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Analysis of polyethylene glycol and derivatives by high-performance liquid chromatography using elevated temperatures and low-wavelength ultraviolet detection, and supercritical fluid chromatography

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ABSTRACT

A gradient reversed-phase high-performance liquid chromatography system is described for the characterisation of polyethylene glycols (PEGs), methoxy polyethylene glycols (MPEGs) and methoxy polyethylene glycol methacrylates. The system enables oligomeric distributions to be obtained for these types of materials with average molecular weights of up to 4000 dalton. The requirements to achieve the necessary detection and separation efficiency have been studied including the column type, operating temperature and solvent composition.

The PEGs have been analysed by capillary supercritical fluid chromatography, using carbon dioxide as the mobile phase. The results obtained from the two chromatographic techniques have been compared and the relative merits discussed.

INTRODUCTION

The uses of polyethylene glycols (PEGs) and their derivatives are widespread and include such applications as the production of emulsifiers, surfactants, detergents, cosmetics as well as their uses in foodstuffs and wood preservation. There are therefore, many requirements for accurate material quality control and product characterisation, paticularly for environmental monitoring.

Although high-performance liquid chromatography (HPLC) is the technique of choice for the separation of ethoxylated oligomers, the subsequent detection and quantification has proved to be difficult. The insensitivity of these compounds towards conventional UV detection is due to the lack of chromaphoric structure. The use of refractive index (RI) detection [1,2], whilst having an inherent low sensitivity, also limits any method to isocratic elution and the range of component polarities which can be chromatographed.

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To overcome these problems derivatisation techniques have been used to produce dibenzoates [2], 3,5-dinitrophenylates [3] and phenyl isocyanates [4], and thereafter allow detection by UV. Berry [5] has shown that low-wavelength UV detection is applicable to the determination of compounds with a low or minimal absorptivity, providing almost universal detection. Berry also reported that at 210 nm 0–100% acetonitrile gradient elution could be used with the addition of sodium azide to balance solvent absorbances. However. at lower wavelengths solvent impurities prohibited sensitive detection.

Snyder and Van der Wal [6] have previously reported the separation of polyethylene glycol oligomers of molecular weights up to 1200 dalton, using 0–35% acetonitrile gradients with UV detection at 185 nm. Again the limiting factor being the purity of the acetonitrile, with gradient "humps" and "ghost" peaks being produced when 40% acetonitrile was exceeded.

With the advent of more pure solvents (HPLC and spectroscopic grades), the use of sodium azide for solvent absorbance equalisation at 190 nm, enables the achievement of full range gradient elution. We have found that the use of this type of system, with coupled columns and elevated temperatures (from ambient up to 80°C), has made possible the separation of PEG oligomers of molecular weights up to 5000 dalton. This technique has also been suitable for PEG derivatives such as methoxy PEGs and methoxy PEG methacrylates with molecular weights up to 2500 dalton.

The ability of supercritical fluid chromatography (SFC) to utilise flame ionisation detection (FID), makes it an attractive technique to use for the characterisation of these types of compounds. The comparison of SFC–FID and HPLC (with low-wavelength detection) made here, demonstrates the limitations and advantages of both techniques for this type of analysis.

EXPERIMENTAL

Equipment

The liquid chromatograph consisted of 2 LKB 2150 HPLC pumps with a LKB 2152 gradient controller. The samples were injected by means of a Valco C6W valve, fitted with a 20-μl loop. The detector was a Kratos Spectroflow 757, which was used with a continuous nitrogen purge of the flow cell to reduce ozone build-up. An Anachem column oven (Gilson, Luton, U.K.) was used for the elevated temperature work, and the data system was a Trivector Trilab 3000 (Vinten Analytical, U.K.).

The HPLC columns used included: (i) the cartridge columns (S-5 ODS2) supplied by Phase Separations (Clwyd, U.K.). These are 250 mm × 4.6 mm I.D. stainless-steel, packed with 5-μm octadecyl silane bonded phase material of 80 Å pore diameter; (ii) a column of the same dimensions but packed (in our laboratory) with 5-μm Hypersil WP-300 (Shandon Southern) octyl bonded phase material, which has a pore diameter of 300 Å.

The SFC system was a Lee Scientific 600 with a Lee Mk III flame ionisation detector. The column was a 10 m × 50 μm I.D. SB-octyl capillary column, also purchased from Lee Scientific. The mobile phase used was carbon dioxide (SFC grade) obtained from Air Products. The samples were prepared in dichloromethane solutions, and were injected using timed split injection over 30 ms with a 200-nl loop. All data were collected on a VG Multichrom PDP11/23 data system.

TABLE I

HPLC ANALYSIS CONDITIONS

Analyte	Gradient profile						Temparature (°C)
	Step 1		Step 2		Step 3		
	%B	Time (min)	%B	Time (min)	%B	Time (min)	
PEGs 200–3400 (Fig. 1)	25–40	15	40–55	45	55–60	45	80
PEG 2000 (Fig. 3)	35–50	40	50	20	–	–	60
PEG 4000 (Fig. 2)	45	45	45–60	90	–	–	80
MPEG 2000 (Fig. 5)	35–50	40	50	20	–	–	60
MPEG 2000 methacrylate (Fig. 4)	35–50	40	50	20	–	–	60

Reagents

The polyethylene glycol materials were purchased from BDH (Poole, U.K.) and the sodium azide from Fisons Labs. (Loughborough, U.K.), the acetonitrile (used as solvent B) was far-UV grade from Romil Chemicals (Loughborough, U.K.), and all water (used as solvent A) was purified by means of a Millipore Milli-Q system. The sodium azide was added to the water at a concentration of 5 μg/l. All solvents were continually degassed with helium, and a total flow-rate of 1 ml/min was used for all analyses.

The HPLC analysis conditions, including the gradient profiles, used for the different analytes are summarised in Table I.

RESULTS AND DISCUSSION

A number of specific procedures have been reported in the literature for the analysis of PEG oligomers. The work by Snyder [7] predicted that convex gradient profiles are the most suitable for PEG oligomeric separations. Whilst this may be a requirement for optimal resolution, the simple linear profiles used in this work were found to be adequate for the characterisation of the materials investigated. The combination of two reversed-phase columns as used here, provides a relatively large number of theoretical plates (*ca.* 40 000). This, together with the linear gradients and the elevated operating temperatures has enabled the higher-molecular-weight PEGs to be resolved into individual oligomers, albeit with an increased analysis time. Figs. 1 and 2 show the scope of the method for oligomeric separations, although to achieve the broad molecular weight range, a high sample loading (5 mg of material injected) was used. This necessitated a relatively high detector sensitivity (0.05 a.u.f.s.) which does show some baseline drift. The separation achieved for the PEG 4000 oligomers would appear to be approaching the separation and detection limits of the technique for these compounds. It was found that a relatively long (45 min) initial isocratic period was required to provide high capacity factors (k'). The resulting long column residence times are more suitable for separating high molecular weight compounds with slow mass transfer characteristics. For the analysis of a "single" material (rather than

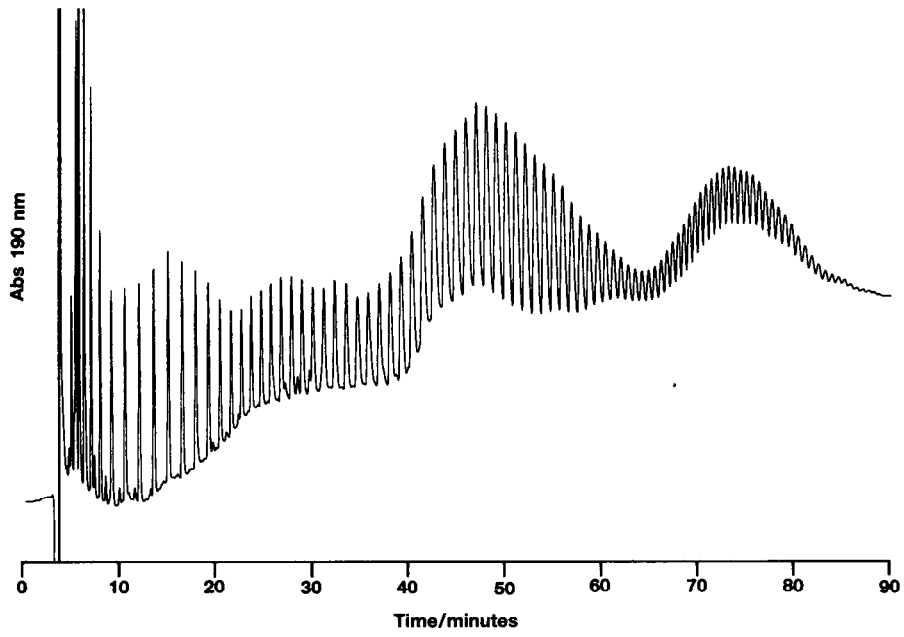

Fig. 1. HPLC analysis of a blended mixture of PEGs (with average molecular weights of 200, 400, 600, 1000, 1500, 2000 and 3400 dalton) carried out on two ODS columns at 80°C and with a detector sensitivity of 0.05 a.u.f.s.

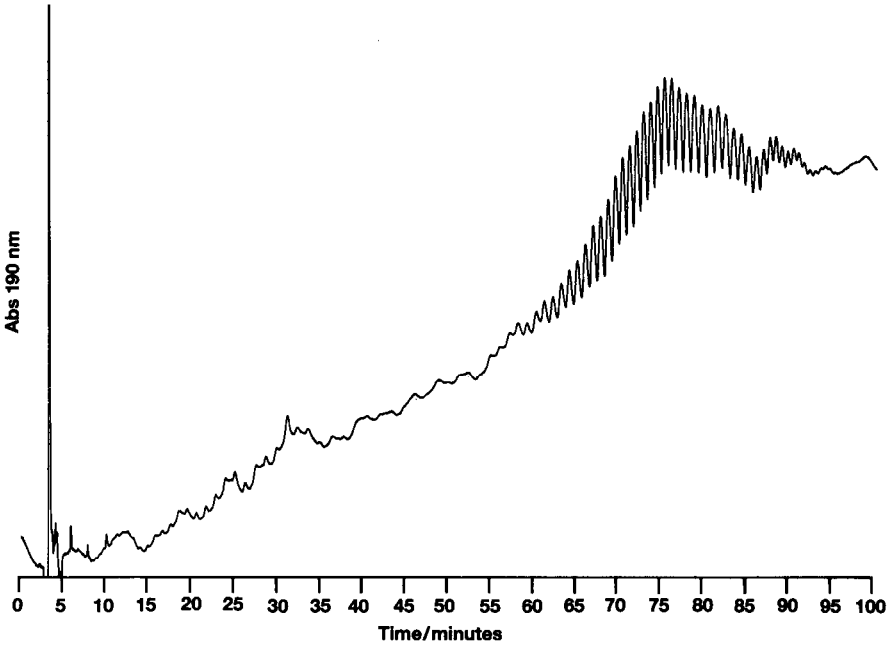

Fig. 2. HPLC analysis of PEG with an average molecular weight of 4000 dalton. The analysis conditions are given in Fig. 1 and Table I.

a blend), a sample size of 1 mg and a detector sensitivity of 0.2 a.u.f.s. provided adequate sensitivity with minimum baseline drift (see Figs. 3–5).

The problems associated with low-wavelength UV detection such as solvent background absorbance and baseline drift, have been documented [5–7]. The minimum useable detection wavelength for current commercial deuterium lamps appears to be 185 nm. Although the sensitivity obtained at this wavelength was significantly better than that at 190 nm, the apparent absorbance of the sodium azide is considerably reduced. This effectively nullifies the solvent absorbance equalisation by the azide and the baseline follows the profile of the gradient used. The use of nitric acid in place of the azide for detection of PEGs at 185 nm has been described [8] and this may provide a more universal detection capability. However, the problems associated with solvent impurities are exacerbated by detection at 185 nm. This has often limited the amount of organic modifier which can be used, and therefore a less polar stationary phase must be selected.

For the system described here, it was found that 100% acetonitrile could be used, if necessary, with adequate sensitivity at 190 nm. However, a maximum of 60% acetonitrile, for the PEGs (Fig. 2) and 50% for the PEG derivatives (Figs. 4 and 5), was all that was required to elute the oligomers up to 4500 dalton. The separations shown here were found to be reproducible, and no interference from "ghost" peaks or "humps" from the gradient profiles were obtained. The background absorbance levels were found to vary with different solvent batches, and hence some baseline drift was obtained without individual solvent absorbance equilisation for each batch.

Gel permeation chromatography (GPC) has also been used, together with

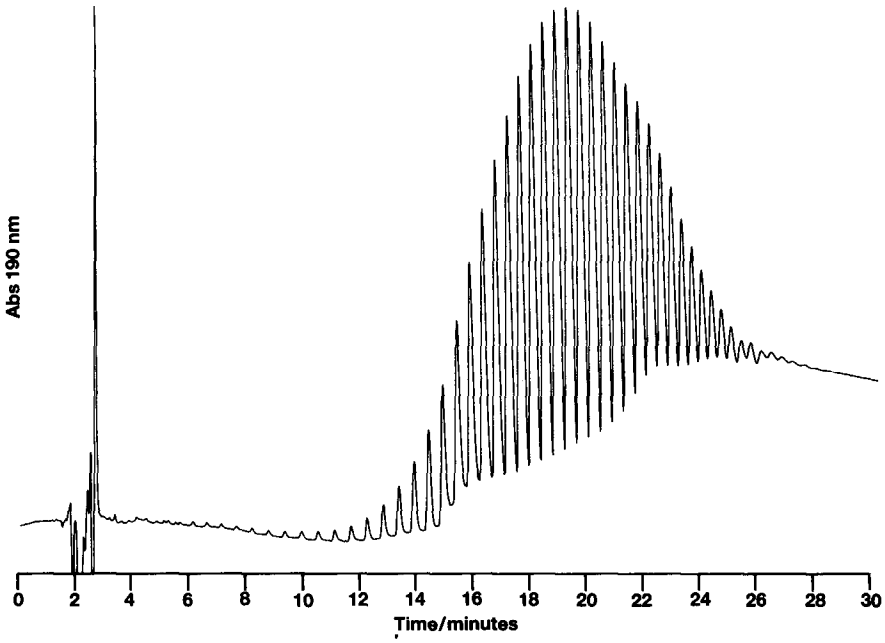

Fig. 3. HPLC analysis of PEG with an average molecular weight of 2000 dalton, carried out on two ODS columns at 60°C and with a detector sensitivity of 0.2 a.u.f.s.

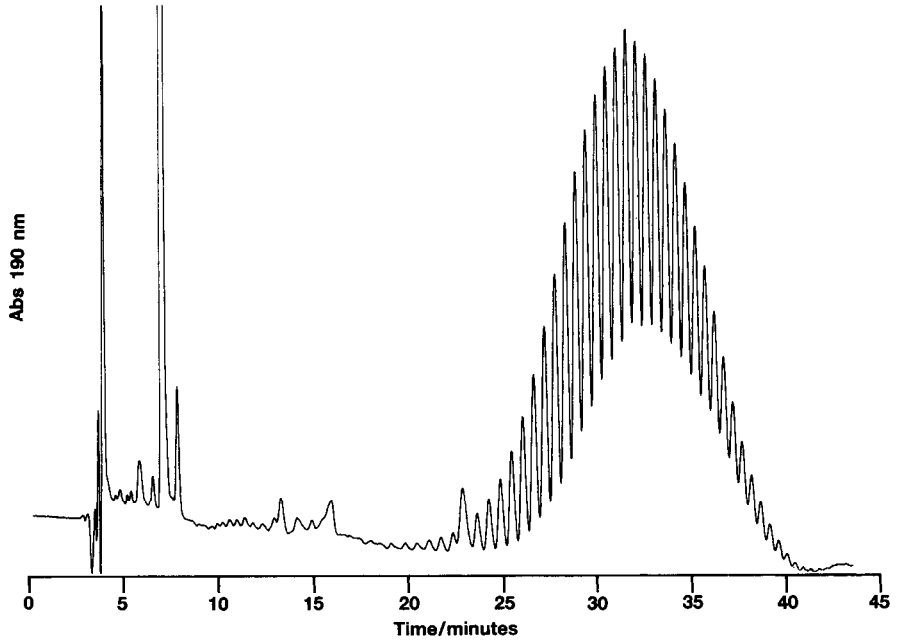

Fig. 4. HPLC analysis of methoxy PEG methacrylate with an average molecular weight of 2000 dalton. The analysis conditions are given in Fig. 3 and Table I.

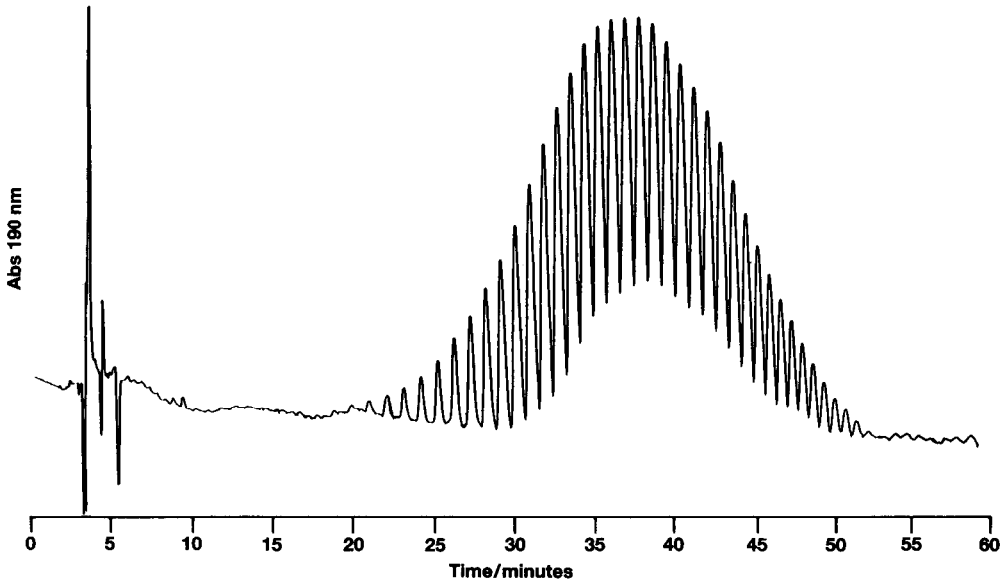

Fig. 5. HPLC analysis of methoxy PEG with an average molecular weight of 2000 dalton, carried out on an ODS column coupled with a 300 Å octyl column at 60°C, and with a detector sensitivity of 0.2 a.u.f.s.

low-wavelength UV detection, for the analysis of PEGs [9]. GPC alone was not able to separate the oligomers of PEGs >2000 dalton, and a mixed mode separation mechanism of GPC and adsorption was postulated with a combination of 100 Å and 300 Å columns. The pore size of the column packing does not appear to have a large effect on the separation obtained by adsorption chromatography. As a part of this work, the results obtained from the analysis of PEG 2000, MPEG 2000 and MPEG 2000 methacrylate achieved on two 80 Å ODS columns (Fig. 3), were not significantly different from those obtained from an 80 Å ODS coupled with a 300 Å octyl column (Fig. 5). Thus it would appear that at elevated temperatures, the large PEG molecules have suitable mass transfer characteristics for interaction with conventional (80 Å pore diameter) bonded phase HPLC columns.

Temperature effects

The operating temperature can have a profound effect on chromatographic resolution. This is due to the temperature dependence of the phase capacity ratio k', which is usually expressed in terms of the ratio of the solute concentration in stationary (C_s) and mobile (C_m) phases:

$$k' = \frac{C_s V_s}{C_m V_m} \tag{1}$$

where V_s and V_m are the volumes of stationary and mobile phases.

The exponential dependence of k' on temperature (T) is such that changes in operating temperature can have a significant effect on component retention and resolution:

$$\ln k' \propto \frac{\Delta H}{RT} \tag{2}$$

where ΔH is the standard enthalpy of sorption and R is the molar gas constant.

As values of ΔH are usually negative, an increase in temperature will result in a decrease in k', *i.e.*, C_m increases producing shorter retention times. A sequence of HPLC analyses for PEG 2000 spiked with four probe compounds, with increasing operating temperature is shown in Fig. 6. The probe compounds (benzamide, phenol, *p*-cresol and anisole) behave in a typical manner with retention decreasing with a rise in temperature. However, the reverse is observed with the PEG 2000 components, the resolution and retention being significantly increased with 20°C increments of temperature. It is clear that the simple equation above (eqn. 2) cannot be applied to PEG materials directly.

The irregular retention behaviour of relatively short chain ethoxylated oligomers has been studied in detail by Melander *et al.* [10], and it has been shown [11,12] that these oligomers can exist in at least two conformational forms. One of these is a compact dihedral helical structure (called the meander form) and the other is an extended open coil (called the zigzag form), each having intrinsically different retention characteristics. An equilibrium exists between these forms which is affected by several factors: (i) the ethoxylate chain length; (ii) temperature; and (iii) solvent composition.

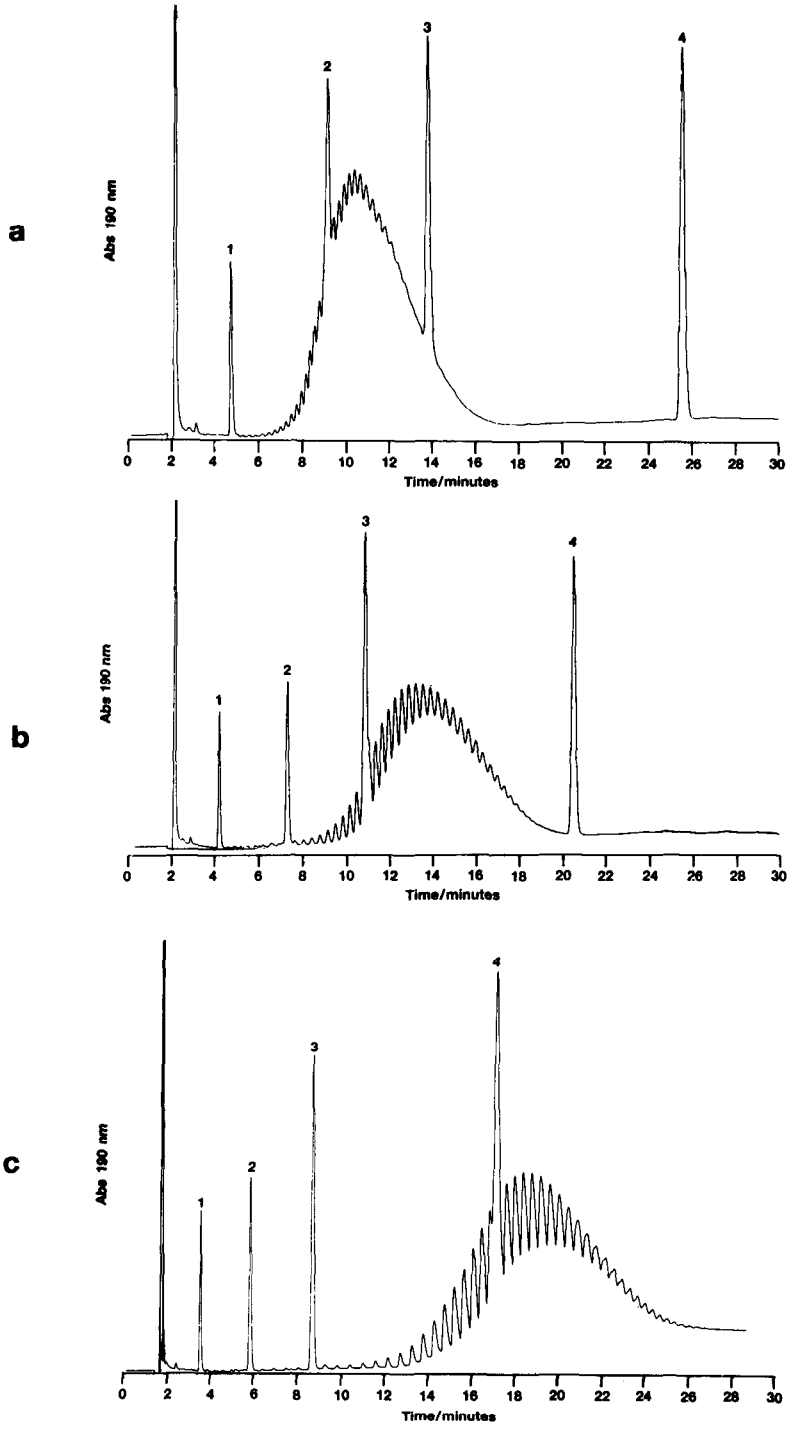

Fig. 6. HPLC analysis of PEG with an average molecular weight of 2000 dalton, spiked with a probe compound mixture: 1 = benzamide; 2 = phenol; 3 = *p*-cresol and 4 = anisole. The two ODS columns were used at (a) ambient temperature, (b) 40°C and (c) 60°C.

The most significant effect, for the analyses of the PEGs and PEG derivatives described here, was that from the column operating temperature. At elevated temperatures the equilibrium is in favour of the zigzag conformation, which has a larger molecular surface area than the corresponding meander form. The work by Melander and co-workers [10,13] has shown that retention factors, observed in reversed-phase chromatography, will increase with increasing molecular surface area, provided all other conditions are identical. The increased resolution obtained is likely to be due to two factors: (i) the higher effective column efficiencies at higher temperatures, which are realised by the decreased viscosities and increased solute diffusion coefficients [14], and (ii) the better interaction of the linear zigzag conformation with the linear alkyl groups of the bonded phases. .

Analysis by SFC

For this work a 10 m × 50 μm I.D. SB-octyl capillary column was used, which provided approximately 60 000 theoretical plates. The chromatograph was operated at a temperature of 100°C with carbon dioxide as the mobile phase, and with a pressure programme from 100 to 400 bar over 30 min. The results obtained showed good resolution for the lower molecular weight PEG oligomers, and PEG 200, 400 and 600 (see Fig. 7) could be fully characterised. The analysis times were significantly less, *e.g.*, 30 min for PEG 600 by SFC, compared to 50 min by HPLC. However, for PEGs with average molecular weights greater than 600, the resolution obtained decreased rapidly. This is illustrated by the results obtained for a blended mixture of PEG 200, 400, 600 and 800 (see Fig. 8). The later eluting higher-molecular-weight oligomers are detected as a very broad unresolved envelope. This could be due in part to the poor solubility of the higher-molecular-weight oligomers in carbon dioxide. Modifiers, such as methanol, could be added to the carbon dioxide to improve the solubility, but the choice and concentration of modifier is somewhat limited by FID.

CONCLUSIONS

The present quality of commercial HPLC and spectroscopic-grade solvents, and

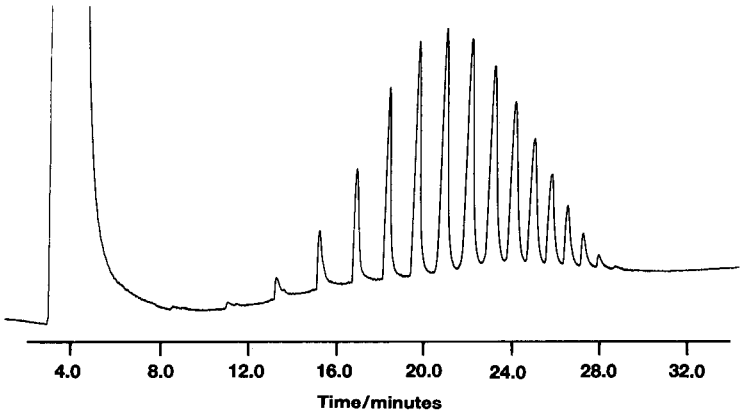

Fig. 7. SFC analysis of PEG with an average molecular weight of 600 dalton.

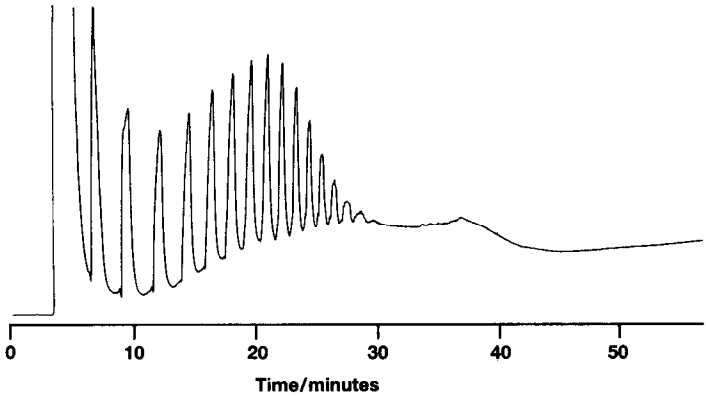

Fig. 8. SFC analysis of a blended mixture of PEGs with average molecular weights of 200, 400, 600 and 1000 dalton.

UV detection systems enables sensitive, full range acetonitrile–water gradient elution to be achieved, with a detection wavelength of 190 nm.

HPLC with low-wavelength detection can be used to characterise polyethylene glycols, methoxy polyethylene glycols and methoxy polyethylene glycol methacrylates in terms of their oligomeric distribution. The system developed has enabled materials containing oligomers of 4500 dalton to be analysed.

For low-molecular-weight materials (<800 dalton), SFC–FID is the preferred technique as it provides a more straighforward procedure and shorter analysis times.

The use of coupled 80 Å, 5-μm reversed-phase columns at elevated temperatures, provides the necessary separation media for these compounds without the use of wide pore or GPC stationary phases. It is postulated that the linear zigzag conformation of ethoxylated materials (which is induced at higher temperatures) can interact more effectively than the meander form, with conventional bonded reversed-phase materials.

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