focus on Mass Spectrometry & Spectroscopy

Electron-Induced Dissociation of Anions

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Tandem mass spectrometry (MS/MS) has become an integral part of chemical analysis within many industries due to its ability to identify known or unknown compounds, often at low abundance, and on a rapid time-scale that is compatible with chromatography. A recent addition to this tandem MS tool-box is a technique called Electron-Induced Dissociation (EID)[1]. An extension of Electron-Capture Dissociation (ECD) which uses low energy electrons to fragment multiply charged cations [2] and Electron Detachment Dissociation (EDD) for multiply charged anions [3], EID is the dissociation of singly charged ions following irradiation with electrons of slightly greater energy than used for ECD. This stems from the very early work by Cody *et al.* who brought forth the benefit of Electron Impact Excitation of Ions from Organics (EIEIO) [4].

Earlier this year, we showed that EID was highly beneficial for the dissociation of small positively charged pharmaceutical-type ions [5]. A comparison between EID and CID demonstrated the complementary nature of the two dissociation methods, with each technique supplying its own unique set of spectral peaks, and only a few peaks common to both. Comparison with electron ionisation (EI) was also drawn, concluding that EID can form a half-way-house resulting in a product ion spectrum that combines information obtainable from both CID and EI. EID proved to be particularly advantageous for the analysis of a set of analogous pharmaceutical compounds, whereby the observation of unique product ions facilitated essential structural characterisation, unachievable by other MS means. Of particular note was the effect the charge-carrying species has on the product ion spectrum and, in fact, for the molecules studied, performing EID on $[M+H]^+$, $[M+NH4]^+$, $[M+Na]^+$ and $[M+K]^+$ greatly enhanced the amount of useful information that could be obtained from any one molecule.

Keen to keep the ball rolling, a BMSS sponsored summer studentship allowed Eleanor Humphrey to join the group for a six-week period to see whether the same degree of success could be achieved for anions. It must be said that reports in the literature, following a metabolite study, suggested this might be a challenge [6]. Håkansson *et al.* used EID for the structural characterisation of phosphate containing metabolites. Whilst they found that EID did result in product ions from the negatively charged precursor ions, CID was found to be far better for analysing the smaller carbohydrates, producing more extensive fragmentation than EID [6]. Not to be daunted, our plan was to analyse a set of fifteen compounds by EID to determine efficacy. The samples chosen cover a reasonable range of chemical classes amenable to electrospray ionisation. Haloperidol, Raffinose, Reserpine, Sulphamethazine, Tazobactam, Allura Red (E129), Brilliant Blue (E133) and Sunset Yellow (E110) were purchased from Sigma-Aldrich. Cediranib plus some un-named compounds, identified here as **A, B, C, D, E** and **F** were kindly provided by AstraZeneca, Macclesfield, UK.

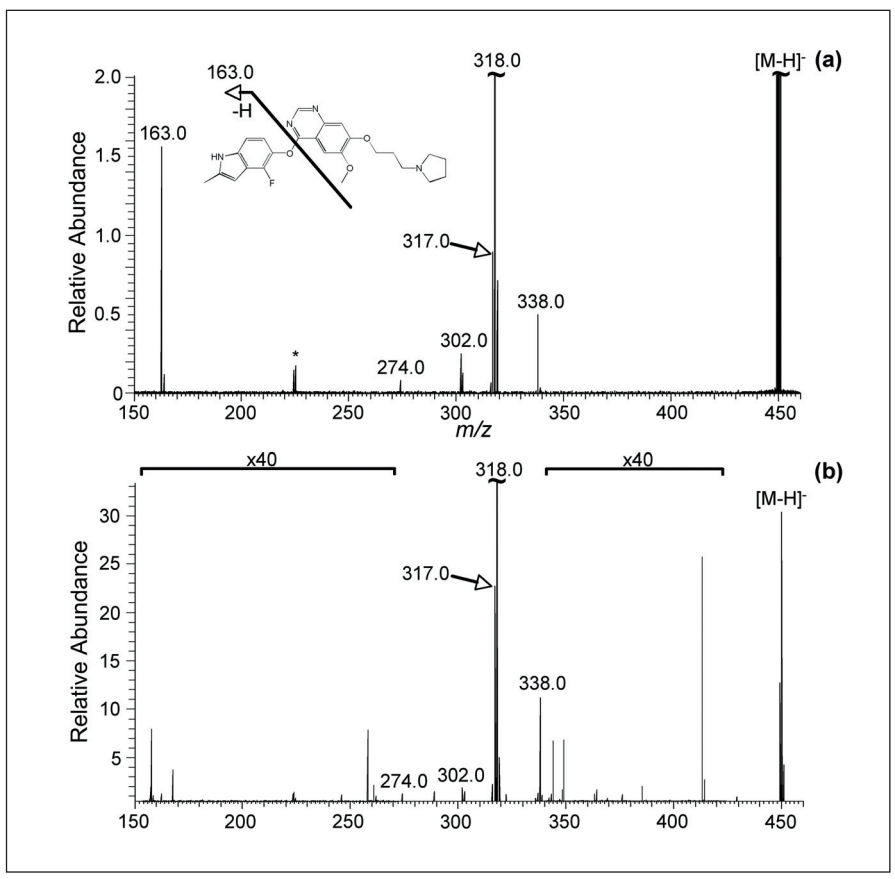

*Figure 1. (a) EID spectrum of Cediranib (inset) at 18.8 eV and 70 ms. (b) CID spectrum with peaks common with EID labelled. Peak marked * correspond to electronic noise.*

The experimental approach was direct infusion of sample solutions into an ESI FTICR MS (Thermo Finnigan, Bremen, Germany). This instrument comprises of two mass spectrometers connected in series. The first is a linear ion trap MS while the second is a FTICR MS. CID was performed in the linear ion trap with the product ions mass measured in the FTICR MS. EID was performed in the FTICR MS from precursor ions that were isolated in the linear ion trap. Further experimental details can be found in Mosely *et al.* [5]. The main parameters known to have an effect on EID product ion spectra are the electron energy and the duration of electron irradiation and so were treated as variables in the hope of optimising EID of negatively charged precursor ions.

Table 1. Summary of product ions generated by EID and CID for each negatively charged molecular ion. All precursor ions are $[M-H]^-$ unless otherwise stated.

Compound		Number of product ions observed		Common Products between EID and CID
		EID	CID	
A		0	15	0
B		6	13	3
C		11	23	7
D		4	8	2
E		0	7	0
F		0	6	0
Cediranib		6	40	5
Haloperidol		0	11	0
Raffinose		1	11	1
Reserpine		1	11	1
Sulfamethazine		2	8	0
Tazobactam		1	10	1
Allura Red AC	$[M-Na]^-$	2	17	2
	$[M-2Na]^{2-}$	16	15	8
Brilliant Blue FCF	$[M-Na]^-$	6	26	4
	$[M-2Na]^{2-}$	14	18	8
Sunset Yellow FCF	$[M-Na]^-$	5	34	5
	$[M-2Na]^{2-}$	17	5	5

The effect of EID on the negatively charged precursor ions has been seen to have a varying degree of success. EID analysis of $[M-H]^-$ for Haloperidol failed to generate any product ions, whereas compound C produced 11 product ions evenly distributed over a wide mass range. A summary of EID product ions is given in *Table 1*, along with comparative CID data. For many of these compounds there is clearly room for improvement. The compound Cediranib was then chosen for a set of EID optimisation studies as the $[M+H]^+$ adduct has previously been the focus of an in-depth EID analysis, generating an impressive 29 product ions by EID compared to 10 by CID. In contrast, EID of Cediranib $[M-H]^-$ generated only 6 product ions with CID much more successful in generating over 40 product ions, although it must be pointed out, many of these CID product ions differ only by the one or two hydrogen atoms and are not structurally helpful. This comparison is shown in *Figure 1*. The peak at *m/z* 163 observed in *Figure 1a* is unique to the EID spectrum and corresponds to the indole portion of the molecule formed by cleavage of the C-O bond shown inset in Figure 1a. Unique peaks such as this can produce that crucial piece of information, however for each of the singly charged anions listed in *Table 1* CID consistently produced a higher degree of fragmentation than EID, and often included the peaks observed in the EID product ion spectra, reversing the trend observed for positive ions [5].

Optimising EID Conditions

Firstly, alteration of the electron energy was carried out in order to determine whether the quality or content of the product ion spectra could be improved. An increase in the electron energy from 8.8 eV to 18.8 eV was found to increase the number of product ions from 1 to 6 for Cediranib $[M-H]^-$, with a slight increase in their relative intensities. Above 18.8 eV there was no increase in the number of product ions observed and signal-to-noise (S/N) ratio of the observed peaks deteriorated. The default electron energy was therefore set to 18.8 eV. Secondly, a study into the effect of electron irradiation time was also carried out on Cediranib $[M-H]^-$ at two different energy levels; 8.8 eV, where there was only one product ion at 318 m/z and 18.8 eV, the previously determined optimum value. Both these energy levels have been investigated over a range of electron irradiation times. For an electron energy of 8.8 eV, increasing the electron irradiation time from 10 ms to 150 ms failed to bring about any additional product ions, however there was a three-fold increase in the relative intensity of the 318 *m/z* product ion as shown in *Figure 2a*. At an electron energy of 18.8 eV, more product ions result, 4 of which are shown in *Figure 2b*. The product ions generated at 18.8 eV are observed at higher relative intensities at 10 ms than was ever achieved at 8.8 eV. It is perhaps not unexpected that increased energy is required for the bond cleavages that lead to these product ions. As the period of electron irradiation is increased, the relative intensity of the product ions show a sharp initial increase that then levels off or decreases after about 50 ms. The product ion at 318 *m/z* shows the greatest response to the alteration of the irradiation time with a 16-fold increase from the minimum at 10 ms duration to the maximum at 130 ms, but for Cediranib, little is gained by going beyond 50 ms of electron irradiation. As, during the six-week project, only one iteration of the optimisation experiments were completed, additional experiments will be performed to refine the observations made from this data.

Application to Sulphonated Food Dyes

The sulphonated food dyes were observed as both singly and doubly charged anions so a comparison was made between $[M-Na]^-$ and $[M-2Na]^{2-}$. Results are shown in *Figure 3* for Sunset Yellow. EID analysis of Sunset Yellow $[M-Na]^-$ resulted in limited fragmentation, primarily the neutral loss of sulphur dioxide and sulphur trioxide *(Figure 3a)*. Aside from these terminal fragmentation sites, cleavage is observed in the centre of the ion from cross-ring cleavage

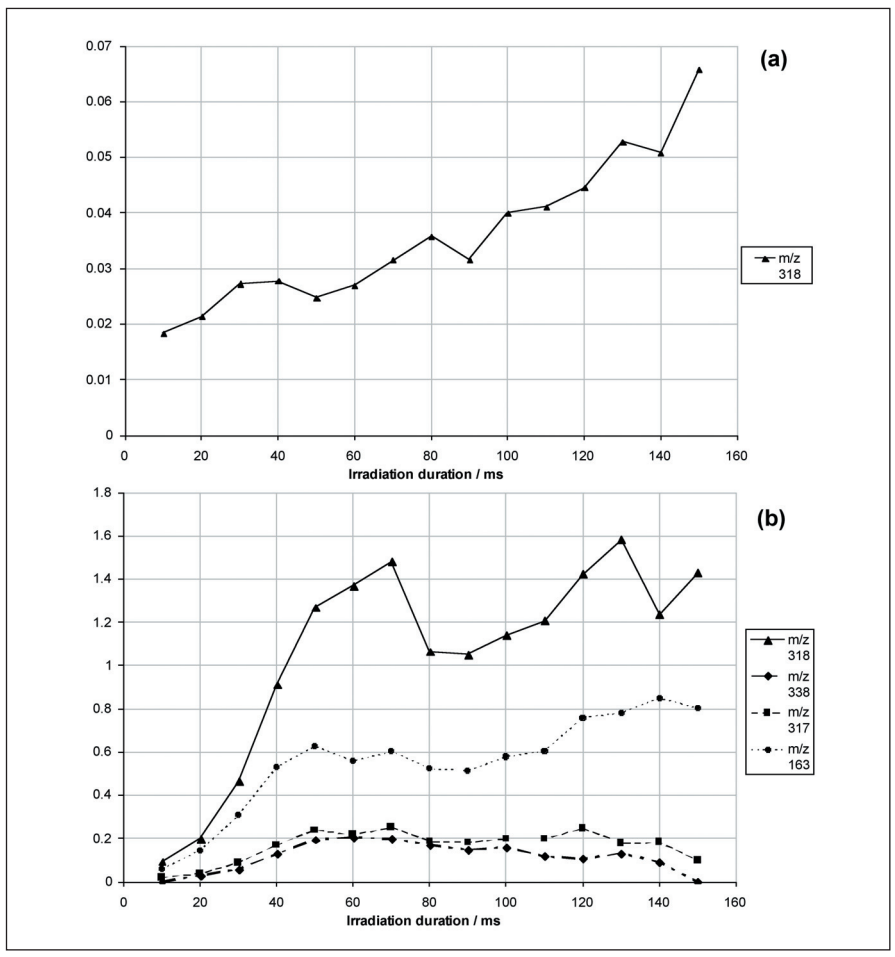

Figure 2. Relative intensity of product ions with increasing electron irradiation time at electron energy (a) 8.8 eV (b) 18.8 eV

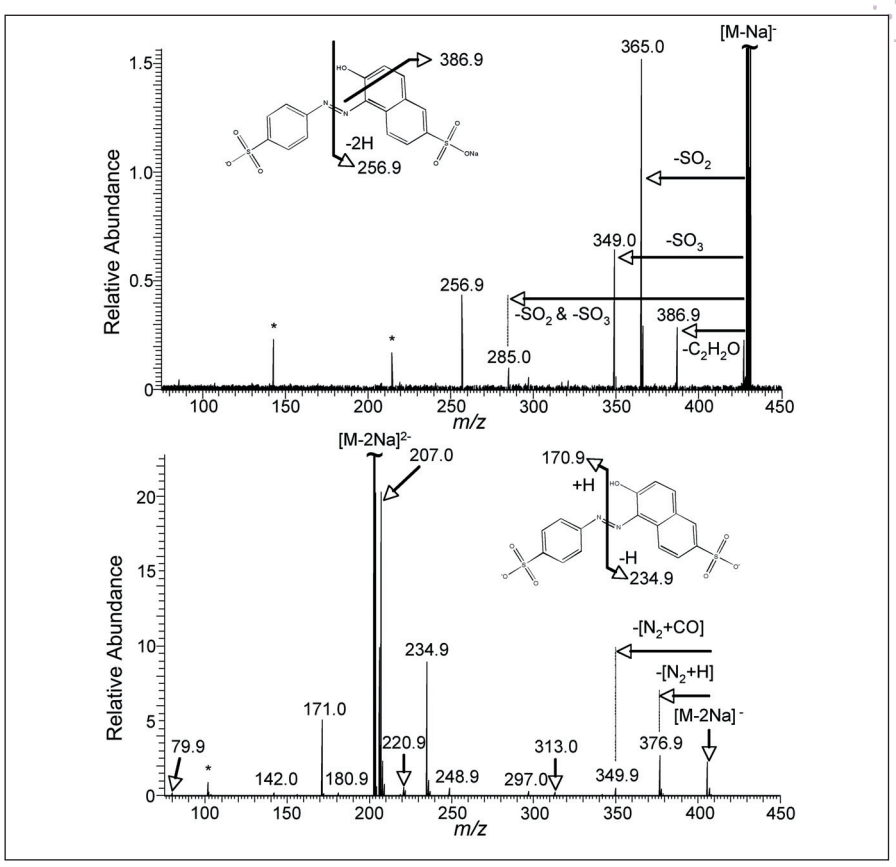

Figure 3. EID of (a) [M-Na]- and (b) [M-2Na]2- for Sunset Yellow.

or the cleavage of the N=N bond *(Figure 3a inset)*. Cleavage of the N=N bond was also the primary fragmentation site for the doubly charged $[M-2Na]^{2-}$ and is accompanied by small neutral losses from the charge-reduced species, formed by electron detachment, occurring in the same region of the molecule *(Figure 3b inset)* indicating this is a very 'active' part of the ion. There is no observed neutral loss of SO_2 or SO_3 from $[M-2Na]2^-$, however there is very weak evidence of SO_3^-. This interesting observation is currently under further investigation. What is apparent from this data, as with the other dyes, Allura Red and Brilliant Blue, is the larger number of product ions formed by EDD of the doubly charged precursor ion than EID of the singly charged, and much improved peak intensities. The same cannot be said for corresponding CID experiments *(Table 1)*, where a greater number of product ions are generated from the singly charged species. Of note is the limited number of product ions common to EDD and CID from the $[M-2Na]^{2-}$ of these three compounds. This ability to maximise information highlights the importance of using all available technologies to tackle analytical challenges.

In general, like Håkansson *et al.* [6], we found that for the singly charged anions employed in this project, CID gave a greater number and more abundant product ions than EID. However the unique nature of some of the product ions observed following EID renders this a useful and desirable tool. The wide variation in EID results across the set of compounds studied warrants further investigation, yet in-roads have been made towards optimising experimental parameters and thus far an electron energy of 18.8 eV and an irradiation time of 50 to 60 ms would seem to be a good starting position. As the set of compounds and compound classes investigated expands we will further our understanding of this technique and refine optimal parameter details.

Acknowledgements

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