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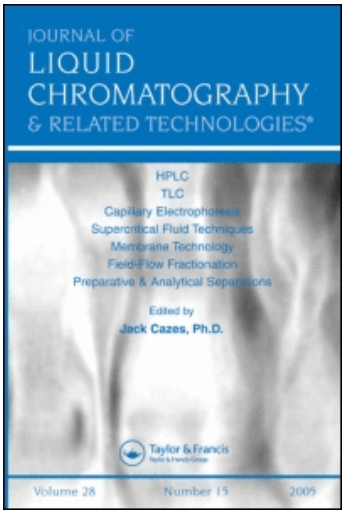

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
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Separation of Polyethylene Glycol Oligomers on Normal-Phase and Reversed-Phase Materials by Gradient High Performance Liquid Chromatography and Detection by Evaporative Light Scattering. A Comparative Study

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To cite this Article Rissler, Klaus , Fuchslueger, Ulf and Grether, Hans Jüurg(1994) 'Separation of Polyethylene Glycol Oligomers on Normal-Phase and Reversed-Phase Materials by Gradient High Performance Liquid Chromatography and Detection by Evaporative Light Scattering. A Comparative Study', Journal of Liquid Chromatography & Related Technologies, 17: 14, 3109 — 3132

To link to this Article: DOI: 10.1080/10826079408013194

URL: http://dx.doi.org/10.1080/10826079408013194

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JOURNAL OF LIQUID CHROMATOGRAPHY, 17(14&15), 3109-3132 (1994)

SEPARATION OF POLYETHYLENE GLYCOL OLIGOMERS ON NORMAL-PHASE AND REVERSED-PHASE MATERIALS BY GRADIENT HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND DETECTION BY EVAPORATIVE LIGHT SCATTERING. A COMPARATIVE STUDY

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Abstract

Separation of the polyethylene glycols PEG-200, PEG-300, PEG-600 and PEG-1000 by gradient high performance liquid chromatography is reported on a C_{18} as well as a Si 80 column in aqueous mixtures of acetonitrile, acetone and methanol as organic solvents. Detection was performed by means of evaporative light scattering. Further, the M_n and M_w values were determined by gel permeation chromatography and refractive index detection. Marked differences in the chromatographic behaviour occured between the two stationary phases with all three modifiers. Whereas PEG-200 and PEG-300 exhibited better peak resolution R_s on the C_{18} matrix, the inverse effect was observed with PEG-600 and PEG-1000, which reveal much better R_s on bare silica gel. Further, a dependence of the R_s of the PEG-600 and PEG-1000 samples (consisting of a substantially higher number of oligomers versus PEG-200 and PEG-300) from the organic modifier was seen, which increases in the range methanol < acetone < acetonitrile. A hypothetical view of the improved separation of

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PEG-600 and PEG-1000 oligomers is given on the basis of prevailing hydrophilic interactions between polar sites of polyethers and silanol groups of the stationary phase. Additionally a mechanistic hypothesis, which considers the superiority of acetonitrile and acetone versus methanol with respect to the R_s of oligomers is discussed by the assumption of different hydrogen bonding mediated solvent-stationary phase interactions implying a preponderance of adsorption (acetonitrile, acetone) versus partition chromatography (methanol).

Introduction

Polyethers of the polyethylene glycol (PEG) type as well as their derivatives (e.g. by substitution of one or both hydroxy groups by alkyl or aryl residues) have a broad application range in many different fields of chemistry. Among them important applications are the use as so-called non-ionic surfactants as well as emulsifiers for pharmaceuticals.

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These facts suggest that elucidation of the molecular composition of polyethylene glycols will be of great scientific and industrial importance. For technical polymers knowledge of the homologous distribution is crucial because the products consist exclusively of oligomeric mixtures. This fact may essentially contribute to the physico-chemical properties of the products. Among chromatographic techniques used for characterisation, in general, gas chromatography (GC) is only applicable for separation of low molecular weight oligomers, whereas supercritical fluid chromatography (SFC) additionally includes measurement of medium sized samples. Although thin layer chromatography (TLC) has been frequently used for polyethylene glycol analysis and covers a wide molecular weight (M_r) range, peak resolution proves to be unsufficient in most cases. Gel permeation chromatography (GPC) covers the whole M_r range of polyethers because solute-matrix interactions can be neglected. Nevertheless satisfactory peak resolution is only achieved with low-molecular weight samples. Owing to the high number of mobile as well as stationary phases reversed-phase high performance liquid chromatography (RP-HPLC) is the preponderably used procedure for the separation of PEG oligomers. On the other hand chromatography has also been described on bare silica gel and so-called bonded phases, such as diol, aminopropyl and cyanopropyl matrices by use of non-aqueous ("normal-phase") solvents. Sufficient separation of the 3,5-dinitrobenzoyl derivatives of monoalkylated PEG oligomers on C_{18} and C_8 stationary phases (1) and of PEG dinitrobenzoyl derivatives on an ion-exchange matrix (2) was

reported. Isocratic elution of PEG mono- and dimethylethers as well as of the native samples on C_{18} materials has also been applied successfully (3,4). Similarly normal-phase partition chromatography of the native monoarylalkyl or monoalkyl substituted oligomers (5-7) on amino- and cyanopropyl bonded silica gel proved to be a reasonable alternative tool, whereas up to 40 oligomers were identified from ethoxylated octylphenol applying isocratic RP-HPLC on a C_1 matrix (8). Further, investigations of Alexander et al. (9) revealed that the R_s of PEG oligomers on C_{18} materials increased with decreasing carbon content, which may presumably be attributable to the influence of "polar sites" on the stationary phase surface. In a recent study we compared the chromatographic separation of different types of polyether with marked differences in polarity on different reversed-phase materials (10). In contrast to polybutylene glycol 1000 and polypropylene glycol 1200, which are well-resolved at least on C_{18} and C_8 stationary phases, unsufficient resolution was observed with PEG-1000. However, it should be remarked that in this case conditions of gradient HPLC had not been optimized for PEG 1000 due to the comparative purpose of the study requiring similar chromatographic conditions for all three types of polyether, which further markedly differ in polarity. For this reason we developed a more suitable chromatographic system for the separation of low-molecular weight PEG samples differing markedly in average molecular weight M_r. We applied gradient HPLC on both normal-phase and reversed-phase adsorbents by use of aqueous organic solvents prepared from different organic modifiers. Signal monitoring was performed by evaporative light scattering detection (ELSD). GPC coupled to refractive index detection (RI) was further carried out to evaluate the M_n, M_w and polydispersity index (M_w/M_n) values.

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Materials

- *Separation media:*

For HPLC Spherisorb Si 80 bare silica (125 x 4.6 mm I.D., 5 μm particle size, 80 Å pore diameter) and Nucleosil $5C_{18}$ (125 x 4.6 mm I.D., 5 μm particle size, 100 Å pore diameter) were purchased from Metrohm-Bischoff (Wallisellen, Switzerland) and Macherey-Nagel (Oensingen, Switzerland), respectively. For GPC a series of four PLgel columns (each 300 x 7.5 mm I.D., 5 μm particle size) with pore diameters in

the range of 10^5 Å, 10^3 Å, 500 Å and 100 Å and a PLgel precolumn (50 x 7 mm I.D., 5 μm particle size, pore diameter 100 Å) for protection of the analytical columns were purchased from Polymer Labs. (Church Stretton, Shropshire, UK).

- *Reagents and solvents:*

Polyethylene glycol samples PEG-200[1)] PEG-300, PEG-600 and PEG-1000 ("pract." quality) were purchased from Fluka (Buchs, Switzerland). Narrow-range polystyrene molecular weight calibration standards for determination of M_n and M_w values were obtained from Polymer Labs. (Church Stretton, Shropshire, UK). Acetonitrile, methanol and acetone (all HPLC grade) were from Fluka. Water for the use in HPLC was purified with a Milli-Q reagent water system from Millipore-Waters (Milford, MA, USA). Tetrahydrofuran ("pro analysi") stabilised with 0.025 % of 2,6-di-tert. butyl phenol (Fluka) was used for GPC.

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Methods

- *Analytical equipment:*

The HPLC apparatus consisted of a combined type SP 8100 system of HPLC pump and autosampler with a 10 μl sample loop, a PC 1000 data acquisition unit, all obtained from Spectra Physics (San Jose, CA, USA). For ELSD a type Sedex 45 apparatus from SEDERE (Vitry sur Seine, France) equipped with a 20 W iodine lamp was applied. For GPC a SP 8810 precision isocratic pump, a SP 8875 autosampler with a 100 μl sample loop, a SP 8430 refractive index detector, a SP 4270 integrator (all from Spectra Physics) and a column thermostat from Henggeler Analytic Instruments (Riehen, Switzerland) was used. A 2 micron filter (Rheodyne, Cotati, CA, USA) was inserted between pump and autosampler in order to avoid clogging of the columns by non-soluble solvent and sample impurities.

- *Chromatographic separation:*

Gradient system I (Table 1) was used for the separations on the C_{18} column, whereas gradient system II (Table 1) was applied for the Si 80 column. Separation

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[1)] The numbers indicate the average molecular weight M_r as specified by the manufacturer.

Table 1: Gradient Systems I and II (Organic Solvents: Acetonitrile, Acetone, Methanol)

Gradient System	*Time (min.)*	*Organic Solvent (%)*	*Water (%)*
I	*0*	*0*	*100*
	40	*50*	*50*
	50	*50*	*50*
	51	*0*	*100*
	65	*0*	*100*
II	*0*	*10*	*90*
	40	*80*	*20*
	50	*80*	*20*
	51	*10*	*90*
	70	*10*	*90*

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was performed at ambient temperature (ca. 22°C) at a flow-rate of 1.5 ml/min. PEG samples (2 %, w/v) were dissolved in methanol and 10 µl aliquots were injected. For detection by means of ELSD the nebulisation chamber was heated to 40°C and the nitrogen flow was adjusted to 4.5 l/min corresponding to an inlet pressure of 200 kPa. GPC was performed at a flow-rate of 1 ml/min and the column temperature was adjusted to 29°C. Aliquots of 100 µl of PEG samples (0.5 %, w/v) were injected and signals monitored at a range of 0.02 x 10^{-3} refractive index units full scale (RIUFS) measured against tetrahydrofuran in the reference cell.

- Calculation of M_n, M_w and M_w/M_n values:

This was performed on the basis of "low-molecular weight" calibration by use of 17 narrow range polystyrene calibration standards covering the M_r range from 104 D (styrene monomer) to 120'000 D (approx. n = 1150).

Results

We have investigated the separation of the low M_r polyethylene glycols PEG-200, PEG-300, PEG-600 and PEG-1000 on both bare silica gel (Si 80) and octadecylsilyl silica gel stationary phases (C_{18}) in aqueous organic solvents with acetonitrile, acetone and methanol as organic modifiers. All tested samples clearly reveal their oligomeric composition and marked differences in the chromatographic behaviour of the samples occurred on the two different stationary phases. The common feature on both sorbents is that the capacity factor values k' for the individual polyether samples show a marked dependence on the type of organic solvent and increase in the range methanol > acetonitrile > acetone. From the chromatographic patterns it is evident that the peak resolution R_s[2)] of PEG-200 and PEG-300 (Figs. 1-3a,b) on octadecylsilyl silica gel is better than that of PEG-600 and PEG-1000 (Figs. 1-3c,d). In contrast, on bare silica gel the R_s of PEG-600 and PEG-1000 oligomers increases substantially in comparison to the reversed-phase material (Figs. 4-6c,d), whereas the R_s of PEG-200 and PEG-300 decreases on this stationary phase (Figs. 4-6a,b). The R_s of PEG-600 and PEG-1000 on the Si 80 column reveals a dependence on the type of organic modifier and increases in the range methanol < acetone < acetonitrile. The protic solvent shows either decreased R_s values for PEG-600 and PEG-1000 or selectivity with respect to the separation of the whole series of tested low M_r PEGs. This means that a better distinction between the different types of polyether in mixtures is achieved with both aprotic modifiers as can be concluded from a superposition of the individual chromatographic patterns. Almost complete base-line separation of the whole quantity of PEG-1000 oligomers is achieved on a silica column with acetonitrile as organic modifier (Fig. 4d). It should be remarked that the R_s for the different oligomers of PEG-1000 on reversed-phases other than C_{18} decreases gradually in the range $C_8 > C_4 > C_{Phenyl} > C_1$ and the peak width of the poorly resolved peak, which covers the whole entity of oligomers becomes more and more diffuse (10). When compared with the elution using acetonitrile and acetone as modifier, differences in the chromatographic patterns of PEGs between bare silica and octadecylsilyl silica gel are less pronounced with

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2) $R_s = (t_2-t_1)/w_2-w_1)$, where t_1 and t_2 are the retention times of two adjacent peaks and w_1 and w_2 their base-widths.

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methanol (Figs. 3a-d, 6a-d). Within the group of investigated PEG oligomers the R_s decreases on both kinds of columns in the series PEG-200 ≅ PEG-300 > PEG-600 > PEG-1000 and the effect is stronger on the reversed-phase material. This general phenomenon can be satisfactorily explained by the higher relative mass difference of individual oligomers of PEG-200 and PEG-300 versus PEG-600 and PEG-1000. This means that in the case of both low M_r samples an increase of the number of repeating units n is associated with a more substantial relative increase of the sample surface and, as a consequence, by higher solute-matrix interactions. It should be remarked that the capacity factor of PEG-200 and PEG-300 is very low on bare silica gel and a decrease in the initial elution strength would surely effect a better resolution. However, the principal goal of this study was to improve separation of the two "high" M_r samples PEG-600 and PEG-1000.

The lack of a normally expected "Gaussian-like" distribution in particular of PEG-200 and partially of PEG-300 oligomers can presumably be ascribed to the high volatility of the low M_r members, which do not yield a response by ELSD. In these cases derivatisation with e.g. 3,5-dinitrobenzoyl chloride and UV detection would provide a more convenient means for signal monitoring. The first peak in PEG 200 seen in some chromatogrammes may be caused by the "residual" ELSD response of a low M_r homologue, which, due its high volatility will not reflect its true amount.

When compared with HPLC a marked lower separation efficiency of the four PEGs is observed by GPC. In our GPC system only PEG-200 and PEG-300 reveal at least partial peak resolution, whereas the PEG-600 and PEG-1000 samples elute as broad and unresolved peaks (results not shown). The values for the polydispersity index M_w/M_n are nearly identical for all four samples ranging from 1.05 to 1.095 (Table 2). Although the M_n and M_w values were determined with polystyrene calibration standards, which may not be an optimum means for an estimation of M_r on the basis of their hydrodynamic volumes, a relatively good agreement between the "true" M_r values of the PEG samples and those measured by GPC was obtained (Table 2).

Discussion

Based on our experience described recently (10), we applied gradient HPLC with a linear solvent strength (LSS) system and signal monitoring by ELSD, which has

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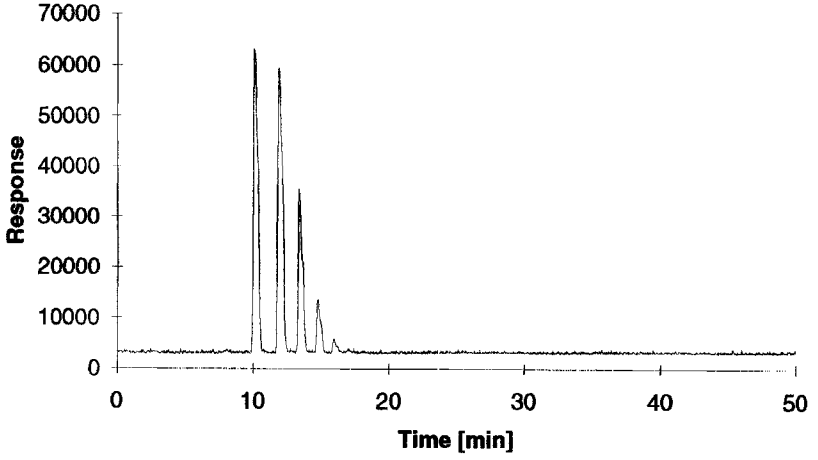

Figure 1a

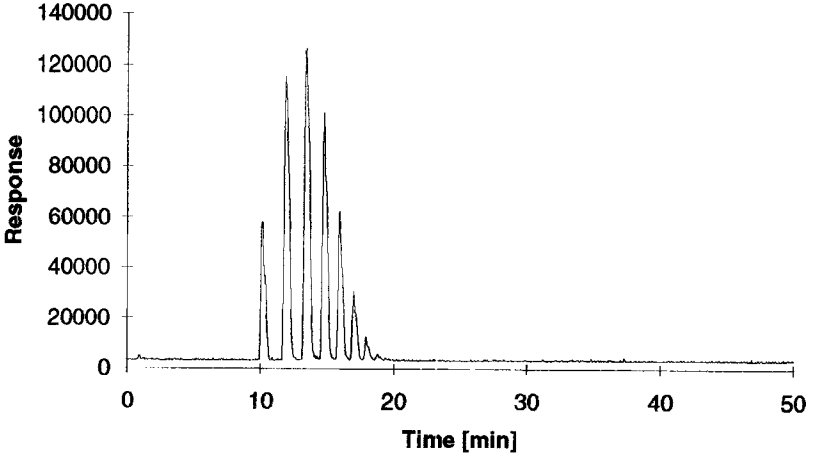

Figure 1b

Figures 1a-d: HPLC-chromatogrammes of a) PEG 200 b) PEG 300 c) PEG 600 d) PEG 1000 on a C_{18} column with acetonitrile as organic modifier

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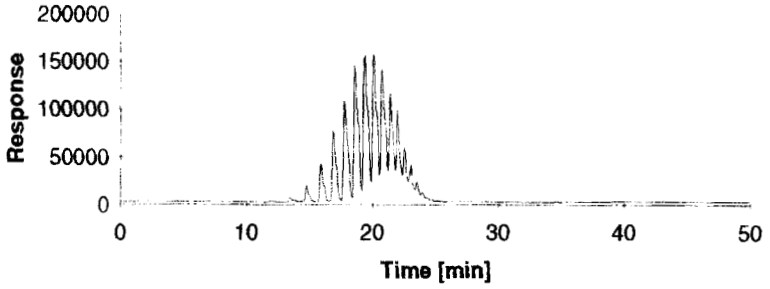

Figure 1c

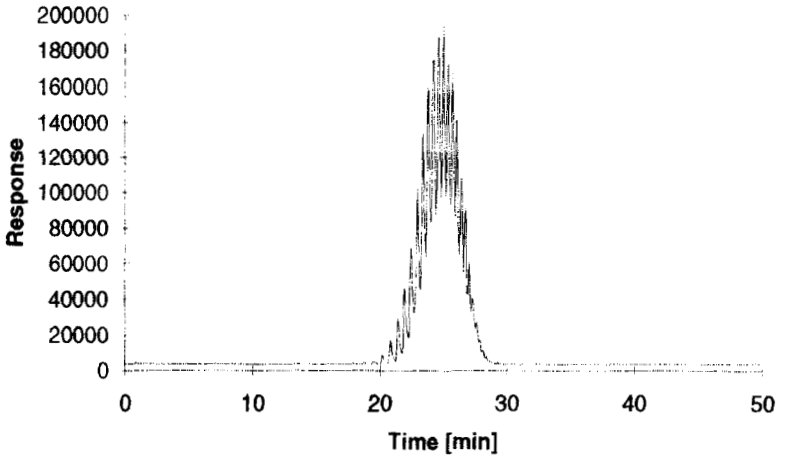

Figure 1d

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been earlier reported for the measurement of signal responses of PEGs. This technique allows the additional use of acetone as organic solvent, which cannot be applied in UV detection due to its vast self-absorption. Further, detection by UV is inferior to ELSD in particular for gradient HPLC even at wavelengths < 200 nm owing to substantial baseline deterioration. Nevertheless several authors have described addition of trace amounts (5 ppm) of nitric acid (11) or sodium azide (12) to the aqueous phase to compensate for the baseline drift invoked by the gradual increase

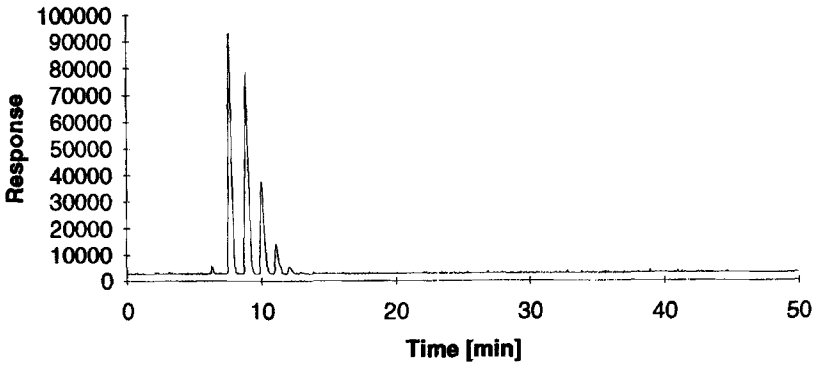

Figure 2a

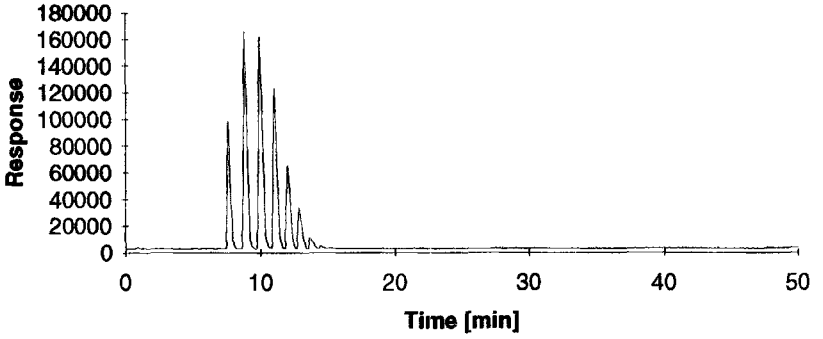

Figure 2b

Figures 2a-d: HPLC-chromatogrammes of a) PEG 200 b) PEG 300 c) PEG 600 d) PEG 1000 on a C_{18} column with acetone as organic modifier

of the concentration of organic modifier. On the other hand pre-chromatographic tagging of the α,ω- dihydroxy groups by a chromophoric agent (1,10,13) would make the polyethers amenable to the detection at the usual wavelength range. However, as shown recently (10), derivatisation of free hydroxy groups of polyethylene glycols with 3,5-dinitrobenzoyl chloride rather decreases the R_s of oligomers and thus contrasts with the results of Desbène et al. (1). Detection of PEG and its alkylated or arylated

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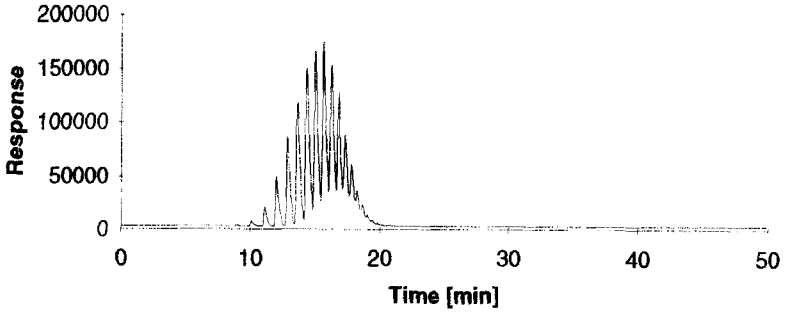

Figure 2c

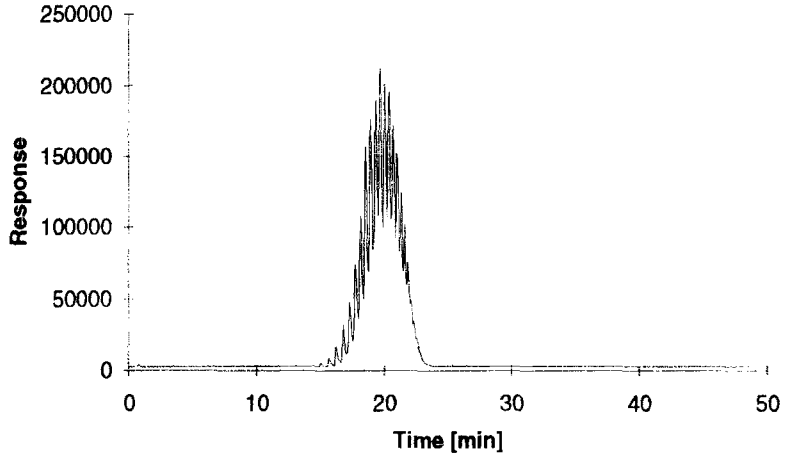

Figure 2d

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derivatives by refractive index measurement is restricted to isocratic LC. Although isocratic reversed-phase HPLC yields excellent separation of low to medium M_r oligomers, the higher M_r sample constituents eluting at higher t_R values exhibit marked peak-tailing as seen from the investigations of Trathnigg et al. (3,4). Further, quantitative elution of PEG samples with M_r > 1000 will be difficultly to achieve in the isocratic mode. Thus the gradient technique promises to be the method of choice in *particular for samples showing a broad M_r distribution of oligomers.*

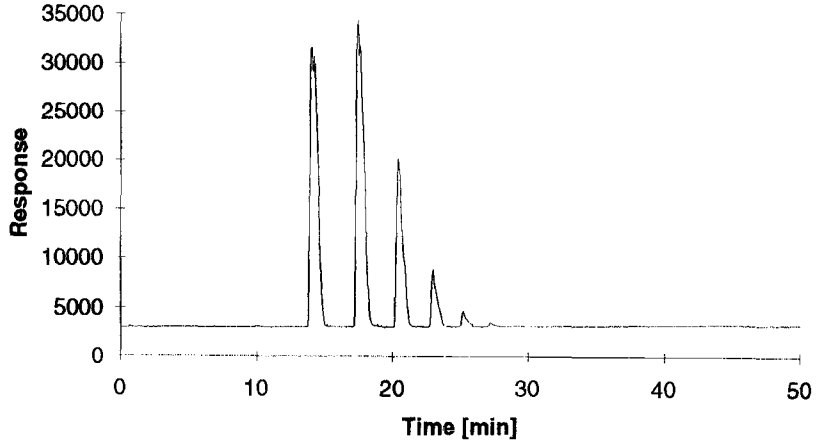

Figure 3a

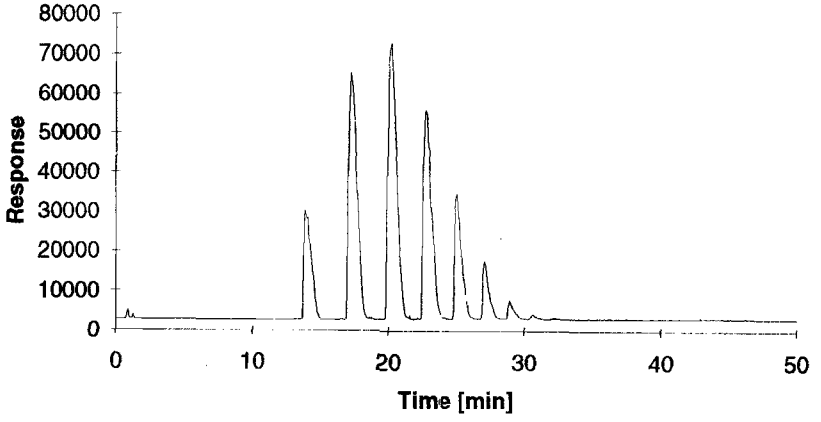

Figure 3b

Figures 3a-d: HPLC-chromatogrammes of a) PEG 200 b) PEG 300 c) PEG 600 d) PEG 1000 on a C_{18} column with methanol as organic modifier

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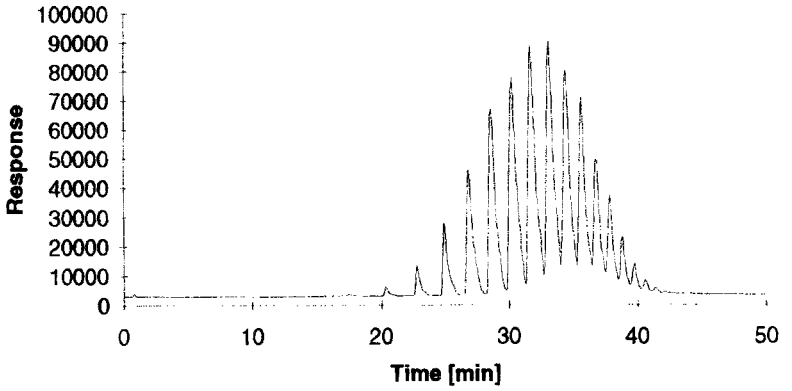

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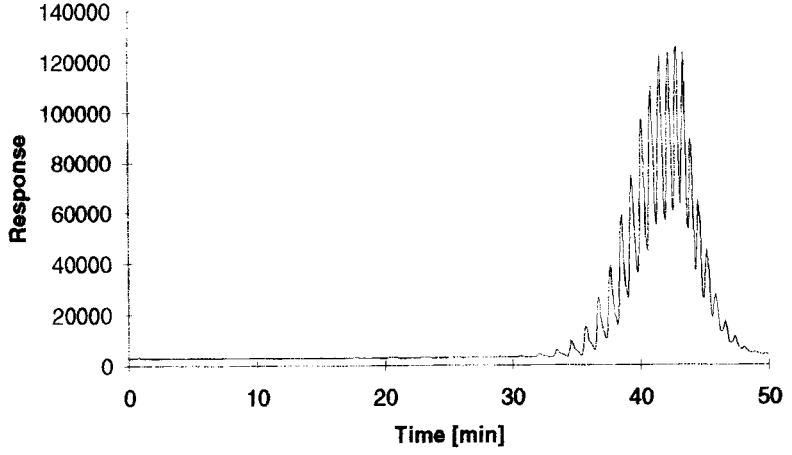

Figure 3d

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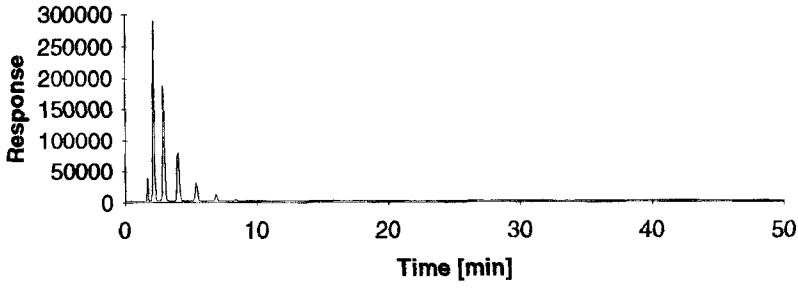

Figure 4a

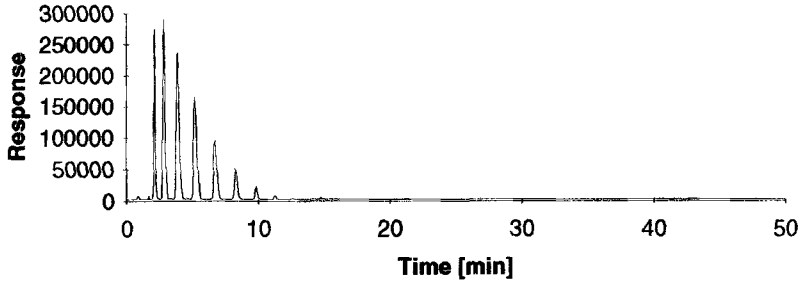

Figure 4b

Figures 4a-d: HPLC-chromatogrammes of a) PEG 200 b) PEG 300 c) PEG 600 d) PEG 1000 on a Si 80 column with acetonitrile as organic modifier

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Our aim in searching for a new powerful technique of an efficient separation of low M_r polyethylene glycols has been stimulated by the investigations of Alexander et al. (9). The authors observed a dependence of the R_s of PEGs on the extent of silica gel matrix coverage by octadecylsilyl groups and found that the lower the "saturation" of silanol groups by alkyl residues the better the peak resolution. Indeed chromatography of PEG-1000 on C_{18} matrices, which have been used for a high number of injections revealed substantial improvement of oligomer separation with respect to a new one (results not shown). This may be attributable to a "column ageing-invoked" depletion of marked amounts of octadecylsilyl substituents from the

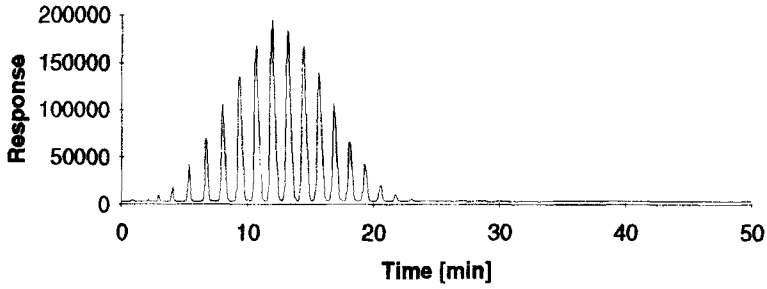

Figure 4c

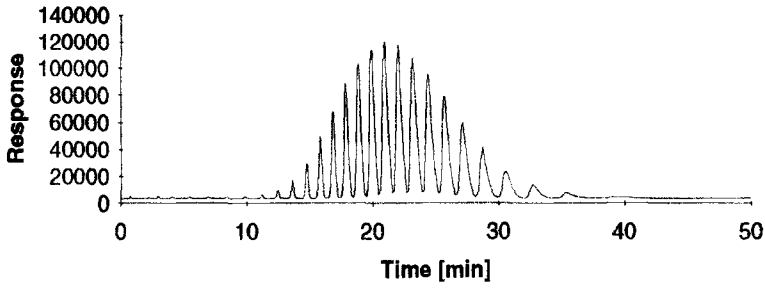

Figure 4d

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silica backbone. Starting from this observation we assumed that a more extended or even complete "dissociation" of octadecylsilyl substituents from the RP matrix should further increase the R_s of PEG-1000 oligomers. However, aqueous organic solvents are rarely used for the separation on "normal-phase" materials, which may primarily be attributed to the relatively long equilibration times postulated after changes of the solvent composition, as is the case particularly in gradient chromatography. For this reason "normal-phase sorbent - reversed-phase solvent" chromatography is usually restricted to isocratic LC and the LSS gradient technique would normally be associated with poor chromatographic performance due to an expected slow adjustment of the sorption-desorption equilibrium during sample elution. On the other hand it is known that even small amounts of water in organic mobile phases are able to deactivate the stationary phase surface and thus increase the rate of adjustment of

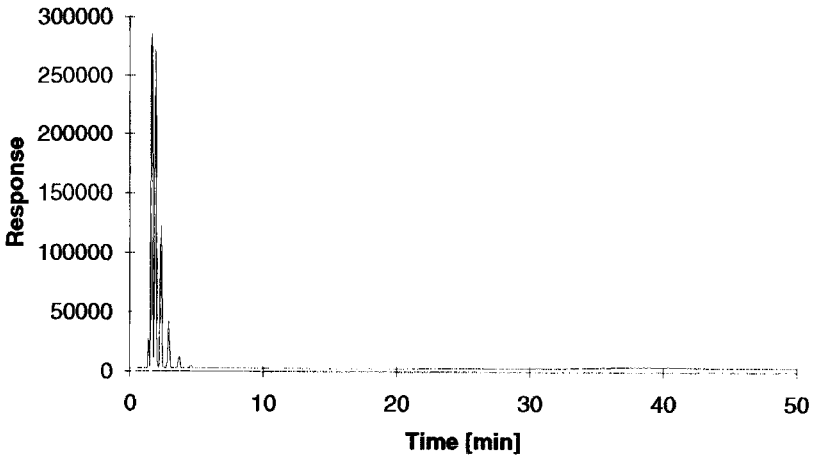

Figure 5a

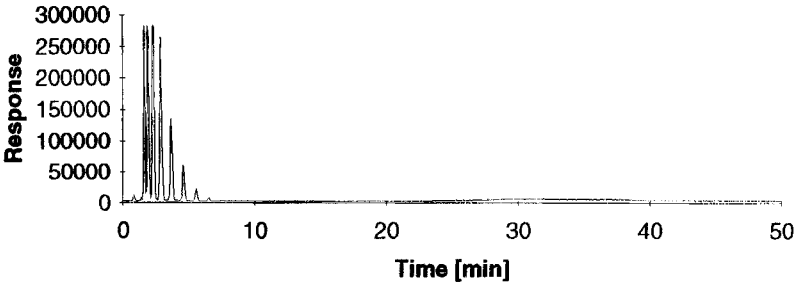

Figure 5b

Figures 5a-d: HPLC-chromatogrammes of a) PEG 200 b) PEG 300 c) PEG 600 d) PEG 1000 on a Si 80 column with acetone as organic modifier

the sorption-desorption equilibrium (14,15). Nevertheless extended reequilibration times should be expected, which would render the HPLC analysis unattractive within an adequate time range at least when LSS gradient HPLC is used. Surprisingly we did not encounter any of these problems and even reestablishment of initial gradient conditions within 1 min (e.g. from 90 % to 10 % of organic modifier) followed by reequilibration for 19 min proved to be sufficient and neither significant changes in t_R

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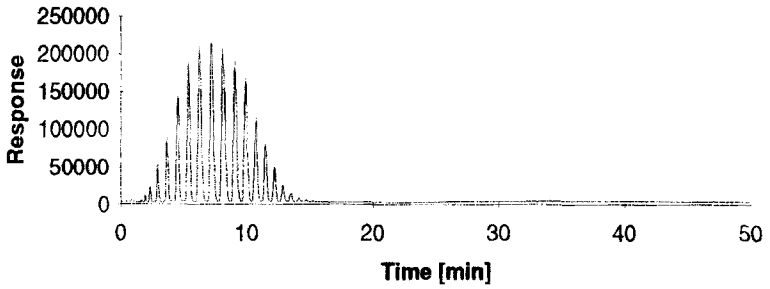

Figure 5c

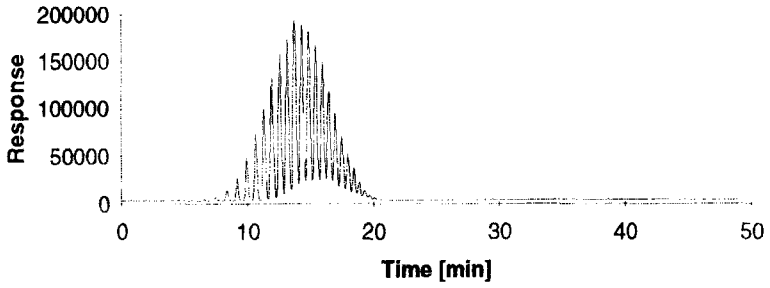

Figure 5d

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values nor base-line deterioration and peak distortion were observed after a multitude of injections of the same sample. The successful use of bare silica gel in LSS gradient RP-HPLC may be astonishing at first sight. However, it must be taken into account that according to Horvath et al. (16,17) reversed-phase chromatography can also be achieved on "naked" silica by use of excessive water in the mobile phase due to its extensive silanol-coating effect. The marked increase in band broadening of PEG-1000 oligomers at higher t_R values in particular by use of methanol and acetonitrile may thus be in accordance with their hypothesis of a typical reversed-phase behaviour of "naked" silica at higher concentrations of water. It may be explained by the continuous replacement of water by organic solvent during gradient elution, which in turn, may lead to a decrease in silanol-coating efficiency. Thus the "normal" chromatographic behaviour of acetone (i.e. negligible band broadening of

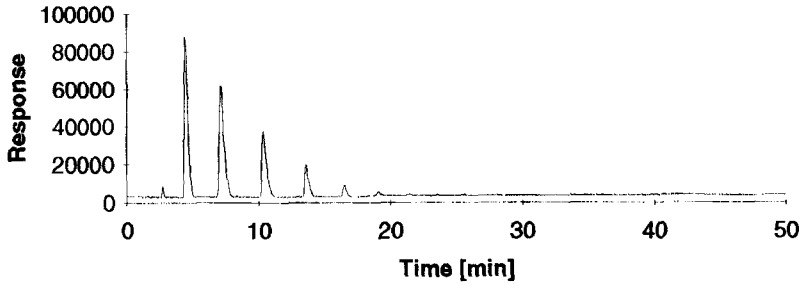

Figure 6a

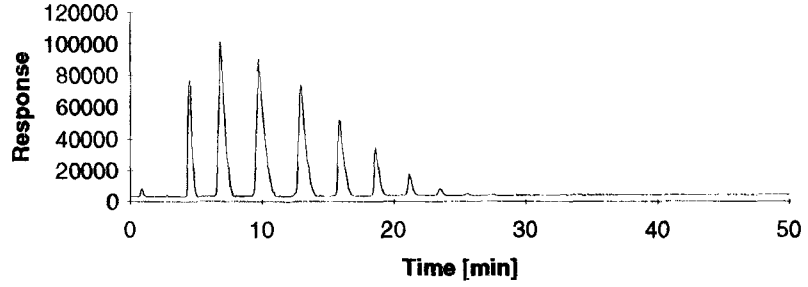

Figure 6b

Figures 6a-d: HPLC-chromatogrammes of a) PEG 200 b) PEG 300 c) PEG 600 d) PEG 1000 on a Si 80 column with methanol as organic modifier

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later eluting homologues) may be attributed to elution of the whole amount of oligomers at a water content already being sufficient to mask the silanol groups. It is worthy to note that isocratic separation of basic drugs was also performed on bare silica gel with "reversed-phase" eluents. In these cases solute-matrix interactions were suppressed by addition of ammonia to the mobile phase and the excellent separation characteristics observed were ascribed to a combination of adsorption and ion-exchange.

Whereas separation improvement of PEG-200 and PEG-300 on a Si 80 column should approximate to the similar pattern obtained with the C_{18} matrix by use of a more shallow gradient starting with e.g. 0 % of organic modifier, substantial

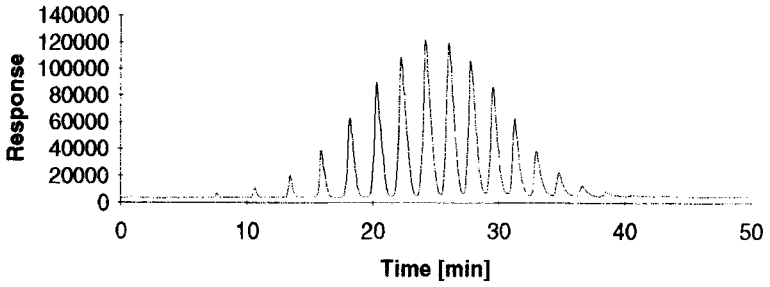

Figure 6c

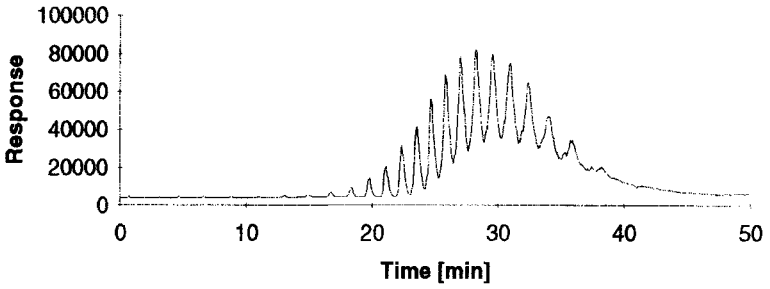

Figure 6d

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refinement of peak resolution of PEG-600 and particularly of PEG-1000 on a C_{18} column, as compared with bare silica, could be achieved with difficulty. Therefore we have concentrated on a possible hypothesis of the excellent separation of PEG-600 and PEG-1000 oligomers on silica gel adsorbents with acetonitrile and acetone as the organic modifier. The observations of Alexander et al. (9) imply that the separation characteristics are essentially governed by the polar superficial layer of free matrix silanols rather than by interactions with ether-like oxygens of the polysiloxane backbone, which in turn, may be responsible for distinct "modulating" effects during the separation process. This view is further corroborated by the observation that a better accessibility of solute molecules to polysiloxane oxygens as it should be the case with the "short-chain" substituted silica gels, such as C_8, C_4, C_{Phenyl} and C_1 matrices did not result in improvement of the R_s of PEG-1000 oligomers (10).

Table 2: M_n, M_w and Polydispersity Index (M_w/M_n) Values of Polyethylene Glycol Samples

	PEG 200	PEG 300	PEG 600	PEG 1000
M_n	244	326	673	1205
M_w	265	357	724	1265
M_w/M_n	1.086	1.095	1.075	1.050

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Further, the R_s seems to be preponderably influenced by interactions between the polyether backbone of the PEGs and the hydrophilic stationary phase and only to a small extent by those between the silica matrix and the hydroxy endgroups of the solute. This is supported by an almost identical chromatographic pattern after conversion of the PEGs to their diacetates (results not shown).

The marked delay of oligomer elution with methanol as organic modifier versus acetone and acetonitrile may be attributed to a partial sorbent-invoked demixing of methanol from the aqueous organic phase. Effects like these, presumably due to strong adsorption of the more polar component of binary mixtures of pure organic solvents to the hydrophilic silica gel matrix are well-known in liquid solid chromatography (LSC) and are often responsible for vast discrepancies from the theoretically calculated t_R values (18). Thus in order to explain the increased retention with methanol both a delay in gradient onset and/or a more shallow gradient profile of organic solvent concentration at the start of sample elution (18) should be considered. On the one hand "selective retention" of methanol on the polar sorbent would be facilitated by very tight hydrogen bonding to either silanol groups or ether-like oxygens of the polysiloxane backbone. On the other hand "extraction" of water should be favoured due to its excess at the gradient starting conditions as well as by the assumption of a better compatibility in polarity towards the stationary phase compared with methanol. However, the marked hydrophobic properties of the siloxane backbone (19,20) should also be taken into consideration, which in turn, imply a preferable interaction with the organic solvent component. Substantially lower

k'- values of PEG 1000 are observed with ethanol and isopropanol as organic modifiers, although it might be expected that their lower polarities with respect to methanol would yield an increase in retention at least on bare silica (results not shown). This observation would thus be in accordance with a preferable "extraction" of methanol and may be ascribed to a lower actual concentration in the mobile phase as calculated from the gradient profile at the start of chromatographic separation. Acetonitrile as well as acetone are also able to undergo hydrogen bonding at least with silanol groups, which implies a "fine-tuning" effect of both modifiers on separation characteristics.

In order to attempt an explanation of the marked separation improvement on bare silica gel with the aprotic modifiers acetonitrile and acetone compared with methanol we have developed a possible mechanistic hypothesis, which will be discussed briefly. As already mentioned above the polar solvents are able to mask residual silanol groups in reversed-phase materials and according to Horvàth et al. (16,17) this effect is stronger with methanol than with acetonitrile. Whereas methanol is a proton acceptor, i.e. in terms of Pearson's classification (21,22) a hard base, acetonitrile and acetone are weak dipolar bases. Methanol exhibits hydrogen bonding by interactions of either its free pair of electrons on the oxygen with silanol protons or by the inverse interaction between its hydroxy proton with free pairs of electrons on the ether-like siloxane oxygens. These synergistic effects may lead to a strong attachment of methanol molecules to the silica gel surface. Thus possible "localisation" of methanol on the silica surface will favour partition of the solute between this dense surface layer and the neighbouring solute transporting aqueous methanolic layer in a corresponding way as stated for normal bonded phase liquid chromatography (NBP-LC) on aminopropyl silica gel (23). Owing to their lack of exchangeable ("acidic") protons, which will cause a less marked coverage of surface silanols, acetonitrile as well as acetone are only able to undergo hydrogen bonding with silanol groups and adsorption chromatography may prevail in this case. A simplified presentation of the hypothetic solvent-stationary phase hydrogen bonding sites is given in Figure 7.

It should be stated that effects quite different from the afore-mentioned may also be of importance. This would include a differential "solvation" of polyether samples in the mobile phase by hydrogen bonding between the hydroxy protons of methanol and

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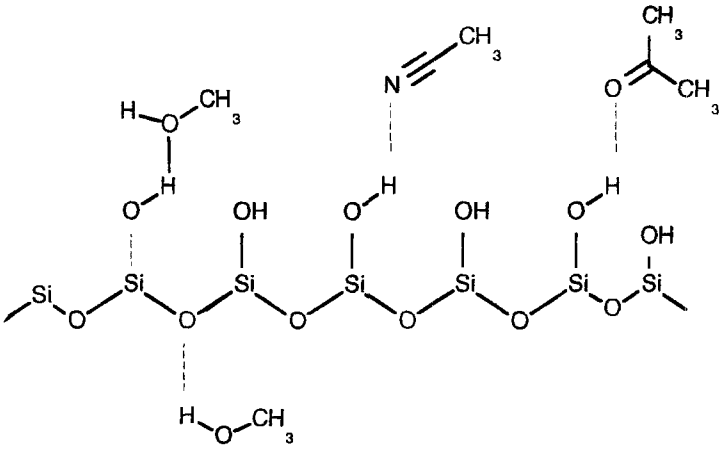

Figure 7: *Simplified model of possible interactions between organic solvent, silanol groups and polysiloxane oxygens at the surface of bare silica gel stationary phases*

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ether oxygens of the solute, which is not achievable with the aprotic solvents and thus additionally may "superimpose" solute-matrix interactions. Further, a continuous transition from a "reversed-phase-like" behaviour to "normal-phase" (adsorption) chromatography owing to the gradual replacement of water by organic solvent during gradient elution may also be operating. Nevertheless at the moment the hypothesis discussed above may represent a reasonable preliminary attempt to explain the separation improvement of PEG 600 and PEG-1000 with the change from a reversed-phase to a normal-phase matrix.

Conclusions

To our knowledge, gradient HPLC of polyethylene glycol samples with aqueous organic solvents typically used in classical RP-HPLC on bare silica gel has not previously been reported. The feasibility of the method with respect to its analytical power has been demonstrated and thus offers an attractive alternative way to the generally used reversed-phase materials. From the investigations it is evident that the

higher the number of oligomers within a PEG sample the better the peak resolution compared with reversed-phase matrices. The main advantage of the silica stationary phase over its octadecylsilyl derivative consists of its higher selectivity with respect to an assigment of the resulting chromatographic patterns to individual PEG samples, which will be of great technical importance due to "recognition" of the type of PEG within mixtures. In contrast to the findings with bare silica the more pronounced overlap of peaks from different samples by use of reversed-phase separation media would make a selective attribution more difficult. Improvement of chromatographic performance on bare silica may presumably be attributed to specific interactions between polar sites on the polyethers and the vast amount of silanol groups on the stationary phase surface. Owing to the high water content at the start of gradient elution a "reversed-phase like behaviour" of the silica gel can additionally be postulated, which is also in agreement with the marked hydrophobic properties of the polysiloxane backbone, whereas, at higher concentrations of organic solvent adsorption may prevail. Taken together the improved separation characteristics of polyethylene glycols on "naked" silica opens up an efficient tool for characterising the oligomeric distribution of polar polyethers.

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Received: October 18, 1993
Accepted: December 1, 1993

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