

On the use of breakdown graphs combined with energy-dependent mass spectrometry to provide a complete picture of fragmentation processes

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Both breakdown graphs and energy-dependent (ED) maps provide a means of visually representing the fragmentation processes resulting from collision-induced dissociation at different energies. The two presentation methods may be combined for a complete picture of the appearance, disappearance and maximum abundance collision potentials of each of the ions involved in the fragmentation process. A demonstration of this approach is presented using two very different classes of compound, an amino acid and a transition metal carbonyl cluster. Copyright © 2002 John Wiley & Sons, Ltd.

Electrospray ionisation mass spectrometry (ESI-MS) is an enormously versatile analytical technique, allowing the collection of molecular weight data on a huge range of compounds hitherto inaccessible to mass spectrometry.¹ Modern electrospray mass spectrometers also incorporate means of fragmenting ions produced in the ESI process, usually by collision-induced dissociation (CID)² either at the skimmer cone, in a collision cell or inside an ion trap or FTICR cell.³ The extent of CID can be smoothly varied depending on the degree of fragmentation required, and the resulting ions provide useful structural information. A huge amount of data may be gathered in this way, and we have recently introduced the technique of energy-dependent electrospray ionisation mass spectrometry (EDESI-MS) as a convenient means of presenting this data.⁴ It essentially involves a 2D projection (collision energy vs. m/z) of a 3D surface (the 3rd dimension, ion intensity, is on the axis perpendicular to the paper). The major advantages of this method are concise illustration of the data from up to 200 spectra and easy visualisation of the fragmentation sequence. EDESI-MS is a comprehensive solution to the problem of data presentation compared with the traditional approach of a stacked series of selected spectra.⁵ We have recently extended the technique to laser-desorption/ionisation mass spectrometry (EDLDI-MS),⁶ and, in the MS/MS mode, the 'energy-dependent' method of data presentation is equally applicable to *any* ionisation technique.

Breakdown graphs (a plot of percentage fragment ion abundance vs. collision energy or cone voltage) have been

used by Harrison and others to obtain energy-resolved fragmentation data from CID experiments at the skimmer cone or in the collision cell of electrospray ionisation mass spectrometers.⁷ These breakdown graphs represent an alternative way of depicting the same data presented in an EDESI map, and may be incorporated into the EDESI-MS presentation in a logical way by including a breakdown graph on the right-hand side of the map. This approach results in a degree of redundancy, as the data illustrated on the map is projected both onto the mass axis (the summation spectrum at the top of the map) and to the cone (or collision) voltage axis (the breakdown graph at the side of the map). Nonetheless, it does provide an aid to interpretation in much the same way as the standard spectra that are typically projected onto two axes of a 2D NMR spectrum.⁸ Furthermore, there is potential in using the data to calibrate different instruments.

EXPERIMENTAL

L-Cysteine was purchased from Aldrich and used as supplied. Os₇(CO)₂₁ was prepared and purified according to the literature procedure,⁹ and derivatised *in situ* with NaOMe to generate the anionic methoxycarbonyl cluster [Os₇(CO)₂₀(COOMe)]⁻. Mass spectra collected using the Micromass Q-TOF used the following settings. The nebuliser tip was set at 3000 V and 80 °C, and nitrogen was used as the bath gas. Samples were introduced directly into the source at 10 µL/min via a syringe pump. Data collection was carried out in continuum mode. For the EDESI mass spectrum, the cone voltage was initially set at 0 V. A scan time of 1 s/spectrum was used. The cone voltage was increased by increments of 1 V every 2 s up to a maximum of 200 V,

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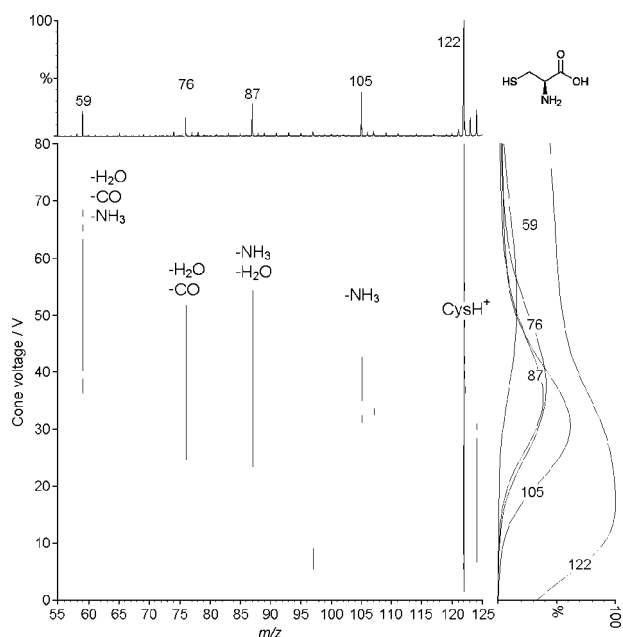


Figure 1. EDESI mass spectrum generated from 81 positive-ion ESI-MS spectra of $[\text{CysH}]^+$ at cone voltage settings of 0–80 V. The top trace is a 1D spectrum generated by combining all 81 spectra together. The side trace is a breakdown graph of all the major ions in the spectrum.

allowing a full scan from 0–200 V to be collected in 7 min. The EDESI-MS/MS spectra were collected in a similar fashion, but by adjusting the collision voltage in the argon-filled collision cell rather than by altering the cone voltage. The collision voltage was increased by increments of 0.1 V/s from 4 V up to a maximum of 40 V. The full scan therefore took approximately 6 min to collect.

Mass spectra collected using the Micromass Quattro LC used the following settings. The nebuliser tip was set at 3100 V and 100 °C, and nitrogen was used as the bath gas. Samples were introduced directly into the source at 8 $\mu\text{L}/\text{min}$ via a syringe pump. Data collection was carried out in continuum mode. For the EDESI mass spectra, the cone voltage was initially set at 0 V. A scan time of 8 s/spectrum and a low resolution setting (peak width at half-height ~ 0.8 Da) was used to maximise the signal-to-noise ratio. The cone voltage was increased by increments of 1 V after every scan up to a maximum of 200 V. A full scan from 0–200 V therefore took approximately 25 min to collect. The EDESI-MS/MS spectra were collected in a similar fashion, but by adjusting the collision voltage in the argon-filled collision cell rather than by altering the cone voltage.

RESULTS AND DISCUSSION

Protonated amino acids, $[\text{AAH}]^+$, have been extensively studied by mass spectrometry using a wide variety of ionisation techniques, although it is fairly recently that the fragmentation mechanisms of electrospray-generated $[\text{AAH}]^+$ ions have been examined in detail.¹⁰ As a member of a well-understood system of compounds, an amino acid seemed ideal as a candidate for demonstrating the utility of

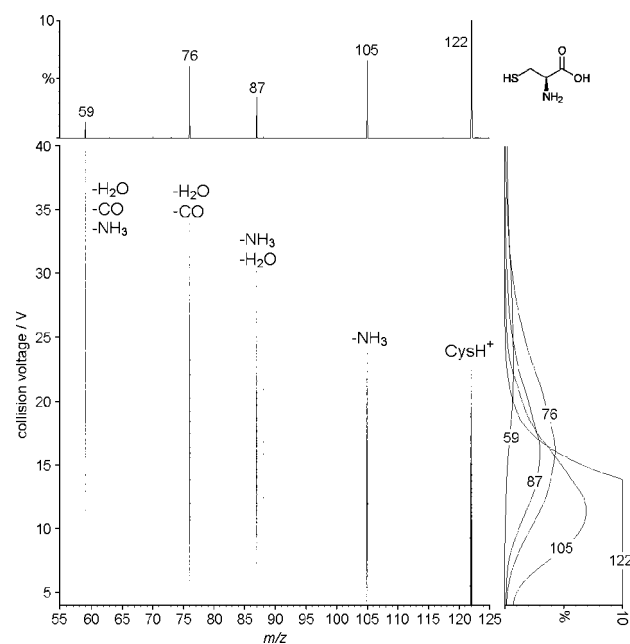


Figure 2. EDESI-MS/MS generated from 360 positive-ion product-ion spectra of $[\text{CysH}]^+$ at collision voltage settings of 4–40 V (increments of 0.1 V). The top trace is a 1D spectrum generated by combining all 360 spectra together. The side trace is a breakdown graph of all the major ions in the spectrum.

the combined EDESI/breakdown graph presentation. Cysteine, (*R*)-(+)- $\text{HSCH}_2\text{CH}(\text{NH}_2)\text{COOH}$ (Cys), provides a satisfactory number of intense fragment ions and was selected for analysis. Figures 1 and 2 show the EDESI-MS and EDESI-MS/MS spectra of $[\text{CysH}]^+$, respectively. The spectra were collected using a quadrupole-TOF instrument, which has the advantage not only of higher resolution than a triple quadrupole, but it also collects spectra much faster and so the entire map can be collected in a matter of a few minutes.

The spectra are in broad agreement with the breakdown graphs previously published; slight differences exist in the relative intensities of the various fragments. The fragmentation of $[\text{CysH}]^+$ (m/z 122) proceeds by loss of neutral molecules: $[\text{CysH-NH}_3]^+$ (m/z 105), $[\text{CysH-NH}_3\text{-H}_2\text{O}]^+$ (m/z 87), $[\text{CysH-H}_2\text{O-CO}]^+$ (m/z 76) and $[\text{CysH-NH}_3\text{-CO-H}_2\text{O}]^+$ (m/z 59). The intensity and range of collision potentials over which each of these fragment ions is present at significant abundance is depicted on the EDESI map and projected on to the y axis in the form of a breakdown graph. The total intensity of each ion is also summed and represented on the x axis, just as in the conventional EDESI presentation. The spectra collected are practically identical to those collected on a triple quadrupole instrument.

It has been reported that cone voltage and quadrupole cell CID produce breakdown graphs that are very comparable,¹¹ and the results here concur with this analysis. The similarities are perhaps most obvious when comparing the two maps rather than between the breakdown graphs, but the latter serve the useful function of better quantifying the intensities of the crosspeaks ('crosspeaks' referring to the

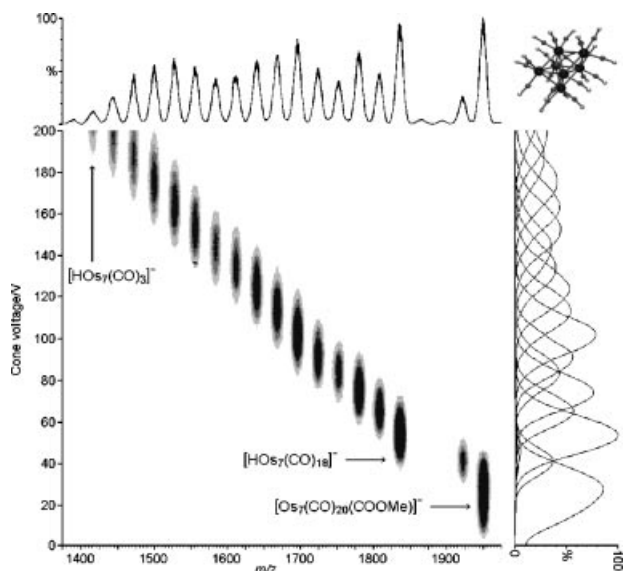


Figure 3. EDESI mass spectrum generated from 201 negative-ion ESI-MS spectra of $[\text{Os}_7(\text{CO})_{20}(\text{COOMe})]^-$ at cone voltage settings of 0–200 V. The top trace is a 1D spectrum generated by combining all 201 spectra together. The side trace is a breakdown graph of all the major ions in the spectrum.

regions of ion intensity in the 2D map). The MS/MS spectrum possesses the advantage of being cleaner and, as such, easier to interpret.

ESI-MS has become a recognised characterisation technique in inorganic and organometallic chemistry, and several reviews are available.¹² As a further demonstration of the combined EDESI/breakdown graph approach, we present the full analysis of the anionic transition metal cluster $[\text{Os}_7(\text{CO})_{20}(\text{COOMe})]^-$ in Figs 3 and 4 (data were collected in this case on a triple quadrupole instrument, a Micromass Quattro LC). The cluster was generated *in situ* by chemical derivatisation of the neutral metal carbonyl cluster $\text{Os}_7(\text{CO})_{21}$, using sodium methoxide as the reagent. This is a standard method of producing $[\text{M}_n(\text{CO})_{m-1}(\text{COOMe})]^-$ ions from $\text{M}_n(\text{CO})_m$ precursors.¹³

The progressive loss of CO from the cluster produces a series of crosspeaks stretching across the full cone voltage range. This pattern is typical of most of the anionic carbonyl clusters we have studied,¹⁴ exceptions only occurring where there is the possibility of structural rearrangement of the core¹⁵ or ligands.¹⁶ Early in the fragmentation sequence, loss of formaldehyde (HCHO) occurs, a phenomenon we have reported elsewhere. MS/MS assists in locating this 30-Da loss (cf. 28 Da for CO), as it eliminates the ambiguity arising from overlap of the very broad isotopomer envelopes. The rearrangement of the ions causes a marked discontinuity in the pattern of CO loss. The first crosspeak (bottom right-hand corner of the map) corresponds to the precursor ion, $[\text{Os}_7(\text{CO})_{20}(\text{COOMe})]^-$, at m/z 1951. The first fragment ion, at m/z 1923, is of appreciably lower intensity, and is the CO loss ion $[\text{Os}_7(\text{CO})_{19}(\text{COOMe})]^-$. The next two fragment ions are of very low intensity (less than 5% of the base peak), and isotope pattern matching shows clearly that both are also CO loss fragments, $[\text{Os}_7(\text{CO})_{18}(\text{COOMe})]^-$ and

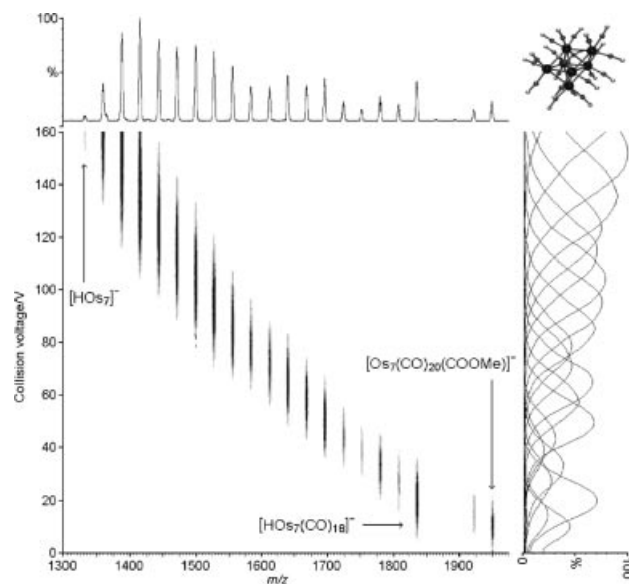


Figure 4. EDESI-MS/MS spectra generated from 161 negative-ion product-ion spectra of $[\text{Os}_7(\text{CO})_{20}(\text{COOMe})]^-$ at collision voltage settings of 0–160 V. The top trace is a 1D spectrum generated by combining all 161 spectra together. The side trace is a breakdown graph of all the major ions in the spectrum.

$[\text{Os}_7(\text{CO})_{17}(\text{COOMe})]^-$. The next crosspeak, at m/z 1837, is of much greater intensity and corresponds to $[\text{HOs}_7(\text{CO})_{18}]^-$, and is derived from the previous ion by loss of formaldehyde from the methoxycarbonyl ligand. The remaining crosspeaks are all derived by CO loss from $[\text{HOs}_7(\text{CO})_{18}]^-$, and their intensities are all similar because no further rearrangement of core or ligands is possible.

Comparing the MS and MS/MS spectra, the main differences are the relative fragmentation energies at which the various ions appear. Even at the highest cone voltage (200 V), the lowest mass ion, at m/z 1417, in the EDESI-MS map (Fig. 3) corresponds to the partially carbonylated ion $[\text{HOs}_7(\text{CO})_3]^-$. In contrast, the completely decarbonylated ion $[\text{HOs}_7]^-$ appears in the MS/MS spectrum at a collision voltage of ~ 160 V. The difference is probably due mainly to the different path lengths in the two collision regions and the gas pressures therein.

CONCLUSIONS

Combining breakdown graphs and energy-dependent ESI-MS (or conventional MS/MS with CID) provides a convenient means of presenting all of the information on the CID processes undergone by a given ion. Because the EDESI map already contains all the data needed for the breakdown graph, inclusion of this information does not require conducting an additional experiment.

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