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MOBILE PHASE EFFECTS IN REVERSED-PHASE CHROMATOGRAPHY

III. CHANGES IN CONFORMATION AND RETENTION OF OLIGO-(ETHYLENE GLYCOL) DERIVATIVES WITH TEMPERATURE AND ELUENT COMPOSITION

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SUMMARY

The retention behavior of homologous oligo(ethylene glycol)s and their derivatives on octadecyl-silica with aqueous eluents was investigated under a wide range of conditions as far as the chemical nature and concentration of organic co-solvent as well as the temperature are concerned. The data revealed numerous irregularities concerning the dependence of retention factors on temperature, eluent composition and the carbon number of the elute molecules. The observed behavior is explained by postulating that each elute can have the extended zigzag or the compact meandering conformation, that the two conformers of an elute have significantly different retention factors in the same chromatographic system and that the relative concentration of the conformers, *i.e.*, the magnitude of the equilibrium constant for the transition, depends on the eluent composition, temperature and the number of ethylene oxide residues in the molecule. The two-state theory allows a separate treatment of the effect of temperature and retention on the equilibrium between the conformers and the predictions of the theory are in qualitative agreement with the experimental results. Thus secondary equilibria which do not involve a complexing agent, but the solvent proper, can give rise to non-linear Van 't Hoff plots, to increase of retention with temperature, to retention decreasing with the carbon number of homologues and to non-linear dependence of the logarithm of retention factor on the volume percent of organic co-solvent in aqueous eluents commonly used in reversed-phase chromatography. Similar phenomena are likely to be encountered in high-performance liquid chromatography when complex molecules, which can exist in two or more significantly different conformations, are chromatographed. Thus, the results of the present work not only can be useful to solve various analytical problems involved in the separation and identification of compounds having a poly(ethylene glycol) moiety, but also can serve as model for study of the chromatographic behavior of biological substances like peptides and oligonucleotides.

INTRODUCTION

Recognizing that the versatility and selectivity of reversed-phase chromatography (RPC) largely depends on mobile phase effects, in the first communication of this series¹ we examined the effect of solvent composition and temperature on retention under regular conditions, *i.e.*, in the absence of secondary equilibria. The second communication², however, demonstrated that the interaction of ionizable elutes with buffer species and the multifarious temperature dependence of protonic equilibria in the eluent can give rise to "irregularities" that, unless understood completely, may preclude prediction of retention behavior.

The present work is an attempt to demonstrate that irregular retention behavior can arise not only from secondary equilibria entailing complexation of the elute but also from solvent or temperature mediated conformational changes of the elute. Whereas such behavior is likely to be observed with many solute molecules having complex molecular structure we have chosen homologous series of oligo(ethylene glycol) derivatives not only because the compounds are known to have two conformations but also because the study of the behavior of a homologous series can give much deeper insight into the physico-chemical phenomena involved than that of a single substance.

Furthermore poly(ethylene glycol)s and their alkyl and aromatic ethers are widely used as non-ionic detergents and surfactants in formulations. The analysis of these materials is important, in part, because "these various [detergent] products all contribute to present-day pollution problems and this aspect now constitutes an important part of the field of detergent analysis"³.

RPC of oligo(ethylene glycol) and its derivatives has received only little attention⁴. The usual method of chromatographic analysis is size exclusion⁵⁻⁷ or high-performance liquid chromatography (HPLC) with polar sorbents^{8,9}. The combination of size exclusion chromatography and infrared spectroscopy allows detection of polyoxyethylene surfactants⁷ at 0.05-0.1 ppm.

On the other hand the physico-chemical properties of this type of compounds have been subjects of a great deal of study. Structural characteristics of the ethoxy chain have been studied in oligo(ethylene glycol) and its derivatives neat and in solution. Poly(ethylene glycol) has been examined by numerous spectroscopic methods, including X-ray, infrared, nuclear magnetic resonance (NMR), and Raman¹⁰⁻¹³. As a result of X-ray investigations the existence of two conformers, called zigzag and meander has been demonstrated^{10,11}. Results obtained from Raman spectra of poly(ethylene glycol) have shown the existence of those forms, but also another intermediate form^{13,14}. Both methods indicate that at a high and low degree of polymerization of the poly(ethylene glycol) the chain assumes the compact dihedral helical structure, meandering form, and an extended open coil, zigzag form, respectively. The transition from extended to compact conformer takes place when the number of ethoxy groups reaches nine in the solute molecule in solution¹¹. The thermal dependence of solubility and adsorption is quite unusual for these species. For instance, the binding of non-ionic surfactants to a variety of surfaces is found to increase with temperature¹⁵⁻¹⁷.

In this study the effect of temperature, solvent composition and number of ethoxy groups in the polymer on retention are investigated by RPC. On the one hand,

the results of such a study may lead to a greater understanding of the physico-chemical behavior of poly(ethylene glycol)s and related compounds. On the other, the observed irregular chromatographic retention patterns caused by conformation changes can produce insights into the chromatographic behavior of large biological molecules such as peptides, proteins and polynucleotides which are also found to exist in more than one conformation.

THEORETICAL

Conformational changes

The "meandering" and "zigzag" conformations of poly(ethylene glycol)s are depicted in Fig. 1. Short oligo(ethylene glycol) chains prefer the zigzag form whereas compounds having a high degree of ethylene oxide polymerization are in the meandering form. The degree of polymerization where the transition occurs is a function of the experimental conditions and may be dependent on the hydrophobic head group in a detergent. For oligo(ethylene glycol) in the zigzag chain the length and diameter

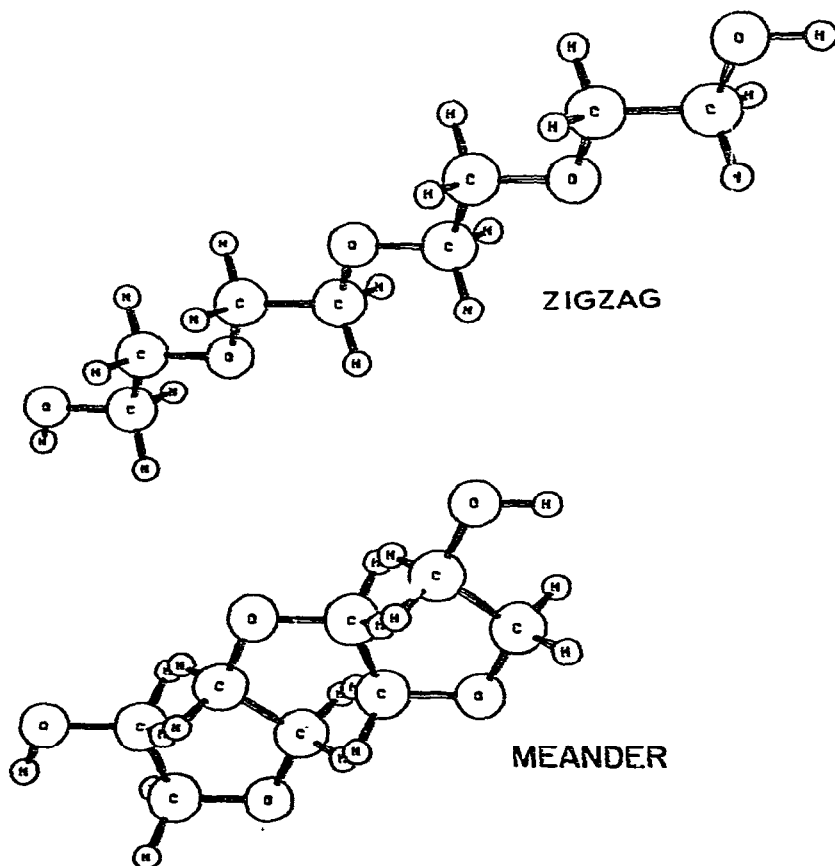
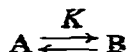


Fig. 1. Perspective views of the zigzag (upper) and meandering (lower) forms of tetra(ethylene glycol) drawn with bond angles and pitch distances given in ref. 10. The figure shows that the zigzag structure is a much more extended form.

of one ethoxy unit, considered as a cylinder aligned along the molecular axis, are 3.5 Å and 2.5 Å, respectively; the corresponding values in the meandering chain are 2 Å and 4 Å (ref. 10). As a consequence, a conformation change from the zigzag to the meandering form decreases the molecular surface area.

Effect of temperature

The simplest case in which secondary equilibria can lead to irregular retention behavior is the isomerization of elute. Consider the reaction



where A and B are isomers in equilibrium described by the equilibrium constant K . Whereas this expression is general, A and B could represent the zigzag and meandering forms in our case. If both forms can bind to a stationary phase the observed retention factor, k , can be written as

$$k = \frac{k_A + Kk_B}{1 + K} \quad (1)$$

where k_A and k_B are the limiting retention factors of pure A and B, respectively.

The temperature dependence of the above parameters can be expressed² by

$$k_A = \varphi \exp(-\Delta H_1^0/RT) \exp(\Delta S_1^0/R) \quad (2a)$$

$$k_B = \varphi \exp(-\Delta H_2^0/RT) \exp(\Delta S_2^0/R) \quad (2b)$$

$$K = \exp(-\Delta H_3^0/RT) \exp(\Delta S_3^0/R) \quad (2c)$$

where ΔH_1^0 , ΔH_2^0 and ΔH_3^0 are the standard enthalpies of binding of A and B and the enthalpy of the isomerization and ΔS_1^0 , ΔS_2^0 and ΔS_3^0 are the corresponding entropy changes. The phase ratio is φ .

The enthalpy observed at any temperature can be obtained by combining eqns. 1-2c and differentiating with respect to reciprocal temperature². The result is given by

$$\begin{aligned} \Delta H_r^0 = & \frac{k_A \Delta H_1^0}{(k_A + k_B K)} + \frac{k_B K \Delta H_2^0}{(k_A + k_B K)} \\ & + \frac{K(k_A - k_B) \Delta H_3^0}{(k_A + k_B K)(1 + K)} \end{aligned} \quad (3)$$

where ΔH_r^0 is the retention enthalpy characteristic for the chromatographic process including secondary equilibria² at temperature T .

Three conclusions can be drawn from eqn. 3. First, the enthalpy observed is a weighted mean of enthalpies associated with each process if secondary equilibria occur in the mobile phase. This result is general and true for equilibria more complex than those of this example². A second conclusion is that Van 't Hoff plots are non-linear unless one works under conditions such that the formation of all isomers but

one is suppressed, or a most felicitous combination of enthalpies and entropies occurs. The third conclusion is that changes in the sign of the enthalpy may be seen. This can be demonstrated by a simple example. Suppose the enthalpy of binding of each form is identical but the entropies differ in such a fashion that the ratio of the capacity factors k_A/k_B is five. Suppose, furthermore, that the enthalpy for isomerization has the opposite sign of that for binding and is three times greater. Then at a temperature at which the equilibrium constant is one, the enthalpy will be zero and at temperatures above and below this temperature, retention enthalpies will have opposite signs.

Effect of solvent composition

Inspection of eqn. 1 shows that changes in solvent composition may affect retention by changing the retention factors of each of the two forms, k_A and k_B and the equilibrium constant K . In RPC retention factors often decrease exponentially with the volume fraction, φ , of the organic co-solvent in hydro-organic eluents. Thus the dependence of the individual retention factors on solvent composition can be expressed as

$$k_A = k_{A,0} \exp(-s_A\varphi) \quad (4a)$$

$$k_B = k_{B,0} \exp(-s_B\varphi) \quad (4b)$$

where the parameters $k_{A,0}$ and $k_{B,0}$ are the retardation factors of the two forms in water and the slopes of the corresponding $\ln k$ vs. φ plots are given by s_A and s_B .

The effect of solvent composition on the equilibrium constant of the isomerization can be conveniently treated by assuming that the conformation change is accompanied by solvation according to the scheme



where S represents solvent molecules and BS_n is the conformer solvated by n solvent molecules. One should note that this formulation can be used also if A is the more highly solvated species, but in this case n would be negative.

As both forms, A and BS_n , can bind to the stationary phase the retention factor can be expressed as:

$$k = \frac{k_{A,0} \exp(-s_A\varphi) + k_{B,0} \exp(-s_B\varphi) K[S]^n}{1 + K[S]^n} \quad (5)$$

Eqn. 5 implies non-linear plots of the logarithms of the retention factor *versus* the volume percent of organic co-solvent. At solvent compositions where the A form is dominant, the limiting slope is given by $-s_A$ whereas in the regime of dominant B form, the limiting slope is $-s_B$. Where A and B are present in approximately equal concentrations, the term $K[S]^n$ is of the order of one and the retardation factor takes values intermediate between the two limits. Consequently, the plots may manifest marked deviations from linearity.

Effect of chain length

Eqn. 1, which expresses the retention factor of molecules, which exist in two states under chromatographic conditions, does not have an explicit dependence on the number of ethyleneoxy groups in the molecule. However, there is ample experimental evidence that in RPC the logarithm of the retention factors for a series of homologues is linear in the number of structural units such as methylene groups¹⁸ or the amino acid units in oligomers of alanine¹⁹ and glutamic acid²⁰. We may, therefore, assume that this behavior is manifested also by oligo(ethylene glycol) derivatives so that the retention factors of the A and B forms can be expressed by

$$k_A = k_D \alpha_A^{N_{EO}} \quad (6a)$$

$$k_B = k_D \alpha_B^{N_{EO}} \quad (6b)$$

where k_D is the retention factor of the parent molecule, for instance *tert.*-octylphenol or nonylphenol in the present study, and α_A and α_B measure the increase in the retention factor due to addition of one ethyleneoxy (EO) group to conformers A and B, respectively. The number of EO residues in the molecule is given by N_{EO} . According to eqn. 6a and 6b, the α values can be regarded as the selectivity for EO group in this type of molecules and, as it will be shown later, α is a function of the length of the poly(ethylene glycol) chain.

The equilibrium constant for the conformational change, K , is expected to depend on the length of the poly(ethylene glycol) chain in the molecule as Raman studies have shown that oligo(ethylene glycol) exists in the more extended form in water if the number of ethylene residues is three or less, whereas the compact form is found in higher oligomers¹⁴. These findings suggest that we may assume that the equilibrium constant depends on the number of the EO units according to

$$K = K_0 \exp[(N_{EO} - \bar{N})S] \quad (7)$$

where \bar{N} is the number of EO units in the molecule for which the probabilities of existence in either A or B form are nearly equal and the value of K_0 is close to unity. The effect of the solvent composition on K is expressed by the magnitude of the solvent parameter S , which serves as a scaling factor as well.

Eqns. 1 and 6a-7 can be combined to determine a relationship between retention factor and chain length. The result is

$$k_N = \frac{k_D \alpha_A^N + k_D \alpha_B^N K_0 \exp[(N_{EO} - \bar{N})S]}{1 + K_0 \exp[(N_{EO} - \bar{N})S]} \quad (8)$$

where k_N is the retention factor of an elute having N_{EO} residues in the oligo(ethylene glycol) moiety. According to eqn. 8 the slopes of $\ln k$ vs. N_{EO} plots are positive when both α_A and α_B are greater than unity. However, the slope can be negative when either α_A or α_B are smaller than unity in agreement with eqn. 6a and 6b.

The selectivity of the chromatographic system toward EO structural units, α_{EO} , is conveniently defined as:

$$\alpha_{EO} = \frac{k_{(N+1)EO}}{k_{NEO}} \quad (9)$$

From eqn. 8 it follows that the magnitude of α_{EO} depends on N_{EO} and the six parameters. If the lower and higher members of a homologous series have different conformations in the eluent used for their chromatographic separation, then a plot of α_{EO} against N_{EO} would be sigmoidal according to eqn. 8. On the other hand, α_{EO} is practically independent from N_{EO} when $N_{EO} \ll \bar{N}$ or S is a large negative number. Curvature is observed when the term $K_0 \exp [(N_{EO} - \bar{N}) S]$ is of the order of unity.

EXPERIMENTAL

Chromatography

A Model 100 (Altex, Berkeley, Calif., U.S.A.) HPLC solvent metering system with a Model 7010 (Rheodyne, Berkeley, Calif., U.S.A.) sample valve with a 20- μ l injection loop and a Kratos-Schoeffel (Westwood, N.J., U.S.A.) Model 770 variable-wavelength UV detector were used. Alkylaryl-oligo(ethylene glycol)s and oligo(ethylene glycol)s were detected at 273 and 200 nm, respectively. Chromatograms were obtained with a Perkin-Elmer (Norwalk, Conn., U.S.A.) Model 56 recorder. A 5- μ m Zorbax ODS column (250 \times 4.6 mm I.D.) (DuPont, Wilmington, Del., U.S.A.) was used in the study of temperature effects. The column temperature was controlled by water circulation through an insulated stainless-steel jacket from Model K-2/R thermostatted water-bath (Messgeraetewerk, Lauda, G.F.R.).

In the study of solvent effects on retention the eluent flow generating and controlling unit consisted of two Altex Model 100A HPLC solvent pumps and an Altex Model 420 system controller. A Rheodyne Model 7010 sample valve with a 20- μ l loop was used for sample introduction. A Perkin-Elmer Model LC-55 variable-wavelength UV detector at 273 nm or at 200 nm was used with a Sargent-Welch (Skokie, Ill., U.S.A.) Model SRG recorder to obtain chromatograms. A 7- μ m LiChrosorb KP-8 (250 \times 4.6 mm I.D.) (Knauer, Berlin, G.F.R.) and 5- μ m DuPont Zorbax ODS (250 \times 4.6 mm I.D.) were used in this study in addition to a column (150 \times 4.6 mm I.D.), packed with 5- μ m Partisil (Whatman, Clifton, N.J., U.S.A.) which had been reacted with dimethyloctadecylchlorosilane and capped with trimethylsilyl functions. The carbon load of the octadecyl-silica thus obtained was 16.5%.

NMR

The base-catalyzed addition of ethylene oxide to alkylphenols is the most common method of preparation of alkylaryl-oligo(ethylene glycol)s and leads to a product which consists of molecules having different numbers of EO units²¹. For NMR studies a molecularly homogenous alkylaryl-oligo(ethylene glycol) was obtained by collecting a single peak from chromatographic separation of such products on a

reversed-phase column. Phenyl-oligo(ethylene glycol)s present in commercial samples of non-ionic detergents were separated on a 7- μm LiChrosorb RP-8 prep-column (Knauer) (250 \times 8 mm) and 5- μm Zorbax ODS (DuPont) (250 \times 4.6 mm) column by using acetonitrile-water (15:85) and tetrahydrofuran-water (40:60) as the mobile phase, respectively. Sample loops of 200 and 20 μl were used on a Rheodyne Model 7010 injection valve with LiChrosorb RP-8 and Zorbax ODS columns, respectively.

The procedure was repeated successively until a total of 2–4 mg of the sample was collected. Thereafter the samples were dried under nitrogen and redissolved in perdeuterated acetone for NMR analysis by $^1\text{H-NMR}$ at 270 MHz in FT mode at the Southern New England High Field NMR Facility. The ratio of the integrated peak intensities due to protons in the oligo-EO-chain to that of aromatic ring protons was used to establish the number of EO units per phenol moiety in the sample molecule.

$^{13}\text{C-NMR}$ at 67.9 MHz in FT mode was run on unchromatographed samples to establish the position of alkyl substitution on the ring relative to the phenolic oxygen. In order to determine the structure of the major alkylaryl-oligo(ethylene glycol) present in the commercial non-ionic surfactant samples NMR spectroscopy was used.

Differential scanning calorimetry

In a Perkin-Elmer Model DSC-2 differential scanning calorimeter 10 μl of sample was scanned in a sealed cell at a rate of 10 $^\circ\text{C}/\text{min}$ and the integral heat of adsorption was recorded.

Theoretical curves to model the effect of temperature in chromatographic retention were calculated from eqn. 1 by using the following parameter values: $\Delta H_1 = \Delta H_2 = -3000$ cal/mol; $k_{1,0} = 1/1000$; $k_{2,0} = 1/10$. Various values of ΔH_3° were assumed and in each case the value of ΔS_3° was obtained from the relationship: $\Delta S_3^\circ = \Delta H_3^\circ/316$. All calculations were done on a PDP 11/10 computer in BASIC language and the output was graphed on Tektronix (Beaverton, Ore., U.S.A.) No. 4662 Interactive Digital Plotter.

Gas chromatography-mass spectrometry (GC-MS)

The mass number of different oligo(ethylene glycol)s in Carbowax 400 was determined by using a Perkin-Elmer RNU-6 mass spectrometer interfaced with a Perkin-Elmer Model 990 gas chromatograph. Oligomers of polyethoxy (PEO)-octylphenol were separated on a Sigma III gas chromatograph (Perkin-Elmer) using an OV-101 column and their empirical formulae were calculated from the mass ratios determined by using an Applied Chromatographic System MPD-850 helium microwave plasma detector (Kratos). The data allowed the number of EO groups in the molecule to be determined.

The number of EO units in higher oligomers of PEO-octylphenol was established from the chromatographic behavior of pauci-disperse samples which differed in the mean number of EO units incorporated per mole. In samples which differed by a few ethylene oxide residues, peaks were observed to overlap and therefore the method could be used to establish the number of EO groups. The values obtained for the lower members agreed with values obtained by NMR, GC-MS and GC-microwave plasma detector (MPD) measurements.

Materials

Non-ionic surfactants, PEO-nonylphenol, *i.e.*, nonylphenoxy-poly(ethoxy-ethanol), and PEO-octylphenol were purchased from Chem Service (Media, Pa., U.S.A.). The samples consisted of a series of compounds having different PEO chain lengths. Poly(ethylene glycol) with three different molecular size distributions having average molecular weights of 200, 400 and 600 were purchased from Fisher Scientific (Fair Lawn, N.J., U.S.A.) under the trade name of Carbowax. Acetonitrile, tetrahydrofuran and methanol were "distilled in glass" from Burdick & Jackson Labs. (Muskegon, Mich., U.S.A.).

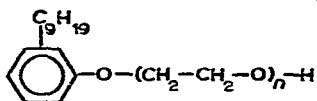
Retention factors have been evaluated from the chromatograms in the usual way¹⁹.

RESULTS AND DISCUSSION

Structure analysis of sample components

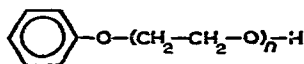
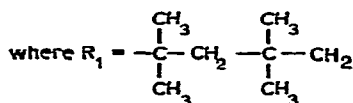
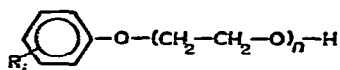
Commercial oligo(ethylene glycol) derivatives are composed of homologous substances differing from each other in the number of EO units. Variations in the structure and position of the alkyl moiety in alkylphenols used in the manufacture of non-ionic detergents give rise to a number of different homologous series.

In the present study the following types of compounds were investigated:

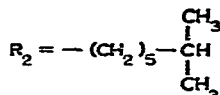


I. Oligo(ethylene) glycol

II. Nonylphenyl-oligo(ethylene glycol)



or



III. Octylphenyl-oligo(ethylene glycol)

IV. Phenyl-oligo(ethylene glycol)

RPC on octadecyl-silica by using gradient elution with acetonitrile-water mixtures over a wide composition range yielded one series of peaks with samples of Carbowax 200, 400 and 600, all of which have the general structure I. On the other hand several series of peaks have been found when commercial samples having the nominal structures of II and III were chromatographed. The first eluting series,

which contain substances having the general structure IV as will be seen later, makes up for an estimated 2–3% of the total sample. About 75% of the sample elutes in the second series which therefore contains the main product. Later eluting series represent minor fractions of the sample and are evidently composed of oligo(ethylene glycol) derivatives having a hydrocarbonaceous moiety more hydrophobic than that of the main product. Although RPC has been found suitable to separate such substances into groups according to non-polar moieties in the molecules, the resolution of homologues differing in EO units is often insufficient. On the other hand rechromatography of a homologous series on silica gel by using hexane containing 20–50% of an organic solvent such as tetrahydrofuran or methanol as the eluent often yields superior resolution of homologues. Therefore, the use of RPC appears to be preferable for separations according to the hydrocarbon moiety of the sample components, *i.e.*, for group separations, whereas chromatography on silica gel may be more advantageous to separate the numbers of homologous series which differ in the number of EO units. Nevertheless, RPC can also be used to obtain high resolution of homologues as shown in Fig. 2. According to our experience RPC yields high-resolution separation only for certain series in terms of the hydrophobic moiety and the average N_{EO} .

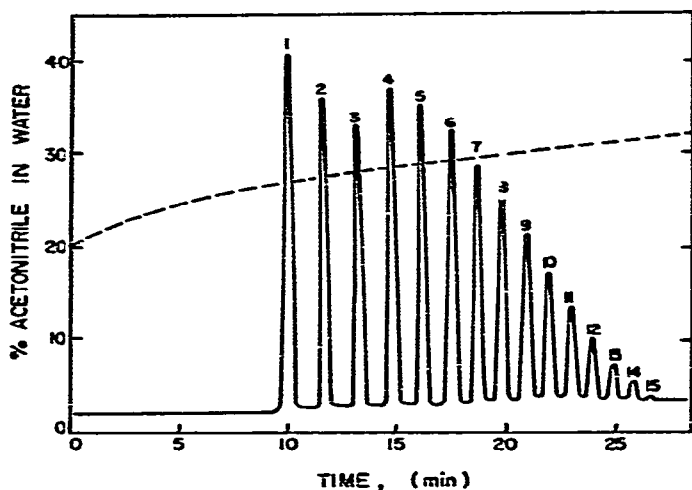


Fig. 2. Chromatogram of phenyl-oligo(ethylene glycol) homologues obtained on octadecyl-silica with gradient elution. The number of EO residues in each sample component is illustrated. Column: 7- μ m LiChrosorb RP-8 (250 \times 4.6 mm); temperature, 25 $^{\circ}$ C; flow-rate, 2.0 ml/min; gradient elution at the acetonitrile–water concentrations indicated; UV detector at 254 nm.

As no specifications concerning the molecular structure of the samples were available, the identification of the individual sample components required the use of a variety of analytical tools and a major effort which is briefly summarized below.

Phenyl-oligo(ethylene glycol)s. RPC of commercial PEO-nonylphenol and PEO-octylphenol samples revealed that both products contain the same series of peaks that elute before the homologous series of the main components and represent a minor contaminant. Co-chromatography with Carbowax 200 and 400 and

the spectra of the peaks confirmed that they are not poly(ethylene glycol)s. Since the spectra suggested that they may be EO-homologues of phenol, a sample was co-chromatographed with phenol and 2-phenoxyethanol on octadecyl-silica with acetonitrile-water (22:78) at room temperature. The logarithm of the retention factors obtained under these conditions gave a straight line when plotted against the peak number. The $\log k$ values of phenol and 2-phenoxyethanol fell on the extrapolated line at peak numbers zero and one, respectively. UV scans of the unknown peaks were very similar to those of the reference substances and showed absorbance maxima and minima at 270 and 240 nm, respectively. From these findings we concluded that the contaminants in the two detergent samples are PEO-phenol homologues. The compounds were isolated by RPC in semipreparative runs and used to demonstrate anomalous temperature and solvent effects. The balance between the hydrophobic and hydrophilic moieties in these substances appears to be such that they are eluted from standard reversed-phase columns with conveniently measurable retention factors by eluents having a composition range in which conformation changes occur.

Octylphenyl-oligo(ethylene glycol). The major components found in commercial PEO-octylphenol were investigated to establish N_{EO} for each peak as well as the structure and the position of the alkyl group attached to the aromatic ring for the homologous series. First the number of ethylene oxide groups was determined by co-chromatography with pauci-disperse standards having a known mean N_{EO} value and a narrow molecular-weight distribution. Two standards were used for each type of homologous series to establish N_{EO} for the individual peaks separated by RPC.

Samples of them were collected, pooled, evaporated by N_2 stream to dryness and subjected to H-NMR spectroscopy with integration of signal intensity. Two symmetric doublets were observed in the region of aromatic proton resonances. Several triplets were observed at *ca.* 1–1.1 kHz and assigned to ethyleneoxy methylenic proton resonances on the basis of the splitting and the chemical shift which is similar to that reported by others²³. The ratio of integrated peak intensities of the ethyleneoxy protons to the aromatic proton was used to estimate number of ethyleneoxy groups. The ratio gives this number since the number of ring protons, four, is the number of protons per ethyleneoxy group. The assignment of ethyleneoxy numbers using this argument was found to be identical to that made by the chromatographic peak matching described above.

Pauci-disperse samples of PEO-octylphenol containing 2–3 EO units were separated by GC with mass plasma detection. The elemental composition of each peak was determined and the structures proposed for the hydrocarbonaceous moiety and the EO number were confirmed on the basis of the C, H and O content. A fourth mode of confirmation of the structures assigned was the use of low-resolution MS of samples which had been fractionated by liquid chromatography and taken to dryness. The mass-to-charge ratios of parent ions were consistent with the structural assignments made on the basis of liquid chromatographic retention factors for pauci-disperse standards and the results of H-NMR analysis.

The splitting and number of H-resonances observed in that study are consistent with a structure in which the alkyl group in III is *para* to the phenolic oxygen. This result was confirmed by ¹³C-NMR on unfractionated samples which had two

equally strong central aromatic resonances and two very weak ones. This is observed with C_2 symmetry and therefore the alkyl group is in *para* position with respect to that of the oligo(ethylene glycol) chain. Concerning the structure of the alkyl substituent in III no assignments could be made on the basis of the ^{13}C resonances in the alkyl region. However, H-NMR eliminated structure R_2 because the expected peak splitting was not observed. On the other hand, the results were consistent with structure R_1 .

PEO-Nonylphenol. The structure of nonylphenol-oligo(ethylene glycols) was elucidated by using the same approach described in the previous section for PEO-octylphenols. Attempts were made to establish the position of alkyl group in the ring with respect to the phenolic oxygen. More resonances were observed in the aromatic region by using both H-NMR and ^{13}C -NMR than could be assigned to any reasonable structure. As a consequence, we believe the PEO-nonylphenol is a mixture containing nearly equal amounts of the *ortho* and *para* isomers. This would account for the excessive number of resonances and is consistent with the fact that technical nonylphenol is a mixture of the two isomers because its synthesis from nonene and phenol proceeds by initial formation of *ortho*-nonylphenol followed by a rearrangement to the more stable *para*-isomer.

Differential scanning calorimetry

Curved Van 't Hoff plots of retention factor data described below have suggested the existence of an endothermic conformation change of the detergents near 30 °C. Because no data were found in the literature supporting this result, it was tested directly by examining the thermal properties of solutions of oligo(ethylene glycol) and PEO-octylphenol. Differential scanning calorimetry (DSC) was employed because of its sensitivity and convenience. This technique directly measures the difference in energy required to raise the temperature of the solution of these substances and that of the solvent proper. The results allow the determination of (i) the heat capacity of the solute; (ii) the enthalpy of any transition which may take place in the temperature interval investigated and (iii) the temperature at which the transition is half complete. Since the equilibrium constant is usually defined as unity at that temperature, the entropy of transition can also be determined from the enthalpy and temperature.

Solutions of oligo(ethylene glycol) (Carbowax 400) and PEO-octylphenol were prepared by dissolving 1.0 g/l of solid in the following hydro-organic mixtures: acetonitrile-water (20:80), tetrahydrofuran-water (13:87) and methanol-water (75:25). The samples were subjected to DSC analysis using the appropriate solvent as the reference and all showed a major transition near 30 °C. The enthalpy change was about the same in all three cases and was estimated as +6 kcal/mol. The results confirmed the existence of two conformers and the transition which were postulated on the basis of chromatographic data. The entropy of transition was also calculated and the results of this investigation are summarized in Table I.

Chromatographic behavior of oligo(ethylene glycol) derivatives

Effect of temperature. Retention factors usually decrease with increasing temperature in RPC and yield linear Van 't Hoff plots^{1,24}. As shown in Fig. 3, such

TABLE I

TRANSITION TEMPERATURE, ENTHALPY AND ENTROPY OF CARBOWAX 400 AND PEO-OCTYLPHENOL

The entropy value is obtained at the transition temperature. Median of EO units per molecule: Carbowax 400, 7.5; PEO-octylphenol, 9.1.

<i>Solute</i>	<i>Solvent</i>	<i>T</i> (°K)	ΔH^0 (kcal/mol)	$\Delta S^0(T)$ (cal/deg·mol)
Carbowax 400	20% Acetonitrile	302.5	2.50	8.3
PEO-octylphenol	20% Acetonitrile	309	6.16	19.9
Carbowax 400	13% Tetrahydrofuran	307	3.75	12.2
PEO-octylphenol	13% Tetrahydrofuran	307.5	6.40	20.8
PEO-octylphenol	75% Methanol	304	3.60	11.8

regular behavior is not exhibited by POE-phenol homologues containing one to fifteen EO groups when they are chromatographed on an octadecyl-silica column with aqueous eluents containing 22, 28 or 33% acetonitrile. It is seen from the three sets of Van 't Hoff plots that the sign of the slopes is dependent on the number of EO units in the elute molecules and most frequently on the temperature as well so that the plots are curved. The irregular behavior displayed in Fig. 3 is not restricted to the retention factors of the detergent molecules but shown also by oligo(ethylene glycol)s proper as illustrated by Van 't Hoff plots of hepta(ethylene glycol) in Fig. 4. The retention factors were measured on octadecyl-silica with acetonitrile-water mixtures of different composition. It is seen in Fig. 4 that with 14% of acetonitrile in the eluent, the retention enthalpy is practically zero, at least at relatively high temperatures, whereas the retention factors obtained with eluents containing 10 and 18% acetonitrile have clearly positive and negative slopes, respectively. Thus, the retention of this ethylene glycol oligomer may increase or decrease with temperature depending on the composition of the eluent.

Literature data on the two conformations, in which oligo(ethylene glycol)s and their derivatives can exist, prompted us to examine the possibility that the anomalous temperature dependence of the retention factors for these compounds be interpreted by evoking the existence of at least two conformers having different intrinsic retention factors. This assumption was made and the treatment, given in Theoretical, yields eqn. 1 which describes the observed retention factor as a function of the retention factors of the two conformers and the equilibrium constant for the transition.

In order to compare the behavior predicted by the theoretical model to the experimental results illustrated above, Van 't Hoff plots were calculated by using eqn. 1 together with eqn. 2a-c. Retention factors were calculated with the assumption that the retention enthalpy of both conformers was -3kcal/mol and that retention entropy of the form favored at high temperatures, A, is 4.55 times less than that of the low temperature form, B. Therefore the ratio k_A/k_B is ten and independent of temperature. The enthalpy chosen is typically observed in RPC^{18,24,25}. Various values of transition enthalpy, ΔH_3^0 , were assumed with the value of transition entropy, ΔS_3^0 , chosen to ensure the equilibrium constant be unity at 43 °C. As can be seen from the theoretical Van 't Hoff plots in Fig. 5, if the enthalpy of the conformation change is small, a few kcal/mol or less, Van 't Hoff plots are linear or apparently so.

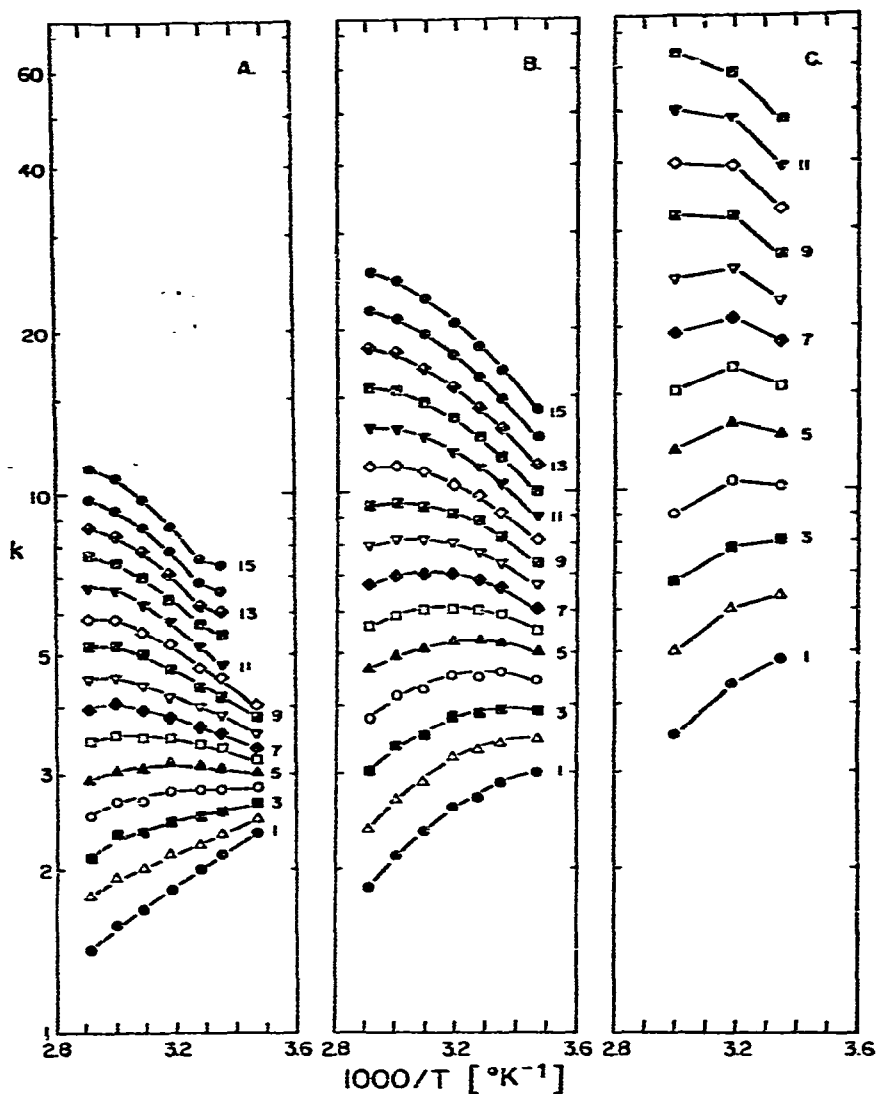


Fig. 3. Van 't Hoff plots of the retention factors for phenyl-poly(ethylene glycol)s containing the number of ethylene oxide units indicated at each curve. The retention data were measured on a 5- μ m Zorbax ODS column (150 \times 4.6 mm) at an eluent flow-rate of 2 ml/min and 273-nm detector setting. The eluents were water-acetonitrile mixtures containing 33% (A), 28% (B) and 22% (v/v) (C) acetonitrile.

However, when the enthalpy change for the transition is 10 kcal/mol or greater, the curvature becomes noticeable. The chromatographic results presented in Figs. 3 and 4 show the same trends as the computer simulated Van 't Hoff plots in Fig. 5 and the similarity supports the simple theoretical model described by eqn. 1.

Nevertheless, regular behavior, *i.e.*, linear Van 't Hoff plots having positive slopes, was observed with the retention factors of phenyl-oligo(ethylene glycol)

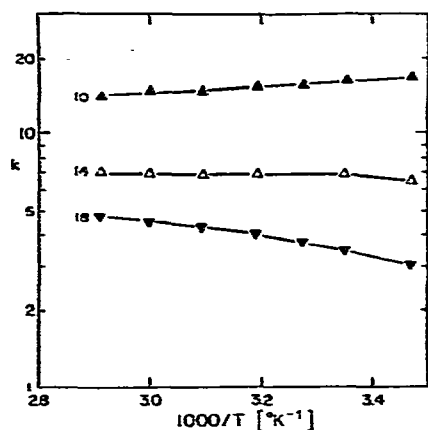


Fig. 4. Van 't Hoff plots of the retention factors for hepta(ethylene glycol), a component of Carbowax 400, as measured in reversed-phase chromatography with three different acetonitrile-water mixtures as the mobile phase. The volume percentage of acetonitrile in the eluent is indicated for each curve. Column: 5- μm Zorbax ODS (250 \times 4.6 mm); flow-rate, 2 ml/min; detector setting, 200 nm.

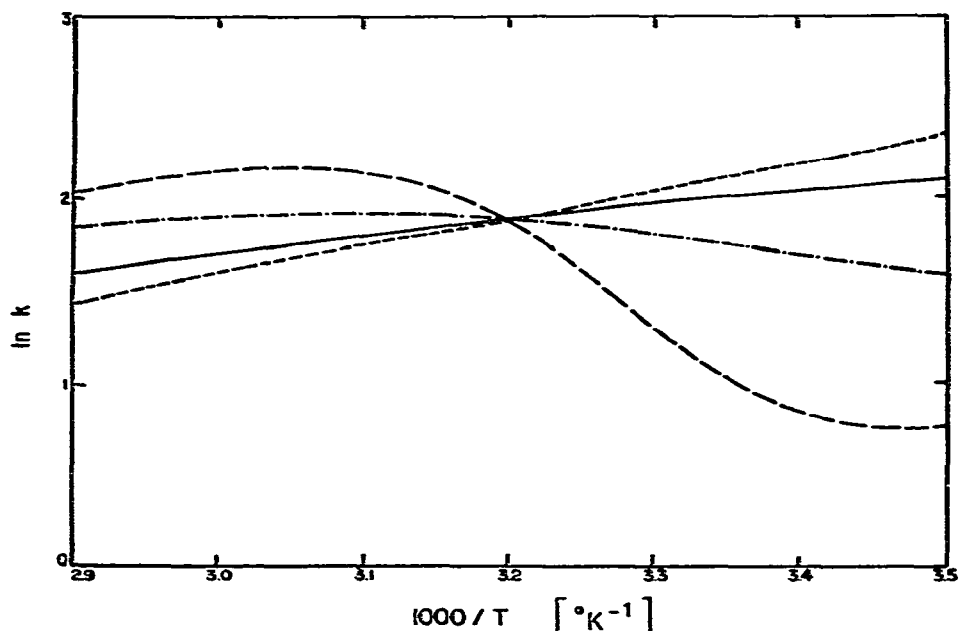


Fig. 5. Van 't Hoff plots of retention factors calculated from eqn. 1 for an elute which can undergo a conformational change in the temperature range of 285–345 $^{\circ}\text{K}$ examined here. It is assumed that the enthalpies of binding for the two limiting forms were identical and equal to -3 kcal/mol, but the ratio of their capacity factors was 10. The enthalpy of the conformational change was assumed to be either zero (—), 1 kcal/mol (---), 10 kcal/mol (-.-) or 30 kcal/mol (---). The associated entropy change was chosen to predict equal amounts of the two forms at 316 $^{\circ}\text{K}$, the temperature at which the curves intersect.

homologues when methanol-water (50:50) was used as the eluent. These observations together with the results shown in Fig. 4 make us to conclude that the temperature dependence of the retention factor for oligo(ethylene glycol) derivatives is also affected by the nature and concentration of the organic co-solvent in the aqueous eluent. Consequently the equilibrium constant for the conformation change is determined by not only the temperature but also the solvent composition. Data obtained with the differential scanning calorimeter also indicates that the zigzag-meandering transition equilibrium is affected by the solvent composition. DSC measurements were carried out with samples of Carbowax 400 and commercial octylphenyl-oligo(ethylene glycol) having a median EO number of *ca.* 7, over the temperature range of 290–400°K in 20 and 13% (at 25 °C) of acetonitrile and tetrahydrofuran, respectively. With the detergent, data was taken also in 75% (at 25°C) methanol. In each case an endothermic transition was observed over a ten degree temperature interval near 30°C. The temperatures at which the transition was half-complete, as well as the enthalpy and entropy of transition are given in Table I. The thermodynamic values should be regarded as illustrative since they represent average values for the transitions of a number of homologues. Nevertheless, it is interesting to note that enthalpy values for the transition are *ca.* 6 kcal/mol, which is expected to cause significant curvatures in Van 't Hoff plots as discussed above with regard to Fig. 4. Enthalpy changes associated with Carbowax 400 are smaller than those observed for the PEO-octylphenol homologues. This result implies, in view of the theory, less curvature in Van 't Hoff plots for Carbowax 400 than for the detergent. This is indeed the case as can be seen for instance, by comparing the plot obtained with hepta(ethylene glycol) at 18% acetonitrile as shown in Fig. 4 to that obtained with the corresponding octylphenyl-hepta(ethylene glycol) shown by curve 7 in Fig. 3.

The differences in the transition entropy changes between PEO-octylphenol and Carbowax 400 are striking in view of the similarity of the median EO number of these mixtures, 9.1 and 7.8, respectively, and that of the distributions. If the two polymers exist in the same, presumably extended, conformation at elevated temperature, this result suggests that at low temperatures the chains of poly(ethylene glycol) proper are more extended than the chains in PEO-octylphenol molecules. This may be explained by "hydrophobic interactions" between the poly(ethylene glycol) chain and the hydrophobic moiety in the latter type of compounds.

Effect of solvent. Data shown in the preceding section demonstrates that the nature and concentration of the organic solvent in aqueous eluents can profoundly affect the retention behavior of oligo(ethylene glycol)s and their derivatives in RPC. In order to gain insight into this solvent effect, the retention behavior of such samples was investigated by using octadecyl-silica columns and hydro-organic eluents containing either acetonitrile or tetrahydrofuran as the organic solvent component. The results are illustrated in Fig. 6. The dependence of the logarithm of the retention factor on acetonitrile concentration is shown for phenyl-oligo(ethylene glycol)s having EO numbers from 1 to 13 in Fig. 6A, whereas a similar plot for the oligo(ethylene glycol) components of Carbowax 400 is presented in Fig. 6B.

In contradistinction to the behavior usually observed in RPC and well established for alkylbenzenes¹, the dependence of the retention factors for oligo(ethylene glycol)s on the volume percent of organic co-solvent in the eluent is non-linear under

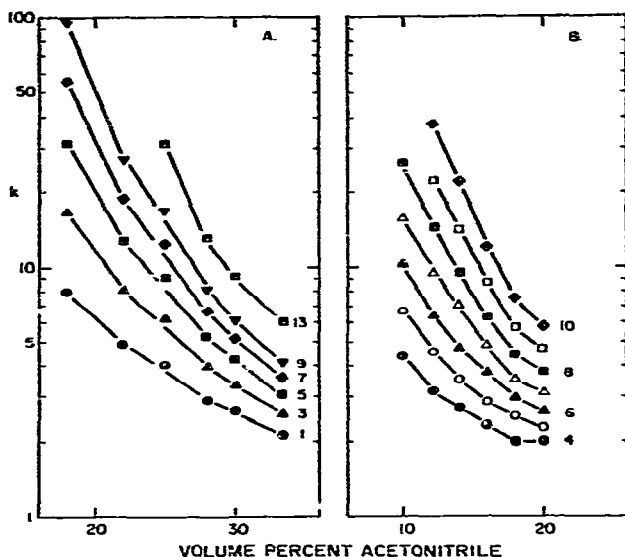


Fig. 6. Graphs illustrating plots of the retention factor on a logarithmic scale against the composition of the acetonitrile-water mixture used as the eluent. The number at each curve indicates the number of EO units contained in the solute molecule. Column: 5- μ m Zorbax ODS (250 \times 4.6 mm); temperature, 25 $^{\circ}$ C; flow-rate, 2.0 ml/min; detector setting, 200 nm. A, Phenyl-oligo(ethylene glycol)s contained in Triton 100X as contaminant; B, oligo(ethylene glycol) components of Carbowax 400.

conditions shown in Fig. 6. The findings suggest that this is the result of special interactions between the solvent and the oligo(ethylene glycol) chains. In view of the conformation changes discussed above, the effect can be considered as a solvation *cum* isomerization equilibrium and, for the simple case treated in the theoretical section, the dependence of the observed retention factor on the solvent concentration can be expressed by the limiting retention factors of the two conformers and the difference between the numbers of solvent molecules associated with the individual conformers according to eqn. 5. For increasing organic co-solvent concentration eqn. 5 predicts the observed retention factor to gradually change from the limiting value of one conformer to that of the other, provided the experimental solvent concentration range is appropriate and wide enough for the system investigated. Under such conditions, graphs depicting $\log k$ vs. solvent concentration would exhibit kinks and "dog-legs". Plots of data obtained with phenyl-oligo (ethylene glycol)s on octadecyl-silica with tetrahydrofuran-water mixtures as the eluent are shown in Fig. 7. The results are in agreement with the predicted behavior. On the other hand the curved plots shown in Fig. 6 suggest that under the conditions illustrated the solvent composition was too narrow to encompass the whole transition region or the solvation energy was too low.

Obviously the solvent composition range in which the transition occurs depends on the number of EO units in the solute molecules and the temperature besides the concentration and nature of the organic solvent. Since the range of conditions used in the chromatographic experiments is constrained by the magnitude of retention factors which can be conveniently measured, the transition may not

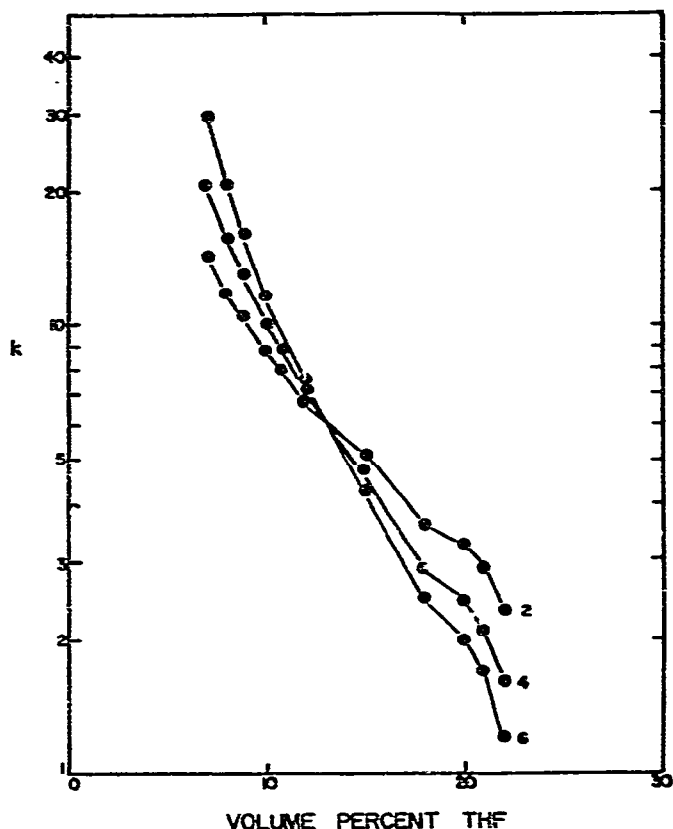


Fig. 7. Graph illustrating plots of the retention factor on a logarithmic scale against volume percent of tetrahydrofuran (THF) in the aqueous eluent for phenyl-oligo(ethylene glycol)s containing 2, 4 or 6 EO units as illustrated. Column: 5- μ m Zorbax ODS (250 \times 4.6 mm); flow-rate, 2.0 ml/min; temperature, 25 $^{\circ}$ C.

all in the range of practical chromatographic operation and the solvent effect may not be observed at all with certain poly(ethylene glycol) derivatives. Under experimental conditions used in this study phenyl-oligo(ethylene glycol)s, which are contaminants in commercial detergents, exhibited the most irregular behavior. From this finding we infer that the substances, which have hydrophobic properties intermediate between those of oligo(ethylene glycol)s and detergents, undergo conformational changes under conditions within the range of the chromatographic experiments. Consequently, these homologues could be used most conveniently to demonstrate the postulated effect of solvent mediated conformational change, an atheric secondary equilibrium, on retention in RPC.

Fig. 8 shows how the logarithm of retention factors for phenyl-oligo(ethylene glycol)s depend on the number of ethylene oxide units at different tetrahydrofuran concentrations in the hydro-organic eluents. Similar behavior has been observed with other types of oligo(ethylene glycol) homologues and organic co-solvents. As displayed in Fig. 8 a complete inversion of the elution order for the members of the

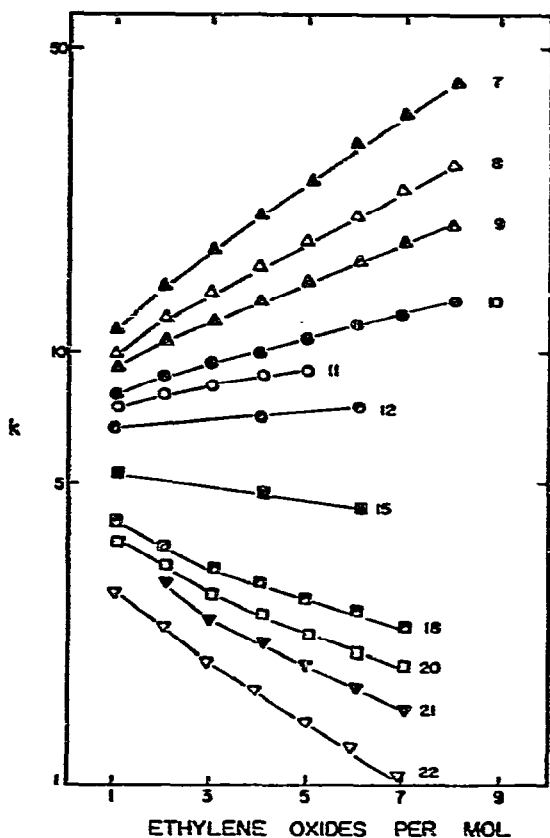


Fig. 8. Graph illustrating plots of the retention factors on a logarithmic scale against the number of EO units in phenyl-oligo(ethylene glycol)s. Column: 5- μ m Partisil ODS-2 (150 \times 4.6 mm); flow rate, 2 ml/min; temperature, 25 $^{\circ}$ C; eluent, tetrahydrofuran-water mixtures having THF concentrations in volume percent as indicated at the individual curves.

homologous series occurs upon changing the concentration of tetrahydrofuran from 12% to 15%. Whereas larger oligomers are eluted before their smaller homologues by eluents rich in organic co-solvent, the opposite elution order is observed with hydro-organic eluents lean in the organic component. In the case illustrated, the reversal takes place when the concentration of tetrahydrofuran reaches *ca.* 13%. With the same column and sample components but with isopropanol-water mixtures as the eluent, the reversal of elution order was found at an isopropanol concentration of *ca.* 20%. With methanol, acetonitrile and tetrahydrofuran the retention factors of oligo(ethylene glycol) derivatives increase with the number of EO groups below the organic solvent concentration at which reversal takes place, whereas retention decreases with increasing EO number at higher organic solvent concentrations.

On LiChrosorb RP-18 the reversal of elution order of PEO-octyl phenols with methanol-water mixtures occurs at 85% methanol. With acetonitrile-water eluents and the same column, however, the reversal of elution order of these compounds takes place below 45% acetonitrile. Thus, the organic solvent concen-

tration, where the homologues elute together depends on the nature of the organic modifier in the eluent, and might be used to characterize the solvation effect by various organic solvents. Neither this nor other findings suggested a behavior contradictory to the model put forward in the Theoretical section.

Further insight into the secondary equilibria involved here can be gained from plots of the EO group, α_{EO} , selectivity against the number of EO units in the members of the homologous series by using data measured under different chromatographic conditions. Families of such selectivity plots for phenyl-oligo(ethylene glycol)s are depicted in Fig. 9. Data obtained by changing the modifier concentration in acetonitrile-water mixtures shows in Fig. 9A that α_{EO} strongly depends on the number of ethylene oxide residues at relatively low acetonitrile concentrations, whereas α_{EO} is almost independent of the EO number when the eluent is sufficiently rich in acetonitrile. The results imply that at high acetonitrile concentrations the oligo-(ethylene glycol) moiety is present predominantly in one conformation for the lower members of the homologous series. In other words, acetonitrile favors one form, the meander conformation, which represents the more compact spatial arrangement. The same compounds, however, show non-linear Van 't Hoff plots of retention factors measured at acetonitrile concentrations as high as 28–33% because increasing temperature appears to shift the conformational equilibria in a direction opposite to that favored by acetonitrile. This implies that temperature shifts the transition equilibria to the extended conformer. In view of the solvophobic theory, which predicts greater retention for molecules having greater surface area²⁶, and eqn. 5, this phenomenon should give rise to non-linear Van 't Hoff plots of the form seen in Figs. 3 and 4.

The effect of temperature upon the dependence of EO group selectivity on the number of ethylene oxide residues with 28% of acetonitrile in the aqueous eluent is illustrated in Fig. 9B. If the increase of both acetonitrile concentration and temperature promotes the formation of the same conformer, then decreasing α_{EO} values with increasing temperature should have been observed at high number of ethoxy groups where the oligo(ethylene glycol) moiety is naturally in the compact form. On the other hand, when tetrahydrofuran is used as the organic modifier (see Fig. 9C) the dependence of α_{EO} on the number of ethoxy groups is opposite to that observed with acetonitrile-water mixtures, shown in Fig. 9A. Thus, at increasing tetrahydrofuran and acetonitrile concentrations the equilibrium of the zigzag and meander conformers is shifted in opposite directions. Since higher temperature favors the same conformation as higher tetrahydrofuran concentration, no anomalous temperature behavior is observed when 16–25% tetrahydrofuran is used in the aqueous eluent.

Length of oligo(ethylene glycol) chain. As discussed earlier the molecular dimensions of the zigzag and meandering conformers of oligo(ethylene glycol)s in water are known. Assuming that they are cylindrical rods we can calculate the molecular surface area for an ethylene oxide unit in zigzag and meandering forms as 27.5 Å and 25.1 Å, respectively. According to the solvophobic theory²⁶ chromatographic retention in RPC increases with the molecular surface area under otherwise identical conditions. Consequently a given oligo(ethylene glycol) derivative is expected to have a greater retention factor when eluted in the zigzag form than when eluted in the meandering form at least by using plain aqueous eluents in RPC.

The presence of organic solvent in the eluent, however, can significantly affect the conformation, as discussed in the preceding section, and can lead to an in-

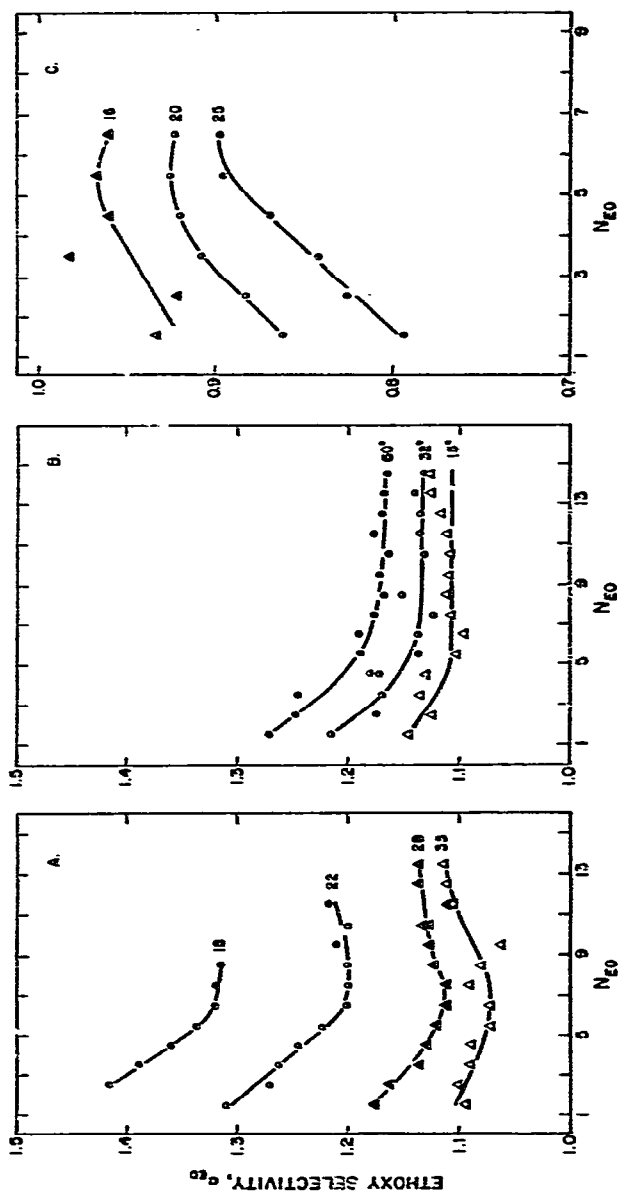


Fig. 9. Graphs illustrating the EO-group selectivity as a function of the number of EO groups in phenyl-oligo(ethylene glycol) homologues. Column: 5- μ m Zorbax ODS (250×4.6 mm). A, Acetonitrile-water mixtures of the compositions indicated were used as eluents at 25°C; B, 28% (v/v) acetonitrile in water was used as the eluent at various temperatures as shown at the individual curves; C, tetrahydrofuran-water mixtures having the compositions indicated were used as eluents at 25°C.

version of the elution order for the members of a given homologous series. Similarly the analysis of ethylene oxide group selectivity as a function of the EO number did shed light on departures from the retention behavior predicted for plain aqueous eluent due to solvent-mediated conformational changes that occur in hydro-organic solvents. The selectivity plots for acetonitrile-water mixtures in Fig. 9A and B, indicate that in the concentration range of 18–28% of acetonitrile selectivity decreases with increasing number of ethoxy groups. This indicates that at high ethoxy numbers the compact conformer is favored. Since with 33 and 28% of acetonitrile and at 25 and 15°C, respectively, selectivity is nearly independent of the number of ethoxy groups, we may infer that at high acetonitrile concentration only the meandering form is present.

However, in aqueous eluents containing 16–25% of tetrahydrofuran, selectivity increases with the number of ethoxy groups as shown in Fig. 9C. The same results were obtained with PEO-octylphenol and PEO-nonylphenol by using the same column and tetrahydrofuran in the concentration range of 36–42%. In each case the retention times decreased with increasing number of ethoxy groups and this observation suggests that with increasing number of EO units the extended conformation is favored in aqueous tetrahydrofuran eluents.

CONCLUSIONS

(1) Reversed-phase chromatography was found suitable for the separation of oligo(ethylene glycol) derivatives.

(2) A variety of analytical tools was employed to identify the individual components of commercial poly(ethylene glycol)s and non-ionic detergents.

(3) In most cases the retention behavior of these substances was irregular: (i) Van 't Hoff plots were non-linear and in some instances the retention increased with temperature; (ii) with increasing carbon number in oligo(ethylene glycol) homologues, a decrease in retention was also observed and (iii) non-linear dependence of the logarithm of retention factor on volume percent of organic solvent in hydro-organic eluents was frequently found.

(4) The observations were consistent with the theoretical model developed by assuming that each elute molecule can be present either in zigzag or meandering conformation, that the equilibrium constant for the transition depends not only on the temperature but also on the solvent composition and the length of the oligo(ethylene glycol) chain as well and that the intrinsic retention factors of the conformers are significantly different under chromatographic conditions investigated.

(5) The two-state theory provides a simple and self-consistent interpretation for the mass of data which can be readily gathered with such commercial products.

(6) The effect of solvent composition and temperature on the equilibrium between the conformers and the retention can be separately treated within the framework of the two-state theory.

(7) The data show that the relative concentration of the two conformers also depends on the number of monomer units in the polymer and this may be true with other homologous series.

(8) The present theoretical approach can be extended to other polymeric systems. It may be necessary in more complex cases to invoke the existence of more

than two conformational states and the treatment of solvent effect on conformation may require modification²⁷ especially if data on the interaction of solvent with polymer is available.

(9) The results demonstrate that the study of the retention behavior of homologous series of substances is a particularly powerful method to understand the physico-chemical underpinnings of the chromatographic process.

(10) It has been shown that a rather simple molecular picture suffices to explain a bewildering collection of chromatographic observations. The philosophy used here can probably be extended to the analysis of the RPC data of complex molecules particularly in the biological field.

(11) An analytical scheme is outlined for the study of the chemical structure and chromatographic separation of oligo(ethylene glycol)s and their derivatives.

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REFERENCES

- 1 W. R. Melander, B.-K. Chen and Cs. Horváth, *J. Chromatogr.*, 185 (1979) 99.
- 2 W. R. Melander, J. Stoveken and Cs. Horváth, *J. Chromatogr.*, 185 (1979) 111.
- 3 G. F. Longman, *Talanta*, 22 (1975) 621.
- 4 L. P. Turner, D. McCullough and A. Jackewitz, *J. Amer. Oil Chem. Soc.*, 53 (1976) 691.
- 5 J. Porath, in C. L. A. Harbourn (Editor), *Gas Chromatography 1968*, Institute of Petroleum, London, 1969, pp. 212-216.
- 6 D. Berek and D. Bakoš, *J. Chromatogr.*, 91 (1974) 237.
- 7 R. M. Cassidy and C. M. Niro, *J. Chromatogr.*, 126 (1976) 787.
- 8 C. F. Allen and L. I. Rice, *J. Chromatogr.*, 110 (1975) 151.
- 9 A. C. Hayman and N. A. Parris, *Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, 1979*, Abstract 294.
- 10 M. Rosch, in M. J. Schick (Editor), *Nonionic Surfactants*, Marcel Dekker, New York, 1979, Ch. 22.
- 11 F. E. Bailey, Jr. and T. V. Koleske, in M. J. Schick (Editor), *Nonionic Surfactants*, Marcel Dekker, New York, 1967, Ch. 23.
- 12 P. S. Sheih and J. H. Fendler, *J. Chem. Soc., Farad. Trans. I*, 73 (1977) 1480.
- 13 K. Kalyanasundaram and J. K. Thomas, *J. Phys. Chem.*, 80 (1976) 1462.
- 14 T. L. Koening and A. C. Angood, *J. Polym. Sci., Part A-2*, 8 (1970) 1787.
- 15 J. M. Corkill, J. F. Goodman and J. R. Tate, *Trans. Faraday Soc.*, 62 (1966) 979.
- 16 Yu. S. Lipatov and L. M. Sergeeva, *Adsorption of Polymers*, Wiley, New York, 1974, Ch. 6.
- 17 M. J. Rosen, *Surfactants and Interfacial Phenomena*, Wiley-Interscience, New York, 1978, Ch. 2.
- 18 W. Melander and Cs. Horváth, in Cs. Horváth (Editor), *Liquid Chromatography*, Academic Press, New York, in press.

- 19 I. Molnár and Cs. Horváth, *J. Chromatogr.*, 142 (1977) 623.
- 20 B. T. Bush, J. H. Frenz, W. R. Melander, Cs. Horváth, A. R. Cashmore, R. N. Dryer, J. O. Knipe J. K. Coward and J. R. Bertino, *J. Chromatogr.*, 168 (1979) 343.
- 21 N. Shachat and H. L. Greenwald, in M. J. Schick (Editor), *Nonionic Surfactants*, Marcel Dekker, New York, 1967, Ch. 2.
- 22 Cs. Horváth, *Methods Biochem. Anal.*, 27 (1973) 82-83.
- 23 R. A. Greff and P. W. Flanagan, *J. Amer. Oil Chem. Soc.*, 40 (1963) 118.
- 24 W. Melander, D. E. Campbell and Cs. Horváth, *J. Chromatogr.*, 158 (1978) 215.
- 25 H. Colin, J. C. Diez-Masa, G. Guiochon, T. Czajkowska and I. Miedziak, *J. Chromatogr.*, 167 (1978) 41.
- 26 Cs. Horváth, W. Melander and I. Molnár, *J. Chromatogr.*, 125 (1976) 129.
- 27 J. Schellman, *Biopolymers*, 17 (1978) 1305.