International Journal of Mass Spectrometry 301 (2011) 184–194

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Tandem Fourier transform mass spectrometry of block and random copolymers

Saša M. Miladinović, Cynthia J. Kaeser, Matthias M. Knust, Charles L. Wilkins*

Chemistry and Biochemistry Department, University of Arkansas, Fayetteville, AR 72701, USA

ARTICLE INFO

Article history:
Received 22 March 2010
Received in revised form 28 August 2010
Accepted 31 August 2010
Available online 15 September 2010

Keywords:
Tandem FTMS
Block copolymer
Random copolymer
Polymethacrylates
Polyethers

ABSTRACT

In recent years, many fragmentation techniques have been developed for use with Fourier transform mass spectrometry (FTMS) in sequencing polymers. Tandem mass spectrometric techniques such as collision-induced dissociation (CID) and electron capture dissociation (ECD) were used to fragment high mass polyether and polyacrylate copolymer ions produced using external electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) sources. High resolution FTMS analysis of fragments produced by collision cell CID (QCID) is available in a new generation hybrid FTMS instrument. Although tandem QCID-TOF techniques have been used heavily to explore copolymer fragmentation, we report the first QCID-FTMS analysis of such systems. The high fragmentation efficiency of QCID combined with the high mass resolution and mass accuracy of FTMS reveals a novel fragmentation pattern of selected oligomers. This method provided evidence of re-combination of fragments in the gas phase, in that some fragments have higher mass and charges than the isolated precursor ions. QCID fragmentation of oligomers of varying size was studied in the presence of Li, Na, or Cs salts. The ECD-FTMS spectra of polyacrylates showed only side group losses. Accordingly, this technique can be used for an unknown polyacrylate sample analysis and has the potential for side group determination.

1. Introduction

After the introduction of soft ionization methods, such as electrospray ionization (ESI) [1] and matrix assisted laser desorption/ionization (MALDI) [2,3] techniques, and mass analyzers, such as time-of-flight (TOF) [4] and Fourier transform mass spectrometers (FTMS) [5], mass spectrometry became the main tool in "top-down" proteomics and polymer analysis [6].

In recent years, many fragmentation techniques have been developed for use with Fourier transform mass spectrometry (FTMS) in sequencing peptides and polymers. The most commonly used methods include sustained off-resonance irradiation collision-induced dissociation (SORI-CID) [7,8], infrared multiphoton dissociation (IRMPD) [9], and electron capture dissociation (ECD) [9,10]. Recently, hybrid FTMS instruments that have the capability of performing collision-induced dissociation in a hexapole collision cell (QCID) which is filled with argon gas and kept at a pressure of 10^{-2} mbar [11] were introduced. This relatively high pressure increases the probability of collisions between selected precursor ion and gas molecules when compared with the SORI-CID technique, where collision occurs in the ion cyclotron cell (ICR) using collision gas pressures of up to 10^{-5} mbar [12]. However, analysis time employing QCID is shorter, since the ICR cell is separated from the collision cell by ion optics and constantly held under high vacuum (10^{-10} mbar). This differs from SORI-CID where analysis time depends on high-vacuum pump efficiency because of the need to rapidly pump the analyzer region down to 10^{-10} mbar after the gas pulse, required for SORI. The disadvantage of QCID when compared to SORI-CID is that the ion's path length during which collisions occur is limited by the collision cell length. In SORI-CID, the path length of precursor ions is basically unlimited. This results from the fact that collisions occur in the trapped ion cell, enabling many collisions with gas molecules resulting in higher collision energies for fragmentation.

Until now, most of the literature on MS/MS analysis has focused on sequencing of biomolecules [8–10]. In contrast, the present research deals with the equally challenging problem of sequencing synthetic polymers. This is a consequence of the stability of the covalent bonds connecting monomer units. Therefore, the tandem MS technique employed must impart high enough energy to the oligomer ions to break those bonds. Often when fragmentation does occur, a limited number of fragments are detected and their low abundances require a large number of measurements, in order to increase the signal to noise ratio of resulting spectra. In the past, such analyses were very time consuming [13]. With development of new hybrid instruments, required analyses time has been reduced significantly [14]. Electron-capture dissociation (ECD) is a technique in tandem MS that produces fragments through a non-ergodic mechanism making this technique unique for frag-

---

* Corresponding author at: 251 Chemistry Bldg., University of Arkansas, Fayetteville, AR 72701, USA. Tel.: +1 479 575 3160; fax: +1 479 575 4049.
E-mail address: cwilkins@uark.edu (C.L. Wilkins).

1387-3806/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijms.2010.08.028

mentation [15]. As most often implemented, ECD requires the use of multiply charged ions produced by electrospray ionization (ESI) [16]. There are a few reports of successful fragmentation of copolymer and polymer samples using ECD-FTMS [17–21]. On the other hand, tandem MS characterizations of the polymers were said to be "unreliable" due to occurrence of recombination processes following molecular ion dissociation. As mentioned above, although ECD fragmentation was applied to analysis of a number of synthetic polymer samples [19,20], including polyethers [16,18], in contrast to its applications to biomolecules [22–24], the analytical utility of ECD for polymers may be less useful.

Polyether CID fragmentation has been investigated using the previously mentioned techniques, providing a source of comparison for this study's results [7,16,18,25–27]. The main focus of the present research is analysis of QCID and ECD-FTMS of polyacrylates [28]. Specifically, the homopolymer poly(methyl methacrylate), the random copolymer methyl methacrylate–butyl methacrylate and the block copolymer methyl methacrylate–butyl methacrylate were studied. A previous study performed on polyacrylates by Jackson, Slade, and Scrivens showed that different ionization agents cause various fragmentation patterns for multiply-charged oligomers fragmented and analyzed using an orthogonal time-of-flight mass spectrometer [29]. For this reason, we also explored the influence of cationizing agents on polyacrylate fragmentation patterns by replicate FTMS analyses in the presence of LiCl, NaCl, and CsCl salts. Lithium and cesium cations were chosen for this study because they are the smallest and largest, respectively, of readily available alkali metal cations, while sodium cation was chosen as the middle ion because, as a ubiquitous element, it often contaminates synthetic polymers during their synthesis. As a consequence, sodiated peaks are often present in spectra even when no sodium has been added [30]. Thus, when sodium chloride is added, and samples analyzed by MALDI, the relative abundances of the sodium catonized oligomers are enhanced. Interestingly, in the present research it appears that the results allow *rapid differentiation of block and random copolymers by suitable analysis of their fragmentation patterns*.

2. Materials and methods

All polymer-cation solutions used in electrospray (ESI) experiments were of a concentration ratio of 8 μM polymer to 200 μM cation in methanol. The methanol used was purchased from EMD Chemicals (Gibbstown, NJ). The poly(ethylene glycol) and poly(methyl methacrylate) have average molecular weights of 3000 g/mol and 5000 g/mol, respectively, and were both purchased from Fluka (now Sigma–Aldrich, St. Louis, MO). Random poly(ethylene oxide-propylene oxide) was obtained from Polysciences (Warrington, PA) ($M_w \sim 2900$) while block copolymers poly(ethylene oxide)-block-poly(propylene oxide)-block-poly(ethylene oxide) ($M_w \sim 2900$) and poly(propylene oxide)-block-poly(ethylene oxide)-block-poly(propylene oxide) ($M_w \sim 3300$) were acquired from Sigma–Aldrich. Block copolymer poly(methyl methacrylate-b-*n* butyl methacrylate) ($M_w \sim 4000$) was purchased from Polymer Standard Service–USA (Warwick, RI) and random poly(methyl methacrylate-*n* butyl methacrylate) ($M_w \sim 3000$) was a generous gift from Dr. William J. Simonsick of DuPont. The lithium chloride and sodium chloride used were purchased from Sigma–Aldrich, while the cesium chloride was purchased from Shelton Scientific (Shelton, CT).

For matrix-assisted laser desorption ionization (MALDI), polymers were mixed with dihydroxy-benzoic acid (Alfa Aesar, Ward Hill, MA) and cationization agent in methanol with an approximate molar ratio of 1:400:30 (analyte:DHB:salt) respectively. Solutions were aerosprayed onto stainless steel plates.

Polymers were analyzed using a Bruker APEX Qe 9.4T FTMS (Billerica, MA) with a dual external ionization ESI and MALDI source. The instrument is equipped with Nd:YAG laser operated at a wavelength of 355 nm. Detailed schematics of the instrument appear elsewhere [11]. For tandem FTMS, a full spectrum of the polymer was obtained and several oligomers of high abundance were selected to isolate and fragment. These were isolated using a quadrupole ion filter and fragmented using the desired method. In this paper, MS/MS data from a representative oligomer is reported. For CID, the pressure of argon gas within the collision cell was held at 10^{-2} mbar. The collision energy ranged from 55 to 80 eV, as calculated in the laboratory frame, and was adjusted to give the highest fragmentation efficiency. Electron energy for electron capture dissociation was between 3 and 4 eV employing an electron pulse length of 50 ms.

3. Results and discussion

3.1. ESI-QCID-FTMS of polyacrylates

For the initial collision cell CID Fourier transform mass spectrometry (QCID-FTMS) experiments, the homopolymer poly(methyl methacrylate) (PMMA, $M_w = 5000$) was chosen. Before looking at the more complicated copolymer samples using electrospray ionization (ESI) QCID PMMA was analyzed. For these measurements, lithium, sodium, and cesium salts were added in order to determine the fragmentation pattern and efficiency for each of the cations. QCID was performed on the doubly-charged oligomers consisting of 37 methyl methacrylate (MMA) monomeric units and on the triply-charged oligomers consisting of 50 MMA monomeric units. The 50 monomer chain was chosen because it represented the average weight of the polymer used. However, the doubly-charged oligomer of that length produced a mass-to-charge ratio too large for the quadrupole to isolate efficiently. Therefore, the abundant and efficiently isolated oligomer containing 37 monomeric units was chosen for the doubly-charged analysis instead.

Fig. 1A represents a full mass range ESI-FT mass spectrum of sodium-cationized PMMA. Fig. 1B–D shows the fragmentation spectra of the doubly-charged 37-mer oligomers obtained in the presence of each of the cations. Table 1 displays all of the fragments present in each of these three spectra. The QCID process of fragmentation is ergodic [31]. In such a case, the energy from the collision is spread out across the oligomer, breaking the weakest bond. The large size and low charge density of Cs would make its coordination bonds with the carbonyl groups along the chain weak [31]. Thus, it is quite favorable for a Cs^+ ion to be removed by a collision. As a result the peak corresponding to the loss of one Cs^+ ion ($m/z = 3842.9$, Fig. 1D) is more abundant than those corresponding to other fragments. In all three spectra, there are fragments that are longer or more highly charged than the precursor ion. This indicates that some of the fragments produced by the collision combine while still in the gas phase. Depending upon the lengths and number of ions present on the fragments, longer, more highly charged oligomers result. This is more frequent when Li^+ and Na^+ are used as the cationizing agents because these cations are not as easily removed as Cs^+ [31–33]. The results of the fragmentation of the triply-charged 50-mer using each cation are shown in Fig. 1E–G and are summarized in Table 2. These are similar to the results of the doubly-charged 37-mer in that cesiated species readily loses the cation and fragment less than oligomers cationized by Li^+ or Na^+. However, there are more fragments present for Cs^+ with the 50-mer than there are for the 37-mer and no peak representing the loss of two Cs^+ ions is present. This is consistent with reports that Cs^+ coordinates readily with longer oligomers

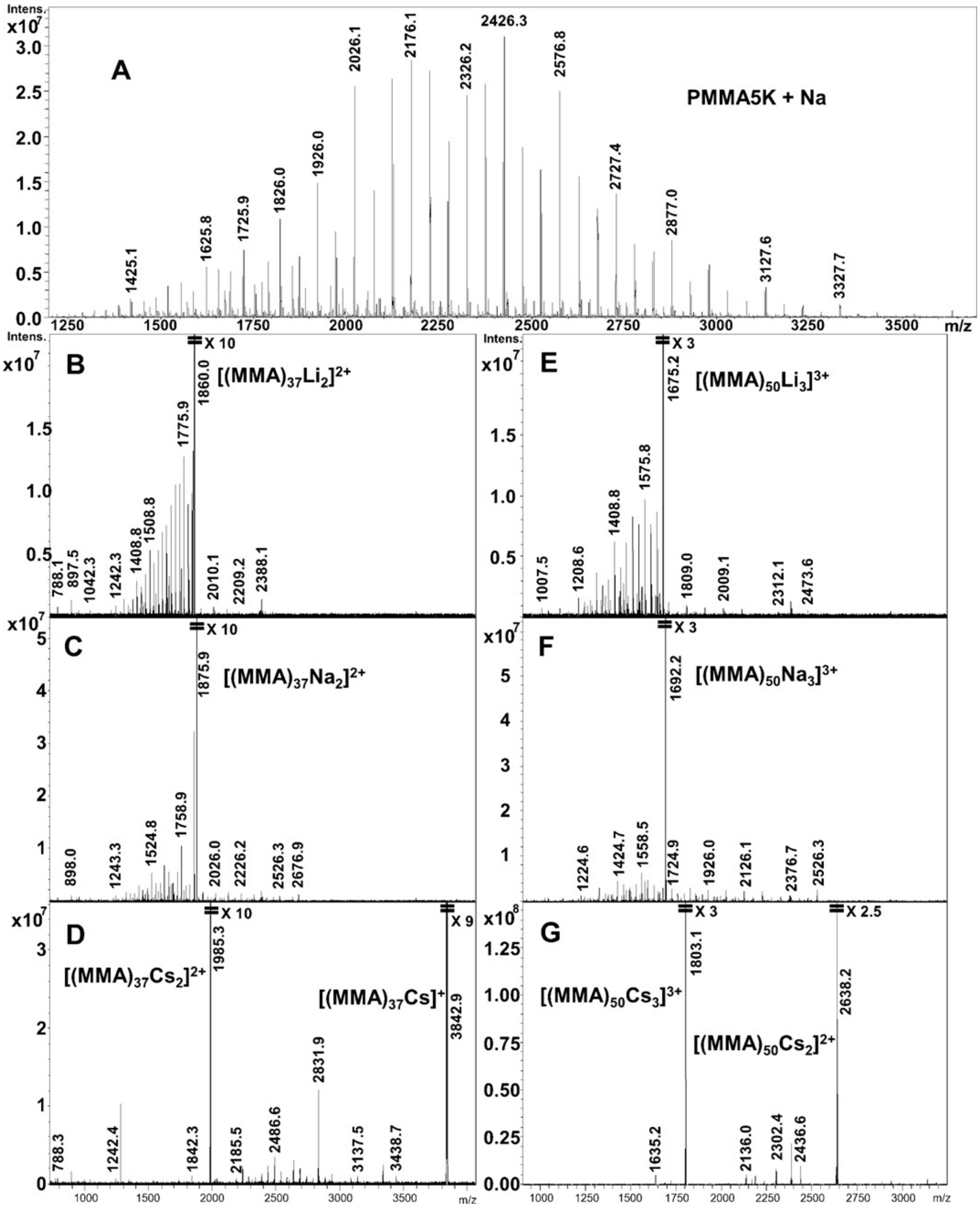

Fig. 1. (A) Full mass range ESI-FT mass spectrum of PMMA; (B) ESI-QCID-FT mass spectrum of $(MMA)_{37}$ doubly-charged oligomer with Li^+ cation, (C) with Na^+ cation, (D) with Cs^+ cation; (E) ESI-QCID-FT mass spectrum of $(MMA)_{50}$ triply-charged oligomer with $3Li^+$ cations, (F) with $3Na^+$ cation, and (G) with $3Cs^+$ cation.

Table 1
Fragmentation pattern of $[H–(C_5H_8O_2)_{37}–H\ M_2]^{2+}$ using QCID; fragment structure: $[H–(C_5H_8O_2)_x–H\ M_z]^{z+}$.

M	Charge (z)	End groups	Loss	Monomer lengths present (x)	Average error (ppm)
Li	1	H	H	13–18	5.26
	2	H,H	–	27–37	5.63
	3	H,H or OH and H	–	39–55	3.78
	4	H,H	–	56, 58, 60–67	3.23
Na	1	H	H	12, 26	2.69
	2	H,H	–	26–33, 35–50, 52–53	4.13
	3	H,H	–	38–62, 64	2.83
	4	H,H	–	54–55, 57–68, 70–71	4.06
Cs	1	H	H	28, 32–33, 37	7.72
	2	H,H	–	42–55, 60	3.84

Table 2
Fragmentation pattern of $[H–(C_5H_8O_2)_{50}–H\ M_3]^{3+}$ using QCID; fragments structure: $[H–(C_5H_8O_2)_x–H\ M_z]^{z+}$.

M	Charge (*z*)	End groups	Loss	Monomer lengths present (*x*)	Average error (ppm)
Li	1	H	H	15–16	3.45
	2	H,H	CH_3OH or H_2O or H	26–27, 29, 31–33	3.07
	3	H,H or H and OH	–	38–50	4.18
	4	H,H or H and OH	–	51–66	5.89
Na	1	H	H or CH_4	11–12, 14–25	2.56
	2	H	H or CH_4	25–50	2.49
	3	H, H	–	37–63, 66	3.55
	4	H, H	–	52–63, 65–66	4.15
Cs	1	H	H	26	2.61
	2	H,H or H	H	30–31, 36–37, 40–46, 50–51, 55–57, 60–61, 65	2.73
	3	H, H	–	50, 60–61, 65	3.05

[21,34]. It has also been shown that longer oligomers require larger cations or more charges and the present results are consistent with that conclusion. Accordingly, there are fewer fragments with Li on the 50-mer than on the 37-mer [35]. The recombination observations have most likely not been reported before, because previous studies used oligomers that were of much lower mass and time-of-flight (TOF) mass analyzers were used [36–39]. In those studies, recombinant products can easily be missed in low resolving power TOF instruments employed since they could overlap with fragment signals. Although lithiated fragments had the highest abundances, doubly and triply-charged sodiated oligomers produced the largest number of backbone fragments. Therefore, sodium was selected to be cationizing agent for copolymer analysis. Backbone fragmentation is important for copolymer analysis because it can be used to determine the sequence of monomeric units in selected oligomers.

For the random poly(methyl methacrylate-*n* butyl methacrylate) (rMMABMA), the oligomer of composition of 12 BMA monomeric units/13 MMA monomeric units with H end groups and two Na^+ cations was isolated from other oligomer signals present in the ESI-FTMS mass spectrum (Fig. 2A). The fragmentation spectrum of this doubly charged oligomer ion is shown in Fig. 2B. The fragment spectrum shows evidence of fragments that are both triply-charged and have extra monomeric units, covering the mass range from m/z ~500 to ~750. These rearranged frag-

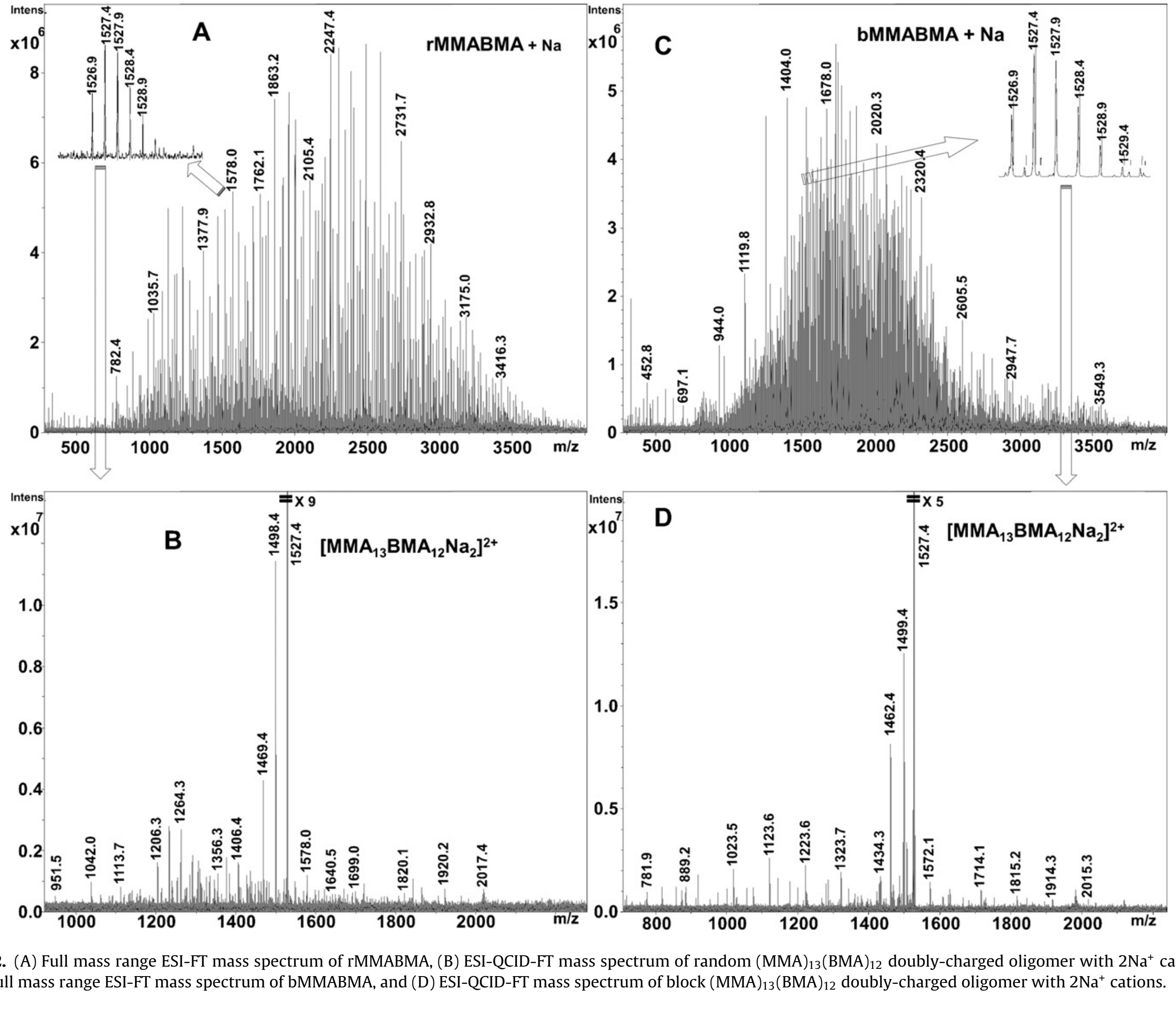

Fig. 2. (A) Full mass range ESI-FT mass spectrum of rMMABMA, (B) ESI-QCID-FT mass spectrum of random $(MMA)_{13}(BMA)_{12}$ doubly-charged oligomer with $2Na^+$ cations, (C) Full mass range ESI-FT mass spectrum of bMMABMA, and (D) ESI-QCID-FT mass spectrum of block $(MMA)_{13}(BMA)_{12}$ doubly-charged oligomer with $2Na^+$ cations.

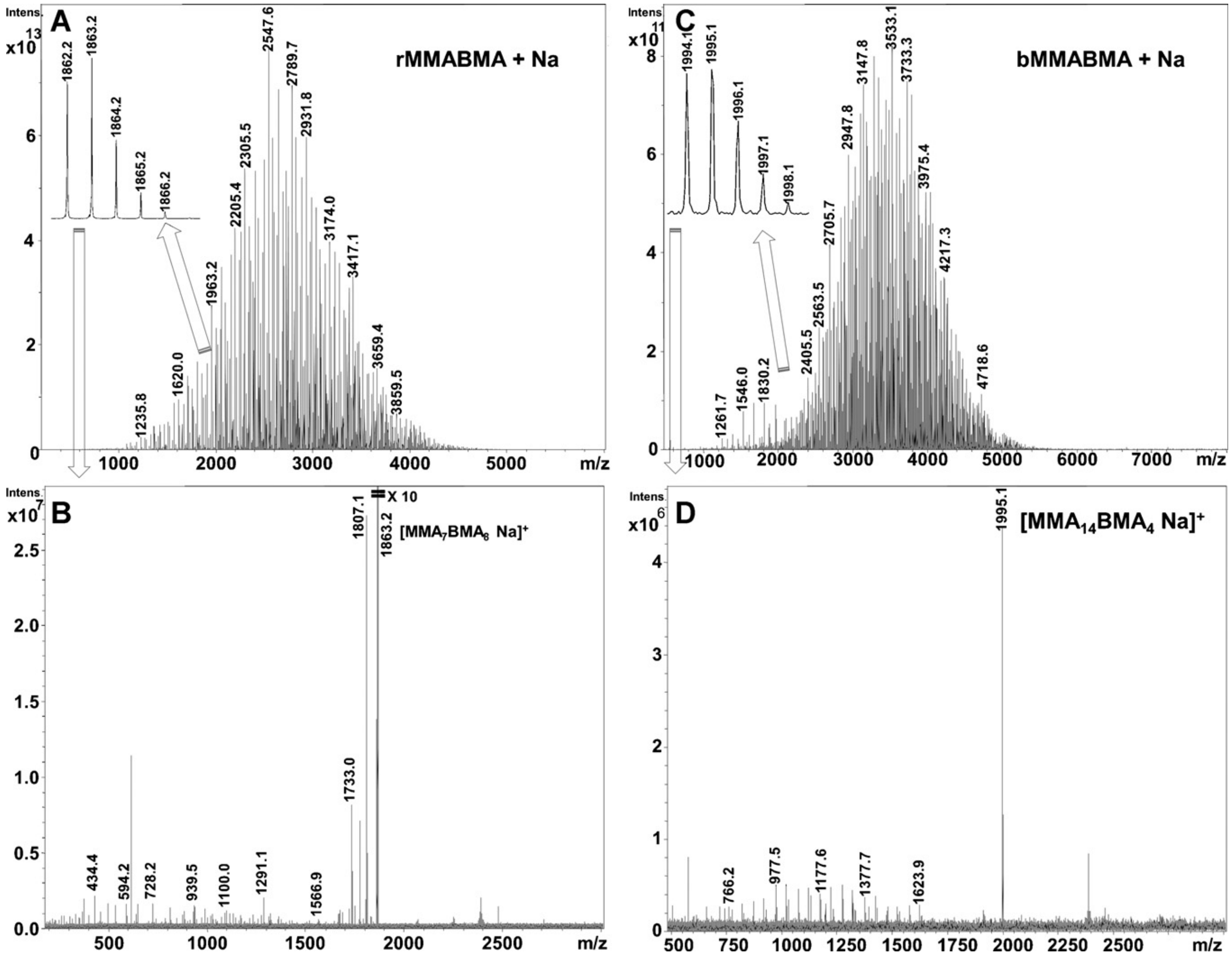

Fig. 3. (A) Full mass range MALDI-FT mass spectrum of rMMABMA, (B) MALDI-QCID-FT mass spectrum of random $(MMA)_7(BMA)_8$ doubly-charged oligomer with $2Na^+$ cations, (C) full mass range MALDI-FT mass spectrum of bMMABMA, (D) MALDI-QCID-FT mass spectrum of block $(MMA)_{14}(BMA)_4$ doubly-charged oligomer with $2Na^+$ cations.

ments produced signals of low abundance and did not overlap with the signals of fragments formed by backbone and side group cleavages. Backbone fragments resulted in MMA or BMA monomeric unit losses from the isolated oligomer where the monomeric unit losses followed a randomized pattern. For example, the most abundant fragment corresponds to loss of 2 BMA and 3 MMA units and no fragments showing loss of a large number of one type of monomeric unit preferentially were observed. Also, most fragments showed a loss of monomeric units in approximately a 1:1 ratio corresponding to the monomer ratio used to synthesize this polymer sample. This occurrence is due to the random monomer sequence of the sample and the fact that the isolated ion with $m/z = 1526.9$ (12 BMA/13 MMA) can be constituted of over 2.6 million different oligomers with the same number of the two monomeric units but with a different sequence.

From the full mass spectrum of the block poly(methyl methacrylate-b-*n* butyl methacrylate) (bMMABMA) sample (Fig. 2C), the oligomer ion with the same *m*/*z* as the random polymer oligomer was isolated and fragmented by QCID (Fig. 2D). Thus, the precursor ion was also constituted of 12 BMA units and 13 MMA units with two Na^+ ions attached. However, the identities of the fragment ions were different from those of the fragment ions formed from random oligomers. This is clear from the different number of monomeric units lost or the MS/MS ion abundances. The block precursor oligomer had a tendency to lose one kind of monomeric preferentially. This difference between random and block oligomers can be represented by a plot of the number of BMA monomeric loses versus the number of MMA monomeric units lost for each oligomer. These plots are shown in Fig. 6A and B where the color coded third dimension of the plot represents the induced current of the fragment ions. It is much easier to see the fragmentation difference between random and block precursor ions in such plots than through constructing tables of fragment ions similar to Table 1. For the random oligomer, the most abundant fragment ions were located in the middle of the plot as a result of losing evenly both monomeric units (Fig. 6A). On the other hand, Fig. 6B shows the plot of the block oligomer where the most abundant ions favored the preferential loss of one of the monomeric units. The explanation for the block oligomer fragmentation behavior is that the isolated ion that has a monomer unit sequence grouped at one end of the copolymer chain with the other monomeric unit sequence grouped at the opposite end. The energy introduced by QCID is most likely sufficient to break only one bond between monomeric units at a time. Therefore, since the adjacent monomeric unit is going to be of the same kind, the fragments will exhibit excessive losses of one monomeric unit. The structure in Fig. 2B represents the arbitrary sequence of rMMABMA while the structure in Fig. 2D represents the sequence of bMMABMA with the assignment of A and B fragment series based on Jackson–Scrivens fragmentation pathway [13]. In the

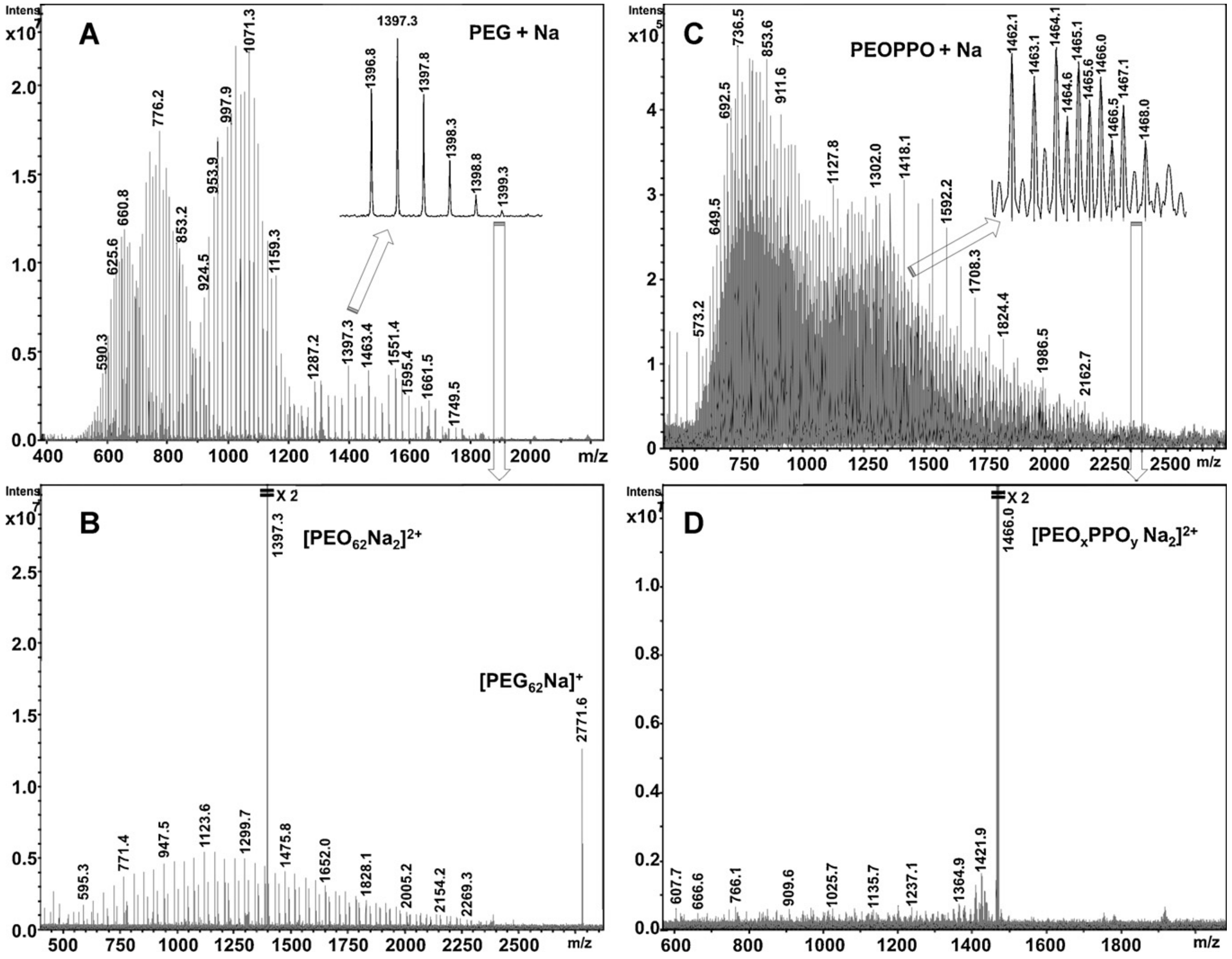

Fig. 4. (A) Full mass range ESI-FT mass spectrum of PEG, (B) ESI-QCID-FT mass spectrum of PEO_{62} doubly-charged oligomer with $2Na^+$ cations, (C) Full mass range ESI-FT mass spectrum of random PEOPPO, and (D) ESI-QCID-FT mass spectrum of doubly-charged group of random oligomers with $2Na^+$ cations.

case of the random copolymer, both A and B series of fragments consist of two monomeric units randomly distributed along the fragmented chain, while in the case of block copolymer A or B fragments will have one of the monomeric units in excess.

This is, to the best to our knowledge, the first rapid analytical technique that can differentiate between random and block copolymer samples. The sample preparation took about half an hour, while ESI-QCID-FTMS analysis was about 20 min long (300 acquisitions). The data analysis was done using Bruker Data Analysis software, and plots were made by Origin 8 software (OriginLab Corp., Northampton, MA).

3.2. MALDI-QCID-FTMS of polyacrylates

Matrix assisted laser desorption ionization (MALDI) is the preferred ionization mass spectrometry technique for polymers because only singly-charged ions are formed, making interpretation and data analysis much simpler. The MALDI-FTMS mass spectrum of rMMABMA sample is shown in Fig. 3A. For example, the singly-charged oligomer with composition of 7 MMA units and 8 BMA units was isolated and fragmented by QCID (Fig. 3B). The collision energy used for the fragmentation of singly-charged ions (around 80 eV) was slightly higher than that used for the electrosprayed multiply-charged ions (around 55 eV). Similar to the ESI-QCID-FTMS study on random copolymers, monomer loss was equally distributed between the two types of monomeric units, an outcome that can be seen from a monomer loss plot (Fig. 6F). On the other hand, an isolated block oligomer (Fig. 3C) with 14 MMA and 4 BMA units, upon collision (Fig. 3D) tends to lose one monomeric unit over the other (Fig. 6G). The reason that the isolated random and block oligomers did not have the same composition of monomers is due to the quadrupole *m*/*z* isolation range of the instrument used and different molecular weights of polymers. It has been noticed that above an *m*/*z* of 2000, the quadrupole tends to stop isolating and starts behaving as an ion guide. For MS/MS, in order to have sufficient ion abundance, isolated oligomers below *m*/*z* of 2000 were examined. Fulfilling these requirements were block oligomer bMMABMA ion that had a composition of 14 MMA and 4 BMA and the oligomer ion from rMMABMA with a composition of 8 MMA, 7 BMA for the random polymer (Fig. 3). Even with different compositions it is obvious that random and block copolymers gave different fragmentation pattern with a conclusion similar to that described in the ESI-QCID-FTMS section.

3.3. ESI-QCID-FTMS of polyethers

For the initial ESI-QCID-FTMS analysis using a poly(ethylene glycol) (PEG) homopolymer and sodium chloride solution, the doubly-charged oligomer consisting of 62 ethylene glycol monomeric units and having end groups of H and OH (*m*/*z* = 1397.3)

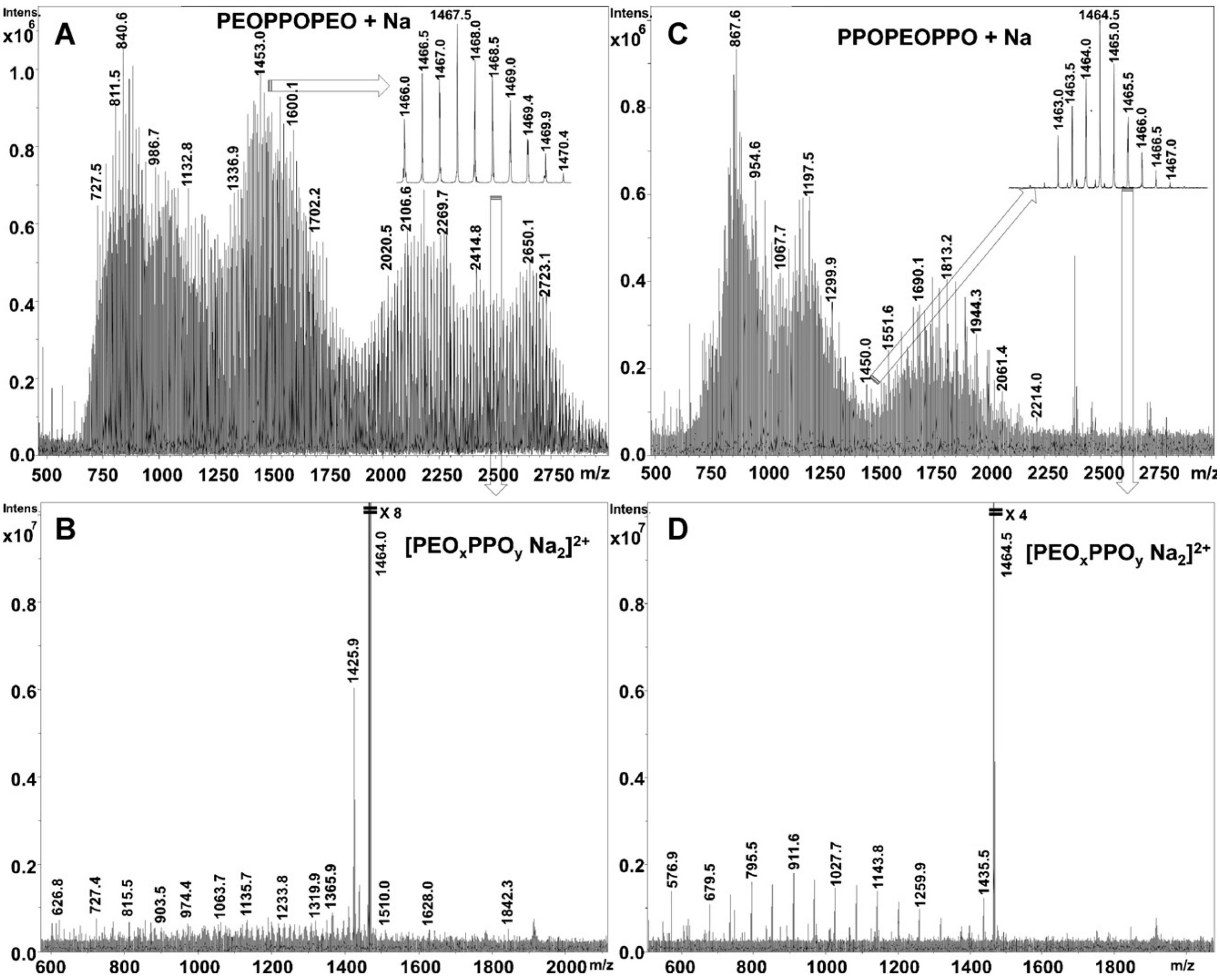

Fig. 5. (A) Full mass range ESI-FT mass spectrum of PEOPPOPEO, (B) ESI-QCID-FT mass spectrum of doubly-charged group of block oligomers with 2Na$^+$ cations, (C) Full mass range ESI-FT mass spectrum of PPOPEOPPO, and (D) ESI-QCID-FT mass spectrum of doubly-charged group of block oligomers with 2Na$^+$ cations.

was selected (Fig. 4A). The spectrum of the fragmented oligomer is shown in Fig. 4B. Ninety-eight of the fragments present were either singly- or doubly-charged and were assigned using monomer units and losses of either H_2 or H_2O with an average error of 4.08 ppm. Six of the doubly-charged fragments show the interesting result is that they have higher mass than the precursor and consist of a longer monomer chain than the parent ion with no hydrogen or water losses. This spectrum is consistent with the previously explained fragmentation mechanism of polyethers [31] where for an A, B and C series of fragments [31], the B and C series are of higher abundance than series A signals.

The polyether copolymers used consisted of ethylene oxide and propylene oxide monomeric units and are one of the most complicated polymer systems that have been investigated by mass spectrometry. The complexity arises from a number of isobaric and overlapped signals [40,41]. It has been shown that unmatchable isolation efficiency of a single isotopic composition can be achieved by the SWIFT method [42,43] inside the ICR cell, but for QCID experiments, oligomers needed to be isolated using the quadrupole, before the collision cell entrance. The isolation efficiency of a linear quadrupole ion trap is much worse than the SWIFT isolation method in the ICR cell. As a result, the isolation of single oligomer without severe loss in sensitivity was not possible [44,45]. Therefore, the ESI-QCID-FTMS study on polyether copolymers was performed using selection of multiple oligomers at the same time. The mass isolation window of the quadrupole was, without loss in sensitivity, as large as a range of 5 m/z units, therefore the m/z of selected oligomers differed by ±5 Da from desired m/z. Differentiation between random and block structures of polyethers by QCID was possible because all of the isolated oligomers had either a random or block sequence of monomeric units. Corresponding fragment ions originating from different oligomers lost monomeric units either randomly or with a preferential order, similarly to ESI-QCID-FTMS experiments described above.

Fig. 4C shows the full ESI-FT mass spectrum of random poly(ethylene oxide-propylene oxide) (PEOPPO) with 40% of ethylene oxide units. For the chosen window, the isolated ions were either singly- or doubly-charged due to the overlap of lower molecular weight singly-charged and higher molecular weight doubly-charged ions (Fig. 4C, inset). The fragment ions were assigned by the number of monomeric units present, unlike the polyacrylate fragments where fragments were assigned by the number of units lost. This assignment was applied due to the inability to isolate a single precursor ion and the incapability to determine a fragment's origin. A plot of the number of PEO units versus PPO units for a selected oligomer is shown in Fig. 6C. The shape of this

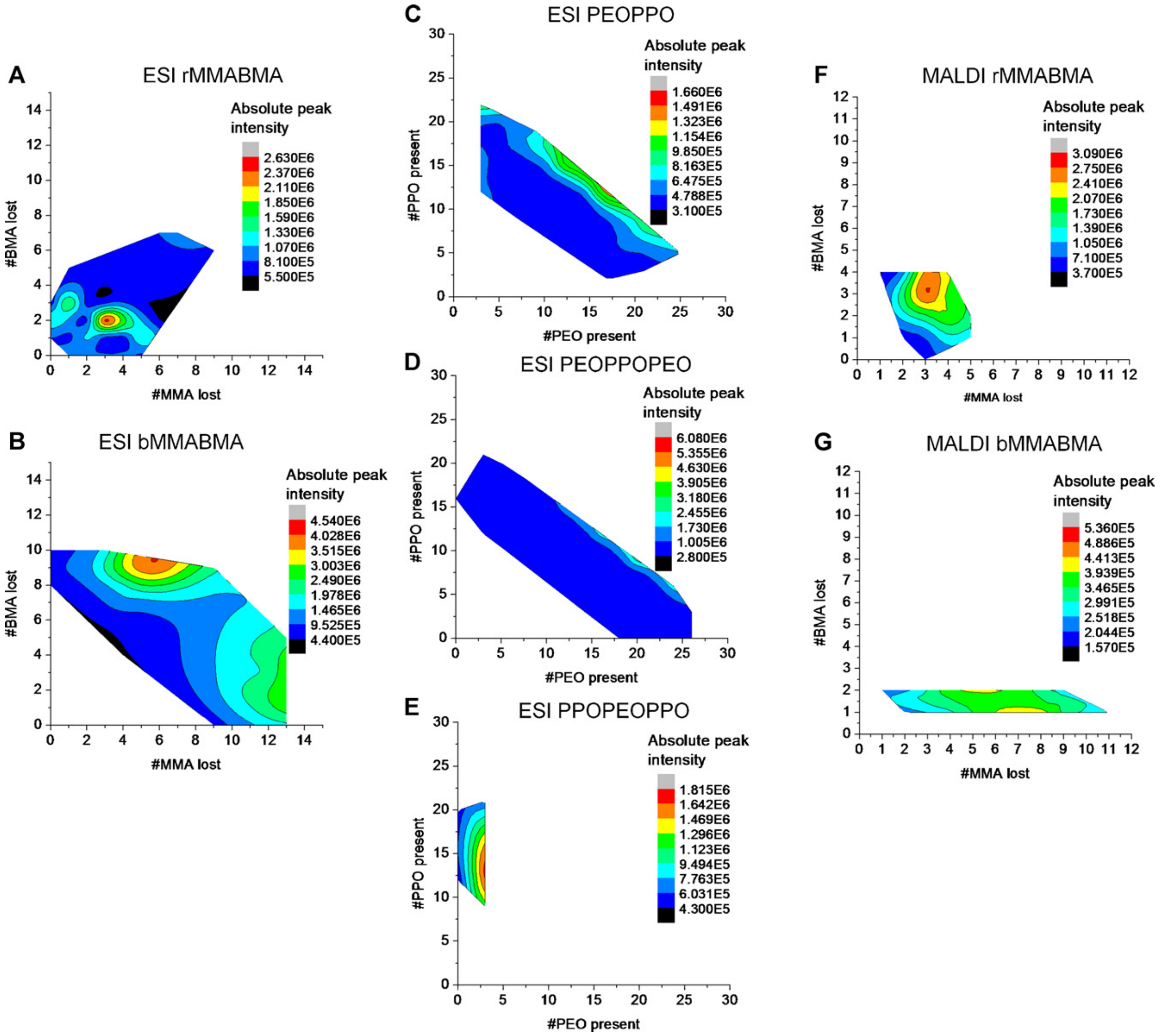

Fig. 6. Plots of MMA vs. BMA monomer fragment losses using ESI-QCID-FTMS for: (A) rMMABMA, (B) bMMABMA; plots of PEO vs. PPO monomers present in fragments using ESI-QCID-FTMS for: (C) PEOPPO, (D) PEOPPOPEO, (E) PPOPEOPPO; plots of MMA vs. BMA monomer fragment losses using MALDI-QCID-FTMS for: (F) rMMABMA, (G) bMMABMA.

contour plot is different than the plot for the random polyacrylate copolymer in that the concentric circles are absent (Fig. 6A and F). The PEOPPO plot's stretched shape is most probably due to the multiple oligomer isolation. The most abundant fragment ions consist of 10–15 PPO units and 15–20 PEO monomeric units. The only conclusive indication that a random copolymer was analyzed was the absence of fragment ions containing only a single monomeric unit.

The signal at $m/z = 1467.5 \pm 5.0$ of diblock copolymer poly(ethylene oxide)-block-poly(propylene oxide)-block-poly(ethylene oxide) (PEOPPOPEO) with 40% of ethylene oxide monomeric units was isolated (Fig. 5A) and fragmented by QCID (Fig. 5b). The resulting plot of monomeric units present in the fragments, shown in Fig. 6D, had a similar shape as the corresponding plot of random PEOPPO fragments. However, there were additional fragments consisting of only ethylene oxide units, unlike the random copolymer. The reason that some of the fragment ions have compositions corresponding to only ethylene oxide monomeric units is that this triblock copolymer was synthesized to have ethylene oxide units at the end and propylene oxide monomers in the middle part of the chain. The most abundant fragment ions had from 15 to 25 ethylene oxide monomeric units and 5–15 propylene oxide monomeric units in their structures.

For poly(propylene oxide)-block-poly(ethylene oxide)-block-poly(propylene oxide) (PPOPEOPPO) with 10% of ethylene oxide present, loss of polyethylene oxide is favored. Precursor ions with m/z similar to PEOPPO and PEOPPOPEO (Fig. 5C) showed many relatively abundant fragments with a complete loss of ethylene oxide units and the presence of 10–20 propylene oxide monomeric units (Figs. 5D and 6E). This occurrence cannot be exclusively related to the diblock nature of the copolymer but rather part of this is due to low percentage (10%) of ethylene oxide in the structure. Therefore, differentiation of random from block polyether copolymers by ESI-QCID-FTMS did not give the conclusive results seen for polyacrylate copolymers.

It can be concluded that the best results for differentiation between the block and random nature of copolymers by QCID-FTMS can be achieved with efficient quadrupole isolation of a single

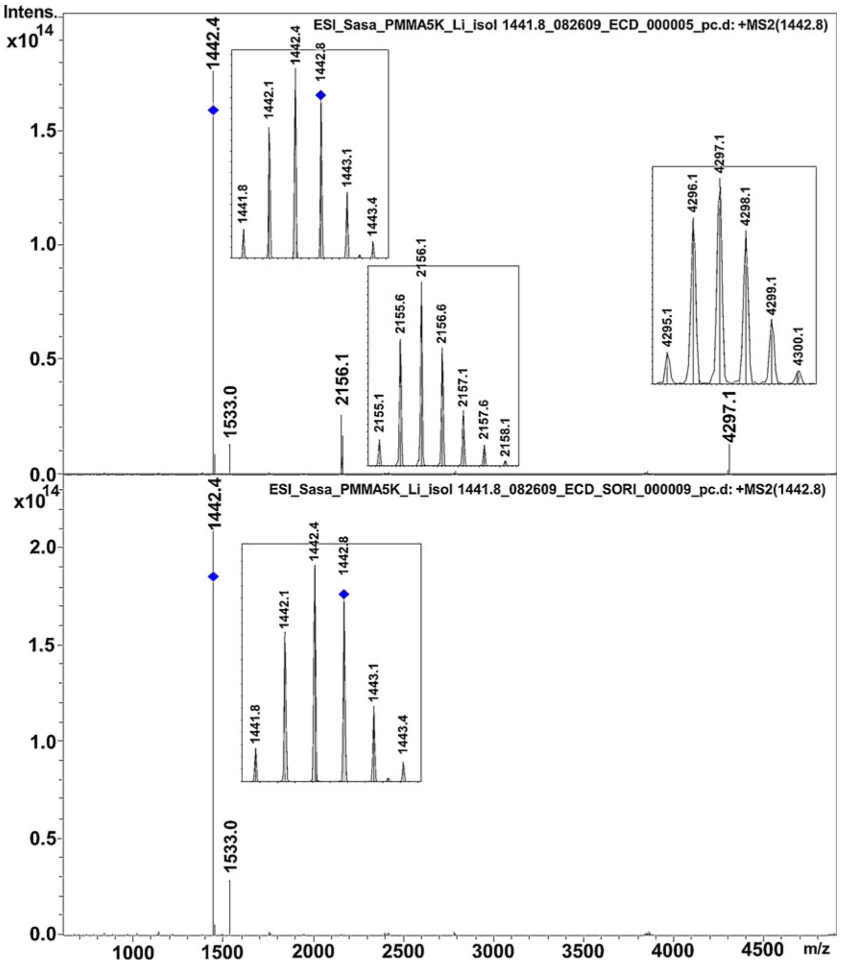

Fig. 7. ESI-ECD-FT mass spectrum of (A) triply-charged $(MMA)_{43}$ oligomer, (B) triply-charged $(MMA)_{43}$ oligomer when SORI pulse was used to eject doubly-charged fragment with proposed fragmentation mechanism.

oligomeric and fragmentation of the oligomer in case where there are similar amounts of the two different monomeric units.

3.4. ESI-ECD-FTMS of polyacrylates

The study performed using electron capture dissociation was done using PMMA and both rMMABMA and bMMABMA copolymers. The cation's influence on fragmentation by ECD was investigated using the homopolymer PMMA. The selected doubly-charged oligomer had 44 monomeric units and after electron irradiation produced only one fragment—a singly-charged ion with a loss of a methyl group (data not shown). The fragment had a surprisingly high abundance compared to ECD-FTMS experiments done on other polymer classes [16,18], probably due to the fact that only one fragment was produced. The abundance of the fragment ion was highest with Li^+ as a cation and decreased with increasing cation size. ECD of the triply-charged oligomer with 43 monomeric units and three Li^+ cations produced one doubly-charged ion with the loss of one CH_3 group and one singly-charged fragment with the loss of two CH_3 groups (Fig. 7A). For the experiment with the triply-charged precursor ion, it was not clear if the oligomer reacted simultaneously with two electrons to give the singly-charged fragment or if the singly-charged ion was formed by the reaction of an electron and a doubly-charged fragment. In order to determine that, above-threshold SORI excitation was applied to eject the doubly-charged fragment from the ICR cell upon its formation by ECD. The resulting spectrum did not show any presence of singly-charged fragment ions, therefore it should be concluded that the removal of the CH_3 group by electron from polyacrylate oligomer happens in a cascade manner (Fig. 7B). A proposed mechanism for this process is shown in Fig. 7B and involves the capture of an electron by the carbonyl group, followed by cleavage of the CH_3 radical and formation of a relatively stable carboxylate anion. All cations from precursor ion are still present in fragment ions and charge reduction happens due to the presence of the carboxylate anion.

The ESI-ECD-FTMS was performed on rMMABMA and bMMABMA polymers with Li^+ attached. The rMMABMA fragment spectrum is shown in Fig. 8A. The doubly-charged precursor ion consisted of 13 MMA units and 11 BMA units. ECD produced two singly-charged fragments with the loss of C_4H_9 and CH_3 radicals. The butyl loss probably occurred from a BMA monomer and the methyl loss from a MMA monomer (similar to ECD fragment ions of PMMA homopolymer). The doubly-charged precursor ion of bMMABMA copolymer had 18 MMA and 12 BMA monomeric units and produced only two singly-charged fragments with butyl and methyl group losses (Fig. 8B). The ECD-FTMS spectra of polyacrylates showed that the only losses the parent ion sustains when it undergoes ECD are those of the possible side-chain groups. While the leaving side group location along the polymer chain is unclear, it is important to note that no fragment recombination was observed when fragmenting multiply-charged oligomers using this method. This indicates that the technique can be

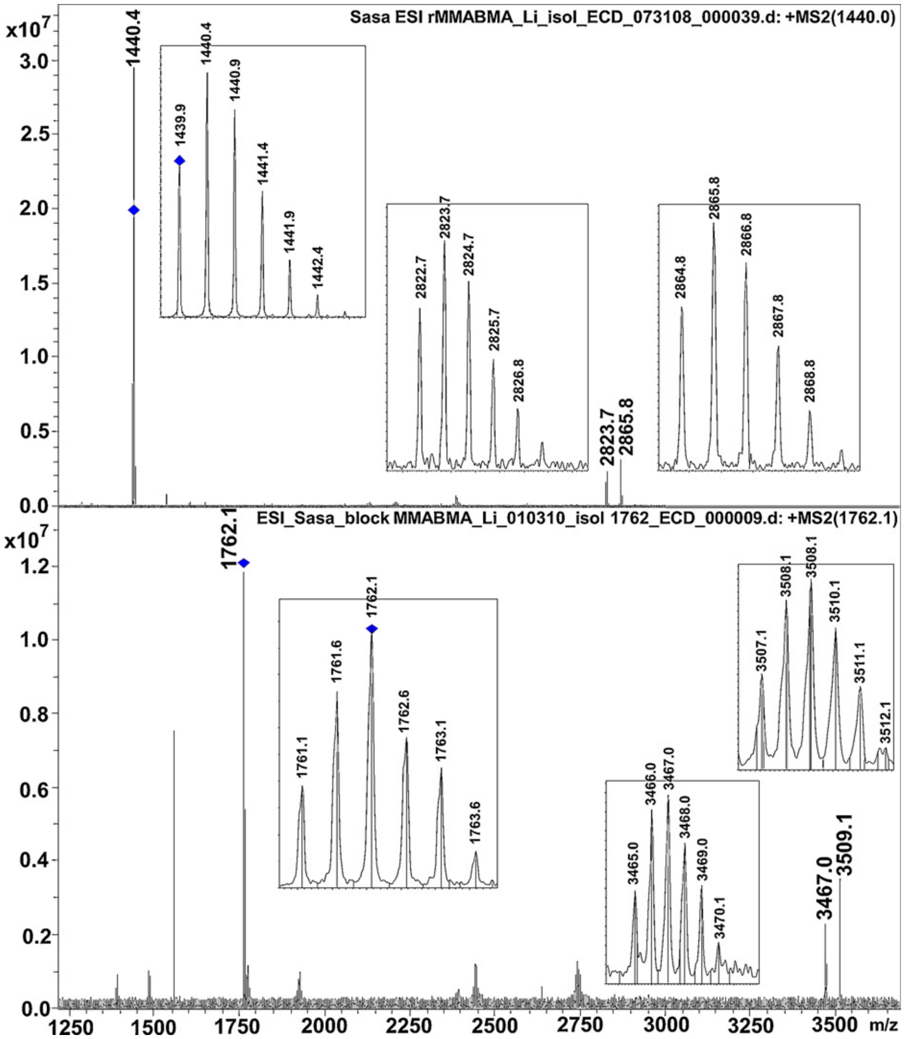

Fig. 8. ESI-ECD-FT mass spectrum of (A) doubly-charged random $(MMA)_{13}(BMA)_{11}$ oligomer, (B) doubly-charged block $(MMA)_{18}(BMA)_{12}$ oligomer.

used for identification of side-groups in unknown polyacrylate samples.

4. Conclusions

Both QCID-FTMS and ECD-FTMS can be used to fragment polyacrylate and polyether oligomer ions formed by ESI or MALDI, although the resulting fragmentation patterns using the two different fragmentation techniques are significantly different. QCID-FTMS produced many fragments corresponding to various combinations of losses of monomeric units and side-groups, when present. This method also showed evidence of the recombination of fragments in the gas phase. These fragments that are longer or more highly charged than the precursor ion and have not, to our knowledge, been reported elsewhere and are under further investigation. Identification of fragments with monomer losses provided insight into each copolymers nature. It appears that random and block copolymers can be differentiated by QCID-FTMS technique. This technique showed the best results when a single oligomer was isolated (i.e. when oligomer isolation is efficient), when CID energy is sufficient to break only one inter-monomer bond, and when the oligomer it composed of approximately equal numbers of two monomeric units.

The ECD-FTMS spectra of the polyacrylates examined showed only losses of single methyl and butyl side-groups for copolymers and single methyl groups for homopolymers. It is possible for a triply-charged polyacrylate ion to react with one electron to form a doubly-charged fragment, subsequently the doubly-charged ion can react with another electron to form a singly-charged fragment. It was established that when doubly-charged ions are continually ejected during ECD, no singly charged ions are observed. This specific behavior of polyacrylates during the ECD process could be used in side-group determination in unknown copolymers.

Acknowledgements

Partial support from NSF CHE-04561324 is acknowledged. We thank Dr. William J. Simonsick (DuPont) for a generous gift of random MMA/BMA copolymer.

References

[1] J.B. Fenn, M. Mann, C.K. Meng, S.F. Wong, C.M. Whitehouse, Electrospray ionization for mass spectrometry of large biomolecules, Science 246 (1989) 64–71.
[2] M. Karas, F. Hillenkamp, Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons, Anal. Chem. 60 (1988) 2299–2301.
[3] K. Tanaka, H. Waki, Y. Ido, S. Akita, Y. Yoshida, T. Yohida, Protein and polymer analyses up to *m/z* 100,000 by laser ionization tme-of-flight mass spectrometry, Rapid Commun. Mass Spectrom. 2 (1988) 151–153.
[4] A.E. Cameron, D.F. Eggers Jr., Ion “velocitron”, Rev. Sci. Instrum. 19 (1948) 605–607.
[5] A.G. Marshall, C.L. Hendrickson, G.S. Jackson, Fourier transform ion cyclotron resonance mass spectrometry: a primer, Mass Spectrom. Rev. 17 (1998) 1–35.

[6] A.I. Nesvizhskii, O. Vitek, R. Aebersold, Analysis and validation of proteomic data generated by tandem mass spectrometry, Nat. Methods 4 (2007) 787–797.

[7] S.J. Pastor, C.L. Wilkins, Sustained off-resonance irradiation and collision-induced dissociation for structural analysis of polymers by MALDI-FTMS, Int. J. Mass Spectrom. Ion Process. 175 (1998) 81–92.

[8] Q. Wu, S. Van Orden, X. Cheng, R. Bakhtiar, R.D. Smith, Characterization of cytochrome *c* variants with high-resolution FTICR mass spectrometry: correlation of fragmentation and structure, Anal. Chem. 67 (1995) 2498–2509.

[9] K. Hakansson, H.J. Cooper, M.R. Emmett, C.E. Costello, A.G. Marshall, C.L. Nilsson, Electron capture dissociation and infrared multiphoton dissociation MS/MS of an N-glycosylated tryptic peptide to yield complementary sequence information, Anal. Chem. 73 (2001) 4530–4536.

[10] A. Kalli, K. Hakansson, Comparison of the electron capture dissociation fragmentation behavior of doubly and triply protonated peptides from trypsin, Glu-C, and chymotrypsin digestion, J. Proteome Res. 7 (2008) 2834–2844.

[11] K. Rajabi, M.L. Easterling, T.D. Fridgen, Solvation of electrosprayed ions in the accumulation/collision hexapole of a hybrid Q-FTMS, J. Am. Soc. Mass Spectrom. 20 (2009) 411–418.

[12] S.M. Miladinovic, S.A. Robotham, C.L. Wilkins, Wide mass range trapping using a 7-T internal source matrix-assisted laser desorption/ionization fourier transform mass spectrometer, Anal. Bioanal. Chem. 392 (2008) 585–594.

[13] A.T. Jackson, H.T. Yates, J.H. Scrivens, M.R. Green, R.H. Bateman, Utilizing matrix-assisted laser desorption/ionization-collision induced dissociation for the generation of structural information from poly(alkyl methacrylate)s, J. Am. Soc. Mass Spectrom. 8 (1997) 1206–1213.

[14] A.V. Tolmachev, M.E. Monroe, S.O. Purvine, R.J. Moore, N. Jaitly, J.N. Adkins, G.A. Anderson, R.D. Smith, Characterization of strategies for obtaining confident identifications in bottom-up proteomics measurements using hybrid FTMS instruments, Anal. Chem. 80 (2008) 8514–8525.

[15] M.A. Kaczorowska, H.J. Cooper, Electron capture dissociation, electron detachment dissociation, and collision-induced dissociation of polyamidoamine (PAMAM) dendrimer ions with amino, amidoethanol, and sodium carboxylate surface groups, J. Am. Soc. Mass Spectrom. 19 (2008) 1312–1319.

[16] B.A. Cerda, D.M. Horn, K. Breuker, B.K. Carpenter, F.W. McLafferty, Electron capture dissociation of multiply-charged oxygenated cations. A nonergodic process, Eur. Mass Spectrom. 5 (1999) 335–338.

[17] B.A. Cerda, K. Breuker, D.M. Horn, F.W. McLafferty, Charge/radical site initiation versus coulombic repulsion for cleavage of multiply charged ions. charge solvation in poly(alkene glycol) ions, J. Am. Soc. Mass Spectrom. 12 (2001) 565–570.

[18] B.A. Cerda, D.M. Horn, K. Breuker, F.W. McLafferty, Sequencing of specific copolymer oligomers by electron-capture-dissociation mass spectrometry, J. Am. Chem. Soc. 124 (2002) 9287–9291.

[19] S. Koster, M.C. Duursma, J.J. Boon, R.M.A. Heeren, S. Ingemann, R.A.T.M. van Benthem, C.G. de Koster, Electron capture and collisionally activated dissociation mass spectrometry of doubly charged hyperbranched poly(ester-amide)s, J. Am. Soc. Mass Spectrom. 14 (2003) 332–341.

[20] S. Lee, S.Y. Han, T.G. Lee, G. Chung, D. Lee, H.B. Oh, Observation of pronounced by cleavages in the electron capture dissociation mass spectrometry of polyamidoamine (PAMAM) dendrimer ions with amide functionalities, J. Am. Soc. Mass Spectrom. 17 (2006) 536–543.

[21] S.Y. Han, S. Lee, H.B. Oh, Comparable electron capture efficiencies for various protonated sites on the third generation poly(propylene imine) dendrimerIons: applications by SORI-CAD and electron capture dissociation mass spectrometry (ECD MS), Bull. Korean Chem. Soc. 26 (2005) 740–746.

[22] R. Bakhtiar, Z. Guan, Electron capture dissociation mass spectrometry in characterization of peptides and proteins, Biotechnol. Lett. 28 (2006) 1047–1059.

[23] H.J. Cooper, K. Hakannson, A.G. Marshall, The role of electron capture dissociation in biomolecular analysis, Mass Spectrom. Rev. 24 (2005) 201–222.

[24] A.J. Crease, H.J. Cooper, Liquid chromatography electron capture dissociation tandem mass spectrometry (LC-ECD-MS/MS versus liquid chromatography collision-induced dissociation tandem mass spectrometry (LC-CID-MS/MS) for the I dentification of proteins, J. Am. Soc. Mass Spectrom. 18 (2007) 891–897.

[25] R. Chen, L. Li, Lithium, Transition metal ions enable low energy collision-induced dissociation of polyglycols in electrospray ionization mass spectrometry, J. Am. Soc. Mass Spectrom. 12 (2001) 832–839.

[26] R. Chen, X. Yu, L. Li, Characterization of poly(ethylene glycol) esters using low energy collision-induced dissociation in electrospray ionization mass spectrometry, J. Am. Soc. Mass Spectrom. 13 (2002) 888–897.

[27] R.P. Lattimer, Tandem mass spectrometry of lithium-attachment ions from polyglycols, J. Am. Soc. Mass Spectrom. 3 (1992) 225–234.

[28] W.J. Simonsick, V.I. Petkovska, Detailed structural elucidation of polyesters and acrylates using fourier transform mass spectrometry, Anal. Bioanal. Chem. 392 (2008) 575–583.

[29] A.T. Jackson, J.H. Scrivens, J.P. Williams, E.S. Baker, J. Gidden, M.T. Bowers, Microstructural and conformational studies of polyether copolymers, Int. J. Mass Spectrom. 238 (2004) 287–297.

[30] J.M. Kaufman, A.J. Jaber, M.J. Stump, W.J. Simonsick, C.L. Wilkins, Interference from multiple cations in MALDI-MS spectra of copolymers, Int. J. Mass Spectrom. 234 (2004) 153–160.

[31] M. Girod, Y. Carrisan, S. Humbel, L. Charles, Tandem mass spectrometry of doubly charged poly(ethylene oxide) oligomers produced by electrospray ionization, Int. J. Mass Spectrom. 272 (2008) 1–11.

[32] A.J. Hoteling, K. Kawaoka, M. Goodberlet, W.-M. Yu, K.G. Owens, Optimization of matrix-assisted laser desorption/ionization tme-of-flight collision-induced dissociation using poly(ethylene glycol), Rapid Commun. Mass Spectrom. 17 (2003) 1671–1676.

[33] S. Okuno, M. Kiuchi, R. Arakawa, Structural characterization of polyethers using matrix-assisted laser desorption/ionization quadrupole ion trap tme-of-flight mass spectrometry, Eur. Mass Spectrom. 12 (2006) 181–187.

[34] A.T. Jackson, H.T. Yates, W.A. MacDonald, J.H. Scrivens, G. Critchley, J. Brown, M.J. Deery, K.R. Jennings, C. Brookes, Time-lag Focusing, Cation attachment in the analysis of synthetic polymers by matrix-assisted laser desorption/ionization-time-of-flight-mass spectrometry, J. Am. Chem. Soc. 8 (1997) 132–139.

[35] O. Laine, S. Trimpin, H.J. Räder, K. Müllen, Changes in post-source decay fragmentation behavior of poly(methyl methacrylate) polymers with increasing molecular weight studied by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, Eur. Mass Spectrom. 9 (2003) 195–201.

[36] A.M. Alhazmi, P.M. Mayer, Protonating polymer oligomers in the gas phase to change fragmentation pathways, J. Am. Chem. Soc. 20 (2009) 60–66.

[37] J. Gidden, A.T. Jackson, J.H. Scrivens, M.T. Bowers, Gas phase conformations of synthetic polymers: poly(methyl methacrylate) oligomers cationized by sodium ions, Int. J. Mass Spectrom. 188 (1999) 121–130.

[38] A.T. Jackson, S.E. Slade, J.H. Scrivens, Characterisation of poly(alkyl methacrylate)s by means of electrospray ionisation–tandem mass spectrometry (ESI–MS/MS), Int. J. Mass Spectrom. 238 (2004) 265–277.

[39] J.H. Scrivens, A.T. Jackson, H.T. Yates, M.R. Green, G. Critchley, J. Brown, R.H. Bateman, M.T. Bowers, J. Gidden, The effect of the variation of cation in the matrix-assisted laser desorption/ionisation–collision induced dissociation (MALDI–CID) spectra of oligomeric systems, Int. J. Mass Spectrom. 165/166 (1997) 363–375.

[40] F.J. Mayer-Posner, V. Sauerland, H. Kaminski, S. Ndoni, W.B. Petersen, S. Hivd, P. Kingshott, Investigation of copolymers by liquid chromatography and matrix assisted laser desorption/ionization, PMSE Preprints 88 (2003) 21–25.

[41] W. Suen, J. Percy, S.L. Hsu, I.A. Kaltashov, H.D. Stidham, Influence of polyether copolymer configuration on polyurethane reaction: a mass spectrometry analysis, Cell. Polym. 22 (2003) 23–42.

[42] S. Guan, A.G. Marshall, Ion excitation in trapped-ion mass spectrometry, Int. J. Mass Spectrom. Ion Process. 157/158 (1996) 5–37.

[43] A.G. Marshall, T.C.L. Wang, T.L. Ricca, Tailored excitation for fourier transform ion cyclotron mass spectrometry, J. Am. Chem. Soc. 107 (1985) 7893–7897.

[44] K.R. Jonscher, J.R. Yates III, The quadrupole ion trap mass spectrometry—a small solution to a big challenge, Anal. Biochem. 244 (1997) 1–15.

[45] S. Guan, A.L. Burlingame, High mass selectivity for top-down proteomics by application of swift technology, J. Am. Soc. Mass Spectrom. 21 (2010) 455–459.