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Electrospray ionization mass spectrometry of the non-covalent complexes of ammonium ions with high molar mass polyethers

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ABSTRACT

The formation of low charge state non-covalent complexes of high molar mass polymers with primary and tertiary amines and quaternary ammonium salts (Quats) was studied by electrospray ionization time-of-flight mass spectrometry (ESI-TOF-MS). The amines studied were of different degree of substitution and size, and were added to the acidic polymer solution prior to MS analysis. A comparison was made between the non-covalent complexes of high molar mass polyethers with various amines and Quats, based on the ability to form low charge state adduct ions, overall MS response, and presence of fragment ions due to up-front fragmentation. Tertiary amines showed the best performance in forming low charge state adduct ions and produced mass spectra with the lowest degree of up-front fragmentation. In both primary and tertiary amines, more simplified mass spectra are produced, since no cluster ions are formed (as in the case of Quats). The comparison of the overall MS response did not reveal a specific trend. An amine and a Quat with an optimal performance for formation of low charge state adduct ions were applied to the mass spectrometric analysis of a poly(lactic acid)-*block*-poly(ethylene glycol)-*block*-poly(lactic acid) triblock copolymer, allowing the detection of low charge state adduct ions and revealing information about the molecular weight distribution. The method demonstrated here shows that addition of primary/tertiary amines and quaternary ammonium salts to the spray solvent facilitates the analysis of high molar mass polyethers by ESI-MS.

1. Introduction

Synthetic polymers, known for their variety of structural and functional properties, are nowadays of crucial importance in a large variety of industrial activities. Their success is partly attributed to the extensive application of analytical tools such as liquid chromatography (LC), nuclear magnetic resonance spectroscopy and MS to characterize their structure. MS can provide structural information such as monomer unit and endgroup type [1–4], molecular weight distribution [5], monomer sequence and composition of copolymers [6–8]. This information is essential for polymer chemists, since knowledge of the microstructure of polymers enables the control of their synthesis and may provide the (cor)relation between structure and physicochemical and mechanical properties [9].

The major challenges in the analysis with MS remain the high molar mass, dispersity and complexity of polymers. In a period of approximately two decades soft-ionization methods such as ESI [10] and matrix-assisted laser desorption/ionization [11] have grown to become the major techniques for the analysis of intact macromolecules. In some cases they even allowed the detection of relatively high molar mass synthetic polymers. The challenge of complexity has been dealt with so far by coupling the MS to separation techniques such as LC [12–14], size-exclusion chromatography (SEC) [15] and capillary electrophoresis (CE) [12]. On the other hand, the challenge of dispersity, when combined with one or both of the above challenges, requires more advanced MS methods. In disperse polymers there is overlap of peak clusters due to the presence of both charge state distributions and molecular weight distributions. The probability of this complicating phenomenon increases with the dispersity of the polymer, and as a result of isotope distribution also with molecular weight. It makes the determination of the molecular weight distribution (MWD) more cumbersome. Some of the advanced MS methods used to deal with this issue are charge state reduction [16–27], high resolution MS [5,28], separation of charge state with ion mobility spectrometry (IMS) MS [29,30] or a combination of those [24,25]. These methods require hardware modification [17,23], use of radioactive material [22] and/or expensive, state-of-the-art MS instrumentation [24,25,28–30]. Another strategy that may overcome the obstacles of dispersity and high molar mass is to employ additives into the sample solution prior to the ESI-MS analysis,

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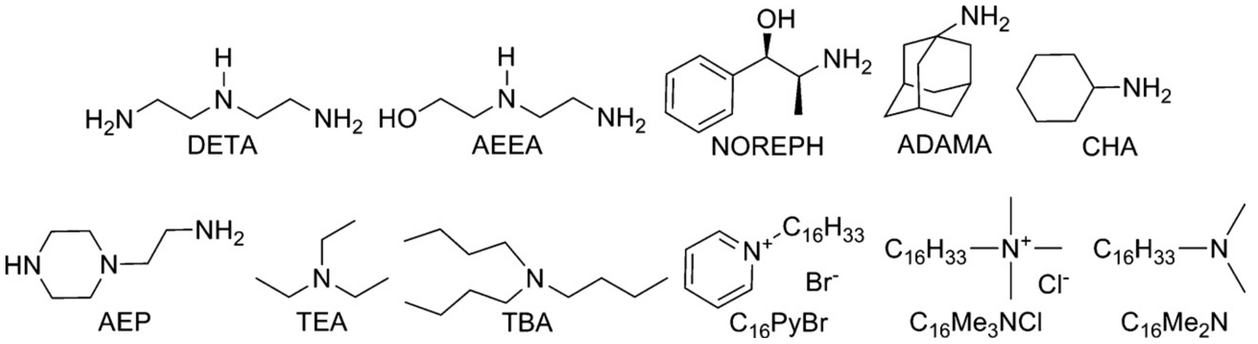

Chart 1. Structures of amines and Quats used in this study.

which can induce charge reduction. This has been demonstrated quite recently by two groups [27,31,32], who used amines and quaternary ammonium salts (Quats) to form low charge state adduct ions in the ESI source. Huang et al. [27] demonstrated the postcolumn addition of amines in the LC effluent prior to MS. With this method, poly(ethylene glycols) (PEGs) and PEGylated proteins till 40 kDa were successfully analyzed. The use of Quats provided similar results and was also shown to be applicable to other polyether systems (e.g., poly(propyleneglycol) and poly(tetramethylene glycol)), as previously demonstrated [31]. In addition, the Quats do not modify the pH of the solution which prevents hydrolysis of sensitive polymeric materials. The drawback of the use of Quats is that the mass spectra are dominated by Quat cluster ions. The high abundance of these cluster ions reduces the overall sensitivity of the method, especially in instruments with narrow dynamic ranges.

This study explores the potential of other amines to provide the same benefits as in previous reports [27,31,32] but without the drawbacks of previously used Quats. The research focus was on amines with different degree of substitution (e.g., primary and tertiary) and size. A comparison was made on the degree of low charge state adduct ion formation, the overall MS response, and the level of up-front fragmentation between various amines and the best performing Quats from our previous study [31]. In addition, the potential of the method for the MS analysis of high molar mass copolymers was investigated for the case of a poly(lactic acid)-*block*-PEG-*block*-poly(lactic acid) (PLA-*block*-PEG-*block*-PLA) triblock copolymer.

2. Materials and methods

PEG 10 kDa, 2-[(2-aminoethyl)amino]-ethanol (AEEA), N-(2-aminoethyl)-1,2-ethanediamine (DETA) and 1-piperazineethanamine (AEP) were synthesized and characterized in house at AkzoNobel (Arnhem, Netherlands). 1-N,N-dibutyl-butanamine (TBA) and cyclohexanamine (CHA) were from Fluka (Steinheim, Germany), N,N-diethyl-ethanamine (TEA) was from J. T. Baker (Deventer, The Netherlands). Adamantan-1-amine (ADAMA), N,N,N-trimethyl-1-hexadecanaminium chloride ($C_{16}Me_3NCl$), 1-hexadecyl-dimethylamine ($C_{16}Me_2N$), 1-hexadecyl-pyridinium bromide ($C_{16}PyBr$), (*1R*,*2S*)-2-amino-1-phenyl-propan-1-ol (NOREPH) and methanol (MeOH) of HPLC grade were purchased from Sigma–Aldrich (St. Louis, MO). The structures of the Quats and amines used in this work are provided in Chart 1. (PLA-*block*-PEG-*block*-PLA) triblock copolymer was synthesized [33] and characterized in the Centre of Polymer and Carbon Materials of the Polish Academy of Sciences (Zabrze, Poland). Cation exchange resin AG MP-50 was purchased from Bio-Rad Laboratories (Hercules, CA). The resin was used to remove the alkali metal ions from the solvents used (especially sodium ions). This was accomplished by mixing the resin with the solvents and subsequent filtration of the solvent before use. Ultra-pure water with a resistivity of 18.2 MΩ cm (at 25 °C) was obtained from a Millipore Direct-Q® 3 water purification system (Molsheim, France). The samples were dissolved in a 50/50 (v/v) water/MeOH mixture or in pure MeOH (after purification with the cation exchange resin) at a concentration of 125–250 μg/mL.

For the comparison of the mass spectra obtained with the addition of different Quats and amines, the calculation of the average charge state value, as used in a previous work [31], was used as another expresion of the charge state shift trend. The equation used was the following:

$$\bar{z} = \frac{\sum_i z_i \cdot I_{i,Z}}{\sum_i I_{i,Z}} \qquad (1)$$

where $\bar{z}$ is the intensity-weighted average charge state, z_i is the charge state of each distribution i, and $I_{i,z}$ is the absolute intensity of the highest peak in each charge state distribution i. It should be stressed, though, that this equation is applied only for semi-quantitative purposes. It does not take into consideration parameters, such as width of the charge state distribution and abundance of the product ions due to up-front fragmentation that could influence its value [31]. An additional complicating factor is that identical values of amine molarities do not necessarily result in the same concentration of ammonium ions, because structurally different amines have different basicities. To avoid this complication, the amount of amine added was optimized so that the pH of the solutions was slightly basic and in the same range (pH 7.5–8). This ensured that the molar concentration of the ammonium ions was almost the same. The optimal concentration for all amines was approximately 1.2 mM (50–100 times higher than the analyte concentration).

MS experiments were performed on a Waters LCT TOF mass spectrometer (Micromass, Manchester, UK) equipped with an ESI source. The transfer of ions from the source to the orthogonal acceleration TOF mass analyzer is made by two hexapole radio frequency lenses. The settings of the LCT were the following: capillary voltage 3–4.25 kV, desolvation temperature 150 °C, desolvation gas flow 395 L/h, and mass range 400–14,500 Da. The cone voltage has a great influence on the MS response [31]. Therefore, its value was optimized every time for maximum MS response. The sample was introduced via a Cole-Palmer syringe pump (Vernon Hills, IL) at a flow rate of 10 μL/min.

3. Results and discussion

3.1. MS analysis of PEG with addition of Quats and amines

Previous results [31] demonstrated that the addition of Quats or amines to a high molar mass polyether solution has a double

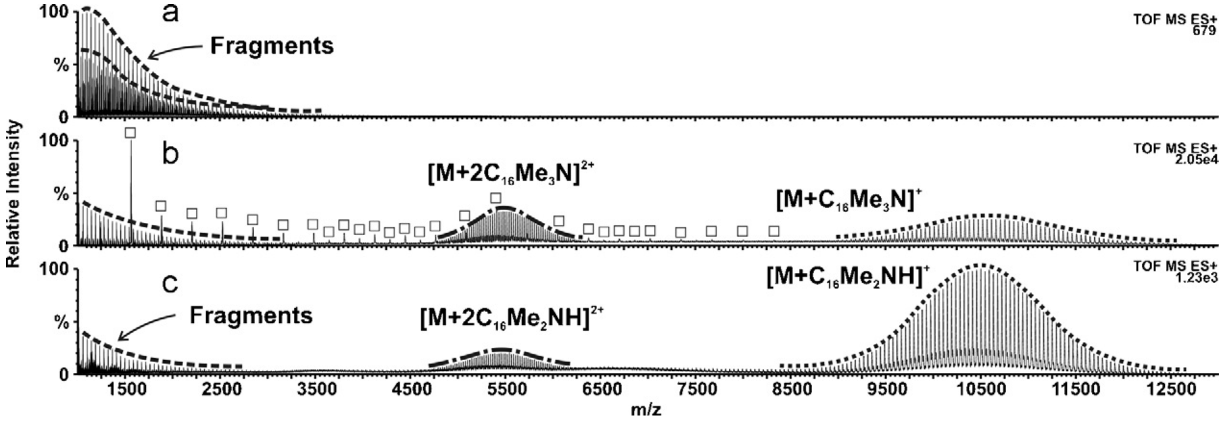

Fig. 1. Mass spectra of 10 kDa PEG obtained by direct infusion (a) without addition of a Quat or amine, (b) with addition of 1.2 mM $C_{16}Me_3NCl$, and (c) with addition of 1.2 mM $C_{16}Me_2N$. *Note*: Peaks indicated with a square (□) are $[(C_{16}Me_3N)_{x+1}Cl_x]^+$ cluster ions and "Fragments" are protonated PEG fragment ions. All mass spectra were obtained at 180 V cone voltage.

benefit: reduction of product ions due to up-front fragmentation and increase of the signal intensity of the low charge state ions. Fig. 1 depicts this effect showing the mass spectra of PEG 10 kDa without amine or Quat (Fig. 1a), with an optimized addition of $C_{16}Me_3NCl$ (Fig. 1b) and with an optimized addition of $C_{16}Me_2N$ (Fig. 1c). Without the addition of any Quat or amine (Fig. 1a), the mass spectrum is dominated by protonated PEG product ions due to up-front fragmentation at high cone voltages. It was demonstrated previously [31] that lowering the cone voltage reduces the intensity of the protonated PEG product ions and allows the detection of multiply charged protonated PEG adduct ions, albeit at very low abundance. The addition of $C_{16}Me_2N$ (Fig. 1c) resulted in an overall improvement of the mass spectra. $[PEG + C_{16}Me_2NH]^+$ and $[PEG + 2C_{16}Me_2NH]^{2+}$ adduct ions, that provide information about the molecular weight distribution of the polymer sample, are observed. Although protonated PEG product ions are still observed, their abundance is lower than that of the observed singly and doubly charged adduct ion distributions. The $C_{16}Me_2N$ tertiary amine maintains the structural requirement (combination of large size with specific charge localization and/or facile interactions with the polymer) that was mentioned previously [31], and has a similar structure as $C_{16}Me_3NCl$. A comparison between the mass spectra obtained after the addition of $C_{16}Me_2N$ and $C_{16}Me_3NCl$ clearly shows the three additional benefits of the use of this tertiary amine. Firstly, the addition of $C_{16}Me_2N$ results in mass spectra with lower average charge state values ($\bar{z} = 1.2$) than with the addition of $C_{16}Me_3NCl$ ($\bar{z} = 1.6$). Secondly, the abundance of the protonated PEG product ions compared with the singly and doubly charged adduct ion distributions is lower than in the case with $C_{16}Me_3NCl$. Thirdly, the cluster ion peaks present in the mass spectra obtained with addition of $C_{16}Me_3NCl$ are not present when $C_{16}Me_2N$ is used, which simplifies the mass spectra. These improvements are attributed to the physicochemical nature of $C_{16}Me_2N$. This tertiary amine functions as a base and reacts with the protons in the solution (these protons are present after the treatment of the solution with the cation exchange resin), producing the $C_{16}Me_2NH^+$ tertiary ammonium ion. The amount of protons in the solution is lowered and, consequently, the peak intensity of the protonated PEG product ions is lowered. A possible explanation for the absence of cluster ions is that there is no counter ion in the solution capable of forming a strong complex with $C_{16}Me_2NH^+$ in the gas phase.

The potential of other amines for their capability to form low charge state adduct ions was investigated. To allow as much as possible a direct comparison between the performance of the different amines, the concentration of the ammonium ions needs to be the same. As demonstrated recently [31], an increase in the Quat concentration influences the mass spectra by favoring the lower charge states. The addition of 1.2 mM of amine was sufficient to react with the protons of the solution (pH measurements showed a shift in pH values from ~6 to ~8) and provide an excess of ammonium ions in all cases. Fig. 2 shows a selection of the mass spectra of PEG 10 kDa after the addition of structurally different amines. The remaining mass spectra of PEG 10 kDa with the other amines of this study are presented in the supplementary information (Fig. A-1, Appendix A). In each case the same number of scans was summed. The calculated average charge state values for all cases are presented in Table 1.

Fig. 2a shows the mass spectrum of PEG 10 kDa with the addition of CHA. The singly and doubly charged distributions were assigned to the $[PEG + (CHA + H)]^+$ and $[PEG + (CHA + H) + H]^{2+}$ adduct ions, respectively. The other multiply charged distributions detected could not be assigned due to the insufficient resolving power of the TOF instrument, but it is suggested that a mixture of one $(CHA + H)^+$ primary ammonium ion with multiple protons is present.

Fig. 2b shows the mass spectrum of PEG 10 kDa after addition of AEEA. AEEA produces adduct ions of lower average charge state ($\bar{z} = 1.7$) than CHA ($\bar{z} = 3.4$). Envelopes assigned to $[PEG + (AEEA + H)]^+$, $[PEG + 2(AEEA + H)]^{2+}$ and $[PEG + (AEEA + H) + 2H]^{3+}$ adduct ions were detected. The detection of $[PEG + (AEEA + H) + 2H]^{3+}$ adduct ions and the absence of the expected $[PEG + 3(AEEA + H)]^{3+}$ and/or $[PEG + 2(AEEA + H) + H]^{3+}$ adduct ions indicate that steric hindrance plays a role in higher charge states complex ions. This is also shown with other primary amines (e.g., AEP and NOREPH), where cations

Table 1
Average charge state values and peak intensities of various 10 kDa PEG-Quat/amine adduct ions.

Quat/amine	Average charge state value $\bar{z}$	Absolute intensity of highest singly charged peak[a]
AEP	3.8	0 (946)
DETA	3.6	0 (915)
CHA	3.4	357
NOREPH	2.1	453
AEEA	1.7	676
ADAMA	1.6	1050
$C_{16}Me_3NCl$	1.6	1840
$C_{16}PyBr$	1.5	186
TEA	1.2	6410
$C_{16}Me_2N$	1.2	425
TBA	1.1	224

[a] For the cases where singly charged ions were not detected, the intensity of the doubly charged distribution is shown in parentheses.

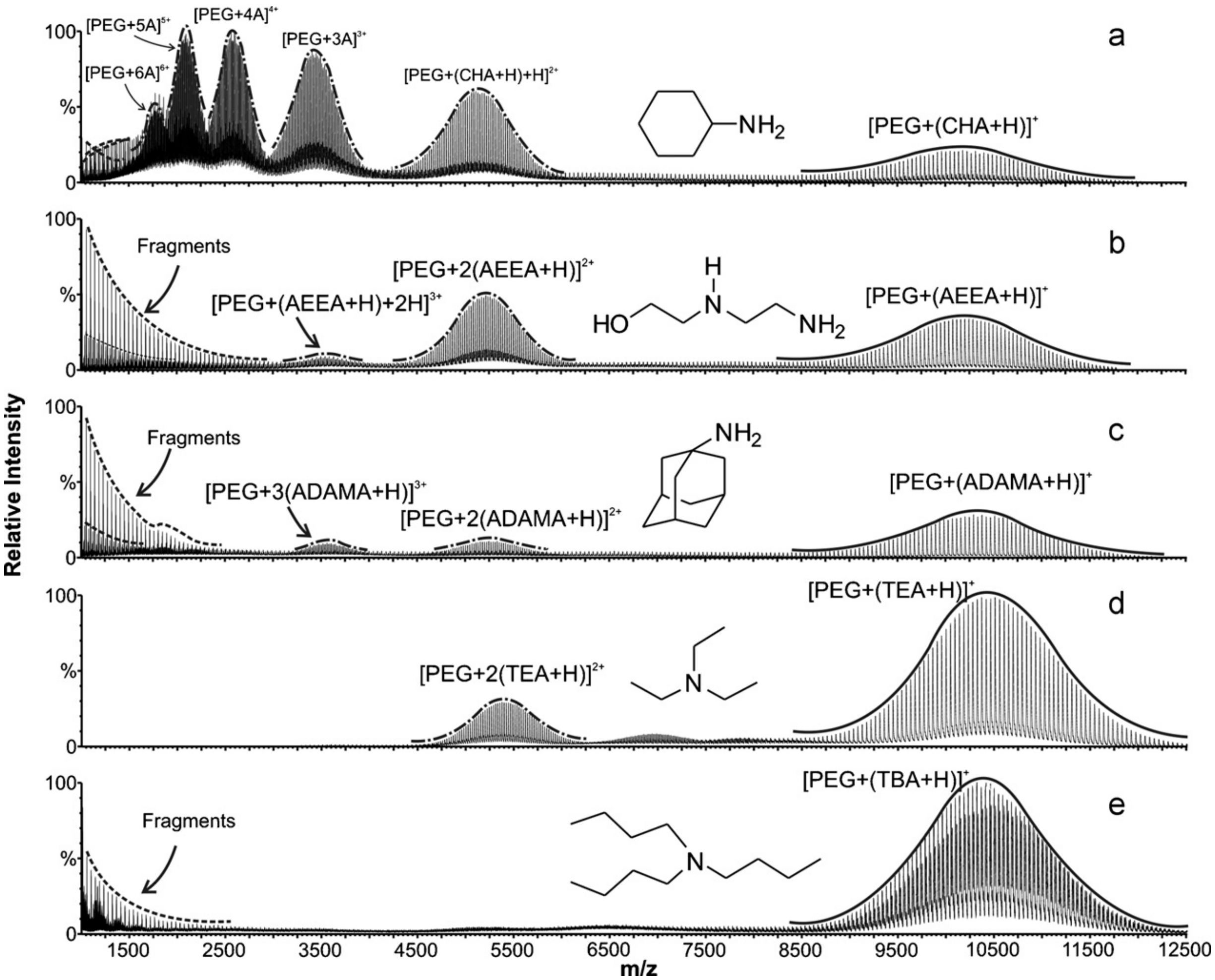

Fig. 2. Mass spectra of 10 kDa PEG obtained by direct infusion with addition of 1.2 mM of: (a) CHA, (b) AEEA, (c) ADAMA, (d) TEA, and (e) TBA. *Note*: "A" refers to an unspecified cation or mixture of adducts (e.g., (CHA + H) or H), because the exact nature of the adduct ion cannot be determined accurately due to the low resolving power of the instrument. "Fragments" were protonated PEG product ions. All mass spectra were obtained under the same ESI conditions.

in the multiply charged adduct ions were a mixture of one primary ammonium ion with multiple protons (Fig. A-1, Appendix A). The addition of ADAMA (Fig. 2c), a primary amine with a large and rigid substituent gave an average charge state value ($\bar{z} = 1.6$) comparable to AEEA and the best performing Quats of the previous study [31], $C_{16}Me_3NCl$ ($\bar{z} = 1.6$) and $C_{16}PyBr$ ($\bar{z} = 1.5$).

The addition of a simple tertiary amine such as TEA results in a further decrease of the average charge state (Fig. 2d). The performance of TEA is comparable with $C_{16}Me_2N$ (both have a $\bar{z}$ value of 1.2), indicating that the structural requirement suggested previously (large size cations with specific charge localization) [31] does not necessarily need to be fully satisfied for effective formation of low charge state ions. Tertiary amines with relatively short chains, such as TEA and diethylmethylamine [27,32] can give comparable results as well. In Fig. 2e the mass spectrum of PEG 10 kDa with the best $\bar{z}$ "performer"-TBA- is presented. TBA has a $\bar{z}$ value of 1.1 and almost exclusively $[PEG + (TBA + H)]^+$ adduct ions are detected. It should be noted also, that a comparison of the singly charged distributions in Fig. 2b and d suggests that the structure of the amine has an influence on the MWD. This suggestion was investigated previously by determining the MWD of PEGs cationized by Quats (with different chain lengths) [31]. In the present case this approach is not functional due to overlap of the fragment ion distribution with the molecular ion distribution. This overlap hinders the accurate determination of the MWD. MWD determinations of the amines studied were not performed and will be part of future work on the MWD determination of ammonium ion cationized polymers by ESI and MALDI.

The average charge state value is directly related to the ability to form low charge state adduct ions, and can, therefore, be used as a parameter for assessing the suitability of amines or Quats to deal with the dispersity challenge. However, other parameters such as absolute peak intensity of these ions and the presence of fragment ions due to up-front fragmentation should also be taken into consideration. These factors are important since they are closely related with the sensitivity of the method. An MS method with high sensitivity may allow the analysis of low analyte concentrations and polymer impurities. In addition, mass spectra with good signal-to-noise ratio can be produced even with a limited number of scans. This can be of great help when hyphenated techniques are used, such as LC or SEC coupled to MS. Table 1 shows the highest peak intensity of the singly charged PEG/(amine or Quat) complex ions. It should be noted that the absolute intensity measurement was made after summing the same number of mass spectra. The highest intensity is observed in the case of TEA (6410 ion counts) followed by $C_{16}Me_3NCl$ (1840 ion counts), which is almost 3.5 times lower than TEA. ADAMA (1040 ion counts) is third in rank, with the rest being below 1000 ion counts.

A relation between the structure of the amines and the presence of protonated PEG product ions has so far not been established. The source of these ions is still under investigation and will be addressed in later work on the fragmentation behavior of the low

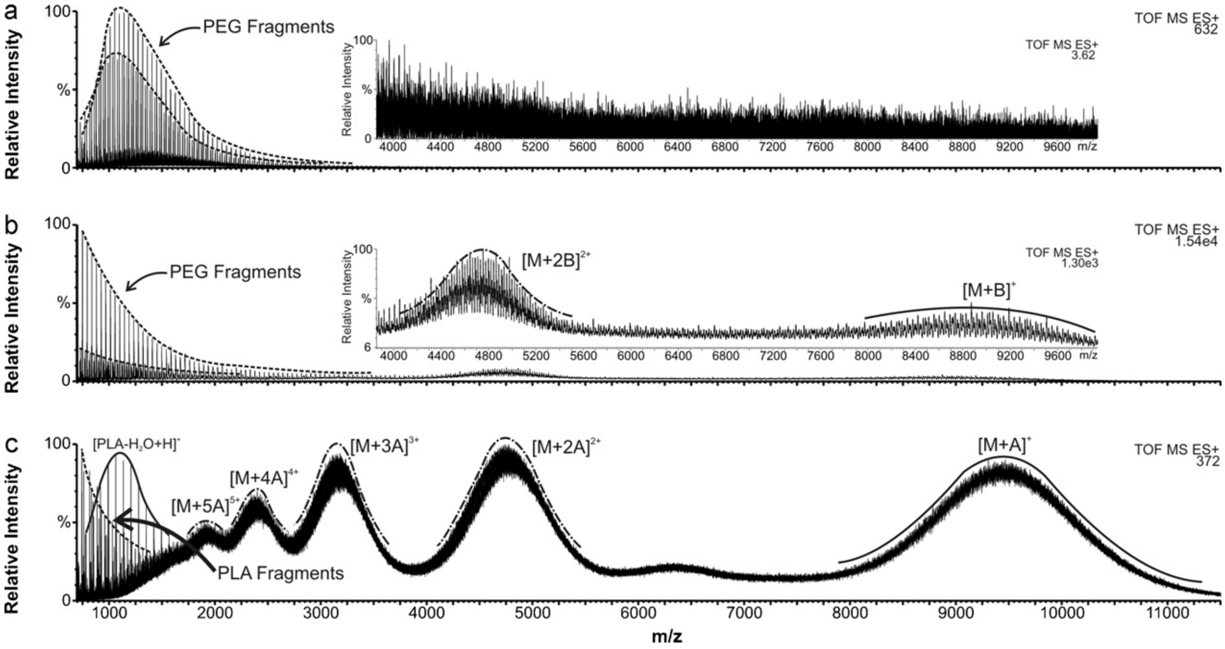

Fig. 3. Mass spectra of 10 kDa PLA-*block*-PEG-*block*-PLA copolymer obtained by direct infusion: (a) without addition of any Quat or amine, (b) with addition of 1.2 mM $C_{16}Me_3NCl$, and (c) with addition of 1.2 mM of TEA. *Note*: "M" refers to the block copolymer. "A" and "B" refer to the $(TEA+H)^+$ and $C_{16}Me_3N^+$ cations, respectively. All mass spectra were obtained under the same ESI conditions.

charge state non-covalent complexes of high molar mass polymers with amines or Quats.

The efficiency of the various amines and Quats is determined by the analytical needs. As a general rule, when the simultaneous analysis of low and high molar mass polymers is needed, the presence of cluster ions and fragments is not wanted, and therefore a tertiary amine should be preferred. When additional information about the structure of the polymer is required, then a primary amine should be preferred so that fragments can still be observed. Quats should be used for more easily hydrolysable or pH sensitive polymeric materials.

3.2. MS analysis of block copolymers with addition of Quats and amines

The applicability of the developed method was previously tested for other homopolymers [31]. From these, only polyethers (e.g., PEG, poly(propylene glycols) and poly(tetramethylene glycols)) were shown to form low charge state adduct ions with Quats. Fig. 3 shows the mass spectra of a PLA-*block*-PEG-*block*-PLA triblock copolymer without and with the addition of TEA or $C_{16}Me_3NCl$. These two compounds were selected based on a good balance between their performance in the formation of low charge state ions (low $\bar{z}$ value) and the intensity of their singly charged ions. They produced average charge state values of 1.2 and 1.6 respectively and the highest absolute intensities of singly charged ions (see Table 1). In addition, this pair allows a comparison between the use of a Quat salt and a tertiary amine.

Fig. 3a depicts the mass spectrum of the block copolymer without the addition of any Quat or tertiary amine. The mass spectrum is dominated by protonated PEG fragment ions produced due to up-front activation. A similar mass spectrum was obtained on a block copolymer solution containing 0.25 mM NaI (Fig. A-2, Appendix A). The addition of $C_{16}Me_3NCl$ (Fig. 3b) leads to an overall signal intensity increase (from 632 ion counts to 15,400 ion counts) and features the presence of low intensity peaks of singly and doubly charged adduct ion distributions of the copolymer. The addition of TEA results in the detection of singly to quintuply charged adduct ions (Fig. 3c). In addition, PLA fragment ions and PLA oligomers are detected. These PLA ions were not detected in the case of $C_{16}Me_3NCl$, possibly due to the oversaturation of the low mass region with protonated PEG fragment ions. The low signal-to-noise ratio and the limited resolving power of the TOF do not allow the confirmation of the various copolymer structures in both cases by direct infusion analysis. A resolving power higher than 170,000 at mass-to-charge ratio 10,000 would be required. The sample is a complex mixture of various copolymer distributions, since the initiating block was a disperse PEG 8 kDa sample. Lee et al. [34] demonstrated the successful separation of the copolymer distributions of a PLA-*block*-PEG-*block*-PLA triblock copolymer (of approximately the same molar mass) by LC at critical conditions. The application of such a separation in our MS method could provide the necessary deconvolution and allow the generation of more detailed information on the copolymer structures. This is, however, beyond the scope of this study.

4. Conclusions

Addition of various amines and Quats to a PEG 10 kDa and PLA-*block*-PEG-*block*-PLA triblock copolymer 10 kDa solution facilitates the formation of low charge state adduct ions. A comparison between the various amines and Quats has been performed based on: (a) the ability to form low charge state adduct ions, (b) the signal intensity of the low charge state adduct ions, and (c) the degree of up-front fragmentation. The performance of the amines and Quats as additives in ESI depends on their structure. Primary amines tend to produce adducts ions with higher average charge state values than tertiary amines and Quats. They also produce mass spectra with fragment ions, due to up-front fragmentation, but they do not produce cluster ions (as in the case with Quats). The use of the tertiary amine $C_{16}Me_2N$ instead of its quaternary equivalent $C_{16}Me_3NCl$ results in a lower average charge state value, reduction of up-front fragmentation and absence of the high intensity peaks from the Quat clusters ions. This example shows the benefit of using tertiary amines instead of primary amines and Quats. These results show that the MS analysis of high molar mass polyethers (homopolymers or copolymers containing large polyether blocks) is facilitated by the use of various amines and Quats. The selection of amines and Quats in an application depends on the research question; the system can be optimized so as to get molecular weight information (and good sensitivity) or additional structure information through up-front activation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijms.2010.12.016.

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