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Analysis of polyethylene glycols with respect to their oligomer distribution by high-performance liquid chromatography

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ABSTRACT

The analysis of average molecular masses and molecular mass distributions by means of liquid chromatography is usually performed with size-exclusion columns. With mathematical methods the average molecular mass of the sample and its distribution are then calculated by comparison with standards. For polyethylene glycols (PEGs), a simple way to separate the oligomer species by isocratic elution with reversed phase columns was found. They can be identified and analysed quantitatively. The measurements were performed for PEGs with molecular masses from 300 up to 2000 g/mol. The results for several commercially available PEGs are presented. A comparison between a large-scale PEG and a PEG standard is shown, which demonstrates that polymer standards must be handled with care.

INTRODUCTION

In recent years, aqueous polymer two-phase systems have become increasingly interesting. These liquid two-phase systems are made by mixing two so-called incompatible polymers such as polyethylene glycol (PEG) and dextran (Dx) or a polymer and a salt in water [1,2]. Two water-rich phases form without the addition of any organic solvents, and these systems have quite interesting properties for the extraction of biochemically manufactured products from fermentation broth. Many efforts have been made to analyse and understand the phase equilibrium behaviour depending on temperature, pH and type of polymer [3–5]. Earlier work reported a fractionation of the polymer between the phases, *i.e.*, the average molecular mass of the polymer used is different in the phases after establishing equilibrium. With a view to the precise analysis of these type of equilibria, a method has been developed to analyse the PEGs with respect to the type and amount of each oligomer species. In contrast to the frequently used size-exclusion columns, from which the average molecular mass and its distribution can only be estimated with the help of polymer standards and mathematical methods [6,7], a reversed-phase column was used to separate and identify the oligomer species from each other. The separation of the oligomer species is achieved by isocratic elution. This is advantageous because the equipment is cheaper and a refractive index (RI) detector can be used without problems. The separation of the oligomer species is important for the development of thermodynamic models for the aqueous two-phase-systems considering the polydispersity of the PEG.

Throughout this paper, polyethylene glycols are specified as PEG300, PEG1000, etc., where the number is the approximate average molecular mass in g/mol, based on mass fractions as given by the distributor. Single oligomers are

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characterized in the same way, but with their precise molecular mass and in addition with the number of their $-CH_2CH_2O-$monomer units; for example, PEG370(8) means octaethylene glycol.

EXPERIMENTAL

Chemicals

All chemicals used were commercially available. The polyethylene glycols were industrial scale products (trade name Lipoxol) and were obtained from Hüls (Hamburg, Germany), except the PEG standard PEG445, which was purchased from Polymer Standard Service (PSS) (Frankfurt, Germany). Mono-, di-, tri- and tetraethylene glycols were obtained from Merck (Darmstadt, Germany). Methanol was purchased from Riedel-de Haën (Seelze, Germany) and had a purity of better than 99.8%. Demineralized water was used after double distillation.

Equipment

An isocratic HPLC system was set up for the analysis. The solvent was methanol–water with different volume fractions of methanol, depending on the type of polymer to be analysed. The HPLC system consisted of the components listed and described in Table I. As PEG cannot be detected by UV absorption, an RI detector had to be used. Integration was performed with a simple integrator.

Chromatographic conditions

A high-precision RI detector was used. It was automatically heated to a fixed temperature of 40°C, so that the whole analysis was performed at this temperature. To ensure maximum baseline stability the injection valve and the column were also held at a constant temperature (40°C). The tube connections from the column to the detector were insulated to avoid disturbance from room temperature changes. Before entering the column a side stream of the solvent, which was pumped at 1 cm^3/min, was separated by a simple T-piece to supply continuously the reference cell of the detector. The flow through the reference cell was controlled by a needle valve. The samples were detected by measuring the refractive index relative to this reference. To ensure reproducible conditions, the pressure at the pump, which depends naturally on the position of the needle valve, was held constant.

The quality of the separation of the oligomer species depends strongly on the methanol-to-water ratio in the solvent. At a constant ratio in an isocratic system small molecules leave the column first and are detected as sharp more or less separated peaks. With increasing chain length the peaks become wider and show tailing effects. Very large molecules are highly diluted and are only detected with difficulty. This limits the described method to PEGs up to PEG2000. Gradient elution was tested but not taken into account, because this causes problems with the

TABLE I

COMPONENTS OF THE HPLC SYSTEM

Component	Model and company	Description/conditions
Degasser	ERC, Type 3312	Automatic online membrane degasser
Pump	Merck–Hitachi, HPLC pump 6000	0–400 bar, 0.01–9.99 cm^3/min
Injection valve	Rheodyne, Type 7215	Standard six-way valve, 20-μl sample loop
Column	Merck, LiChrospher 100 RP-18	Standard reversed-phase column
Thermostat	Merck–Hitachi, Type T-6300	Air thermostat for columns, 30–99°C
Detector	ERC, Type 7511	High-precision RI detector, automatic temperature control at 40°C
Integrator	Merck–Hitachi, Type D-2500	Standard HPLC integrator

RI detector and is not necessary for the smaller PEGs. For every polymer analysed the optimum methanol-to-water ratio had to be found and these are given in Table II. Samples were prepared by dissolving the polymer in an amount of 1–5% (w/w) in the ready-mixed solvent.

Ethylene and di-, tri- and tetraethylene glycols were available as pure substances. Analysing lower-molecular-mass PEG, e.g., PEG300, and comparing the retention times of the peaks with those of the pure components led easily to the identification of these special species in the PEG. From this the other species were identified by sequentially counting the peaks. The retention times increase regularly with increasing chain length. Oligomer species in higher molecular mass PEG were then found by comparison of the retention times with those from PEG300, and so on.

The amount of each oligomer was calculated from the peak area. It was found that the detector signal is linearly dependent on the mass fraction of polymer in the sample, but independent of the molecular mass in the studied range. This could be verified by injecting equally concentrated solutions of different molecular masses and solutions of different concentration and the same molecular mass. Here only a simple calibration relating the total area of all peaks of an analysis to the overall mass fraction of PEG in the sample was needed.

RESULTS AND DISCUSSION

Table II gives the calculated average molecular masses from the analysis and the range of oligomers found. In PEG300 twelve species of oligomers were detected from ethylene glycol to dodecaethylene glycol. The number of species increases with increasing average molecular mass M_r. The error is about 2%. It is convenient to do the calibration runs several times and take the average number of counts to minimize the error.

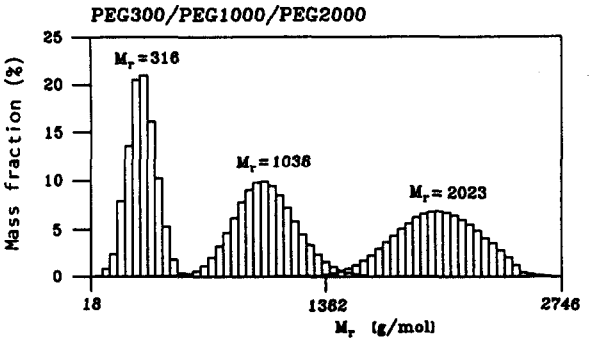

Fig. 1. Histogram of amounts of oligomer species in PEG300, PEG1000 and PEG2000.

Fig. 1 demonstrates the distribution of the molecular mass of the oligomers from PEG300, PEG1000 and PEG2000 in the form of histograms. The mass fraction of each species in the polymer (not in the sample solution) is shown as the function of molecular mass. These are results for what we call "clean" polymers. In the chromatograms only a sequence of consecutive oligomer peaks with increasing and subsequently, after a maximum, decreasing peak areas are found. Fig. 2 shows such a run for PEG2000.

TABLE II

METHANOL-TO-WATER RATIOS FOR THE DIFFERENT POLYMERS ANALYSED

Polymer	Methanol in solvent (vol.%)	M_r (g/mol)	Range of oligomers
PEG300	20	316	PEG62(1)–PEG546(12)
PEG400	20	417	PEG150(3)–PEG590(13)
PEG445 (standard)	20	344	PEG194(4)–PEG590(13)
PEG550	45	1034	PEG150(3)–PEG1954(44)
PEG1000	50	1038	PEG502(11)–PEG1602(36)
PEG2000	50	2023	PEG1118(25)–PEG2658(60)

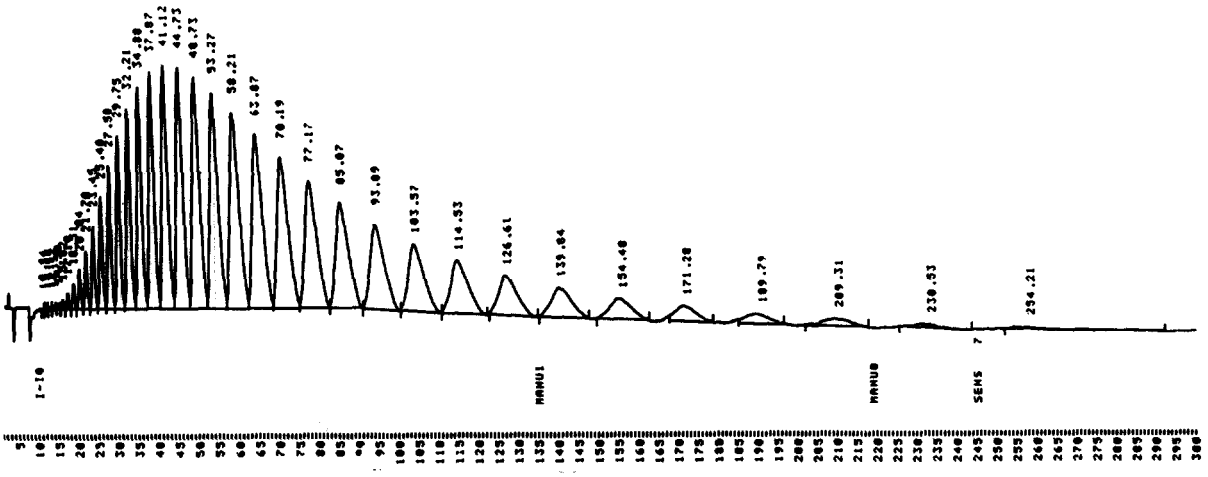

Fig. 2. Chromatogram for PEG2000.

Most of the chromatograms from the Hüls polymers analysed in this work were similar to that in Fig. 2.

Some surprising results were obtained for a few types of polymers. Fig. 3 shows an analysis of PEG550 from Hüls. Here obviously a polymer with a lower molecular weight of around 450 g/mol has been blended with an amount of high-molecular-mass polymer (around 1500 g/mol). As shown in Table II, the obtained weight-average molecular mass of 1034 g/mol differs significantly from 550 g/mol. To verify this surprising result, the average molecular mass of the PEG sample was determined by the viscosimetric method of Staudinger [8]. With PEG600 and PEG2000 as standards it was found that the average molecular mass of the PEG550 sample was *ca.* 960 g/mol. The molecular mass estimated by the Staudinger method is nearly a weight-average value. An inquiry at Hüls con-

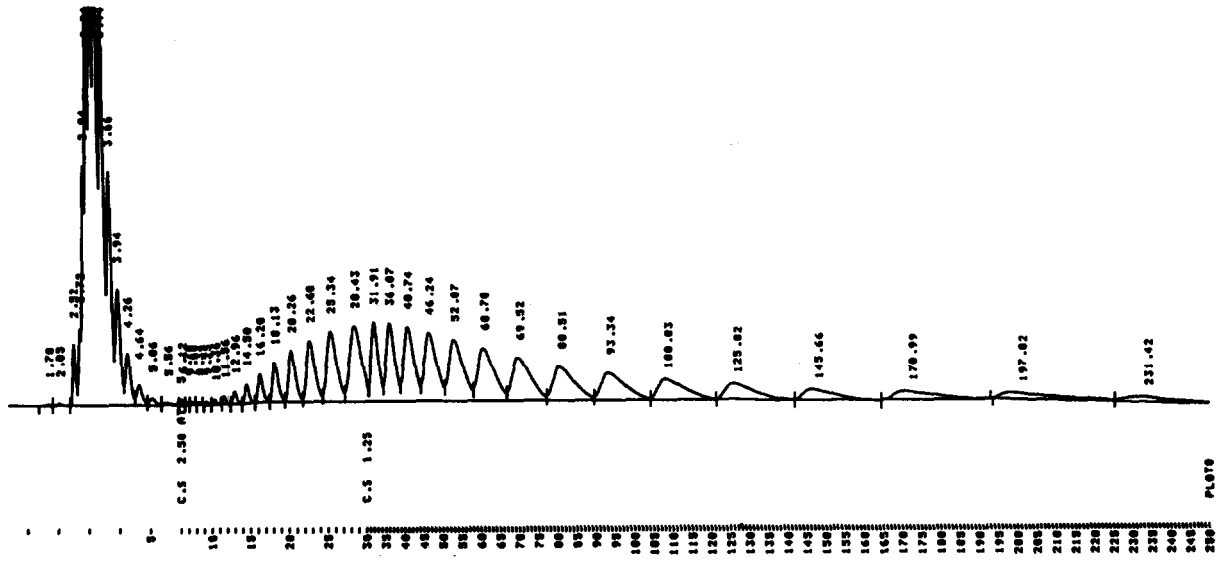

Fig. 3. Chromatogram for PEG550.

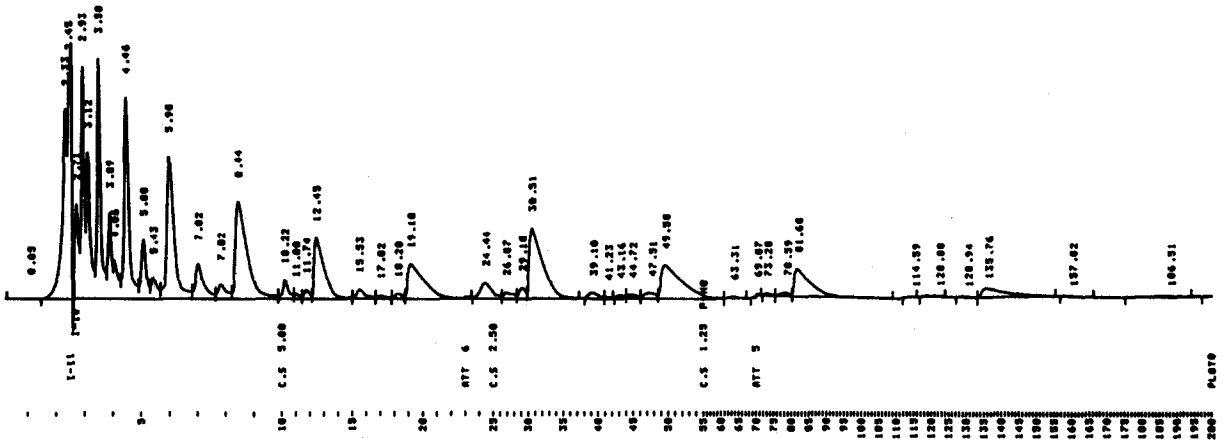

Fig. 4. Chromatogram for PEG445 from PSS.

firmed our measurements. Their PEG550 is a mixture of two different PEGs.

Another example is shown in Fig. 4, where a commercially available PEG445 standard from Polymer Standard Service (PSS) was analysed. Polymer standards are expected to have a narrow molecular mass distribution. Instead, this sample had nearly the same distribution as the Hüls PEG400 (see Table II). In addition, the analysis showed the presence of impurities, which could not be identified. It should be pointed out that an industrial-scale PEG costs less than US$5 per 1000 g, whereas the price for 1 g of a PEG standard is *ca.* US$50.

These examples show that even with basic HPLC equipment the composition of polyethylene glycols can be analysed with good accuracy in the range investigated. It is obvious that large amounts of impurities or the use of blends of polymers instead of "clean" products can have significant effects on the behaviour of the aqueous two-phase systems. This and the differences between specified and real molecular masses explain the difficulties that arise when trying to reproduce phase equilibrium measurements published by other workers. Also, researchers who want to develop a thermodynamic model to describe these systems often find large deviations when comparing their calculation results with data from different sources. The average molecular mass and the weight distribution of the polymers depend strongly on the method of production and can change from batch to batch.

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