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Sequencing of Specific Copolymer Oligomers by Electron-Capture-Dissociation Mass Spectrometry

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Abstract: Although a poly(ethylene/propylene glycol) (PEG/PPG) copolymer mixture is far too complex (~150 oligomeric formulas) for conventional purification, oligomer ion compositions of <1% abundance can be separated by Fourier transform mass spectrometry and dissociated into sequence-specific fragment ions. Using collisionally activated dissociation (CAD) or other conventional energetic methods, we found that misleading rearrangements are common; however, these are negligible with electron capture dissociation (ECD), consistent with its nonergodic mechanism. Despite the lack of reference compounds, ECD of five oligomers ranging from PEG_1PPG_{18} to PEG_9PPG_{15} shows that ~80% of their isomers have all PEG units at one end, while CAD gave lower values because of an ~21% rearrangement loss of internal monomer units. In contrast to the indicated triblock "PEG/PPG/PEG" sample designation of this commercial surfactant, all of these oligomers are found to consist primarily of diblock PEG/PPG structures, so that their termini differ significantly in hydrophobicity, as expected for a surfactant.

Introduction

Despite the high commercial importance of synthetic polymers, most methodologies for their molecular characterization can only examine mixtures of their oligomeric components and provide little information on copolymer sequences. Individual oligomers of larger homopolymers (>2 kDa) can be isolated in high purity only with specialized techniques, and copolymers can represent far more complex mixtures.[1] However, these oligomers after ionization can be readily separated by mass spectrometry (MS).[2−5] Even isotopic separation can be achieved by Fourier transform (FT) MS, identifying 50 oligomers of >20 kDa poly(ethylene glycol) (PEG)[5a] and ~200 oligomers of the copolyester dipropoxylated bisphenol A/adipic acid/isophthalic acid (DAI 12).[5f] The mass values of these molecular ions can provide monomer identification, molecular weight distributions, and end group determinations,[2−5] and conformational data have been reported.[6] For a linear copolymer (e.g., EP_2), however, the sequence ordering of its monomers (e.g., EPP, PEP, or PPE) is also a critical determinant of its properties, but MS characterization has been unreliable because molecular ion dissociation often leads to rearranged products (e.g., PEP → PP + E).[2−5,7]

For a linear protein, tandem MS (MS/MS) structural characterization of a mass separated molecular ion by energetic cleavage (e.g., collisionally activated dissociation, CAD) has been extensively exploited to yield reliable sequence data, as the backbone cleavage products retain their original amino acid connectivities, without rearrangement.[8] For common polymers, however, this is often not true, even for CAD at 8 keV.[4] Such rearrangements are especially competitive for even-electron ions,[9] such as those produced by MS sample introduction

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methods for nonvolatile compounds (e.g., electrospray ionization, ESI).[5,10] A recent article on MS/MS of copolyesters[5f] cogently illustrates the problem with DAI 12, in which the Ω-diol (D) can be bound to either of the diacids, adipic (A) or isophthalic (I). ESI followed by CAD of the isolated major ion $[(DA)_2DI]Na^+$ gave firm evidence for the isomers DIDADA and DADADI, but no evidence for the third possibility, DADIDA; sequencing would be more extensive "if the bonds could be broken more selectively".[5f] MS/MS of alkylpolyisocyanate copolymers formed cyclic trimer product ions that indicated block microstructures, but not their location in the polymer.[11] Although conventional matrix-assisted laser desorption/ionization (MALDI)/time-of-flight is poorly suitable for MS/MS sequencing of separated oligomeric ions,[2a,c] details of diblock copolymer structures have been derived from extensive analysis of MALDI molecular weight distribution data.[5b,12]

Polyglycols are the polymers most studied by MS;[1,4−7,10,13,14] CAD spectra of PEG, poly(propylene glycol) (PPG), and other polyether even-electron ions show fragment ions containing both termini, demonstrating rearrangement association through internal monomer loss.[7] We report here that electron capture dissociation (ECD, the nonergodic dissociation of an odd-electron ion)[13,15] causes minimal rearrangement during dissociation of PEG/PPG copolymer ions to provide useful sequence information from oligomer components representing <1% of the total. Such "Pluronics" copolymers are commonly used as nonionic surface-active agents for which the PEG and PPG blocks are assumed to provide contrasting hydrophilicity and hydrophobicity, respectively.[16]

Experimental Section

Ions from nanoelectrospray of 46:46:8 H_2O/MeOH/acetic acid solutions (20−40 μM) of PEG, PPG, and PEG-block-PPG-block-PEG samples (Aldrich) were introduced into a modified 6T Finnigan FTMS instrument and isolated by SWIFT[17] as a $^{13}C_0$ and $^{13}C_1$ (Δ1 Da) pair of peaks, unless noted otherwise.[5a,10] Their CAD[18] and ECD[13,15] spectra were measured with data collection starting at *m/z* 500 and with data reduction by the THRASH program.[19]

Results and Discussion

Rearrangement Minimization. CAD of MS isolated $[HO(C_2H_4O)_xH + nH]^{n+}$ ($x = 24, 44, 95, 100$) ions formed by ESI[10] of PEG produces (eq 1, * = +, R = H) the complementary **A** and **B** fragment ions by cleavage of a backbone ether bond.[7]

$$[R(OC_2H_4)_xOR + nH]^{n+} \xrightarrow[\text{ECD: } + e^-]{} [R(OC_2H_4)_y\overset{\overset{\cdot\cdot H\cdot\cdot}{|}}{\underset{*}{O}}(C_2H_4O)_{(x-y)}R + (n-1)H]^{(n-1)+}$$

$$\longrightarrow [R(OC_2H_4)_yOH + mH]^{m+} + [*(C_2H_4O)_{(x-y)}R + (n-m-1)H]^{(n-m-1)+} \quad (1)$$

A **B**

C: $[R(OC_2H_4)_yOR + mH]^{m+}$

ECD is similar (eq 1, * = ·, an unpaired electron, and R = H) in yielding **A** ions, but **B** products are negligible.[13] For CAD spectra, product **C** was demonstrated previously[7] to be formed by the rearrangement elimination of internal C_2H_4O units through the cleavage of two bonds. For R = H, product **A** is identical to product **C**, but these products can be distinguished using methoxy end-capped PEG (eq 1, R = CH_3). CAD of these $(PEG_{24}Me_2 + H)^{1+}$ ions produces 21% **C** rearranged ions (Figure 1), similar to the yield shown by deuterium labeling data from CAD of smaller PEG oligomers.[7a] ECD of $(PEG_xMe_2 + nH)^{n+}$ gives **A** ions (eq 1, R = CH_3) that represent >98% of the fragment ions for $x = 24$ (Figure 1). Rearrangement products $\mathbf{C}_{23}$ and $\mathbf{C}_{22}$ (direct loss of one and two monomer units) represent <1% of the products. For ECD of the 3+ and 4+ ions of $x = 42$, 45, and 48 (not shown), only **A** products are visible, and no products represent the loss of both methoxy termini. The absence of **C** ions from ECD is consistent with the proposal[15] that its 5−6 eV neutralization energy causes immediate (nonergodic, $<10^{-12}$ s) dissociation of one bond without appreciably changing the internal energy of the rest of the ion; products requiring the dissociation of two bonds, such as rearrangements, are also negligible in the ECD spectra of proteins.[15]

Copolymer Characterization. ESI of the commercial copolymer "PEG-block-PPG-block-PEG" gave a complex spectrum whose molecular ions indicate 33% (charge-normalized ion abundance) PPG_{10-22} homopolymer oligomers, plus ~130 copolymer $PEG_{1-11}PPG_{10-22}$ oligomers whose highest abundance values of the 2+ ions are ~0.5% (Figure 2). The 1+ ion spectrum shows a remarkable resemblance to the published[5b] MALDI/FTMS spectrum of BASF Pluronic L31, suggesting a similar source for both their and our samples. Theirs was described as a triblock PEG/PPG copolymer "with the structure $HO-(C_2H_4O)_x-(C_3H_6O)_y-(C_2H_4O)_z-H$", matching the "PEG-block-PPG-block-PEG" description of our sample. For the Figure 2 data, the PEG/PPG compositions (E_xP_y or *x*, *y*) were assigned to the Figure 2 data as described by van Rooij et al.[5b] They provide an extensive discussion of the distribution of the 130 oligomer compositions identified, and their data fully support a random coupling of C_2H_4O units onto the PPG reactants.[5b]

Copolymer Sequencing. Despite abundances of only ~0.5% for the oligomer 2+ ion precursor, ECD spectra were obtained for PEG_4PPG_{18} (Figure 3), PEG_4PPG_{16}, PEG_1PPG_{18}, PEG_5PPG_{15} (Figure 4),[20] and PEG_9PPG_{15}.[21] For the fragmentation

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(20) Some of the selected mass values will contain contributions from $^{13}C_2$ ions 2 Da lower; PEG_4PPG_{18} will be contaminated with 11% PPG_{21}, PEG_4PPG_{16} with 15% PPG_{19}, and PEG_5PPG_{15} with 13% PEG_1PPG_{18}.

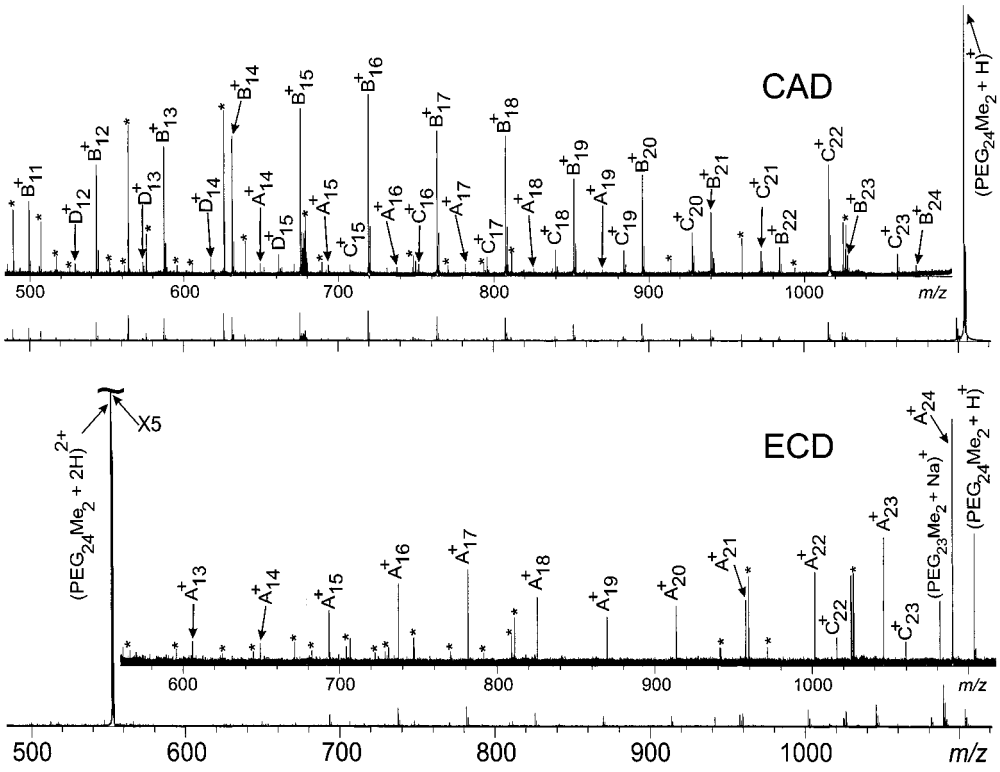

Figure 1. CAD spectrum of $(PEG_{24}Me_2 + H)^{1+}$ and ECD spectrum of $(PEG_{24}Me_2 + 2H)^{2+}$. Inset: ECD of the monoisotopic precursor ions. **C** ions, internal C_2H_4O loss; **D** ions, possible CH_3OH loss from **A**; *, noise (no isotope peaks) or background peak. ECD of the isobaric $(PEG_{23}Me_2 + 2Na)^{2+}$ yields the $(PEG_{23}Me_2 + Na)^{1+}$ ions.[14]

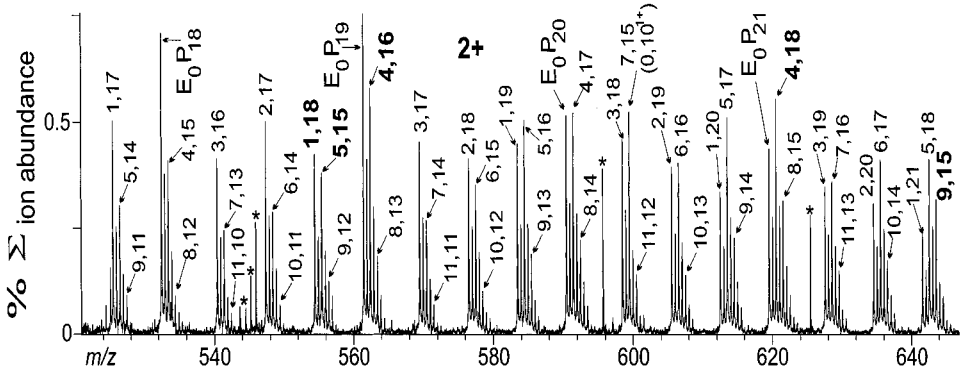

Figure 2. Partial (2+ region) ESI mass spectrum of "PEG-block-PPG-block-PEG" copolymer. E_xP_y or *x*, *y*, ions whose exact mass (±5 ppm) corresponds to the protonated monoisotopic molecular ion $[H(C_2H_4O)_x(C_3H_6O)_yH + nH]^{n+}$; the peak 1 Da higher is that containing one ^{13}C atom.

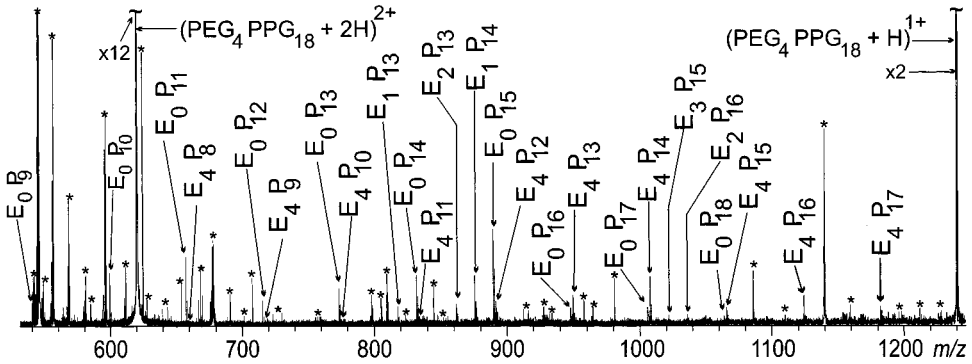

Figure 3. ECD mass spectrum of the MS separated $(PEG_4PPG_{18} + 2H)^{2+}$ ions of Figure 2.

behavior expected of a specific homopolymer block at a polymer chain terminus, the reference ECD spectra of $(PEG_{24} + 2H)^{2+}$ and $(PPG_{18} + 2H)^{2+}$ show significant differences in their **A** fragments (Figure 4, solid bars, top left and top right spectra), with the most abundant corresponding to the loss of four C_3H_6O units for PPG versus loss of two C_2H_4O units for PEG. Each

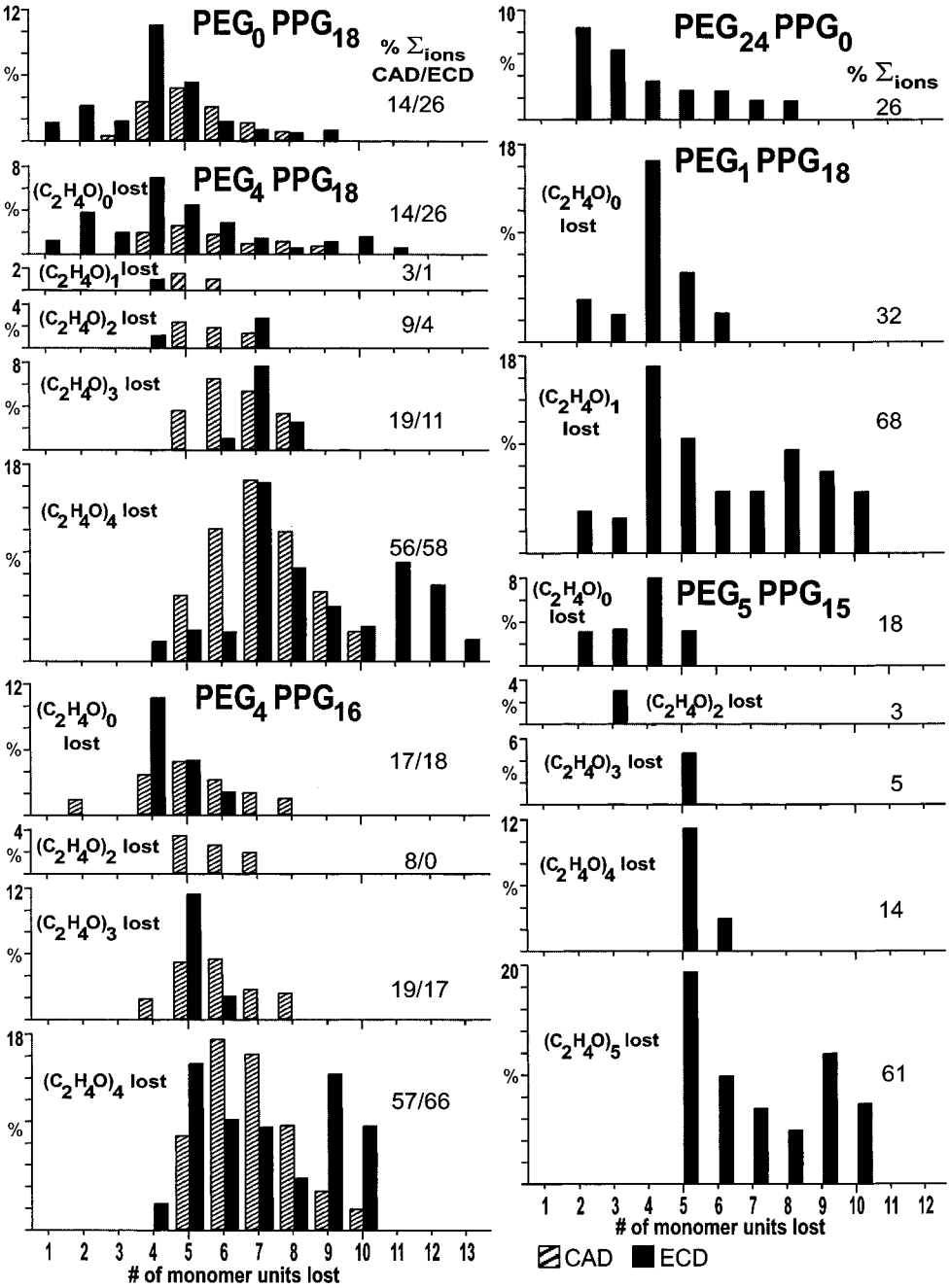

Figure 4. CAD (**A**, **C** ions, striped bars) and ECD (**A** ions, solid bars) spectra of MS separated $(PEG_xPPG_y + 2H)^{2+}$ ions of Figure 2 showing relative ion abundances as a function of monomer units lost; separate bar graphs are shown for each x value for $(C_2H_4O)_x$ lost.

proton will be repelled by the other toward the end of the chain, where the terminal hydroxyl can be one of approximately seven groups that participate significantly in H^+ solvation;[13−15] apparently, −OH participation is far less important in the PPG terminus than in the PEG terminus because of the higher cation binding affinity of the ether units $-CH_2OCH(CH_3)-$ versus $-CH_2OCH_2-$.

These correlations can be used to interpret the ECD spectrum of the simplest copolymer formula, PEG_1PPG_{18}. Irrespective of where the one C_2H_4O monomer unit is placed, at least a terminus of nine C_3H_6O units must have no C_2H_4O units. The bar graph "$(C_2H_4O)_0$ lost" (Figure 4 right column, second spectrum) must represent fragmentation of the C_2H_4O free terminus, and it does match well the spectrum of PEG_0PPG_{18}. The "$(C_2H_4O)_1$ lost" spectrum of PEG_1PPG_{18} is consistent with a terminal C_2H_4O unit; its close resemblance to that of PEG_0-PPG_{18} (and not of $PEG_{24}PPG_0$) shows that one C_2H_4O unit near the terminus has not allowed the cation binding affinity of the terminal hydroxyl to affect the spectrum. However, the greater abundance (68%) of the "$(C_2H_4O)_1$ lost" products suggests that the C_2H_4O unit has significantly increased the H· atom affinity of this terminus.[15]

From the "PEG-block-PPG-block-PEG" designation, the other oligomers should have C_2H_4O at both termini; yet their "$(C_2H_4O)_0$ lost" spectra are also of significant abundance (18−

(21) A very low signal/noise ECD spectrum of PEG_9PPG_{15} (not shown) had a majority of peak intensities for the formulas $PEG_0PPG_{14,15}$ (all C_2H_4O lost) and PEG_9PPG_{11-14} (no C_2H_4O lost).

26%), with the most intense peaks in each representing the losses of $(C_3H_6O)_4$ and $(C_3H_6O)_5$ like that of PEG_0PPG_{18} and PEG_1PPG_{18}.[21] Because of the low H· atom affinity of the all-PPG terminus, even lower than for PEG_1PPG_{18}, the "$(C_2H_4O)_0$ lost" abundances should indicate that the majority of the PEG_4PPG_{18}, PEG_4PPG_{16}, and PEG_5PPG_{15} oligomers have one terminus with at least five (C_3H_6O) units.

This major diblock structure is also consistent with the more abundant ECD fragment ions that have lost all of their PEG monomers [bar graphs "$(C_2H_4O)_x$ lost" for PEG_xPPG_y].[21] These represent 58−66% of the ECD products, and they must have been formed by a single ECD cleavage[15] that removed from one end of the polymer all of the C_2H_4O units plus a varying number of C_3H_6O units. The relative abundances in these vary much more than those of the "$(C_2H_4O)_0$ lost" bar graphs; detailed sequence interpretation would be helped by reference ECD spectra of pure isomers. Even with four or five terminal C_2H_4O units, proton solvation must also involve the neighboring C_3H_6O units, in keeping with the many additional C_3H_6O units lost in these "$(C_2H_4O)_x$ lost" spectra.

Triblock Impurities. Although the "$(C_2H_4O)_0$ lost" and "$(C_2H_4O)_x$ lost" spectra are consistent with diblock HO−$(C_2H_4O)_x$−$(C_3H_6O)_y$−H structures for PEG_4PPG_{18}, PEG_4PPG_{16}, and PEG_5PPG_{15} (and PEG_9PPG_{15}),[21] this cannot be true for the 16, 17, and 22%, respectively, **A** fragments that represent simultaneous loss of both monomers (but less than PEG_x) from a single ECD cleavage, as such products are negligible from rearrangement (vida supra). The "$(C_2H_4O)_1$ lost" product indicates that one of the PEG units is on the terminus lost, while the "$(C_2H_4O)_{x-1}$ lost" product indicates that all but one of the PEG units is on one terminus; again, the much more abundant products are those losing more C_2H_4O units, the terminus that should thus have the greater H· atom affinity. Note also that each of the three "$(C_2H_4O)_{x-1}$ lost" spectra, despite low signal/noise, is similar to its counterpart "$(C_2H_4O)_x$ lost" spectrum; the most intense peak is formed by loss of the same number of monomer units. Again, replacing a C_2H_4O unit with a C_3H_6O unit has had little apparent effect on the proton solvation of the original dication.[13−15]

The Copolymer Structure. Thus, most isomers of these oligomers must have no C_2H_4O units on one terminus, contrary to the sample label. For anionic polymerization of C_2H_4O onto PPG, the growing chain at the $-OCH(CH_3)CH_2O^-$ end apparently is favored for C_2H_4O addition over that at the $-OCH_2CH(CH_3)O^-$ end, resulting in chain ends $HOCH(CH_3)-$ and $-CH_2OH$. Proton NMR could only confirm the **H**OCH_2- group in this sample; the complex NMR spectrum, indicating 1PEG:4PPG, has a small 3.1 ppm triplet consistent with **H**OCH_2-, but this is adjacent to a complex CH_2 region at 3.2−3.7 ppm that obscures the expected **H**$OCH(CH_3)-$. In a careful study of the distribution of oligomer formulas of the molecular ion spectrum from the closely similar "triblock PEG/PPG/PEG" sample, this absence of a PEG block at one end was not discernible.[5b] However, their analysis was consistent with a random coupling hypothesis, indicative of a very small, or a very large, difference in reactivity between the ends of the PPG polymer. The ECD data for PEG_4PPG_{18} and PEG_4PPG_{16} (Figure 4) indicate that >84% of the products result from adding four C_2H_4O units to one end of the PPG chain without adding any C_2H_4O units to the other end, and for PEG_5PPG_{15}, 79% from adding five C_2H_4O units to one end.

CAD Rearrangement. Characterization by CAD, however, of PEG_4PPG_{18} and PEG_4PPG_{16} indicates (Figure 4, striped bars) that the products whose termini contain only zero or four C_2H_4O units represent 70 and 74%, respectively, versus the 84 and 84% found by ECD. The difference should represent **C** rearrangement ions for which a 21% yield was found for the Figure 1 data.

Conclusions

For ECD applications, polyglycols were the first macromolecules investigated[13] after proteins;[15] for other copolymers to be amenable to such structural characterization, they must give multiply charged molecular cations for which ECD effects backbone dissociation. Here ECD/FTMS was effective in eliminating sequence-misleading rearrangements and in characterizing low abundance oligomers; with ∼150 different formulas for oligomer ions, ∼40 are still of sufficient abundance for ECD. The five ECD spectra measured are consistent with a large reactivity difference for chain extension at the two ends of the PPG reactant, so that ∼80% of the isomeric structures for this surfactant are diblock, critically different than the label "PEG-block-PPG-block-PEG". These oligomers do not have ends of similar hydrophilicity outside a hydrophobic center, but instead have the more common surfactant structure with ends of contrasting solubilities. Even without reference copolymers of established isomeric structure, the extensive structural details obtainable by ECD MS/MS from such complex copolymer mixtures should provide a far more powerful method for relating synthetic variables to the physical properties that are critical to specific applications.

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