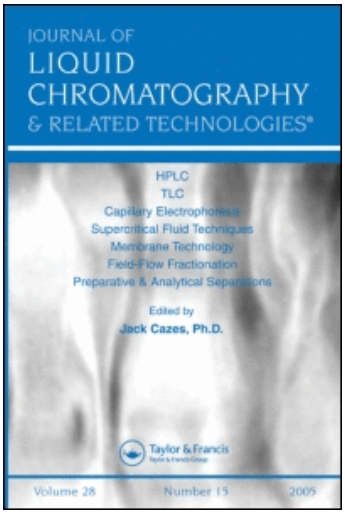

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Separation of Polybutylene Glycols on C_{18} and C_4 Stationary Phases

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SEPARATION OF POLYBUTYLENE GLYCOLS ON C_{18} AND C_4 STATIONARY PHASES

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ABSTRACT

Polybutylene glycol (PBG) samples largely differing in molecular weight M_r are separated by gradient reversed-phase high performance liquid chromatgraphy on C_{18} and C_4 stationary phases by use of either acetonitrile or methanol as organic modifiers and signal monitoring by means of evaporative light scattering detection. Neither acetonitrile nor methanol are sufficient for the quantitative elution of all samples from the extremely hydrophobic C_{18} matrix. However, the elution potency of methanol is markedly better compared with acetonitrile presumably attributable to a solubility increase of sample molecules via hydrogen bonding between its hydroxy group and the ether oxygens of the solutes. Marked lower retention of high M_r oligomers is observed on the more polar C_4 matrix and the elution power of acetonitrile is now sufficient for the quantitative release of all investigated polyether samples. A substantial increase of signals eluting at higher retention times and thus representing oligomers with higher M_r becomes evident in the range PBG 650 < PBG 1000 < PBG 2000 < PBG 3000. Although the PBGs exhibit a broad "within-sample" oligomeric M_r distribution and thus substantial peak overlapping by superposition of the individual chromatograms a clear assignment to individual samples can be done on the basis of the different chromatographic patterns.

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INTRODUCTION

Besides the polypropylene glycols the polytetrahydrofurans, i.e. the polybutylene glycols (PBGs) play a more and more increasing role for the use as flexibiliser and toughener in formulated epoxy-based systems. Further, they are used as long-chain α,ω- dialcohol components in the synthesis of either polyurethane fibres, polyester and polyurethane plastics or as starting materials in the synthesis of cross-linked polyurethane casting elastomers. In many applications PBGs are first reacted with diisocyanates to form isocyanate prepolymers, which are subsequently converted to polyurethanes. For quality control as well as for discussion of structure-property relationships there is a need to know the oligomeric composition of PBGs. Structural assignment of the PBGs can be easily accomplished by gradient reversed-phase high performance liquid chromatography (RP-HPLC) of the cleavage products obtained after acid or base catalysed hydrolysis of the samples. Signal monitoring by evaporative light scattering detection (ELSD) offers the advantage to use the native hydrolysis products without prior derivatisation with a chromophoric agent. Recently we reported on our chromatographic investigations of PBG 1000 by use of different stationary phases and different organic modifiers (1,2). In this paper an improved and very efficient separation system for the analysis of samples largely differing in M_r is described, which further permits a selective attribution to the type of PBG on the basis of the individual chromatographic patterns. For comparative purpose we have applied a C_{18} as well as a C_4 stationary phase and both acetonitrile and methanol as the organic modifiers.

EXPERIMENTAL

- Separation media

The polybutylene glycol samples PBG 650, PBG 1000, PBG 2000 and PBG 3000 ("technical quality") were obtained from BASF (Ludwigshafen, Germany). Nucleosil $5C_{18}$ and $5C_4$ (each column 125 x 4.6 mm I.D., 5 µm particle size, 100 Å pore diameter) from Macherey-Nagel (Oensingen, Switzerland) were used as the stationary phases for RP-HPLC. For gel permeation chromatography (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, 100 Å) for column protection were purchased from Polymer Labs. (Church Stretton, Shropshire, UK).

- *Chemicals and solvents*

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 and methanol (both HPLC grade) were from Fluka (Buchs, Switzerland). Tetrahydrofuran ("pro analysi") stabilised with 0.025 % of 2,6-di-tert. butyl-4-methyl phenol (Fluka) was used for GPC. Water for the use in HPLC was purified with a Milli-Q reagent water system from Millipore-Waters (Milford, MA, USA).

- *Analytical equipment*

The HPLC apparatus consisted of a SP 8800 ternary HPLC pump, an SP 8880 autosampler equipped 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 equipped 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*

The gradient system depicted in Table 1 was used for the separation with either acetonitrile or methanol as organic modifier. Chromatography was performed at ambient temperature (ca. 22°C) and a flow-rate of 1.5 ml/min. Aliquots of 10 μl of ca. 2 % methanolic solutions (w/v) of the polyethers were injected onto the HPLC column. 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 done at a flow-rate of 1 ml/min and the column

Table 1: Gradient Programme for the Separation of PBGs

Time (min.)	Organic Solvent (%)	Water (%)
0	20	80
40	100	0
75	100	0
76	20	80
90	20	80

temperature was adjusted to 29°C. Aliquots of 100 µl of PBG samples (0.5 %, w/v) were injected and signals were 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 and M_w 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

From the chromatographic patterns it is obvious that the different samples show a broad M_r distribution. This view is also corroborated by calculation of the polydispersity index M_w/M_n of the different PBGs from the GPC measurements using polystyrene calibration, which yields values markedly differing from unity and thus attributable to substantial sample heterogeneity (Table 2). Although the choice of polystyrene calibration standards may not be an optimum means of M_r determination and therefore the true may differ markedly from the measured values (see Discussion below), it is nevertheless evident that the individual M_n and M_w values within the range of investigated PBGs differ at a factor of about 8 (Table 2). When measured by HPLC an optimum peak resolution R_s[1)] of the low M_r oligomers of the PBG 650 and

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

Table 2: *M_n, M_w and M_w/M_n Values of Polybutylene Glycols*

	PBG 650	PBG 1000	PBG 2000	PBG 3000
M_n	1070	1552	3938	6095
M_w	2089	3452	12073	15796
M_w/M_n	1.95	2.28	3.07	2.61

PBG 1000 samples was achieved on a C_{18} stationary phase with a linear solvent strength (LSS) system consisting of acetonitrile and water but the medium-to-high M_r sample constituents were not eluted. This view becomes evident from the vast decrease of the signal responses for the oligomers in the range PBG 650 > PBG 1000 > PBG 2000 > PBG 3000 (Figures 1a-d), though equal amounts had been injected and thus only the low M_r "impurities" from PBG 2000 and PBG 3000 were eluted with this system. It is further remarkable that the t_R values of all elutable oligomers of PBG 650, PBG 1000, PBG 2000 and PBG 3000 coincide completely and the last peak leaving the column appears at ca. 40 min. The only difference between the chromatographic patterns consists in a substantial relative increase of peak areas attributable to oligomers with higher M_r in the range PBG 650 < PBG 1000 < PBG 2000 < PBG 3000 (Figures 1a-d). As already shown previously (1), methanol as organic modifier strongly enhances elution of medium-to-high M_r PBG oligomers and nearly complete elution was at least observed for PBG 650 and PBG 1000 (Figures 2a,b). In contrast, marked amounts of PBG 2000 and in particular PBG 3000, which preponderably consist of high molecular weight oligomers, still remain on the stationary phase (Figures 2c,d). Nevertheless after replacement of the strongly hydrophobic C_{18} by the more polar C_4 matrix elution of the whole quantity of oligomers of PBG 650, PBG 1000, PBG 2000 and PBG 3000 was already effected with acetonitrile affording excellent peak resolution R_s (Figures 3a-d). Base-line separation of 38 PBG 650 and 40 PBG 1000 oligomers, as well as of 47 and 51, respectively, sufficiently resolved PBG 2000 and PBG 3000 oligomers (the most of them exhibiting base-line separation) are observed on the C_4 matrix with acetonitrile as modifier. In contrast resolution of signals attributable to high M_r oligomers is much

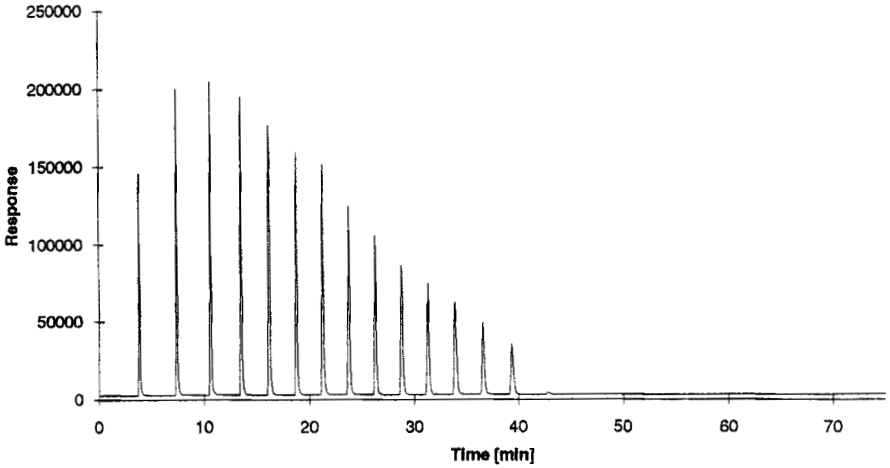


Figure 1a

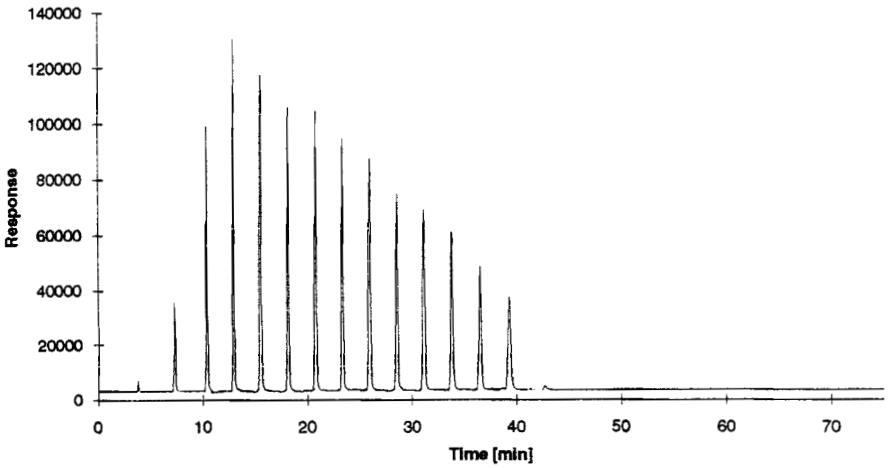


Figure 1b

Figures 1a-d: Chromatogrammes of a) PBG 650, b) PBG 1000, c) PBG 2000 d) PBG 3000 on a C_{18} column and acetonitrile as organic modifier

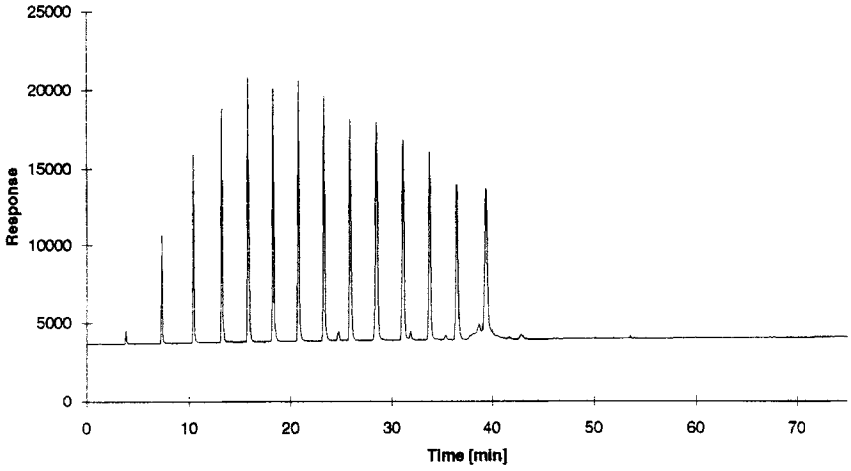


Figure 1c

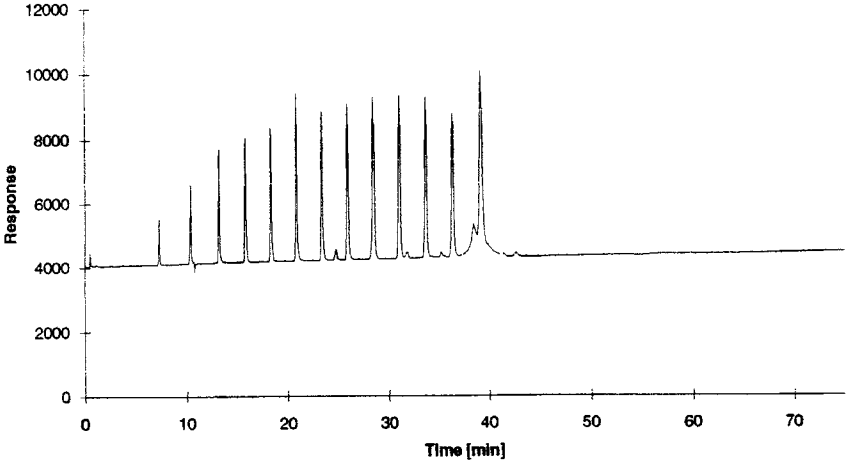


Figure 1d

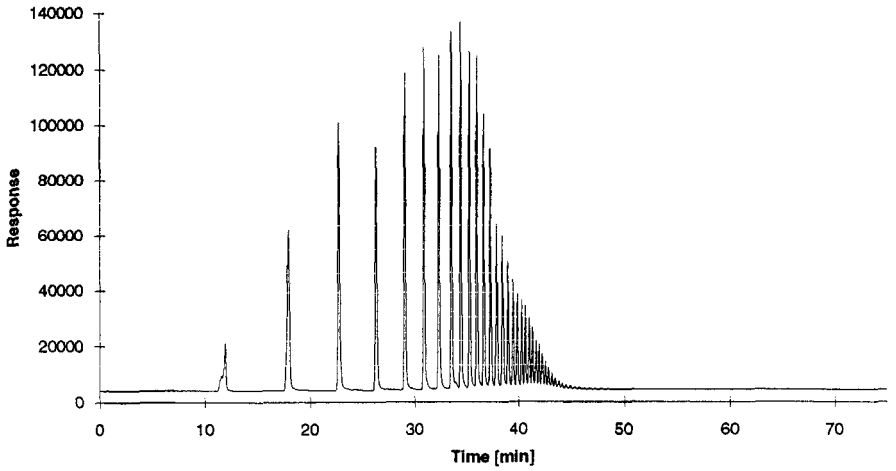


Figure 2a

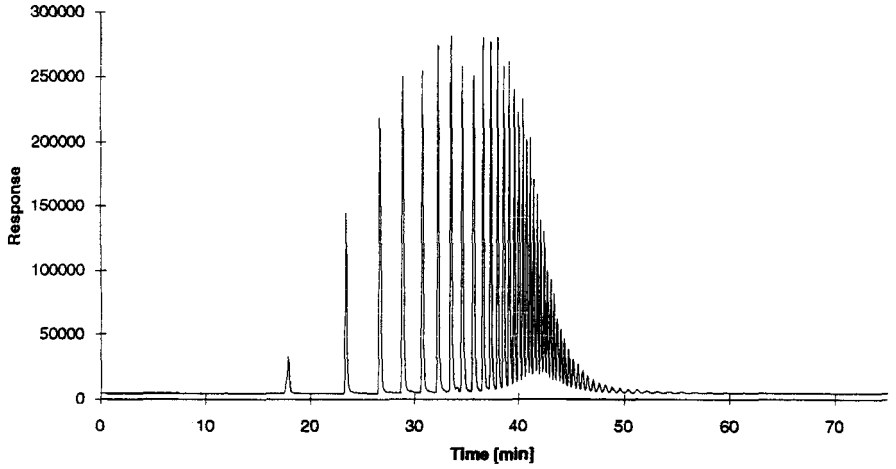
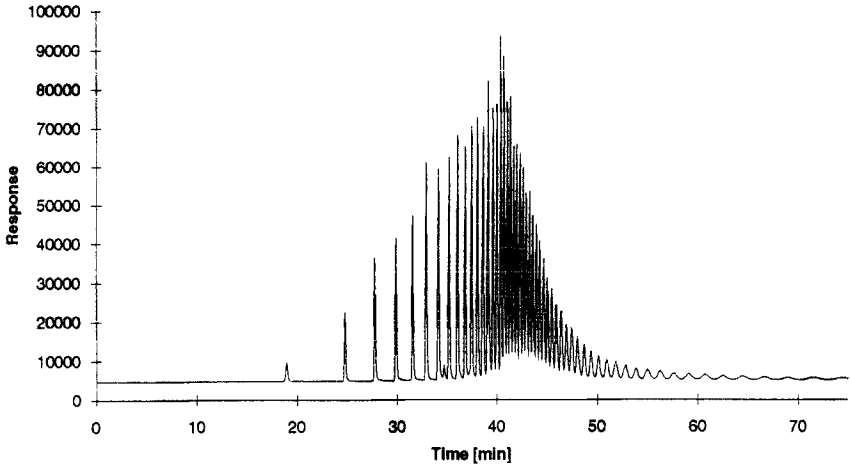


Figure 2b

Figures 2a-d: Chromatogrammes of a) PBG 650, b) PBG 1000, c) PBG 2000 d) PBG 3000 on a C_{18} column and methanol as organic modifier



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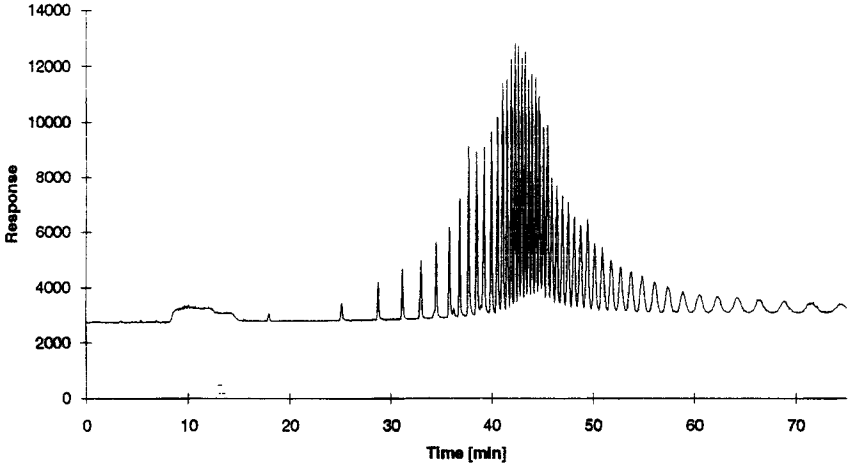

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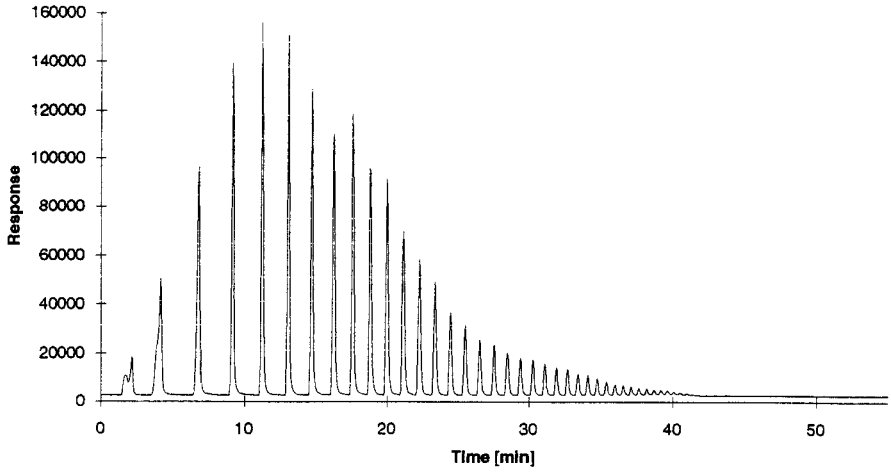

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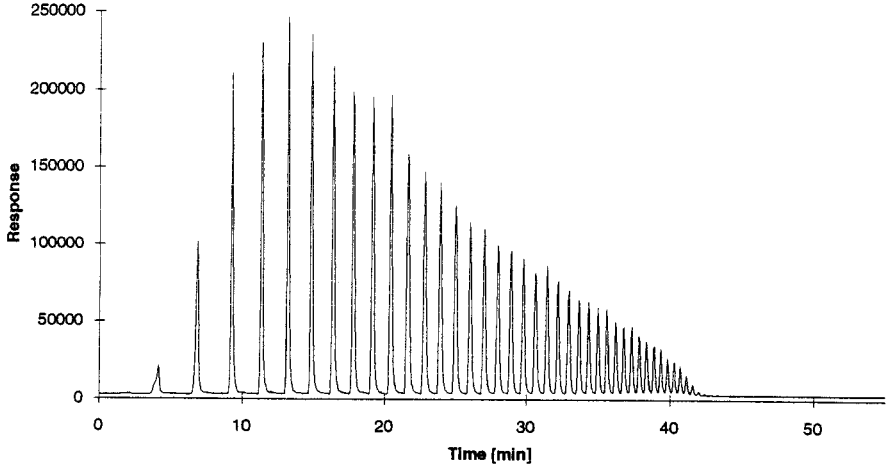
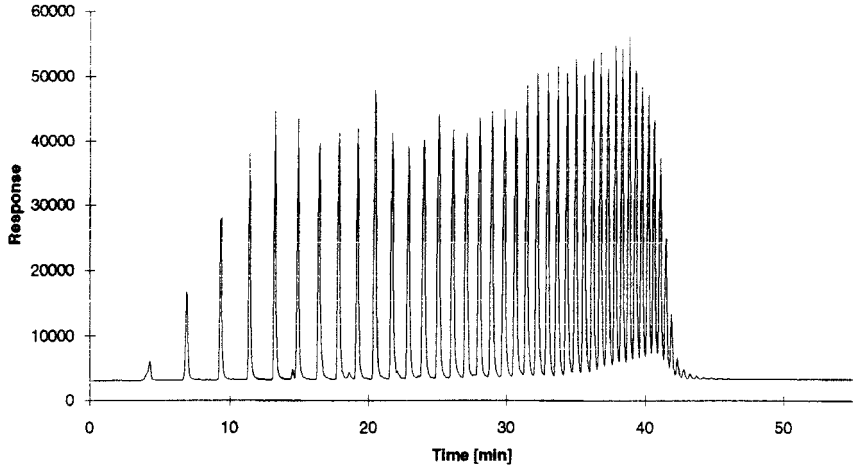


Figure 3b

Figures 3a-d: Chromatogrammes of a) PBG 650, b) PBG 1000, c) PBG 2000 d) PBG 3000 on a C_4 column and acetonitrile as organic modifier



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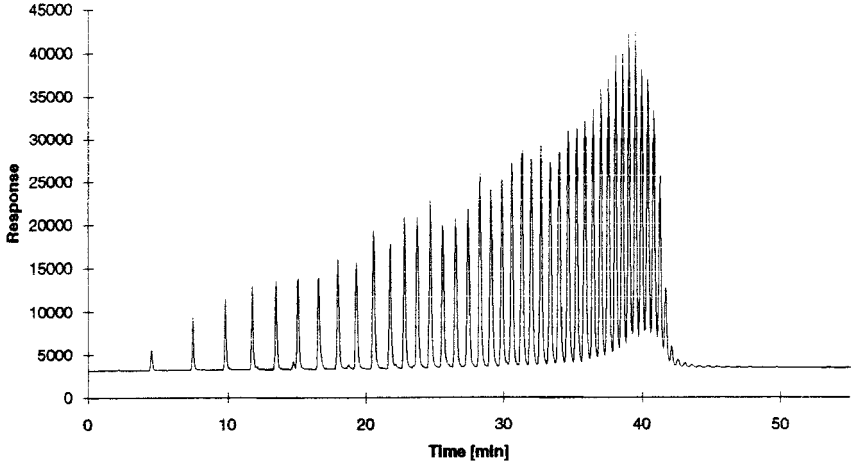

Figure 3d

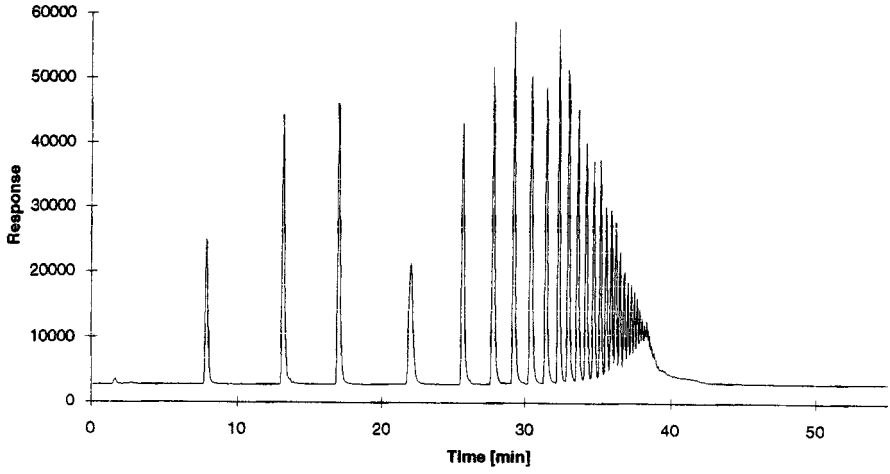

Figure 4a

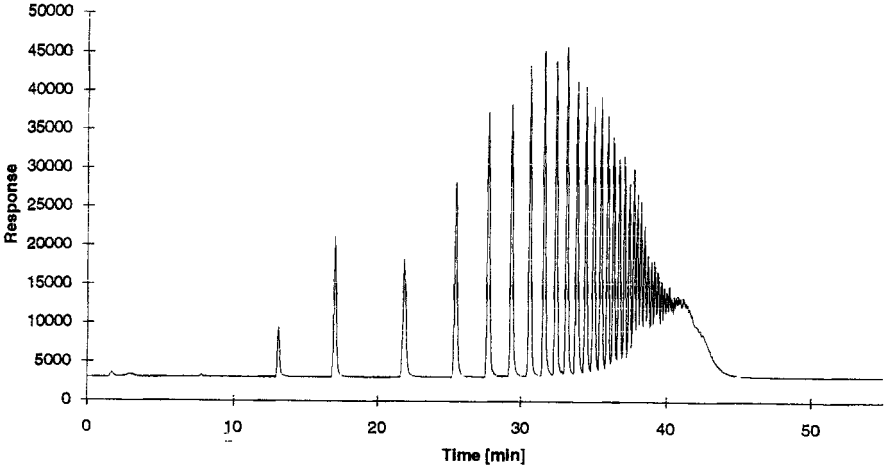

Figure 4b

Figures 4a-d: Chromatogrammes of a) PBG 650, b) PBG 1000, c) PBG 2000 d) PBG 3000 on a C_4 column and methanol as organic modifier

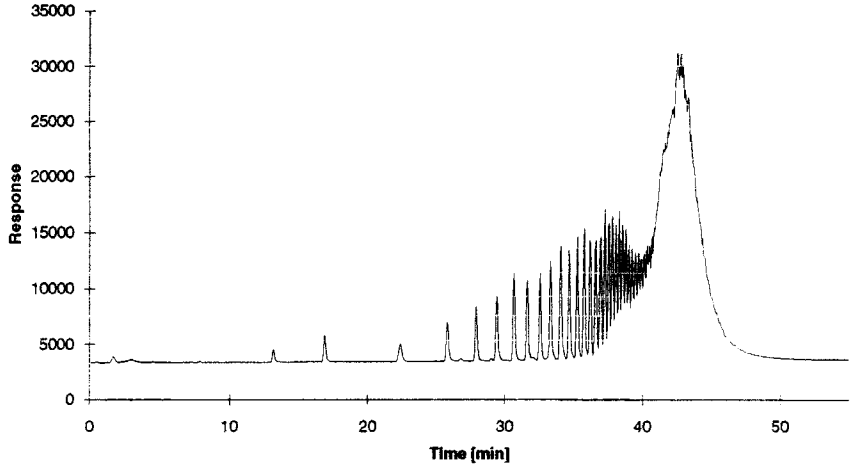

Figure 4c

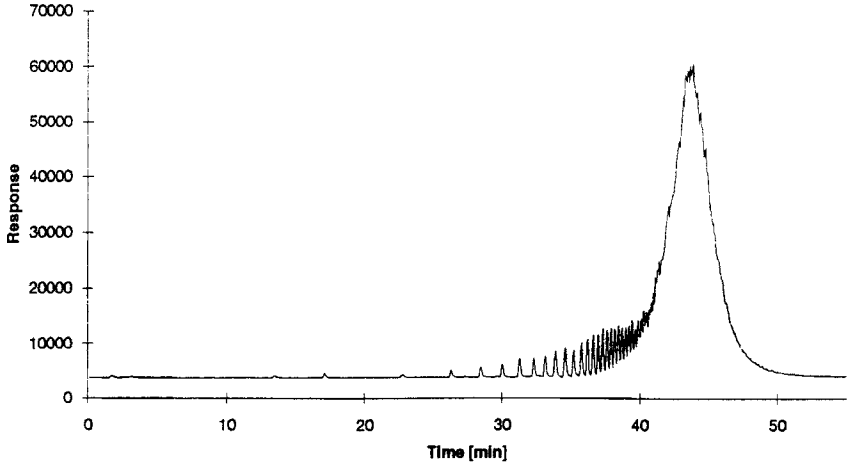

Figure 4d

lower with methanol as modifier resulting in a marked "signal compression" in the range PBG 650 < PBG 1000 < PBG 2000 < PBG 3000 (Figures 4a-d).

DISCUSSION

The native polyethers are not detectable by UV in the usual wavelength range between 210 and 280 nm due to the lack of a chromophor. When wavelength's below 200 nm are used severe baseline deterioration has to be taken into account in particular in gradient chromatography. Nevertheless several authors have described addition of trace amounts (5 ppm) of nitric acid (3) or sodium azide (4) to the aqueous phase to compensate for the baseline drift invoked by the gradual increase of the concentration of organic modifier. On the other hand tagging of the hydroxyl endgroups with a chromophoric agent (1,5,6) makes the polyethers amenable to detection in the usual wavelength range. Signal monitoring on the basis of refractive index (RI) measurement cannot be applied when a solvent gradient is used. In order to avoid an additional derivatisation step we have chosen ELSD primarily based on its wide application range for nonvolatile components, which easily form solid particles after loss of the surrounding solvent shell by nebulisation and subsequent heating of the resulting droplets in the drift tube (7-18). In contrast to low-wavelength UV detection a stable baseline is now obtained, which is either independent on the type of organic modifier or gradient shape (8-10,17). Optimisation of the conditions for signal monitoring of polyethers were described recently (1,2) and used throughout this study.

In most cases of oligomer separation gradient HPLC is required due to the broad M_r distribution of oligomers. Isocratic elution, on the one hand, will yet effect sufficient R_s of low-to-medium M_r oligomers but already a fraction of medium M_r sample constituents will show more and more increased peak broadening and thus neither unsatisfactorily detected nor quantitatively eluted from the stationary phase. On the other hand a mobile phase with stronger elution potency often provides sufficient resolution of high M_r oligomers but signals attributable to low-to-medium M_r oligomers are often unsufficiently separated or even coincide owing to their small retention.

In previous papers (1,2) we have shown that acetonitrile yet provides excellent peak resolution of PBG 1000 oligomers on a C_{18} stationary phase but unfortunately the higher molecular weight sample constituents are not eluted from the matrix. In contrast, methanol as organic modifier effected an almost quantitative "release" of PBG oligomers. We assume that the substantially hydrophobic polyether samples undergo strong interactions with the highly hydrophobic C_{18} chains of the stationary phase. It is known that in n-alkyl bonded silicas the high mobility of the n-alkyl chain results in a liquid-like behaviour of the hydrophobic ligands as was concluded from cross-polarisation magic angle spinning nuclear magnetic resonance experiments (CP-MAS NMR) (19). As a logical consequence the more hydrophobic this "liquid layer", i.e. the longer the n-alkyl chain covalently bound to silanol groups on the surface of the solid silica support, the better its solvation potency towards the relatively hydrophobic PBG samples. Starting from this point of view it will now be easily comprehensible that the power of acetonitrile as a polar and aprotic solvent will be too small to effect elution of medium to high M_r PBG homologues. In contrast, the marked increase of the elution potency of methanol may be ascribed to an "affinity-shift" of solute molecules away from the hydrophobic layer on the silica surface towards the protic solvent methanol due to its ability to undergo hydrogen bonding with PBG ether oxygens (1). This effect may thus provide a substantial solubility increase of the solutes in the mobile phase, which cannot be achieved with the aprotic modifier due to its lack of "exchangeable" protons and, as a consequence, the inability to undergo hydrogen bonding. These conclusions can be drawn from the elution profiles depicted in Figures 1a-d and 2a-d, respectively.

It is clearly shown that methanol effects a marked decrease in retention on a C_{18} matrix compared with acetonitrile but unfortunately a part of the medium and the whole amount of high M_r PBG-2000 and PBG-3000 oligomers still remain on the stationary phase. As reported recently (2) ethanol and isopropanol yet further improve elution of PBG 1000 from a C_{18} phase but the high M_r oligomers coincide and elute as a broad and poorly resolved signal. Hence it might be possible to effect either sufficient R_s or complete elution by combining the separation efficiency of acetonitrile and the excellent eluotropic properties of the protic modifiers ethanol and isopropanol by use of a ternary solvent gradient programme. This would include the start of chromatography with a binary gradient of acetonitrile and water for several

minutes to separate the low M_r oligomers, subsequent admixture of the protic solvent in the gradient mode and a concomitant decrease of the concentration of the aprotic modifier at higher t_R values to achieve quantitative elution of the sample. It should, however, be emphasised that ternary solvent (gradient) systems are only easily applicable when the percentage of one mobile phase component is kept constant, i.e. when small amounts (between 0 and 10 %) e. g. of "modulating" solvent like tetrahydrofuran or dioxan are used (20). In contrast, optimisation of elution conditions would be tremendously complicated when all three components of the solvent mixture are changed simultaneously.

In a former study we tested solute retention with the aprotic modifier acetonitrile on less hydrophobic, i.e. more polar stationary phases like C_8, C_4, C_{phenyl} and C_1 matrices (1). As expected, solvophobic solute-matrix interactions are markedly suppressed in the range $C_{18} > C_8 > C_4 > C_{Phenyl} \cong C_1$ and PBG 1000 was quantitatively eluted from a C_4 matrix. This fact prompted us to apply a gradient-based chromatographic system for the separation of PBGs markedly differing in average M_r and further showing a broad within-sample oligomeric M_r distribution (high polydispersity index M_w/M_n) on a C_4 column by further comparing the effects of the two modifiers acetonitrile and methanol on chromatographic characteristics. On this short-chain matrix the elution power of acetonitrile is now sufficient to afford a complete "release" of the whole amount of oligomers of investigated PBG samples from the stationary phase. This effect may primarily be attributed to the marked decrease in solute-matrix interactions resulting in a relative solubility shift of the solutes towards the aprotic modifier. Fortunately these interactions are still sufficiently high to allow excellent separation of medium-to-high M_r oligomers (Figures 3a-d) without impairment of the R_s of low M_r homologues. For this reason the C_4 column offers a compromise between solute solubility in the stationary and the mobile phase, by which either complete release or separation of all PBG samples is achieved. Further, the substantial differences in the chromatographic patterns allow "recognition" of individual PBG samples within mixtures. In contrast, with methanol the "solvation equilibrium" of solutes in the stationary and mobile phase is markedly shifted towards the protic solvent, as can be concluded from Figures 4a-d. In this case it is assumed that the vast solubility increase with methanol suppresses those interactions between the individual PBG oligomers and the matrix, which are

indispensable for sufficient peak resolution. As the consequence a "levelling" effect occurs affording co-elution of medium-to-high M_r sample constituents in particular for PBG 2000 and PBG 3000 (Figures 4c,d), which now appear in the chromatogramme as broad and unresolved signals. Nevertheless the low and at least a part of the medium M_r oligomers are well-separated and a selective assignment of individual PBG samples within mixtures can be achieved in a similar manner as with acetonitrile (see above).

Further, on the basis of the impressive high resolution chromatographic patterns obtained with acetonitrile on a C_4 matrix, it seems reasonable that HPLC would provide a better means for the determination of the M_n, M_w and M_w/M_n values of polybutylene glycols than GPC as proposed recently by Trathnigg et al. (21) after their HPLC investigations of polyethylene glycols. In this context it may be worthy to note that the M_r measurements of PBGs with polystyrene calibration standards (Table 2) far exceeds the manufacturer's classification, which may be interpreted by a different solvation of calibrator and sample by the solvent tetrahydrofuran. For this reason the similar polarities of solvent and samples will favour formation of solute-solvent associates, which in turn markedly extends the hydrodynamic volume of the PBG samples yielding elevated M_n and M_w values.

CONCLUSIONS

Polybutylene glycol oligomers, which markedly differ in molecular weight as well as in polydispersity can be efficiently separated on a C_4 stationary phase with acetonitrile as organic modifier. Although methanol elicits only poor resolution of high M_r oligomers on this material it nevertheless permits a selective attribution of solutes to an individual PBG sample. On the other hand, the power of acetonitrile is unsufficient to elute the whole amount of medium-to-high M_r homologues of all investigated PBG samples from a C_{18} column and methanol only effects the "release" of the total quantity of PBG 650 and PBG 1000 oligomers from this stationary phase. This observation can be ascribed to a marked "solvation" of the solute by the highly hydrophobic matrix, by which it is tightly retained in the "liquid layer" of n-octadecyl chains covalently bound to the silica gel surface. In contrast, the

short-chain C_4 column affords complete elution of all investigated PBG samples due to lower hydrophobic solvation and a concomitant solubility shift of the solutes towards both modifiers. The "levelling" effect of methanol, which tremendously decreases the R_S of medium-to-high M_r PBG oligomers compared with acetonitrile can be satisfactorily explained by its hydrogen bonding capability, which further enhances the solubility shift towards the protic modifier. As the consequence solute-matrix interactions responsible for satisfactory oligomer separation are substantially suppressed.

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