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Note

Simple method for gas—liquid chromatographic analysis of polyethylene glycol 400 in biological fluids

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Polymers of ethylene glycol (PEGs) are used extensively in the study of intestinal physiology, since their high solubility in water and low toxicity [1—6] make them useful as non-radioactive tracers. A wide range of molecular weights, 200—20,000, is available; the polymers used commonly include PEG 400, 600, 1500, 4000 and 6000 (numbers refer to the average molecular weight of commercial mixtures, which usually consist of 9—15 polymers). The PEG 4000 polymer has been found to be essentially inert in and not absorbed by the intestine [5, 6]; thus, it has been the standard aqueous marker for studies of intestinal absorption and transit [7—9]. Polymers of lower molecular weight are also useful: PEG 400, which is a common food additive, has been used in physiological studies as a marker of intestinal permeability [10, 11].

We have previously described [10] a method for the analysis of PEG 400 by gas—liquid chromatography (GLC) in biological fluids, and have applied the technique to the study of human physiology and disease [11]. However, wide application of these approaches is restricted potentially by methods available for the analysis of PEG 400 in biological fluids, particularly urine. Our previous method [10] involved acetylation and extraction of the samples, a rather tedious and lengthy process. Also, some samples produced by this method appear to be stable for only a few hours.

Here we report a simpler method for the analysis of PEG 400 by GLC. The method does not require extensive sample preparation and, consequently, a slow and somewhat difficult assay is rendered faster and easier.

STANDARDS AND REAGENTS

Polyethylene glycol 400 (stated by the manufacturer to be of "average molecular weight 380—420") was purchased from Matheson, Coleman and Bell (Norwood, OH, U.S.A.). The internal standard used was tetraethylene glycol, molecular weight 194.23, (No. A8A) from Eastman Kodak Company (Rochester, NY, U.S.A.); it was assessed for purity by repeated GLC analysis, which always yielded a single peak. The ion-exchange resin, used for urine samples, was Amberlite MB-3 (H^+ OH^-) from Mallinckrodt (St. Louis, MO, U.S.A.).

Urine standards were used when analyzing urine samples and deionized water or saline (8.5 g of NaCl per liter) standards were used when analyzing water or saline solutions. The standards were prepared to yield concentrations of PEG 400 ranging from 2 to 16 mg/ml. All solutions were frozen until needed.

PROCEDURE

Standards and samples

After thawing, 10-ml aliquots of the standards and samples were pipetted into 50-ml Pyrex tubes containing 10 ml of MB-3 resin. Tubes were covered with Parafilm®, mixed well, allowed to stand for 5 min and mixed again. After settling, 5 ml of the deionized sample were pipetted into 20-ml scintillation vials. One milliliter of the internal standard solution (25 mg of tetraethylene glycol per ml of water) was added to both the standard and sample. The vials were capped, mixed well, frozen and then lyophilized. When dry, the samples were reconstituted in acetone and transferred to injection vials.

Gas—liquid chromatography

Two glass columns (50 cm × 2 mm) were silanized, then packed with 1% PolyS-179 on Gas-Chrom Q 100—120 mesh (Applied Science Labs., State College, PA, U.S.A.). They were conditioned overnight at 310°C with a helium flow-rate of 20 ml/min.

Analyses were performed on a Hewlett-Packard 5840A gas chromatograph in the dual column (bleed-correction) mode. The injector temperatures were 275°C and detector temperatures were 350°C. The temperature program started at 125°C and was isothermal for the first 4 min. Temperature was increased at 5°C/min until a final temperature of 310°C was reached. This higher temperature was maintained for 6 min, whereupon the analysis was considered complete and the oven cooled for the next analysis.

The data system was calibrated with duplicate standards to yield a report based on the internal standard. The absolute amount of PEG 400 in the sample was obtained from the 5840A report, and the percentage composition of the PEG 400 was calculated from the peak areas.

RESULTS

Fifteen urine standards of varying concentrations (prepared from the same lot number of PEG 400) were analyzed on different days using different columns. An average coefficient of variation of less than 10% was obtained from

these data. This compared to an average coefficient of variation of 8% for data that were generated from multiple samples analyzed on the same column on the same day. Multiple injections of the same sample onto multiple columns yielded data with a coefficient of variation of less than 5%. The graph of the percentage composition of PEG 400 is shown in Fig. 1. There is a Gaussian distribution of the components according to their molecular weights.

Table I gives the least-squares fit of the standard curves for all components relative to tetraethylene glycol internal standard. The relationships for all the PEG 400 components are linear in the range of concentrations used.

Fig. 2 shows a chromatogram of a deionized urine sample, showing the Gaussian distribution of the PEG 400 components. Also, the lack of any added peaks illustrates the specificity of the method for polyethylene glycol.

PEG 400 was administered in doses of 5 g, dissolved in 20 ml water. Urine was collected in 6-h periods following administration. An average of 23% of the PEG 400 dose was recovered in the first 6 h of urine collection, confirming earlier quantitative recoveries [10].

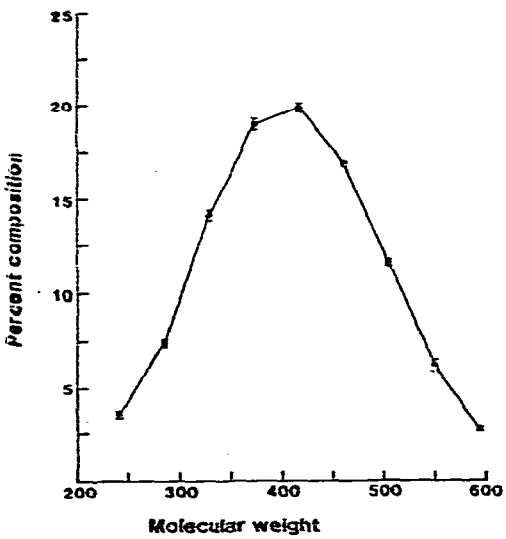

Fig. 1. The percentage composition of PEG 400, measured by GLC. Mean (± S.E.M.) for 15 estimations of samples from the manufacturer's same lot are shown, illustrating the Gaussian distribution of molecular weights.

TABLE I

LEAST-SQUARES ANALYSIS OF STANDARD CURVES FOR INDIVIDUAL PEGs

Ethylene oxide units	Correlation coefficient	Slope	Intercept
5	0.999	0.848	4.85
6	1.000	1.80	− 0.56
7	1.000	3.53	− 8.13
8	1.000	4.70	−12.3
9	0.999	4.89	−16.2
10	0.998	4.05	−21.9
11	0.994	2.63	−24.2

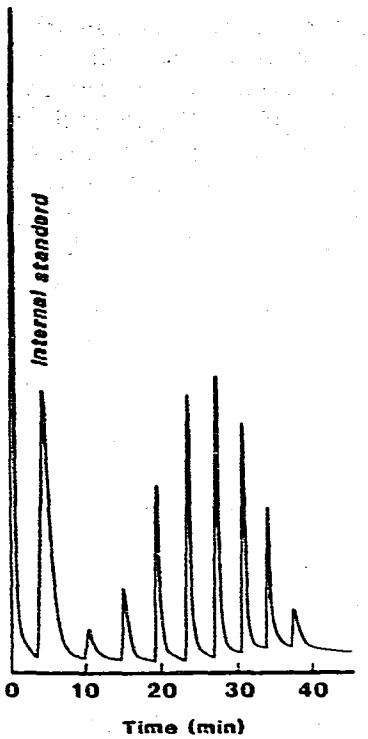

Fig. 2. Chromatogram of PEG 400 in urine using a 50 cm × 2 mm I.D. glass column packed with 1% PolyS-179. Injector temperature was 275°C, detector temperature 350°C. The initial temperature of 125°C was increased after 4 min at a rate of 5°C/min to 310°C. The internal standard was tetraethylene glycol.

DISCUSSION

PEG 400 is a clear viscous liquid that dissolves readily in water to form a colorless solution. It is tasteless in solutions of low concentration and is nontoxic [3, 4]. Moreover, PEG 400 is excreted rapidly and quantitatively in urine [10, 11], and thus recovery from urine can be used to quantify absorption after oral administration. These factors and the availability of an easy, accurate method for simultaneously quantifying several components of the mixture make PEG 400 useful as a differential permeability marker in the study of intestinal absorption.

The number and amount of each component polymer of PEG 400 varies with the batch (for example, different lot numbers). PEGs are prepared by hydroxide-initiated polymerization of ethylene oxide; therefore, there is a batch-to-batch variation in the number of PEG homomers, depending on the exact conditions under which the reaction was performed. The manufacturer labels the bottle of PEG 400 as containing "average molecular weight 380—420" because of these lot-to-lot variations. The data presented in this paper were obtained from standards and samples all containing the same lot number. However, in all experiments, it is essential to characterize the particular lot being used.

The differences in the coefficients of variation for groups of data collected from multiple samples analyzed on different columns, multiple samples analyzed on the same column, and a single sample analyzed on multiple columns, indicate that the differences in columns do not appear to affect the reproducibility of this method as much as does the sample handling. It is likely that the major variant is the degree of redissolution achieved for each sample. Since high volatility of the solvent and free solubility of PEG 400 are desired, acetone was selected to fulfill these qualifications among the solvents commonly available.

The standard curve showed linearity for each PEG 400 species in the concentration range 2–16 mg/ml based on tetraethylene glycol, the internal standard. However, the y-intercept values listed in Table I show that the standard curves of constituent molecules do not intercept the y-axis at zero; intercepts begin at 4.85 and decrease to negative values with increasing molecular weight. The possible causes for this phenomenon include decreasing detector response with increasing molecular weight, an increased boiling point with increasing molecular weight, or optimal efficiency of the column for a particular polymer (hexaethylene glycol had a y-intercept closest to zero, −0.56). Since the biological application is usually a ratiometric one, the non-zero intercept is of no practical concern. Our method is capable of detecting accurately amounts of PEG 400 as low as 1 mg/ml of urine. Samples below this limit could be handled by lyophilizing more than 5 ml of urine; we have used amounts of up to 10 ml.

The other constituents of urine do not appear to affect the GLC analysis of PEG 400. Treatment with ion-exchange resin removes most of the components that would cause unwanted peaks. The first few centimeters of column also trap other nonvolatile components and will darken upon injection of urine samples. Column life can be extended by replacing the darkened region with fresh packing when needed. As many as 75 samples have been analyzed before the column needed complete repacking.

Since we believe interest in the use of PEG 400 as an intestinal permeability marker will be stimulated by the work of Chadwick et al. [10, 11], a more convenient method for the analysis of PEG 400 in urine should have general use. The present approach appears to be much faster and more convenient, has an acceptable sensitivity and gives reproducible results.

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