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Prediction of the doubly charged ion pattern by modelling the high- and low-resolution mass spectra of isotopomeric forms

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Abstract The presence of doubly charged ions in mass spectra is detected only occasionally because their clusters are observed more rarely than singly charged ones. The patterns connected with doubly charged ions are located in the spectrum below M/2. The narrow shapes of such patterns as well as overlapping with other bands generate significant problems in their interpretation. The method described here is based on modelling of the isotopomeric form of single- and double-charged mass ion clusters. The present work attempts to explain the generation of the double charge disotopomeric patterns of high- as well as low-resolution spectra. Predicting the high-resolution mass cluster is simpler than calculations of the low-resolution cluster. The high-resolution cluster may represent the initial form of low-resolution pattern formation.

Keywords Doubly charged ions · Cluster modelling · Computer assisted interpretation

Introduction

Isotopomeric structure of the pattern in mass spectra

The occurrence of poly-isotopic elements (PIE) [1] in a molecule results in a more complex cluster of mass ions [2]. The band of an ion containing only a few PIE atoms can be composed of many peaks corresponding to many mass isotopomers. The isotopomeric cluster is a graphic distribution of the isotopomers related to ions with the same element content [3]. Position isotopomers affect peak intensity, but are not directly detectable in mass spectra [4].

Patterns of doubly charged ions in mass spectra

Multiple charging complicates mass spectra interpretation [5] because the charge state of each ion must be assigned to enable determination of the ion mass, and ions of a given mass typically exhibit several charge states.

For a low-resolution mass-to-charge ratio spectrum (i.e. when adjacent members of an isotopomeric multiplet are unresolved) containing only a few multiply charged species, the charge of each ion may be derived from the mass-to-charge ratio values of ions of the same uncharged mass but different charge [6]. However, for more complex spectra (e.g. many components or fragments), charge state deconvolution by this simple algorithm is difficult. Mann et al. [7] proposed the least-squares based charge state deconvolution algorithm. Hagen and Monning [8] used a multiplicative correlation algorithm to extract molecular weight information from spectra with poor signal-to-noise ratio; however, at the cost of significant peak distortion and unreliable ion relative abundances.

The presence of doubly charged ions is often noticeable because their clusters are observed less often than those of single-charged ions [9]. The bands[1] connected with doubly charged ions are located in the spectrum below M/2. The narrow shapes of such patterns as well as overlapping with other bands generate significant problems in their interpretation [4]. Isotopomer band modelling proves to be a

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[1] cluster = pattern = band

solution to this difficult problem. The present work attempts to explain the generation of doubly charged isotopomeric clusters of high- and low-resolution spectra. Using this approach, the occurrence of doubly charged ions may enhance accurate interpretation of mass-spectral data.

Clusters of high-resolution spectra (accurate mass spectra)

Mass isotopomers are attributed to ions that show little difference in mass but a different isotopic composition, which can be elucidated by high resolution mass spectrometry [10]. The structure of their isotopomeric patterns may be explained by high-resolution forms of the single-charged ion of the same formula [10]. Regardless of their molecular masses, the mass spectral ions of organic compounds show single isolated peaks or narrow sub-clusters [11]. In the isotopomeric pool, the position isotopomers, which are not separated in the spectrum, are dominant. The mass isotopomers, in contrast, are not numerous. The accurate location (m/z) of the peak allows all the possible mass isotopomers contained in the isotopometric cluster to be presented [12]. A resolution of 0.0001 u is needed in this case; such precision precludes overlapping of closely located signals of the mass isotopomers [10].

The most abundant peak stands for 100% (or 9999) and all others are calculated with respect to this peak. The mass isotopomers are important for peak locations; each mass isotopomer of a given ion has its unique position in the isotopomeric pattern. The high-resolution mass spectrum explains the mass isotopomer composition; however, it is not possible to determine the width of the isotopometric cluster (WIC) [12].

The number of peaks observed in the graphic representation is restricted to the dominant mass isotopomers of each sub-pattern corresponding to the specific ion.

Clusters of low-resolution spectra

The patterns of high- and low-resolution spectrum clusters differ substantially [4]. The low-resolution ion cluster does not provide information about a specific isotopomer located in the m/z range. A cluster in the low-resolution mass spectra contains closely located peaks, usually separated by 1 u. The peaks represent all position isotopomers of the same m/z value. The determination of mass isotopomers by low-resolution mass spectrometry is not efficient. The number of peaks in an ionic cluster does not correspond to the number of main mass isotopomers related to the ion investigated.

The low-resolution ionic cluster of a single-charged ion can be predicted from the exact mass model [10]. The location of the respective peak is the integer value of m/z [13]. However no details about the number of mass and

position isotopomers and their intensities can be obtained from experimental low-resolution ionic clusters due to the dense packing of peaks in the (m/z±0.5) range.

The low-resolution cluster of a doubly charged ion contains ca. two times fewer peaks and is moved to the left of the spectrum in the area below m/z < M/2. The distances between peaks are decreased two-fold. Peaks of P^+ located at even m/z values occur only in the P^{2+} cluster, since only integer m/z values are active, other peaks are omitted regardless of their intensities.

However, the peak location in low-resolution spectra is determined with precision 0.1 u, leading to simple detection of doubly charged ion patterns.

Investigating incorrectly interpreted complex patterns can lead to erroneous explanation of fragmentation details. Therefore, identification of the components of such clusters may be an important procedure for mass spectra interpretation. Isotopic cluster modelling can be applied as a helpful tool in the determination of such components.

Methods

The method presented in this work is based on the modelling of a low-resolution single-charged mass ion cluster presented at a recent Modelling and Design of Molecular Materials (MDMM) conference [14]. Each doubly charged ion is related to real or virtual (not observed, but predicted) single-charged ions. The locations of peaks must agree with the following relationship: m_i/z for a single-charged ion, $m_i/2z$ for a doubly charged ion.

The high-resolution isotopomeric cluster of an ion charged ±1 can be predicted from the accurate masses and abundances of the stable isotopes. The cluster consists of several sub-patterns[2] each of which is composed of signals located nearby. The sub-band contains peaks located according to the accurate mass of isotopomers; it can be predicted from isotopic compositions and isotopic masses, factoring in the mass defects. The range of the sub-cluster usually does not exceed 0.005 u. Let us assume that a parent single-charged ion is isotopically described as $(E_1)n_1(E_2)n_2(E_3)n_{3...}(E_z)n_z^+$. The accurate m/z value is calculated from the following formula:

$$m_j = \sum_{i=1}^{z} \left(n_i \cdot m_{E_i} \right) \qquad (1)$$

where n_i denotes the number of the isotope atoms, and n_E the exact mass of the isotope.

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[2] Sub-cluster, sub-pattern or sub-band—in high resolution mass spectra, this also refers to a well-separated part of the mass spectrum containing several peaks located in the range ±0.05 u.

The fractional abundance of this isotopomer is:

$$A_j = \prod_{i=1}^{z} A_i^{n_i} \qquad (2)$$

The mechanism of transformation is shown in Fig. 1.

The next procedure concerns moving the cluster to the left side of the spectrum by transformation of the peak locations to values two times lower ($m/2z$). The precision decreases due to the simultaneous successive reduction in peak number and change in intensities. The peak intensity I_p is related to the sum of abundances of such isotopomers A_{pi}

$$I_p = f \left(\sum_{i=1}^{n} A_{pi} \right)_{m/z = const} \qquad (3)$$

This value concerns mass isotopomers only. Position isotopomers show the same m_j value and so they are not separable in the mass spectrum, and therefore the abundance observed for peaks of the same m_j is more complex.

The theoretical modelling appears simple; each step is logical and so easy that any spreadsheet program can be applied for calculations. Relative peak abundances are normalised by the rule that the intensity of the main peak of the cluster must be 100%, and other values are calculated relative to this. In each step of modelling, the precision of m/z values is reduced to 0.1 u or 1 u.

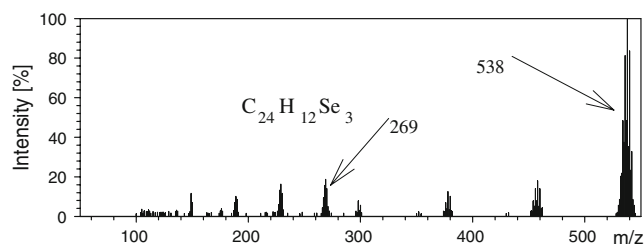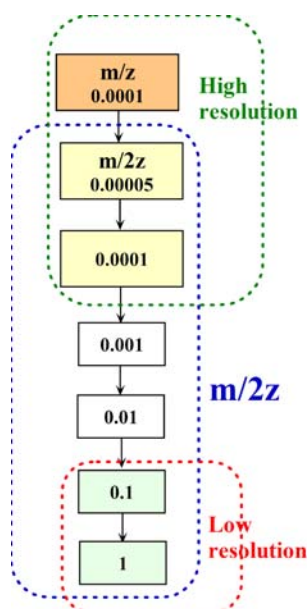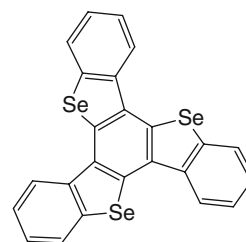**Fig. 1** Scheme of high-resolution cluster transformation to the low-resolution pattern of the doubly charged ion

Fig. 2 Mass spectrum of tris(benzo[b]selenopheno) [2,3:2′,3′:2″,3″] benzene $C_{24}H_{12}Se_3$ [15]

Example

Tris(benzo[b]selenopheno) [2,3:2′,3′:2″,3″]benzene $C_{24}H_{12}Se_3$

The mass spectrum of tris(benzo[b]selenopheno) [2,3:2′,3′:2″,3″]benzene (Fig. 2) contains [15] the high molecular cluster (D_{538}^{+}) located around 538 u and the pattern near 269 u, which is related to the doubly charged ion D_{538}^{2+} [16]. These two bands can be employed for modelling of the doubly charged ion cluster.

Three selenium atoms generate a very complex cluster containing 18 peaks; a good example for isotopomeric modelling. The experimental 'low-mass' band with eight peaks is a good example for comparison with the predicted cluster (Fig. 3). All steps of the transformation of the high-resolution pattern to the low-resolution pattern described above and shown in Fig. 1 have been calculated. Is it possible to predict the low-resolution cluster from natural isotopic abundances?
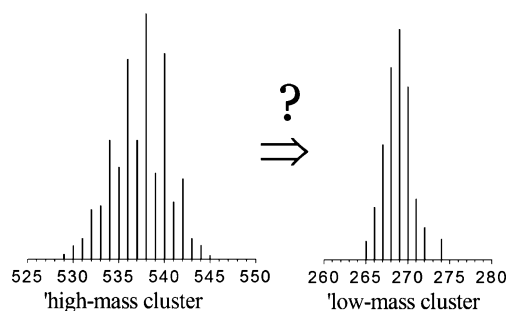

Fig. 3 Experimental clusters of the investigated ions

Fig. 4 Method of transformation of closely located sub-clusters of single-charged high-resolution cluster to the doubly charged ion isotopomeric pattern, and finally into the low-resolution pattern
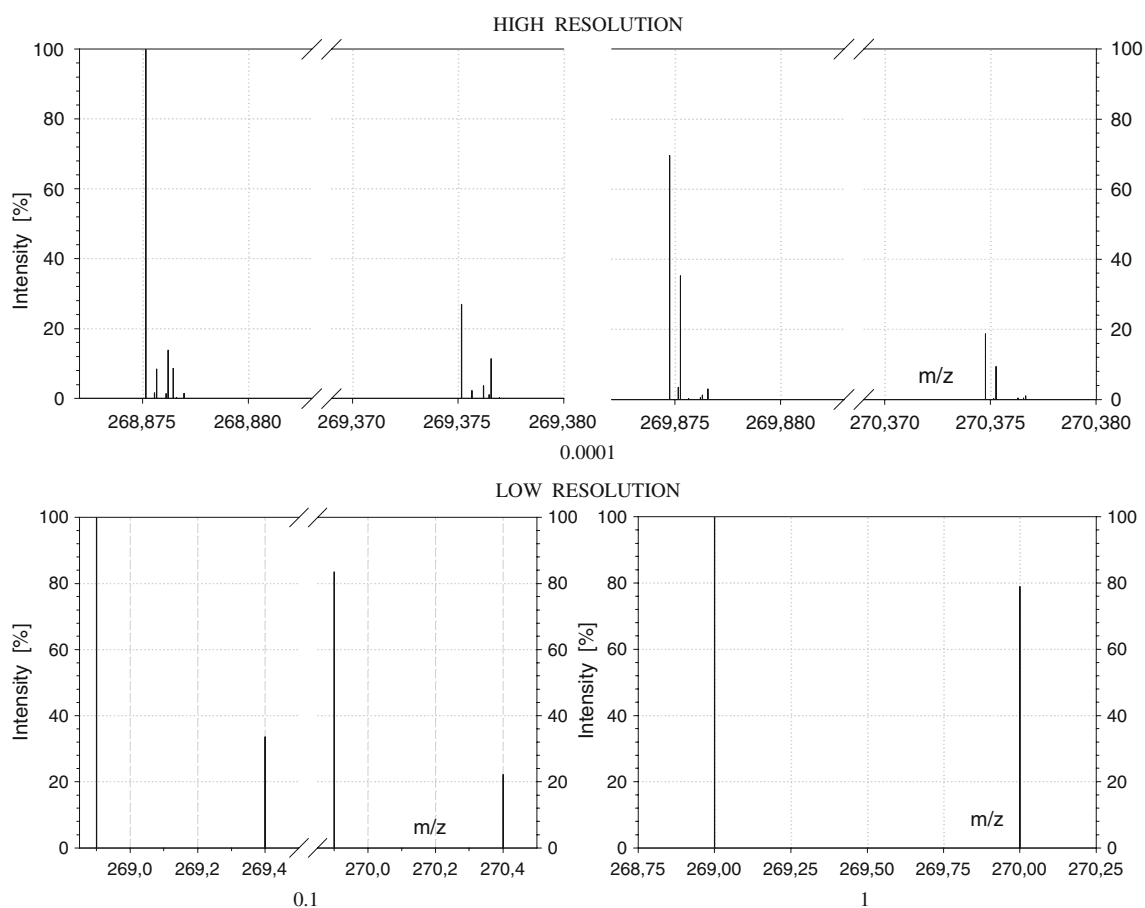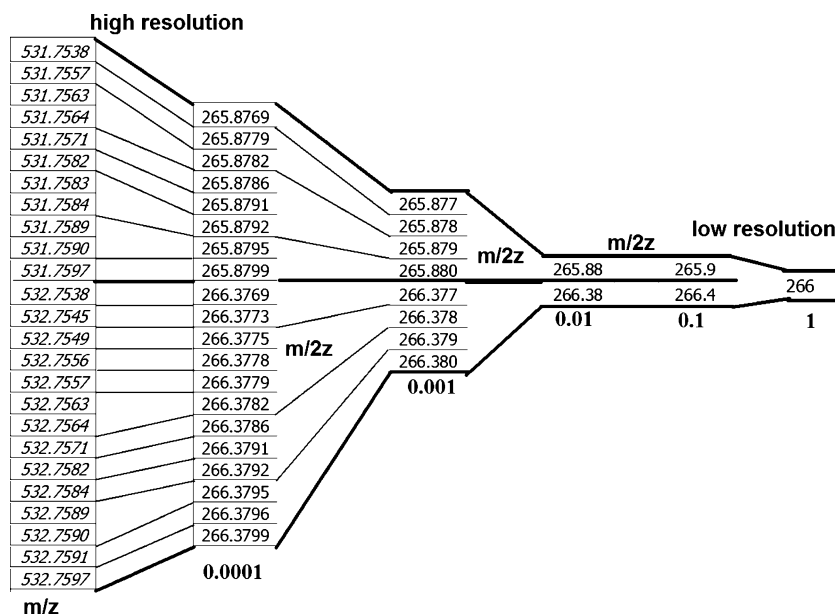

Fig. 5 Transformation of the nearby located sub-clusters of single-charged high-resolution cluster into the doubly charged ion isotopomeric pattern (low-resolution spectra)

Table 1 Prediction of the high-mass cluster of the ion M^+ into the low-mass pattern of M^{2+}

Experimental clusters					Predicted cluster
'High-mass'		'Low-mass'			'Low-mass'
D_{538}		d_{269}			t_{269}
m/z (u.)	P_i (%)	m/z (u.)	P_i (%)	P_{norm} (%)	P_i (%)
528	1.48				
529	2.11				
530	5.62	265	1.56	5.35	8.32
531	8.59				
532	20.31	266	4.30	18.28	22.93
533	21.87				
534	48.44	267	9.38	45.69	50.03
535	37.50				
536	81.25	268	15.62	78.7	83.31
537	48.44				
538	99.99	269	18.75	100	100.00
539	35.16				
540	83.59	270	14.06	78	74.99
541	23.44				
542	32.81	271	5	32.22	26.67
543	8.59				
544	5.70	272	2.66	9	14.19
545	1.56				

A picture of the high-resolution sub-cluster contains the local dominant peaks according to the formula:

$$I_{l-r} = \overset{(m/z+0.49)}{\underset{i=(m/z-0.5)}{MAX}} (I_p) \qquad (4)$$

All other peaks are invisible as they are masked by the dominant peak.

The detailed effects of the transformation are shown in Figs. 4–5. The peak intensities at each step of the procedure are integrated in the area depending on the spectrometer resolution. The clusters are simultaneously moved to $m_i/2$ values.

These two transformations generate significant problems for interpretation of doubly charged ions. The effects are presented graphically in Fig. 5. A mass spectral detector works as an integrator as shown by comparison of the high- and low-resolution spectrum. Some nearby-positioned mass isotopomers from the high-resolution pattern are converted in the low-resolution cluster into the mass and position isotopomers. The good agreement of the experimental and predicted 'low-mass' clusters support the working hypothesis. The data for all the clusters considered are presented in Table 1. The similar intensities indicate that the predicted model is comparable to the experimental model. The low

value of the variance parameter calculated according to Hertz et al. [17], $s^2=16.12$, demonstrates good agreement with the experimental data and confirms the adequacy of the working hypothesis.

The results are confirmed by a graphical comparison of experimental and calculated 'low-mass', low-resolution patterns (Fig. 6).

Surprisingly, predicting the high-resolution mass cluster is simpler than predicting the low-resolution one. Perhaps
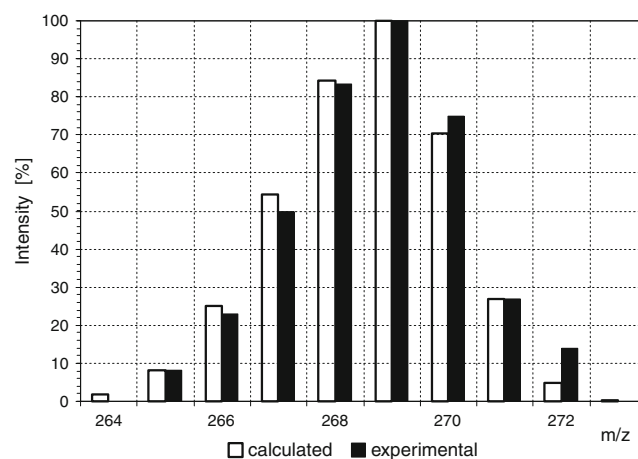
Fig. 6 Modelling effects of the $C_{24}H_{12}Se_3^{2+}$

the mechanism of low-resolution pattern formation proceeds through an initial high-resolution form.

Conclusions

Doubly charged ions can be observed at a location lower than M/2 in the mass spectrum. It is assumed that the structure of their isotopomeric patterns might originated from the high-resolution forms of the single-charged ion of the same formula. The method described here was applied to the modelling of a low-resolution mass ion cluster, which was presented at a previous MDMM conference [14].

The starting points for the prediction are the mass defect values and summary formula of the ion charged ±1. The first step in the procedure generates an accurate mass of the isotopomeric pattern of the 'high-mass' pattern. The next stage in the modelling predicts the form of a doubly charged ion located in the low-mass range of the spectrum. A significant problem is the purity of the experimental cluster of the doubly charged pattern.

Calculation of the high-resolution mass cluster is simpler than predicting the low-resolution one, and the mechanism of low-resolution pattern formation has at its source the high-resolution initial form.

References

1. doLago CL, Kascheres C (1991) New method of isotope pattern analysis. Comput Chem 15:149–155
2. Henderson W, McIndoe JS (2005) Mass spectrometry of inorganic, coordination and organometallic compounds. Wiley, Chichester, pp 236–246
3. Gorączko AJ (2005) Molecular mass and location of the most abundant peak of the molecular ion isotopomeric cluster. J Mol Model 11:271–277
4. Gorączko AJ (2007) Isotope patterns. In: Gross ML, Capriolli RM (eds) Encyclopedia of mass spectrometry, vol 6. Elsevier, Amsterdam, pp 62–76
5. Vekey K (1995) Multiply charged ions. Mass Spect Rev 14:195–225
6. Senko MW, Beu SC, McLafferty FW (1995) Automated assignment of charge states from resolved isotopic peaks for multiply charged ions. J Am Soc Mass Spectrom 6:52–56
7. Mann M, Meng CK, Fenn JB (1989) Interpreting mass spectra of multiply charged ions. Anal Chem 61:1702–1708
8. Hagen JJ, Monnig CA (1994) Method of estimating molecular mass from electrospray spectra. Anal Chem 66:1877–1883
9. Smith RM, Busch KL (1999) Understanding mass spectra—a basic approach. Wiley, New York, pp 46–48
10. Gorączko AJ (2007) Modeling of isotopomeric molecular clusters in electron ionisation mass spectra. J Mol Model 13:813–821
11. Gorączko AJ, Szymura JA (1999) The prediction of relative abundance of isotopic clusters in mass spectrometry of coordination and organometallic compounds. Comput Chem 23:132–142
12. Gorączko AJ (1998) Simplification of mass spectra of organometallic and coordination compounds by cluster profile analysis. Comput Chem 22:499–508
13. Silverstein RM, Webster FX, Kiemle DJ (2007) Spektroskopowe metody identyfikacji związków organicznych. PWN, Warszawa
14. Gorączko A (2006) Modeling of isotopomeric molecular clusters in electron ionisation mass spectra. In: Proceedings of "Modelling and design of molecular materials" 10–15 September 2006, Wrocław, Poland
15. NIST-2000 #112461, CAS 107210822, ID#61932, Egestad B, Dept Physiol Chem Karolinska Inst Stockholm, Sweden
16. Goraczko A, Szewczykowska K (2008) Detection of symmetrical decomposition of the molecules – isotopometric analysis of the M/2 clusters. In: Proceedings of "Modelling and Design of Molecular Materials" 23–28 June 2008, Piechowice
17. Hertz HS, Hites RA, Bieman K (1971) Identification of mass spectra by computer searching a file of known spectra. Anal Chem 43:681–691