Discrimination between charge-catalyzed and charge-independent fragmentation processes of cationized poly(n-butyl acrylate).

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Discrimination between Charge-Catalyzed and Charge-Independent Fragmentation Processes of Cationized Poly (*n*-Butyl Acrylate)

Junkan Song[1], Antony Memboeuf[2,3], Ron Heeren[4], Károly Vékey[2], *Oscar F. van den Brink[1],

1 Research, Development and Innovation, AkzoNobel, Deventer, The Netherlands,

2 Chemical Research Center, Hungarian Academy of Sciences, H-2515 Budapest, P.O.Box 17, Hungary

3 Université de Brest, UMR CNRS 6521, Laboratoire CEMCA, 6Av. Le Gorgeu, 29238 Brest Cedex 3, France.

4 FOM Institute of Atomic and Molecular Physics, Amsterdam, The Netherlands

*corresponding authors

Oscar F. van den Brink, oscar.vandenbrink@akzonobel.com

Dear Sir,

   When performing structural investigation of macromolecular systems using tandem mass spectrometry, an important matter is the choice of the cation and how that does affect the tandem mass spectra. Moreover, when studying mechanistic aspects, the role of the adduct ions in the mechanism of the fragmentation processes is often discussed: Is the process charge-induced (more accurately described as charge-catalyzed) or charge-remote (more accurately described as charge-independent). In a previous study[1], we shed light on the catalytic effect of lithium in the case of cationized poly(ethylene glycols) (PEGs). The activation energy of the main fragmentation process for the trimer was shown, by means of Density Functional Theory calculations, to decrease by as much as 0.3 eV in the presence of lithium.
   To further sketch our picture of the role of cation adducts on the fragmentation of even electron ions, we present in this communication the results obtained on the fragmentation of poly (*n*-butyl acrylate), PBA, with different alkali ions and various degrees of polymerization. Thus, we present a method to clarify the role of the charge on the fragmentation processes.
   The experiments were performed on a Bruker Esquire 3000plus ion trap mass spectrometer with PBA from 4 up to 12 monomeric units (polymerization degree varies with different cations). The experimental setting is identical to the one described in the previous study[2] and the structure of PBA selected can be found in the supporting information. PBA oligomers with positively charged alkali ion

adducts including Li^+, Na^+, K^+ or Cs^+ were studied in the system. The main fragmentation processes observed are the loss of 56 Da and 130 Da in the cases of lithium and sodium adducts. Using high resolution and high accuracy mass spectrometry, the elemental compositions of these losses were identified to be C_4H_8 and $C_8H_{16}O$. Backbone cleavage was not observed for PBA; all neutral losses originate from side chain cleavages. The PBA used here (i.e. with a butylmethyl carboxylate and a hydrogen end group) was specifically selected for this work in order to avoid any end group fragmentation.

When potassium and caesium were used for adduct formation, no significant fragmentation was observed. This is most probably due to cation detachment, which was shown, e.g. in the case of cationized PEGs, to become preferential as the size of the cation increases[3]. This observation supports previous studies claiming the value of low mass cations for structural investigation of oligomers using tandem MS: stable complexes are formed with Li^+ and Na^+ cations that enable the macromolecule to dissociate into various fragments carrying specific structural information.

To study the energetics of polymer fragmentation, the survival yield (SY)[2, 4-7] is a convenient quantitative measure. It is defined according to Eq.1:

$$SY = \frac{I_M}{I_M + \sum I_F} \qquad (Eq.1)$$

in which I_M is the intensity of the cationized molecule and $\sum I_F$ is the sum of all product ion intensities. The survival yield curve is typically of the sigmoid-type (continuously decreasing from 1, at low energy, to 0 at high energy). Similarly the intensity of the individual product ions can be used as numerator in Eq1 and the corresponding curves can then be plotted. This way, a breakdown diagram is obtained. These diagrams were plotted in the case of lithium and sodium cationized PBA with 10 monomeric units and are shown in Figure 1. Only two of the most intense fragmentation processes (i.e. neutral loss of 56 and loss of 130 Da) are shown here for the sake of clarity and as they represent altogether between 80-90% of the product ions observed. An example of the spectra underlying the data presented in Figure 1 is shown in the supporting information. In the case of the lithium adduct, the two curves of the product ions are running closer and more parallel to each other, while they are much more separated in the case of the sodium adduct. Hence, the relative importance of the respective fragmentation pathways (loss of 56 and 130Da) changes significantly with the adduct cations[8].

Figure 1. Breakdown diagrams of (a) lithium and (b) sodium cationized poly (*n*-butyl acrylate) with 10 monomeric units.

To further investigate this effect, the relative intensities of the two reaction channels were compared as a function of polymer size. For a given oligomer the ratio changes significantly with collision energy, as shown in Figure. 1. At high energy the abundance ratio of the two fragmentation process becomes fairly constant; so this was measured corresponding to ca. 95% fragmentation (SY=0.05). The

abundance ratio measured for the two processes, -56/-130, was measured as a function of molecular size, and this is shown in Figure. 2; both for lithium and sodium cationized polymers. The figure shows that the relative intensities of the two main fragmentation processes are approximately independent of the degree of polymerization; especially for hexamers and above. The asymptotic values calculated are 1.27 ± 0.11 for PBA oligomers with Li^+ adduct and 1.99 ± 0.12 with Na^+ adduct. (The method of error estimation is presented in supporting information).
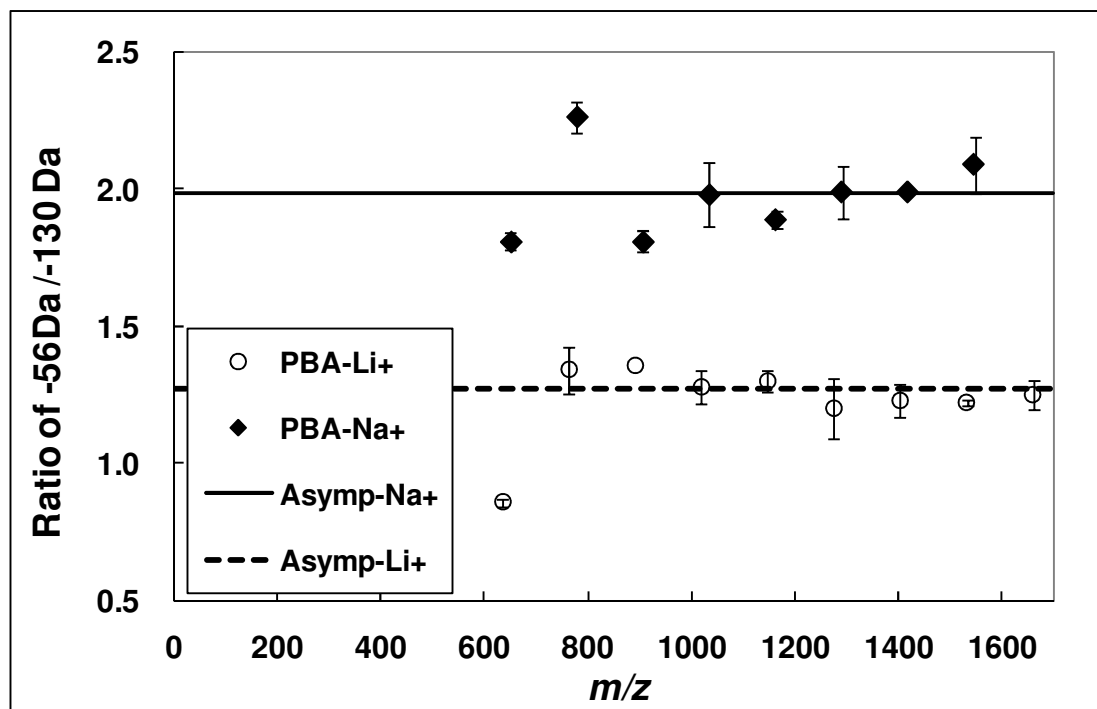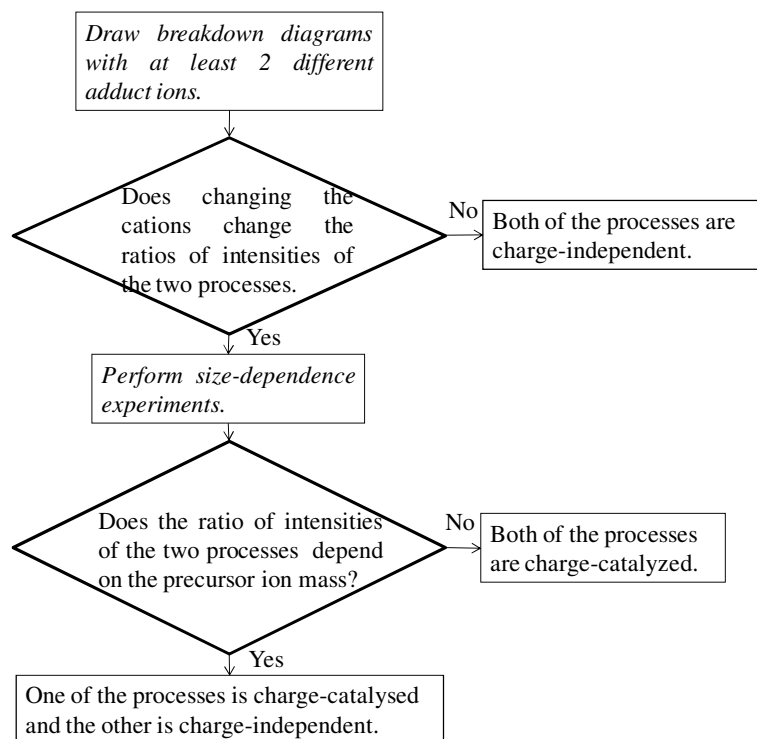

Figure 2. Ratios of signal intensities for the two main fragmentation processes (losses of 56 Da / 130 Da) observed at 95-100% fragmentation of the precursor ion, plotted versus *m/z* for lithium and sodium cationized PBAs. Full lines are asymptotic values obtained after averaging the last 8 values for Li^+ and the last 6 values for Na^+.

Figure 1 shows that the breakdown curve changes when the cation is changed from Li^+ to Na^+. This clearly suggests that at least one of the two (major) fragmentation processes is charge-catalyzed. There are two possibilities; only one of the processes is charge-catalyzed (and the other is charge independent) or both processes are charge-catalyzed. As mentioned above, both fragmentation channels involve side chain losses. If the process is charge-catalyzed, there is only one possible reaction channel in the cationized molecule (i.e. that close to the charged site). If the process is independent of the charge; the number of possible reaction channels will increase with the degree of polymerization (possibly linearly with polymerization degree). As a consequence, the abundance of charge independent processes should increase with molecular size. The abundance of a charge-catalyzed process, however, will not be affected by varying the molecular size. Therefore, under conditions of similar degrees of fragmentation, a combination of a charge-catalyzed and a charge-independent process will lead to a change in the intensity ratio of product ions when the mass of the precursor ions is changed. If both processes are charge-catalyzed the intensity ratio will not be affected on change of the precursor ion's mass. Figure 2 clearly demonstrates that the ratio of the two processes reaches a constant value at n=6. Therefore, both of the fragmentation processes, *viz.* the loss of 56 Da and the loss of 130 Da, are charge-catalyzed.

The flow chart shown in Scheme 1 can be used to formalise the above reasoning in a procedure for determining whether processes are charge-catalyzed or charge-independent. The validity of this procedure is, at this stage, limited to polymers with a periodic structural pattern that show two (main)

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fragmentation products. There are two critical steps in the procedure: The first one is drawing breakdown diagrams with different cations, which reveals whether there is at least one charge-catalyzed fragmentation process. The second one is performing a size-dependence analysis, which provides information about the similarity of the nature of the fragmentation processes.

```
┌─────────────────────────┐
│ Draw breakdown diagrams │
│ with at least 2 different│
│ adduct ions.            │
└─────────────────────────┘
            │
            ▼
        ╱────────────╲
       ╱ Does changing╲         No    ┌──────────────────────┐
      ╱  the cations    ╲──────────────│ Both of the processes│
      ╲  change the     ╱              │ are charge-independent.│
       ╲ ratios of     ╱               └──────────────────────┘
        ╲intensities  ╱
         ╲of the two ╱
          ╲processes╱
            │ Yes
            ▼
┌─────────────────────────┐
│ Perform size-dependence │
│ experiments.            │
└─────────────────────────┘
            │
            ▼
        ╱────────────╲
       ╱ Does the ratio╲        No    ┌──────────────────────┐
      ╱  of intensities  ╲─────────────│ Both of the processes│
      ╲  of the two       ╱            │ are charge-catalyzed.│
       ╲ processes depend╱             └──────────────────────┘
        ╲on the precursor╱
         ╲ ion mass?    ╱
            │ Yes
            ▼
┌──────────────────────────────┐
│ One of the processes is      │
│ charge-catalysed and the     │
│ other is charge-independent. │
└──────────────────────────────┘
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Scheme 1. Determination of the cation influence on two fragmentation processes (charge-catalyzed/independent).

This paper demonstrated that changing the size of the adduct ions can be used to increase the degree of fragmentation of polymers in tandem mass spectrometry. In fact similar to the case of PEG oligomers, tandem MS spectra of PBA oligomers ionised with Li^+ and Na^+ provide more structural information than those of PBA oligomers ionised with larger cations[3]. Although the cation doesn't qualitatively change the fragmentation pattern (the same product ions are observed), the nature of the cation changes the relative abundance of the respective fragmentation processes. This illustrates well the catalytic effect the cation can exert on the fragmentation processes observed[9]. Moreover, we have also shown that performing a simple size-dependence analysis using breakdown diagrams with at least two different cations, the catalytic influence of the cation on the fragmentation processes observed can be clearly demonstrated. We have also delineated a simple procedure for determination on the presence of an effect of the cation on the fragmentation (charge-catalyzed or charge-independent). This is important information for investigations by means of quantum chemical modelling. Although the MS/MS spectra obtained for PBA on ion trap instrument are rather simple (only two product ion peaks are observed), this procedure should also be generally applicable to homopolymers (or alternating copolymers). The limitation is however that there should be more than one product ion. The applicability of the method to tandem MS experiments that produce more than two different product ions should be investigated. A particularly interesting subject of research would be fragmentations in which the backbone of the polymer is cleaved in two ways, producing a multitude of product ions that can be grouped as two types.
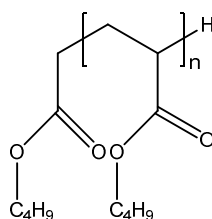
Acknowledgements

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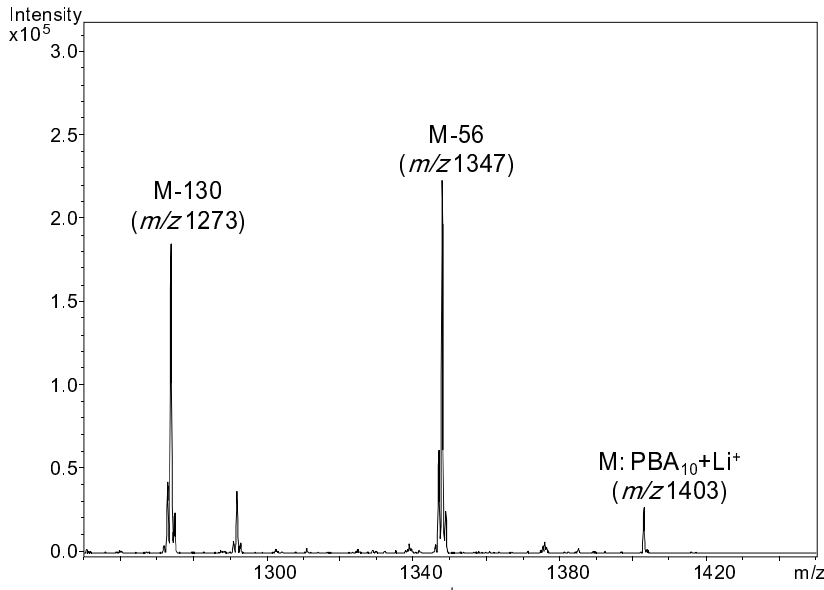
Supporting Information

The PBA sample was obtained from AkzoNobel Car Refinishes, Sassenheim, The Netherlands. It was prepared by radical polymerization using tert-butyl peroxy-3,5,5-trimethylhexanoate as initiator at 140 $^{\circ}$C in xylene. The number average molecular weight is 2,800 and the weight average molecular weight is 5,510. These values were measured by gel permeation chromatography (GPC) calibrated with polystyrene standards. The structure selected for this study is shown in Scheme S1.
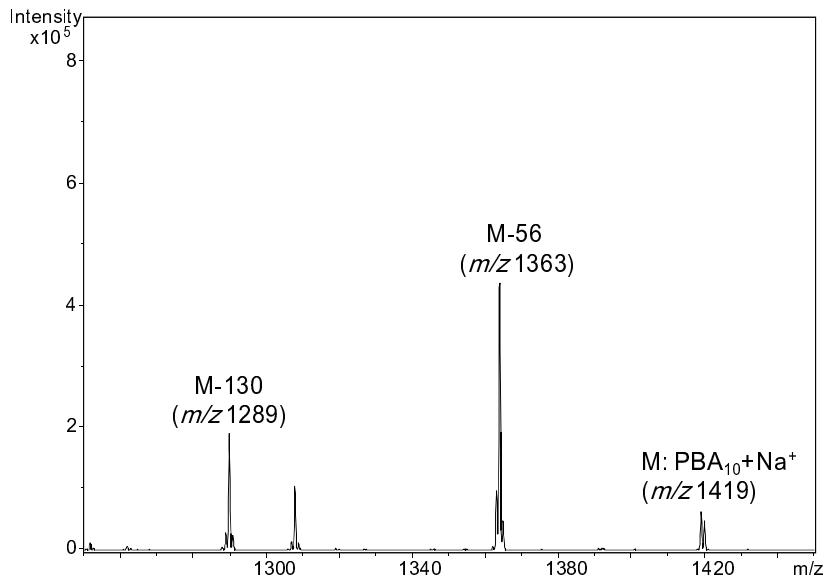

S1. The PBA selected for the present study.

Two examples of tandem MS/MS spectra underlying the data presented in Figure 1 are shown in S2 and S3.

S2. MS/MS spectrum of m/z 1403 (PBA$_{10}$+Li$^+$) at excitation voltage of 0.64 V

S3. MS/MS spectrum of m/z 1419 (PBA$_{10}$+Na$^+$) at excitation voltage of 0.64 V

The error bar value s presented in Figure 2 is the standard deviation of the ratio of the -56 and -130 product ion peaks. It is calculated according to SEq.1.

$$s = \frac{\overline{I(-56)}}{I(-130)} \sqrt{(\frac{s_{I(-56)}}{\overline{I(-56)}})^2 + (\frac{s_{I(-130)}}{\overline{I(-130)}})^2} \qquad \text{(SEq.1.)}$$

where $\overline{I(-x)}$ is the mean value of the intensities of the product ion peak (-x), $s_{I(-x)}$ corresponds to the standard deviation of the ion peak (-x) intensities.