RESEARCH ARTICLE

Electrospray Ionization Tandem Mass Spectrometry of Ammonium Cationized Polyethers

Andreas Nasioudis,¹ Ron M. A. Heeren,² Irene van Doormalen,³ Nicolette de Wijs-Rot,³ Oscar F. van den Brink¹

¹AkzoNobel Research, Development and Innovation, P.O. Box 10, 7400 AA, Deventer, The Netherlands

²FOM Institute for Atomic and Molecular Physics, P.O. Box 41883, 1009 DB, Amsterdam, The Netherlands

³Merck Sharp and Dohme, P.O. Box 20, 5340 BH, Oss, The Netherlands

Abstract

Quaternary ammonium salts (Quats) and amines are known to facilitate the MS analysis of high molar mass polyethers by forming low charge state adduct ions. The formation, stability, and behavior upon collision-induced dissociation (CID) of adduct ions of polyethers with a variety of Quats and amines were studied by electrospray ionization quadrupole time-of-flight, quadrupole ion trap, and linear ion trap tandem mass spectrometry (MS/MS). The linear ion trap instrument was part of an Orbitrap hybrid mass spectrometer that allowed accurate mass MS/MS measurements. The Quats and amines studied were of different degree of substitution, structure, and size. The stability of the adduct ions was related to the structure of the cation, especially the amine's degree of substitution. CID of singly/doubly charged primary and tertiary ammonium cationized polymers resulted in the neutral loss of the amine followed by fragmentation of the protonated product ions. The latter reveals information about the monomer unit, polymer sequence, and endgroup structure. In addition, the detection of product ions retaining the ammonium ion was observed. The predominant process in the CID of singly charged quaternary ammonium cationized polymers was cation detachment, whereas their doubly charged adduct ions provided the same information as the primary and tertiary ammonium cationized adduct ions. This study shows the potential of specific amines as tools for the structural elucidation of high molar mass polyethers.

Key words: Synthetic polymers, Tandem mass spectrometry, CID, ESI, Non-covalent complexes

Introduction

The formation of noncovalent complexes between ammonium ions [e.g., quaternary ammonium salts (Quats) or primary or tertiary ammonium ions] and polymers has been

shown to be a versatile tool that can address various analytical challenges in mass spectrometry (MS). This versatility derives from the fact that these noncovalent complex ions can be used to study either the neutral polymer or the cation itself.

It has been demonstrated that polyethers and crown ethers can be used as shift reagents for ion mobility spectrometry MS (IMS-MS) and separate isobaric and/or isomeric ammonium ions (e.g., protonated peptides, small ammonium molecules) [1–4]. This is accomplished by the formation of noncovalent complexes between the ether and the ammo-

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Correspondence to: Oscar F. van den Brink; e-mail: Oscar.vandenBrink@akzonobel.com

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nium ions. The complex ions exhibit a different mobility behavior than their naked peptide ions or ammonium ions (in the case of small amine molecules). As a result, they extend the ability to separate components. In addition, the intensity of the complex ions is significantly increased in relation to the intensity of other polymer adduct ions present, indicating a possible stabilization effect.

The use of this technique for the analysis of polymers is still in an early stage. Obtaining information, such as monomer composition, endgroup type, and molecular weight distribution [5–8] by MS is well established for relatively pure and narrow dispersed polymers of relatively low molecular weight, and it is sufficient for a number of applications. The current challenge in polymer analysis is to get the same type of information for complex mixtures with broad distributions and high molar mass. To some extent, liquid chromatography (LC) and size exclusion chromatography (SEC) coupled to electrospray ionization (ESI) MS can face this challenge of complexity [9–12]. When disperse and high molar mass polymers are analyzed, convolution of the multiple charge state distributions with the size distribution is a common phenomenon. Various attempts to deal with this challenge have been reported. In summary, methods such as charge reduction [13–23], high resolution MS [24, 25], and separation of ions with different charge-state with IMS-MS [26, 27] have been used. All of these strategies require advanced and/or custom made MS instruments. Another approach, which requires only relatively simple MS instrumentation, is to facilitate the formation of polymer–ammonium ion complexes. Recent studies showed that the MS analysis of high molar mass (2–40 kDa) poly(ethylene glycols) (PEGs) [28, 29], synthetic copolymers [30], and PEGylated proteins [28, 31] can be performed by forming complex ions of these polymers with primary, tertiary and quaternary ammonium ions.

Although the use of various ammonium ions for the formation of low charge state adduct ions has been demonstrated [28–31], the behavior of these ions upon excitation has not been thoroughly studied. To the best of our knowledge, only Howdle et al. [32] presented a tandem MS (MS/MS) spectrum of a PEG/amine complex ion. The amine used was an active pharmaceutical ingredient, lamivudine (a primary amine). Activation of the complex ion resulted in dissociation of the complex to its constituents. Fragment ions that could provide structural information (e.g., sequence, monomer unit, end group) about the polyether were not reported.

This work targets the identification of the type of polyether/amine complex ions that can best be used as a source of structural information of the polyether. A comparative study is made of ammonium ion adducts with different degree of substitution, structure, and size, focusing on their stability upon excitation and on product ion mass spectra. Results of excitation of complex ions of polymers with structurally different Quats are also presented. In addition, breakdown diagrams are created from the energy-

dependent analysis by collision-induced dissociation (CID) of selected complex ions. These diagrams provide insight in the complex ions' stability and potential use for structural elucidation of high molar mass polyethers.

Experimental

Materials

PEG 2 kDa was synthesized and characterized in house at AkzoNobel (Arnhem, The Netherlands). Poly(propylene glycol) (PPG) 2 kDa from Waters Associates (Framingham, MA, USA) and 1-*N,N*-dibutyl-butanamine (TBA) was from Fluka (Steinheim, Germany). Poly(tetramethylene glycol) (PTMEG) 2 kDa, adamantan-1-amine (AD), hexadecyltrimethylammonium chloride ($C_{16}Me_3NCl$), 1-hexadecyl-pyridinium bromide ($C_{16}PyBr$), hexadecyldimethylamine ($C_{16}Me_2N$), and methanol (MeOH) of HPLC grade were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cation exchange resin AG MP-50 was purchased from Bio-Rad Laboratories (Hercules, CA, USA). Ultra-pure water with a resistivity of 18.2 $M\Omega\cdot cm$ (at 25 °C) was obtained from a Millipore Direct-Q3 water purification system (Molsheim, France). The samples were dissolved in a 50/50 (vol/vol) water/MeOH or pure MeOH (after purification with the cation exchange resin) at a concentration of 25–50 $\mu g/mL$ containing 0.25–0.75 mM of the amines and Quats studied. The concentration of the samples for the experiments in the LTQ Orbitrap hybrid mass spectrometer was 2–5 $\mu g/mL$. The concentration of the amines remained the same as previously.

ESI-MS and ESI-CID-MS-MS of Complex Ions

MS and MS/MS experiments were performed in a Waters Synapt G1 IMS time-of-flight (TOF) mass spectrometer (Waters, Manchester, UK), a Bruker Esquire 3000^{plus} quadrupole ion trap (QIT) mass spectrometer (Bruker Daltonics, Bremen, Germany) and an LTQ Orbitrap hybrid Fourier transform mass spectrometer (FTMS) (Thermo Scientific, San Jose, CA, USA). The settings of the IMS-TOF mass spectrometer were the following: capillary voltage 3 kV, desolvation temperature 150 °C and desolvation gas flow 600 L/h. The value of the sampling cone was adjusted in each case to prevent upfront fragmentation and never exceeded 45 V. The isolation settings in the quadrupole (LM and HM resolution) were optimized to maximize the intensity of the precursor ion (indicative values were 20 and 10 arbitrary units for LM and HM, respectively). Isolation and fragmentation of the precursor ions was performed in the trap cell. The mobility cell was inactive during these measurements. Hence, the IMS-TOF mass spectrometer was used as a regular quadrupole TOF (q-TOF) mass spectrometer. The settings of the QIT mass spectrometer were the following: capillary voltage 3 kV; nebulizer pressure 76 kPa; dry gas flow 4 L/min; dry gas

temperature 250 °C; the collision gas was He. The number of trapped ions was approximately 5000 and was kept constant by using the ion charge control (ICC) option throughout all measurements. It was observed that when the abundance of the precursor ions was low, the necessary accumulation time to obtain an ICC of 5000 was too long, and resulted in space charge effects. Therefore, the maximum accumulation time was set between 3 and 5 ms, depending on the precursor ion's abundance. The isolation parameters (e.g., isolation width and isolation mass) were optimized to increase the abundance of the isolated precursor ion. A cooling period of 50 ms was inserted between the isolation and excitation steps. The excitation time was set to 60 ms and the level of excitation was controlled by changing the fragmentation amplitude. The "Smart Frag" feature was disabled. The settings of the LTQ Orbitrap FTMS were the following: source voltage 4 kV; capillary temperature 300 °C; tube lens voltage 70–200 V; capillary voltage 50 V. The MS/MS experiments were performed in the linear ion trap (LIT). The isolation parameters (e.g., isolation width and isolation mass) were optimized to increase the abundance of the isolated precursor ion. The excitation time was set to 30 ms and the Q value at 25%. The level of excitation was controlled by changing the normalized collision energy (NCE). The "wide band activation" feature was disabled. The sample was introduced via a Cole-Palmer syringe pump (Vernon Hills, IL, USA) at a flow rate of 10 μ L/min in the case of QIT. For the other instruments, the onboard syringe pump was used at the same flow rate as for the QIT.

Results and Discussion

q-TOF MS/MS of Singly Charged Complex Ions

Figure 1a depicts the product ion mass spectrum of the $[\text{PEG}_{41} + \text{C}_{16}\text{Me}_3\text{N}]^+$ ion generated in a *q*-TOF mass spectrometer. The three peaks observed are assigned to the

precursor ion, $\text{C}_{16}\text{Me}_3\text{N}^+$ and the quat dimer $[(\text{C}_{16}\text{Me}_3\text{N})_2\text{Cl}]^+$. The $[(\text{C}_{16}\text{Me}_3\text{N})_2\text{Cl}]^+$ dimer is not a product of the activation of the precursor ion, because it is also present in the isolation step. This phenomenon may result from the relatively high Quat concentration used in this experiment. It should be noted that this was only observed in the *q*-TOF MS/MS experiment; it was not seen in QIT and LIT MS/MS experiments (*vide infra*). The absence of product ions of polymer chain cleavage shows that the predominant fragmentation process is loss of the cation. This is expected since the charge is retained by the quat cation. The same behavior was observed in the product ion mass spectra of $[\text{PEG}_{41} + \text{C}_{16}\text{Py}]^+$ and $[\text{PEG}_{41} + (\text{Bu})_3\text{NH}]^+$ (Figure A-1, Appendix A). In both cases, the only product ion observed was the corresponding cation due to cation detachment. The C_{16}Py^+ cation retains the charge, as in the case of $[\text{PEG}_{41} + \text{C}_{16}\text{Me}_3\text{N}]^+$, hence no other product ions are observed. However, in the case of $[\text{PEG}_{41} + (\text{Bu})_3\text{NH}]^+$, the proton charge can migrate to the polymer and induce fragmentation. The fact that also in this case only cation detachment is observed suggests that the proton affinity (PA) of the amine as well as the strength of interactions between the polymer and the cation do play a crucial role. $(\text{Bu})_3\text{N}$ has a high PA and will not allow the proton to easily migrate to the polymer. In addition, the bulky alkyl chains of $(\text{Bu})_3\text{N}$ hinder the formation of strong interactions between the cation and the polymer, as indicated by the observation of low intensity adduct ions of this type in previous work [30].

Figure 1b shows the product ion mass spectrum of $[\text{PEG}_{41} + \text{C}_{16}\text{Me}_2\text{NH}]^+$ ion, where the most dominant peak is the tertiary ammonium ion $\text{C}_{16}\text{Me}_2\text{NH}^+$ as a result of cation detachment. The $[\text{PEG}_{41} + \text{H}]^+$ ion formed after the neutral loss of the amine $\text{C}_{16}\text{Me}_2\text{N}$ was also detected (see respective panel in Figure 1b), but its abundance was very low in relation to the abundance of the precursor ion and the dominant product ion $\text{C}_{16}\text{Me}_2\text{NH}^+$. In addition, six series of ions spaced by 44 u were identified as product ions. From

Figure 1. Product ion mass spectrum of (a) $[\text{PEG}_{41} + \text{C}_{16}\text{Me}_3\text{N}]^+$ and (b) $[\text{PEG}_{41} + \text{C}_{16}\text{Me}_2\text{NH}]^+$ in a *q*-TOF mass spectrometer. Asterisks (*) indicate the respective precursor ions. Inset figures are magnifications of areas of the product ion mass spectrum of $[\text{PEG}_{41} + \text{C}_{16}\text{Me}_2\text{NH}]^+$. Product ion notation based on Wesdemiotis et al. [33] (see also Table 1)

Table 1. List of identified product ions for the respective polymer

Polymer Type	Structure of product ion	Notation of product ion ^a
PEG		$c_n^{''(X)}$
		$c_n^{(X)}$
		$b_n^{(X)}$
		$J_n^{EV(X)}$
		$J_n^{CE(X)}$
		$J_n^{CC(X)}$
		$J_n^{CV(X)}$
PPG		$c_n^{''(X)}$
		$c_n^{(X)}$
		$b_n^{(X)}$
PTMEG		$e_n^{''(X)}$
		$d_n^{(X)}$

^aThe notation of the product ions was based on the fragmentation nomenclature proposed by Wesdemiotis et al. [33] Superscripted “(X)” indicates the type of cation adduct.

these, two series are of the same type (b ions) but with different cations. To avoid confusion in the annotation of these ions, the addition of a new notation rule in the nomenclature proposed by Wesdemiotis [33] is suggested (see also Chart A-1, Appendix A). The addition of superscripted ^(X) (where X is the cation) to the product ions, indicates the type of cation present in each ion. Based on this amendment, five series were identified as product ions of fragmentation of $[\text{PEG}_{41} + \text{H}]^+$. These ions were identified as the $c_n^{''(H)}$, $b_n^{(H)}$, $J_n^{CC(H)}$, $J_n^{CV(H)}$ and $J_n^{EV(H)}$ product ions. Table 1 lists these product ions along with their structures.

The sixth series of peaks with the same spacing (44 u) was identified as product ions after the direct dissociation of the precursor ion. These were $b_n^{(A+H)}$ type of ions and hence tertiary ammonium $\text{C}_{16}\text{Me}_2\text{NH}^+$ adducts (for simplification indicated as “A + H” in the ion notation; “A” stands for amine, and consequently “A + H” is the ammonium ion). The presence of $x_n^{(A+H)}$ (where x is b , c , c'' , etc) type of ion with $\text{C}_{16}\text{Me}_2\text{N}$ but not with $(\text{Bu})_3\text{N}$ (also a tertiary amine) is

attributed to differences in the binding energies of the cation with the oxygens’ lone pairs in the polymer chain and in the PAs. The shorter substituents of $\text{C}_{16}\text{Me}_2\text{N}$ (two methyl groups) may cause higher binding energies than the large butyl groups of $(\text{Bu})_3\text{N}$ and allow the detection of $x_n^{(A+H)}$ type of product ions. In addition, differences in the PAs of $\text{C}_{16}\text{Me}_2\text{N}$ and $(\text{Bu})_3\text{N}$ could explain why protonated PEG product ions are detected in the former case.

Figure 2 presents the product ion mass spectrum of the same PEG oligomer cationized with a primary ammonium ion, $(\text{AD} + \text{H})^+$ in a q-TOF. Figure 2a presents the product ion mass spectrum of the singly charged $[\text{PEG}_{41} + (\text{AD} + \text{H})]^+$ and Figure 2b of the doubly charged equivalent $[\text{PEG}_{41} + 2(\text{AD} + \text{H})]^{2+}$. The fragmentation behavior of the singly charged adduct ion partly resembles the one of $[\text{PEG}_{41} + \text{C}_{16}\text{Me}_2\text{NH}]^+$. Protonated AD, $[\text{AD} + \text{H}]^+$ and $[\text{PEG}_{41} + \text{H}]^+$ were the main product ions. The carbocation $[(\text{AD} - \text{NH}_3) + \text{H}]^+$ is possibly formed on consecutive fragmentation of $[\text{AD} + \text{H}]^+$ by loss of ammonia. In addition, low intensity peaks of six

Figure 2. Product ion mass spectra of $[\text{PEG}_{41} + n(\text{AD} + \text{H})]^{n+}$ complex ions obtained in a q-TOF mass spectrometer. (a) Singly charged and (b) doubly charged. Asterisks (*) indicate the respective precursor ions. Inset figures are magnifications of areas of the respective product ion mass spectra. Product ion notation based on Wesdemiotis et al. [33] (see also Table 1)

different series of PEG product ions were detected. These were the $c_n^{n(H)}$, $c_n^{(H)}$, $b_n^{(H)}$, $J_n^{CC(H)}$, $J_n^{CV(H)}$, and $J_n^{EV(H)}$ series (see insets in Figure 2a). Product ions that maintain the ammonium adduct, like in the case of $\text{C}_{16}\text{Me}_2\text{NH}^+$, were not detected. These were observed only when the doubly charged PEG oligomer $[\text{PEG}_{41} + 2(\text{AD} + \text{H})]^{2+}$ was activated (see Figure 2b). The product ion mass spectrum of the doubly charged adduct ion shows intense peaks of $[\text{AD} + \text{H}]^+$ and $[(\text{AD}-\text{NH}_3) + \text{H}]^+$. The singly charged adduct ion $[\text{PEG}_{41} + (\text{AD} + \text{H})]^+$ results from loss of one ammonium ion from the precursor ion. $[\text{PEG}_{41} + \text{H}]^+$ is

also observed (possibly from consecutive fragmentation of $[\text{PEG}_{41} + (\text{AD} + \text{H})]^+$). Low intensity $[\text{PEG}_{41} + (\text{AD} + \text{H}) + \text{H}]^{2+}$ and $[\text{PEG}_{41} + 2\text{H}]^{2+}$ product ions as a result of direct amine loss from the precursor ion and low intensity peaks of $c_n^{n(H)}$ and $b_n^{(H)}$ product ion series were also detected. Ammonium ion cationized product ions were also present. Their type was $c_n^{n(A+H)}$. It is not clear why $x_n^{(A+H)}$ type product ions are not observed on activation of the singly charged ion but only in the doubly charged. The suggestion that PA plays a crucial role in the detection of $x_n^{(A+H)}$ type product ions cannot fully explain this phenomenon. Additional experiments are necessary with

Figure 3. Product ion mass spectrum of $[\text{PEG}_{44} + 2(\text{AD} + \text{H})]^{2+}$ obtained in an LTQ Orbitrap. Asterisk (*) indicates the precursor ion. Inset figure is a magnified area of the product ion mass spectrum. The peak label is the measured m/z value along with the proposed elemental composition and mass error. Product ion notation based on Wesdemiotis et al. [33] (see also Table 1)

Figure 4. Product ion mass spectrum of $[\text{PPG}_{38} + 2(\text{AD} + \text{H})]^{2+}$ obtained in an LTQ Orbitrap. Asterisk (*) indicates the precursor ion. Inset figure is a magnified area of the product ion mass spectrum. Product ion notation based on Wesdemiotis et al. [33] (see also Table 1)

various primary, secondary and tertiary amines with known PAs. These experiments will be part of a future work.

LIT and QIT MS/MS of Singly and Doubly Charged Complex Ions

For comparison, the fragmentation behavior of $[\text{PEG}_{41} + (\text{AD} + \text{H})]^+$ and $[\text{PEG}_{41} + 2(\text{AD} + \text{H})]^{2+}$ was also investigated in a LIT and a QIT mass spectrometer. QITs and LITs are known for fragmentation under low collision energy regimes. This is reflected also in the product ion mass spectra of $[\text{PEG}_{41} + (\text{AD} + \text{H})]^+$ generated in the LIT and QIT instruments. In the case of LIT (Figure A-2, Appendix A), the major product ions observed were the “charge-catalyzed” $c_n^{(H)}$ and $b_n^{(H)}$ and the $J_n^{CV(H)}$ type of product ions. The high mass accuracy of the LIT instrument (LTQ Orbitrap) confirmed the above attribution. In addition two other (unconfirmed) series of product ions were detected. These could be product ions retaining the ammonium ions such as $c_n^{(A+H)}$ and $J_n^{HE(A+H)}$ but the accurate mass measurement could not confirm this attribution. The spectrum produced in the QIT (Figure A-3, Appendix A) shows a similar pattern to the one produced in a LIT. The major product ions observed were the “charge-catalyzed” $c_n^{(H)}$ and $b_n^{(H)}$. Low intensity “charge-independent” $c_n^{(H)}$ type of product ions were also detected, while $J_n^{CC(H)}$, $J_n^{CV(H)}$ and $J_n^{EV(H)}$ type are absent. On the other hand, the product ion mass spectrum of $[\text{PEG}_{44} + 2(\text{AD} + \text{H})]^{2+}$ resembles the one generated in a q-TOF (Figure 3). The major product ions detected derived from fragmentation processes dealing with loss of the ammonium ion or amine. The detection of $[\text{PEG}_{44} + (\text{AD} + \text{H})]^+$ and $[\text{PEG}_{44} + \text{H}]^+$ is explained as previously. The higher abundance of $[\text{PEG}_{44} + (\text{AD} + \text{H}) + \text{H}]^{2+}$ product ion, in comparison with its abundance in the product ion mass spectrum from the q-TOF, suggests that it is favorably formed in low collision energy regimes. In addition, $c_n^{(H)}$, $b_n^{(H)}$ and $c_n^{(A+H)}$ product ion series were detected and confirmed by accurate mass measurement. A fourth ion with m/z 919.5610 could be a $c_n^{(A+H)}$ type of

product ion, but the accurate mass measurement does not confirm this attribution. It should be noted that the cut-off selection in LIT does not allow the detection in the low mass region (actually ions with m/z lower than 25% of the precursor ion’s m/z). A comparable fragmentation pattern with similar type of product ions was also observed in the product ion mass spectrum of $[\text{PEG}_{41} + 2(\text{AD} + \text{H})]^{2+}$ produced in the QIT (Figure A-4, Appendix A). A distinct difference is that in the QIT product ion mass spectrum the product ion $[\text{PEG}_{41} + 2\text{H}]^{2+}$ was detected. This ion results from neutral loss of two amines from the precursor ion and is explained as previously. In addition, $c_n^{(H)}$ and $b_n^{(A+H)}$ type of product ions were detected.

To establish if the observation of the $x_n^{(A+H)}$ type of product ions is related to the cation’s and/or the polymer’s structure, the CID of ammonium adduct ions of PPG and PTMEG was explored in both instruments (i.e., QIT and LIT). The activation of $[\text{PPG}_{39} + (\text{AD} + \text{H})]^+$ in the QIT did not result in the detection of any product ions. In the LIT one product ion resulting from the neutral loss of the amine was detected (Figure A-5, Appendix A). The activation of $[\text{PPG}_{38} + 2(\text{AD} + \text{H})]^{2+}$ in the LIT, however, resulted in a product ion mass spectrum (Figure 4) with five series of product ions. These were $c_n^{(H)}$, $c_n^{(H)}$, $b_n^{(H)}$, $c_n^{(A+H)}$ and $b_n^{(A+H)}$ type of product ions. Their structures are presented in Table 1. The product ion mass spectrum of the same ion produced in a QIT was similar to the one in Figure 4 (Figure A-6, Appendix A). Six series of product ions were observed. These were $c_n^{(H)}$, $c_n^{(H)}$, $b_n^{(H)}$, $c_n^{(A+H)}$, $c_n^{(A+H)}$, and $b_n^{(A+H)}$ type of product ions. The activation of $[\text{PTMEG}_{25} + (\text{AD} + \text{H})]^+$ (Figures A-7 [QIT] and A-8 [LIT], Appendix A) resulted in a product ion mass spectrum with main ions generated by combined neutral loss of the amine and fragmentation, forming $d_n^{(H)}$ and $e_n^{(H)}$ type product ions (see also Table 1 for structures of the product ions). CID of $[\text{PTMEG}_{29} + 2(\text{AD} + \text{H})]^{2+}$ (Figure A-9 [QIT] and A-10 [LIT], Appendix A) resulted in a product ion mass spectrum comparable to the singly charged PTMEG/ammonium complex with as only difference the presence of product

Figure 5. Survival yield versus collision energy of PEG₄₁ with different adduct ions. Survival yield was calculated as the ratio of the precursor ion peak intensity to the sum of peak intensities of the precursor and all product ions

ions retaining the ammonium cation (i.e., $e_n^{(A+H)}$). These experiments show that the presence of $x_n^{(A+H)}$ type of product ions is related to the cation's structure and its binding energy and not to the polyether structure.

Energy-Dependent Analysis of Singly Charged Complex Ions

To test the hypothesis that the structure of the cation influences the binding energy, and consequently the detection of product ions retaining the cation, the stability of the complex ions in CID was investigated. Figure 5 depicts the survival yield of the precursor adduct ions as a function of the laboratory frame collision energy. The survival yield is the ratio of the intensity of the precursor ion to the sum of the intensities of the precursor and product ions at a given collision energy. Comparison of the curves is done using the characteristic point of 50% survival yield. The rationale for use of this reference point is described in previous work [34]. The ranking based on this point is: $(AD + H)^+$ (40.1 eV) > $C_{16}Me_2NH^+$ (35.7 eV) > $C_{16}Me_3N^+$ (21.8 eV) > $C_{16}Py^+$ (18.5 eV) > Bu_3NH^+ (16.4 eV). The curves show that the complex ions of PEG with quaternary ammonium ions and tertiary amines with bulky substituents need less energy to fragment than the complex ions with primary amines and short-substituent tertiary amines. This ranking indicates a trend that confirms with the above-mentioned hypothesis.

Conclusions

Complex ions of PEGs with structurally different ammonium ions can be formed by ESI and activated at both low energy (e.g., QIT and LIT) and high energy (e.g., q-TOF)

collision regimes. The amount and quality of the information obtained from the product ion mass spectra is strongly influenced by the structure of the ammonium ion. Quaternary and tertiary ammonium ions with bulky substituents do not have enough binding energy to prevent their expulsion during dissociation. Hence, in those cases only cation detachment is observed. With tertiary and primary ammonium ions that contain only one long chain substituent, however, product ions from charge-catalyzed reactions and homolytic bond cleavages are observed. These include product ions that retain the tertiary and primary ammonium and protonated product ions. This behavior is not limited to PEG, as similar results were obtained on PPG and PTMEG. The results show that these complex ions can be used as a source of polymer structural information, and in combination with their ability to form low charge state adduct ions, allow the structural elucidation of high molar mass polyethers by ESI-MSⁿ.

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