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Combining energy-dependent electrospray ionisation with tandem mass spectrometry for the analysis of inorganic compounds

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The recently developed technique of energy-dependent electrospray ionisation mass spectrometry (EDESI-MS) has been implemented on a triple quadrupole mass spectrometer such that fragmentation occurs in the collision cell rather than at the skimmer cone. This modification enables a superior two-dimensional map of the collision voltage versus mass-to-charge ratio to be generated, providing unambiguous peak assignments. This latest enhancement to the technique is referred to as energy-dependent electrospray ionisation tandem mass spectrometry (EDESI-MS/MS). In the present work the technique has been applied to investigate the sequential removal of ligands from the inorganic mixed-metal anionic cluster compound $[Ru_5CoC(CO)_{16}]^-$, which serves to illustrate the advantages of this approach. Copyright © 2001 John Wiley & Sons, Ltd.

Electrospray ionisation mass spectrometry (ESI-MS)[1] has proved to be an enormously versatile analytical technique, providing molecular weight information for a huge range of compounds in areas as diverse as polymer,[2] biological,[3] organic,[4] and inorganic chemistry.[5] Because ESI is a soft ionisation technique the fragmentation tends to be minimal and therefore structural information is limited. Nonetheless, fragmentation may be easily brought about in ESI sources by increasing the voltage between the skimmer cones (referred to as the cone voltage),[6] which increases the energy of ion-neutral collisions (the neutral species being the bath gas, typically nitrogen). The collision-induced dissociation (CID), or fragmentation, therefore depends on the applied cone voltage. By collecting spectra at different cone voltages and plotting them in a two-dimensional format (cone voltage versus *m/z*), the entire fragmentation pattern for a given compound may be viewed at a glance, providing important structural information. This approach forms the basis of energy-dependent electrospray ionisation mass spectrometry (EDESI-MS).[7] In this communication we report an extension of the EDESI-MS technique in which fragmentation does not occur at the skimmer cone of the ESI interface, but rather in the collision cell of a triple quadrupole mass spectrometer. The technique is christened energy-dependent electrospray ionisation tandem mass spectrometry (EDESI-MS/MS). We have used this technique to study a range of inorganic compounds and the EDESI-MS/MS of a transition metal carbonyl cluster anion is used to illustrate the method herein. Transition metal carbonyl clusters are important compounds since they are widely used to bring about organic transformations, either in stoichiometric or catalytic amounts.[8] In general, they are difficult to analyse by mass spectrometry[9] and we have an ongoing program in this area using LDI[10] and ESI-MS[11] with quadrupole,[12] TOF,[13] and, more recently, Fourier transform mass spectrometers.[14] Neutral metal carbonyl compounds are not generally amenable to study by ESI due to the low basicity of the carbonyl ligands preventing protonation. While this problem may be overcome with the use of alternative forms of chemical derivatisation,[15] using an anionic cluster eliminates this difficulty entirely as the target compound is already charged.

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EXPERIMENTAL

All mass spectra were collected using a Micromass Quattro LC instrument, in negative-ion mode, with methanol as the mobile phase. The nebuliser tip was set at 3100 V and 90 °C, and nitrogen was used as the bath gas. Samples were introduced directly into the source at 4 μLmin^{-1} 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 7 s per spectrum and a low resolution setting (peak width at half-height ~0.8 Da) were used to maximise the signal-to-noise ratio. The cone voltage was manually 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 spectrum was collected by selecting the *m/z* 1024.5

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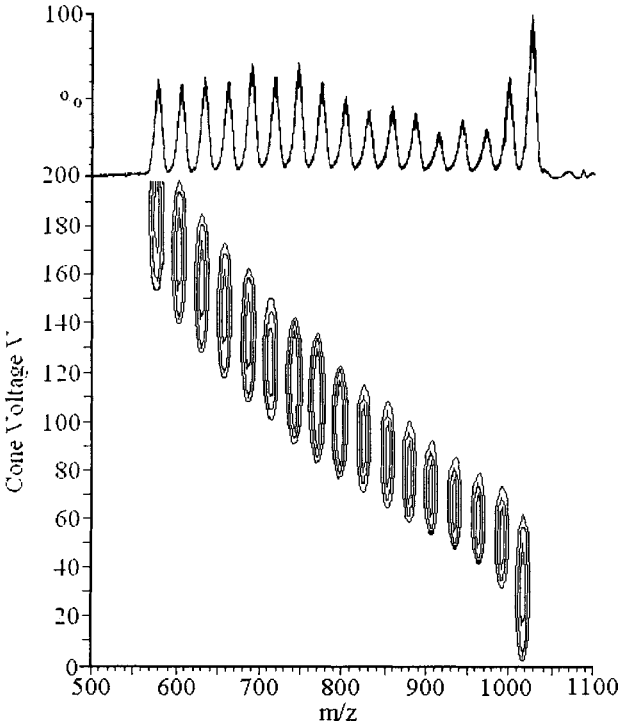

Figure 1. Two-dimensional EDESI-MS map generated from 201 negative-ion ESI-MS spectra of $[Ru_5CoC(CO)_{16}]^-$ at cone voltage settings of 0–200 V. The top trace is a 1D spectrum generated by combining all 201 spectra together.

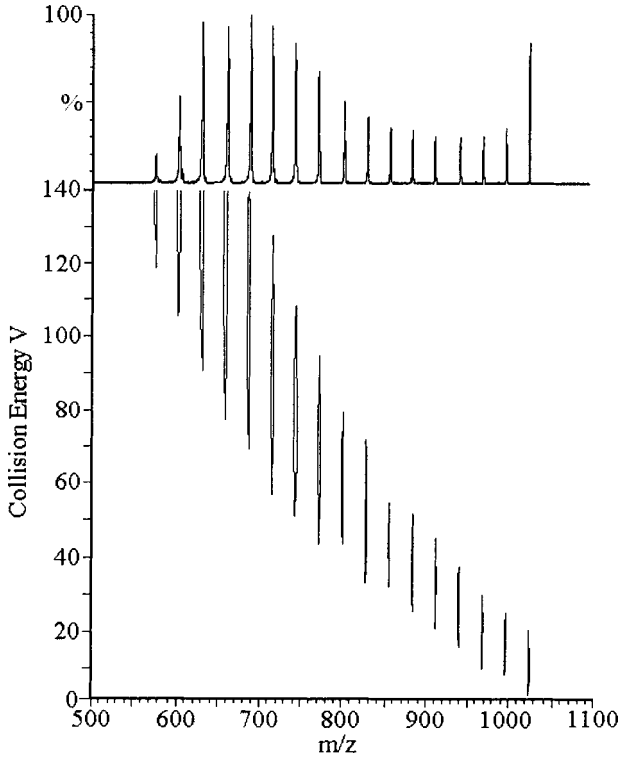

Figure 2. Two-dimensional EDESI-MS/MS map generated from 141 negative-ion daughter ion ESI-MS/MS spectra of $[Ru_5CoC(CO)_{16}]^-$ at collision voltage settings of 0–140 V. The top trace is a 1D spectrum generated by combining all 141 spectra together.

isotopomer of the $[Ru_5CoC(CO)_{16}]^-$ parent ion at a cone voltage of 15 V, at which value the ion current for that peak was at a maximum. The collision cell voltage was manually increased by increments of 1 V after each scan from 0 V up to 140 V, at which voltage the naked metal core is observed. The daughter ion spectra, measured by the second quadrupole mass analyser, were used to generate the EDESI-MS/MS map. Again, a scan time of 7 s per spectrum and a low resolution setting (peak width at half-height ~0.8 Da) were used. $[Ru_5CoC(CO)_{16}]^-$ was prepared and purified according to a literature procedure.[16]

RESULTS AND DISCUSSION

The conventional method of displaying fragmentation data from ESI-MS is to stack a series of spectra gathered at different cone voltages.[17] Each spectrum provides a snapshot of the ligand stripping process as a function of increasing cone voltage, and presentation of all the possible data sets in this fashion is clearly not practical. However, the entire fragmentation pattern can be easily visualised using EDESI-MS. The method involves collecting mass spectra across a range of cone voltages and combining the data into a map, with mass-to-charge ratio on the horizontal axis and cone voltage on the vertical axis. The 'map' function on the MassLynx™ software, designed for use with LC/MS, was adapted for this purpose (the axis labelling has been changed from retention time to cone voltage). Presentation of 201 spectra (~15 MB of data) as a map results in a 300-fold decrease in storage demands. In the map, all the daughter ions generated from successive fragmentation of the parent ion(s) may be observed simultaneously, thereby providing a clear and compact overall picture of the dominant fragment ions. Furthermore, combining the spectra that generate the map into a single, 1D spectrum provides an instant comparison between the relative intensities of all the ions across the full cone voltage range. By presenting this 1D spectrum aligned with the 2D spectrum, all of the information contained in the contributing spectra can be represented in a single image. Figure 1 shows the composite 1D/2D EDESI-MS for $[Ru_5CoC(CO)_{16}]^-$. Due to the timespan of the experiment, good signal-to-noise is obtained at the expense of resolution. A similar approach is taken when collecting two-dimensional NMR spectra, as decreasing the collection time and increasing the signal-to-noise ratio are more important than obtaining high resolution when it is the pattern of cross-peaks that is important rather than pin-point accuracy. The fragment peaks in the spectrum simply correspond to consecutive loss of CO from the central $[Ru_5CoC]^-$ core.

Daughter ion ESI-MS/MS spectra can be obtained by selecting an ion with the first mass spectrometer and then inducing fragmentation in the collision cell. The collision voltage may be altered in an analogous fashion to the cone voltage, and as it is increased the selected ion is fragmented by essentially the same mechanism (CID). The EDESI-MS/MS map for $[Ru_5CoC(CO)_{16}]^-$ generated by stacking 201 spectra, collected at collision voltages of 0–200 V, is shown in Fig. 2. Comparison between the two EDESI maps reveals the expected similarities, but also some marked

differences. In particular, fragmentation of the cluster occurs at consistently lower voltages in the MS/MS example. For example, at 25 V, the only ion apparent in the EDESI-MS map is the parent ion, $[Ru_5CoC(CO)_{16}]^-$, whereas, at the same voltage in the MS/MS map, the ions $[Ru_5CoC(CO)_x]^-$ ($x = 11 - 15$) are observed. Similarly, the carbonyl ligand-free metal core $[Ru_5CoC]^-$ makes its first appearance at ~150 V in the EDESI-MS map compared to ~120 V in the MS/MS map. These differences can largely be attributed to the use of argon as a collision gas in MS/MS, whereas fragmentation at the skimmer cone involves nitrogen. Essentially the same pattern of intensities for each daughter ion is observed, best represented by the summed spectrum at the top of each map.

In conclusion, we have extended the application of EDESI-MS to enable viewing of ESI-MS/MS fragmentation data in an informative and compact fashion. We expect the technique to find broad application for the structural analysis of any compound which undergoes fragmentation under collision-induced dissociation. Like all MS/MS techniques, EDESI-MS/MS is well suited to the analysis of mixtures. A further advantage of the MS/MS approach is the reduction of a broad isotopomer envelope down to a single peak, enabling more accurate identification of fragment ions.

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