



Characterisation of end groups in poly(2-hydroxyethyl methacrylate) by means of electrospray ionisation-mass spectrometry/mass spectrometry (ESI-MS/MS)

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ABSTRACT

Poly(2-hydroxyethyl methacrylate) (poly(HEMA)) has been characterised by means of electrospray ionisation-mass spectrometry/mass spectrometry (ESI-MS/MS), in order to evaluate this technique for the generation of end group information. Low energy collision-induced dissociation (CID) data from poly(HEMA) enabled information on both end groups of the polymer chain to be gleaned, in a similar fashion to that proposed previously for other methacrylate polymer systems. Exact-mass CID information was employed to aid the understanding of the dissociation mechanism of the polymer. Some additional fragmentation pathways, compared to other methacrylate polymer systems, are proposed. An example of how software can aid the interpretation of the MS/MS data is also shown.

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1. Introduction

Polymers containing 2-hydroxyethyl methacrylate as the base monomer or co-monomer are used in applications such as contact lenses [1,2] and for dental treatments [3]. The homopolymer of poly(2-hydroxyethyl methacrylate) (poly(HEMA)) was first developed in the 1950's by a group working in Czechoslovakia [1]. Polymer hydrogels based on HEMA have recently been developed for biocompatible applications [4,5].

Many methacrylate based polymers and copolymers have been characterised by means of matrix-assisted laser desorption/ionisation time-of-flight (MALDI-ToF) mass spectrometry [6–22] and also tandem mass spectrometry (MS/MS) [20,22–25], in order to generate structural information. These techniques are typically used to characterise the end groups of methacrylate homopolymers, such as poly(methyl methacrylate), but sequence information can be gleaned from copolymers by means of MS/MS [19,26]. Acrylic polymers with fragile end groups can sometimes be more

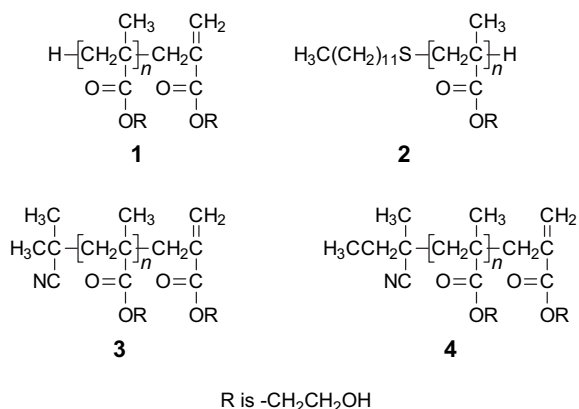
readily characterised by electrospray ionisation-mass spectrometry (ESI-MS) when compared to MALDI-ToF, as fragment ion peaks may be detected rather than molecular species in the spectra using the latter technique [20–22].

The advantage of MS/MS techniques, over either ESI-MS or MALDI-ToF, is the ability to generate information on the masses of the individual end groups. This has been shown for a number of methacrylate based polymers, including poly(methyl methacrylate) [20,22–25], poly(ethyl methacrylate) [24] and poly(n-butyl methacrylate) [24,27]. Furthermore, similar end group information was also obtained from a block copolymer of methyl methacrylate/butyl methacrylate, as well as information on the sequence of the backbone [19,26].

A number of homopolymers of poly(HEMA) (1–4) have been synthesised by standard free radical polymerisation techniques and characterised by means of ESI-MS/MS, in order to evaluate the capabilities of the latter for end group identification of this methacrylate. In addition, software, that was recently developed in order to aid the interpretation of MS/MS data from synthetic polymers [28], has been used for the resulting data. Exact mass-ESI-MS/MS has also been employed to aid structural identification and understanding of the fragmentation pathways for this polymer system.

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2. Experimental

2.1. Polymer synthesis

All polyHEMA samples were prepared by solution polymerisation of HEMA in degassed, refluxing, isopropyl alcohol at 82 °C, using either 2,2'-azobis (isobutyronitrile) (AIBN) or 2,2'-azobis (2-methyl butanenitrile) (AMBN) as initiator. Chain transfer agents (bis(boron difluorodiphenylglyoximate) cobalt (II) (COBF) and *n*-dodecyl mercaptan (DDM)) were used for two of the polymers. The solvent was then removed in vacuo and the polymers were analysed without further purification. The amounts of initiator and chain transfer agent employed in the preparation of each polymer are shown in Table 1. Confirmation of structural integrity was generated using nuclear magnetic resonance (NMR) spectroscopy.

2.2. Tandem mass spectrometry

Experiments were performed in a hybrid quadrupole time-of-flight mass spectrometer (Q-ToF Ultima Global, Waters MS Technologies, Manchester, UK). The instrument was equipped with a Lockspray™ ion source and operated at a source temperature of 80 °C in the positive mode of ionisation, with a capillary voltage of 3.5 kV. The cone voltage was optimised between 100 and 200 V for MS and tandem mass spectrometry (MS/MS) experiments and the collision energy used for MS/MS experiments ranged between 45 and 100 eV (see figure legends for details). MS/MS was carried out using argon as the collision gas. The ToF mass analyser was tuned in W-optic mode for an operating resolution of 15,000 (FWHM). Mass spectra were acquired at an acquisition rate of 1 spectrum/s with an inter-scan delay of 100 ms. Data acquisition and processing were carried out using MassLynx™ (V4.0).

2.3. Sample preparation

Sodium acetate, leucine enkephalin and methanol were obtained from Sigma–Aldrich (Gillingham, UK). All poly(HEMA) samples were dissolved in methanol to a concentration of 2 mg/mL.

Table 1
Recipes for Synthesis of Poly(HEMA) Samples.

Polymer	Initiator	Mol% Initiator	Chain Transfer Agent	Mol % Chain Transfer Agent
1	AMBN	2	COBF	0.5
2	AMBN	5	DDM	7
3	AMBN	5	–	–
4	AIBN	5	–	–

Sodium acetate was dissolved in methanol to a concentration of 100 ng/μL. MS and MS/MS analysis of Poly(HEMA) samples was performed by infusing a mixture of sample and lithium acetate at a ratio of 1:1. Leucine enkephalin (150 ng/μL in 50:50 methanol:–water) was introduced at a flow rate of 1 μL/min via the reference sprayer of the Lockspray ion source for exact-mass experiments.

2.4. Software

The Polymerator software [29] was written in Java v1.4.2. (Sun Microsystems). The graphical user interface of the software is used to enter the information required in order to calculate the polymer fragments, as described previously [28]. The MS/MS data to be annotated are read from an ASCII text file containing mass intensity information. Given a user selected mass accuracy error (in Da), fragments are computed by the software and are matched to experimentally observed peaks. Each matched peak is displayed with a colour corresponding to each fragment type and annotated with the fragment information. Matched peaks are also displayed in a table. The user can interact with the table and edit the peak annotation, add annotation to peaks not matched by the software or delete the annotation from incorrectly assigned peaks.

3. Results and discussion

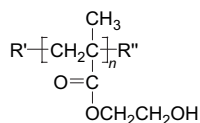
The use of COBF as a catalytic chain transfer (CCT) agent has been demonstrated to generate methacrylate polymers with unsaturated end groups and well controlled molecular weights [30]. The unsaturated terminating end group is proposed to result from abstraction of a hydride radical from a growing polymer chain (effectively terminating the oligomer) and initiating a new chain with this radical [30]. A polymer of HEMA made with this CCT agent (**1**) was characterised by means of ESI-MS/MS and the resulting spectrum from the sodiated undecamer (i.e. **1** with *n* is 11, $[M + Na]^+$ is *m/z* 1583) is shown in Fig. 1. The spectrum contains a number of series of peaks that are separated by *m/z* 130, which is equivalent to the mass of a monomer unit of HEMA. These ions are all proposed to retain the ionising cation (sodium ion, Na⁺). Two series of peaks at low mass-to-charge ratios, annotated **A_n** and **B_n**, are proposed to retain the ω- and α-end groups respectively of the polymer and may be used to generate information about these functionalities. This is analogous to that noted for other methacrylate polymers that have been characterised by means of MS/MS [20,22–25,27], with these odd electron, possibly distonic radical cations, noted to be present at significant intensities at low mass-to-charge ratios. The **A_n** series (**A₁** at *m/z* 268 and **A₂** at *m/z* 398) are proposed to retain the ω-end group and the **B_n** series (**B₁** at *m/z* 154 and **B₂** at *m/z* 284) contains the α-end group functionality.

The proposed fragmentation is summarised for these two series in Fig. 1. Equations that enable the masses of the end groups of the polymer to be calculated can be derived, similarly to that described for other methacrylate polymer systems [24]:

$$m/z(\mathbf{A}_n) = M(R'') + (n-1)M(\text{HEMA}) + M(\text{C}_5\text{H}_8\text{O}_3) + M(\text{Na}^+) \quad (1)$$

$$m/z(\mathbf{B}_n) = M(R') + nM(\text{HEMA}) + M(\text{Na}^+) \quad (2)$$

where *m/z*(**A_n**) and *m/z*(**B_n**) are the mass-to-charge ratios of peaks from the **A_n** and **B_n** series respectively, *M*(*R'*) and *M*(*R''*) are the masses of the initiating (α) and terminating (ω) end groups respectively, *M*(HEMA) is the mass of the monomer, *M*(C₅H₈O₃) is the mass of a portion of the monomer and *M*(Na⁺) is the mass of the cation (Na⁺).



Furthermore, the relationship between the mass-to-charge ratios of ions from the **A_n** and **B_n** series can be described as shown below:

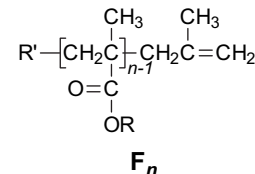
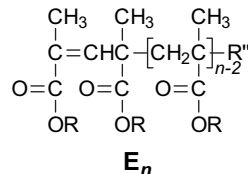
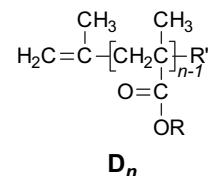
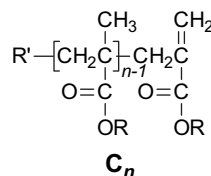
$$m/z([M + \text{Na}]^+) = m/z(\mathbf{A}_n) + m/z(\mathbf{B}_n) + nM(\text{HEMA}) + M(\text{CH}_2) - M(\text{Na}^+) \quad (3)$$

where $m/z([M + \text{Na}]^+)$ is the m/z of the precursor ion ($[M + \text{Na}]^+$). The proposed mass-to-charge ratios of a series of peaks from the **A_n** or **B_n** series can therefore be calculated if the m/z of a peak from the other series is known.

The data from **1** (Fig. 1) are consistent with that expected for this polymer, indicating that the oligomers contain an unsaturated terminating functionality, validating the proposed termination mechanism [30].

The other peaks of significant intensity in the spectrum are proposed to result from losses of both of the end groups and a number monomer units (**G_n** series in Fig. 1), again analogous to that from other methacrylate systems [24]. These ions are even electron species and can be clearly differentiated from the **A_n** and **B_n** series by the fact that the former are seen at odd mass-to-charge ratios and the latter pair of series at even mass-to-charge ratios (when the end groups do not contain odd numbers of nitrogen atoms, *vide supra*). These ions may be formed by similar mechanisms to that proposed for analogous species noted in MS/MS spectra from poly(styrene)s [31].

An expansion (m/z 400–1600) of this spectrum, magnified to show some of the peaks of low intensity, is shown in Fig. 2. Three series of peaks, labelled **D_n**, **E_n** and **F_n** in Fig. 2, are proposed to be analogous to those seen in spectra from other alkyl methacrylate polymers [24]. A fourth series of peaks (**C_n**), also typically seen for methacrylate polymers [24], have equivalent mass-to-charge ratios with that of the **G_n** series for this sample and presumably also make



R is -CH₂CH₂OH

a contribution to the significant intensity of these peaks. These peaks are all even electron species that are proposed to retain the sodium cation. Proposed structures for ions of the **C_n**, **D_n**, **E_n** and **F_n** series for poly(HEMA) are shown above.

Peaks noted at m/z 1539 and 1477 are proposed to result from sequential loss of ethenyl alcohol (probably rearranges to acetaldehyde) and ethylene glycol from side chains of the poly(HEMA) backbone. It is suggested that these sequential losses could be from adjacent side chains, such that a six-membered cyclic anhydride functionality is formed on the backbone, as shown by the indicative structures in Fig. 2. The formation of similar structures was previously proposed to happen on degradation of poly(methacrylic acid) by dehydration [32]. Formation of analogous fragment ions was recently proposed from MS/MS of acrylate polymers [33]. Additional peaks, which result from similar losses from ions of the **G_n** series, are also annotated in Fig. 2.

The addition of dodecyl mercaptan as a CTA is commonly employed for methacrylates to give a polymer with good molecular weight control. Poly(HEMA) made with this CTA has been characterised by means of ESI-MS/MS. The resulting spectrum from the

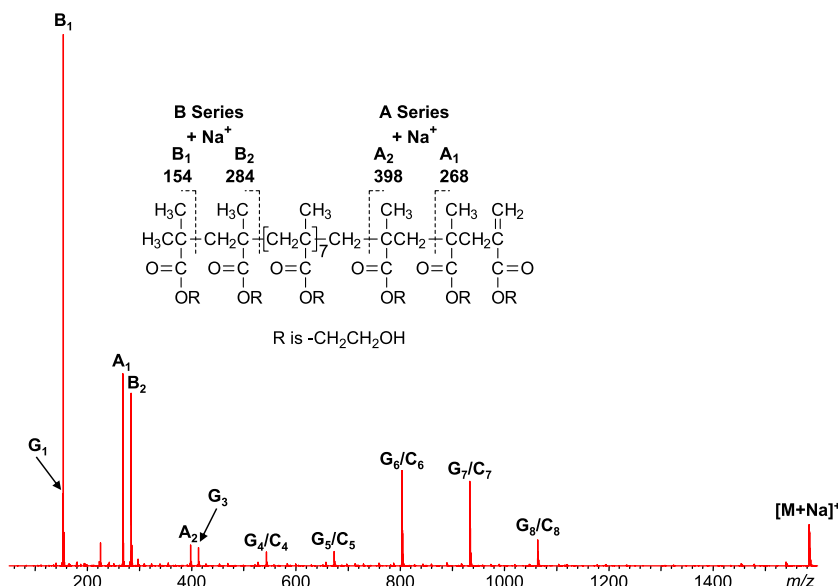


Fig. 1. ESI-MS/MS spectrum of sodiated undecamer of 1, m/z 1583 ($E_{\text{coll}} = 100$ eV). A schematic of the proposed fragmentation to form ions of the **A_n** and **B_n** series is also shown.

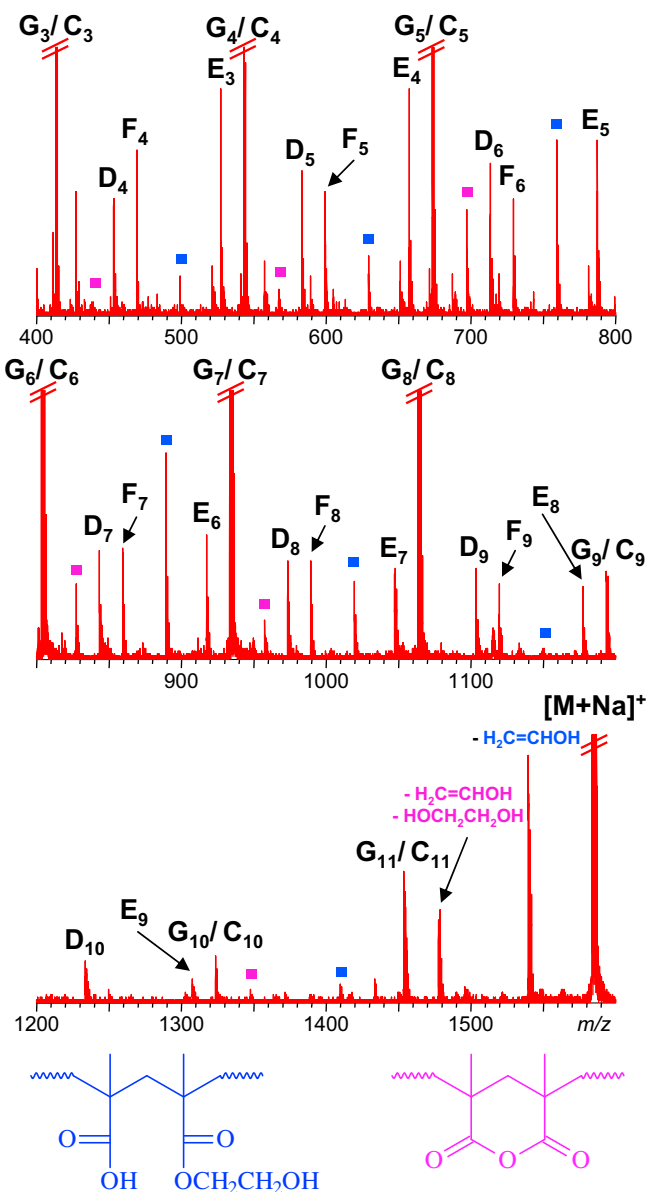


Fig. 2. Partial (m/z 400–1600), magnified, ESI-MS/MS spectrum of sodiated undecamer of **1**, m/z 1583 ($E_{\text{coll}} = 100$ eV). Indicative partial structures for some of the fragment ions are shown. Squares represent fragments that are proposed to have lost ethenyl alcohol (or acetaldehyde) (■) or this molecule and ethylene glycol (■) from an ion of the G_n series.

sodiated heptamer (m/z 1135) is displayed in Fig. 3. The expected series from the A_n series Eq. (1) is noted to be present (A_1 at m/z 140 and A_2 at m/z 270), indicating that the terminating functionality is that proposed. The lack of peaks from the B_n series (B_1 expected at m/z 354 and B_2 at m/z 484) is proposed to be a result of the lability of the thio-ether group at the α -end of the polymer chain. It was noted that the analogous expected peaks from an thio-ether end-capped poly(styrene) were also not observed [31]. The fragmentation scheme in Fig. 3 indicates how end group masses can be calculated from the peaks of the A_n series for the terminating functionality.

It was previously proposed, however, that confirmatory evidence on the masses of the end groups could be inferred from the mass-to-charge ratios of the C_n , D_n , E_n and F_n series [24]. The C_n and F_n series are proposed to retain the α -end group and the D_n and E_n series

retain the ω -end functionality. It is proposed that for this sample, the peaks of the C_n and F_n series, noted to be present in Fig. 3, can provide information on the α -end group functionality in the absence of the B_n series. A similar phenomenon was noted for a poly(methyl methacrylate) polymer with the same end group functionality as **2** [34]. The peaks from sequential losses of ethenyl alcohol and ethylene glycol (m/z 1091 and 1029 respectively) from the precursor ion are again noted at significant intensities, along with other minor peaks resulting from these losses from other fragments (peaks not annotated in Fig. 3). Peaks from the G_n series (indeed G_1 at m/z 153 is the most intense peak in the spectrum) are also noted to be present, again not containing either end group functionality.

Polymers of 2-hydroxyethyl methacrylate generated with an azo-based initiator such as 2,2'-azobis(isobutyronitrile) (AIBN) and no CTA would be expected to be terminated either by disproportionation and/or combination of chains. The former would result in oligomers containing a portion of the azo-initiator as the α -end group and a mixture of unsaturated and saturated end group functionality at the terminating end, which is the case for the polymer analysed here. This poly(HEMA) contains unsaturated end groups (**3**) as well as the corresponding saturated functionality (as shown by NMR spectroscopy, data not shown). Polymers made by this route typically have less molecular weight control than those produced by a CTA, which is probably partially a cause of the low signal-to-noise for ions of **3** seen in the ESI-MS spectrum (data not shown). The resulting ESI-MS/MS spectrum from the sodiated tetramer of **3** (m/z 740) is shown in Fig. 4, at notably lower signal-to-noise than from polymers of **1** and **2**. One peak from the A_n and B_n series is noted to be present at significant intensity, which allows information on the masses of the two end groups to be gleaned (as indicated by the fragmentation scheme in Fig. 4). This is expected for lower m/z precursors, with more ions from the A_n and B_n series commonly noted to be present for higher m/z precursor ions of alkyl methacrylates [25,27]. The peak from the B_n series (B_1 at m/z 221) is present at an odd m/z , which is an indicator of where the nitrogen atom is located in the oligomeric structure (known to be present as the precursor has an even m/z). An ion from the G_n series (G_1 at m/z 153) is again the most abundant fragment ion, with peaks from others in this series noted to be present at lower intensity in the ESI-MS/MS spectrum.

Peaks from the C_n , D_n , E_n and F_n series are again noted to be present, providing confirmatory evidence for the masses of the end groups. Peaks from the C_n and F_n series are observed at even mass-to-charge ratios, as expected for ions containing one nitrogen atom from the initiating group of the poly(HEMA). Peaks at m/z 696 and 634 are again proposed to result from sequential loss of ethenyl alcohol (or acetaldehyde) and ethylene glycol (*vide supra*).

It was recently shown that software can aid the interpretation of MS/MS data from a number of polymer systems, including methacrylates [28]. To demonstrate that the software can reduce the time taken to interpret ESI-MS/MS data from poly(HEMA), a text file (intensity versus m/z) from the spectrum shown in Fig. 4 was used to import these data. The software automatically annotates peaks from known fragmentation pathways and creates a table of these annotated fragment ion peaks, when values for the repeat unit formula ($C_6H_{10}O_3$), number of repeat units (4), end groups formulae (C_4H_6N and $C_6H_9O_3$) and cation (Na) are entered [28]. The resulting output for the spectrum from **3** is shown in Fig. 5. The annotation for peaks from losses of ethenyl alcohol (or acetaldehyde) and ethylene glycol from the precursor were manually added in the software. The different series shown to be present (Fig. 4) are annotated in different colours with the proposed assignments. The table shown in Fig. 5(b) indicates that good mass accuracy was obtained in these experiments, giving confidence in the assignments. All of the assigned major fragments were noted to be within

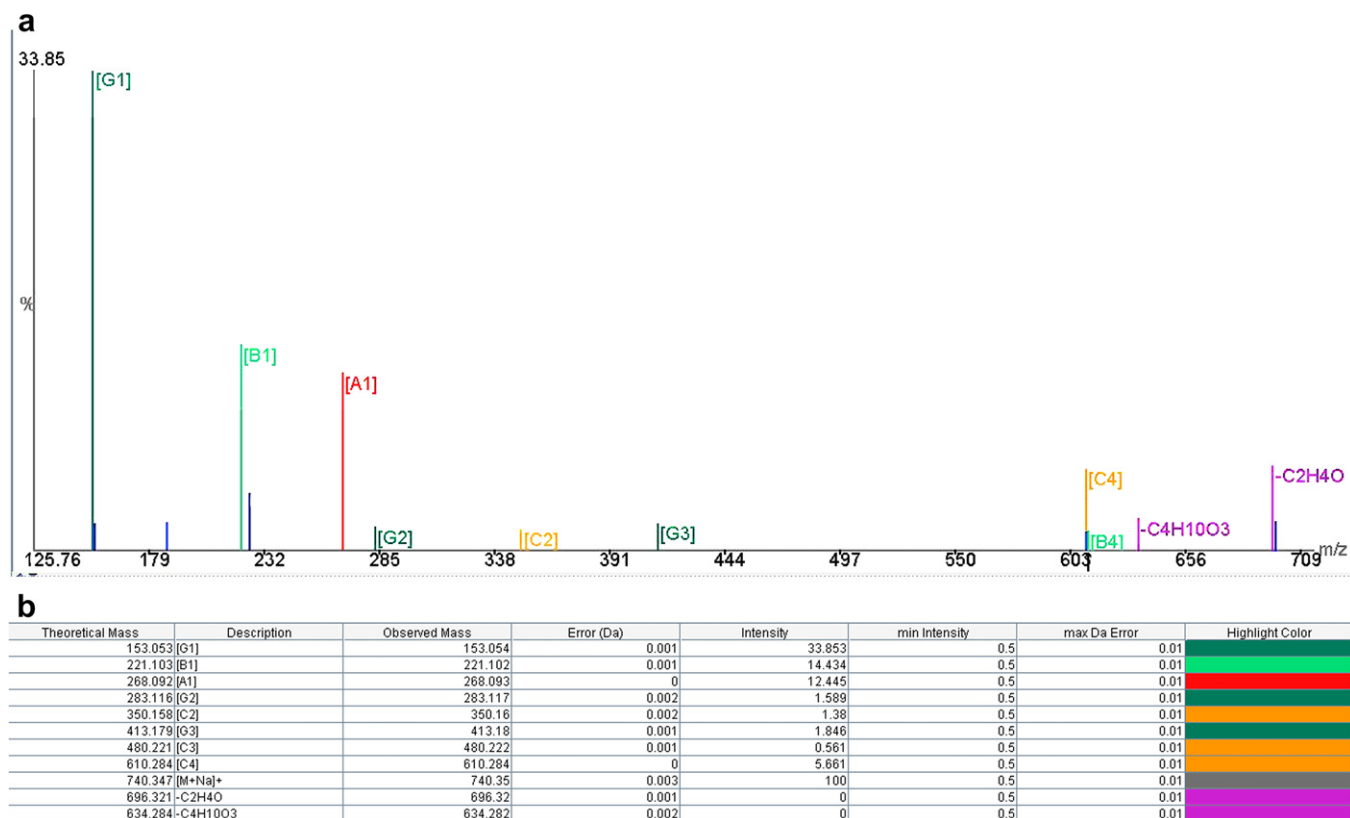


Fig. 5. (a) Screenshot from Polymerator software of annotated ESI-MS/MS spectrum from the sodiated tetramer of **3** (see Fig. 4 for original spectrum) and (b) Details of annotated fragment ions are displayed by the Polymerator software in the table (screenshot).

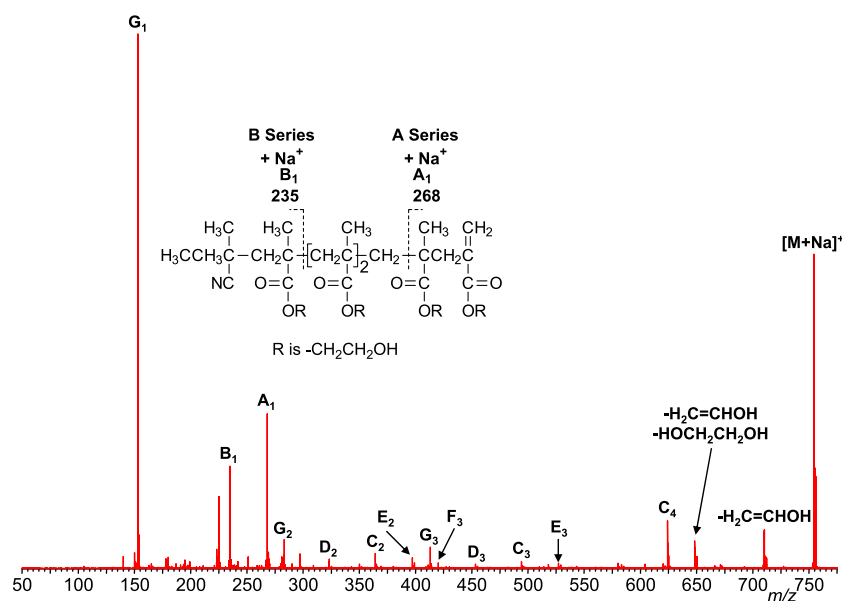


Fig. 6. ESI-MS/MS spectrum of sodiated tetramer of **4**, m/z 754 ($E_{\text{coll}} = 50$ eV). A schematic of the proposed fragmentation to form ions of the A_n and B_n series is also shown.

4. Conclusions

These results, along with data from other methacrylate polymers [23,24], show that MS/MS is a powerful methodology for fast end group determination from these systems. MS/MS typically also provides more definitive end group information for methacrylates

than can be inferred from using techniques such as MALDI-TOF. Characteristic intense fragment ion peaks at low mass-to-charge ratios in MS/MS spectra are primarily used to infer end group information from oligomers of poly(HEMA), as well as other methacrylate systems. Two series, as noted for other methacrylate polymers [23,24], of intense peaks in the spectra may be used to

infer information about the end groups for most samples. One exception is for a polymer made with dodecyl mercaptan as chain transfer agent, where only one dominant low mass-to-charge ratio series that is characteristic of the terminating end group was proposed to be observed, as noted for poly(styrene) with a similar α -end group functionality [31]. Peaks of lower intensity, however, in the ESI-MS/MS spectrum from this poly(HEMA) are indicative of the thio-ether functionality.

Additional peaks, resulting from losses of one or more side chains from the backbone of the polymer, are proposed to be characteristic of this polymer, with six-membered rings possibly generated from loss of segments of two adjacent units. The use of software, that aids the interpretation of the MS/MS spectra [28], was demonstrated for one of the poly(HEMA) samples.

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