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Polymer analysis using size-exclusion chromatography with coupled density and refractive index detection

VI. Molecular mass dependence of preferential solvation of polyoxyethylenes in chloroform with different ethanol contents

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ABSTRACT

It is shown that preferential solvation of polyoxyethylenes with hydroxy and methoxy end-groups in chloroform containing ethanol takes place mainly at the hydroxy end-groups, but also along the main chain. A method is described that allows a separate determination of the adsorbed ethanol molecules per repeating unit and per end-group.

INTRODUCTION

It is well known that in a solution of a polymer in a mixed solvent, the composition of the latter within the polymer coils will be different from outside because of different interactions of the polymer chains with the components of the solvent. This effect, which is called preferential solvation [1–5], is often neglected in size-exclusion chromatography (SEC) because most chromatographers consider their mobile phase to be pure.

If one takes into account that HPLC solvents are, however, typically less than 99.9% pure, *e.g.*, some of them, such as tetrahydrofuran (THF), are hygroscopic [4] and others contain a stabilizer, such as chloroform, which contains up to 1% of ethanol (Aldrich, Fluka, Zinsser) or 2-methylbutene (Merck, Janssen, Riedel-de Haën), then the concentration of a second component can be considerably higher than the concentration of a sample. In the chromatographic column, the zone of "dialysed solvent" [1] is separated from the solute molecules and a "ghost" or vacant peak [1,2] appears (vacant peaks can, however, arise for other reasons also [2]).

If a non-specific detector, such as a refractive index (RI) detector, is used, its response will not only represent the concentration of the eluted polymer, but will also contain a contribution of the preferential solvation. Hence the response factor of the polymer will be only an apparent value. As long as the contribution of preferential solvation is the same over the entire peak, the accuracy of the molecular mass averages will not be affected.

As has already been shown [6], the response factors vary within a homologous series of poly-

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mers with molecular mass owing to different contributions of the repeating unit and end-groups. It is very likely that also the interaction of the repeating unit and the end-groups with the components of the solvent can differ considerably, hence the preferential solvation will also depend on molecular mass.

In this work, we tried to separate these effects in order to evaluate the influence of molecular mass on the true response factors and on the preferential solvation.

RESPONSE FACTORS AND MOLECULAR MASS

Within a homologous series of polymers, specific properties, such as partial specific volume, refractive index and refractive index increment, vary with molecular mass because they are composed of the different contributions of the repeating unit and end-groups. This dependence can be described by the relationship

$$x_M = x_R + M_E(x_E - x_R)/M \quad (1)$$

where x_M, x_R and x_E are the properties of a polymer chain with the molecular mass M, of the repeating unit R and the end-groups E, and M_E is the molecular mass of the end-groups [7–13]. With

$$M_E(x_E - x_R) = k \quad (2)$$

one may write

$$x_M = x_R + k/M \quad (3)$$

This equation has been demonstrated to describe the molecular mass dependence of specific properties of polymers [7–13].

As the response factors in density and RI detection are closely related to specific properties (apparent specific volume and refractive index increment, respectively), their variation with molecular mass can be described by

$$f_P = A_P/m_P = f_R + K/M_P \quad (4)$$

where f_P is the response factor and A_P is the area obtained for each interval of the peak, within which the mass m_P is eluted [6] (the molecular mass M_P of the fraction is obtained from the SEC calibration); f_R is the response factor of the repeating unit and K is a constant reflecting the difference between the repeating unit and end-groups.

For a sample with infinitely high molecular mass, the term K/M_P disappears. Hence the response factor f_∞ of such a sample equals the response factor f_R of the repeating unit (in a plot of f_P *vs.* $1/M_P$, f_R is the intercept and K the slope of the regression line).

In a previous paper [6], we described different approaches for the determination of the parameters f_R and K and the effect of compensation of response factors on the dependence of their molecular mass on the molecular mass averages, and the effect of compensating the molecular mass dependence of response factors on the molecular mass averages.

PREFERENTIAL SOLVATION IN CHROMATOGRAPHY

When a polymer is dissolved in a mixed solvent, the polymer coils will be solvated preferentially as the components of the solvent have a different affinity for the polymer.

In chromatography, the absorbed solvent will move together with the polymer chain. Hence one has to consider two different situations. (a) If the solution passes the detector directly without separation, the detected area will reflect only the concentration of the polymer sample, whether preferential absorption occurs or not, and *true response factors* will be obtained. (b) On a chromatographic column the polymer peak will be separated from zone of "dialysed" solvent [1,2], hence the detected area will contain contributions from the polymer sample and the preferentially absorbed solvent. Hence *apparent response factors* will be found.

MOLECULAR MASS DEPENDENCE OF PREFERENTIAL SOLVATION

If a polymer is separated on a chromatographic column, preferential solvation will have different effects on the parameters f_R and K in eqn. 4, whether it takes place at the end-groups or at the repeating units (or both): preferential solvation of the end-groups will only influence the slope K without affecting the intercept f_R; preferential solvation of the repeating units will influence both the slope K and the intercept f_R.

If a mass m_P of a polymer is eluted together

with a mass m_S of a preferentially adsorbed solvent, the apparent peak area A_P^* of the polymer is given by

$$A_P^* = m_P f_P + m_S f_S \quad (5)$$

where f_P and f_S are the true response factors for the polymer and solvent, respectively. If other sources of ghost peaks (*e.g.*, evaporation) can be excluded, the sum of the apparent polymer peak area A_P^* and the area of the solvent peak A_S should equal the true peak area A_P, which is obtained when the polymer is injected into the bypass (without a column):

$$A_P^* + A_S = A_P \quad (6)$$

From the apparent peak area A_P^*, an apparent response factor f_P^* will be obtained:

$$f_P^* = A_P^*/m_P = f_P + f_S m_S/m_P \quad (7)$$

Combination of eqns. 4 and 7 yields

$$f_P^* = f_R + K/M_P + f_S m_S/m_P \quad (8)$$

or, in terms of the numbers of polymer and solvent molecules (n_P and n_S, respectively), and the corresponding molecular masses M_P and M_S:

$$f_P^* = f_R + K/M_P + f_S n_S M_S/(n_P M_P) \quad (9)$$

If one considers the different possible sites of preferential solvation, the total number n_S of preferentially adsorbed solvent molecules consists of the molecules adsorbed at the end-groups ($n_{S,E}$) and along repeating units of the chain ($n_{S,R}$):

$$n_S = n_{S,E} + n_{S,R} \quad (10)$$

Hence one may write

$$f_P^* = f_R + K/M_P + f_S M_S(n_{S,E} + n_{S,R})/n_P M_P \quad (11)$$

As the number of repeating units n_R is given by their molecular mass M_R, the number of polymer molecules n_P, their molecular mass M_P and the molecular mass M_E of the end-groups, one may write

$$n_R = n_P(M_P - M_E)/M_R \quad (12)$$

and

$$f_P^* = f_R + K/M_P + (f_S M_S n_{S,E}/n_P M_P) + [f_S M_S n_{S,R}(M_P - M_E)/n_R M_R M_P] \quad (13)$$

It should be mentioned that M_E is the sum of both end-groups: for polyethylene glycols it is 18 (H–OH), for polyoxyethylene monomethyl ethers 32 (CH_3–OH) and for dimethyl ethers 46 (CH_3–OCH_3).

Rearrangement of eqn. 13 yields

$$f_P^* = f_R + f_S M_S n_{S,R}/(n_R M_R) + \{K + f_S M_S[(n_{S,E}/n_P) - (n_{S,R} M_E)/(n_R M_R)]\}/M_P \quad (14)$$

In a linear polymer, the number of end-groups n_E will equal the number of polymer chains n_P:

$$n_E = n_P \quad (15)$$

Introduction of the ratios r_R and r_E of the numbers of adsorbed solvent molecules to the numbers of repeating units and end-groups:

$$r_R = n_{S,R}/n_R \quad (16)$$

and

$$r_E = n_{S,E}/n_E = n_{S,E}/n_P \quad (17)$$

yields

$$f_P^* = f_R + r_R f_S M_S/M_R + \{K + f_S M_S[r_E - r_R M_E/M_R]\}/M_P \quad (18)$$

If one plots the response factors (from column and bypass measurements) *versus* $1/M_P$, r_R can be calculated from the difference in the intercepts:

$$r_R = (f_P^* - f_P)M_R/f_S M_S \quad (19)$$

and r_E from the difference in the slopes:

$$r_E = (K_{col.} - K_{byp.})/f_S M_S + r_R M_E/M_R \quad (20)$$

EXPERIMENTAL

For these investigations, a DDS 70 density detection system (Paar, Graz, Austria) was used, which was developed in our group. It was combined with a Bischoff 8110 RI detector and connected to an MS-DOS computer for data

acquisition and processing. The entire system has been described in detail in previous papers [14–17]. Data acquisition and processing were performed using the software package CHROMA, which was developed for the DDS 70.

SEC measurements were performed on a set of four Phenogel 300 × 4.6 mm I.D. columns (2 × 500 + 2 × 100 Å) and in bypass. Columns could be selected using two Rheodyne Model 7660 switching valves. A flow-rate of 1.00 ml/min was maintained with a Gynkotek 300 C HPLC pump. A Sicon LCD 201 RI detector was coupled to the density detector for these measurements. Samples were injected using a VICI injection valve equipped with a 100-μl loop; the concentration range was 5–7 g/l. A measuring temperature of 25.0°C was maintained with a Lauda MS3 thermostat, which was coupled to the column box of the DDS 70. Each sample was injected into the SEC system twice at the bypass and connected columns.

Polyethylene glycol (PEG), polyethylene glycol monomethyl ether (MME-PEG) and polyethylene glycol dimethyl ether (DME-PEG) samples and the lower oligomers (DP 2–6) were purchased from Fluka and dried carefully *in vacuo* at 50°C for 40 h. The solvents used were of HPLC grade (Merck, LiChroSolv). Chloroform was dried over molecular sieve prior to use.

RESULTS AND DISCUSSION

Before studying the preferential solvation of the samples in chloroform containing ethanol, we had to ensure that no other component (such as 2-methylbutene, which is added as a stabilizer, or moisture) in the solvent used for these investigations would also show preferential solvation. Hence we injected polyoxyethylenes with different end-groups (diols and mono- and dimethyl ethers) in dry, ethanol-free chloroform both on to the columns as also in bypass. Because the lowest oligomers of polyethylene glycols showed strong tailing in this mobile phase, they could not be analysed under these conditions. In the column measurements no solvent peak was observed, which proves the absence of any preferential solvation.

In Fig. 1 the response factors of polyoxyethylene monomethyl ethers as obtained from column and bypass measurements with RI detection are shown. The regression lines for column and bypass measurements agree very well, whether the polymer had been separated from the "dialysed" zone or not.

Fig. 2 shows a comparison of the regression lines for diols and mono- and dimethyl ethers. The corresponding intercepts, slopes and correlation coefficients are given in Table I. All

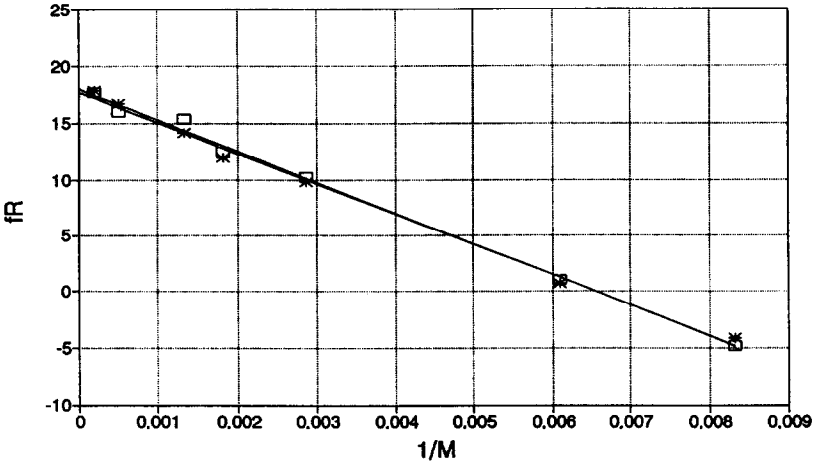

Fig. 1. Response factors of polyoxyethylene monomethyl ethers in dry, ethanol-free chloroform from (□) column and (*) bypass measurements with RI detection.

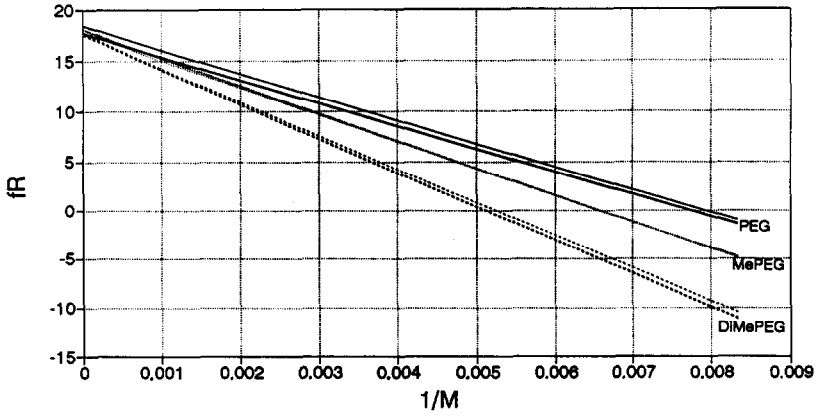

Fig. 2. Response factors of polyoxyethylenes with different end-groups in dry, ethanol-free chloroform from (thick lines) column and (thin lines) bypass measurements with RI detection.

regression lines had a similar intercept but a different slope, according to the different end-groups.

When 1.1% (v/v) of ethanol was added to the mobile phase, the chromatograms obtained on the column showed a vacancy peak (Fig. 3), the sign of which indicated that ethanol had been preferentially adsorbed by the polymer. As expected, the sum of the areas of polymer and solvent peaks on the column was equal to the area of the peak in bypass measurements.

In order to confirm the nature of the vacancy peak, the same analysis was performed without drying the chloroform. As can be seen from Fig. 4, the chromatogram obtained showed two vacancy peaks, which indicates that preferential solvation with both ethanol and water had occurred. (With density detection, the response factors of polyethylene oxide, ethanol, and water are negative, hence the polymer peak is negative and the vacancy peaks are positive. In Figs. 3 and 4 the sign of the density trace has been changed. With RI detection, the response factors of the polymer and solvents, ethanol and water, have opposite signs, hence all the peaks have the same sign.)

TABLE I

INTERCEPTS, SLOPES AND CORRELATION COEFFICIENTS OF THE REGRESSION LINES OBTAINED FROM BYPASS AND COLUMN MEASUREMENTS (WITH DENSITY AND RI DETECTION) OF MONO- AND DIMETHYL ETHERS OF POLYOXYETHYLENE IN DRY, ETHANOL-FREE CHLOROFORM

Parameter	MePEG		DiMePEG	
	Column	Bypass	Column	Bypass
$f_{D,\infty}$	−17.73	−17.08	−17.11	−16.69
K_D	−1600.3	−1683.1	−2635.8	−2623.0
r	0.9373	0.9979	0.9999	0.9991
$f_{R,\infty}$	18.00	17.69	17.57	17.60
K_R	−2747.6	−2694.5	−3441.9	−3371.1
r	0.9978	0.9975	0.9984	0.9983

The results obtained in dry $CHCl_3$ containing 1.1% of EtOH are shown in Figs. 5 and 6 and the corresponding slopes, intercepts and correlation coefficients of the regression lines are given in Table II. For the dimethyl ethers, the intercepts obtained from bypass and column measurements were different, but the slopes were similar, which indicates that preferential solvation took place at the repeating units.

For monomethylethers and diols, both different slopes and intercepts were observed, which indicates that preferential solvation took place both at the end-groups and along the chain, but to different extents (depending on the nature of the end-groups). Using eqns. 19 and 20, we calculated r_E and r_R for different homologous series, as shown in Table III.

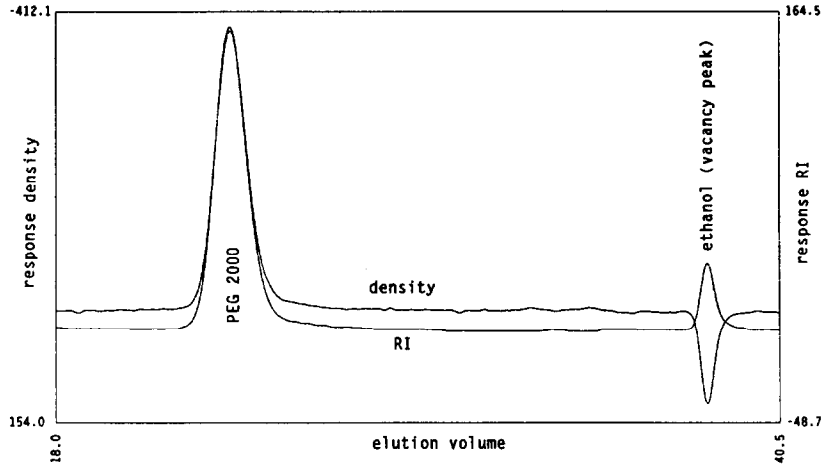

Fig. 3. Chromatogram of PEG 2000, as obtained with density and RI detection, in dry chloroform containing 1.1% (v/v) of ethanol.

From these data the following conclusions can be drawn:

(1) At a given ethanol content, all homologous series showed approximately the same value of r_R, as expected.

(2) For the dimethyl ethers, the slopes obtained in column and bypass measurements were almost the same, which indicates that the methoxy end-group behaves very much like the repeating unit.

(3) The preferential solvation of methoxy groups is small. Exchange of each methoxy group for a hydroxy group increased the r_E value by roughly the same amount.

(4) The preferential solvation of the hydroxy groups was found to be much stronger than that of the repeating unit.

(5) Preferential solvation depends strongly on molecular mass for polyethylene glycols.

CONCLUSIONS

In the liquid chromatography of polymers, preferential solvation has to be taken into account even in "pure" mobile phases, because even a very small amount of a second component in the mobile phase can be preferentially adsorbed, thus contributing to the detector signal.

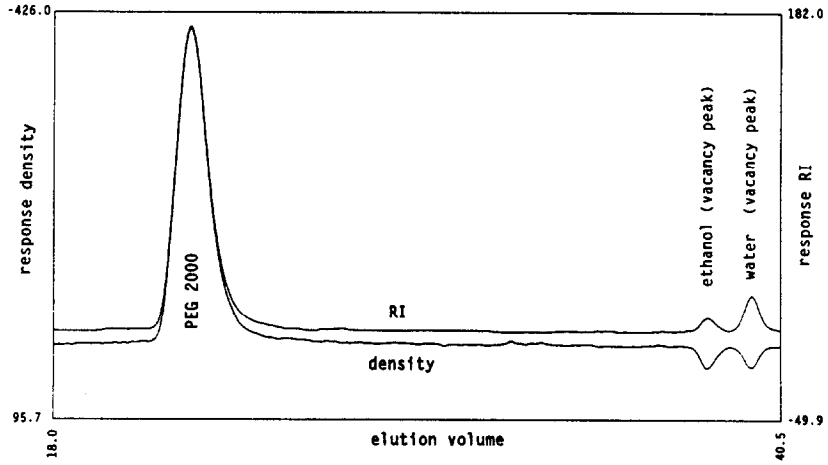

Fig. 4. Chromatogram of PEG 2000, as obtained with density and RI detection, in chloroform containing ethanol and water.

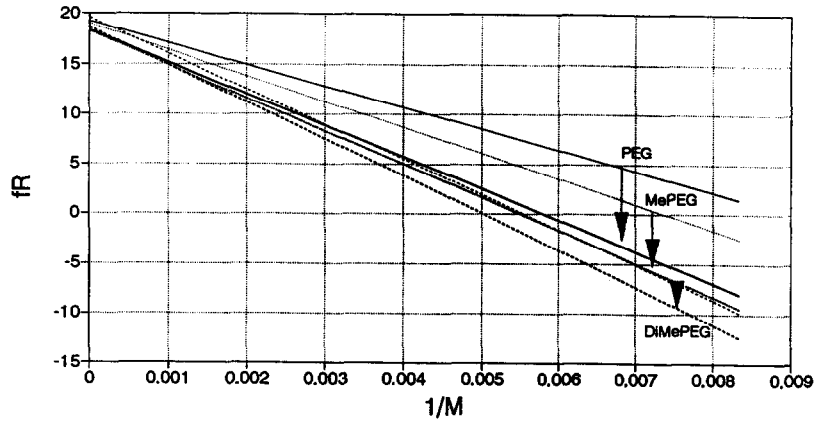

Fig. 5. Response factors of polyoxyethylenes with different end-groups in dry chloroform containing 1.1% (v/v) of ethanol from (thick lines) column and (thin lines) bypass measurements with RI detection.

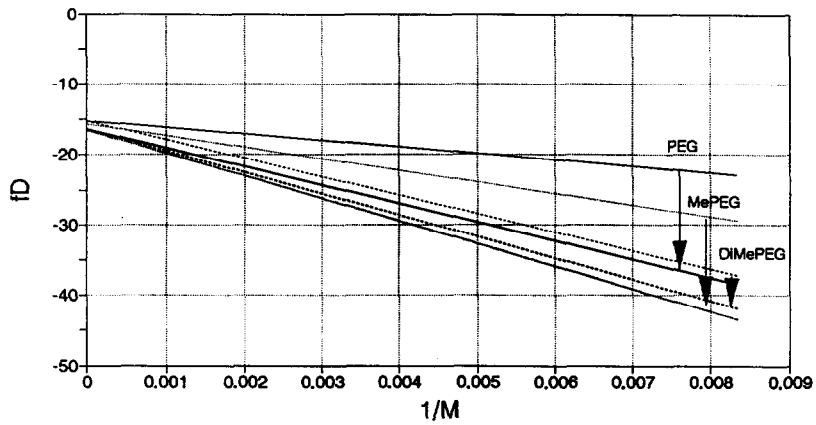

Fig. 6. Response factors of polyoxyethylenes with different end-groups in dry chloroform containing 1.1% (v/v) of ethanol from (thick lines) column and (thin lines) bypass measurements with density detection.

TABLE II

INTERCEPTS, SLOPES AND CORRELATION COEFFICIENTS OF THE REGRESSION LINES OBTAINED FROM BYPASS AND COLUMN MEASUREMENTS (WITH DENSITY AND RI DETECTION) OF POLYETHYLENE GLYCOLS AND THEIR MONO- AND DIMETHYL ETHERS IN DRY CHLOROFORM CONTAINING 1.1% (v/v) OF ETHANOL

Parameter	PEG		MePEG		DiMePEG	
	Column	Bypass	Column	Bypass	Column	Bypass
$f_{D,\infty}$	−16.36	−15.27	−16.46	−15.59	−16.12	−15.29
K_D	−2658.7	−912.4	−3239.1	−1653.4	−3020.3	−2627.5
r	0.9926	0.9942	0.9937	0.9957	0.9984	0.9983
$f_{R,\infty}$	18.32	19.21	18.27	18.95	18.85	19.64
K_R	−3165.9	−2141.9	−3334.7	−2576.7	−3740.3	−3539.0
r	0.9986	0.9993	0.9992	0.9983	0.9992	0.9995

TABLE III

AVERAGE NUMBERS OF ETHANOL MOLECULES ADSORBED PER REPEATING UNIT (r_R) AND PER END-GROUP (r_E) FOR DIFFERENT HOMOLOGOUS SERIES OF POLYOXYETHYLENES IN CHLOROFORM CONTAINING 1.1% (v/v) OF ETHANOL, AS OBTAINED WITH DENSITY AND RI DETECTION

Derivative	r_R		r_E	
	Density	RI	Density	RI
Diol	0.0164	0.0200	0.603	0.531
Monomethyl ether	0.0131	0.0153	0.312	0.398
Dimethyl ether	0.0164	0.0200	0.167	0.113

If the end-groups of the polymer are considerably different from the repeating units, preferential solvation will depend on molecular mass, thus leading to errors in the molecular mass distributions determined by SEC if no compensation is performed. The response factors of non-specific detectors are apparent values, which will not affect the accuracy of the molecular mass averages as long as the composition of the mobile phase is kept constant.

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