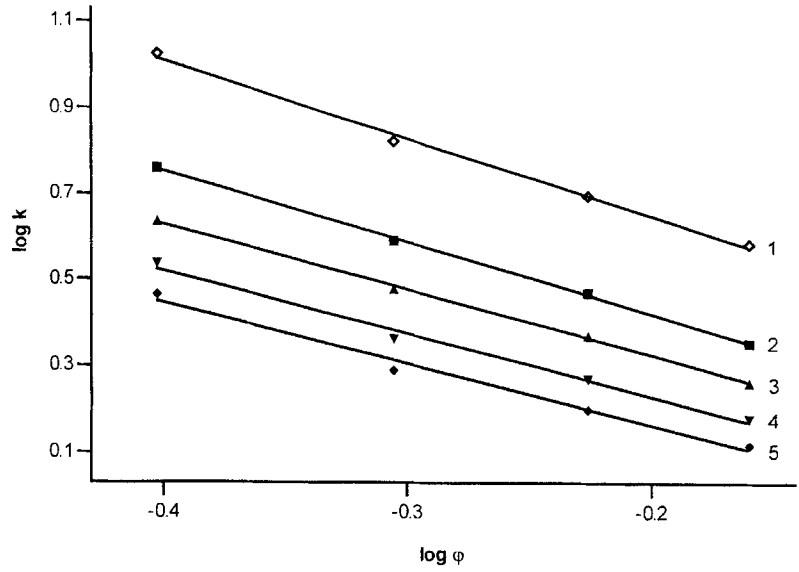

FIGURE 3 Dependency of the retention factor k of the EO–PO oligomers with 12 oxyethylene units in a Novanik 600/20 sample on the concentration, φ (%vol. 10^{-2}), of aqueous acetonitrile (with 1% water) in acetonitrile–dichloromethane–water mobile phases on a Separon SGX Amine column, 10 μm, (150 × 3.2 mm I.D.). The numbers of the plots correspond to the numbers of oxypropylene units in the individual species. The numbers of the peaks correspond to the numbers of oxyethylene units in the oligomers.

nonpolar solvent and decrease with increasing solvent strength (polarity) of the solvent B. The coefficients m_0 and m_1 characterize the effect of the unit concentration change of the polar solvent B in the mobile phase on the contributions of the end groups and of the repeat unit to k, respectively and increase with decreasing ratio of the adsorbent surface area occupied by a molecule of the polar solvent to the area of the surface occupied by an adsorbed repeat monomer unit (m_1) or of the surface occupied by the end groups (m_0). Equations (16) and (17) show that the relative effect of increasing concentration of different polar solvents on decreasing retention is directly proportional to the ratio of the areas on the adsorbent surface occupied by the structural element to the surface occupied by a molecule of the polar solvent B. These equations can be interpreted in the following way: The larger is the area of the adsorbent surface occupied by an

adsorbed molecule, the weaker is the force of its interaction per the unit of adsorbed area. Consequently, the average number of molecules of the solvent B necessary to displace the structural unit from the adsorbent (proportional to the concentration of B, φ) increases as the area occupied by a repeat monomer unit (A_i) or by the end group(s) (A_0) decreases or as the area occupied by a molecule of the polar solvent (n_b) increases.

This model predicts the repeat unit selectivity α depending on the composition of the mobile phase. The logarithms of the retention factors k of polymers and oligomers in IC usually increase in linear manner with increasing number of repeat monomer units n.[6, 19, 20] According to Eq. (13) this is the case if $\alpha > 1$, *i.e.*, if the effect of the coefficient a_1 on the retention of a repeat monomer unit is larger than the effect of the coefficient m_1. This occurs when the oligomers possess small polar repeat monomer units showing relatively high energy of adsorption Q_i in pure nonpolar solvent and occupy relatively low area on the surface of the adsorbent A_i. However, from Eq. (13) it can be deduced that the retention in an oligomer series can even decrease with increasing n in chromatographic systems where the combination of the coefficients a_1 and m_1 for a polymer or oligomer series is such that $\alpha < 1$, *i.e.*, with relatively bulky and nonpolar repeat monomer units which are characterized by low energy of adsorption, Q_i and occupy large area on the adsorbent surface, A_i.[9] Equation (13) also predicts that the repeat unit selectivity in normal-phase interactive chromatography should decrease with increasing concentration of the polar solvent B and, at the same concentration φ, it should increase if polar solvents with lower polarities and larger molecules occupying greater area of the adsorbent are employed.

Table II shows several examples demonstrating the validity of the present model for polystyrenes,[11] oligoethylene glycol nonylphenyl ethers[6, 10] and oligoethylene glycol hexadecyl ethers[16] in normal-phase chromatography on silica gel, bonded nitrile, diol and amine columns in binary mobile phases containing tetrahydrofuran, dioxane, 2-propanol and ethanol in *n*-hexane and in hydrophilic interaction chromatography on a bonded-amine column in aqueous acetonitrile–dichloromethane mobile phases. The retention of all oligomers increases with increasing number of repeat monomer units in the useful mobile phase composition range in the chromatographic systems

TABLE II Examples of the coefficients of Eq. (12) for various oligomers (O) in NP systems

O	*Repeat unit*	*C, S*	a_0	a_1	m_0	m_1	φ *range*	*Retention*
PS	C_6H_5—CH—CH_2—	1,T	−1.35	0.0	0.50	0.00	< 100%	↑
PS	C_6H_5—CH—CH_2—	1,D	−0.92	0.0	0.30	0.11	< 100%	↑
OEP	—CH_2—CH_2—O—	1,P	−1.47	0.4	1.00	0.10	< 100%	↑
OEP	—CH_2—CH_2—O—	1	−2.46	0.3	3.30	−0.30	> 8%	↑
OEP	—CH_2—CH_2—O—	2,P	−1.81	0.0	0.90	0.14	< 100%	↑
OEP	—CH_2—CH_2—O—	3,P	−1.60	0.2	0.60	0.00	< 100%	↑
OEP	—CH_2—CH_2—O—	4,P	−1.78	0.2	1.30	0.00	< 100%	↑
OEA	—CH_2—CH_2—O—	4,P	−1.07	0.2	0.15	0.10	< 100%	↑
OEA	—CH_2—CH_2—O—	4,ADW	−1.01	0.1	1.40	0.00	> 1%	↑

O-oligomer series: PS-polystyrenes, OEP-oligoethylene glycol nonylphenyl ethers, OEA-oligoethylene glycol hexadecyl ethers; C-columns: (1) Separon SGX (silica), (2) Separon SGX Nitrile, (3) Silasorb Diol and (4) Separon SGX Amine, binary mobile phases containing various polar solvents, S: tetrahydrofuran-T, dioxane-D, 2-propanol-P, ethanol-E in *n*-hexane; ADW-acetonitrile: water 99:1 in CH_2Cl_2; ↑-retention increases in the order of increasing number of repeat monomer units.

studied. The data in Table II illustrate the dependence of the repeat unit selectivity characterized by the coefficients a_1 and m_1 on the experimental conditions in normal-phase HPLC. In agreement with Eq. (15), the values of a_1 for oligoethylene glycol nonylphenyl ethers in propanol–hexane mobile phases increase with increasing polarity (activity, α') of the adsorbent (nitrile < diol ≈ amine < silica gel stationary phase) and are lower in mobile phases containing more polar solvent *B* (0.29 in mobile phases with ethanol and 0.35 in mobile phases with propanol), but are independent of the end groups (0.16 for nonylphenyl and 0.15 for hexadecyl ethers) in the same chromatographic system. Further, a_1 values are significantly lower in the series with less polar (lower Q_i) repeat phenylethylene units than in the series with more polar oxyethylene units. The values of m_1 are more difficult to interpret, as little is known about the actual size a repeat monomer unit occupies on the surface of different adsorbents in different mobile phases. Negative values of m_1 in aqueous–organic (ADW) mobile phases could possibly be attributed to a more complex, probably mixed adsorption–partition, mechanism (this possibly applies also to mobile phases with ethanol which could contain small amounts of water). Figure 4 illustrates increasing retention of oligoethyleneglycol nonylphenyl ethers with increasing number of oxyethylene units in a normal-phase system, in contrast to the behavior observed in reversed-phase systems (Fig. 2).

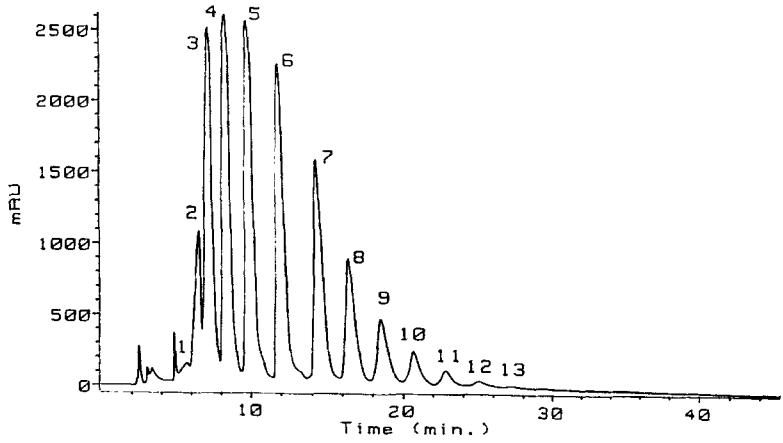

FIGURE 4 Gradient–elution normal-phase separation of a Serdox NNP 4 sample containing oligoethylene glycol nonylphenyl ethers with 1–13 oxyethylene units on a Separon SGX Amine column. Linear gradient 0–90% 2-propanol in heptane in 60 min. Flow rate 1 mL/min, UV detection at 230 nm.

GRADIENT ELUTION POLYMER CHROMATOGRAPHY

In isocratic chromatography, only a limited number of oligomers can be separated as the retention usually increases excessively at high n, so that gradient elution is necessary for successful separation of samples with broader molar mass distribution.[21] The retention in gradient elution depends on the initial concentration of the solvent B, A, on the gradient time t_g (gradient volume, V_g) – or steepness, $B = (\varphi_g - A)/V_g$, (φ_g is the concentration of the solvent B at the end of the gradient where the volume of the eluate, $V = V_g$) and on gradient shape.[22,23] Based on Eqs. (6) and (12) describing the dependencies of the retention factors on the number of repeat units n and on the concentration of the solvent B, φ, equations were derived for calculations of the retention data in dependence on the parameters characterizing the gradient profile.[22,24] These equations can be used for optimization of the gradients for reversed-phase or normal-phase separations of lower polymers or oligomers to achieve a desired repeat unit selectivity and minimum time of separation. In gradient–elution HPLC with linear gradients, the increase of the concentration φ with increasing volume of the eluate V from the start of the gradient is controlled by a linear

"gradient function".[22]

$$\varphi = A + B \cdot V \tag{18}$$

The retention volumes in reversed-phase systems where Eq. (6) applies can be calculated from Eq. (19).

$$V_R = [1/(m \cdot B)] \log [2.31 m B V_0 k_0 / (10^{m \cdot A}) + 1] + V_0 \tag{19}$$

For linear gradient elution in normal-phase systems where the retention can be described by Eq. (12) the retention volumes can be calculated from Eq. (20).

$$V_R = (1/B)[(m+1)Bk_0V_0 + A^{(m+1)}]^{1/(m+1)} - (A/B) + V_0 \tag{20}$$

In Eqs. (19) and (20),

$$m = m_0 + m_1 n, \quad \log k_0 = a_0 + a_1 n \tag{21A, 21B}$$

It is well known that nonlinear gradients often provide improved peak spacing and higher peak capacity of the separation. A convenient gradient function can describe nonlinear gradients in normal-phase chromatography, with the gradient shape characterized by a curvature constant $\kappa(\kappa > 0)$.

$$\varphi = (A^{(1/\kappa)} + BV)^{\kappa} \tag{22}$$

where

$$B = [\varphi_g^{(1/\kappa)} - A^{(1/\kappa)}]/V_g \tag{23}$$

The gradient profile is linear if $\kappa = 1$, concave if $\kappa > 1$ and convex if $\kappa < 1$. The elution volumes in normal-phase HPLC where the Eq. (12) applies can be calculated using a relatively simple equation

$$V_R = (1/B)[(\kappa m + 1)Bk_0V_0 + A^{(\kappa m+1)/\kappa}]^{1/(\kappa m+1)} - (A^{1/\kappa}/B) + V_0 \tag{24}$$

Equations (19), (20) and (24) can be used for the predictive optimization of the separation conditions for polymers and oligomers in normal-phase and in reversed-phase chromatography.

Both in linear and nonlinear gradient elution, the retention volumes V_R and the resolution R_s decrease with increasing A and B, *i.e.*, with decreasing gradient time t_g. For nonlinear gradients, the optimum initial concentration A does not depend significantly on the gradient time, t_g. The optimization of gradient elution consists in the calculation of minimum (optimum) values of the curvature parameter κ and of the gradient time t_g for minimized retention volume V_R of the last eluted compound at a pre-set desired value of resolution R_s.

With increasing parameter κ the gradient profile becomes less convex and the analysis time increases. Optimized nonlinear gradients may decrease the analysis time (or increase the peak capacity) up to 10–25% with respect to optimized linear gradients. This improvement increases with increasing number of individual species, *i.e.*, with the range of repeat monomer units in a polymer or oligomer sample. More details on this topic can be found elsewhere.[24]

The necessity for using gradient elution in IC separations of polymers can be derived directly from Eqs. (6) and (12), whose coefficients a and m regularly increase with increasing number of repeat monomer units because of the additivity of the structural contributions to log k and may have very large values for higher polymers. As a consequence, only a very narrow composition range of the mobile phase is available for the elution of large molecules. For example, from the data in Table I it can be predicted that a polystyrene sample with molecular mass 10 000 (with approximately 100 repeat units) has $k = 2$ on a C18 column in 86.9% dioxane in water (best elution conditions), but $k = 300$ in 85% dioxane (very strong retention) and $k = 0.3$ (very low retention) in 88% dioxane. This means that this sample is either completely retained or completely non-retained almost over the whole composition range of the mobile phase. The elution range is even more limited for higher molar mass samples. Consequently, the application of gradient elution is a prerequisite in order not to miss the narrow mobile phase "composition window" available for elution and to accomplish the elution of a sufficiently large number of individual species.

Further, the gradient profile should be adjusted with great care and relatively shallow gradients should be preferred to obtain good resolution. It should be noted that the width of the mobile phase "composition window" depends on the value of the coefficient m_1,

which is a measure of the effect of the solvent B on the repeat unit selectivity. This means that correct selection of the type of the solvent B is essential for successful separation.

The present model also predicts, in agreement with numerous experimental results, that there is a certain molar mass, depending on the polymer or oligomer type and on the components of the chromatographic system used, which limits possibilities of separation of higher polymers by IC. This can be again illustrated by the example of reversed-phase separation of polystyrenes in dioxane–water mobile phase: The polymer with 1000 units (with molecular mass approximately 100 000) has $k = 1.89$ in 87.45% dioxane, but the next polymer with 1001 units has $k = 1.892$, so that the repeat unit selectivity characterized by the separation factor α is only 1.001 and a column with 36 million theoretical plates would be required for complete separation of these two species.

END-GROUP DISTRIBUTION

In HPLC of polymers on a porous active column packing, size-exclusion effects and retentive interactions can sometimes exactly compensate each the other at the "critical conditions",[25–30] so that species with different molar masses coelute in a single peak at the same elution volume V_e close to the column hold-up volume and can be separated from polymers with other type of repeat units or end groups. This approach is often applied to the separation of polymer blends. According to the present model, the coelution of the oligomers with different numbers of repeat monomer units can occur also in pure interaction-controlled separations at a "critical" concentration of the efficient eluting component (solvent B), φ_0 in the mobile phase if

$$\varphi_0 = a_1/m_1 \text{(in RP systems) or } \varphi_0 = 10^{a_1/m_1} \text{(in NP systems)} \qquad (25A, 25B)$$

Under these chromatographic conditions, mixtures of polymers with the same monomer units theoretically can be separated only on the basis of the structural differences in the end groups.

In reversed-phase systems, this approach can be applied to oligomers with polar repeat monomer units and less polar end groups, *e.g.*, on a C18 column oligomers with different numbers of oxyethylene units are coeluted and the elution order in gradient elution with methanol in water is controlled only by the polarity of the end groups, so that the compounds are eluted in the order of decreasing polarities of the end groups: oligoethylene glycols < oligoethylene glycol alkylphenyl ethers < oligoethylene glycol alkyl ethers.[9]

Normal-phase systems are suitable for the separations of the oligomers with moderately polar repeat monomer units according to the polar end group distribution. This is illustrated by the data describing the retention behavior of polyesters in Table III. The experimental data used as the source of the coefficients in this table were measured by Philipsen.[31] The present model predicts that dipropoxylated bisphenol *A*–adipic acid polyesters with mono-acid end groups can be separated from the products with diol end groups on a silica gel column in 50% tetrahydrofuran in *n*-heptane with relatively low retention and on a polyamine column in mobile phases containing 70% tetrahydrofuran in *n*-heptane, with higher retention and in reversed elution order of the two types of oligomers. For tetrahydrofuran–dichloromethane mobile phases, critical conditions are predicted at 4–4.5% tetrahydrofuran, but without sufficient separation selectivity for the end groups on the silica gel column and at

TABLE III Examples of the coefficients of Eq. (12) and of "critical" conditions for coelution of oligomers with different numbers of repeat units in NP systems

	S, I		*S, II*		*PA, I*		*PA, II*	
	Diol	*MA*	*Diol*	*MA*	*Diol*	*MA*	*Diol*	*MA*
a_0	−0.80	−1.00	−0.90	−2.00	−0.11	0.17	−0.21	−0.71
a_1	−0.20	−0.21	−0.48	−0.35	−0.23	−0.16	−0.44	−0.16
m_0	3.00	3.00	1.20	2.00	3.00	6.00	0.54	1.95
m_1	0.65	0.70	0.35	0.26	1.44	1.04	0.27	0.05
φ_0	50%	50%	4%	4.5%	70%	70%	2%	0.1%
k_0	1.26	0.79	5.70	5.80	2.25	12.6	60	340

Polymers: dipropoxylated bisphenol *A*–adipic acid polyesters with mono-acid (MA) and diol end groups, columns: Nucleosil–100–5 silica, 5 μm, 100 Å, 200 × 4.0 mm (S) and Jordi Gel DVB Polyamine, 5 μm, 100 Å, 250 × 4.6 mm (PA), binary mobile phases tetrahydrofuran–heptane (I) and tetrahydrofuran–dichloromethane (II). φ_0–"critical concentration" and k_0–corresponding retention factor for the coeluted species (Eq. (25B)). The values of the coefficients were calculated from the experimental data measured by Philipsen.[31]

0–2% tetrahydrofuran, but with too strong a retention of the two types of oligomers on the polyamine column.

CHEMICAL COMPOSITION DISTRIBUTION

Using similar models as for the description of retention of oligomers with a single repeat monomer unit, the dependence of the retention of the individual species in block copolymers with two different types of repeat monomer units, A and B, on the number of the two units n_A and n_B can be described by Eq. (26).[17]

$$\log k = \log \beta + n_A \log \alpha_A + n_A^2 \log \gamma_A + n_B \log \alpha_B + n_B^2 \log \gamma_B \quad (26)$$

Equation (26) describes the retention of block copolymers and cooligomers both in reversed-phase and in normal-phase interactive HPLC systems. Here, α_A and α_B are the repeat unit separation selectivities for each oligomer block. The constants α, β and γ depend on the composition of the mobile phase, *e.g.*, in reversed-phase systems depend in linear manner on the concentration of organic solvent B. The values of log α_A and log α_B can be either positive or negative and the retention may either increase or decrease with increasing number of repeat monomer units A and B, as it is illustrated by the plots in Figures 5 and 6 for Slovanik EO–PO block copolymers in reversed-phase systems and in Figures 7 and 8 for Novanik EO–PO block copolymers in normal-phase systems. In reversed-phase systems, the retention increases with increasing number of PO units, but slightly decreases with increasing number of oxyethylene units, while significant increase of retention both with increasing number of EO units and decreasing number of PO units is observed in normal-phase systems.

The terms with n_A^2 and n_B^2 in Eq. (26) can often be neglected. In such a case, the dependence of the retention factor on the number of the individual repeat monomer units in the block cooligomer and on the composition of the mobile phase can be described by Eq. (27) for reversed-phase and by Eq. (28) for normal-phase systems:

$$\log k = a_0 + a_{1A} n_A + a_{1B} - (m_0 + m_{1A} n_A + m_{1B} n_{1B})\varphi \quad (27)$$

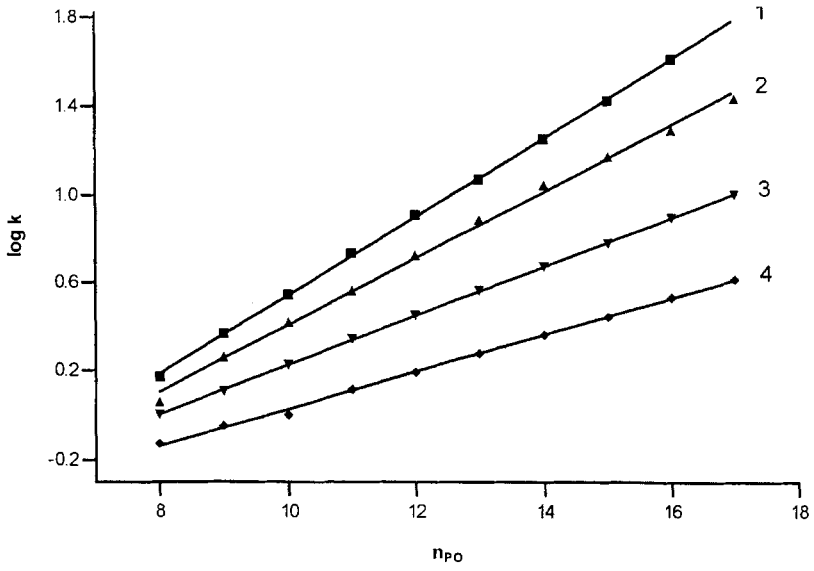

FIGURE 5 Dependency of the retention factor k of the EO–PO oligomers with two oxyethylene units in a Slovanik 310 sample on the number of oxypropylene units in the individual species in different mobile phases: 50% (1), 60% (2), 70% (3) and 80% (4) acetonitrile–water on a Separon SGX C18 column, 10 μm, (150 × 3.2 mm I.D.).

$$\log k = a_0 + a_{1A}n_A + a_{1B} - (m_0 + m_{1A}n_A + m_{1B}n_{1B}) \log \varphi \qquad (28)$$

The coefficients a_{1A}, a_{1B}, m_{1A}, m_{1B} in Eqs. (27) and (28) have the same meaning as the coefficients a_1 in Eqs. (10) and (15), m_1 in Eqs. (11) and (17), but with respect to the individual blocks A and B. The coefficients a_0, m_0 relate to the contribution of the end groups to the retention, as in Eqs. (8), (14) and (9), (16).

Similar to the LC under "critical conditions" often used for separation of oligomer blends, conditions can be found in some interactive HPLC systems that could facilitate the analysis of block copolymers. For this purpose, the separation according to the distribution of the repeat monomer units in one block is intentionally suppressed and the separation selectivity according to the distribution of the repeat monomer units in the other block is enhanced. The separation solely according to the distribution of one block i can be achieved if the deviations from the Martin rule are not very significant, *i.e.*, if $\gamma_i \approx 1$, at a "critical concentration" φ_i of solvent B.[17]

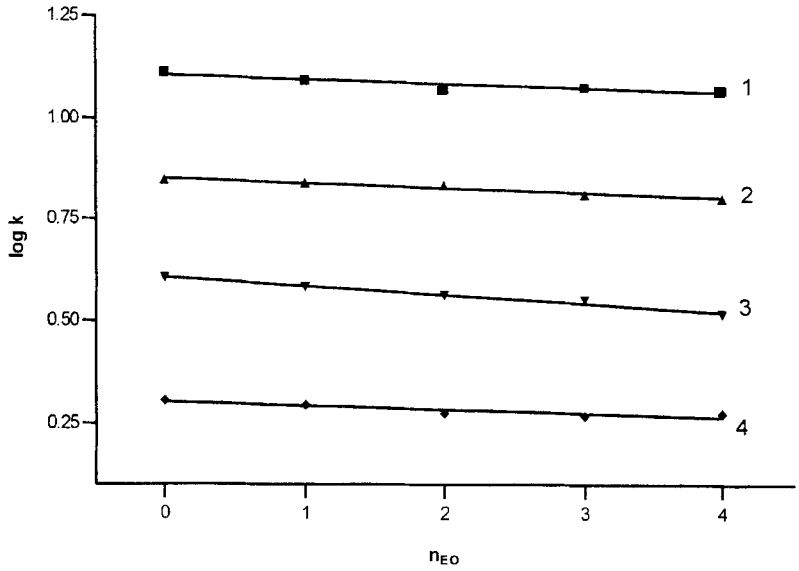

FIGURE 6 Dependency of the retention factor k of the EO–PO oligomers with 13 oxypropylene units in a Slovanik 310 sample on the number of oxyethylene units in the individual species in different mobile phases: 50% (1), 60% (2), 70% (3) and 80% (4) acetonitrile–water on a Separon SGX C18 column, 10 μm, (150 × 3.2 mm I.D.).

$$\varphi_i = a_i/m_i(\text{in RP systems}) \text{ or } \varphi_i = 10^{ai/mi}(\text{in NP systems}) \quad (29A, 29B)$$

Size-exclusion effects (if significant at all) contribute to the second power terms with γ coefficients – see discussion of Eq. (1), but real SEC–IC compensation is not necessary for the coelution of the cooligomer species with different numbers of repeat monomer units in one block. Rather, the coelution of the species with different numbers of repeat monomer units in one block occurs under pure IC conditions, if the composition of the mobile phase is adjusted so that the interactions of the repeat monomer units of one type with the mobile phase more or less compensate their interactions with the stationary phase. Such chromatographic conditions result in (almost) null contribution of one type of the repeat monomer units to the retention of the individual species in a block copolymer or cooligomer. In practice, adjusting the conditions for coelution of one block in pure interactive chromatography usually is possible only if there are significant differences in the polarities of the repeat monomer units in

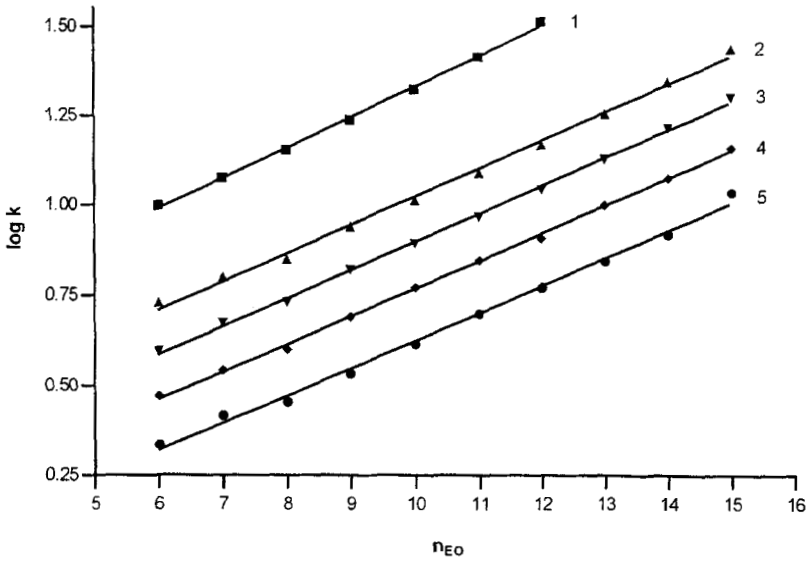

FIGURE 7 Dependency of the retention factor k of the EO–PO oligomers in a Novanik 600/20 sample on the number of oxyethylene units in the individual species in 30% 2-propanol–hexane mobile phase on a Separon SGX Amine column, 10 μm, (150 × 3.2 mm I.D.). Numbers of the plots correspond to the numbers of oxypropylene units in the individual species.

the two blocks. If so, such conditions in IC can be used to simplify the separation not only of block copolymers. Similar approach could be probably used also to the separation of polymer blends.

The separation with coelution of species with different numbers of polar repeat monomer units and good resolution of cooligomers with different numbers of less-polar repeat monomer units should be attempted at in reversed-phase systems, whereas the separation of cooligomers into groups with the same number of nonpolar repeat monomer units, but with different numbers of more polar repeat monomer units in the second block usually requires normal-phase systems.

EO–PO block copolymers are frequently used surfactants or as emulsifiers and solubilizers of flavors and fragrancies in cosmetic products. The oxypropylene chain $-[CH_2-CH(CH_3)-O]_m-$, *i.e.*, $(PO)_m$, is significantly less polar than the oxyethylene chain $-[CH_2-CH_2-O]_n-$, *i.e.*, $(EO)_n$. Two types of block co-polymers

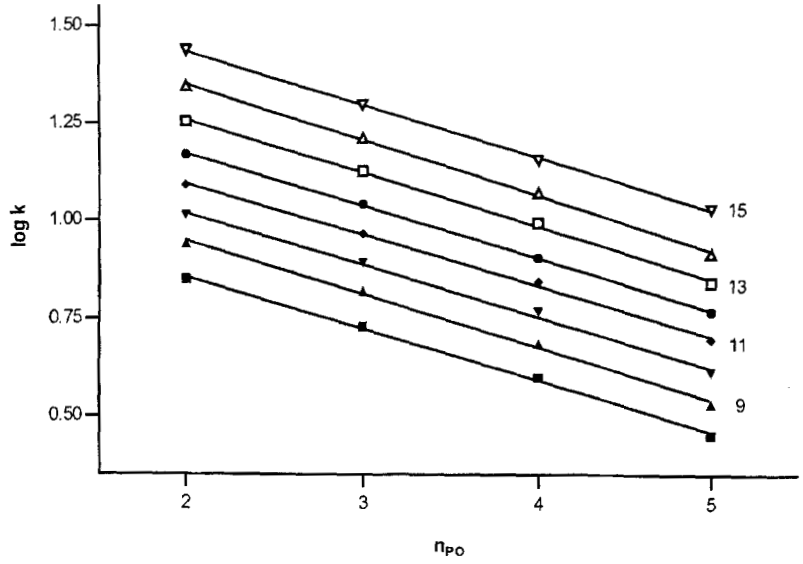

FIGURE 8 Dependency of the retention factor k of the EO–PO oligomers in a Novanik 600/20 sample on the number of oxypropylene units in the individual species in 30% 2-propanol–hexane mobile phase on a Separon SGX Amine column, 10 μm, (150 × 3.2 mm I.D.). Numbers of the plots correspond to the numbers of oxyethylene units in the individual species.

were studied, (1):$(EO)_n$–$(PO)_m$–$(EO)_n$(Slovanik) and (2):$(PO)_m$–$(EO)_n$–$(PO)_m$(Novanik). Increasing average molecular mass of the industrial products enhances gelation and with increasing number of PO units, the wetting properties are improved. The products with higher numbers of EO units are better soluble in water, but show poorer wetting properties. Type 2 (Novanik) copolymers are characterized by reduced foam formation and gelation with respect to the type 1 (Slovanik) copolymers.[32] Consequently, molar mass distribution, chemical composition distribution and block sequence distribution are all important for the characterization of the industrial products of this type.

Table IV gives the values of the coefficients of Eqs. (27) and (28) characterizing the separation of oligoethylene glycol ethers of alcohols C12—C16[16] and of ethylene oxide–propylene oxide (EO–PO) block cooligomers.[17] The interactive HPLC systems investigated in Table IV include: (1) reversed-phase systems with a Separon SGX

TABLE IV Coefficients of Eq. (27) for a C18 column and Eq. (28) for a bonded-amino column and oligomer series of EO-alcohols and three-block copolymers $(EO)_n-(PO)_m-(EO)_n$(Slovanik, NH_2 column) and $(PO)_m-(EO)_n-(PO)_m$(Novanik, C18 column)

	EO alcohols				***EO–PO cooligomers***		
	A = EO groups, B = n_c in alcohols				*A = EO groups, B = PO groups*		
Column: ***Mobile phase:***	*C18 ACN—H_2O*	*C18 MeOH—H_2O*	*NH_2 PrOH—hexane*	*NH_2 ADW*	*C18 ACN—H_2O*	*NH_2 PrOH—hexane*	*NH_2 ADW*
a_0	−1.03	−0.80	−1.07	−1.01	−1.96	−1.23	−0.25
m_0	0.14	0.29	0.15	1.35	−1.53	1.56	0.64
a_{1A}	−0.09	0.04	0.15	0.13	−0.05	−0.02	0.13
m_{1A}	−0.12	0.04	0.08	−0.07	−0.05	0.28	0.12
a_{1B}	0.32	0.51	−0.01	−0.02	0.32	0.00	−0.02
m_{1B}	0.24	0.45	0.00	0.00	0.29	0.00	0.00
φ_{iA}	95%	78%	–	1%	–	82%	–

Coelution conditions for EO-alcohols: on a Separon SGX C18 column (Eq. (29A)) for EO units–95% methanol–water [k(C12) = 0.84, k(C14) = 1.23, k(C16) = 1.81, k(C18) = 2.65] and 78% acetonitrile–water [k(C12) = 3.21, k(C14) = 6.03, k(C16) = 11.33, k(C18) = 21.30]; on a Separon SGX NH_2 column (Eq. (29B)) for EO units – in 1% aqueous acetonitrile–dichloromethane and for the number of carbon atoms in the alkyl chains – in any propanol–hexane phase. Coelution conditions for $(EO)_n-(PO)_m-(EO)_n$ cooligomers on a Separon NH_2 column: in 82% propanol–hexane for EO units (too low a retention, $k = 0$) and in any propanol–hexane mobile phase for PO units.
Coelution conditions for $(PO)_m-(EO)_n-(PO)_m$ cooligomers on a Separon C18 column: in any acetonitrile–water mobile phase for EO units and in no mobile phase for PO units.
(ADW: ACN + 1%H_2O—CH_2Cl_2).

C18 column, methanol–water and acetonitrile–water mobile phases; (2) normal-phase systems with a Separon SGX NH_2 bonded amino phase column and (a) 2-propanol–hexane, (b) acetonitrile–water–dichloromethane mobile phases. (The chromatographic system (2b) corresponds to the conditions of hydrophilic interaction chromatography often used for separations of biopolymers.) Partial dependencies of the retention on the number of oxyethylene and oxypropylene units calculated with the coefficients of Eqs. (29A) and (29B) are illustrated by the plots in Figures 5–8, which illustrate good agreement with the experimental data (points).

From the coefficients in Table IV, the conditions for coelution of species with different numbers of repeat monomer units in one of the blocks can be estimated. In agreement with the experimental data, Eqs. (29A) and (29B) predict the mobile phase composition for the coelution of oligoethylene glycol ethers with various numbers of repeat oxyethylene units and separation only according to the alkyl lengths distribution on a C18 column either in 95% methanol or in 78%

acetonitrile in water, or on the bonded amino column in 1% aqueous acetonitrile in dichloromethane, which agrees with the experimental results. Equation (29B) predicts that with 82% propanol in hexane as the mobile phase coelution occurs of ethylene oxide–propylene oxide block cooligomers with different numbers of oxyethylene repeat units on the bonded amino column, but the retention of the individual species is too low in this mobile phase, so that it is not possible to accomplish any separation of the cooligomers with different numbers of oxypropylene repeat units.

Equations (29A) and (29B) cannot be employed for the calculations of the mobile phase composition suitable for the separation according to the distribution in one block only, if the values of the two coefficients for one block are close to zero. In such an instance, the coelution of the species with different numbers of repeat monomer units in this block occurs over a broad composition range of the mobile phases. From the data in Table IV it follows that on the bonded-amino column the composition range of propanol–hexane or acetonitrile–dichloromethane (+1% water) mobile phases suitable for coelution of the ethers with different alkyl lengths is very broad and that coelution of cooligomers with different numbers of oxypropylene units occurs practically at any composition of propanol–hexane mobile phases. On the contrary, very low values of a_{1A}, m_{1A} on the octadecyl silica column mean that coelution of EO–PO cooligomers with different numbers of oxyethylene units occurs over a very broad range of concentrations of acetonitrile in water, likely the coelution of EO alcohols with different numbers of oxyethylene units is found over a broad composition range of methanol–water mobile phases. In such a case, it is possible to use gradient elution to speed-up the elution of the sample with the separation according to the distribution in one block suppressed in the whole course of gradient elution, as it is illustrated by an example of reversed-phase separation of a Novanik sample of EO–PO block cooligomers only according to the number of oxypropylene units in Figure 9 and by an example of normal-phase separation on a Separon Amine column of a Slovanik sample of EO–PO block cooligomers only according to the number of oxyethylene units in Figure 10. Surprisingly, the repeat unit selectivity depends significantly on the sequence of the blocks, as it is discussed in the next paragraph.

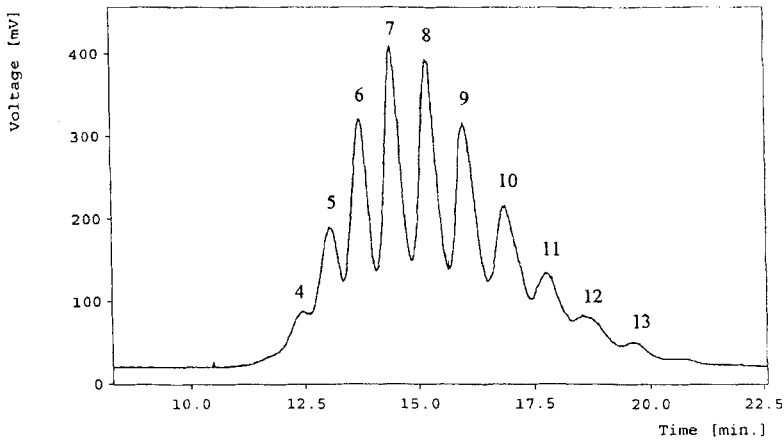

FIGURE 9 Gradient–elution reversed-phase separation of a Novanik 600/20 sample on a Separon SGX C18 column. Linear gradient, 15–100% acetonitrile in water in 25 min. Flow rate 0.5 mL/min, light-scattering detection. Peak numbers correspond to oligomers with 5–13 oxypropylene units at the conditions of coelution for oxyethylene units.

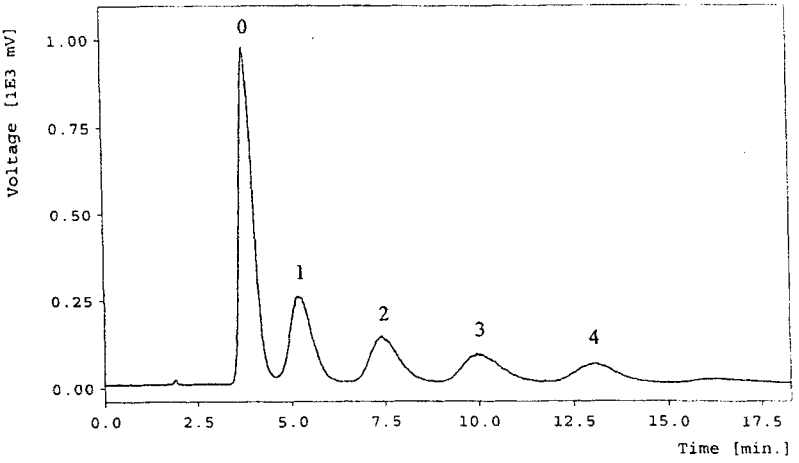

FIGURE 10 Gradient-elution normal-phase separation of a Slovanik 320 sample on a Separon SGX Amine column. Linear gradient, 10–30% 2-propanol in a-hexane in 30 min. Flow rate 0.5 mL/min, light-scattering detection. Peak numbers correspond to oligomers with 0–4 oxyethylene units at the conditions of coelution for oxypropylene units.

SEQUENCE DISTRIBUTION

The effect of the sequence distribution of the individual blocks on the chromatographic behavior can be demonstrated on the example of reversed-phase and normal-phase separation of the EO–PO block cooligomers. We found that the possibilities for a successful separation according to the chemical composition distribution, *i.e.*, according to the number of either oxyethylene or oxypropylene units, strongly depend on the sequence distribution of the blocks in these products. In reversed-phase systems, we did not succeed with the separation of the products with the inner oxypropylene and the outer oxyethylene blocks (Slovanik) according the oxypropylene unit distribution. For their characterization, selected ion chromatograms using mass-spectrometric detection can be used after peak deconvolution.[17] However, good separation according to this type of distribution is possible for the products with inner oxyethylene and outer oxypropylene blocks (Novanik), using gradient elution with acetonitrile in water, as it is illustrated by the chromatogram in Figure 9.

However, only partial separation of the products with the inner oxyethylene and the outer oxypropylene blocks (Novanik) according to the ethylene oxide units distribution was accomplished on the bonded-amino column in propanol–hexane mobile phases. Much better separation of the products with the oxypropylene inner blocks and the oxyethylene outer blocks (Slovanik) can be achieved on this column using elution with a gradient of 2-propanol in hexane, as illustrated in Figure 10.

The effect of the sequence distribution on the chromatographic behavior in both reversed-phase and normal-phase systems obviously follows the rule that the separation selectivity according to the "outer" block repeat units is enhanced (probably because of better possibility of the orientation towards the surface of the column packing material) and the separation selectivity according to the "inner" block is suppressed. In other words, the outer blocks shield the inner block and allow only limited access of this block to the surface of the stationary phase, so that differences between the stationary phase interactions of cooligomers with different numbers of repeat monomer units in the inner block are suppressed. Hence, chromatography on a C18 column

provides better separation of PO–EO–PO (Novanik) than of EO–PO–EO (Slovanik) samples, whereas on a bonded amino column in propanol–hexane mobile phases better separation of Slovanik than of Novanik samples is achieved.

Lesser effect of the block sequence on the separation of the EO–PO block cooligomers on a bonded-amino column with aqueous acetonitrile–dichloromethane mobile phases can be possibly explained by mixed retention mechanism, including partition between the mobile phase and the adsorbed liquid phase more rich in water. In the adsorbed layer of liquid on the adsorbent surface in these systems, the orientation of the blocks can be expected to show minor effect on the retention than in "regular" nonaqueous normal-phase systems. In aqueous acetonitrile–dichloromethane mobile phases, the separation selectivity for the EO units distribution in EO–PO–EO samples and for the PO units distribution in PO–EO–PO samples increases with respect to the propanol–hexane mobile phases, which results in a strong coelution of the individual species with different numbers of EO and PO units with both sample types. Further, under these conditions significant contribution is observed of the $(n_{EO})^2 \log \gamma_{EO}$ term to the retention of the EO–PO–EO cooligomers and of the $(n_{PO})^2 \log \gamma_{PO}$ term to the retention of the PO–EO–PO cooligomers.

CONCLUSION

The separation selectivity for oligomers according to the molar mass distribution is often better in interactive chromatography (IC) than in SEC. Reversed-phase chromatography is preferred for separations of oligomers with nonpolar repeat units and normal-phase chromatography for separations of oligomers with polar repeat units. In hydrophilic interaction chromatography, the selectivity is often higher than in RPC or in NPC. Gradient elution is generally required to achieve adequate peak capacity in IC of polymers and oligomers. The separation can be optimized by adjusting the initial mobile phase composition, the gradient time and shape.

"Critical conditions" in IC, where the separation according to the molar mass distribution is suppressed to allow better separation according to the functionality type distribution in oligomers can be

occasionally achieved for some samples, but the "critical" composition of the mobile phase strongly depends on the type of the column. Similarly, in many cases it is possible to adjust the separation conditions for cooligomers so that the separation according to the distribution of the number of repeat monomer units in one block is intentionally suppressed and on the contrary, the separation according to the distribution in other block is enhanced. This optimization approach can be employed for two-dimensional separations according to the chemical composition distribution in cooligomers. Some block cooligomers such as EO–PO products show significant dependence of the retention behavior on the block sequence distribution.

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