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Multi-stage Mass Spectrometry for the Isolation and Structure Elucidation of Components of a Crude Extract

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Multi-stage mass spectrometry (MS^n) (up to $n = 6$) using a combination of electrospray ionization with a quadrupole ion trap was applied to the gas-phase isolation and direct structure elucidation of a complicated surfactant molecule in a crude product extract. Doubly unsaturated ions were found to arise from neutral, saturated fatty amide side chains via a unique fragmentation in the positive ion mode. Negative ion experiments revealed the substructure of the anionic portion of the surfactant. Strategies for software-controlled acquisition and mapping of MS^n data are discussed. © 1997 John Wiley & Sons, Ltd.

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INTRODUCTION

In the first major volume devoted to the subject of tandem mass spectrometry (also referred to as mass spectrometry/mass spectrometry or MS/MS), McLafferty[1] stated, 'As suggested to me by at least a dozen individuals, after MS/MS we must consider MS/MS/MS or even $(MS)^n$.' Almost 15 years later, the quadrupole ion trap has become particularly useful for executing sequential product ion multi-stage mass spectrometric (MS^n) experiments in the time domain. Its well known simplicity of design, operation at relatively high pressures (10^{-4} Torr (1 Torr = 133.3 Pa)) and high degree of software control facilitate MS^n experiments. Our specific application of ion trap MS^n is the direct, structure elucidation of even minor components in an unpurified, complex mixture. Electrospray ionization followed by substructural probing using sequential product ion experiments was the focus of this study.

Unfortunately, perhaps, the *general* MS^n acronym is currently in popular use to describe *specifically* sequential product ion experiments, usually involving collision-induced dissociation (CID) of a particular parent ion, selected at each '*n*th' stage of mass spectrometry. However, it is worth noting that the MS^n acronym is broadly descriptive, in that for any value of $n \geqslant 1$ there are >1 unique experiments. For instance, associative ion–molecule reactions may be included in MS^n experiments.[2] Another example involves the individual MS^3 experiments theoretically possible using a pentaquadrupole instrument.[3] Eight fundamental MS^3 experiments were identified, only one of which was the sequential product ion experiment. Several of the suggested scan modes were actually demonstrated and showed unique specificities for certain components of specially prepared mixtures of standards. These studies culminated in the development of MS^n symbology, which was advanced for systematically classifying the various experiments possible for any value of n.[4] Thus the term MS^n should only be used with a specific description of the type of experiment being carried out.

The limitations of sequential product ion MS^n experiments involving CID and using so-called beam instruments have been discussed and contrasted with the advantages of CID, using resonant excitation in quadrupole ion traps.[5] A particular advantage of the quadrupole ion trap compared with beam instruments is the relatively high *observed* CID efficiency (defined as the sum of the product ion peak intensities divided by the intensity of the unattenuated parent ion beam, at the collector). For any individual 'nth' experiment, this efficiency is more than 50% in many cases of 'smaller' organic molecules (e.g. <1000 Da). This result is a consequence of many factors that favor the ion trap in comparison with beam instruments, including (i) the longer path length of the parent ions (tens of meters), (ii) the longer time frame of the experiment (tens of milliseconds), (iii) the continuous excitation of the parent ions during CID, (iv) many low-energy collisions that accumulate internal energy in the ions, (v) low scattering losses, (vi) the efficient trapping of product ions formed at every '*n*th' stage and (vii) efficient product ion transmission to the detector. Thus, the dwindling signal intensity associated with sequential product ion MS^n experiments using a beam instrument (especially from scattering and transmission losses) is not as severe by comparison.

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An early commercial instrument, the ion trap mass spectrometer, featured a programmable scan function, allowing many sequential product ion isolations and dissociations to be carried out. These early experiments relied on a combination of r.f. and d.c. voltages to effect monoisotopic isolation of parent ions.[6] Thus, cumulative activation energies up to 17 eV were achieved in the MS^{10} analysis of polyaromatic hydrocarbons.[7] Combinations of CID and subsequent additive reactions of product ions with neutral organic molecules (also a 'stage' of mass spectrometry) allowed even higher values of n to be demonstrated.[2] Forward and reverse r.f. voltage scans with an additional a.c. voltage applied across the trap endcaps were used for parent ion isolation in an MS^4 experiment to demonstrate sequencing a peptide.[8] Finally, various fundamental aspects of sequential product ion experiments have been systematically defined.[9]

Early examples of quadrupole ion trap MS^n experiments focused more on the development and demonstration of the technique than on solving 'unknown' organic structures. As the prototype of the now commercially available electrospray ion trap was tested, our focus was understanding possible product ion structures, rationalizing fragmentation pathways and connecting them back to the structure of the parent molecule,[10] particularly solving unknowns. Fully automated MS^n now begins to make feasible the total structure elucidation of unknown components in complex mixtures. The ionization method helps to decrease the complexity of the mixture by producing mainly pseudo-molecular ions. Although ESI is a relatively dim ion source and ion injection losses are significant, parent ions are integrated to useful abundances by the ion trap. Efficient isolation of specific parent ions at high values of q_z, using tailored waveforms[11] simplifies the mixture to a monoisotopic species of the component of interest. Thus, sample pre-purification and handling are minimized. The software supports up to nine sequential MS/MS experiments.

A recent example of using the LCQ instrument for structure elucidation dealt with a drug and several hydroxylated metabolites. The parent compound's fragmentation behavior had been well defined in other types of mass spectrometric experiments. Owing to the nature of the sequential product ion experiment, the established product-ion linkages helped pinpoint the locus of oxidation in the metabolites.[12] The example reported here shows the total elucidation of the structure of a surfactant, presented as an unknown to us, in a complex mixture, using only ESI/MS^n of a dissolved product formulation. Since the amount of data generated in a complete MS^n map can be rather cumbersome, recommendations are made for data display and software development.

EXPERIMENTAL

Sample preparation

All solvents (HPLC grade) and chemicals were from standard suppliers. A 20 mg sample of shampoo was dissolved in 2 ml of acetonitrile–methanol–water (50:25:25) in a 100 × 13 mm i.d. culture tube (Corning, VWR Scientific, Cleveland, OH, USA). The sample was vortex mixed (1 min) and centrifuged at high speed (5 min, Dynac II benchtop model, Clay Adams). The supernatant was removed and acidified with 2 μl of 88% formic acid (positive ion analyses) or basified with 2 μl of concentrated (8.5 M) ammonia solution (negative ion analyses).

Instrumentation

A prototype LCQ electrospray (ES) ion trap mass spectrometer and a commercial LCQ instrument (Finnigan-MAT, San Jose, CA, USA) were used for MS^n experiments. The ES interface was operated at 220 °C, the potential on the needle was typically held at 4–6 kV and samples were infused at 5 μl min^{-1}. The co-axial gas flow rate was ~2 l min^{-1}. The tube lens and r.f. (760 kHz) voltage amplitude used during ion injection were optimized automatically by the data system, based on maximizing the intensity of a specified m/z value or range. Parent ion isolation (monoisotopic) was carried out at a q_z of 0.83, by application of a tailored waveform with a zero-amplitude notch at the z-axial frequency of the ion of interest. For CID (He, 1 mTorr), the q_z value was typically 0.2–0.3 and the tickle time and amplitude were adjusted to optimize the fragmentation of the ion of interest. Typical values used were 30 ms and 500 mV, respectively, but do vary. The same sample was also analyzed using the Perkin-Elmer SCIEX API-III triple-quadrupole instrument. The ES needle was held at 4 kV, the co-axial gas flow rate was about 2 l min^{-1}, the orifice potential was 70 V and the ES interface was at 60 °C. CID was carried out at various collision energies up to $E_{cm} = 8.3$ eV (Ar^0, 2.2×10^{14} molecules cm^{-2} indicated target gas thickness).

RESULTS AND DISCUSSION

Triple quadrupole mass spectrometric results

The electrospray analysis of surfactant-based products generally produces a family of protonated, natriated and ammoniated molecules characteristic of each component. It is not unusual for several compound classes to be present (e.g. neutral, acidic, basic, amphoteric, quaternary ammonium surfactants). The electrospray mass spectrum of a solubilized shampoo product, obtained on a conventional triple-quadrupole instrument, is a qualitative 'snapshot' of many of the components (Fig. 1). Some families are easily identified (alkylated polyethylene glycols and fatty alkyl amides) by comparison to tables of molecular masses for common components and confirmed by readily interpreted tandem mass spectra.

The particular sample shown in Fig. 1, however, had a spectrum containing a signal of over 90% relative intensity at m/z 345, not encountered in any of our numerous electrospray analyses of complex mixtures

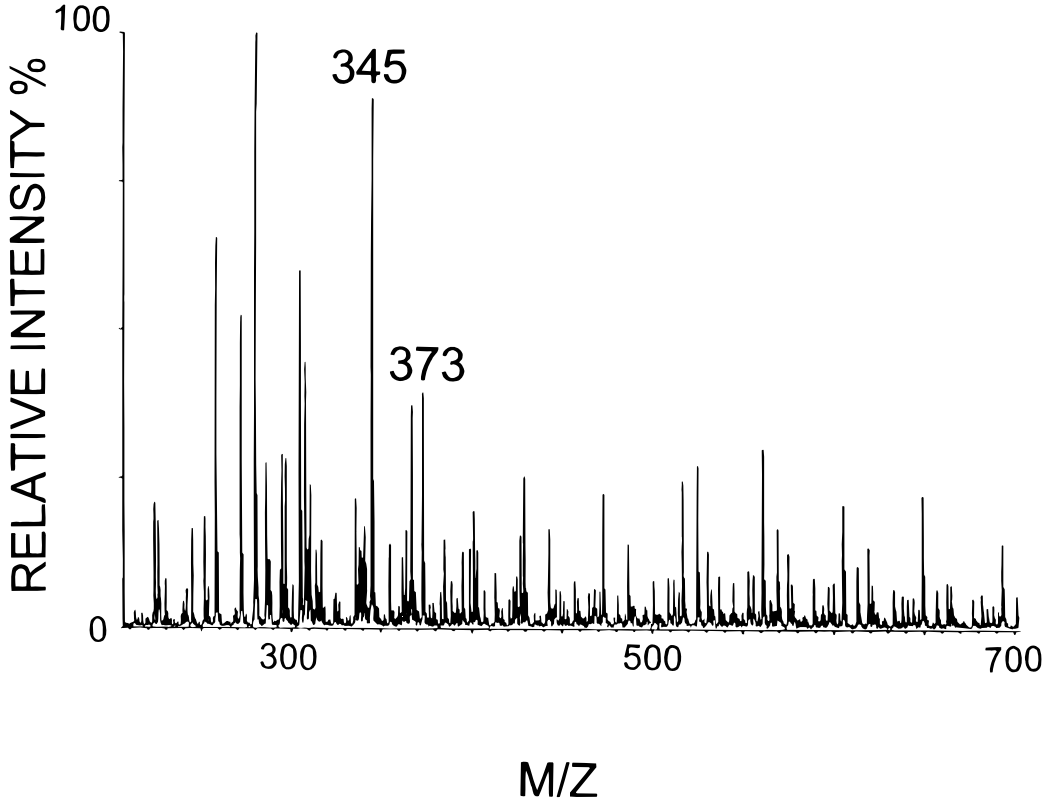


Figure 1. Electrospray positive ion mass spectrum of the infused (5 μl min^{-1}) shampoo extract, obtained using a triple-sector-quadrupole mass spectrometer.

(unpublished data). MS/MS using a triple quadrupole instrument and collision energies as high as E_{cm}(center-of-mass energy relative to Ar^0) = 3.1 eV produced one abundant fragment ion (base peak) at *m/z* 226 (Fig. 2), with low abundance (<5%) fragment ions whose genesis and interrelationships were not obvious, between *m/z* 60 and 125. At higher collision energies, hydrocarbon $(C_nH_{2n+1})^+$ product ions were dominant, along with *m/z* 44 (probably the $CH_2 = NHCH_3^+$ ion for nitrogen-containing surfactants). In this case, refragmentation of *m/z* 226, using an MS^n device, was an obvious choice. Such a scenario was one MS^n application envisioned years ago.[13]

Before advancing to the ion trap MS^n experiment, several initial hypotheses could be made. A peak at *m/z* 373 (+ 28 u relative to *m/z* 345) fragmented similarly to give *m/z* 254 (+ 28 u relative to *m/z* 226). In surfactant products, such similarity is usually due to a saturated hydrocarbon side chain, different by two methylene units. This difference arises from the natural source of fatty raw materials (e.g. coconut oil) used in surfactant synthesis on an industrial scale. The constituent fatty acids arise from the acetyl-CoA biosynthetic pathway, which builds the hydrocarbon chain in steps of two methylene units. Thus, the first hypothesis is that *m/z* 226 contains a fatty chain, which is consistent with the abundant hydrocarbon fragments at higher E_{cm} in CID experiments. In this type of product, the carbon chain length is probably >10.

Second, application of the nitrogen rule shows that the compound must have an even number of nitrogen centers, assuming that the parent ions are protonated molecules or that they contain a quaternary nitrogen. The MS/MS data showed no *m/z* 23 (common for natriated parent ions) or loss of ammonia (common for M + ammonium ion complexes). Further, since the more dominant CID processes of electrosprayed ions are often, although not exclusively, even-electron in nature, it follows that the neutral fragment (119 u) contained at least one nitrogen center and the charged fragment likewise contained at least one nitrogen center (viz. the total nitrogen atoms equal two, at least). It was likely that the fragmentation occurred at a heteroatom site (possibly N) by protonation and displacement, based on our experience in analyzing other surfactants. Finally, negative ion analysis of the extract was more selective and revealed abundant parent ions at *m/z* 343 (100%) and 371 (40%), respectively, so the data suggest at least one acidic site, leading to the formation of $[M - H]^-$ ions.

Ion trap MS^n results

The electrospray spectrum of the shampoo extract, obtained using the ion trap (*n* = 1), is shown in Fig. 3. Because a single amplitude of r.f. voltage was used during the ion injection step, a somewhat selective accumulation of ions over a range of *m/z* values occurred, centered around the ions of interest (*m/z* 345 and 373). Isolation of a particular parent ion for CID (i.e. MS^2) is performed based on its unique, *z*-axial frequency of oscillation in the trap. An excitation wave form (amplitude 20 V p–p), containing all frequencies *except* that of the desired ion, can be added to the experiment, resulting in the ejection of unwanted ions. By trapping

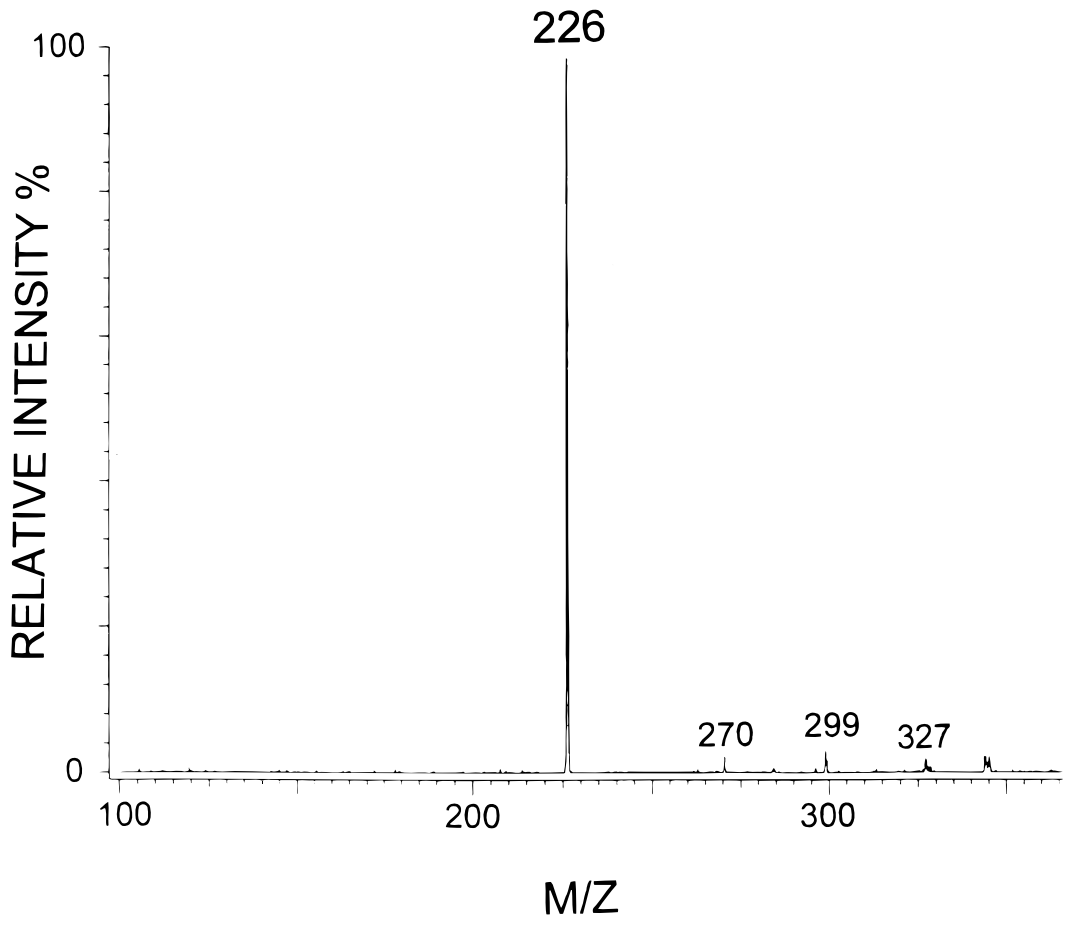


Figure 2. Electrospray positive ion product ion spectrum of *m/z* 345, obtained on the sample whose spectrum is shown in Fig. 1. The collision energy (Ar^0) was 25 eV in the laboratory frame of reference.

the parent ions at a high q_z value (0.83) during this step, a higher *z*-axial frequency resolution from adjacent ions is achieved, versus performing ion isolation at lower values of q_z. This result is predicted by a plot of the dimensionless ion parameters b_z vs q_z, which shows large non-linear increases in b_z beyond a q_z of 0.4.[14] For instance, at *m/z* 1000, $q_z = 0.83$, *m/z* 1001 is removed over 540 Hz using the LCQ instrument. The lineshapes are relatively narrow, so resolution of these adjacent masses is clean.

The isolation of the *m/z* 345 ion from the electrosprayed crude extract is shown in the inset of Fig. 3. The isolation efficiency was $> 90\%$, a result typical in most experiments. Note that the width of the isolation notch may often be opened to several mass units after monoisotopic isolation for MS^2, since there are usually no 'interfering' product ions within that range in subsequent experiments ($n > 2$).

The MS^n experiment is further extended by adding a step of resonant excitation of parent ions (a 500 mV amplitude is typical), effecting their CID. The product ion of *m/z* 226 is obtained almost exclusively. All other product ions are observed at higher *m/z* values and with $<2\%$ relative abundance. The observed CID efficiency was 33%. CID of *m/z* 226 (MS^3) at a q_z of 0.2 produced a unique family of ions at *m/z* 81, 95, 109 and 123 (Fig. 4). The observed CID efficiency was 6.5%. The overall observed CID efficiency through three stages of MS was 2.1%. Finally, fragmentation of *m/z* 95 (MS^4) showed a loss of 28 u. The same experiment on *m/z* 109 gave losses of 28 and 42 u, with about 50% observed CID efficiency. A spectrum showing the actual signal-to-noise ratio (i.e. no smoothing of the data) is shown in Fig. 5.

Interpretation of MS^3 and MS^4 ions

The ions observed in the MS^3 spectrum are methylene homologs, with three $-CH_2-$ in the series *m/z* 81–123. Some assumptions were introduced and tested at this point. Our experience led us to believe that the elemental composition was limited to C, H, O, N and S. (We did not obtain accurate mass data, as we were interested in an ion trap-based approach to solving the structure.) Sulfur is generally found as a sulfonate or sulfate in surfactants, but there were no characteristic ions (viz. *m/z* 80, 97) in the negative ion CID spectra. The composition was then limited to C, H, O and N. Since the nitrogen count had to be an even number, two nitrogens were deemed more likely than four, which would have been unusual for a surfactant in this class of product. If these assumptions proved true, mass tables showed that the ions produced from *m/z* 226 had to be very unsaturated. This postulate created a conflict, since the ions

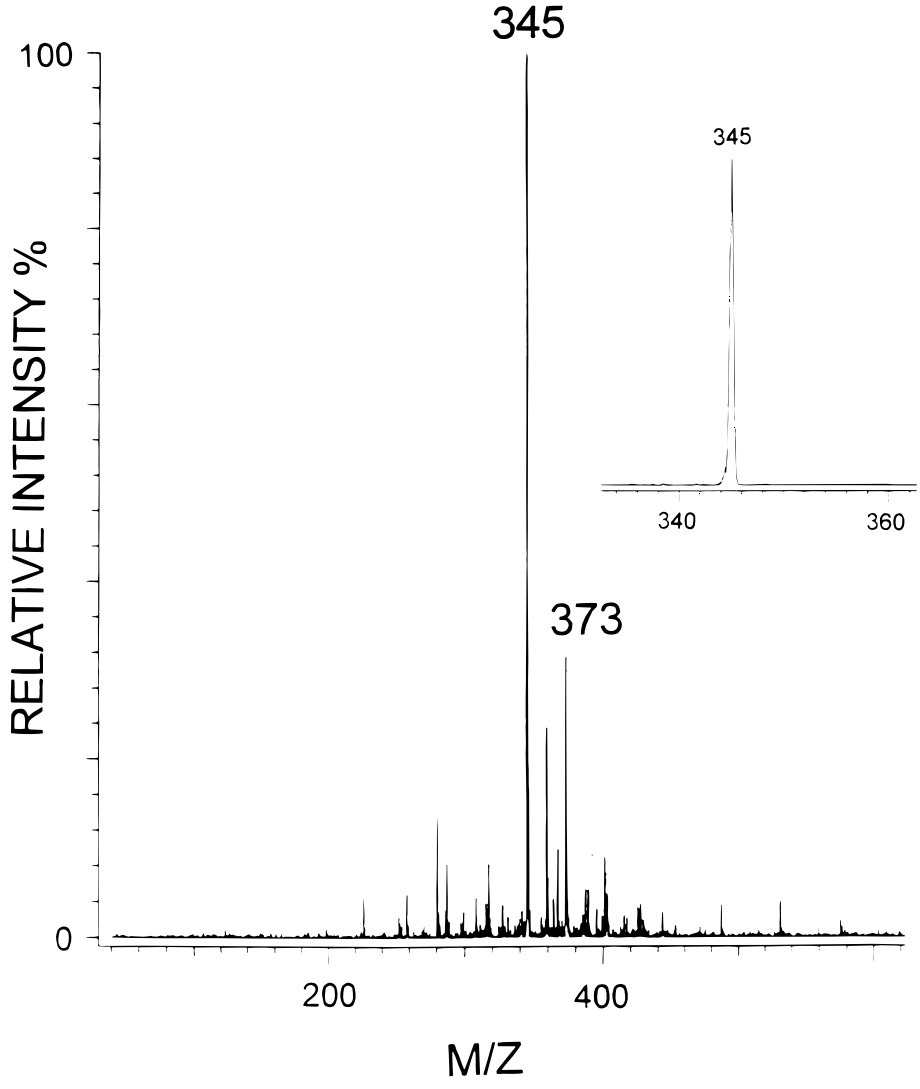


Figure 3. Electrospray positive ion mass spectrum of the infused shampoo extract (5 μl min^{-1}). The spectrum was obtained using the quadrupole ion trap with ion injection. The inset shows the result of application of an isolation waveform for *m/z* 345.

were probably derived from a long alkyl chain, which in surfactants is almost exclusively saturated.

Further studies of known surfactants,[15] and a fundamental ion structural study, have led us to propose that ions of the type $[R{-}COX{-}(CH_2)_nCH_2]^+$, where R is a saturated alkyl chain and X = N or O, rearrange and fragment to produce the series $[C_nH_{2n-3}]^+$ in the ion trap. For instance, in an independent study, the cyclic acetal synthesized by reaction of ethylene glycol and 2-tridecanone (Scheme 1) was analyzed by capillary gas chromatography (GC)/electron ionization MS, using the Finnigan ITMS instrument.[15,16] Alpha cleavage of the cyclic acetal leads to ions of the type $[R{-}COX{-}(CH_2)_nCH_2]^+$, and GC/MS/MS analyses showed these ions fragment primarily to *m/z* 67, 81, 95 and 109 (Scheme 1).

Furthermore, electrospray ionization MS^6 analysis of the surfactant laurylbetaine supports the hypothesis (Scheme 2). The MS^2 result for laurylbetaine was similar to that of the 'unknown' with a single abundant ion at *m/z* 240, a probable methylene homolog of *m/z* 226. MS^3 yielded the acylonium ion $[C_{11}H_{23}CO]^+$at *m/z* 183. This ion must undergo extensive H-rearrangement, with the charge moving down the chain, for the next observed loss (MS^4) is a water molecule, to yield *m/z* 165. We propose that *m/z* 165 is a dienyl carbonium ion, $[C_nH_{2n-3}]^+$. MS^5 and MS^6 yield successive alkene losses to produce *m/z* 123, 109, 95, 81 and 67. Details of these studies will be reported elsewhere. Thus, the unknown was proposed to have a partial structure of $C_{11}H_{23}CONCH_2CH_2{-}$. This is not the only conceivable structure supported by the data—it is a practical suggestion based on the well known use of laurylamide backbones in shampoo surfactants and the data obtained on compounds of known structure.

Negative ion results

Several of the product ions produced by CID of the deprotonated molecule (*m/z* 343) produced new information complementary to the positive ion result (Fig. 6). The observed collision efficiency was 40%. Loss of 18, 44 and (18 + 44) u suggested alcohol and carboxylic acid moieties. The ions of *m/z* 224 and 198, although relatively weak, were supportive of the suggested amide.

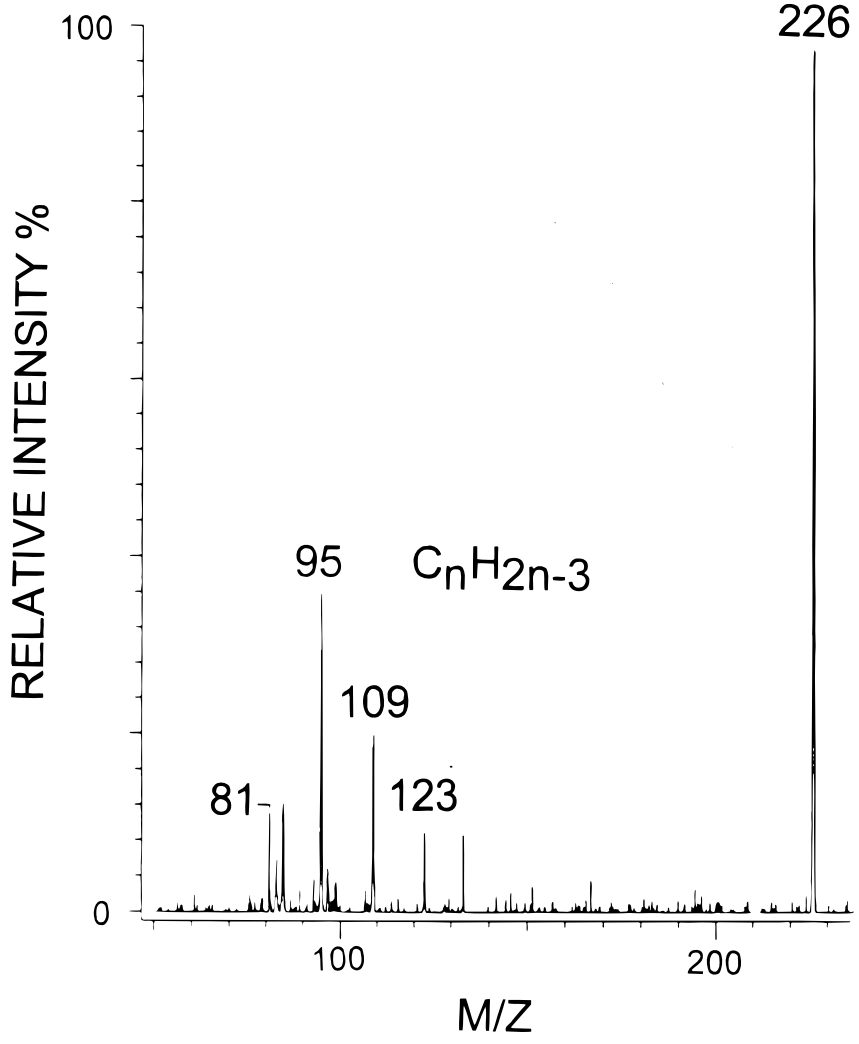


Figure 4. Electrospray positive ion MS^3 analysis (345^+–226^+–product ions) of the infused shampoo extract.

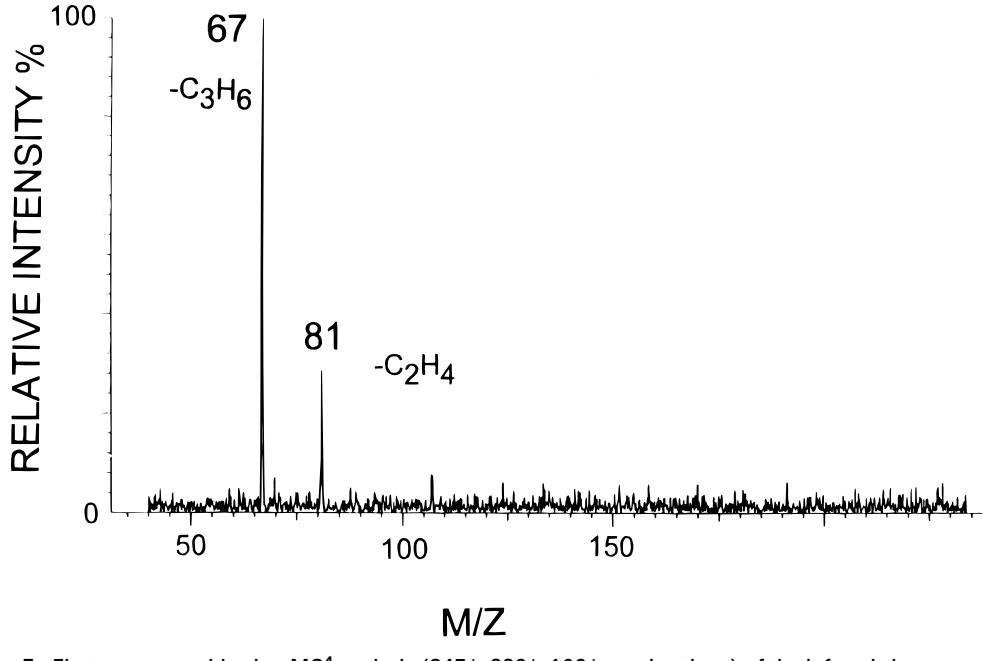
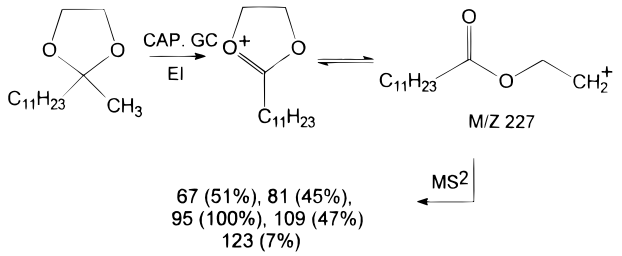
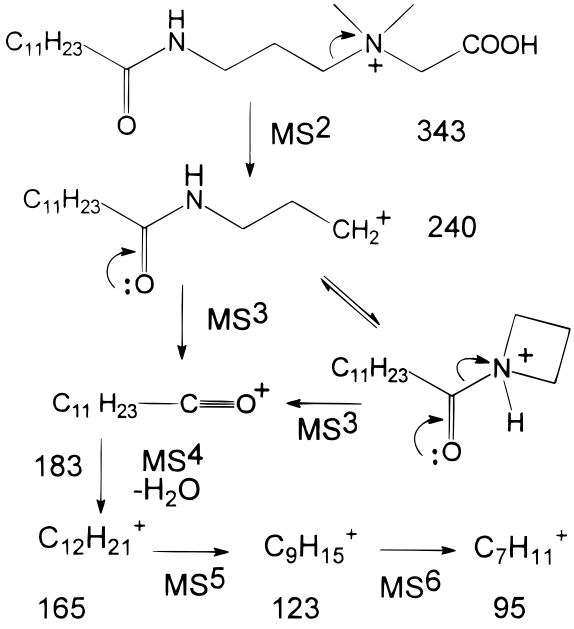


Figure 5. Electrospray positive ion MS^4 analysis (345^+–226^+–109^+–product ions) of the infused shampoo extract.



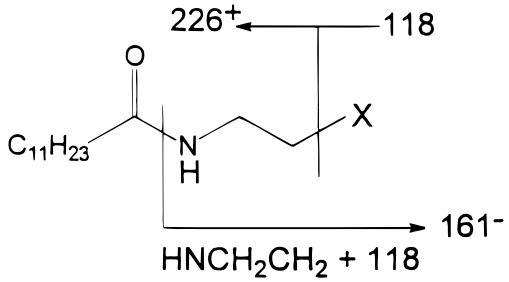
Scheme 1. Independent method for generation of a carbonium ion of the class $[R{-}COX{-}(CH_2)_nCH_2]^+$ by capillary GC/electron ionization MS/MS, where X = O and $n = 1$, with resultant product ions and relative abundances listed.



Scheme 2. One positive ion MS^6 pathway of decomposition observed for electrosprayed laurylbetaine, with *m/z* values and suggested structures of the ions.

A key product ion to the structural puzzle appeared at *m/z* 161. Considering the proposed structure of the positive ion of *m/z* 226, it occurred to us that *m/z* 161 could be considered as the $NHCH_2CH_2$ (43 u) terminus of the proposed fragment of *m/z* 226, plus 118 u (Scheme 3). That is, the missing neutral fragment in the positive ion data (119 u) now appeared as part of a charged ion (*m/z* 161) in the negative ion data. Therefore, negative ion MS^n could be used to probe the rest of the structure. Moreover, the second necessary nitrogen atom was proposed to be the linking atom for the group denoted X, being protonated and directing the loss of 119 u observed in the positive ion data. Finally, the ion of *m/z* 161 did not shift in the product ion spectrum of *m/z* 371^-, the $-CH_2CH_2-$ homolog of *m/z* 343^-, whereas all of the other MS^2 product ions (Fig. 6) shifted by 28 u. Thus, *m/z* 161 did not contain the saturated alkyl chain.

MS^3 of *m/z* 161 produced losses of 18, 44, 61 (18 + 43) and 62 (18 + 44) u. Hence the proposed alcohol and acid moieties were probably located in this fragment. Since we also knew that N-acetates were common in shampoos, a partial structure for the fragment ion was proposed, namely $H_2N(CH_2CH_2)N(CH_2COO^-)$, 116 u. The remaining



Scheme 3. Summary of possible structural content of *m/z* 161^- and *m/z* 226^+.

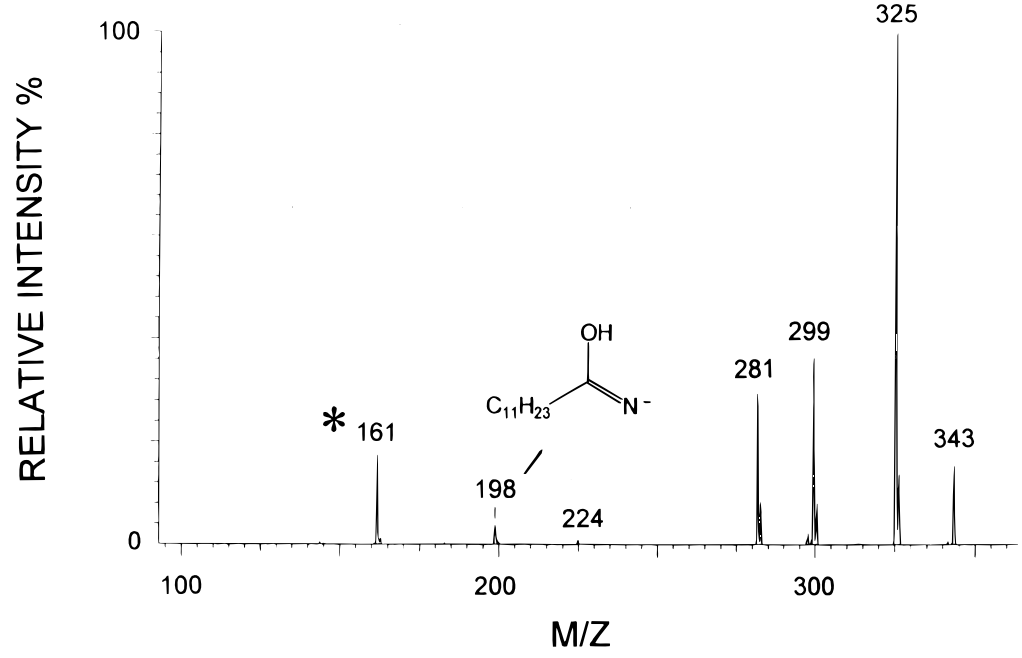


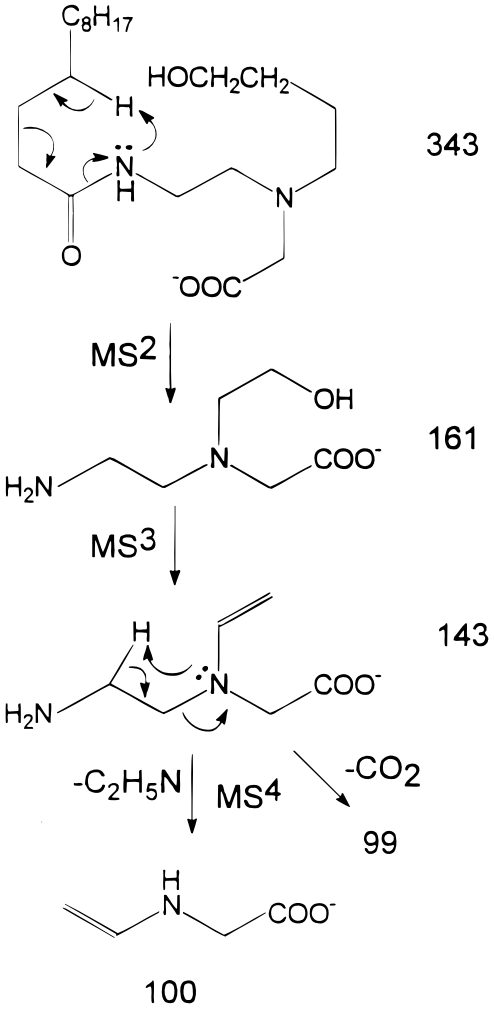
Figure 6. Electrospray negative ion product ion mass spectrum of *m/z* 343 from the infused shampoo extract (5 μl min^{-1}). The peak marked with an asterisk contained the structural elements not found in positive ion analysis.

45 u to be accounted for were proposed to correspond to the alcohol as a $-CH_2CH_2OH$ group, since ethanolamines are common shampoo components. The proposed structure and genesis of *m/z* 161 and its fragment ions are shown in Scheme 4. Note that at the MS^4 stage either CO_2 or ethylenamine can be lost. In fact, in the MS^3 data from *m/z* 161, a mass doublet at *m/z* 99 and 100 can be seen.

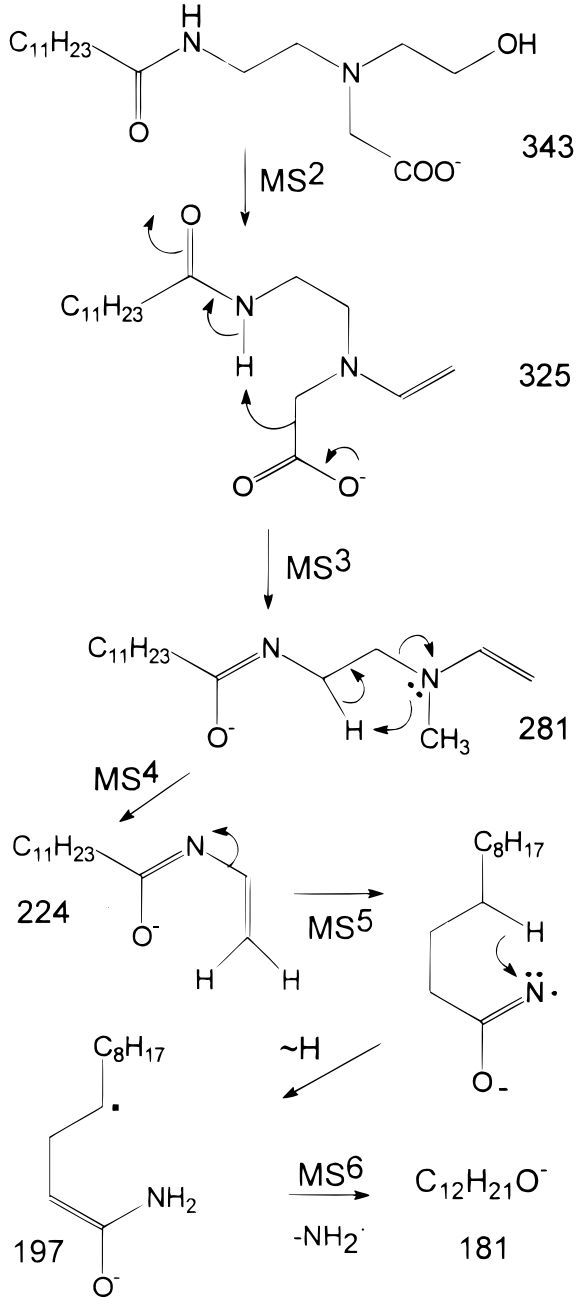
The final proposed structure,

$C_{11}H_{23}CONHCH_2CH_2N(CH_2COOH)(CH_2CH_2OH)$

was confirmed by comparison of MS^n spectral data of an authentic sample. The surfactant is known as 'coco amphomonoacetate' in the surfactant trade, but had not been commonly used in the type of product under analysis. An MS^6 fragmentation pathway of this standard is shown in Scheme 5. An interesting feature is a violation of the so-called 'even-electron rule' where a radical anion is formed at MS^5, to yield *m/z* 197. It was pointed out some time ago that such 'violations' are common.[17] We propose that extensive rearrangement of the fragment leads to loss of an NH_2 radical to give finally the even-electron anion of *m/z* 181. No exact structure for this anion is proposed, but we suspect the existence of cyclic enolate anions. Incorporation of ^{18}O and ^{15}N is straightforward synthetically for this molecule and MS^n studies of labeled compounds would help verify particular hypotheses.



Scheme 4. Proposed generation of *m/z* 161^- and explanation of its subsequent MS^n behavior.



Scheme 5. One negative ion MS^n pathway of decomposition observed for electrosprayed lauryl amphoacetate, with *m/z* values and suggested structures of ions.

Some important summary points are as follows. (i) Comparison with an authentic standard is necessary to confirm fully the structural assignment based on MS^n only. (ii) In this case, previous experience with MS/MS structural interpretation of shampoo components provided the basis for assumptions and hypotheses that proved true. In other words, experience and further elucidation of MS^n fragmentations of various compound classes in the future will be keys to structure elucidation based on MS^n data only. (iii) While proposal of exact fragmentation processes always carries a degree of speculation (e.g. MS/MS data only), the sequential nature of these MS^n experiments contributes to the support of a particular hypothesis. (iv) Some compounds, notably

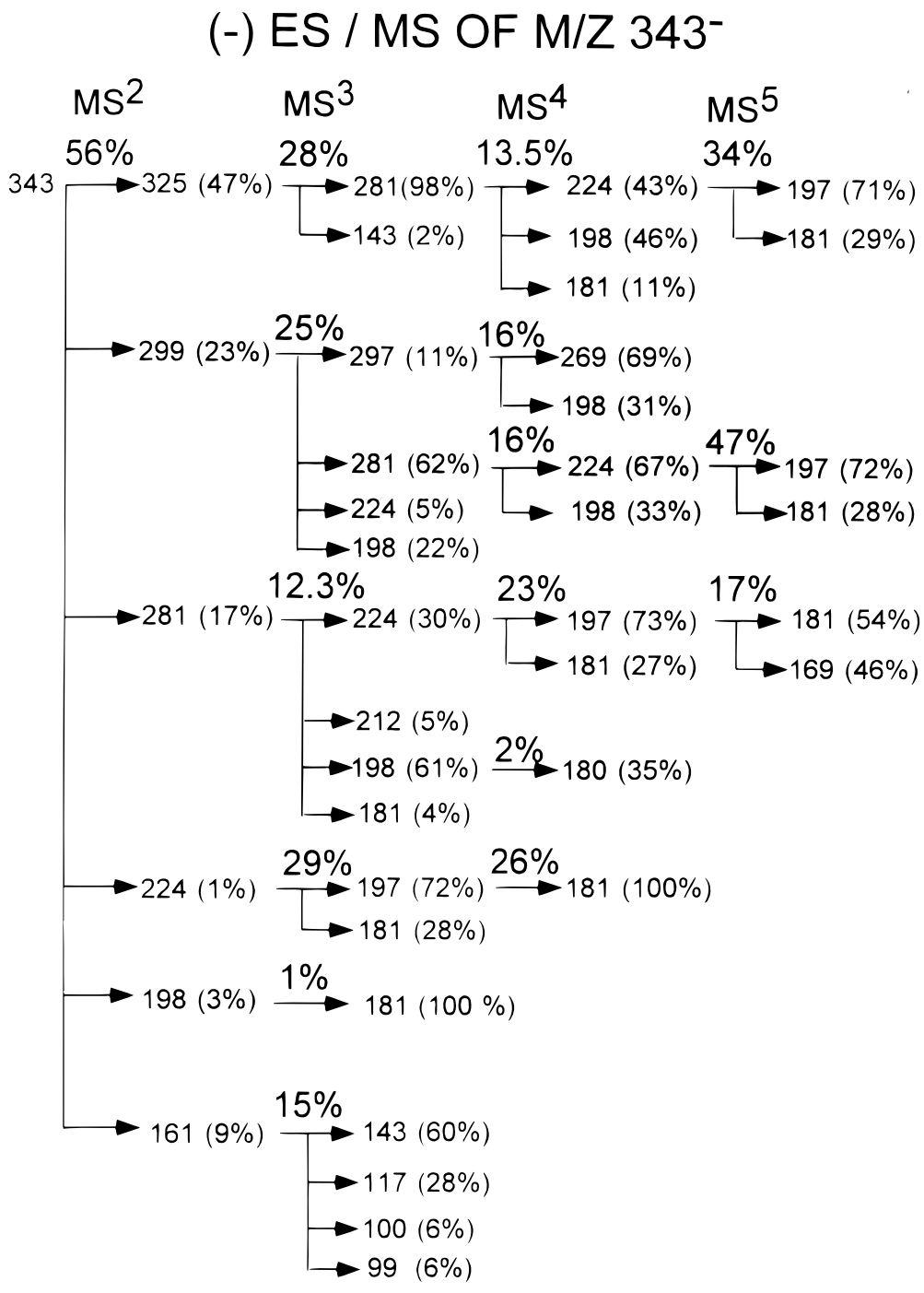


Figure 7. Data display summarizing the negative ion MS^n behavior of *m/z* 343 from electrospray analysis of lauryl amphoacetate.

linear alkyl sulfonates, have negative ion spectra dominated by low-mass fragments derived from the sulfate group. While the compounds may not appear at first to be a good case for MS^n, schemes to derivatize or cationize the molecule may be useful for positive ion MS^n. (v) Finally, we find that in general about 0.3 ml of a solution containing analyte at about 10 nmol ml^{-1} is sufficient for construction of an MS^n map (5 μl min^{-1} flow rate).

Accurate mass assignments at any '*n*th' stage would reveal the formulae of product ions. Such information could reduce the amount of foreknowledge (e.g. likely compound class) required for a sample of an unknown. In principle, this experiment should be possible using Fourier transform ion cyclotron resonance mass spectrometry (FT-ICRMS), if there is a sufficient number of ions in the trapping cell and the formula of the parent ion (used as the reference peak) is known. Although MS^8 has been shown using FT-ICRMS,[18] a similar experiment with accurate mass measurement has not been demonstrated, to our knowledge. Accurate mass errors less than 3 ppm have been claimed through $n = 4$, but we are not aware of published data showing measured mass accuracies and standard deviations derived from several trial measurements at values of $n \geqslant 4$.

Further problems and data reduction

As we have performed further MS^n studies, it has become obvious, in our opinion, that software development is critical. It is not unusual, in our experience, to generate several independent fragment ion paths at the MS^2 or MS^3 stage, and the exponential growth of information can quickly become the rate-limiting step in problem solving. In many cases we obtain information out to MS^6 easily. In structure elucidation, no significant pathway should be ignored. In practice, we use an arbitrary standard of investigating product ions of over 10% relative abundance at each stage.

A fairly simple display of MS^n data in an expanding tree is shown in Fig. 7 (negative ion MS^n). The observed collision efficiency (%) is noted for each branch and the percentage of the total product ion current carried by a particular fragment is listed next to the m/z value. Thus, the overall efficiency for any cumulative MS^n process can be easily calculated. Since parent ion isolation efficiencies are generally $> 90\%$, and often 100%, this factor is not included, so that the display is less cluttered.

Since automated parent ion selection for generally defined LC/MS^n experiments has been incorporated in the LCQ (i.e. 'data-dependent' MS/MS or MS/MS/MS experiments as an LC peak elutes), it should be possible to apply the same principle for infusion MS^n experiments. For instance, intelligent software control of the MS^n experiment should be possible with the generation of a one-page, summary map as in Fig. 7. The use of small pop-up windows could reduce the clutter of such a display. For example, 'pointing and clicking' on an m/z number could show potential formulae for the mass losses. A window displaying a particular product ion spectrum could be similarly accessed at branch points on the grid. Others have suggested a two-dimensional display to summarize a total, particular MS^n path in a single view, also very useful in our opinion. However, one display appears necessary for each path.[19] We have also recently used more complicated displays, similar to metastable ion mapping, to summarize all MS^n information in a single plot.[20]

CONCLUSIONS

In the determination of the structure of an 'unknown' surfactant, MS^n data provided greater insight into the molecular structure than MS/MS alone by (i) clearly defining the genealogy of observed product ions, especially those at lower m/z ratios, (ii) allowing refragmentation and enhancing energy deposition for substructural studies and (iii) helping to locate specifically certain functionalities, because ion relationships could be strictly defined. A current limitation is that interpretation, of necessity, must be experience based.

REFERENCES

1. F. W. McLafferty (Ed.), *Tandem Mass Spectrometry*, p. 7. Wiley, New York (1983).
2. J. N., Louris, J. S. Brodbelt, R. G. Cooks, G. L. Glish, G. J. Van Berkel and S. A. McLuckey, *Int. J. Mass Spectrom. Ion Processes* **96**, 117 (1990).
3. J. C. Schwartz, K. L. Schey and R. G. Cooks, *Int. J. Mass Spectrom. Ion Phys.* **101**, 1 (1990).
4. J. C. Schwartz, A. P. Wade, C. G. Enke and R. G. Cooks, *Anal. Chem.* **62**, 1809 (1990).
5. J. M. Charles and G. L., Glish, in *Practical Aspects of Ion Trap Mass Spectrometry*, edited by R. E. March and J. F. J. Todd, Vol. III, Ch. 3. CRC Press, Boca Raton, FL (1995).
6. R. J. Strife, P. E. Kelley and M. Weber-Grabau, *Rapid Commun. Mass Spectrom.* **2**, 105 (1988).
7. B. D. Nourse, K. A. Cox, K. L. Morand and R. G. Cooks, *J. Am. Chem. Soc.* **114**, 2010 (1992).
8. R. E. Kaiser, Jr, R. G. Cooks, J. E. P. Syka and G. E. Stafford, Jr, *Rapid Commun. Masss Spectrom* **4**, 30 (1990).
9. S. A. McLuckey, G. L. Glish and G. J. VanBerkel, Int. *J. Mass Spectrom. Ion Processes* **106**, 213 (1991).
10. R. J. Strife, J. C. Schwartz, M. Bier and J. Zhou, in *Proceedings of the 43rd ASMS Conference on Mass Spectrometry and Allied Topics*, Atlanta, GA, May 21–26, 1995.
11. J. N. Louris and D. M. Taylor, US Pat. 5324939 (1994).
12. L. C. E. Taylor, R. Singh, S. Y. Chang, R. L. Johnson and J. C. Schwartz, *Rapid Commun. Mass Spectrom.* **9**, 902 (1995).
13. M. L. Gross and D. H. Russell, in *Tandem Mass Spectrometry*, edited by F. W. McLafferty, Ch. 12. Wiley, New York, (1983).
14. R. E. March and R. J. Hughes, *Quadrupole Storage Mass Spectrometry* (Chemical Analysis Series, Vol 102), pp. 196–200. Wiley–Interscience, New York (1989).
15. R. J. Strife and M. M. Ketcha, in *Proceedings of the 44th ASMS Conference on Mass Spectrometry and Allied Topics*, Portland, OR, May 12–16, 1996.
16. R. J. Strife, in *Practical Aspects of Ion Trap Mass Spectrometry*, edited by R. E. March and J. F. J. Todd, Vol. III, Chapt. 1B, CRC Press, Boca Raton, FL (1995).
17. M. Karni and A. Mandelbaum, *Org. Mass Spectrom.* **15**, 53 (1980).
18. T. Lin and G. Glish, in *Proceedings of the 45th ASMS Conference on Mass Spectrometry and Allied Topics*, Palm Springs, CA, June 1–5, 1997.
19. P. Sander, in *Proceedings of the 44th ASMS Conference on Mass Spectrometry and Allied Topics*, Portland, OR, May 12–16, 1996.
20. M. S. Farncombe, R. S. Mason and K. R. Jennings, *Int. J. Mass Spectrom.* Ion Phys. **44**, 91 (1982).