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Note

High-performance liquid chromatographic analysis of polyethylene glycols

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Polyethylene glycols (PEGs) and their derivatives are used widely in the cosmetic and food industries[1], and are the active components of many surfactants and detergents[2]. Native PEGs have also been used in medical research, as inert fluid phase recovery markers[3] or membrane permeability probes[4,5] because of their physicochemical and biological properties and low toxicity[6,7]. Consequently, the determination of concentrations of PEGs in chemical formulations or biological fluids has attracted a great deal of attention.

The classical methods for extraction of PEGs[8,9] have been supplemented by faster and more reproducible column chromatographic techniques on chemically modified silica[10,11] and by partition chromatography on cellulose supports[12].

More recently, high-performance liquid chromatography has been applied to this analytical problem. Medium- and high-molecular-weight PEGs (mol.wt. 1000–40,000 daltons) have been analysed by size exclusion chromatography on a variety of derivatized silica supports[13] and low-molecular-weight PEGs have been analysed by reversed-phase chromatography on octadecyl silica[14] and macroporous organic sorbents[15].

Medium-sized PEGs (mol.wt. 4000 daltons), used as fluid phase markers in biological experiments[3], have been measured by a turbidometric technique[16]. More recently, low-molecular-weight PEGs (400 daltons), used as permeability markers in intestinal permeability studies in man[5,17] have been analysed by a gas chromatographic technique[4].

The current study describes both the analysis of a spectrum of PEGs of low and medium molecular weight (400–4000 daltons), by high-speed gel permeation (GPC) and reversed-phase chromatography, and a rapid and quantitative method of derivatization of PEGs in order to introduce a UV-absorbing group and thus increase the limits of detection of these compounds.

EXPERIMENTAL

Apparatus and materials

Analyses were performed on a Waters Assoc. (Milford, MA, U.S.A.) series 200 gradient liquid chromatograph, consisting of two M6000A solvent delivery systems, a U6K universal injector, and an M660 solvent programmer. Column effluent was

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monitored with either a Waters R401 differential refractometer or a Waters M440 absorbance detector operating at 254 nm. Detector output was monitored with a Pye-Unicam AR25 linear chart recorder, and a Trivector Systems (Sandy, Great Britain) Trilab-2 computing integrator. The GPC columns used were a μStyragel column (10 μm, 300 × 7.8 mm I.D., 500 Å porosity), purchased from Waters Assoc., and a PLgel column (10 μm, 600 × 7.7 mm I.D., 1000 Å porosity), purchased from Polymer Laboratories (Shawbury, Great Britain). These columns were used in series, the 1000-Å column being connected to the injector. The reversed-phase columns used were Spherisorb 10-ODS and 5-ODS (both 250 × 4.4 mm I.D.) purchased from HPLC Technology (Wilmslow, Great Britain).

Tetrahydrofuran (analytical-reagent grade, stabilized with 0.1% hydroquinone), benzoic anhydride (analytical-reagent grade), triethylamine, perchloric acid (72%, analytical-reagent grade), Dowex AG-50W cation-exchange resin, triethylene glycol (Trigol), and PEGs 400, 1500, and 4000 were purchased from BDH (Poole, Great Britain). Methanol (analytical-reagent grade) was purchased from Rathburn Chemicals (Walkerburn, Great Britain). All solvents were degassed with helium prior to use.

Methods

Samples of native PEGs 400, 1500 and 4000 for analysis by GPC were made up in tetrahydrofuran at concentrations of 10 mg/ml of each component (*i.e.*, weight ratio 1:1:1), and injection volumes were 100 μl, giving a column loading of 1.0 mg of each component. Elution from the coupled gel columns was accomplished with tetrahydrofuran at a flow-rate of 1.0 ml/min. Components of the mixture were identified by comparison of their elution volumes with those of individually injected components, and by spiking with these components.

Derivatization of PEG mixtures for detection at 254 nm was accomplished as follows. A standard solution of PEGs 400, 1500 and 4000 was made up, in dichloromethane to give final concentrations of 10 mg/ml for PEG 400, 37.5 mg/ml for PEG 1500 and 100 mg/ml for PEG 4000 (*i.e.*, molar ratio 1:1:1). A sample (0.1 ml) of this solution was transferred to a dry test tube and the solvent was evaporated in a stream of dry nitrogen to leave an intimate mixture of the PEGs. Benzoic anhydride (100 mg) and anhydrous triethylamine (1.0 ml) were then added to the test tube, and the resultant mixture was incubated for 60 min at 60°C. Residual reagent was then destroyed by the addition of freshly-prepared 4 *N* perchloric acid (1.0 ml) and incubating at 60°C for a further 30 min. The mixture was then diluted with water (2 ml) and acidified by the addition of Dowex AG 50W cation-exchange resin (2 ml). The derivatization mixture was then extracted with dichloromethane (5 ml) and the extract was concentrated under a stream of dry nitrogen. The syrupy residue was then adjusted to 2 ml with dichloromethane and 20 μl of this final solution was injected into the chromatograph. This represents 10 μg of PEG 400, 37.5 μg of PEG 1500 and 100 μg of PEG 4000 injected, as their dibenzoates.

Analysis on Spherisorb 10-ODS was accomplished with a solvent gradient from 65% solvent B (pure methanol) in solvent A (methanol–water, 1:9) to 100% solvent B over 12 min on M660 programme 7 (exponent curve, $m = 2$). Analysis on Spherisorb 5-ODS was accomplished with a solvent gradient from 55% Solvent B in solvent A to 100% solvent B over 80 min on M660 programme 8 (exponent curve, $m = 3$).

Component PEG dibenzoates were identified by comparison with retention times of authentic samples (prepared by the method of Carey and Persinger[18], using benzoyl chloride in pyridine) and by spiking with these samples.

RESULTS AND DISCUSSION

Analysis of a mixture of native PEGs 400, 1500 and 4000 on the coupled gel permeation columns gave good separation of the mixture, with each component PEG eluting as a sharp, well-resolved peak (Fig. 1, I). Unfortunately, the limits of detection (by differential refractometry) in this system were only in the sub-milligram range, and attempts to improve on this were thwarted by the inherent instability of the detection system.

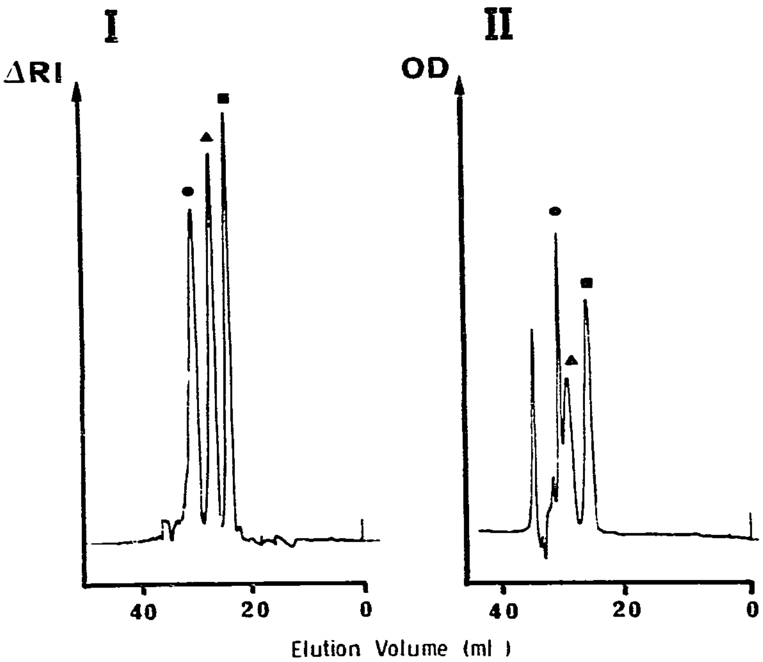

Fig. 1. GPC analysis, on coupled 500-Å and 1000-Å columns (void volume, 18 ml; total elution volume, 36 ml) of (I) native PEGs (detection by differential refractometry; $2 \cdot 10^{-4}$ RI units full scale) and (II) benzoylated PEGs (detection at 254 nm; 0.2 A.U.F.S.) for PEG 400 (●), 1500 (▲) and 4000 (■). Eluent: tetrahydrofuran; flow-rate 1.0 ml/min.

Dibenzoate derivatives of the PEGs were synthesized (by the method of Carey and Persinger[18]) and analysed by the GPC system, but with UV-absorbance detection. Unfortunately, whilst these derivatives had the potential of extending the limits of detection, the integrity of the chromatography was lost (Fig. 1, II). It would appear that the gel columns were no longer operating in purely a size-exclusion mode, and indeed such organic polymer columns have been used in a reversed-phase mode[19].

A rapid method for the derivatization of mixtures of small amounts of PEGs was developed and is described fully in the *Methods* section. Briefly, it consisted of incubating the PEG mixture with benzoic anhydride in the presence of triethylamine at 60°C for 60 min, followed by quenching of excess anhydride with 4 *N* perchloric acid. After acidification with a cation-exchange resin (this improves storage stability of the derivatized PEGs at −15°C), the reaction mixture was extracted with dichloromethane, and the extract was concentrated under a stream of dry nitrogen prior to injection into the chromatograph. The derivatized mixture of PEGs was analysed on either a 10- or 5-μm octadecyl reversed-phase column.

Analysis, on Spherisorb 10-ODS, of a derivatized mixture of PEGs 400, 1500 and 4000 gave three well-resolved peaks with retention times of 13.7, 16.2 and 18.0 min, respectively (Fig. 2, I). External standardization of the amounts of derivatives with previously prepared standards showed the method to be quantitative. The chromatogram also showed a consistent pattern of early peaks (up to 12 min) which were ascribed to reaction by-products of the derivatization procedure (*Note:* these by-product peaks were also present in "blank" derivatization mixtures, *i.e.* without any PEG).

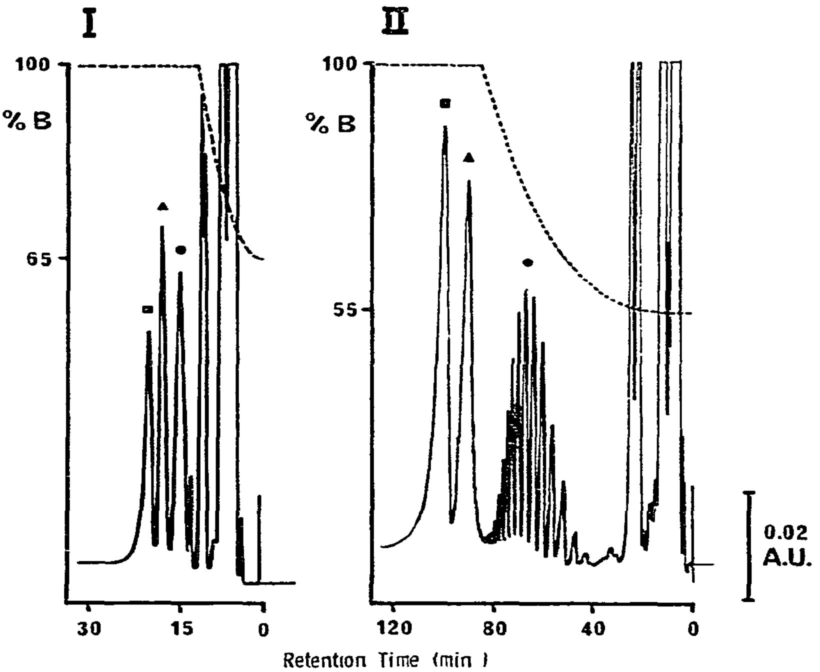

Fig. 2. Reversed-phase analysis of PEG 400 (●), 1500 (▲) and 4000 (■) dibenzoates on (I) Spherisorb 10-ODS, and (II) Spherisorb 5-ODS. Gradient profile denoted by dashed lines [solvent A, methanol–water (1:9); solvent B, methanol]. Detection at 254 nm (0.1 A.U.F.S.). Flow-rate 1.0 ml/min.

The same derivatization mixture, when analysed on Spherisorb 5-ODS with a much longer gradient time, again gave the derivatives of PEGs 1500 and 4000 as single, well-resolved peaks, with retention times of 80.0 and 88.0 min, respectively. The PEG 400 dibenzoate derivative was, however, split into its component oligomers, ranging in molecular weight from PEG 150 (triethylene glycol) to PEG 722 (hexadecylethylene glycol), as shown in Fig. 2, II. The retention times of these oligomers are listed in Table I. The molecular weight of PEG 150 was assigned by co-chromatography with an authentic sample of triethylene glycol dibenzoate, and the ensuing peaks were assigned as homologous oligomers with stepwise increase in molecular weight of 44 daltons. The identity of three of the largest peaks, corresponding to dibenzoates of PEGs 370, 414 and 458, was confirmed by mass spectral analysis of fractions collected from the chromatograph (University of London, Mass Spectrometry Service).

TABLE I

RETENTION TIMES OF PEG 400 DIBENZOATE OLIGOMERS [HO(CH_2CH_2O)$_n$H] ON SPHERISORB 5-ODS

Gradient elution as per Fig. 2, II.

Retention time (min)	*PEG mol.wt.*	*n*	*Retention time (min)*	*PEG mol.wt.*	*n*
35.0	150*	3	61.5	458**	10
39.0	194	4	64.5	502	11
43.0	238	5	66.5	546	12
47.5	282	6	68.0	590	13
51.5	326	7	70.0	634	14
55.5	370**	8	71.5	678	15
58.5	414**	9	72.5	722	16

* Assigned by comparison with derivatized triethylene glycol.
** Confirmed by mass spectrometry.

The limit of detection of the dibenzoate derivatives of PEGs when analysed in this mode is in the range of 5–10 nmoles (the UV response, being a molar dimensional response, varies with molecular weight) and consequently, coupled with the rapid derivatization procedure described, provides a sensitive and accurate method for detecting small amounts of PEGs of low to medium molecular weight.

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REFERENCES

1 Carbowax, *Polyethylene Glycols for Pharmaceuticals and Cosmetics*, Union Carbide Chemicals, New York, 1959.
2 G. F. Longman, *Talanta*, 22 (1975) 621.
3 B. Borgstrom, A. Dahlqvist and G. Lundh, *J. Clin. Invest.*, 36 (1957) 1521.
4 V. S. Chadwick, S. F. Phillips and A. F. Hofmann, *Gastroenterology*, 73 (1977) 241.

5 V. S. Chadwick, S. F. Phillips and A. F. Hofmann, *Gastroenterology*, 73 (1977) 247.
6 H. F. Smyth, C. P. Carpenter and C. B. Shaffer, *J. Pharm. Sci.*, 34 (1945) 172.
7 H. F. Smyth, C. P. Carpenter and C. S. Weil, *J. Pharm. Sci.*, 44 (1955) 27.
8 K. Bürger, *Z. Anal. Chem.*, 196 (1963) 22.
9 B. Weibull, *3-ème Congrès Internationale de la Détergence, Cologne*, Vol. III, 1960, 121.
10 J. Pollerberg and E. Heinerth, *III. International Kongresse Grenzfläche Akt. Stoffe*, 3 (1960) 89.
11 K. F. Blomeyer, *Fette, Seifen, Anstrichm.*, 71 (1969) 240.
12 E. A. Taylor, *J. Chromatogr.*, 64 (1972) 71.
13 H. Engelhardt and D. Mathes, *J. Chromatogr.*, 185 (1979) 305.
14 W. R. Melander, A. Nahum and Cs. Horváth, *J. Chromatogr.*, 185 (1979) 129.
15 M. Čoupková, K. Janě, J. Sanitrák and J. Čoupek, *J. Chromatogr.*, 160 (1978) 73.
16 S. Hyden, *Ann. R. Agr. Coll. Sweden*, 11 (1955) 139.
17 E. A. Fagan, D. Bentley, R. Murphy and V. S. Chadwick, *Clin. Sci.*, 60 (1981) 5p.
18 M. A. Carey and H. E. Persinger, *J. Chromatogr. Sci.*, 10 (1972) 537.
19 M. J. Causer, Polymer Laboratories, Shawbury, personal communication.