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METHODS FOR CHARACTERIZATION OF SELECTIVITY IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

IV. RETENTION BEHAVIOUR OF OLIGOMERIC SERIES

PAVEL JANDERA

University of Chemical Technology, Department of Analytical Chemistry, Leninovo nám. 565, 532 10 Pardubice (Czechoslovakia)

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SUMMARY

The retention behaviour of oligomeric series in reversed-phase systems with respect to the number of repeat structural units and the mobile phase composition can be described by the same set of equations as for homologous series, but the constants of these equations are strongly dependent on the structure of both the repeat structural unit and the structural residue in the molecule. The separation selectivity for the individual oligomers is determined mainly by the size (molar volume) and polarity (interaction index) of the repeat units and of the structural residue, but it also depends on the type and concentration of the organic solvent in the mobile phase. If the repeat structural unit increases both the size and polarity of the oligomer molecules, the selectivity may vary as a function of the structural parameters of the structural residue and of the mobile phase composition, and the retention may even decrease with increasing number of repeat structural units in a given series. This behaviour can be explained, characterized and even predicted (at least qualitatively) from the structural constants V_x , I_x on the basis of the interaction indices model. This retention behaviour was investigated and verified for oligostyrenes, oligoethylene glycols and oligoethylene glycol nonylphenyl ethers on various C_{18} and C_8 columns in mobile phases containing methanol, 1,4-dioxane or 2-propanol in water.

The effectiveness of gradient elution for the separation of oligomeric series depends strongly on their structure. The presence of cetyltrimethylammonium bromide (CTAB) in the mobile phase has only a minor influence on the separation of non-sulphated oligoethylene glycol nonylphenyl ethers, but it leads to an increase in the retention of sulphated ethers, with a separation selectivity similar to that of non-sulphated ethers. The individual oligomers in mixtures containing sulphated and non-sulphated surfactants of this type can be separated in a single run using CTAB-containing mobile phases.

INTRODUCTION

The retention mechanism in reversed-phase liquid chromatography still remains controversial. The solvophobic theory of Horváth *et al.*^{1,2}, the molecular statistical theory of Martire and Boehm³ and the models using molecular connectivity⁴, Hildebrand solubility parameters⁵⁻⁷ and interaction indices^{8,9} have been proposed to describe and characterize retention in reversed-phase systems. Various scales of retention indices based on alkyl aryl ketones^{10,11}, alkan-2-ones^{12,13} or polyaromatic hydrocarbons¹⁴ have been suggested for calibrating retention in reversed-phase systems. The scale of interaction indices based on various simple aromatic compounds was intended for the same purpose^{8,9,15}. A disadvantage of these methods is that they do not take full account of the selectivity changes with changing mobile phase composition.

A calibration method using alkylbenzene calibration series and two indices to characterize the retention of each solute over a wide range of mobile phase compositions has been introduced recently¹⁶. One index, n_{ce} , accounts for the hydrophobicity of a solute and the other, q_i , for the polarity of its functional group(s) interacting with mobile phase components. This approach makes it possible to predict the selectivity changes induced by changes in the mobile phase composition and may be used to predict relative and absolute retentions under isocratic^{17,18} or gradient elution¹⁹ conditions in binary and ternary mobile phases. The lipophilic and polar indices may be calculated from the additive contributions of structural elements¹⁷.

As with homologous series²⁰, a regular increase in the logarithms of the capacity factors with increasing number of repeat structural units was observed for some oligomeric series studied systematically in reversed-phase systems, such as for styrene oligomers²¹ and for oligoethylene glycol phenyl ethers²², in agreement with the Martin rule.

Lower styrene oligomers have been separated on octadecylsilica columns by elution with pure acetonitrile²³, or using gradient elution with increasing concentration of tetrahydrofuran in water²⁴⁻²⁶, tetrahydrofuran in methanol^{24,27} or dichloromethane in methanol^{28,29}. Isocratic separations of styrene oligomers on phenyl-bonded phases using elution with tetrahydrofuran-water, acetonitrile-water or tetrahydrofuran-*n*-hexane mobile phases were also successful²¹. Separations on nitrile-bonded phases³⁰ and on silica gel³¹⁻³⁴ have also been reported.

Reversed-phase chromatography has only exceptionally been employed to separate the individual oligomers in oligoethylene glycol series³⁵ and in samples of their adducts with phenol²². Rather, normal-phase chromatography on silica^{36,37}, nitrile-bonded³⁷, amino-bonded³⁸ or diol-bonded³⁹ phases is used for the separation of the oligomers in various commercial oligoethylene glycol adducts.

It was the purpose of this work to investigate the possibilities of the characterization and prediction of selectivity and retention in oligomeric series, using the approach employed earlier for homologous series²⁰ and the calibration method based on the lipophilic and polar indices¹⁷. To verify the theoretical approach, various series were selected, including oligostyrenes, oligoethylene glycols and sulphated and non-sulphated oligoethylene glycol nonylphenyl ethers. A further aim was to investigate and to attempt to explain possible differences between the chromatographic behaviour of homologous series and that of oligomeric series and to attempt to

formulate some general rules, which could make it possible to predict (at least qualitatively or semi-quantitatively) the selectivity in oligomeric series on the basis of some structural indices in a given oligomeric series.

THEORETICAL

Retention equations for oligomeric series

The description of retention and selectivity of the members of an oligomeric series can be derived using the same approach as that reported earlier for homologous series²⁰, which is based on the model of interaction indices^{8,9}. In both types of series, the molar volumes V_x and the interaction indices I_x , which are a measure of the solute polarities, increase regularly with increasing number of repeat structural units, *i.e.*, the degree of polymerisation, n :

$$V_x = V_{0x} + \Delta V_x n \quad (1)$$

$$I_x = I_{0x} + \Delta I_x n \quad (2)$$

The starting point of the derivation is the basic equation of the interaction indices model for reversed-phase systems. For chromatography in binary mobile phases composed of water (interaction index I_{H_2O}) and an organic solvent (interaction index I_{org}) it reads as follows⁸:

$$\begin{aligned} \log k' = \log \Phi + \frac{V_x c_M I_{H_2O}}{2.3 RT} (c_M I_{H_2O} - c_x I_x) - \frac{V_x c_M}{2.3 RT} (2c_M I_{H_2O} - c_x I_x) \times \\ \times (I_{H_2O} - I_{org})\varphi + \frac{V_x c_M^2}{2.3 RT} (I_{H_2O} - I_{org})^2 \varphi^2 \quad (3) \end{aligned}$$

where k' is the capacity factor of a sample solute with molar volume V_x and interaction index I_x , Φ is the phase ratio, V_s/V_M (V_s and V_M are the volumes of the stationary and mobile phases, respectively, in the column used), R is the gas constant, T is temperature (K), c_M and c_x are proportionality constants relating to the mobile phase and to the solute, respectively, and φ is the concentration of the organic solvent in the mobile phase [% (v/v) $\cdot 10^{-2}$].

The combination of the eqns. 1, 2 and 3 yields the following expressions for k' of the members of a homologous or oligomeric series²⁰:

$$\log k' = \log \beta + (\log \alpha)n + (\log \gamma)n^2 \quad (4)$$

and

$$\log k' = a - m\varphi + d\varphi^2 \quad (5)$$

where n is the number of the repeat structure units in a given member of a homologous or oligomeric series. Eqn. 4 predicts a quadratic dependence of $\log k'$ on the number of oligomeric or homologous units, in contrast to the Martin rule, but as long as the

product of the increments $\Delta V_x \Delta I_x$ is low enough, the quadratic term ($\log \gamma$) can be neglected and the retention is described by the well known expression²⁰

$$\log k' = \log \beta + (\log \alpha)n \quad (4a)$$

Eqn. 5 for the dependence of $\log k'$ on φ is generally quadratic, in agreement with both the interaction index⁸ and solubility parameter^{6,7} theories, but often simple linear expression may be used for description of reversed-phase systems:

$$\log k' = a - m\varphi \quad (5a)$$

where

$$a = a_0 + a_1n \quad (6)$$

$$m = m_0 + m_1n \quad (7)$$

$$\log \beta = \beta_0 - \beta_1\varphi \quad (8)$$

$$\log \alpha = \alpha_0 - \alpha_1\varphi \quad (9)$$

Using eqn. 3, it can be shown that the constants of eqns. 4a and 5a depend on the interaction indices of water (I_{H_2O}) and of the organic solvent used in the mobile phase (I_{org}), on the contribution of a structural repeat unit to the interaction index of a given oligomer (ΔI_x), on an analogous contribution to the molar volume of the oligomer (ΔV_x), and on the contribution of the structural residue to the interaction index (I_{0x}) and to the molar volume (V_{0x}) of the oligomer:

$$a_0 = \beta_0 = \log \Phi + c_1 V_{0x} I_{H_2O} (c_M I_{H_2O} - c_x I_{0x}) \quad (10)$$

$$m_0 = \beta_1 = c_1 V_{0x} (2c_M I_{H_2O} - c_x I_{0x}) (I_{H_2O} - I_{org}) \quad (11)$$

$$a_1 = \alpha_0 = c_1 I_{H_2O} [(c_M I_{H_2O} - c_x I_{0x}) \Delta V_x - c_x \Delta I_x V_{0x}] \quad (12)$$

$$m_1 = \alpha_1 = c_1 (I_{H_2O} - I_{org}) [(2c_M I_{H_2O} - c_x I_{0x}) \Delta V_x - c_x \Delta I_x V_{0x}] \quad (13)$$

where c_1 is a constant depending on temperature and on the nature of the components of the mobile phase:

$$c_1 = \frac{c_M}{2.3RT} \quad (14)$$

Oligomeric series differ from homologous series in the character of the increments ΔI_x and ΔV_x , which are identical for various homologous series in which a methylene group is the repeat structural unit, but may differ considerably between the individual oligomeric series. Thus, the retention behaviour in homologous series represents a special case of the behaviour in oligomeric series. As in homologous series, the following equations apply also for oligomeric series, provided that the linear eqns. 4a and 5a are valid:

$$m = q + pa \quad (15)$$

$$\log k' = (a_0 + a_1n)(1 - p\varphi) - q\varphi \quad (16)$$

The constants p and q can be defined as the correlation parameters between the slope and the intercept of $\log k'$ versus φ dependences for the individual members of a given homologous or oligomeric series. These constants depend on the organic solvent used in the mobile phase and on the polarities and sizes of the structural increment and of the structural residue in a given series²⁰.

The structural dependence of the correlation parameter p can be described by the following equation, obtained after combination of eqns. 5a-7, 12, 13 and 15:

$$p = \left(1 - \frac{I_{\text{org}}}{I_{\text{H}_2\text{O}}}\right) \left(1 + \frac{1}{1 - \frac{c_x}{c_M I_{\text{H}_2\text{O}}} \cdot Q}\right) \quad (17)$$

where Q is a combined structural parameter including the size (ΔV_x , V_{0x}) and polarity (ΔI_x , I_{0x}) parameters of both the repeat structural unit and the structural residue in a given oligomeric series:

$$Q = \frac{V_{0x}\Delta I_x + I_{0x}\Delta V_x}{\Delta V_x} \quad (18)$$

The numerator in eqn. 18 represents a combination term, which can be distinguished in eqns. 12 and 13 for the constants $\alpha_0 = a_1$ and $\alpha_1 = m_1$ and which characterizes the magnitude of the interaction between the sizes and polarities of the repeat structural unit and of the structural residue in a given series. Hence the combined structural parameter Q has the physical meaning of the relative magnitude of the intramolecular repeat unit-structural residue interaction with respect to the size increment of the repeat unit.

Separation selectivity in oligomeric series

The selectivity of separation in a given oligomeric series is understood here as the relative retention of the neighbouring oligomers. If the quadratic term $\log \gamma$ in eqn. 4 can be neglected, the selectivity is constant in a given series and chromatographic system (under isocratic conditions) and is given by the term $\log \alpha$. The combination of eqns. 9, 12, 13 and 18 yields the following expression for the selectivity:

$$\log \alpha = c_1 c_x \Delta V_x \left\{ \left(2 \cdot \frac{c_M}{c_x} \cdot I_{\text{H}_2\text{O}} - Q\right) [I_{\text{H}_2\text{O}} - (I_{\text{H}_2\text{O}} - I_{\text{org}})\varphi] - \frac{c_M}{c_x} \cdot I_{\text{H}_2\text{O}}^2 \right\} \quad (19)$$

eqns. 12 and 13 can be further rearranged:

$$\alpha_0 = c_1 c_x I_{\text{H}_2\text{O}} \Delta V_x \left(\frac{c_M}{c_x} \cdot I_{\text{H}_2\text{O}} - Q \right) = K_1 (K_3 - Q) \Delta V_x \quad (20)$$

$$\begin{aligned} \alpha_1 &= c_1 c_x (I_{\text{H}_2\text{O}} - I_{\text{org}}) \Delta V_x \left(2 \cdot \frac{c_M}{c_x} \cdot I_{\text{H}_2\text{O}} - Q \right) \\ &= K_2 (2K_3 - Q) \Delta V_x \end{aligned} \quad (21)$$

According to eqn. 19, the separation selectivity in a given oligomeric series depends on the molar volume of the structural increment, ΔV_x , on the combined structural parameter Q , on the polarity (I_{org}) and concentration (φ) of the organic solvent in the mobile phase and on temperature. The possible influence of the stationary phase on selectivity, which is of relatively minor importance, is not considered in eqn. 19 but, if present, it could modify the values of the constants c_1 and c_x to some extent.

From eqn. 19, it follows that the selectivity, $\log \alpha$, in an oligomeric series may be either positive or negative, which means that the retention may either increase or decrease with increasing number of the repeat structural units, n . The former case occurs more often and it can be derived from eqn. 19 that $\log \alpha > 0$ if the following condition applies:

$$Q < \frac{c_M}{c_x} \cdot I_{\text{H}_2\text{O}} \left[2 - \frac{1}{1 - \left(1 - \frac{I_{\text{org}}}{I_{\text{H}_2\text{O}}}\right) \varphi} \right] \quad (22)$$

On the other hand, the retention would decrease with increasing n ($\log \alpha < 0$) if

$$Q > \frac{c_M}{c_x} \cdot I_{\text{H}_2\text{O}} \left[2 - \frac{1}{1 - \left(1 - \frac{I_{\text{org}}}{I_{\text{H}_2\text{O}}}\right) \varphi} \right] \quad (23)$$

The physical meaning of eqns. 22 and 23 can be interpreted as follows. The combined structural parameter Q is low if ΔI_x is low or negative, *i.e.*, if the repeat structural unit is relatively non-polar (such as methylene groups in homologous series, where $\Delta I_x < 0$). In such a case, eqn. 22 applies and the retention increases with increasing n . If $\Delta I_x < 0$, the actual values of the structural parameters $V_{0,x}$ and $I_{0,x}$ in eqn. 18 should have only a minor effect on selectivity. Hence ΔV_x and ΔI_x of the repeat structural unit are almost independent of the type of homologous series and the constants $V_{0,x}$, $I_{0,x}$ of the structural residue often tend to compensate the influence of each other. This means that if one homologous series has a bulkier structural residue than the other series, this residue is often less polar. Consequently, the selectivity, $\log \alpha$, is nearly constant in various homologous series, as has been found experimentally²⁰.

The structural combined parameter Q in oligomeric series becomes larger with increasing positive ΔI_x and decreasing ΔV_x , *i.e.*, with increasing polarity and decreasing size of the repeat structural unit the probability increases that the eqn. 23 applies and elution occurs in order of decreasing number of oligomeric units. This is more likely in mobile phases containing high concentrations of relatively low-polarity organic solvents, as the right-hand sides of eqns. 22 and 23 decrease with decreasing I_{org} and increasing φ . This also means that for some oligomeric series a concentration φ_0 could be found for which Q equals the right-hand sides of eqns. 22 and 23 and $\log \alpha = 0$, *i.e.*, all the members of this series would be eluted in a single peak. In mobile phases where $\varphi > \varphi_0$, the elution order of the oligomers would be the reverse of that when $\varphi < \varphi_0$.

Eqn. 19 predicts that the selectivity ($\log \alpha$) should depend also on the character of the structural residue in the oligomeric series if $\Delta I_x > 0$. The combined structural

parameter Q and the probability of elution in order of decreasing number of repeat structural units, n , increase with increasing size (molar volume, V_{0x}) and polarity (interaction index, I_{0x}) of the structural residue (eqn. 23). Consequently, if two oligomeric series contain the same repeat structural unit, but the structural residue in one oligomeric series is significantly bulkier or more polar than the residue in the other series, important differences between the selectivities in the two series may be expected according to eqn. 19 as the combined structural parameters Q of the two oligomeric series differ significantly in this instance. According to eqn. 18, these differences in selectivities should become more significant for oligomeric series in which the repeat structural unit is relatively bulky and polar, as Q increases with increasing ΔI_x and ΔV_x and the possible influence of the structural residue on selectivity increases with increasing Q (eqn. 19). Otherwise, Q is small and the separation selectivity should be almost independent of the structural residue.

The dependence of the selectivity on the character of the structural residue may appear strange at first glance, but it can be understood as follows. The mobile phase, which forms a part of the environment of repeat structural units, has an important influence on the separation selectivity in homologous and oligomeric series. The structural residue is also part of the environment of the repeat structural units. Its influence on the separation selectivity, although less apparent, manifests itself as the result of the interaction between the contributions of the polarities and of the sizes of both the repeat structural units and of the structural residue. This conclusion follows from the assumption that both the molar volume and the polarity of an oligomer change regularly with increasing number of the repeat structural units, n , and is expressed mathematically by eqns. 12 and 13.

The above-derived equations apply if the quadratic terms in $\log k'$ versus φ and in $\log k'$ versus n relationships can be neglected. Otherwise, additional terms are required for more accurate predictive calculations.

Calibration of retention in oligomeric series

Using the method for calibration of retention introduced recently¹⁶⁻¹⁹, k' of each oligomer can be calculated from two characteristic indices:

$$\log k' = (a_{0B} + a_{1B}n_{ce})(1 - p_B\varphi) - q_i\varphi \quad (24)$$

where n_{ce} is the lipophilic index, *i.e.*, the number of methylene groups in a (hypothetical) member of a calibration homologous series (n -alkylbenzenes) with an equal retention to the sample solute in the absence of an organic solvent in the mobile phase, q_i is the polar index, which characterizes the magnitude of the polar interactions of the solute with the mobile phase containing a given organic solvent, φ is the concentration of the organic solvent in the mobile phase and a_{0B} , a_{1B} and p_B are the characteristic constants a_0 , a_1 and p in eqn. 16 for the reference calibration homologous series (n -alkylbenzenes) on a given column, which are determined by regression analysis of the retention-composition plots for several members of the calibration homologous series. The constants n_{ce} and q_i can be calculated from the constants a and m in eqn. 5a for each solute, using eqn. 24 and the known constants a_{0B} , a_{1B} and p_B ¹⁶.

After combination of eqns. 16, 26, 6 and 7, we find that

$$n_{ce} = \frac{a_0 - a_{0B}}{a_{1B}} + \frac{a_1}{a_{1B}} \cdot n = n_{ce0} + \Delta n_{ce}n \quad (25)$$

$$q_i = q + (a_0 + a_1n)(p - p_B) = q_{0i} + \Delta q_i n \quad (26)$$

This means that the indices n_{ce} and q_i should regularly increase (or decrease) with increasing number of oligomeric units in a given series; n_{ce0} and q_{0i} are the n_{ce} and q_i indices of the structural residue in this series, and Δn_{ce} and Δq_i are the contributions of the repeat structural unit to the n_{ce} and q_i indices of the individual oligomers. It should be kept in mind that these indices have been defined as relative values with respect to the calibration homologous series (*n*-alkylbenzenes). In various homologous series, the contribution of the methylene group to retention and selectivity is approximately constant (approximately constant ΔI_x and ΔV_x and small values of the combined structural constant Q). Consequently, $a_1 \approx a_{1B}$ and $p \approx p_B$, which means that the increments $\Delta n_{ce} \approx 1$ and $\Delta q_i \approx 0$ in various homologous series. This need not necessarily apply for various oligomeric series with different repeat structural units (and possible significant influence of the constant Q on selectivity) and a regular change of n_{ce} and q_i according to the eqns. 25 and 26 may be generally expected.

EXPERIMENTAL

Some experiments were performed using an M 6000A pump (or two M 6000A pumps and an M 660 gradient controller), an U6K injector and an M 440 UV detector operated at 254 nm (all from Waters Assoc., Milford, MA, U.S.A.) and a TZ 4221 line recorder (Laboratory Instrument Works, Prague, Czechoslovakia). An HP 1090M liquid chromatograph equipped with a UV-VIS diode-array detector, automatic sample injector, 3DR solvent delivery system, thermostated column compartment, a Series 79994A workstation, an HP 2225 Think-Jet printer and a 7475A plotter (Hewlett-Packard, Avondale, PA, U.S.A.) was used for other experiments. For chromatography of oligoethylene glycols, Waters Assoc. instrumentation was employed with an R 401 refractometric detector instead of the M 440 UV detector.

Stainless-steel columns were packed in the laboratory with spherical octadecylsilica gel (Silasorb C₁₈ SPH, 10 and 7.5 μ m) and with spherical octylsilica gel (Silasorb C₈ SPH, 10 and 7.5 μ m) (both from Lachema, Brno, Czechoslovakia) using a high-pressure slurry packing technique. A stainless-steel column packed with spherical silica gel (Separon SIX C₁₈, 5 μ m) was purchased from Laboratory Instrument Works.

Column dead volumes, V_M , were determined using methanol and ²H₂O as dead volume markers and refractometric detection with an R 401 differential refractometer.

The compounds used as sample solutes included *n*-alkanes and *n*-alkylbenzenes (various sources), oligoethylene glycols (Carbowax 200, Michrome, Gurr, London, U.K.), oligostyrenes (a polystyrene standard of a nominal molecular weight 2350, Waters Assoc.), oligomeric ethoxylated nonylphenols (Serdox NNP 4, Servo, Delden, The Netherlands) and oligomeric anionic surfactants prepared by sulphation of

ethoxylated nonylphenols⁴⁰. The solutes were dissolved in mobile phases at appropriate concentrations to yield a good UV or RI detector response.

The mobile phases were prepared by mixing water (doubly distilled in glass with addition of potassium permanganate) with the organic solvents [and with cetyltrimethylammonium bromide (CTAB)] in the required volume ratios. Methanol, 2-propanol and 1,4-dioxane were of spectroscopic or analytical-reagent grade (Lachema). CTAB was purchased from Serva (Heidelberg, F.R.G.). All the solvents were filtrated using a Millipore 0.45- μm filter and the mobile phases were de-gassed by ultrasonication before the use, or were prepared directly in the HP 1090M instrument from the pure solvents continuously stripped by a stream of helium.

Evaluation of the data

The retention volumes, V_R , of the members of the homologous and oligomeric series tested were measured at different mobile phase compositions. The capacity factors, k' , were calculated from the mean V_R value of three repeated experiments under given conditions ($k' = V_R/V_M - 1$). The order of elution was checked and the individual oligomers were identified by comparison of the chromatograms of the monomers and of the samples of different nominal degrees of polymerization, *i.e.*, of different average molecular weights (oligoethylene glycols 200 and 400, oligostyrenes 2350 and 3500 and oligomeric ethoxylated nonylphenols NNP4, NNP8 and NNP12) under the conditions where the low oligomers were best separated. This identification of the peaks was compared with previous experimental peak identification in normal-phase systems³⁴ and, with the oligomeric ethoxylated nonylphenols, it was further checked using mass spectrometry (further details are given under Results and Discussion).

Linear regression analysis was used to calculate the constants $\log \alpha$ and $\log \beta$ in eqn. 4a from the experimental plots of $\log k'$ versus n at different mobile phase compositions and the constants a and m in eqn. 5a from the experimental plots of $\log k'$ versus φ for the individual homologues or oligomers. Some $\log k'$ versus φ plots in propanol-water and in dioxane-water mobile phases were slightly curved at the lower concentrations of the organic solvent in the mobile phase; the parts of the plots that deviated more than 5% from linearity were not considered in the regression analysis. The plots of the constants a and m in eqn. 5a versus n for the individual oligomers and the plots of the constants $\log \alpha$ and $\log \beta$ in eqn. 4a versus φ in the mobile phase were subjected to linear regression analysis to determine the constants $a_0, a_1, m_0, m_1, \alpha_0, \alpha_1, \beta_0, \beta_1, q$ and p (eqns. 6-9 and 15). The values of these constants for the series and chromatographic systems studied are given in Tables I-IV, VI and VII.

RESULTS AND DISCUSSION

Comparison of the chromatographic behaviour of the members of homologous and oligomeric series

The validity of the present retention model in reversed-phase systems was tested on the chromatographic behaviour of *n*-alkanes, *n*-alkylbenzenes, oligostyrenes, oligoethylene glycols and ethoxylated nonylphenols on several C_{18} and C_8 columns in various mobile phases, containing water and methanol, acetonitrile, 1,4-dioxane or 2-propanol as the organic solvents. For each series, the validity of eqns. 4a, 5a, 6-9, 15,

25 and 26 was tested. The values of the constants a , m , $\log \alpha$, $\log \beta$, $a_0 = \beta_0$, $m_0 = \beta_1$, $a_1 = \alpha_0$, $m_1 = \alpha_1$, p , q , n_{ce} , q_i , n_{ce0} , Δn_{ce} , q_{0i} and Δq_i of these equations and the corresponding correlation coefficients, which indicate the validity of the above equations, are given in Tables I–IV. The constants a_{0B} , a_{1B} , m_{0B} , m_{1B} , q_B and p_B of the calibration homologous n -alkylbenzenes series, necessary for calculations of the indices n_{ce} and q_i (eqns. 24–26), were determined in an analogous way from the experimental k' values of C_2 – C_5 alkylbenzenes measured at four or five different mobile phase compositions and are also given in Tables I–IV. The indices n_{ce} and q_i were calculated from the following equations:

$$n_{ce} = \frac{a - a_{0B}}{a_{1B}} \quad (27)$$

$$q_i = m - p_B a \quad (28)$$

and the constants n_{ce} , Δn_{ce} , q_{0i} and Δq_i in eqns. 25 and 26 were calculated using linear regression of the n_{ce}^0 versus n and q_i versus n plots. The calculated values of these constants (Tables I–IV) were in all the instances virtually identical with the values determined directly from the constants a_0 , a_1 and p in eqn. 16 applying for a given

TABLE I

EXPERIMENTAL VALUES OF THE PARAMETERS α_0 , α_1 , β_0 , β_1 , a_0 , a_1 , m_0 , m_1 , q , p , n_{ce0} , Δn_{ce} , q_{0i} AND Δq_i IN EQNS. 6–9, 15, 25 AND 26 FOR n -ALKANES ON A C_8 COLUMN IN METHANOL–WATER AND ACETONITRILE–WATER MOBILE PHASES, CALCULATED USING LINEAR REGRESSION OF THE EXPERIMENTAL DATA ACCORDING TO THE EQUATIONS

R = correlation coefficient. Column: Silasorb C_8 , 7.5 μ m (300 \times 3.8 mm I.D.).

Mobile phase: methanol–water; $n = 5$ –10:				Mobile phase: acetonitrile–water; $n = 5$ –10:			
Methanol (%, v/v)	Log β	Log α	R	Acetonitrile (%, v/v)	Log β	Log α	R
60	–0.506	0.236	0.9999	50	–0.332	0.192	0.9999
70	–0.617	0.179	0.9999	55	–0.328	0.169	0.9998
80	–0.763	0.131	0.9990	60	–0.405	0.154	0.9999
90	–0.979	0.091	0.9995	70	–0.530	0.131	0.9998
				80	–0.714	0.114	0.9997
Log $\alpha = 0.521 - 0.483\varphi$;			$R = 0.9974$	Log $\alpha = 0.311 - 0.252\varphi$;			$R = 0.9818$
Log $\beta = 0.456 - 1.563\varphi$;			$R = 0.9886$	Log $\beta = 0.376 - 1.329\varphi$;			$R = 0.9801$
$a = 0.618 + 0.494n$;			$R = 0.9996$	$a = 0.518 + 0.287n$;			$R = 0.9979$
$m = 1.757 + 0.450n$;			$R = 0.9991$	$m = 1.527 + 0.219n$;			$R = 0.9939$
$m = 1.193 + 0.912a$;			$R = 0.9999$	$m = 1.249 + 0.766a$;			$R = 0.9985$
$n_{ce} = -1.85 + 0.99n$;			$R = 0.9996$	$n_{ce} = -2.04 + 0.98n$;			$R = 0.9980$
$q_i = 1.18 - 0.01n$;			$R = 0.9000$	$q_i = 1.08 - 0.03n$;			$R = 0.9454$
Calibration series of n -alkylbenzenes:				Calibration series of n -alkylbenzenes:			
$a_{0B} = 1.542$; $a_{1B} = 0.501$;		$R = 0.9982$		$a_{0B} = 1.114$; $a_{1B} = 0.293$;		$R = 0.9994$	
$m_{0B} = 2.635$; $m_{1B} = 0.466$;		$R = 0.9966$		$m_{0B} = 2.082$; $m_{1B} = 0.254$;		$R = 0.9990$	
$q_B = 1.195$; $p_B = 0.932$;		$R = 0.9997$		$q_B = 1.118$; $p_B = 0.864$;		$R = 0.9998$	

TABLE II

EXPERIMENTAL VALUES OF THE PARAMETERS α_0 , α_1 , β_0 , β_1 , a_0 , a_1 , m_0 , m_1 , q , p , n_{ce0} , Δn_{ce} , q_{0i} AND Δq_i IN EQNS. 6-9, 15, 25 AND 26 FOR OLIGOSTYRENES ON A C₁₈ COLUMN IN 1,4-DIOXANE-WATER MOBILE PHASES, CALCULATED USING LINEAR REGRESSION OF THE EXPERIMENTAL DATA ACCORDING TO THE EQUATIONS

R = correlation coefficient. Column: Silasorb C₁₈ SPH, 7.5 μ m (300 \times 4.1 mm I.D.).

Mobile phase: 1,4-dioxane-water; n = 3-11:

Dioxane (% v/v)	Log β	Log α	R
75	0.149	0.148	0.9980
77	0.099	0.134	0.9992
80	-0.012	0.102	0.9988
83	-0.116	0.082	0.9984
85	-0.155	0.062	0.9984
86	-0.176	0.058	0.9988

$$\text{Log } \alpha = 0.768 - 0.826\varphi; \quad R = 0.9992$$

$$\text{Log } \beta = 2.487 - 3.116\varphi; \quad R = 0.9932$$

$$a = 2.487 + 0.767n; \quad R = 0.9980$$

$$m = 3.116 + 0.826n; \quad R = 0.9979$$

$$m = 0.437 + 1.077a; \quad R = 0.9999$$

$$n_{ce} = 2.00 + 2.30n; \quad R = 0.9980$$

$$q_i = 0.82 + 0.12n; \quad R = 0.9964$$

Calibration series of n-alkylbenzenes:

$$a_{0B} = 1.820; \quad a_{1B} = 0.334; \quad R = 0.9980$$

$$m_{0B} = 2.724; \quad m_{1B} = 0.308; \quad R = 0.9966$$

$$q_B = 1.041; \quad p_B = 0.923; \quad R = 0.9998$$

oligomeric series, using eqns. 25 and 26. The two calculation methods are identical as far as the $\log k'$ versus φ , $\log k'$ versus n , n_{ce} versus n and q_i versus n plots are linear and the agreement between the values calculated using the two methods can be considered as evidence for the applicability of the linear forms of the above relationships in the systems studied.

Homologous n-alkylbenzenes and n-alkanes

Both $\log k'$ versus φ and $\log k'$ versus n plots for these two series are linear in methanol-water mobile phases and only slightly curved outside the concentration range 50-80% (v/v) of acetonitrile in acetonitrile-water mobile phases. The selectivity constants $\alpha_0(a_1)$ and $\alpha_1(m_1)$ are almost the same for the two series in mobile phases containing a given organic solvent and are higher in methanol-water than in acetonitrile-water mobile phases (Table I). $\Delta n_{ce} \approx 1$ and $\Delta q_i \approx 0$, which is in agreement with general theoretical predictions for homologous series; q_i and p are also almost the same for the two series in the mobile phases studied. From the comparison of n_{ce0} values it follows that the contribution of the benzene ring to lipophilic selectivity in the n -alkylbenzene series corresponds to *ca.* two methylene groups.

TABLE III

EXPERIMENTAL VALUES OF THE PARAMETERS $\alpha_0, \alpha_1, \beta_0, \beta_1, a_0, a_1, m_0, m_1, q, p, n_{ce0}, \Delta n_{ce}, q_{0i}$ AND Δq_i IN EQNS. 6-9, 15, 25 AND 26 FOR OLIGOETHYLENE GLYCOLS ON A C_{18} COLUMN IN METHANOL-WATER MOBILE PHASES, CALCULATED USING LINEAR REGRESSION OF THE EXPERIMENTAL DATA ACCORDING TO THE EQUATIONS

R = correlation coefficient. Column: Silasorb C_{18} , 10 μm (300 \times 4.2 mm I.D.).

Mobile phase: methanol-water; $n = 3-9$:

Methanol (% v/v)	Log β	Log α	R
10	-1.233	0.330	0.9994
15	-1.278	0.308	0.9999
20	-1.310	0.258	0.9975
30	-1.338	0.188	0.9977
Log $\alpha = 0.409 - 0.734\varphi$;			$R = 0.9944$
Log $\beta = -1.195 - 0.507\varphi$;			$R = 0.9601$
$a = -1.061 + 0.363n$;			$R = 0.9938$
$m = 0.614 + 0.598n$;			$R = 0.9863$
$m = 2.358 + 1.655a$;			$R = 0.9978$
$n_{ce} = -5.31 + 0.66n$;			$R = 0.9494$
$q_i = 1.56 + 0.28n$;			$R = 0.9678$
Calibration series of <i>n</i> -alkylbenzenes*:			
$a_{0B} = 1.915$;	$a_{1B} = 0.536$;	$R = 0.9985$	
$m_{0B} = 2.628$;	$m_{1B} = 0.466$;	$R = 0.9971$	
$q_B = 0.960$;	$p_B = 0.871$;	$R = 0.9999$	

* Measured in 70-90% (v/v) methanol-water.

Oligostyrenes

Because oligostyrenes are not soluble in methanol, it was not possible to investigate their behaviour in methanol-water mobile phases. Fig. 1 shows the plots of log k' versus n for oligostyrenes on a C_{18} column in 1,4-dioxane-water mobile phases. The log k' versus φ plots are linear in the range 75-87% dioxane; outside this range curvature is observed and the absolute retention increases rapidly in mobile phases containing less than 75% dioxane. The plots of increasing log k' with degree of polymerization n are linear and only the k' for the first members of the series show slight negative deviations. An example of the isocratic separation of oligostyrenes in 83% dioxane is shown in Fig. 2. The separation achieved is approximately comparable to that in chromatography on a silica gel column³⁴.

The repeat structural unit $[-\text{CH}_2(\text{phenyl})-\text{CH}_2-]$ shows a contribution to lipophilic selectivity approximately equivalent to 2.3 methylene groups. Both lipophilic, Δn_{ce} , and polar, Δq_i , indices for the repeat unit are positive, in contrast to the behaviour in homologous series (Table II); $n_{ce0} = 2$, which corresponds to the contribution to lipophilic selectivity of the structural residue equivalent to four methylene groups, in agreement with the structure of the butyl end-group for an anionically polymerized polystyrene sample. The constant p in the oligostyrene series is only slightly (*ca.* 10%) higher than the corresponding values for homologous *n*-alkylbenzenes and *n*-alkanes in methanol-water mobile phases.

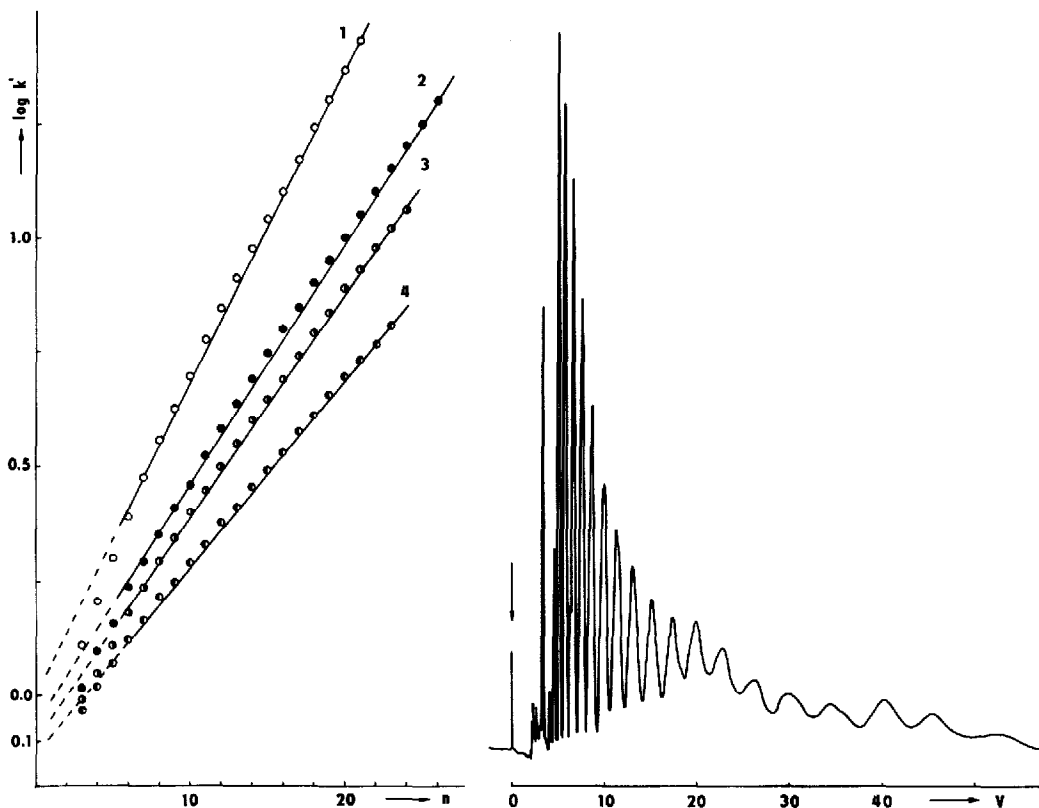


Fig. 1. Dependence of retention (k') of oligostyrenes on the number of structural repeat units, n , in mobile phase composed of 1,4-dioxane and water; (1) 83:17; (2) 85.3:14.7; (3) 86:14; (4) 87:13. Column: Silasorb C_{18} SPH, 7.5 μm (300×4.1 mm I.D.).

Fig. 2. Separation of a polystyrene standard, nominal molecular weight 2350, on a Silasorb C_{18} SPH column in 1,4-dioxane-water (83:17). Flow-rate, 0.5 ml/min; detection, UV (254 nm); sample volume, 10 μl . V = volume of eluate (ml).

Oligoethylene glycols

Oligoethylene glycols are readily soluble in methanol and a linear decrease in $\log k'$ with increasing concentration of methanol in the mobile phase is observed up to *ca.* 40% (v/v). At higher methanol concentrations, the retention of all the oligomers is low and these are eluted as a single peak with a retention volume close to the column dead volume. The $\log k'$ values increase linearly with increasing number of oligomeric units, n (Table III).

The increase in retention with increasing n at lower methanol concentrations was confirmed experimentally by comparison of the elution pattern of two oligoethylene glycol samples with different average molecular weights. The elution pattern of the sample with higher molecular weight was shifted towards larger elution volumes than that of the lower molecular weight sample.

The repeat structural unit $-\text{CH}_2\text{CH}_2\text{O}-$ contributes to the lipophilic selectivity (Δn_{cc}) as the equivalent of 0.7 methylene units, whereas its contribution Δq_i to polar

selectivity is about twice that for a repeat unit in the oligostyrene series. n_{ce0} is much lower and q_{01} is larger than the corresponding values for *n*-alkanes, *n*-alkylbenzenes and oligostyrenes. The values of these constants in Table III may be subject to some error, as it was necessary to use the calibration constants for *n*-alkylbenzenes determined in the region with much larger concentrations of methanol (in 10–30% methanol mobile phases, alkylbenzenes are too strongly retained). The constant p is almost twice that for homologous *n*-alkanes and *n*-alkylbenzenes. Consequently, an equivalent change in methanol concentration has a larger effect on the selectivity of oligoethylene glycols than on that of *n*-alkanes or *n*-alkylbenzenes.

Ethoxylated nonylphenols

Log k' of the individual oligomers decrease linearly with increasing concentration of 2-propanol in the mobile phase in the concentration range 40–60% (v/v) of 2-propanol for C_{18} columns and in the range 30–50% of 2-propanol for C_8 columns. Outside these ranges the plots are slightly curved. In the mobile phases studied, the retention decreases with increasing number of repeat oligoethylene units. This behaviour, which could appear strange at first glance, was confirmed in two independent ways: (i) fractions containing the individual oligomers were collected and subjected to gas chromatography–mass spectrometry, which confirmed that the molecular weights of the oligomers decrease with increasing elution volumes of the fractions, and (ii) several samples with different average molecular weights were subjected to high-performance liquid chromatography under identical conditions and it was found that the maxima of the elution patterns for higher molecular weight samples are shifted towards lower elution volumes. The same behaviour was observed

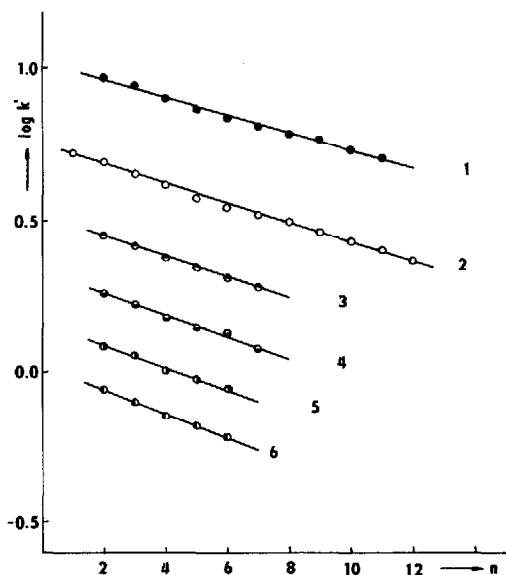


Fig. 3. Dependence of retention (k') of oligoethylene glycol nonylphenyl ethers on the number of structural repeat units, n , in mobile phases composed of 2-propanol and water: (1) 40:60; (2) 45:55; (3) 50:50; (4) 55:45; (5) 60:40; (6) 65:35. Column: Silasorb C_{18} SPH, 7.5 μm (300 \times 3.6 mm I.D.).

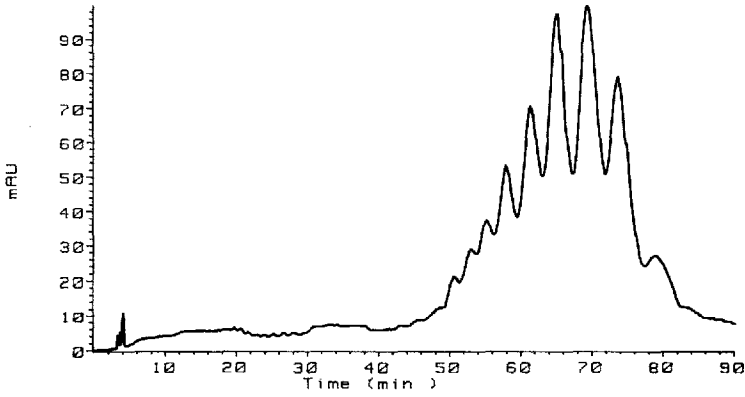


Fig. 4. Separation of a commercial sample of Serdox NNP 4, containing oligoethylene glycol nonylphenyl ethers on a Silasorb C_{18} SPH column in 2-propanol-water (35:65). Flow-rate, 0.5 ml/min; detection, UV (230 nm); instrument, HP 1090M; sample volume, 5 μ l.

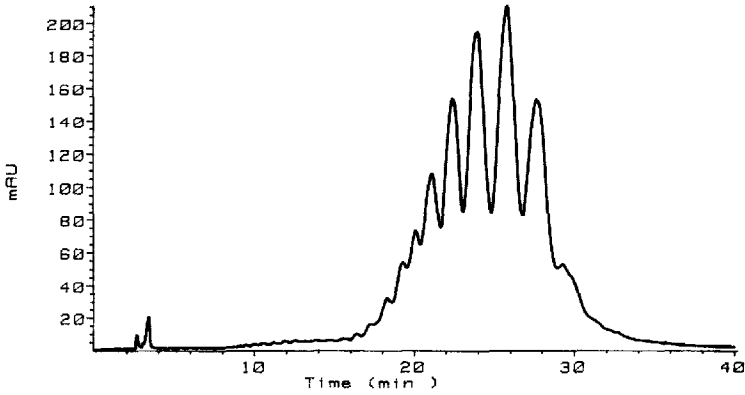


Fig. 5. Separation of Serdox NNP 4 in 2-propanol-water (45:55). Other conditions as in Fig. 4.

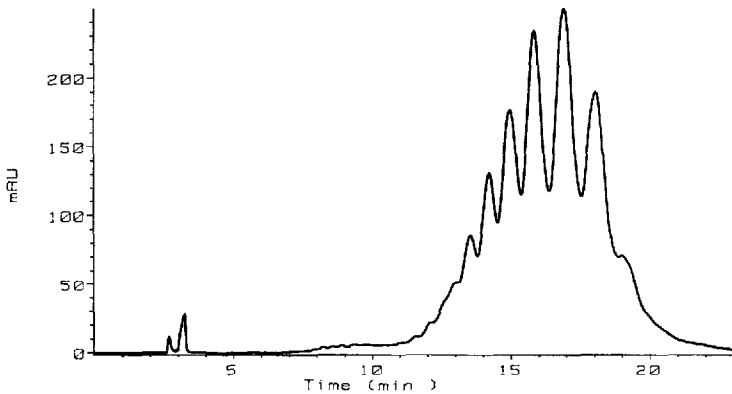


Fig. 6. Separation of Serdox NNP 4 in 2-propanol-water (50:50). Other conditions as in Fig. 4.

on different C_{18} and C_8 columns (Table IV) and $\log k'$ decreased linearly with increasing number of repeat structural units (Fig. 3). A chromatogram of a commercial sample of oligoethylene glycol nonylphenyl ethers is shown in Fig. 4.

The experimental selectivity constants α_0 are negative, whereas α_1 are positive; the constants p are negative. This means that the absolute value of the selectivity for neighbouring oligomers increases with increasing concentration of the organic solvent in the mobile phase, in contrast to the behaviour of all the other series tested. The experimental values of $\alpha_0(\alpha_1)$ and $\alpha_1(m_1)$ are very close to zero, which means that the selectivity is low. The slight increase in selectivity with increasing concentration of propanol in the mobile phase is compensated for by a decrease in the contribution of the capacity term to resolution. The separation of the lower oligomers (the later eluted ones) is approximately independent of the mobile phase composition, whereas the separation of the earlier eluted higher oligomers is slightly impaired as the concentration of propanol in the mobile phase increases, as is demonstrated in Figs. 4–6, showing the separation achieved with 35, 45 and 50% (v/v) propanol in water, respectively, as the mobile phases.

The repeat structural unit in the ethoxylated nonylphenol series is the same as that in oligoethylene glycols but, in contrast to the latter series, the lipophilic index n_{ce} decreases with the number of repeat structural units ($\Delta n_{ce} < 0$) and the polar index q_i is approximately constant, *i.e.*, Δq_i is relatively very small. The contribution of the structural residue to q_i , q_{0i} , is similar to that in the oligoethylene glycol series, but the contribution to the lipophilic index, n_{ce0} , is much higher, equivalent to approximately ten methylene units.

As the absolute retention of oligoethylene glycol nonylphenyl ethers decreases with increasing number of repeat structural units, the separation selectivity in this homologous series is expected to decrease with increasing polarity of the organic solvent in the mobile phase (see Theoretical) and the experimental behaviour agrees with this prediction. The separation selectivity is much lower in methanol–water than in propanol–water mobile phases and all the individual oligomers are eluted in a single common peak (Fig. 7). The absolute retention increases as the concentration of methanol decreases, but the resolution does not improve.

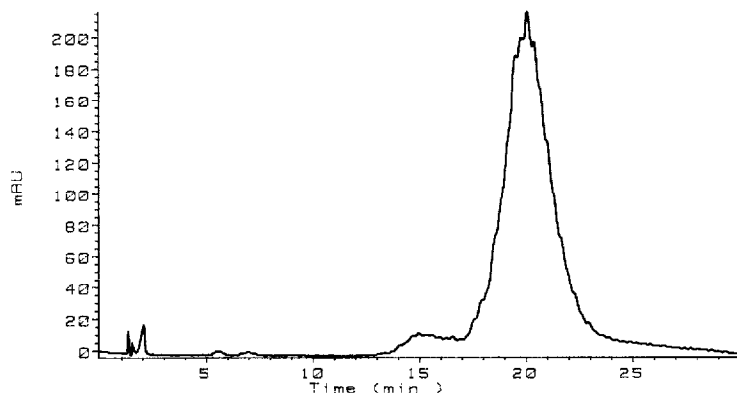


Fig. 7. Separation of Serdax NNP 4 in methanol–water (75:25). Other conditions as in Fig. 4.

Structural dependence of separation selectivity

The experimental values of the selectivity constants $\alpha_0(\epsilon)$ and $\alpha_1(\epsilon)$ (eqns. 12 and 13) for the homologous and oligomeric series tested are summarized in Table V. These values vary significantly for different series, which is understandable, as the structures of the repeat oligomeric units and also the solvents used in the mobile phases differ for the individual series studied. The differences in selectivity and in the constants α_0 and α_1 between the individual oligomeric series could be explained, at least qualitatively, on the basis of eqns. 20 and 21.

TABLE V

EXPERIMENTAL AND ESTIMATED STRUCTURAL INDICES V_{0x} , ΔV_x , I_{0x} , ΔI_x (EQNS. 1 AND 2), COMBINATION TERMS ($V_{0x}\Delta I_x + I_{0x}\Delta V_x$), COMBINED STRUCTURAL PARAMETERS Q (EQN. 18) AND EXPERIMENTAL [$\alpha_0(\epsilon)$ AND $\alpha_1(\epsilon)$] AND CALCULATED [$\alpha_0(\epsilon)$ AND $\alpha_1(\epsilon)$] (CALCULATED FROM THE EXPERIMENTAL OR ESTIMATED STRUCTURAL INDICES AND Q USING EQNS. 20 AND 21) SELECTIVITY CONSTANTS α_0 AND α_1 (EQNS. 12 AND 13)

The experimental data relate to a Silasorb SPH C₁₈ column, except for *n*-alkanes (Silasorb C₈ column). The constants $K_1 = 0.315$, $K_2 = 0.166$, $K_3 = 10.72$ in eqns. 20 and 21 were calculated using the following data from ref. 8 (LiChrosorb RP-18 column, methanol-water mobile phases): $I_{H_2O} = 47$, $I_{CH_3OH} = 22.2$, $c_M = 0.291$, $c_x = 1.274$. The calculated selectivity constants $\alpha_0(\epsilon)$ and $\alpha_1(\epsilon)$ apply for methanol-water mobile phases.

The constants V_{0x} , ΔV_x , I_{0x} and ΔI_x for alkylbenzenes and *n*-alkanes were calculated directly from the linear regression analysis of V_x and I_x as a function of *n* (eqns. 1 and 2). For other series, they were roughly estimated as follows:

(1) For oligostyrenes: V_{0x} , I_{0x} for *n*-butyl group (anionically polymerized polystyrene sample): $I_{0x} \approx 1.40 - 4 \cdot 0.32 = 0.12$; $V_{0x} \approx 0.35 + 4 \cdot 0.16 = 0.99$, $\Delta V_x \approx V_x$ (ethylbenzene) = $0.90 + 2 \cdot 0.16 = 1.22$; $\Delta I_x \approx I_x$ (ethylbenzene) = $2.94 - 2 \cdot 0.25 = 2.44$.

(2) For oligoethylene glycols: $V_{0x} \approx V_x$ (water) = 0.18; $I_{0x} \approx 2I_x$ (OH group) - I_x (-O- group) $\approx 2[I_x$ (benzyl alcohol) - I_x (toluene)] - [I_x (di-*n*-butyl ether) - $2I_x$ (*n*-butane)] = $2 \cdot (7.44 - 2.69) - (2.35 - 2 \cdot 1.40 + 8 \cdot 0.32) = 7.39$. $\Delta V_x \approx V_x$ (di-*n*-butyl ether) - V_x (*n*-hexane) = $1.70 - 0.35 - 6 \cdot 0.16 = 0.39$; $\Delta I_x \approx I_x$ (di-*n*-butyl ether) - I_x (*n*-butane) - I_x (ethane) = $2.35 - 0.12 - 0.76 = 1.47$.

(3) For oligoethylene glycol nonylphenyl ethers: $V_{0x} \approx V_x$ (phenol) + $9\Delta V_x$ (CH₂) = $0.88 + 9 \cdot 0.16 = 2.32$; $I_{0x} \approx I_x$ (*p*-cresol) + $8\Delta I_x$ (CH₂) = $6.60 - 8 \cdot 0.25 = 4.60$. ΔV_x and ΔI_x as for oligoethylene glycols.

Parameter	Series				
	Alkylbenzenes	<i>n</i> -Alkanes	Oligostyrenes	Oligoethylene glycols	Ethoxylated nonylphenols
Structural repeat unit	-CH ₂ -	-CH ₂ -	-CH ₂ C(C ₆ H ₅)H-	-CH ₂ CH ₂ O-	-CH ₂ CH ₂ O-
Structural residue	C ₆ H ₅ , H	H, H	C ₄ H ₉	OH, OH - (-O-)	C ₉ H ₁₉ C ₆ H ₄ , OH
V_{0x}	0.90	0.35	0.99	0.18	2.32
ΔV_x	0.16	0.16	1.22	0.39	0.39
I_{0x}	2.94	1.40	0.12	7.39	4.60
ΔI_x	-0.25	-0.32	2.44	1.47	1.47
$(V_{0x}\Delta I_x + I_{0x}\Delta V_x)$	0.24	0.11	2.56	3.15	5.20
Q	1.53	0.70	2.10	8.07	13.34
$\alpha_0(\epsilon)$	0.46	0.51	3.31	0.33	-0.32
$\alpha_1(\epsilon)$	0.53	0.55	3.92	0.87	0.52
$\alpha_0(\epsilon)$	0.54	0.52	0.77	0.41	-0.015
$\alpha_1(\epsilon)$	0.47	0.48	0.83	0.78	0.052
Mobile phase for experimental data	Methanol-water	Methanol-water	1,4-Dioxane-water	Methanol-water	2-Propanol-water

Each of the constants $\alpha_0(e)$ and $\alpha_1(e)$ has almost the same value for different homologous series (*n*-alkylbenzenes and *n*-alkanes) in methanol–water mobile phases. The structural incremental indices ΔV_x and ΔI_x for the methylene group (eqns. 1 and 2) are approximately equal in different homologous series and the polarity increments are negative (Table V). Consequently, the combination term $\Delta V_x I_{0x} + \Delta I_x V_{0x}$ (the numerator in eqn. 18) is close to zero and the combined structural parameter Q is relatively small (0.7 for *n*-alkanes and 1.53 for *n*-alkylbenzenes), so that they have no significant effect on the separation selectivity, *i.e.*, on the values of the selectivity constants α_0 and α_1 . This leads to approximately equal separation selectivities and α_0 and α_1 values for various homologous series. The selectivity in homologous series is controlled primarily by the size (molar volume, ΔV_x) of the methylene group and by the polarity (I_{org}) and concentration (φ) of the organic solvent in the mobile phase.

Similar conclusions should obviously apply also for the oligomeric series with relatively non-polar repeat structural units. The oligomeric unit in the oligostyrene series has a relatively high positive polarity contribution index ΔI_x and an almost eight times larger volume contribution index ΔV_x than the methylene group (Table V). Consequently, the combination term ($\Delta V_x I_{0x} + \Delta I_x V_{0x}$) and the combined structural parameter Q (eqn. 18) are greater than in homologous series, but the influence of the size, ΔV_x , of the repeat oligomeric unit on the separation selectivity obviously prevails over the influence of the combination term. This leads to a greater retention and selectivity [and constants $\alpha_0(e)$ and $\alpha_1(e)$] than in homologous series (Table V). Therefore, only a limited concentration range of the mobile phase is available for the practical separation of oligostyrenes, the separation selectivity changes more significantly with changing mobile phase composition and the correct choice of the mobile phase composition is more critical than for the separation of the other series studied.

In the oligoethylene glycol series and in the series of ethoxylated nonylphenols, the repeat structural unit $-\text{CH}_2\text{CH}_2\text{O}-$ contributes significantly to the polarity (ΔI_x), but its contribution to the molar volume (ΔV_x) is about three times lower than ΔV_x in the oligostyrene series and about three times higher than ΔV_x of the methylene group (Table V). The combination term ($\Delta V_x I_{0x} + \Delta I_x V_{0x}$) and the structural parameter Q (eqn. 18) are relatively large, which means that their influence on the separation selectivity may become important.

As the repeat structural unit $-\text{CH}_2\text{CH}_2\text{O}-$ is the same in these two oligomeric series, the selectivity $\log \alpha$ should also be the same in a mobile phase of given composition, provided that the structural residue has no influence on the separation selectivity. The retentions of the two types of oligomers are very different, so that it was not possible to compare directly the separation selectivities for the two series in the same mobile phase. Table V shows the experimental selectivity constants $\alpha_0(e)$ and $\alpha_1(e)$ for oligoethylene glycols in water–methanol mobile phases. The constant α_0 is lower and the constant α_1 is higher than the corresponding values for the homologous series tested. The separation selectivity for ethoxylated nonylphenols is very low in water–methanol mobile phases and the individual oligomers are eluted in a single common peak with very bad resolution (Fig. 7). A change in methanol concentration does not influence the bad quality of the separation and only causes a shift of the elution volume of the common peak. Hence it was not possible to evaluate quantitatively the selectivity and the constants α_0 and α_1 (eqns. 12 and 13) in

water-methanol mobile phases, but it can be concluded that they are close to zero; otherwise, the separation could have been improved by a change in methanol concentration. This means that the α_0 and α_1 values for ethoxylated nonylphenols are different to those for oligoethylene glycols, which suggests an important influence of the structural residue on the separation selectivity for the oligomeric series with $-\text{CH}_2\text{CH}_2\text{O}-$ repeat units.

In dioxane-water mobile phases, it was also impossible to resolve the individual oligomeric ethoxylated nonylphenols. These compounds could be separated in propanol-water mobile phases. The selectivity constants $\alpha_0(\text{e})$ and $\alpha_1(\text{e})$ applying for ethoxylated nonylphenols in these mobile phases are very low and a negative value of α_0 means that the oligomers are eluted in order of decreasing number of oligomeric units, n (Table V). The selectivity constant α_0 is (theoretically) independent of the organic solvent used in the mobile phase and the constant α_1 is expected to be larger in mobile phases containing less polar organic solvents (eqn. 21). This further confirms the essential differences between the selectivity constants α_0 and α_1 of ethoxylated nonylphenols and the corresponding values for oligoethylene glycols, which are eluted in order of increasing number of the oligomeric units in propanol-water mobile phases.

This behaviour may be explained by the influence of the combination term ($\Delta I_x V_{0x} + \Delta V_x I_{0x}$) and of the combined structural parameter Q (eqn. 18) on the separation selectivity. These terms have higher values for the series with $-\text{CH}_2\text{CH}_2\text{O}-$ repeat units than for the other series tested and are higher for ethoxylated nonylphenols ($Q = 13.34$) than for oligoethylene glycols ($Q = 8.07$). If the combination term is large, the structural parameter Q may be even greater than the term involving the interaction index of water in eqns. 20 and 23 applies, which means that α_0 is negative, whereas α_1 may still be positive, as has been observed experimentally for ethoxylated nonylphenols.

Eqns. 20 and 21 make it possible to calculate the selectivity $\log \alpha$ and the selectivity constants α_0 and α_1 from the structural indices I_{0x} , ΔI_x , V_{0x} and ΔV_x , provided that the constants K_1 , K_2 and K_3 in these equations are known. To calculate these selectivity constants, K_1 – K_3 were taken from earlier work⁸ and the indices I_{0x} , ΔI_x , V_{0x} and ΔV_x necessary for the calculations were either measured or estimated from the structural indices of other compounds and are given in Table V together with the calculated parameters Q and selectivity constants $\alpha_0(\text{c})$ and $\alpha_1(\text{c})$. The agreement between the predicted and experimental α_0 and α_1 values is relatively good for the homologous series of n -alkylbenzenes and n -alkanes and for oligoethylene glycols, the retention data of which were measured in methanol-water mobile phases. For the oligostyrene series, where the retention data were measured in dioxane-water mobile phases, and for the ethoxylated nonylphenols, where propanol-water mobile phases were employed, large differences between the numerical values of $\alpha_0(\text{c})$ and $\alpha_0(\text{e})$ and of $\alpha_1(\text{c})$ and $\alpha_1(\text{e})$ were found (Table V), which can be attributed to the following possible sources of errors: (a) the experimental data are compared with the data calculated using the constants K_1 – K_3 in eqns. 20 and 21, which were determined in water-methanol mobile phases; (b) slight curvatures of the $\log k' - \varphi$ plots in the range of lower φ in propanol-water and dioxane-water mobile phases were neglected to a first approximation; and (c) the structural indices for the oligomeric series studied were calculated from the values measured for other compounds, assuming additivities for various structural elements, which possibly is not strictly obeyed.

In spite of these significant differences in the numerical values of the experimental and calculated selectivity constants α_0 and α_1 , their comparison may be interesting, as the following qualitative agreement between the calculated and experimental values is observed: (1) both the experimental and the calculated values of α_0 for ethoxylated nonylphenols are negative, whereas all the other experimental and calculated α_0 and α_1 have positive values; and (2) both the calculated and experimental α_0 and α_1 values for oligostyrenes are higher than the corresponding values for the other series.

Gradient elution chromatography

Gradient elution reversed-phase liquid chromatography is well suited to improving the separation of homologous series, where approximately equal distances between the neighbouring peaks can be easily achieved. The situation is not so simple for oligomeric series, where the dependence of retention and selectivity on mobile phase composition is very important.

In the oligostyrene series (Table II), the absolute retention, selectivity and their concentration dependences are greater than in homologous series. Generally, the retention volumes in gradient elution chromatography decrease with increasing initial concentration A of the organic solvent in the mobile phase at the start of the gradient and with increasing product mB [B is the slope of the gradient expressed as the change in φ , in % (v/v) $\cdot 10^{-2}$, per unit volume of the eluate]⁴¹. This implies that low values of B are required to compensate for large m and a relatively high A should be used in gradient elution separations of oligostyrenes in order to keep the separation time within reasonable limits. Fig. 8 shows the reversed-phase separation of a sample of

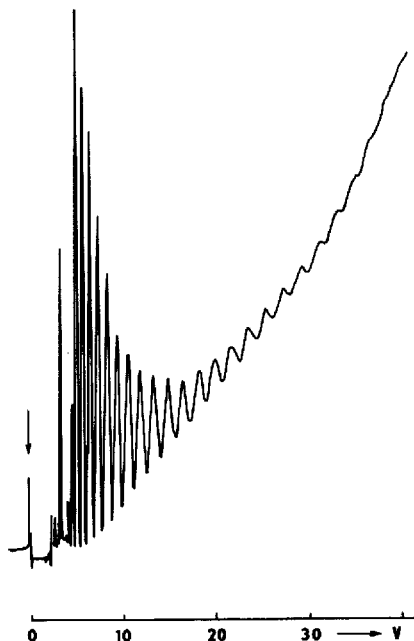


Fig. 8. Separation of a polystyrene standard (MW 2350) using gradient elution with linear gradient from 83 to 90% of 1,4-dioxane in water in 2 h. Other conditions as in Fig. 2.

oligostyrenes using such a shallow gradient. The improvement in the separation in comparison with isocratic conditions (Fig. 2) is not as significant as is usual in reversed-phase gradient elution separations of homologous series, for two reasons: (1) shallow gradients cause less band compression than steeper gradients and (2) restricted diffusion within the pores of the column packing and possible effects of molecular conformation contribute to additional band broadening of the large molecules of higher oligomers⁴².

Gradient elution chromatography is suitable for improving the separation of oligoethylene glycols, but it is incompatible with refractometric detection and UV detection at 185 nm³⁵ requires the use of very pure solvents as mobile phase components.

The retention of ethoxylated nonylphenols is considerably higher than that of oligoethylene glycols under identical conditions and, as expected, it decreases with increasing concentration of the organic solvent (propanol) in the mobile phase. However, as α_0 is negative and α_1 is positive (Table V), the selectivity decreases slightly with decreasing concentration of the organic solvent in the mobile phase, in contrast to the other series studied (see Fig. 3). A decrease in selectivity with decreasing elution strength of the mobile phase has an important consequence for gradient elution separations of ethoxylated nonylphenols. Generally, a decrease in the gradient slope or initial concentration of the stronger eluting component in the mobile phase leads to increased selectivity. However, a decreased gradient slope and initial concentration of propanol cause an increase in the elution volumes of the ethoxylated nonylphenols, but also a slightly impaired separation selectivity and resolution of the individual oligomers (Fig. 9B in comparison with Fig. 9A).

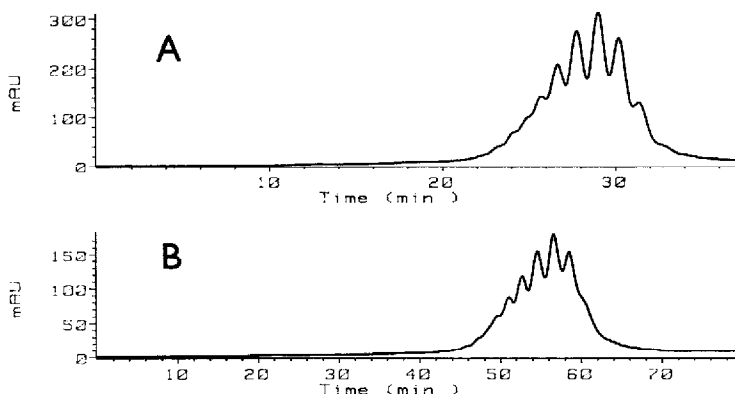


Fig. 9. Gradient elution separation of Serdcox NNP 4 using linear gradients (A) from 35 to 65% 2-propanol in water in 1 h and (B) from 30 to 60% 2-propanol in water in 2 h. Other conditions as in Fig. 4.

Chromatographic behaviour of non-sulphated and sulphated oligoethylene glycol nonylphenyl ethers in mobile phases containing CTAB

Sulphated oligoethylene glycol nonylphenyl ethers are commonly used anionic surfactants prepared by sulphation of oligoethylene glycols. As the sulphated oligomers contain strongly acidic groups, they are eluted in a single non-resolved peak

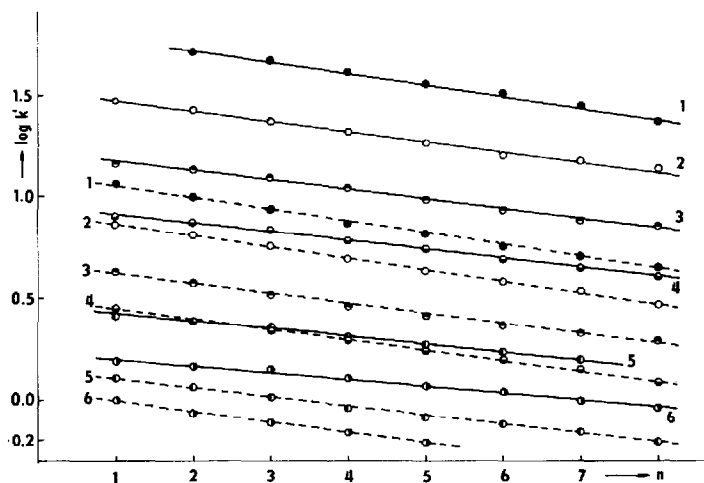


Fig. 10. Dependence of retention (k') of non-sulphated (broken lines) and sulphated (full lines) oligoethylene glycol nonylphenyl ethers on the number of structural repeat units, n , in mobile phases containing 0.04 M CTAB with different ratios of 2-propanol to water: (1) 35:65; (2) 40:60; (3) 45:55; (4) 50:50; (5) 60:40; (6) 65:35. Column: Silasorb C_{18} SPH, 7.5 μm (300×3.6 mm I.D.).

near the column dead volume in reversed-phase systems using mobile phases composed of an organic solvent and water. Addition of an ion-pairing reagent such as CTAB to the mobile phase increases the retention and the individual oligomers can be separated. In propanol–water mobile phases containing CTAB, the retention of both sulphated and non-sulphated ethers decreases linearly with increasing concentration of propanol in the mobile phase and with increasing number of repeat structural units, as in the chromatography of oligoethylene glycol nonylphenyl ethers in mobile phases without CTAB (Fig. 10). The retention of a sulphated ether is always considerably higher than that of the corresponding non-sulphated ether, which can be utilized for the separation of the two groups of compounds (substrates and reaction products) in a single run⁴⁰ (Fig. 11).

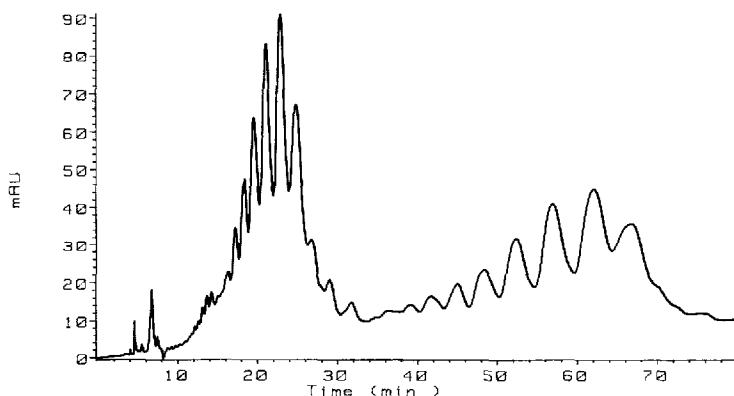


Fig. 11. Separation of partially sulphated Serdox NNP 4 in 2-propanol–water (45:55) + 0.04 M CTAB. Other conditions as in Fig. 4. The first group of peaks are non-sulphated oligoethylene glycol nonylphenyl ethers and the second group of peaks are oligomers in the sulphated product.

Even though the retention behaviour and separation selectivity of the non-sulphated ethers on C_{18} and C_8 columns in mobile phases containing 0.04 M CTAB were similar to those in mobile phases without CTAB, some differences are apparent (Table VI): the constants α_1 (m_1) are negative in CTAB-containing mobile phases and positive in mobile phases without CTAB, whereas the opposite holds true for the constants p . The decrease in n_{cc} with increasing number of repeat structural units, n , is more significant and q_i values are higher in CTAB-containing mobile phases. This means that the separation selectivity improves slightly with decreasing concentration of propanol in the mobile phase, in contrast to mobile phases without CTAB, but this difference is not very significant in practice. The influence of 0.04 M CTAB in the mobile phase on the retention and selectivity of the separation of the non-sulphated ethers is of only minor importance, but it might be useful to investigate the behaviour of non-ionic surfactants in CTAB-containing mobile phases in more detail.

The sulphated oligoethylene glycol nonylphenyl ethers are retained much more strongly and their separation selectivity is similar to, but slightly lower than, that of the non-sulphated ethers (Table VII). The contributions of the structural residue to both the hydrophobic (n_{cc0}) and polar (q_{0i}) selectivities are higher with the sulphated ethers whereas the decrease in n_{cc} with the number of repeat structural units, n , (Δn_{cc}) is similar and q_i is almost independent of n for both series. The larger q_{0i} for sulphated

TABLE VI

EXPERIMENTAL VALUES OF THE PARAMETERS α_0 , α_1 , β_0 , β_1 , a_0 , a_1 , m_0 , m_1 , q , p , n_{cc0} , Δn_{cc} , q_{0i} AND Δq_i IN EQNS. 6-9, 15, 25 AND 26 FOR ETHOXYLATED NONYLPHENOLS ON C_{18} AND C_8 COLUMNS IN 2-PROPANOL-WATER MOBILE PHASES CONTAINING CTAB, CALCULATED USING LINEAR REGRESSION OF THE EXPERIMENTAL DATA ACCORDING TO THE EQUATIONS

R = correlation coefficient.

Column: Silasorb C_{18} SPH, 7.5 μ m (300 \times 3.6 mm I.D.); Column: Silasorb C_8 SPH, 10 μ m (300 \times 4.2 mm I.D.);
Mobile phase: 2-propanol-water + 0.04 M CTAB; Mobile phase: 2-propanol-water + 0.04 M CTAB;
 $n = 1-7$ $n = 2-7$:

2-Propanol (% v/v)	Log β	Log α	R	2-Propanol (% v/v)	Log β	Log α	R
35	1.090	-0.055	0.9962	30	1.100	-0.051	0.9958
40	0.894	-0.050	0.9942	35	0.851	-0.047	0.9979
45	0.670	-0.049	0.9969	40	0.636	-0.042	0.9986
50	0.489	-0.046	0.9968	45	0.436	-0.041	0.9987

$$\text{Log } \alpha = -0.074 + 0.056\phi;$$

$$\text{Log } \beta = 2.514 - 4.066\phi;$$

$$a = 2.258 - 0.076n;$$

$$m = 3.523 - 0.054n;$$

$$m = 1.908 + 0.716a;$$

$$n_{cc} = 3.93 - 0.31n;$$

$$q_i = 1.48 + 0.02n;$$

$$R = 0.9661$$

$$R = 0.9993$$

$$R = 0.9983$$

$$R = 0.9911$$

$$R = 0.9953$$

$$R = 0.9983$$

$$R = 0.9503$$

$$\text{Log } \alpha = -0.071 + 0.070\phi;$$

$$\text{Log } \beta = 2.411 - 4.414\phi;$$

$$a = 2.335 - 0.070n;$$

$$m = 4.247 - 0.066n;$$

$$m = 2.046 + 0.942a;$$

$$n_{cc} = 4.54 - 0.33n;$$

$$q_i = 1.71 + 0.01n;$$

$$R = 0.9726$$

$$R = 0.9987$$

$$R = 0.9999$$

$$R = 0.9970$$

$$R = 0.9970$$

$$R = 0.9995$$

$$R = 0.9160$$

Calibration series of n -alkylbenzenes:

$$a_{0B} = 1.313; \quad a_{1B} = 0.238; \quad R = 0.9999$$

$$m_{0B} = 2.319; \quad m_{1B} = 0.213; \quad R = 0.9999$$

$$q_B = 1.147; \quad p_B = 0.893; \quad R = 0.9998$$

Calibration series of n -alkylbenzenes:

$$a_{0B} = 1.356; \quad a_{1B} = 0.217; \quad R = 0.9987$$

$$m_{0B} = 2.927; \quad m_{1B} = 0.236; \quad R = 0.9974$$

$$q_B = 1.454; \quad p_B = 1.086; \quad R = 0.9997$$

TABLE VII

EXPERIMENTAL VALUES OF THE PARAMETERS $\alpha_0, \alpha_1, \beta_0, \beta_1, a_0, a_1, m_0, m_1, q, p, n_{cc0}, \Delta n_{cc}, q_{0i}$ AND Δq_i IN EQNS. 6-9, 15, 25 AND 26 FOR SULPHATED ETHOXYLATED NONYLPHENOLS ON C_{18} AND C_8 COLUMNS IN 2-PROPANOL-WATER MOBILE PHASES CONTAINING CTAB, CALCULATED USING LINEAR REGRESSION OF THE EXPERIMENTAL DATA ACCORDING TO THE EQUATIONS

R = correlation coefficient.

Column: Silasorb C_{18} SPH, 7.5 μm (300 \times 3.6 mm I.D.); Column: Silasorb C_8 SPH, 10 μm (300 \times 4.2 mm I.D.);
Mobile phase: 2-propanol-water + 0.04 M CTAB; Mobile phase: 2-propanol-water + 0.04 M CTAB;
 $n = 1-7$; $n = 2-7$;

2-Propanol (% v/v)	Log β	Log α	R	2-Propanol (% v/v)	Log β	Log α	R
45	1.218	-0.047	0.9975	35	1.414	-0.042	0.9996
50	0.951	-0.043	0.9971	40	1.142	-0.042	0.9999
60	0.461	-0.038	0.9971	55	0.338	-0.033	0.9996
$\text{Log } \alpha = -0.073 + 0.059\varphi$; $R = 0.9921$ $\text{Log } \beta = 3.471 - 5.022\varphi$; $R = 0.9998$ $a = 3.471 - 0.073n$; $R = 0.9972$ $m = 5.022 - 0.059n$; $R = 0.9948$ $m = 2.233 + 0.803a$; $R = 0.9986$ $n_{cc} = 9.07 - 0.31n$; $R = 0.9972$ $q_i = 1.92 + 0.01n$; $R = 0.9089$				$\text{Log } \alpha = -0.058 + 0.045\varphi$; $R = 0.9656$ $\text{Log } \beta = 3.295 - 5.377\varphi$; $R = 0.9999$ $a = 3.421 - 0.060n$; $R = 0.9990$ $m = 5.712 - 0.050n$; $R = 0.9913$ $m = 2.881 + 0.828a$; $R = 0.9960$ $n_{cc} = 9.19 - 0.22n$; $R = 0.9634$ $q_i = 1.91 + 0.03n$; $R = 0.8720$			
<i>Calibration series of n-alkylbenzenes</i> $a_{0B} = 1.313$; $a_{1B} = 0.238$; $R = 0.9999$ $m_{0B} = 2.319$; $m_{1B} = 0.213$; $R = 0.9999$ $q_B = 1.147$; $p_B = 0.893$; $R = 0.9998$				<i>Calibration series of n-alkylbenzenes:</i> $a_{0B} = 1.356$; $a_{1B} = 0.217$; $R = 0.9987$ $m_{0B} = 2.927$; $m_{1B} = 0.236$; $R = 0.9974$ $q_B = 1.454$; $p_B = 1.086$; $R = 0.9997$			

ethers may be attributed to the presence of the sulphuric acid ester end-group and larger n_{cc0} values (equivalent to about five methylene units) to the formation of ion pairs between the sulphated ethers and CTAB. Hence the mobile phase composition and structural effects on the retention of compounds such as the sulphated oligoethylene glycol nonylphenyl ethers in mobile phases containing CTAB can be explained in a closely analogous manner to the reversed-phase mechanism of the retention of the non-sulphated oligoethylene glycol nonylphenyl ethers.

CONCLUSIONS

In reversed-phase chromatography, the retention of oligomeric series as a function of the number of repeat structural units and of the mobile phase composition can be described formally by the same set of equations as the retention in homologous series. The constants of these equations have different values, depending on the structures of the oligomeric repeat unit and of the structural residue in the molecule. The retention decreases with increasing concentration of the organic solvent in the mobile phase, φ , and the $\log k' - \varphi$ plots are linear at least over a limited range of mobile phase compositions. The dependences of $\log k'$ on the number of repeat structural units, n , at different φ are also linear for a more or less limited number of oligomers.

The selectivity of separation for a pair of neighbouring oligomers is constant in an oligomeric series under given separation conditions and depends on the contributions of both the repeat structural units and the structural residue to the lipophilic and polar selectivity, characterized by the indices n_{ce0} , Δn_{ci} , q_{0i} and Δq_j . If a repeat structural unit is non-polar, the retention increases with n , but if it contributes to both the size and polarity of oligomer molecules, the retention may, under certain circumstances, even decrease with n , such as in the oligoethylene glycol nonylphenyl ether series. This behaviour depends also on the concentration and type of the organic solvent in the mobile phase and can be explained and predicted, at least qualitatively, from the molar volumes and interaction indices of repeat units and the structural residue, using the theoretical model of interaction indices.

Gradient elution reversed-phase chromatography significantly improves the separation of homologous series and it usually also improves the separation of oligomeric series with relatively non-polar oligomeric units; the individual oligomers of such series are relatively strongly retained and have large values of m in eqn. 5a. Relatively shallow gradients are usually required for separation of these oligomeric series, such as the lower oligostyrenes. However, the application of gradient elution may have little advantage in the separation of oligomeric series, where the structural repeat unit is relatively polar and the structural residue is relatively large and non-polar. In such series, the separation selectivity may not change significantly or may even decrease with decreasing elution strength of the mobile phase, such as in the reversed-phase chromatography of ethoxylated nonylphenols, where the application of gradient elution is not successful.

In mobile phases containing CTAB, the retention behaviour of non-sulphated ethoxylated nonylphenols is similar to that in mobile phases without CTAB, but the presence of CTAB may influence the selectivity and its dependence on concentration to a certain extent. Anionic oligomers, such as sulphated ethoxylated nonylphenols, form ion pairs with CTAB, which leads to a very significant increase in retention. The separation selectivity for the neighbouring sulphated oligomers and the dependence of selectivity on mobile phase composition and on the number of repeat structural units are similar to those for the non-sulphated oligomers. In propanol-water mobile phases containing CTAB, the separation of the individual oligomers in both non-sulphated and sulphated ethoxylated nonylphenols may be achieved in a single chromatographic run.

SYMBOLS

a	extrapolated $\log k'$ in pure water as the mobile phase, <i>i.e.</i> , the intercept in eqns. 5 and 5a;
a_0	a of the structural residue in a homologous or oligomeric series, <i>i.e.</i> , the intercept in eqn. 6;
a_{0B}	a_0 for the calibration n -alkylbenzene homologous series;
a_1	slope of the a versus n relationship (eqn. 6);
a_{1B}	a_1 for the calibration n -alkylbenzene homologous series;
c_1	temperature- and organic solvent-dependent constant in eqns. 10–13 (eqn. 14);

c_M	temperature- and organic solvent-dependent constant in eqns. 3 and 10–13;
c_x	temperature- and solute-dependent constant in eqns. 3 and 10–13;
d	second-power term constant in eqn. 5;
k'	capacity factor of solute;
m	first-power term constant in eqns. 5 and 5a, <i>i.e.</i> , the slope of the log k' versus φ relationship, equivalent to the term S introduced by Snyder ⁴³ ;
m_0	m of the structural residue in a homologous or oligomeric series (intercept in eqn. 7);
m_1	slope of the m versus n relationship, <i>i.e.</i> , contribution of one repeat structural (oligomeric or homologous) unit to m (eqn. 7);
n	number of repeat structural units in a given homologue or oligomer;
n_{ce}	lipophilic structural index, <i>i.e.</i> , the number of methylene groups in a hypothetical member of the calibration homologous n -alkylbenzene series with the same retention as the solute in water (eqn. 24);
n_{ceo}	n_{ce} of the structural residue in an oligomeric series (eqn. 25);
Δn_{ce}	contribution of one oligomeric unit to n_{ce} in a given series (eqn. 25);
p	slope of the linear dependence of m on a , applying for a given homologous or oligomeric series and organic solvent (eqn. 15);
p_B	p applying for n -alkylbenzene homologous calibration series;
q	intercept of the linear dependence of m on a , applying for a given homologous or oligomeric series and organic solvent in the mobile phase (eqn. 15);
q_i	polar structural index, characterizing the magnitude of the polar interactions of the solute with the mobile phase (eqn. 24);
q_{0i}	q_i of the structural residue in an oligomeric series (eqn. 26);
Δq_i	contribution of one oligomeric unit to n_{qi} in a given series (eqn. 26);
A	concentration of the stronger eluting solvent in a binary mobile phase at the start of gradient elution;
B	gradient slope, <i>i.e.</i> , the change in φ [% (v/v) · 10 ⁻²] per unit volume (1 cm ³) of the eluate during gradient elution;
I	interaction index as a quantitative measure of polarity ⁸ ;
I_{H_2O}	I of water;
I_{org}	I of the organic solvent in a binary mobile phase;
I_x	I of solute;
I_{0x}	I_x of the structural residue in a given homologous or oligomeric series (eqn. 1);
ΔI_x	contribution of one repeat structural (homologous or oligomeric) unit to I_x (eqn. 1);
K_1, K_2, K_3	temperature- and organic solvent-dependent constants in eqns. 20 and 21;
Q	combined structural parameter in a given homologous or oligomeric series (eqn. 18);
R	gas constant;
T	absolute temperature;
V_M	volume of the mobile phase in the column;
V_S	volume of the stationary phase in the column;
V_x	molar volume of solute (cm ³ mol ⁻¹ · 10 ⁻²);

V_{0x}	V_x of the structural residue in a given homologous or oligomeric series (eqn. 2);
ΔV_x	contribution of one repeat structural (homologous or oligomeric) unit to V_x (eqn. 2);
α	relative retention, <i>i.e.</i> , selectivity in a given homologous or oligomeric series, the ratio of k' of the neighbouring members (eqn. 4);
α_0	selectivity constant, <i>i.e.</i> , intercept of $\log \alpha$ versus φ relationship (eqns. 9 and 12);
α_1	selectivity constant, <i>i.e.</i> , slope of $\log \alpha$ versus φ relationship (eqns. 9 and 13);
β	$\log k'$ of the zeroth member of a given homologous or oligomeric series (eqn. 4);
β_0	intercept of $\log \beta$ versus φ relationship (eqns. 8 and 10);
β_1	slope of $\log \beta$ versus φ relationship (eqns. 8 and 11);
γ	second-power term constant in eqn. 4;
φ	concentration of the organic solvent in the mobile phase [$\%(\text{v/v}) \cdot 10^{-2}$];
φ_0	φ at which the selectivity $\alpha = 0$;
Φ	phase ratio in column, $\Phi = V_S/V_M$.

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