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The incorporation of metal cations into polymer backbones: An important consideration in the interpretation of ESI-MS spectra

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ABSTRACT

Poly(acrylic acid) (polyAA), poly(*N*-isopropylacrylamide) (polyNIPAAm) and poly(diisopropyl vinyl phosphonate) (polyDISP) samples were subjected to electrospray ionisation-mass spectrometry (ESI-MS) analysis employing quadrupole-ion trap as well as high resolution quadrupole time-of-flight (Q-TOF) detectors, and the resulting spectra screened for the detection of polymers in which metal cations have become incorporated into the chain backbone following deprotonation reactions during the ESI process. Adducts formed from such polymer-metal salts were detected in spectra produced from each of the samples under investigation, indicating that the formation, ionisation and detection of polymer-metal salts can be readily achieved under typical ESI-MS experimental conditions. Specifically (under conditions of MeOH containing 0.1 mM sodium acetate) polyAA is most susceptible to the incorporation of metal ions (up to 3 replacements), followed by polyNIPAAm with two replacements and polyDISP with one replacement. The results obtained from the present investigation clearly demonstrate that for synthetic polymers capable of undergoing deprotonation under ESI conditions, the potential detection of polymer-metal salt adducts must be considered during spectral data interpretation to avoid erroneous species assignments.

1. Introduction

Numerous analytical techniques have been used to investigate the characteristic features of synthetic polymer samples; however, a characterisation of individual chains within a given sample using absolute molecular weight information is only available via high resolution mass spectrometry (MS). The ability for MS to gain access to such individual chain molecular weight data was markedly influenced by the 'soft' ionisation protocols developed in the mid to late 1980s: electrospray ionisation (ESI) [1] and matrix-assisted laser desorption/ionisation (MALDI),[2] coupled to variable mass analysers. These techniques readily allow intact polymer chains to be ionised into the gas phase and subjected to mass analysis. The continual refinement of these ionisation methodologies [3], along with the development of ever more sophisticated mass analyser technologies [4,5], has seen soft ionisation MS mature into one of the most potent methods available for the characterisation of synthetic polymers.

The data obtained from synthetic polymer samples using ESI- or MALDI-MS are frequently employed to gain insight into polymer formation processes [6–8], as well as to gain insight into the mechanisms of polymer degradation [6,8]. For these purposes, the characterisation of different end-group, molecular weight, molecular architecture, and/or copolymer distributions can be of vital importance. In order to maximise the amount of mechanistic information capable of being obtained using these techniques, it is generally favourable to identify as many of these different distributions as possible in mass spectra generated from a given polymer sample; however, various factors can serve to complicate and/or limit the identification of analyte [9] molecules. An example of one such factor can be found in the generation of unwanted ions during the acquisition of mass spectra. That is, if secondary means of ion identification such as tandem mass spectrometry (MS/MS) are not implemented, the assignment of certain peaks in a given mass spectrum may be considered virtually impossible if these peaks consist of isobaric signals due to the presence of unwanted ions,

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and if the instrument's mass resolving power is insufficient to allow for differentiation of these signals.

A potentially important but still poorly investigated means by which unwanted ions can be generated in ESI-MS derived spectra involves the incorporation of metal cations into polymer backbones. The presence of metal cations, which are introduced during ESI-MS sample preparation either as impurities or through the deliberate addition of metal salts, are often a necessity if polymer ions are to be detected in the resulting mass spectra. This is because the ionisation of polymer chains using ESI frequently proceeds via the formation of analyte-metal cation adducts [6]. If the polymer chains of interest act as acids in the solvent used to prepare the ESI sample solution, it is therefore possible that metal cations can become bound to the sites in which deprotonation has occurred on the polymer backbone, resulting in the formation of polymer-metal salts. Thus, depending upon the quantity of polymer-metal salt formed in the given ESI sample solution and the efficiency by which these compounds are ionised during the ESI process, it is possible that adducts generated from these materials may be detected in ESI derived mass spectra.

The formation of gas phase ions from polymer-metal cation salts from acrylic acid (AA) and methacrylic acid (MAA) derived polymers has been observed to be readily achieved via MALDI [10,11]; indeed, some attention has been devoted to minimising the detection of these compounds through the use of ion-exchange resins [12,13] or derivatization [10]. However, the impact of the generation, and potential ionisation and detection, of polymer-metal salts during ESI-MS remains poorly examined. Arnould et al. demonstrated that polymer-sodium salts generated from carboxylic acid end-group containing polyesters composed of isophthalic acid neopentyl glycol are readily detected using ESI, increasing the complexity of spectra obtained from these materials without providing additional structural information [14]. More recently, studies have indicated that polyMAA-metal cation salts are capable of being detected via ESI [15,16]. With the aim of further substantiating the importance of polymer-metal cation salt formation in ESI-MS analyses, the present contribution describes the ESI-MS analyses of samples of polyAA, poly(*N*-isopropylacrylamide) (polyNIPAAm) and poly(diisopropyl vinyl phosphonate) (polyDISP), all polymers which can be expected to undergo deprotonation to varying extents in the organic solvents typically employed in ESI-MS analyses, with an emphasis upon screening the resulting spectra for the detection of polymer-metal salts. Clearly the abundances in which metal cation salts are formed will be heavily influenced by the pK_a values of the polymers in varying ESI-MS sample solutions, and upon metal cation concentrations. The present communication does not aim to provide a systematic exploration of such experimental conditions (including solvent type); rather, the scope of the investigation is to demonstrate that the formation, ionisation and detection of polymer-metal salts can indeed be readily achieved under typical ESI-MS conditions, and hence that the detection of these materials constitutes an important consideration – to avoid the potential incorrect assignment of species – in the interpretation of ESI-MS derived spectra of synthetic polymers that carry labile protons.

2. Experimental part

2.1. Materials

Acrylic acid (AA, Aldrich, 99%) was distilled under vacuum prior to use, and *N*-isopropylacrylamide (NIPAAm, Aldrich, 99%) was purified by two recrystallisations in *n*-hexane. Diisopropyl vinyl phosphonate (DISP) was synthesised following the procedure described by Pike *et al.* [17] 2,2′-Azobis(isobutyronitrile) (AIBN, Fluka, 98%) was used as received. *S*,*S*-bis(α,α'-dimethyl-α''-acetic acid)trithiocarbonate (TRITT) was synthesised according to the method described by Lai *et al.* [18].

2.2. Polymerisation procedure

NIPAAm and AA polymerisations were initiated via γ-irradiation, with the symmetrical reversible addition fragmentation chain transfer (RAFT) agent TRITT employed as a controlling agent. NIPAAm (1.5 mol L^{-1}) or AA (2.5 mol L^{-1}) was dissolved with TRITT in pure water at a monomer/chain transfer agent (CTA) ratio of 200. After the complete dissolution of monomer and RAFT agent, the stock solutions were frozen, placed under vacuum and re-thawed (repeated 5–10 times) until the solutions were completely deoxygenated. Sealed glass sample vials containing the deoxygenated solutions were placed in an insulated room and exposed to a ^{60}Co γ-irradiation source at a dose rate of 0.13 kGy hr^{-1}. PolyDISP was synthesised by weighing DISP (1.0 mL, 5.0×10^{-3} mol) and initiator (AIBN, 0.5 mg, 3.0×10^{-3} mmol, 0.5 gL^{-1}) into a Schlenk flask, which was evacuated and backfilled with nitrogen prior to the polymerisation. The reaction mixture was heated to 60 °C for 12.5 h.

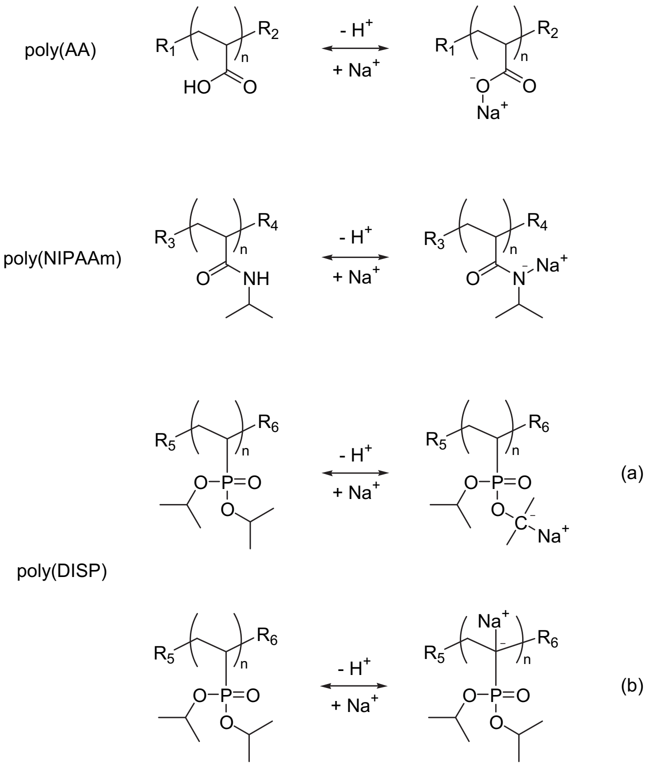
2.3. ESI-MS

ESI-ion trap mass spectra were recorded on a Thermo Finnigan LCQ Deca ion trap mass spectrometer (Thermo Finnigan, San Jose, CA). The instrument was equipped with an atmospheric pressure ionisation source operating in the nebuliser assisted electrospray mode and was used in positive ion mode. Mass calibration was performed using caffeine, methionine-argenine-phenylalanine-alanine (MRFA) and Ultramark 1621 (Aldrich) in the mass-to-charge ratio (*m/z*) range 195 – 1822 Da. All spectra were acquired within the *m/z* range of 150 – 2000 Da with spray voltages ranging from 3 to 5 kV, capillary voltages ranging from 25 to 45 V, a capillary temperature of 275 °C and a flow rate of 3.50 μL/min. Nitrogen was used as sheath gas (flow: 50% of maximum) and helium was used as auxiliary gas (flow: 5% of maximum).

ESI quadrupole time-of-flight (Q-TOF) data were acquired using an Ultima hybrid Q-TOF instrument (Micromass, Altrincham, Cheshire, UK) equipped with a ZSpray sample introduction system in a nanoflow electrospray ion source. Spectra were acquired using a positive ion mode with a source temperature of 200 °C. Mass calibration was performed using the fragment ions of Glu-fibrino peptide (amino acid sequence EGVNDNEEGFFSAR). Samples were delivered into the ion source from nanospray emitters (Proxeon, Odense, Denmark). Cone voltage off-sets ranged from 35 to 50 V, and capillary voltages ranged from 800 to 1200 V. All spectra were acquired via the TOF analyser and were integrated every 2.4 s over the *m/z* range 50 – 2000 Da. Data were recorded and processed using the MassLynx™ software, version 4.0.

For each of the three polymers under investigation, a solvent mixture of methanol (MeOH) with a sodium acetate concentration of 0.1 mM was used for ESI-MS sample preparation. For the polyDISP sample, additional solvent mixtures of MeOH with a potassium iodide concentration of 0.03 mM and MeOH with a lithium iodide concentration of 0.03 mM were also employed. For each solvent mixture, ca. 5 mg mL^{-1} of polymer sample was added.

In assigning chemical structures to the ions detected during the ESI-MS analyses, all theoretical monoisotopic molecular weights were calculated using the exact mass as provided by the software package CS ChemDraw Ultra® version 5.0 for the lightest isotope of the product of interest. Theoretical isotopic peak patterns were generated using the software package Xcalibur™ version 1.4.



Scheme 1. Possible deprotonation and sodium cation binding reactions upon dissolution of polyAA, polyNIPAAm or polyDISP in MeOH containing 0.1 mM sodium acetate. Note that two possible deprotonation pathways, labelled (a) and (b), are proposed for polyDISP.

3. Results and discussion

Scheme 1 illustrates likely sites by which deprotonation and sodium atom binding can occur when solutions of each of the three polymers under investigation are prepared in MeOH containing 0.1 mM sodium acetate. PolyAA is well known to undergo pH-responsive behaviour through its facile donation and acceptance of carboxylic acid group protons [19], and significant carboxylic acid deprotonation can be expected to occur in MeOH. To a lesser extent, deprotonation of the secondary amide functionalities of solutions of polyNIPAAm in MeOH can also be expected to occur. With regards to polyDISP, previous studies tentatively suggest that products formed from deprotonation and metal cation binding can be detected via ESI-MS in identical sample solutions to those employed in the present investigation [20]. If these reactions do indeed take place to a significant extent, a possible deprotonation site is represented by the methine functionalities of the repeat unit pendant groups as depicted in reaction (a) of Scheme 1. However – and possibly more likely and in analogy to the Horner-Wadsworth-Emmons (HWE) reaction – deprotonation may occur at the methylene group of the polymer backbone directly bonded to the phosphorous (reaction (b) of Scheme 1). This group features an acidic –CH, as the electron-withdrawing properties of the phosphonate ester (and an additional electron-withdrawing group in the HWE reaction) stabilise the carbanion.

Fig. 1 shows ESI-ion trap spectra obtained from the sample solutions of the three polymers in question. The dotted lines correspond to m/z ratios of sodium adducts formed from polymer chains in which sodium atoms have become bound to one, two and three deprotonation sites (labelled "+1 Na", "+2 Na" and "+3 Na", respectively); i.e., m/z ratios corresponding to equation (1):

$$m/z(+(n+1)\mathrm{M}) = m/z(+n\ \mathrm{M}) - m/z(\text{singly charged H}) + m/z(\text{singly charged M}) \qquad (1)$$

where n refers to the number of metal cations incorporated into the chain backbone and M refers to a metal atom (in this instance, sodium). From Fig. 1, it can be seen that peaks corresponding to polymer-sodium salts can be observed for each of the samples under investigation. For polyAA, these peaks correspond to chains in which up to three sodium cations have been incorporated into chain backbones; for polyNIPAAm, these peaks correspond to the incorporation of up to two sodium cations; and for polyDISP, only peaks corresponding to the incorporation of one sodium cation are observed. If only using the criterion of matching theoretical and experimentally observed m/z's, it can not be ruled out that several of these peaks can also arise from analyte ions (P_{HTZ} and P_{HTH}) potentially generated from the samples under study [20,21] (see Table 1 and footnote [22]). Though an analysis of the fragmentation pathways generated during collision induced dissociation (CID) in MS/MS product scans can be used to provide additional structural information for these ambiguously assigned peaks, the fragmentation of RAFT end-groups can be expected to dominate the CID of both P_{HTZ} and polymer-sodium salt ions (see footnote [22]), making differentiation of these ions difficult. Additionally, though the analysis of experimentally observed isotopic peak patterns can also provide insight into the structural composition of ions, the distortion of isotopic peak patterns via relatively high noise levels makes such an analysis difficult in the polyAA derived spectrum. It can therefore be seen that if polymer-sodium salts are indeed being detected in the given ESI-ion trap derived spectra, potential exists for the incorrect assignment of ions. Thus, to help confirm that these peaks do indeed arise from polymer-sodium salts and not analyte ions, alternate metal cation doping studies were performed and ESI-Q-TOF spectra were obtained. These experiments are described below.

In confirming the detection of polymer-metal salts, additional mass spectra were acquired from the polyDISP sample using dopant cations other than Na^+; namely K^+ and Li^+. The mass spectra obtained with the various dopant cations are shown in Fig. 2. In these spectra, peaks confidently assigned to adducts formed from analyte molecules are marked with vertical dashed lines (black dashed lines for sodium adducts, and grey dashed lines for hydrogen adducts). A detailed description of these ion assignments in these spectra can be found in our previous contribution [20]. For each of the sodium adduct peaks, an equivalent potassium adduct peak should arise at a m/z 16 Da higher in the K^+ doped sample, and an equivalent lithium adduct peak should arise at a m/z 16 Da lower in the Li^+ doped sample. Such additional peaks are indeed observed, and are henceforth referred to as parent peaks. Importantly, if polymer salts are indeed formed from the cations present within the ESI-MS sample solutions, adducts formed from these salts should be observed at m/z's relating to these parent peaks according to equation (1), with M referring to sodium, potassium or lithium.

Under each of the parent peaks observed in Fig. 2, a horizontal bar is illustrated to demonstrate the expected location of peaks arising from polymer salts according to the relation described in Eq. (1); blue lines for lithium salts, red lines for potassium salts, and black/grey lines for sodium salts. For every parent peak observed at a sizeable relative abundance it can be seen that corresponding peaks arising from the expected polymer-metal salts are indeed observed, lending substantial support to the hypothesis that polymer-metal salts are indeed being detected in the ESI-ion trap derived spectra.

To further confirm the detection of polymer-metal salts, ESI-Q-TOF spectra were produced from the Na^+ doped polymer samples.

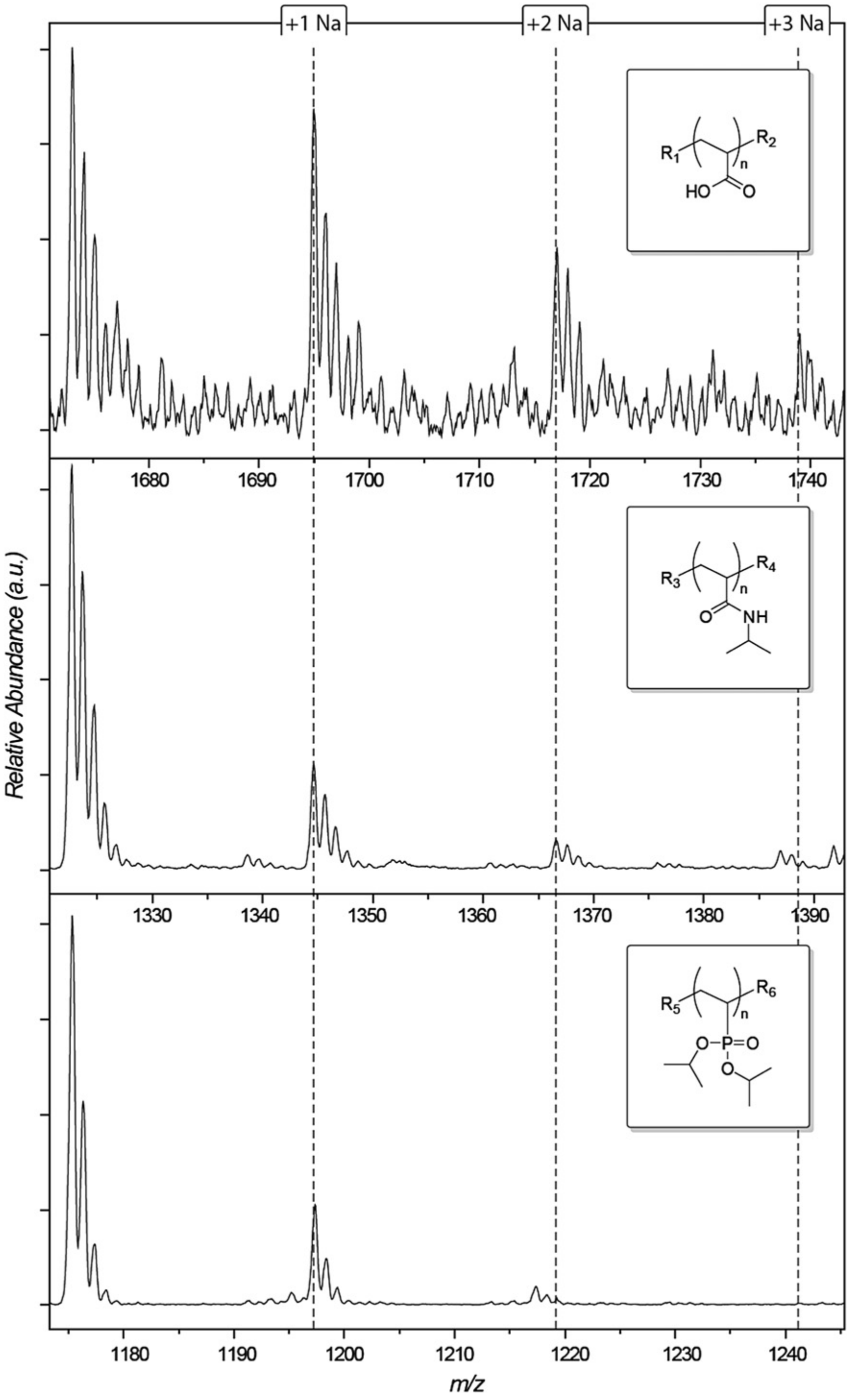


Fig. 1. ESI-ion trap spectra obtained from samples of polyAA, polyNIPAAm and polyDISP in MeOH containing 0.1 mM sodium acetate. The dotted lines represent *m/z* ratios corresponding to sodium adducts formed from polymer chains in which sodium atoms have become bound to one (+1 Na), two (+2 Na) and three (+3 Na) deprotonation sites.

For spectra generated employing the ESI-Q-TOF instrument, an ion assignment was only considered for a given peak if its apparent mass accuracy deviation was less than 50 ppm; this is contrasted with a maximum mass accuracy deviation of 300 ppm for the ESI-ion trap instrument [23]. In each of the ESI-Q-TOF derived spectra, peaks associated with polymer-sodium salts featuring one sodium cation incorporated into the chain backbone can be identified. Importantly, analyte ions capable of being assigned to the ESI-ion trap derived spectra are ruled out as potential ion assignments in the ESI-Q-TOF derived spectra, suggesting that the peaks of interest in both the ESI-ion trap and ESI-Q-TOF spectra arise predominantly, if not entirely, due to adducts formed from polymer-sodium salts (see Table 2).

From the above experimental data it can be confidently concluded that polyAA, polyNIPAAm and polyDISP-metal salts are readily capable of being generated, ionised and detected in ESI-MS derived mass spectra. With regards to the efficiency of the ionisation of these compounds it is noteworthy that the relative abundances of the peaks observed for the polymer-metal salt adducts decrease as the apparent degree of sodium atom incorporation increases (see Fig. 1). This qualitatively reflects the expected abundances in which the various polymer salts in question are

Table 1
Ion assignments for the peaks labelled in the ESI-ion trap derived spectra shown in Fig. 1. #NaP$_{RAFT}$ refer to sodium salts generated from RAFT mediated dormant chains initiated via an R-group radical, where # represents the number of sodium atoms incorporated into the chain backbone. NaP$_{MM}$ refers to a sodium salt generated from the dominant species expected from a DISP free radical polymerisation [20], in which a single sodium atom has been incorporated into the chain backbone. Details of the listed analyte compounds, P$_{HTZ}$ and P$_{HTH}$, are described in footnote [22].

Peak label	Ion assignment	$m/z_{theo.}$	$m/z_{exp.}$	Mass accuracy (ppm)
PolyAA +1 Na	[NaP$_{RAFT}$ + Na]$^+$	1695.38	1694.93	265
	[P$_{HTZ}$ + Na]$^+$	1695.28		206
PolyAA +2 Na	[2NaP$_{RAFT}$ + Na]$^+$	1717.36	1717.00	210
	[P$_{HTH}$ + Na]$^+$	1717.39		227
PolyAA +3 Na	[3NaP$_{RAFT}$ + Na]$^+$	1739.35	1739.00	201
PolyNIPAAm +1 Na	[NaP$_{RAFT}$ + Na]$^+$	1344.73	1344.53	149
	[P$_{HTZ}$ + Na]$^+$	1344.63		74
PolyNIPAAm +2 Na	[2NaP$_{RAFT}$ + Na]$^+$	1366.72	1366.33	285
PolyDISP +1 Na	[NaP$_{MM}$ + Na]$^+$	1197.52	1197.33	159

Table 2
Ion assignments derived from ESI-Q-TOF data obtained from the polyAA, polyNIPAAm and polyDISP samples under investigation. Peaks analogous to those labelled in Fig. 1 are named following the same conventions. Details of the naming conventions for the listed ions are presented in the caption for Table 1. Analyte ions that are capable of being assigned to the ESI-ion trap derived spectra but are ruled out as potential ion assignments in the ESI-Q-TOF derived spectra have been marked with a strike-through.

Peak label	Ion assignment	$m/z_{theo.}$	$m/z_{exp.}$	Mass accuracy (ppm)
PolyAA +1 Na	[NaP$_{RAFT}$ + Na]$^+$	1191.21	1191.27	50
	~~[P$_{HTZ}$ + Na]~~$^+$	1191.13		118
PolyNIPAAm +1 Na	[NaP$_{RAFT}$ + Na]$^+$	666.23	666.23	<1
	~~[P$_{HTZ}$ + Na]~~$^+$	666.13		150
PolyDISP +1 Na	[NaP$_{MM}$ + Na]$^+$	1005.43	1005.42	10

formed, indicating that the ionisation efficiencies of these compounds are not dramatically increased by their metal atom content. In addition, the abovementioned results substantiate the premise that the potential detection of polymer-metal salts must be considered when assigning analyte ions to spectra derived from polymers capable of undergoing deprotonation during ESI sample preparation. Indeed, in light of these observations, certain ESI-MS derived signals assigned to analyte ions in our previous studies of polyAA and polyNIPAAm [21] may be re-interpreted as arising (at least in part) from polymer-metal salts. Such a re-interpretation of data inevitably impacts upon the mechanistic conclusions drawn from these studies, i.e. with regards to the reaction pathways described in Scheme 8 of reference [21], where the likelihood that pathways (1a, initiation by homolytic RAFT agent cleavage) and (1b, radiolysis of water) are influential is reduced.

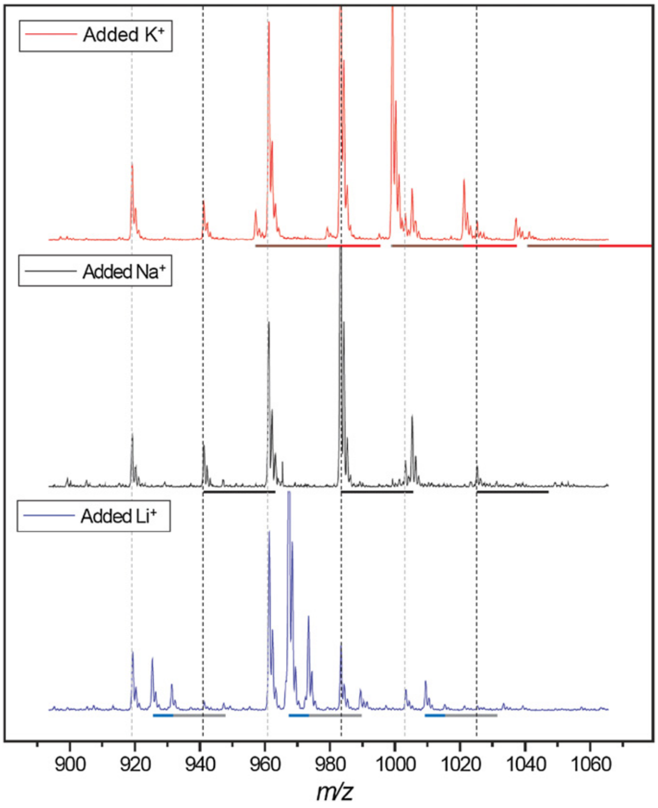


Fig. 2. ESI-ion trap derived spectra generated from a sample of polyDISP, obtained using sample solutions of MeOH containing 0.1 mM sodium acetate (middle), 0.03 mM potassium iodide (above) or 0.03 mM lithium iodide (below). Black vertical dashed lines correspond to m/z's associated with sodium adducts formed from analyte molecules, and grey vertical dashed lines correspond to hydrogen adducts formed from analyte molecules. Peaks arising from suspected polymer-metal salts are located with grey/black bars (sodium salts), red bars (potassium salts) and blue bars (lithium salts).

4. Conclusions

The detection of polymer-metal salts, generated via the incorporation of metal cations into deprotonation sites formed on polymer backbones during ESI-MS sample preparation, was demonstrated in mass spectra produced from polyAA, polyNIPAAm and polyDISP using a positive ion mode. These results indicate that the formation, ionisation and detection of polymer-metal salts can be readily achieved under typical ESI-MS experimental conditions, and must be considered in the interpretation of mass spectra of synthetic polymers capable of undergoing deprotonation under typical conditions of ESI to avoid potentially incorrect ion assignments.

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[22] The compounds represented as P_{HTZ} and P_{HTH} describe analyte molecules potentially formed during the AA and NIPAAm polymerisations, in which proton (H) and/or dormant RAFT (Z) end-groups are generated, and trithioester moieties (T) are present within the chain backbone. The potential generation of trithioester moieties within chain backbones stems from the symmetrical nature of the employed RAFT agent, in which two identical R-group functionalities are capable of fragmenting off the CTA during the RAFT process.

[23] Maximum allowable mass accuracy values were estimated from the *m/z*'s observed for dormant R-group radical initiated chains over different mass spectra produced from RAFT mediated polymer samples; these chains are established as the dominant products produced in the majority of RAFT mediated polymerisations, hence can be assigned to the generated mass spectra with a significant degree of confidence.