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Chromatographic investigations of oligomeric α,ω-dihydroxy polyethers by reversed-phase high-performance liquid chromatography and evaporative light scattering and UV detection*

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ABSTRACT

The separation of the three hydroxy-terminated polyethers, polyethylene glycol 1000, polypropylene glycol 1200 and polybutylene glycol 1000, on reversed-phase separation media differing markedly in the length of the alkylsilyl chain is described. Underivatized polyethers were measured by evaporative light scattering detection, whereas the corresponding 3,5-dinitrobenzoyl derivatives were monitored by UV detection at 254 nm. The detection limits were *ca.* 5, 10 and 20 μg for native polyethylene glycol 1000, polypropylene glycol 1200 and polybutylene glycol 1000, respectively, and 0.5, 1 and 2 μg for the corresponding 3,5-dinitrobenzoyl esters. A clear dependence of t_R and R_s values on the polarity of the analyte was observed in the sequence polyethylene glycol 1000 < polypropylene glycol 1200 < polybutylene glycol 1000 for each adsorbent used. Marked attenuation of analyte retention occurred with increasing polarity of the stationary phase in the sequence $C_{18} > C_8 > C_3 > C_{Phenyl} \approx C_1$ and concomitant loss of peak resolution of high-molecular-mass oligomers became increasingly evident. The solvent strength of acetonitrile was not sufficient for complete elution of polybutylene glycol and polypropylene glycol oligomers with higher molecular mass from the strongly hydrophobic C_{18} stationary phase, which however was markedly improved with methanol as organic modifier. Different possible alternatives are discussed in order to give a reasonable explanation of the separation mechanism.

INTRODUCTION

Hydroxy-terminated polyethers find a wide range of application in different fields of chemistry. They are applied as macropolymers for the synthesis of graft polymers [1] and polyurethanes [2]. Further, they have been used as non-ionic detergents [3–11] and as stabilizers and property modifiers in pharmaceutical and biochemical technology [4,12,13]. A new area of polyethylene glycol chemistry was recently opened up by the synthesis of so-called poly-rotaxanes [14,15], consisting of a polyether axis threaded with a multitude of ring systems. For this reason the development and improvement of chromatographic methods in order to obtain more insight into oligomeric distribution and characterization of polyethers is a further expanding area of analytical research.

Polyethers have been investigated by high-performance liquid chromatography (HPLC) in their native structure [16–29] and after derivatization of their hydroxy end-groups with alkyl or aryl functions [3–10,16,21,22,30]. Thin-layer chromatography (TLC) [11,31–33] and super-

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critical fluid chromatography (SFC) have also been used for characterization [21,22,34–36].

The detection of underivatized polyethers is associated with severe problems owing to the poor response at the wavelength range usually applied for UV detection. Significant responses can only be measured below 200 nm, where the high background noise invoked by self-absorption of the solvent system is a strongly limiting factor for routine applications. Nevertheless, detection of oligomeric polyethylene glycol at wavelengths below 200 nm has been described using either isocratic [18,21] or gradient elution [25,37]. Unfortunately, refractometric detection [4,7,17,23,26–29] cannot be used for the gradient HPLC of complex mixtures of multifunctional polyether oligomers. For this reason, evaporative light scattering detection (ELSD) [8,10,22,38–41] represents a powerful tool for the detection of native polyethers. Additionally, UV detection of their 3,5-dinitrobenzoyl esters opens up an efficient alternative method, yielding high sensitivity, reliability and reproducibility of results.

The aim of this study was to optimize the chromatographic conditions for the separation of α,ω-dihydroxy polyethers. For this purpose, a reversed-phase (RP) HPLC system with the gradient elution technique was developed. Polyethylene glycol (polyoxyethylene), polypropylene-1,2-glycol (1,2-polyoxypropylene) and polybutylene-1,4-glycol (1,4-polyoxybutylene or polytetrahydrofuran) were chosen as model compounds:

$HO(CH_2CH_2O)_nH$
polyethylene glycol (PEG)

$HO[CH(CH_3)CH_2O]_nH$
polypropylene-1,2-glycol (PPG)

$HO(CH_2CH_2CH_2CH_2O)_nH$
polybutylene-1,4-glycol (PBG)

The three types of α,ω-hydroxy-terminated polyether differ substantially in their polarities, which decrease markedly in the sequence PEG > PPG > PBG. Therefore, different interactions would be expected between different kinds of polyethers and different stationary and mobile phases. In order to obtain an assessment of these influences, the following parameters were changed stepwise:

stationary phase: C_{18} (octadecylsilyl)-, C_8 (octylsilyl)-, C_4 (butylsilyl)-, C_{Phenyl} (phenylpropylsilyl)- and C_1 (methylsilyl)-silica gel;

mobile phase: acetonitrile–water or methanol–water gradients;

temperature of the stationary phase: ambient temperature (*ca.* 23°C) and 60°C.

For optimum comparison of results, oligomeric mixtures with an approximately identical average molecular mass (1000 for PEG, 1200 for PPG, 1000 for PBG) were used. When this study was made, PPG was not available in the M_r 1000 form.

It is well known that chromatographic behaviour depends additionally on the space-filling properties of analyte molecules and thus can be regarded as a function of the pore size of the stationary phase [42]. However, owing to the choice of polyethers of $M_r < 1500$, interpretation of the results should not be markedly impaired by influences arising from differences in molecular size [19].

EXPERIMENTAL

Separation media

Nucleosil $5C_{18}$ (125 × 4.6 mm I.D., 5 μm particle size), Nucleosil $5C_8$ (125 x 4.6 mm I.D., 5 μm), Nucleosil $5C_4$ (100 X 4.0 mm I.D., 5 μm) and Nucleosil 7Phenyl = C_{Phenyl} (250 x 4.6 mm I.D., 7 μm) adsorbents were purchased from Macherey-Nagel (Oensingen, Switzerland) and Spherisorb $5C_1$ (125 x 4.6 mm I.D., 5 μm) from Metrohm-Bischoff (Wallisellen, Switzerland).

Reagents and solvents

Polyethylene glycol 1000 and polypropylene-1,2-glycol (both of "pract." quality) were obtained from Fluka (Buchs, Switzerland) and polybutylene-1,4-glycol (technical quality) from BASF (Ludwigshafen, Germany). Acetonitrile (HPLC grade) and methanol (HPLC grade) from either J.T. Baker (Deventer, Netherlands) or Merck (Darmstadt, Germany) were used. Water for use in HPLC was purified with a Milli-Q reagent water system from Millipore–Waters (Milford, MA, USA). Pyridine (puriss. p.a. > 99%) and 3,5-dinitrobenzoyl chloride (DNBCl) (pm-urn > 98%) were obtained from Fluka.

Analytical equipment

The HPLC apparatus consisted of a combined SP 8100 system of HPLC pump and autosampler, an SP 8450 UV detector and a Spectra Station data acquisition unit, all obtained from Spectra-Physics (San Jose, CA, USA). For ELSD, a Sedex 45 apparatus from Sedere (Vitry, France) equipped with a 20-W iodine lamp was applied. Separations at elevated temperature were performed with a π type column oven purchased from Portmann Instruments (Therwil, Switzerland).

Derivatization procedure

About 10 mg of polyether and 10 mg of DNBCl (corresponding to an approximately 100% excess of reagent on a molar basis) were dissolved in 100 μl of pyridine and heated at 60°C for about 30 min. After addition of 2 ml of methanol to the slightly brown liquid, excess of reagent was reacted to the corresponding methyl 3,5-dinitrobenzoate for about 30 min at 60°C. An aliquot of 10 μl of the resulting solution was injected on to the HPLC column without further purification.

Chromatographic separation

The time programme of the chromatographic procedure using the solvent gradient technique is depicted in Table I. For the separation of the hydrophilic PEG-1000 in either its native form or as the 3,5-dinitrobenzoyl (DNB) derivative, a gradient starting with 100% of water was chosen (gradient programme I). The significantly more hydrophobic native samples of PPG-1200 and PBG-1000 were eluted with a gradient starting with 80% of water and 20% of organic modifier (gradient programme II). For chromatography of DNB derivatives the terminal isocratic column rinsing with 100% of pure organic solvent was extended from 55 to 75 min in order to achieve extensive elution (gradient programme III). A flow-rate of 1.5 ml/min was chosen. For detection of native polyethers by means of ELSD the nebulization chamber was heated to 40°C and the nitrogen flow-rate was adjusted to 4.5 l/min, corresponding to an inlet pressure of 200 kPa. DNB derivatives were measured at a wavelength of 254 nm.

TABLE I

GRADIENT PROGRAMMES

Gradient programme	Time (min)	Organic solvent (acetonitrile or methanol) (%)	Water (%)
I	0	0	100
	40	100	0
	50	100	0
	51	0	100
	65	0	100
II	0	20	80
	40	100	0
	55	100	0
	56	20	80
	70	20	80
III	0	20	80
	40	100	0
	75	100	0
	76	20	80
	90	20	80

RESULTS

It should be noted that either the length or diameter of the C_4 and C_{Phenyl} columns differ from those of the C,, C_8 and C_{18} materials. Therefore, substantial deviations from the normally expected k' and R_s values are observed, which were taken into account in the interpretation of the results. Nevertheless, the general chromatographic trend remains unchanged.

The detection limits for native samples of PEG-1000, PPG-1200 and PBG-1000 are *ca.* 5, 10 and 20 μg, respectively, whereas the values for the corresponding DNB derivatives are *ca.* 0.5, 1 and 2 μg.

In the following sections the results obtained from different separation experiments are described.

Gradient of acetonitrile-water

With all the separation media used in the study a dependence of either capacity factor k' or peak resolution R_s on the polarity of the polyethers was observed. Both parameters increase in the sequence PEG-1000 < PPG-1200 < PBG-1000. Further, the chromatograms of PPG-1200 (Fig. la-e) and PBG-1000 (Fig. 2a–e) reveal a significant dependence on either the k' or R_s values on

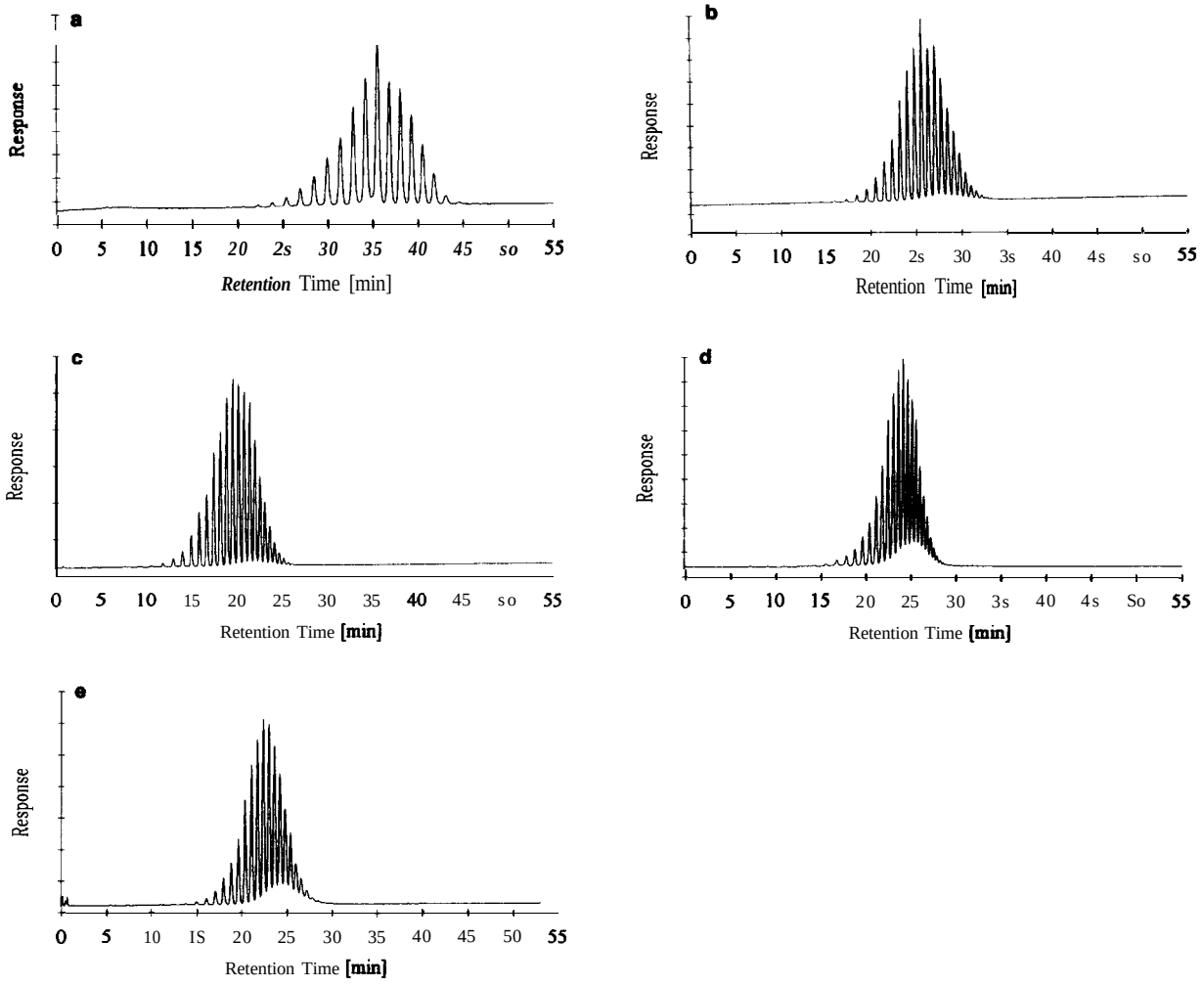


Fig. 1. Chromatograms with PPG-1200 and acetonitrile as organic solvent. (a) C_{18}; (b) C,; (c) C_4; (d) C_{Phenyl}; (e) C,.

the polarity of the stationary phase. An increase was observed in the sequence $C_1 \approx C_{Phenyl} < C_4 < C_8 < C_{18}$. It is remarkable that substantial amounts of high-molecular-mass oligomers of PBG-1000 are not completely eluted from a C_{18} stationary phase either at room temperature (RT) or when the column is held at 60°C. However, an elevated temperature markedly improves the elution potency of acetonitrile (Fig. 3a) and complete release of oligomers is achieved at 60°C on a C_8 column (Fig. 3b). In contrast, the significantly less hydrophobic PPG-1200 elutes quantitatively from a C_{18} matrix at 60°C despite its higher average molecular mass (Fig. 4).

Complete elution of PBG-1000 is effected at RT on the more polar C_4, C_{Phenyl} and C_1 materials (Fig. 2c–e). Unlike PPG-1200 and PBG-1000, the PEG-1000 oligomers exhibit incomplete peak resolution on all the separation media tested (Fig. 5a–e). In comparison with PPG-1200 and PBG-1000, the k' and R_s values are substantially decreased, although elution was started with a solvent of lower elution potency (gradient programme I, see Table I) and complete elution of PEG-1000 oligomers is achieved at RT on a C_{18} column. The incompletely resolved peak multiplet of the oligomeric mixture of PEG-1000 becomes larger and more diffuse in the sequence $C_{18} < C_8 < C_4 < C_{Phenyl} \approx C_1$.

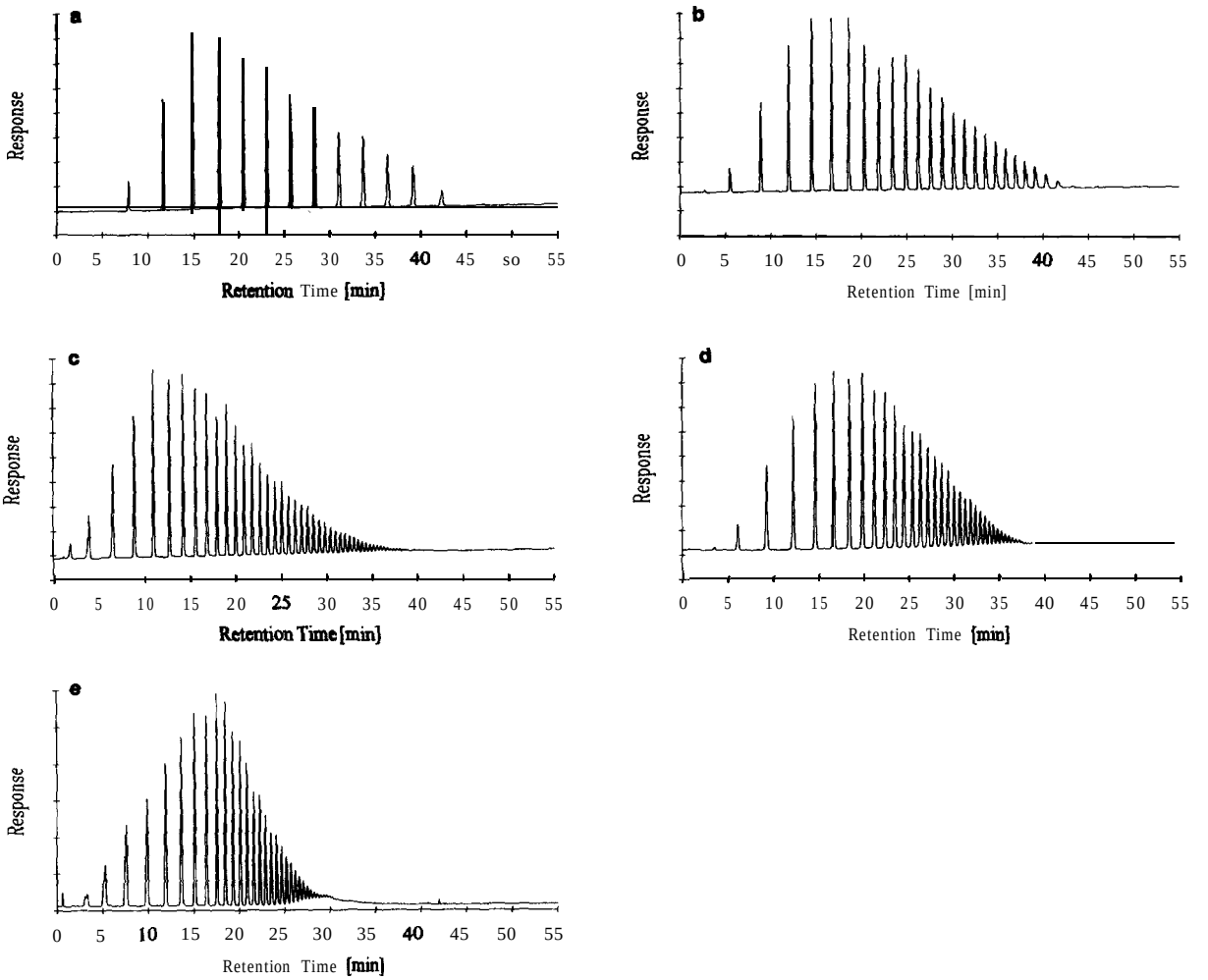


Fig. 2. Chromatogams with PBG-1000 and acetonitrile as organic solvent. (a) C_{18}; (b) C,; (c) C,; (d) C_{Phenyl}; (e) C,.

Derivatization of PPG-1200 and PBG-1000 yielded a similar chromatographic pattern (Figs. 6a–e and 7a–e). As a consequence of the significant increase in hydrophobicity over the native samples, increased k' values are observed. Despite an extension of the isocratic run with pure acetonitrile from 55 to 75 min (gradient programme III), complete elution of DNB-PBG-1000 is only achieved on a C_8 column at 60°C (Fig. 8).

In contrast to these findings, the chromatographic differences between a native polyether and its corresponding DNB derivative are more marked with PEG-1000. The signals of individual oligomers coincide and partial peak resolution, which is observed with the native sample, vanishes completely (results not shown).

Gradient of methanol-water

Whereas acetonitrile as an organic modifier proved to be more efficient in eluting PPG-1200 and PBG-1000 oligomers with low to intermediate molecular masses, methanol is more suitable for elution of high-molecular mass oligomers. With increasing polarity of the stationary phase either the retention or resolution of peaks attributable to high-molecular-mass material decreases dramatically and the signals coincide more and more. These results are shown in Fig. 9a and b for PPG-1200 and Fig. 10a and b for

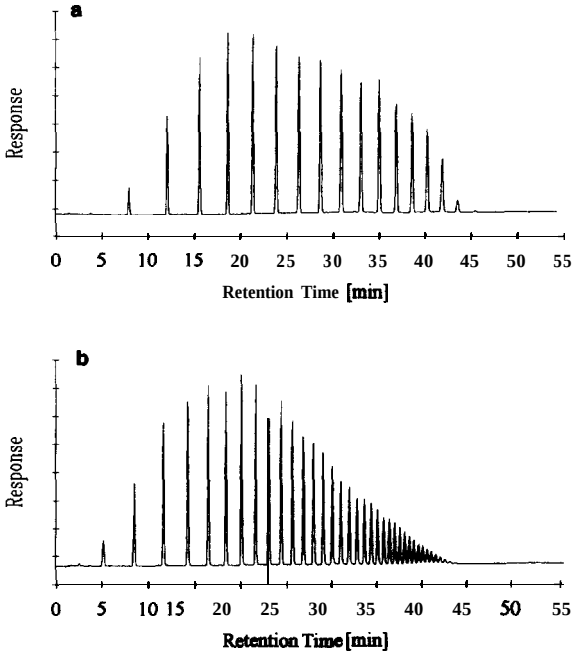


Fig. 3. Chromatograms with PBG-1000 and acetonitrile as organic solvent at a column temperature of 60°C. (a) C_{18}; (b) C_8.

PBG-1000 on C_{18} and C_8 materials, respectively. Complete elution of PPG-1200 oligomers is achieved at RT on a C_{18} column. Under identical conditions, approximately twice the number of PBG-1000 oligomers elute from the column (Fig. 10a) compared with acetonitrile, whereas complete elution of high-molecular-mass material is observed on a less hydrophobic C_8 matrix (Fig. 10b). Elution at a column temperature of 60°C does not influence the elution pattern of either low to intermediate or high-molecular-mass

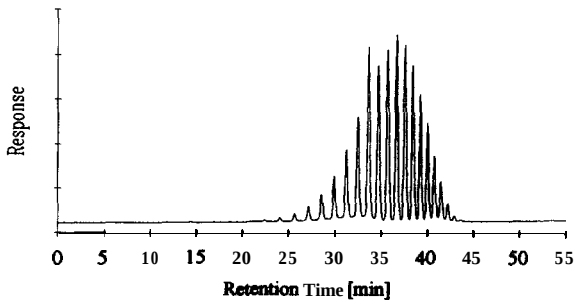


Fig. 4. Chromatogram with PPG-1200 and acetonitrile as organic solvent at a column temperature of 60°C on a C_{18} matrix.

oligomers of PPG-1200 and PBG-1000 (results not shown).

In comparison with acetonitrile, methanol causes a substantially increased retention of PEG-1000 oligomers (Fig. lla-d). The signals of homologues exhibit a significantly larger and more diffuse peak shape but no improvement in peak resolution over acetonitrile is observed.

Analogous results were obtained with DNB-PPG-1200 (Fig. 12) and DNB-PBG-1000 (Fig. 13) on a C_{18} matrix with respect to the native samples. DNB-PPG-1200 is completely eluted on a C_{18} column at RT, whereas quantitative elution of the more hydrophobic DNB-PBG-1000 is achieved on a C_8 matrix (results not shown). Separation of DNB-PEG-1000 on C_{18}, $C_{,,}$ C_{Phenyl} and C_4 materials yields an elution pattern similar to that obtained with acetonitrile as modifier (results not shown), whereas a marked improvement in peak resolution is observed on a C_1 column. The differences between acetonitrile and methanol on the C_1 stationary phase are depicted in Figs. 14 and 15.

DISCUSSION

For minimization of influences arising from individual manufacturing processes of stationary phases on the chromatographic properties, separation media from one supplier were used whenever possible. However, the C_1 column required for completion of the study was only available from a different producer. Further, it should be emphasized that the determination of the detection limits of polyethers (see Results) gives rise to some problems owing to the contribution to the total response by a large number of individual values. They must, in the strictest sense, be ascertained individually for each oligomer. Therefore, oligomers only present in low concentrations will be considered insufficiently. For this reason, the given values may be considered as an approximative set of data relating to minimum sample amounts, necessary for an unambiguous identification from the "fingerprint".

The conditions of gradient elution for the chromatography of PBG-1000 and PPG-1200 and their DNB derivatives could not be directly applied to PEG-1000 and its DNB ester owing to

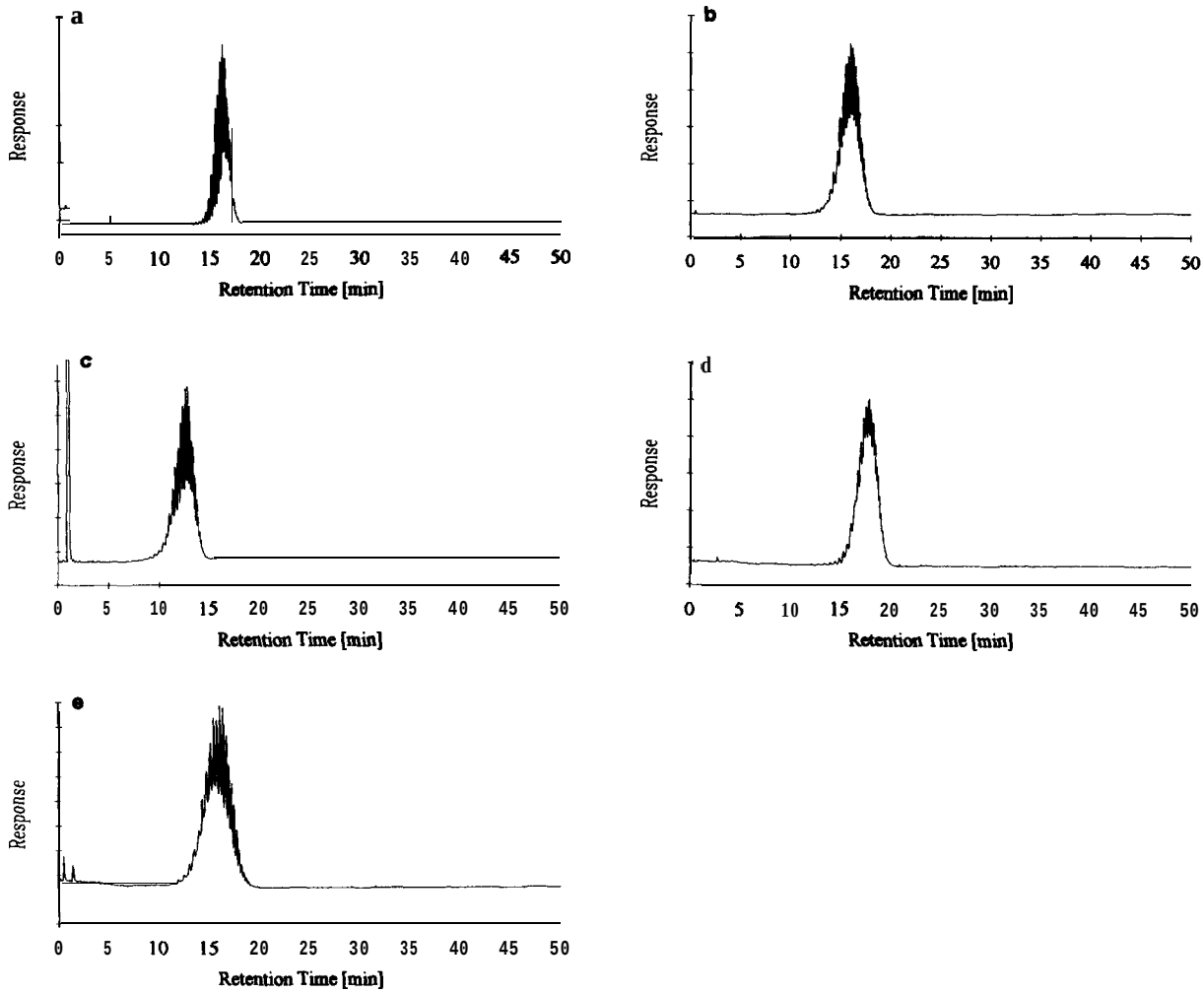


Fig. 5. Chromatograms with PEG-1000 and acetonitrile as organic solvent. (a) C_{18}; (b) C,; (c) C,; (d) C_{Phenyl}; (e) C,.

the very low *k'* values. For comparison of the results with those from PBG-1000 and PPG-1200, gradient elution was started with a mobile phase of lower solvent strength (see Table I, gradient programme I). The HPLC separation of both native polyethers and DNB derivatives with signal monitoring by means of ELSD and UV detection yielded high sensitivity and no substantial baseline deterioration occurred even at elevated temperature (60°C). The main advantage of ELSD over UV detection is that the detector response is independent of the mobile phase composition and allows the use of solvents that generally cannot be applied in HPLC owing to strong UV absorption, such as acetone or methyl ethyl ketone. Nevertheless, the sensitivity of UV detection after derivatization with DNBCl is about ten times higher and the reaction takes place either rapidly or quantitatively. No significant amounts of interfering by-products are observed and samples can be injected directly without further purification.

Marked hydrophobic interactions between the hydrocarbon backbone of both native and derivatized PPG-1200 and PBG-1000 oligomers and the alkyl chains of the C_{18} matrix favour efficient separation with sharp and well resolved peaks (Figs. la, 2a, 6a and 7a), whereas in particular the *k'* and R_s values of high-molecular-mass oligomers decrease significantly on separation

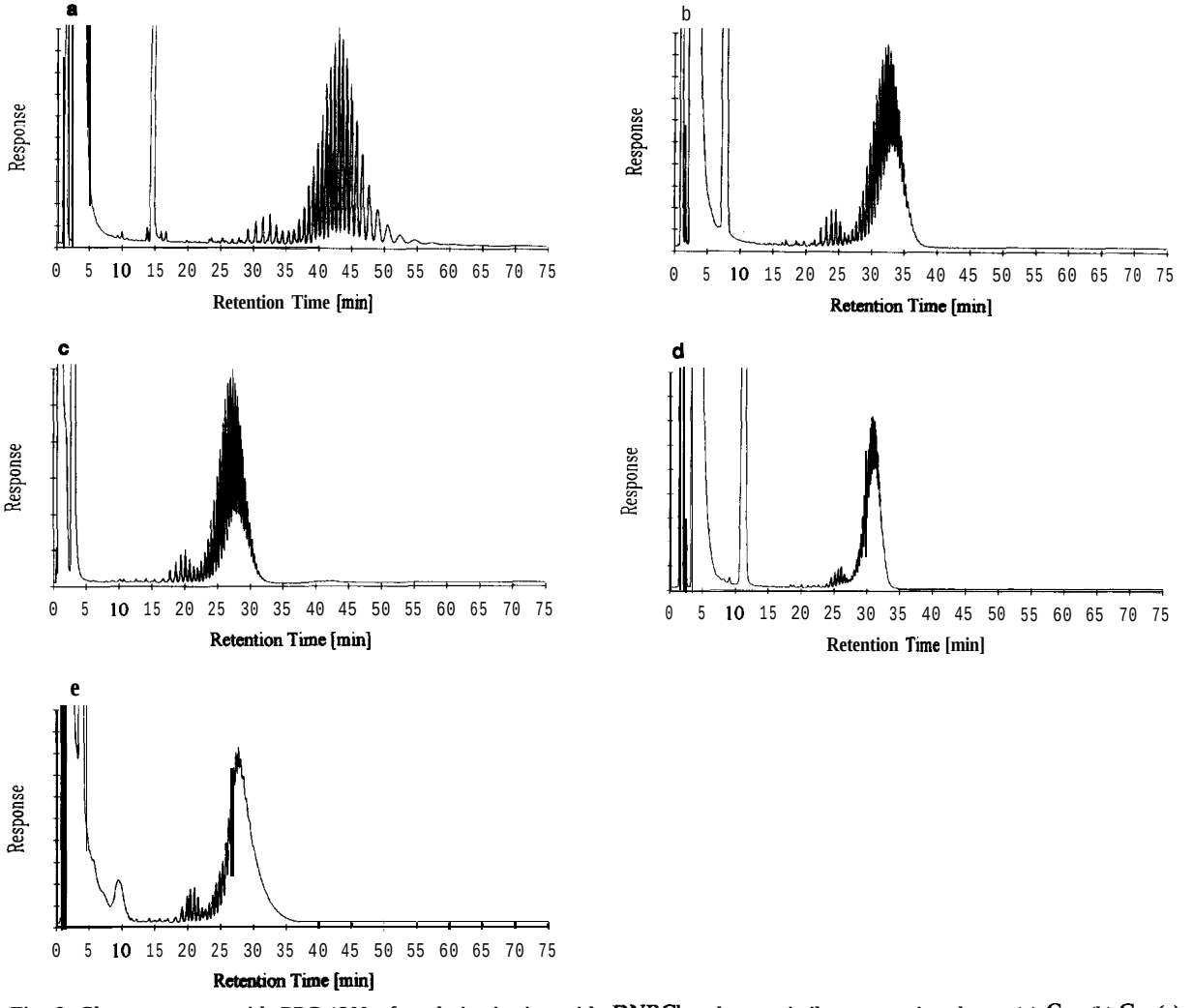


Fig. 6. Chromatograms with PPG-1200 after derivatization with DNBCl and acetonitrile as organic solvent. (a) C_{18}; (b) C_8; (c) C_4; (d) C_{Phenyl}; (e) C_1.

media of intermediate hydrophobicity (e.g., C_8 and C,) or more polar adsorbents (C_{Phenyl} and C,) owing to weakened solvophobic interactions (see Figs. lb-e, 2b–e, 6b–e and 7b–e).

A mechanism of the separation of these polyethers on the basis of their precipitation at the column head and elution of the oligomers in the sequence of their stepwise "redissolution" as a consequence of the gradual increase in the amount of organic solvent [43,44] seems improbable. The solubility of the investigated polyethers in the chosen solvent systems seems to be sufficient. However, precipitation of oligomers with substantially higher molecular mass than those tested may occur. Significant precipitation has been postulated in the normal-phase gradient HPLC of polystyrenes and copolymers of styrene and acrylonitrile [45–48].

Participation of silanophilic interactions [49-52] as described in the HPLC of cyclic polyethers [49] should presumably also be ruled out. This view is supported by the observation that low to intermediate molecular mass oligomers are eluted more rapidly with acetonitrile than with

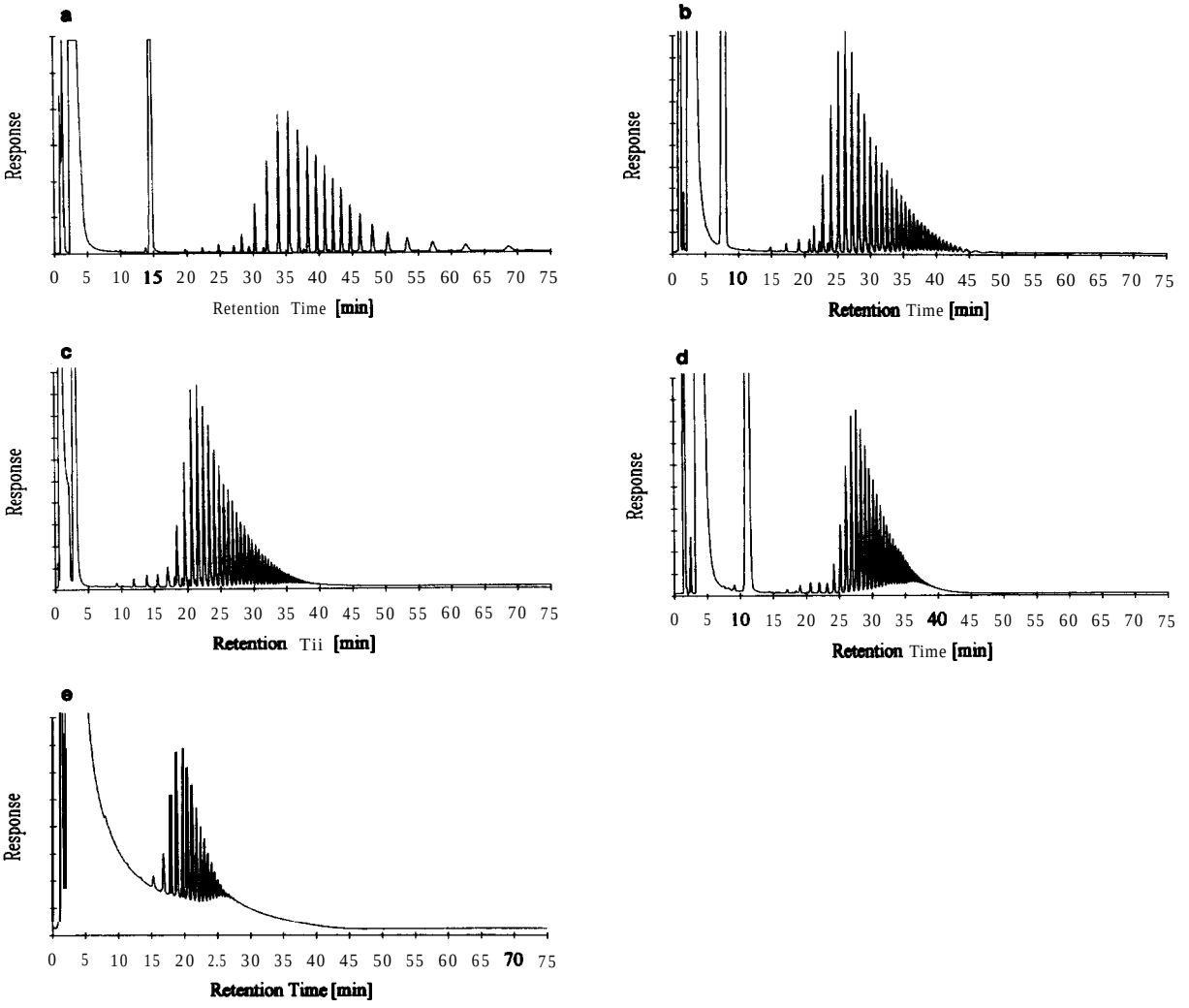


Fig. 7. Chromatograms with PBG-1000 after derivatization with DNBCl and acetonitrile as organic solvent. (a) C_{18}; (b) C,; (c) C_4; (d) C_{Phenyl}; (e) C_1.

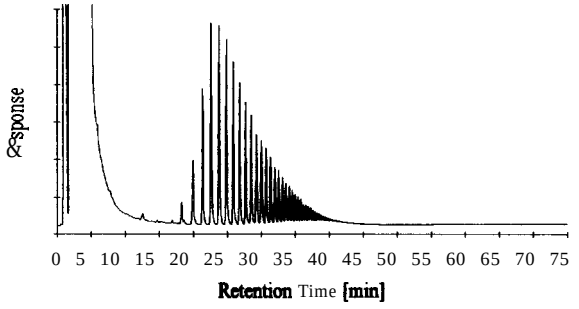


Fig. 8. Chromatogram with PBG-1000 after derivatization with DNBCl and acetonitrile as organic solvent on a C_8 matrix at a column temperature of 60°C.

methanol, although the potency of the protic solvent to cleave hydrogen bonds between ether oxygens and matrix silanols is much higher [49].

Further, it does not seem reasonable that high-molecular-mass sample constituents of PBG-1000 are preferentially able to penetrate the layer of octadecylsilyl chains of the stationary phase in order to reach free silanols. This interpretation is additionally corroborated by the complete elution of high-molecular-mass material from RP materials such as C_4, C_{Phenyl} and C,, because more facile access to residual silanols should be

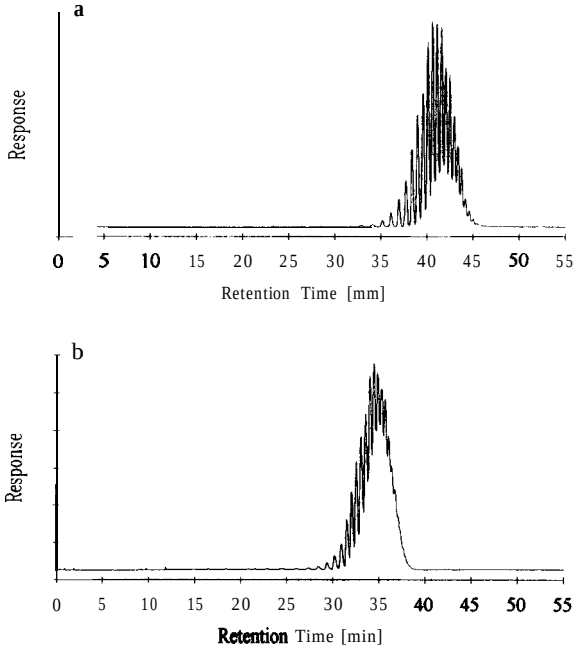


Fig. 9. Chromatograms with PPG-1200 and methanol as organic solvent. (a) C_{18}; (b) C_8.

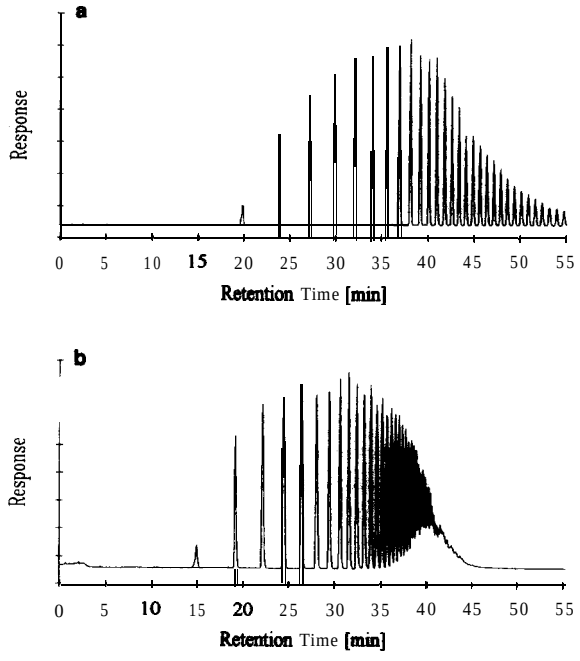
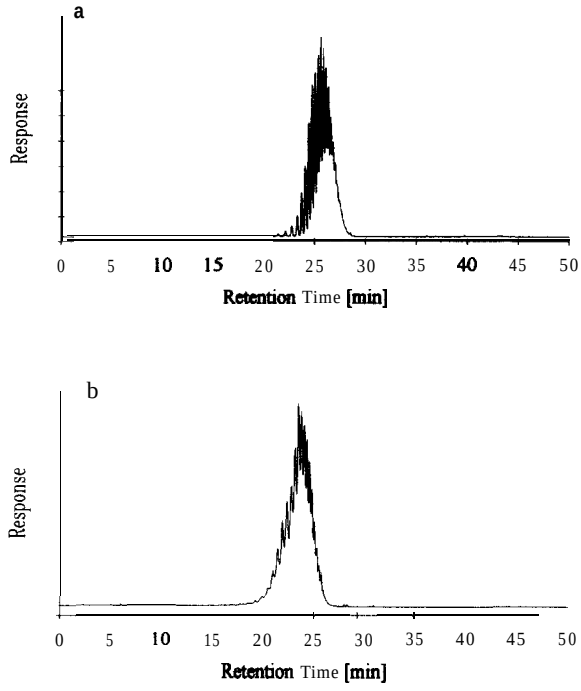


Fig. 10. Chromatograms with PBG-1000 and methanol as organic solvent. (a) C,,; (b) C_8.



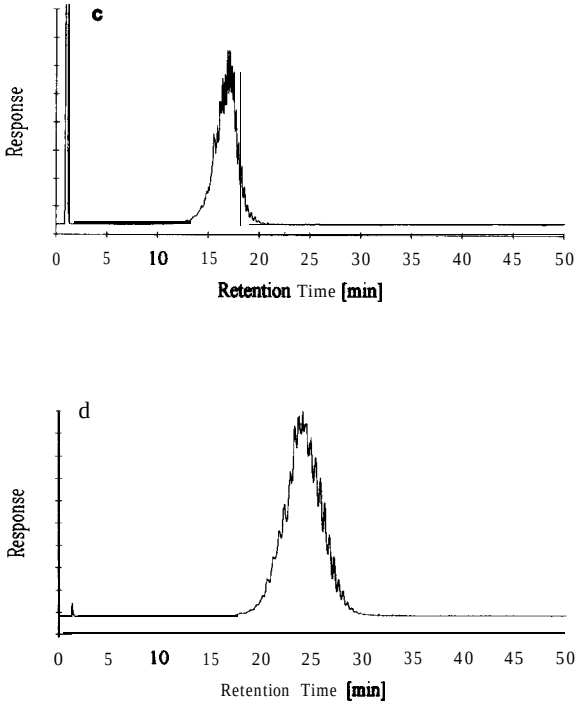


Fig. 11. Chromatograms with PEG-1000 and methanol as organic solvent. (a) C,,; (b) C,; (c) C,; (d) C,.

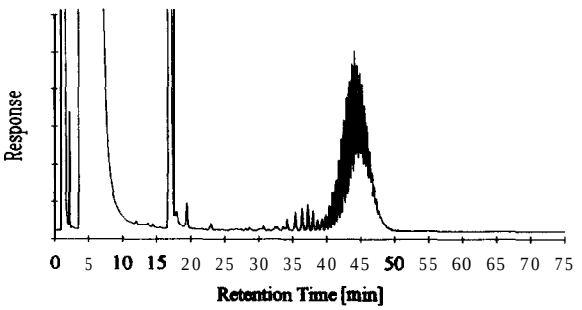


Fig. 12. Chromatogram with PPG-1200 after derivatization with DNBCl and methanol as organic solvent on a C_{18} column.

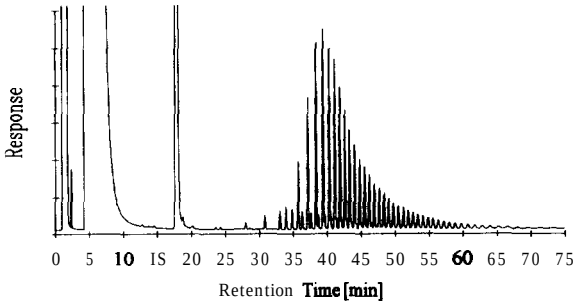


Fig. 13. Chromatogram with PBG-1000 after derivatization with DNBCl and methanol as organic solvent on a C_{18} column.

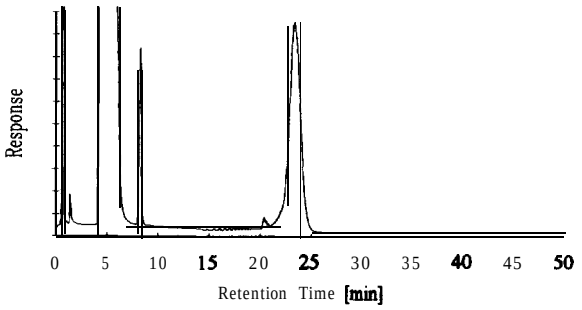


Fig. 14. Chromatogram with PEG-1000 after derivatization with DNBCI with acetonitrile as organic solvent on a C_1 column.

expected in comparison with C_{18} phases (Figs. 2c–e and 7c–e). Therefore, the decrease in retention of polyether oligomers on short-chain alkyl-substituted stationary phases can probably be attributed to either lower hydrophobic solute-matrix interactions or increased repulsive forces between free pairs of electrons on either

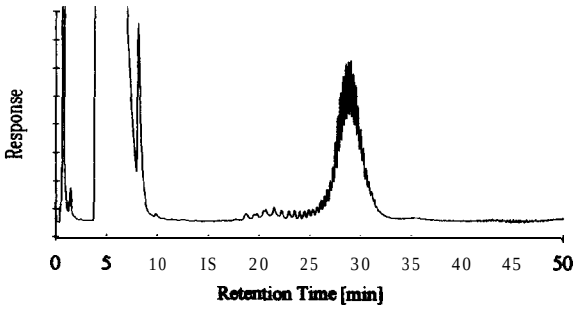


Fig. 15. Chromatogram with PEG-1000 after derivatization with DNBCl with methanol as organic solvent on a C_1 column.

oxygen atoms of the analyte or the polysiloxane backbone of the matrix. Repulsive interactions should be markedly attenuated on C_{18}-modified silica gel by a "shielding effect" of the large alkyl chains.

In addition to a hypothesis like this, a solubility effect seems to be more reasonable, which is further supported by the marked improvement in the elution potency of acetonitrile at elevated temperature. By comparison of the different effects of the two modifiers on the elution behaviour, two mechanisms become evident. On the one hand it can be assumed that the distribution of PBG-1000 between the non-polar stationary phase and the polar but aprotic solvent acetonitrile will be shifted towards an increased concentration of high-molecular-mass oligomers within the layer of C_{18} substituents. Thus decreased retention at elevated temperature can be explained by weakened solvophobic interactions and a concomitant solubility shift towards a higher concentration of analyte in the mobile phase. On the other hand, methanol will exert a solubility increase on the basis of hydrogen bond formation between its hydroxy protons and polyether oxygens. This hypothesis is in accordance with a complete lack of influence of temperature on the k' and R_s values. Similar observations were obtained with PPG-1200 but the differences are much lower owing to its lower hydrophobicity.

The poor peak resolution for PEG-1000 on all the columns tested is indicative of a decrease in hydrophobic solute-matrix interactions due to a significant increase in polarity. Therefore, RP

materials are not suitable stationary phases for separation of polar polyethers. On the other hand, satisfactory separation of alkylphenyl-oligo(ethylene) glycol was reported for "bonded-phase" materials such as 2,3-propanediol- and aminopropyl-substituted silica gel or silica gel alone with normal-phase eluents [22,30]. Further, a dependence of either k' or R_s values on the carbon load of C_{18}-substituted silica gels was established, and the lower the concentration of octadecylsilyl chains the more marked is the peak resolution [30]. Nevertheless, complete loss of peak resolution after derivatization is surprising because, in general, one would expect more marked hydrophobic interactions between the different oligomers and reversed-phase adsorbents at least with a C_{18} column. Conformational changes of analyte molecules may be responsible for this observation, as described for the cyclic polyethers dibenzo-18-crown-6 and dibenzo-24-crown-6 which induced a complete inverse of the usually observed chromatographic behaviour [16,49].

It is assumed that effects such as these will favour a levelling of solute-matrix interactions for the different oligomers. In contrast, the marked separation improvement with methanol on a C_1 column may be attributable to marked interactions between the analyte and methanol on the one hand and methanol and the stationary phase on the other. Either a "coating" effect of silyl ether oxygens by the protic solvent methanol and/or stereochemical factors induced by the special properties of the trimethylsilyl layer may be operating. Obviously the butyl chains of the C_4 column seem to be too large in order to bring about similar interactions (results not shown). In order to give a reasonable explanation of the separation mechanism, investigations on C_3 and C_2 matrices would be useful.

With a phenyl-substituted silica gel stationary phase as used in this study, the possibility of matrix-analyte $\pi-\pi$ interactions [53–56] should be taken into account. Nevertheless, the retention of DNB-polyethers on a C_{Phenyl} column was not markedly affected compared with results with a C_1 stationary phase, which is known to possess a similar matrix polarity. Therefore, an influence of potential $\pi-\pi$ interactions may be considered to be negligible.

CONCLUSIONS

To our knowledge, chromatography of polybutylene-1,4-glycol had not previously been performed. For this reason, this study represents the first report of the RP-HPLC of polybutylene-1,4-glycol. Owing to the characteristic chromatographic "fingerprint", it is possible to identify unambiguously the different types of polyethers. Methanol proved to be the mobile phase of choice especially for the separation of more hydrophobic high-molecular-mass polyether mixtures on a C_{18} stationary phase. This material is superior to the more polar short-chain alkyl-substituted adsorbents owing to its markedly better peak resolution. Overall, liquid chromatography of polyethers offers many advantages over competing methods such as SFC and TLC owing to the multitude of possible experimental approaches and its capability to separate and identify individual kinds of polyethers within complex mixtures with either marked differences in hydrophobicity or molecular masses far above 5000.

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