Proton NMR Characterization of Poly(ethylene glycols) and Derivatives

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ABSTRACT: Derivatives of poly(ethylene glycol) (PEG) are of importance in a variety of applications. Often it is difficult to determine the identity of the end groups and the purity of these derivatives. In the present work we show that proton NMR spectra of PEGs dissolved in deuterated dimethyl sulfoxide (but not other solvents) reveal a hydroxyl peak at 4.56 (±0.02) ppm, which does not shift or broaden with variation in concentration of the PEG, water, or impurities and which is well separated from the large backbone resonance. The hydroxyl resonance in other solvents does not show this independence, and thus it is difficult to locate and utilize. The PEG hydroxyl resonance for samples in dimethyl sulfoxide is, therefore, well suited for quantifying percent substitution and molecular weight of PEG and derivatives. Use of this method is illustrated.

The introduction of new poly(ethylene glycol) derivatives (PEG-X)[1,2] has led to their extensive use as phase-transfer agents,[3,4] as reagents for the formation of PEG–protein adducts with enzymes,[5] and as affinity partitioning ligands.[6] Furthermore, PEG-Xs have found applications as coatings in the preparation of protein-rejecting surfaces,[7] in the control of electroosmosis,[8] and as tethers linking reagents to surfaces.[9]

In the search for new PEG-Xs[10,11] there is an ongoing need to rapidly characterize the new polymer derivatives. In general, the PEGs of interest are solids with average molecular weights between 1500 and 20 000. Derivatives formed by reaction at the hydroxyl termini contain few substituent groups relative to the polymer backbone, thus effectively "diluting" the end groups and complicating analysis. In particular, small amounts of impurities must be distinguished from end groups with the same functionality.

In the present work we investigate the use of proton NMR to determine the molecular weight (MW) and degree of substitution of PEG-Xs. Both proton NMR and carbon-13 NMR have been applied extensively to direct analysis of PEG derivatives.[1a,12-16] Also, several groups have used NMR to quantify indirectly the number of hydroxyl termini through formation of derivatives ((trichloroacetyl)-carbamate,[17,18] phenylcarbamate,[17a] and hexafluoroacetal[19]) with NMR resonances in regions of the spectrum well separated from interfering peaks. Direct determination of the number of hydroxyl termini by proton NMR is complicated by the small size of the peak and by concentration-dependent shifting and broadening of the peak through interaction with other PEG and impurity molecules (including water). We have found that ^{1}H NMR spectra of PEG derivatives in deuterated dimethyl sulfoxide (DMSO-d_6) show a clean triplet (at 4.56 ppm) for the hydroxyl protons (OH) at the polymer terminus, which is well separated from the large backbone peak (OCH_2CH_2; 3.0–4.0 ppm at base, centered at 3.51 ppm) and which does not shift or broaden as in other solvents. The area of this hydroxyl peak can be compared to the areas of other peaks in the spectrum to provide a simple, direct method for determining the degree of conversion of hydroxyls to other groups. This same approach is also useful for estimating the MW of relatively low-MW PEGs in which the ratio of backbone protons to hydroxyl protons is small to moderate (<200:1).

In the present work we illustrate the use of this method in determining (a) the degree of substitution in PEG tosylate formed from PEG (molecular weight 1500), (b) the amount of PEG present in commercial monomethoxy ethers of PEG (M-PEGs), and (c) the molecular weight (MW) of some low-MW PEG standards.

Experimental Section

Materials and Instruments. Methoxypoly(ethylene glycols) (M-PEGs) were obtained from Polysciences and Aldrich and were used without further purification, except for the removal of water by drying over 3A molecular sieves in DMSO-d_6, the NMR solvent. Poly(ethylene glycol) MW standards were purchased from Polymer Laboratories or Polysciences and were dried as above, but otherwise used without further purification. *p*-Toluenesulfonyl chloride (Aldrich) was purified by repetitive recrystallization (twice) and treatment with activated carbon (once), using carbon tetrachloride as solvent. Triethylamine (Baker) was distilled at atmospheric pressure and stored over KOH pellets. DMSO-d_6 was purchased from Aldrich and stored over 3A molecular sieves in a septum-capped bottle. Standard base (ca. 0.1 M NaOH) was obtained from Aldrich.

Liquid chromatographic (HPLC) analysis was accomplished with a Bio-Rad LC system fitted with either a TSK 2000 size-exclusion column (Toya Soda) or a SOTAPhase (Rainen) size-exclusion column (pH 7.0 phosphate buffered saline or 0.5% aqueous NaN_3 eluent, 1.0 mL/min flow, RI detector). The columns were calibrated with MW standards from either Polysciences or Polymer Laboratories covering the MW range from 586 to 23 000. The column efficiencies were between 58 000 and 70 000 theoretical plates based upon ethylene glycol. ^{1}H FT-NMR spectra were recorded on a IBM-Bruker AM-200 spectrometer operating at 200 MHz. ^{1}H NMR data are reported in ppm relative to TMS or to DMSO-d_5 at 2.500 ppm relative to TMS. The spectrometer was routinely adjusted as follows: spin rate, 30 rpm; pulse width, 1.5 μs; sweep width, 5000 Hz; line broadening (for exponential multiplication), 0.305 Hz with 16K data points. Between 120 and 256 transients per fid sufficed to produce the results herein reported. Sufficient time was allowed between sampling pulses for complete relaxation of the protons.

Poly(ethylene glycol)-1500 Ditosylate (PEG-(OTs)$_2$). The preparation of PEG-(OTs)$_2$ was adapted from that previously published;[1a] the main change is the rigorous purification to remove any triethylammonium salts and the use of triethylamine rather than pyridine as base. Pyridinium salts have been implicated in the catalyzed decomposition of PEG tosylates.[20] To a stirred solution of PEG-1500 (15 g, 0.01 mol) in dichloromethane (ca. 35 mL) was added freshly distilled triethylamine (6.0 g, 0.05 mol). This solution was cooled in an ice bath and a N_2 atmosphere maintained. Tosyl chloride (10.4 g, 0.05 mol) was added as a solid piecemeal to the solution over the course of 30 min. After the

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addition the mixture was permitted to stir at ambient temperature overnight. Workup consisted of removing the triethylammonium chloride by filtration, concentration of the solution by reduced-pressure distillation, and dissolution of the syrup in a minimum of benzene. More triethylammonium chloride precipitated and was filtered off. The filtrate was warmed, treated with Norit and neutral alumina (or silica gel, 70–120 mesh), and filtered through a Celite bed, and the crude product was precipitated by dropwise addition of the filtrate to cold diethyl ether. The solid thus obtained was dissolved in dichloromethane, stirred with Norit and alumina (silica gel), and filtered, and the ditosylate was again precipitated by addition to copious volumes of cold diethyl ether. The purified ditosylate was collected by vacuum filtration and placed in a vacuum desiccator (<0.1 Torr) to dry. ¹H NMR: 7.84 and 7.19 (4 H, A_2B_2 dd, J = 8.8 Hz, aryl ring), 4.07 (2 H, t, J = 6.2 Hz, CH_2OTs), 3.51 (s, polymer backbone), 2.47 ppm (3 H, s, CH_3 of tosylate). A similar ¹H NMR spectrum has been reported in $CDCl_3$.[21] The lack of a visible triplet (4.57 ppm) ascribable to the OH of residual PEG suggested a degree of substitution approaching 100%; titration (below) indicates 97.2% substitution. Yield: 12 g. HPLC analysis showed that the initial PEG had a MW of 1500 and that the MW of PEG obtained from PEG-OTs hydrolysis was also 1500; i.e., there was no change in retention volume.

Titration of PEG-$(OTs)_2$-1500. Analysis was accomplished by immediate titration of a PEG-$(OTs)_2$ solution (ca. 0.150 g in distilled water) with standard NaOH (Aldrich; 0.1050 M, phenolphthalein indicator) to determine the amount of free tosyl chloride/*p*-toluenesulfonic acid present. Since the amount of free tosyl chloride/tosyl acid was generally low (ca. 1–2 mol %), it was found necessary to use a semimicro buret (5-mL total volume) to obtain reproducible results. The degree of substitution was determined from the titration (standard base) of samples that had been boiled in water for at least 4 h, corrected for the amount of free tosyl chloride/tosyl acid present. The percent substitution obtained from this procedure was 97.2%.

Poly(ethylene glycol)-4000 Bis(2,2,2-trifluoroethanesulfonate) (PEG-$(OTrs)_2$). PEG tresylate was prepared according to the method of Nilsson and Mosbach[2b] with the exception that freshly distilled triethylamine was substituted for pyridine in the procedure and a nitrogen atmosphere was maintained during the reaction. Microanalysis (Galbraith) gave a percent substitution of 95% based upon sulfur content. Yield (from 4 g): 3.0 g. ¹H NMR: 4.94 (2 H, q, J = 9.6 Hz, $O_3SCH_2CF_3$), 4.57 (t, J = 5.2 Hz, residual OH), 4.46 (t, ill-resolved, $TrsOCH_2CH_2$), 3.51 ppm (s, PEG backbone). The PEG-$(OTrs)_2$ contained no free tresyl acid, as shown by the lack of a singlet for the tresyl acid proton (ca. 14 ppm) and the methylene multiplet (3.70 ppm, q, J = 10 Hz) of the acid in the spectrum. Further, no tresyl chloride was present as shown by the absence of a methylene quartet at 5.62 ppm. The degree of substitution by this NMR method was 68%.

¹H NMR Experiments. In all experiments a weighed sample of the dry PEG or PEG-X (ca. 0.5 g) was dissolved in dry DMSO-d_6 (ca. 1 mL) that had been injected into a 1-mL volumetric tube through the septum cap. The volumetric tube contained some activated 3A molecular sieves. The sample stood in contact with the sieves for at least 1 h and, typically, overnight. An aliquot of the sample (500 μL) was injected into a septum-capped, standard (5 mm, Norell) NMR tube that had previously been swept out with N_2. The spectrum was acquired (120–256 scans) and then plotted. At a minimum, one full spectrum (0–10 ppm, usually) and two expansions of the relevant region were plotted and integrated. Thus, the area determinations represent, at least, three separate integrations of the spectrum.

(a) Degree of Substitution. A test of the suitability of the NMR method for determining the degree of substitution of a PEG derivative was performed by mixing PEG-OTs (97.2% OTs end groups and 2.8% OH end groups (from titration)) and unmodified PEG in varying amounts and subjecting these samples to analysis by hydrolysis/titration and NMR. The samples were prepared by mixing weighed quantities of the PEG-$(OTs)_2$ and purified, dried PEG-1500 in methylene chloride and reprecipitating these mixtures with ether; this procedure was undertaken to ensure the uniformity of the titration samples. Control experiments showed no significant difference in solubility in diethyl ether between PEG and PEG-$(OTs)_2$. The final composition determined from titration was in agreement with that calculated on the basis of the initial ratio of weighed components.

The degree of substitution from NMR was calculated from the ratio of the average of the integrals for the downfield tosylate aryl doublet (7.84 ppm) as compared to the average of the integrals for the OH triplet (4.56 ppm). The integrals were compared after normalization of the tosylate integral (from 2 protons to 1 proton). The percentage of substitution was calculated from the following equation:

$$\% \text{ sub} = (\text{integral OTs}/2)/[(\text{integral OTs}/2) + (\text{integral OH})] \times 100 \quad (1)$$

The upfield tosylate aryl doublet (7.18 ppm) was exaggerated slightly in intensity by apparent overlap with one of the aryl doublets of entrained tosyl acid. The integral for this tosylate doublet was, therefore, unsuitable for use in the degree of substitution measurements.

For PEG-$(OTrs)_2$, a sample of the polymer was prepared as above. The degree of substitution was determined by direct comparison of the ratio of the average of integrals for the methylene triplet of the tresylate moiety (4.94 ppm) as compared to the OH triplet (4.56 ppm), in accord with the following equation:

$$\% \text{ sub} = (\text{integral OTrs}/2)/[(\text{integral OTrs}/2) + (\text{integral OH})] \times 100 \quad (2)$$

(b) Molecular Weight Determination. The samples were prepared as above and the spectra acquired as described. The integral for the OH triplet (4.56 ppm) was normalized to a single proton and compared to the average of the integrals for the polymer backbone (3.51 ppm). This ratio was equal to the number of protons in the backbone. The MW was then calculated by the following equation:

$$M_n = [(\text{integral backbone})/(\text{integral OH}/2)]/4 \times 44 + 18 \quad (3)$$

The errors reported were derived from the standard deviations of the integrals and standard propagation-of-errors methods.[22]

(c) Methoxy/Hydroxyl Ratios. The samples were prepared and the spectra acquired as above. The ratio of the OH integral (at 4.56 ppm) to the integral of the normalized (i.e., divided by 3) methoxy singlet (at 3.26 ppm) should be one for the pure monomethyl ether of PEG. The presence of PEG impurity would produce a ratio greater than one. Dividing this ratio by 2, to account for the two hydroxyl groups of PEG, and multiplying by 100 gives the percentage of PEG in the commercial ether samples.

$$\% \text{ PEG} = \{[(\text{OH integral})/(\text{methoxy integral})/3] - 1\}/2 \times 100 \quad (4)$$

Results and Discussion

The basis of the present method is the well-known observation that hydroxyl protons of alcohols do not exchange rapidly on the NMR time scale in aprotic solvents such as DMSO, even in the presence of exchangeable protons associated with other species (amines, carboxylic acids, etc.). As a consequence, the hydroxyl proton gives a sharp peak and it couples with protons on an adjacent carbon.[23] In the case of PEG the OH appears as a triplet (4.56 ppm, J = 5.2 Hz) as a result of coupling with the adjacent methylene. This triplet is readily distinguishable from spinning side bands or other peaks not only on the basis of the observed multiplicity but also as a result of the chemical shift. Whereas in other solvents (e.g., CCl_4 or $CDCl_3$) the OH would appear as a singlet at a position and of a sharpness highly dependent upon the solvent, PEG concentration (due to intramolecular hydrogen bonding), and concentrations of acidic or basic impurities in the solvent,[23,24] in DMSO the OH triplet of PEG (or as a residual component of PEG-X_2s) is confined to a limited chemical shift range (4.56 ± 0.05 ppm).

Table I
NMR Determination of MW for Some PEG MW Standards[a]

NMR[b]	standard[c]
155 (±21)	150 (1.00)
245 (±23)	202 (1.03)
592 (±37)	586 (1.05)
1030 (±230)	960 (1.07)
1508 (±258)	1470 (1.05)
4495 (±532)	4250 (1.03)
6968 (±1871)	12600 (1.05)

[a] Dried in DMSO-d_6 solution with 3A molecular sieves. [b] Average of three spectral determinations; estimated errors in parentheses (standard deviations). [c] M_w/M_n is listed in parentheses as given by the supplies, except in the case of the MW 150 sample, which is triethylene glycol monomethyl ether.

In the following examples we show how the area of the hydroxyl PEG peak in DMSO-d_6 can be used to obtain information on PEG MW and percent substitution, and we explore the limits of the method. These methods all depend upon the quantitative comparison of the normalized integral for the OH triplet with that for some other suitable peak or group of peaks including the polymer backbone (MW determination), the methoxy group of an M-PEG (determination of excess PEG in commercial M-PEG), and the peak due to an end group (degree of substitution).

Molecular Weight Determinations. The MW of a PEG can be determined by comparing the ratio of the OH triplet integral, normalized to that for a single proton, to the average integral for the polymer backbone (Table I). In a sense this is a type of end-group analysis since the MW determined in this fashion is highly dependent upon the integral assigned to the hydroxyl proton. As such, the MW measured is the number-average MW, M_n.[25] The MW would also be expected to be highly dependent upon the presence or absence of low-MW impurities (e.g., ethylene glycol), as with other M_n determinations,[26] and this NMR method will be limited by the relative sizes and overlap of the small OH peak and the large backbone peak. Further, for MW determinations, the DMSO-d_6 solutions should be carefully dried so that the central peak of the PEG backbone is not augmented in area by overlap with the peak for water (3.33 ppm).

The limit of practical determination of the MW of an unsubstituted PEG by this NMR method is reached close to MW 5000; at MW 12 600 large errors are found (Table I). Errors in the integrals, upon which the method rests, arise from overlap of the small OH peak with the broad shoulder of the large central singlet of the polymer backbone (3.51 ppm). Also, as the central singlet of the PEG repeating units increases in intensity, spinning sidebands of the peak begin to interfere. Nevertheless, the correlation between the NMR and standard MWs is excellent; the regression equation is M_n(NMR) = 1.06(MW standards) − 2.76, with a correlation coefficient of 0.998 (for MWs less than 12 600 (Figure 1)).

By way of comparison, secondary measurements of MWs of PEGs and PEG-Xs are frequently made by size-exclusion chromatography by comparing with a standard curve derived from retention volumes of readily available PEG standards.[27-29] A drawback to this approach is that elution volumes of PEG *derivatives* are frequently affected by adsorption effects.[30] For example, in our preparation of PEG tosylate for the present work we observed that the tosylate had a much greater retention volume on size-exclusion chromatography than did PEGs of comparable MWs; hydrolysis back to the PEG, followed by chromatography, showed that the PEG tosylate in fact had a much larger MW than indicated by retention volume of the tosylate. Craven and co-workers[31] recently enumerated partition, adsorption, and solvation effects in the study of oligo(oxyethylenes) and noted a dependence of elution volume on end-group size, polarity, and structure.

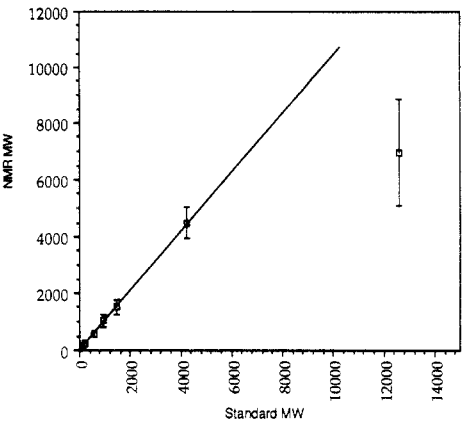

Figure 1. NMR vs standard MWs.

Table II
Percentages of Unmodified PEG in Commercial M-PEGs

supplier	MW	normalized OH/MeO ratio[a]	% PEG[b]
Polysciences	350	1.02	1.2
Aldrich	550	1.14	7.2
Aldrich	750	1.18	9.4
Aldrich	1900	1.23	12
Aldrich	5000	1.21[c]	11[c]

[a] Methoxy integral divided by 3. [b] Calculated from eq 4. [c] Estimate. Determination of the integral for OMe was difficult for this sample because of overlap of the PEG backbone peak with the OMe peak.

Therefore, although the present ^{1}H NMR method is limited to lower MW PEGs, it would appear to be suitable for many applications. It is a primary method, dependent only on the PEG itself rather than on outside standards. It is relatively rapid and easy to apply. And it yields results that are of comparable quality to those obtainable from liquid chromatography.

PEG Contaminant in the Monomethyl Ether of PEG (M-PEG). Commercial samples of M-PEG contain varying amounts of PEG produced by hydroxide initiation of ethylene oxide polymerization.[1a] We have previously estimated the percentage of PEG in commercial M-PEG-1900 and -5000 to be 25% (based on size-exclusion chromatography).[1a] De Vos and Goethals employed the trichloroacetyl isocyanate derivatization route to determine the percentage of PEG for several M-PEGs from MW 750 to MW 5000 and found from 1 to 26% PEG.[17e]

Application of the NMR method to several M-PEGs (Table II) shows, as expected, that the hydroxyl peak is larger than one-third the methoxy peak (a representative spectrum of M-PEG-1900 is shown in Figure 2). Application of eq 4 permits calculation of the percentage of PEG in the M-PEG samples (Table II). Note that the amount of PEG increases with M-PEG MW; this same trend was observed in the earlier works.[1a,16e] Although the amount of PEG determined by this method is slightly smaller than that determined in the earlier works, the numbers are in the same range, and we are of the opinion that our method is reasonably accurate and the differences are simply a result of variation in quantity of PEG impurity present from batch to batch of M-PEG.

Degree of Substitution. *Determination of degree of conversion of PEG hydroxyl to some other functional group is often difficult, and in some cases no suitable*

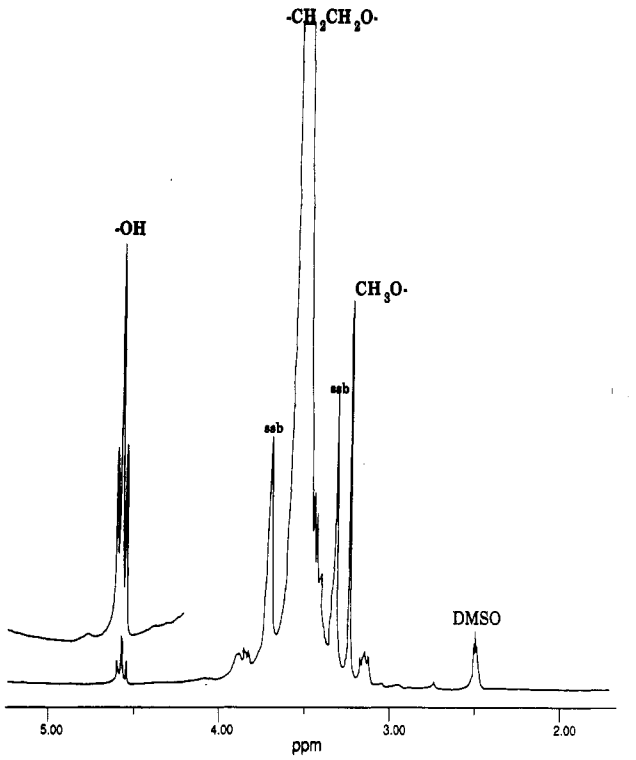

Figure 2. ¹H NMR spectrum of the monomethyl ether of poly(ethylene glycol)-1900.

Table III
Comparison of Degrees of Substitution Determined by Hydrolysis–Titration and by NMR (Eq 1) for Synthetic Mixtures of PEG-$(OTs)_2$-1500 and PEG-1500

titration, %	NMR, %	% difference[a]
97.2 ± 0.1	100 ± 0	−2.8 ± 0.1
87.6 ● 0.3	91.0 ± 5.1	−3.4 ± 5.1
78.1 ± 0.3	66.3 ± 3.9	11.8 ± 3.9
67.3 ± 0.2	58.2 ± 4.5	9.1 ± 4.5
56.7 ± 1.9	49.4 ± 6.4	7.3 ± 6.7
48.6 ± 0.2	41.6 ± 2.9	7.0 ± 2.9
38.3 ± 0.7	31.9 ± 1.1	6.4 ± 1.3
30.2 ± 0.2	23.3 ± 0.9	6.9 ± 0.9
21.7 ± 0.7	15.0 ± 2.6	6.7 ± 2.8

[a] Errors represent standard deviations of, at least, three determinations.

method has been found. Application of the present NMR method to this problem is straightforward for those large number of derivatives in which the new functional group has an NMR absorption well separated from the backbone region of the spectrum (ca. 3.0–4.0 ppm). To illustrate this approach and to explore its limits, we have prepared a series of PEG tosylate samples of varying degrees of "substitution" by mixing PEG tosylate and unsubstituted PEG (as described in the Experimental Section). To apply the NMR method in this case, the area of the tosylate aryl peak at 7.84 ppm (2 H) is compared to the area of the hydroxyl peak at 4.56 ppm (according to eq 1). Although the actual "percent substitution" is known from weights of tosylate and PEG in each sample, we have also determined percent substitution by hydrolysis/titration as an additional control and to simulate determination of the degree of substitution of an unknown sample (Table III). Hydrolysis/titration gave results in essentially perfect agreement with those obtained from known weights of the two components of the mixture.

As can be seen from the data, the NMR results are reasonable, with the method generally underestimating the percent substitution.

As an additional test of the NMR method for percent substitution, we have prepared a sample of the 2,2,2-trifluoroethanesulfonate (or "tresylate") of PEG-4000, and we have determined its degree of substitution by elemental analysis for sulfur and by NMR. The tresylate has a methylene quartet in its NMR spectrum at 4.94 ppm, which is quite suitable for comparison to the hydroxyl triplet at 4.56 ppm. Elemental analysis indicates 95% substitution (based on sulfur) while the NMR method indicates 68% substitution. As with the tosylate, the NMR method again underestimates percent substitution.

As an additional point, we have recently reported the preparation of the picryl ether of M-PEG-1900 and found 11 and 53% substitution for two samples by nitrogen analysis and 8 and 59% substitution by the NMR method.[10] Finally, it should be noted that the NMR method proved unsuitable for determining the degree of substitution for cyanuric chloride activated PEGs because of overlap of the residual OH and the terminal methylene of the derivative.

Conclusions

We have seen from the above experiments that the NMR method provides reasonable estimates of percent substitution and MW for PEG derivatives. The value of the method should be viewed in terms of the alternatives available. Hydrolysis of tosylates, tresylates, and cyanuric chloride derivatives followed by titration, direct titration of end groups (e.g., amines and acids), and combustion analyses on derivatives that contain a good "marker" element (such as sulfur or nitrogen) are excellent methods, but it is critical in applying these methods that very small amounts of impurities be quantified (generally by chromatography). Also there are derivatives for which such methods are not applicable (e.g., ethers). IR analysis and ^{13}C NMR spectroscopy are excellent methods for identifying functional groups, but application in a quantitative fashion is more difficult. We have found that the present NMR method provides a valuable adjunct to these traditional methods. It is easy and rapid to apply, and frequently it reveals the presence of impurities. Also, it is important to note that the tendency of the NMR method to underestimate degree of substitution is frequently not a serious problem, since often it is less important to discover whether a derivative is exactly 23 or 30% substituted than whether the degree of substitution is relatively high or low.

In summary, we have described a straightforward ¹H NMR method for determining MWs of low- and moderate-MW PEGs, estimating the amount of PEG in commercial M-PEGs, and measuring degrees of substitution for a variety of PEG derivatives. These procedures yield results of comparable accuracy to alternative methods in the literature, avoid derivatization, and utilize a readily available ¹H NMR spectrometer of medium resolution. The methods described should find wide use and application in PEG-X analysis.

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