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Journal of Bioactive and Compatible Polymers 2001; 16; 206

DOI: 10.1106/V7G8-R36H-BX1R-647G

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Preparation of Monomethyl Ethers of Poly(ethylene glycol)s Free of the Poly(ethylene glycol)

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ABSTRACT: In this report, poly(ethylene glycol) (PEG) was quantitatively removed from commercial monomethyl ethers of PEG (MPEG) by column chromatography on silica gel on a preparative scale. Most of commercial MPEG, with molecular weights as high as 5000, and containing only up to 20% of PEG, were effectively purified by this technique. The content of PEG in MPEG obtained is comparable or even lower than that in samples of MPEG prepared by anionic polymerization of ethylene oxide under strictly controlled conditions. An analytical approach combining H^1 NMR and chromatography techniques indicates that the PEG content in the chromatographically purified MPEG samples is less than 1%.

INTRODUCTION

Monomethyl ethers of poly(ethylene glycol)s (MPEG)s of various molecular weights are often used as precursors in further syntheses of macromolecular systems, particularly in biomedical and biological fields [1,2]. An important example is the synthesis of conjugates with biologically active compounds, such as polypeptides [3]. The terminal hydroxy groups are used as the reactive sites; it is, therefore, important to have strictly one hydroxy group per macromolecule.

Commercially available MPEG (in the M_n range from 350 to 5000),

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however, is contaminated (up to 25%) with poly(ethylene glycol) (PEG). The percentage of PEG increases usually with the M_n of MPEG [1,4,5]. PEG is probably formed when traces of water are present in the polymerization mixture, acting as an initiator and/or chain transfer agent in the ethylene oxide (EO) polymerization. Recently, companies began providing MPEG with much higher purity. The presence of α,ω -dihydroxy poly(ethylene oxide) (PEO) can lead to branched or even cross-linked products when reacted with multifunctional biological macromolecules.

Anionic polymerization of EO proceeds as a living process without chain transfer and termination if the polymerization mixture is devoid of impurities. This feature permits the preparation of PEO with predetermined chain length and terminal functionality [6–9]. Therefore, starting from properly prepared initiator and excluding active impurities, MPEG of high purity can be prepared. These processes require, in order to be efficient, high-vacuum and sealed polymerization systems that are usually not available in the biochemical laboratories. Thus, we attempted to remove PEG from MPEG by chromatographic methods that could be easily adapted in most laboratories.

To compare the purity of the products obtained by this separation to the products prepared by anionic polymerization of EO, the same analytical methods were applied to both series of products. ^1H NMR, thin layer chromatography (TLC) and high-performance liquid chromatography under critical conditions (CC-HPLC) were used as analytical methods. CC-HPLC is based on the ability of macromolecules under specific conditions to be separated according to the end-group structure rather than on their molecular weight [10]. This method was formerly used only for monoethers of PEG with larger alkyl end groups [11]. For methyl ethers, stricter conditions have to be adhered to in order to determine low concentration of PEG in MPEG, because the shorter the alkyl end group is, the more difficult it is to separate PEG from its monoether derivative. The TLC method, as shown in the present work, can be used as a fast method routine to obtain a rough estimation of the presence of PEG in MPEG, even for low PEG content.

One report claims the possibility of determining even 0.6% PEG in MPEG 5000 (using DMSO- d_6 and a 250 MHz ^1H NMR) [12]. We were not successful in achieving that high accuracy. In fact, none of the analytical methods we applied were reliable enough to determine, with satisfactory accuracy, better than $1 \pm 0.5\%$ PEG content in MPEG.

Chromatographic separation described in this work can be considered as a relatively fast and effective method to remove PEG from a given commercial MPEG, down to approximately 1% of PEG in MPEG [13].

MATERIALS AND METHODS

Material

The MPEGs used were of commercial origin. Besides PEG, they also contained various amounts of water, the presence of which influences the results of both purification and analysis. Some commercial MPEGs were contaminated up to 25% with PEG. A few other ones contained less than 3% PEG (see the following).

$\text{CH}_3(\text{OCH}_2\text{CH}_2)_3\text{OH}$ (Aldrich) was distilled three times under vacuum (b.p. $90^\circ/4$ mm Hg) (a small amount of sodium was added before distillation) and kept in a glass ampoule closed with a Rotaflo[®] stopcock. Only one peak was observed in the GLC under conditions that would reveal $\geq 0.4\%$ of impurities. Hewlett Packard Type 5890 apparatus and Hewlett Packard column FFAP 10 m, ID = 0.53 mm, heated 50–220°C (10°C/min) were used.

Synthesis of MPEGM 2000

The dimethyl ether of poly(ethylene glycol) 2000 (MPEGM 2000), that was used as a reference in the TLC analysis, was synthesized from PEG 2000 (Fluka) according to a previously described method [14,15]. $M_n = 2060$ (calculated from ^1H NMR spectra) and 2005 (by VPO method; in CH_2Cl_2) with a TLC: R_f 0.44 (CHCl_3 – CH_3OH ; 10:15) were determined for this product.

Purification of MPEG by Chromatography of Silica Gel

Commercial MPEG 2000 (Fluka, ~80% purity, cf. Table 1) (10 g) was dissolved in 40 mL of dry CHCl_3 and introduced into a glass column ($h = 20$ cm, $\Phi = 4$ cm) packed with silica gel as received (230–400 mesh, Merck). A CHCl_3 – CH_3OH mixture was used as an eluent with increasing proportions of CH_3OH [13]. Fractions of 100 mL volume were collected. For fractions 1–13, the content of CH_3OH was linearly increased up to 2.5% vol. and then kept on this level for fractions 14–46. Finally, for fractions 47–57, 10% vol. of CH_3OH was used (to remove the residual PEG from column).

Fast, approximate analysis of the PEG content was performed by a TLC method on silica gel. Chromatograms were developed with CHCl_3 – CH_3OH (10:1.5) (for details, see the corresponding Analysis part). "Pure" MPEG 2000 (only one spot in TLC) was observed in fractions 6–46. Fractions 47–51 contained mixtures of MPEG and PEG (two spots), whereas fractions 51–57 contained mostly PEG. MPEG from

Table 1. Some properties of commercial MPEG.*

No.	Supplier	M_n				Ratio $\frac{[\text{OH}]^a}{[\text{OCH}_3]}$		Content of MPEG		
		Original	VPO ^a	¹ H NMR ^b		TFAA	TAIC	NMR Method ^c [mol%]		HPLC ^d [wt%]
				TFAA	TAIC			TFAA	TAIC	
1.	Jansen	550	634	569	578	1.06	1.08	97.3	95.9	94.0
2.	Fluka	750	965	921	930	1.23	1.27	89.4	87.8	—
3.	Fluka	2000	2443	2294	2272	1.48	1.44	80.5	82.0	81.4
4.	Aldrich	2000	2323	2130	2036	1.12	1.07	94.3	96.5	93.2
5.	Sigma	2000	2368	2105	1920	1.23	1.28	89.4	86.0	87.2
6.	Fluka	5000	5187	5461	5373	1.12	1.14	94.0	93.6	94.4
7.	Aldrich	5000	5254	4968	5348	1.03	1.13	98.5	93.9	98.9
8.	Sigma	5000	5103	5208	4913	1.60	1.72	76.9	73.5	75.1

*Measured in this work; "original" label given by the supplier.

^aIn CH₂Cl₂ at 26°C.

^bAverage values calculated from normal and expanded spectra obtained with two reagents [trifluoroacetic anhydride (TFAA) and trichloroacetyl isocyanate (TAIC)] for methylene protons in the α - position to the esterified hydroxyl end groups (RD = 10 s).

^cCalculated from the ratio [OH]/[OCH₃].

^dAnalysis under critical conditions.

^eIf MWD and M_n for MPEG and PEG are the same, then mol% and wt% are identical. If, however, the molecular weight of PEG is twice as high as the M_n of MPEG, then our calculated wt% is to be converted to molar%. However, both values are very close to one another, because of the low content PEG.

fractions 6–46 was precipitated from CHCl_3 solution with $(\text{C}_2\text{H}_5)_2\text{O}$, giving 3.5 g (35% yield) of MPEG of ~99% purity. 5.2 g of MPEG/PEG 3:2 mixture was isolated from fractions 47–51; fractions 52–57 contained 0.9 g of MPEG/PEG 1:4 mixture.

Commercial MPEG 5000 (Fluka, ~90% purity) (10 g) was dissolved in 40 mL of dry CHCl_3 and introduced onto the column as described above for MPEG 2000. For fractions 1–20 the content of CH_3OH was linearly increased up to 2% vol. and then kept on this level for fractions 21–51. For fractions 52–89, the CH_3OH content was increased up to 10% vol. “Pure” MPEG 5000 (one spot in TLC, developed with CHCl_3 – CH_3OH (5:1 v/v) was observed in fractions 23–61 and after isolation as described for MPEG 2000 7.8 g of MPEG 5000 (yield 78%) with purity approximately 99% (Table 2) was obtained. The yield of pure MPEG depends of course on the purity of the starting material. Single spots were observed in TLC: R_f :0.35 for MPEG 5000, R_f :0.38 for MPEG 2000, and R_f :0.39 for MPEG 550 (CHCl_3 – CH_3OH ; 10:1.5). The R_f for the corresponding PEG are: R_f :0.29 for PEG from MPEG 5000, R_f :0.31 for PEG from MPEG 2000 and R_f :0.32 for PEG from MPEG 550.

In addition, samples were analyzed by ^1H NMR and HPLC under critical conditions (see Analysis part); the content of PEG in the purified MPEG is near the level of the error of our determination of PEG in synthesized MPEG; $1 \pm 0.5\%$.

Analysis of Commercial and Purified MPEG

Some products were purified by ultrafiltration in water using an Amicon Ultrafiltration stirred cell (200 mL) with a 1000 molecular weight cut off Diaflo[®] ultrafiltration membrane. Molecular weights were determined with Knauer VPO apparatus (1993) in CH_2Cl_2 solution at 26°C.

^1H NMR spectra were recorded on a Bruker AC-200 or MSL 300 apparatus operating at 200 or 300 MHz, respectively. Best results for $\text{CH}_3(\text{OCH}_2\text{CH}_2)_3\text{OH}$ were obtained for $\text{RD} = 10$. Acquisition time (AQ) was equal to 2.7 s. The determination of the concentration of hydroxy end-groups was based on the analysis of the ^1H NMR spectra of the reaction product of PEG and its derivatives with trifluoroacetic anhydride (TFAA) [16–18,20] or trichloroacetyl isocyanate (TAIC) [5].

The TLC analyses were performed on silica gel aluminium plates (Merck 60 F₂₅₄); developing system: chloroform-methanol (10:1.5). I_2 or Dragendorff reagent [19] were used for detection. With the Dragendorff reagent the limit of detection for PEG, present as an impurity in MPEG,

Table 2. Characterization of MPEG purified by precipitation from *i*-PrOH (A), ultrafiltration (B), precipitation from $\text{CHCl}_3/\text{Et}_2\text{O}$ (C), and liquid chromatography (D) on silica gel and in samples prepared by anionic polymerization [20]. All the data (except for "Original") given for samples purified as indicated in the last column.

No.	Supplier	M_n			[OH] ^a		Content of MPEG		
		Original	¹ H NMR ^a		Ratio $\frac{[\text{OH}]}{[\text{OCH}_3]}$		NMR Method ^{b,c} [mol%]		Method of Purification
			TFAA	TAIC	TFAA	TAIC	TFAA	TAIC	
1.	Fluka	2000	2584	2659	1.32	1.39	86.0	83.7	A
2.	Fluka	2000	2338	2430	1.43	1.46	82.3	81.1	B
3.	Fluka	2000	3015	2578	1.62	1.74	76.5	72.8	C
4.	Jansen	550	512	637	~1.00	1.06	~100	96.9	D
5.	Fluka	2000	2234	2239	1.06	1.01	97.1	99.4	D
6.	Sigma	2000	1911	1988	~1.00	~1.00	~100	~100	D
7.	Aldrich	2000	1893	2038	~100	~1.00	~100	~100	D
8.	Fluka	5000	5796	5227	1.01	1.03	99.5	98.6	D
9.	Sigma	5000	5193	6243	~1.00	1.05	~100	97.8	D
10.	Aldrich	5000	5565	5563	1.02	1.003	98.8	99.9	D
11.	*	2135 ^d	2280	2303	1.01	1.16	99.3	97.7	94.0 ^e
12.	*	5211 ^d	5115	5156	1.04	1.20	98.2	91.0	95.7 ^e

*Anionic polymerization.

^aAverage values calculated from normal and expanded spectra obtained with two shifting reagents [trifluoroacetic anhydride (TFAA) and trichloroacetyl isocyanate (TAIC)] for methylene protons in the α - position to the esterified hydroxyl end groups (RD = 10 s).

^bCalculated from the ratio [OH]/[OCH₃].

^cHPLC at critical conditions gave for all products purified by chromatography (method D) ~ 100% of MPEG.

^dCalculated from the known ratio of [monomer]/[initiator]; $M_n = 44 [M]_0/[I]_0$.

^eContent of MPEG determined by HPLC under critical conditions.

was approximately 1% as determined with samples containing known amounts of PEG.

The HPLC analyses under critical conditions (CC-HPLC) were carried out on a LKB chromatograph (2150 HPLC pump) with a LDC RI detector and LKB Ultropac Column (Lichrosorb RP 18, 5 μm , 4 \times 250 mm); eluent $\text{CH}_3\text{CN-H}_2\text{O}$ (60:40 v/v), 1.0 mL min^{-1} ; samples ~ 2 mg mL^{-1} , 20 μL . Retention volumes of PEG and MPEG were 2.79 and 3.02 mL, respectively. These conditions were elaborated in Reference [20].

Previously, reversed phase HPLC was used to determine the chain length distribution in MPEG: with gradient elution (water-acetonitrile) for MPEG 2000 [21] and under isocratic conditions ($\text{H}_2\text{O/CH}_3\text{OH}$) for MPEG 550 [22]. An HPLC column filled with RP-18 was used for the analysis of functionality type distribution (FTD) in the characterization of functional PEG; $\text{CH}_3\text{CN-H}_2\text{O}$ (43–57 [23] and 42–58 v/v [11]) was used as the mobile phase.

RESULTS AND DISCUSSION

Preparation of Pure MPEG by Chromatographic Purification

As described in the experimental part, commercial MPEGs were purified by chromatography on a silica gel column using $\text{CHCl}_3\text{-CH}_3\text{OH}$ mixture as the eluent with increasing proportions of CH_3OH . It should be mentioned that for silica gel differing from the one used and solvents with different impurities (especially water content) used in this work would perhaps need another optimal gradient.

The purity of our best samples is at the level of or below the accuracy of our measurements $1 \pm 0.5\%$. The yield of the purified product depends on both the PEG content in MPEG and its M_n . For instance, for commercial MPEG of $M_n = 2000$ containing approximately 20% PEG, we obtained 35% pure MPEG with $\sim 1\%$ of PEG left, whereas for another sample with the same M_n but contaminated with 6% PEG, we obtained 68% MPEG with similar purity. The reliability of the various analytical methods used are discussed further in this paper.

Chromatography on a silica gel column was previously used for removing the unreacted PEG from the disubstituted PEG of higher alkyl ethers of PEG [24,25]. In those systems, separation was easy because of the much larger difference in the polarities of both disubstituted and nonsubstituted products. We also tested other purification methods for commercial MPEG, such as crystallization and precipitation from

$i\text{-C}_3\text{H}_7\text{OH}$ or $\text{CHCl}_3/(\text{C}_2\text{H}_5)_2\text{O}$. According to our analytical methods, the purity of the resulting products was inferior to those purified by chromatography; we could not decrease the PEG content in MPEG below 5% by those methods. Similarly, the repeated precipitations with diethyl ether from $\text{CH}_2\text{Cl}_2/\text{C}_2\text{H}_5\text{OH}$ solutions [26] did not give any better results than chromatography.

Critical Discussion of the Analytical Methods Used

The expected accuracy of ^1H NMR is only \pm a few percent. In addition, for macromolecules both the acquisition time (AQ) and relaxation delay (RD) influence the measured ratios of various end groups. This effect, which is related to the differences in segmental mobilities, is well known for ^{31}P [27] and ^{13}C NMR [28,29]. However, we, surprisingly, observed this phenomenon in ^1H NMR as well.

Indeed, analysis of the ^1H NMR spectrum of the model compound, monomethyl ether of triethylene glycol (purified by distillation) and containing less than 1% of impurities (according to GLC analysis) revealed that for the standard instrument setup for recording the ^1H NMR spectrum (AQ = 2.7 s; RD \cong 0 s) the molar ratio of $[\text{OH}]/[\text{OCH}_3]$ obtained (after reaction with $(\text{CF}_3\text{CO})_2\text{O}$, Figure 1) was higher than 1. However, by increasing the relaxation delay (RD) to 10 s, it was possible to obtain better values of $[\text{OH}]/[\text{OCH}_3]$. It was also found that this ratio does not change for RD over 10 s. For monomethyl ether of triethylene glycol we obtained:

RD, s	0	5	10	15	20
$[\text{OH}]/[\text{OCH}_3]$	1.07	1.02	1.00	1.00	1.00

Whatever the reasons are for these observations (association of the $-\text{OH}$ ends?) the ^1H NMR analysis of the end groups of the esterified MPEG/PEG mixture was performed with AQ = 2.7 s and RD = 10 s. A typical ^1H NMR spectrum of MPEG is shown in Figure 2. Chemical shifts for the groups (in ppm δ) are: (a) $\text{CH}_3\text{O}-$ 3.37; (b) $-\text{CH}_2-\text{CH}_2-\text{O}$ 3.64; (c) $-\text{CH}_2\text{CH}_2\text{OC}(\text{O})\text{CF}_3$ (d): 3.75–3.80 $-\text{CH}_2\text{CH}_2\text{OC}(\text{O})\text{CF}_3$ (e): 4.45–4.50. The symmetrical multiplets observed for protons d and e (Figures 1 and 2) are characteristic for a AA'BB' type spectrum. Using a computer simulation of this spectrum we estimated the values of the coupling constants as $J_{\text{AA}} = -10$ Hz, $J_{\text{AB}} = 4.7$ Hz, $J_{\text{AB}} = 1.3$ Hz, $J_{\text{BB}} = 12.0$ Hz.

Direct analysis of the content of PEG in MPEG using ^1H NMR was not practical, because the $-\text{CH}_2\text{OH}$ protons of this end group have almost identical chemical shifts (~ 3.60 ppm δ) as the $-\text{CH}_2\text{CH}_2\text{O}-$ protons of the

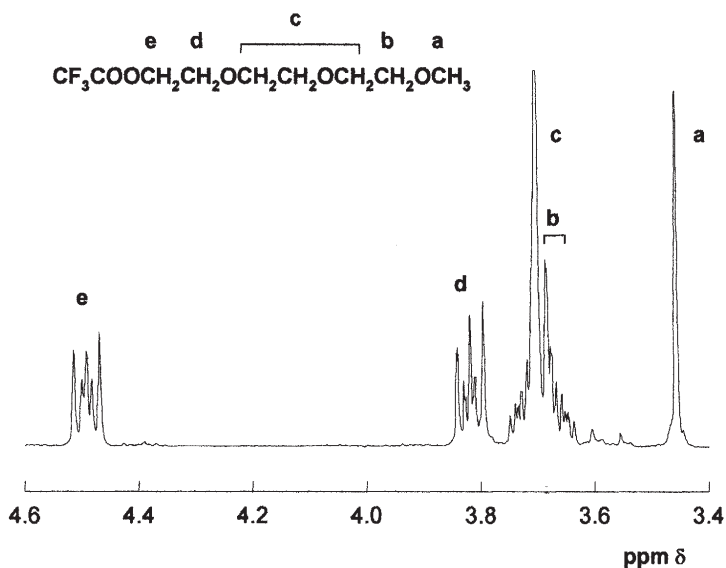


Figure 1. The expanded ^1H NMR spectrum of $\text{CF}_3\text{CO}(\text{OCH}_2\text{CH}_2)_3\text{OCH}_3$ in CDCl_3 .

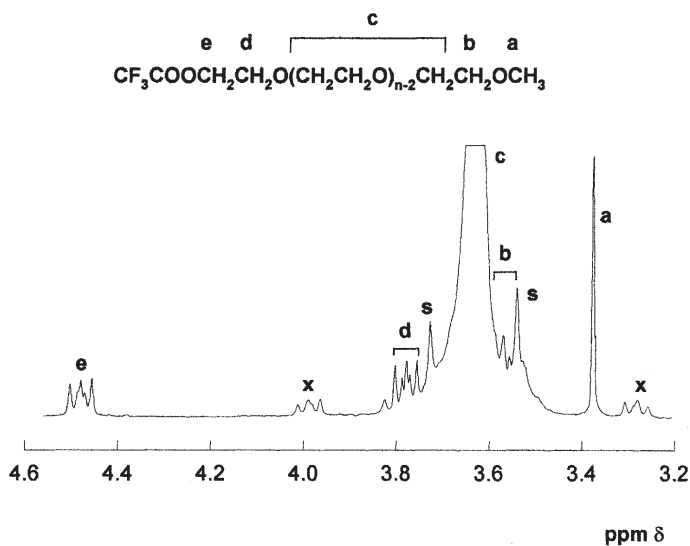


Figure 2. The expanded ^1H NMR spectrum of trifluoroacetate of MPEG 2000 (No. 2 in Table 2) in CDCl_3 ; s: side bands; x: ^{13}C satellites.

main chain (3.64 ppm δ). Therefore, the determination of the purity of MPEG by ^1H NMR was performed after the $-\text{CH}_2\text{OH}$ end groups were transformed into their respective trifluoroacetate esters. The peak for the $-\text{CH}_2\text{OC}(\text{O})\text{CF}_3$ protons was shifted approximately 0.8 ppm downfield. This allowed us to observe these end groups in a region free of other absorptions. A five-fold excess of the esterifying agent was used just before the ^1H NMR spectrum was measured. These methods have been published for trifluoroacetic anhydride [16–18,20] and trichloroacetyl isocyanate [5].

The polymer molecular weights were calculated by comparing the intensities of the methylene protons on the main chain to the sum of 1/3 intensity of methoxy protons (3.37 ppm) and 1/2 of intensity of methylene protons (4.50 ppm) at the chain ends. The purity and the molecular weights of the polymers were calculated from normal and expanded ^1H NMR spectra; the average values are given in Tables 1 and 2. The data from the expanded spectra are much better. In the case of TAIC, used to determine the purity of MPEG, the differences between calculated values from normal and expanded spectra were much larger than for the TFAA method (nevertheless the average values remained close).

The data for the ^1H NMR analyses of commercial MPEG, purified in various ways, including the chromatographic method, are given in Table 2. The analysis of two samples obtained by anionic polymerization [20] is also included. These data indicate that purification by chromatography can provide MPEG containing less than 1% of PEG.

We have checked the accuracy of the ^1H NMR method by preparing mixtures of MPEG 2000 by adding different known amounts of PEG 4000 (1–5%). These mixtures were then esterified with TFAA and the ^1H NMR spectra of mixtures recorded. Analysis of the ^1H NMR spectra of these known mixtures indicate that the ^1H NMR determined PEG was systematically higher than the actual ones. However, this difference for the lowers PEG content was within the experimental error of determination ($\sim 1\%$).

The purity of MPEG was reported earlier on the basis of the analysis of ^1H NMR spectra determined in dry $\text{DMSO}-d_6$ [4,12]. The following signals from the end groups were observed: singlet at $\delta = 3.24$ ppm from $\text{CH}_3\text{O}-$ and triplet at $\delta = 4.57$ ppm from $-\text{OH}$. We repeated these measurements and observed similar chemical shifts; however, we failed in obtaining consistent results, at least for MPEG containing $\sim 1\%$ of PEG. A comparison of the three ^1H NMR methods, namely with TFAA, TAIC and $\text{DMSO}-d_6$ (not given in the table) indicates that the most reliable data were obtained, at least in our hands, with TFAA (measured with $\text{RD} \geq 10$ s) as the end-capping reagent (Tables 1 and 2).

Purity of Samples as Determined by the TLC Method

TLC was found to be a very useful method for the analysis of the purity of MPEG. Previously this method was applied to the nonionic surfactants based on PEG [30–32]. The TLC analysis on silica gel plates of chromatographically purified samples indicates ~99% purity. Only one spot was observed for MPEG ($M_n = 550, 2000$ and 5000) (Figure 3). The threshold for the determination of PEG is ~1%, as determined by a series of independent experiments with samples containing known added amounts of PEG (for the detection of PEG, Dragendorff reagent was applied) [19].

Purity Determination with HPLC Method under Critical Conditions

The HPLC method was applied previously to analyze nonionic surfactants [33–37] and MWD in PEO [22,35–38]. HPLC under critical conditions was not previously used for MPEG/PEG mixtures. We applied this method, following the procedure that Gorshkov et al. described for



Figure 3. TLC chromatogram of MPEGs. Silica gel (Merck 60 F₂₅₄): eluent: CHCl₃ - CH₃OH (10:1.5). MPEG 2000: (a) commercial (Table 1, No. 3), (b) after chromatography (Table 1, No. 5); MPEG 5000: (c) commercial (Table 1, No. 8), (d) after chromatography (Table 2, No. 9).

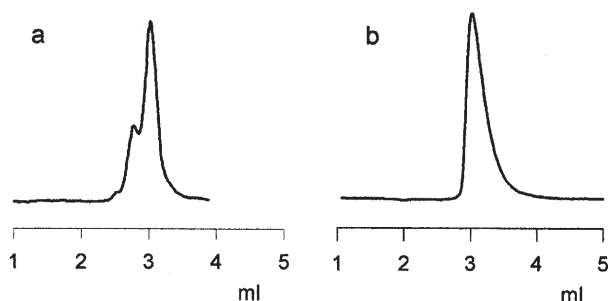


Figure 4. CC-HPLC chromatograms of MPEG 2000: a) commercial (Fluka) (Table 1, No. 3), b) after chromatographic purification of the commercial MPEG/PEG mixtures (No. 5 in Table 2). Column, RP18; eluent, CH₃CN-H₂O (60:40 v/v); 1.0 ml.min⁻¹; 20 μL; refractometric detection.

higher alkyl end groups in PEO [11]. Under specific conditions polymer separation occurs according to the polymer endgroups, irrespective of the polymer M_w [10]. The HPLC analyses were carried out on Lichrosorb RP-18 columns, using as eluent a mixture: CH₃CN-H₂O (60:40 v/v), analogous to that described previously [20]. Typical reversed phase HPLC chromatograms (under critical conditions) of commercial MPEG (a) ($M_n = 2000$ and 5000) and MPEG prepared by chromatographic purification of the commercial MPEG/PEG mixtures (b) are shown in Figures 4 and 5.

The purity of MPEG samples was either based on the analysis of chromatograms with well resolved peaks or analysis of chromatograms

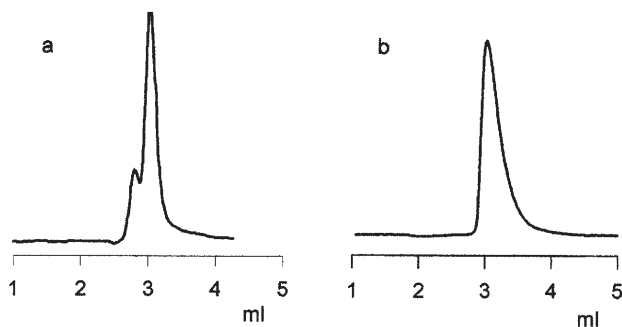


Figure 5. CC-HPLC chromatograms of MPEG 5000: a) commercial (Sigma) (Table 1, No. 8), b) after chromatographic purification of the commercial MPEG/PEG mixtures (No. 9 in Table 2). Conditions as in Figure 4.

with broad peaks. For both instances curve deconvolution was performed; the results are listed in [Tables 1](#) and [2](#). The purity of all of the MPEG samples purified by chromatography was close to 100%, as indicated in [Table 2](#). We found that even small changes in the experimental conditions (e.g., temperature, water content in solvent and samples) could influence the outcome of the analysis. Particularly, the water content is important. Standardization of the conditions for the purification of the solvents used gave more consistent results. Samples, if stored for longer time, should be carefully sealed and protected from contact with the atmosphere.

CONCLUSIONS

A simple and effective method of chromatographic purification of commercial MPEG with up to 25% PEG down to ~1% PEG is described. The purified samples contain approximately 1% (or less) of PEG and in this respect are comparable or even better than MPEG prepared by living anionic polymerization [20]. The purity of the MPEG was determined with an accuracy of $1 \pm 0.5\%$ of PEG in MPEG. This was established by analyzing mixtures with a known amount of PEG in MPEG samples.

The most reliable analytical results were obtained with CC-HPLC and ^1H NMR of the trifluoroacetates of MPEG. In ^1H NMR measurements, the $\text{RD} = 10$ s was used, which gave better CH_3 to OH groups balance than the standard conditions ($\text{AQ} = 2.7$ s, $\text{RD} = 0$ s). TAIC modified samples and measurements in DMSO-d_6 gave, at least in our hands, more scattered results.

A fast and reliable analysis by TLC with Dragendorff developing reagent and HPLC in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ mixture at the critical conditions were developed. The methods require great care in drying samples and using high purity solvents. None of the other methods evaluated gave results at the 1% PEG level in MPEG; the chromatographically purified samples were at least as good as samples prepared by anionic living polymerizations conducted under vacuum conditions with well dried starting compounds [20]. VPO gave scattered results, perhaps due to the high hydrophilicity of MPEG and difficulties in eliminating water.

ACKNOWLEDGMENT

This work was supported financially by the Polish State Committee for Scientific Research (KBN); grant No.: 3 T09A 071 16.

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