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Mass spectrometry for chemists and biochemists

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INTRODUCTION

'Trout made into fish cakes is still trout'
Muggles in *The Minnipins*, Carol Kendall (1959)

The basis of mass spectrometry is the production of ions from neutral compounds and the examination of the subsequent decomposition of those ions. In mass spectrometry, a substance is characterized by investigating the *chemistry* of ions resulting from that substance. Because the technique involves a chemical reaction, the sample being investigated is not recoverable; however, only very small quantities of material are required for the analysis. Most other physical methods of analysis deal with a narrowly defined property of a molecule, but this is not so in mass spectrometry. As with any chemical reaction, the precise outcome is dependent on a considerable number of factors, such as temperature, concentration, effects of the medium and so on. It is this uncertainty which lends mass spectrometry its versatility, intricacy and charm.

Chemical reactions in solids, liquids and the gas phase are usually discussed in terms of isolated molecules whereas the actual behaviour is the result of 'group' effects arising from collisional activation of molecules. Most mass spectra are measured at low pressures when collisions between ions and molecules are rare so that interpretation of the mass spectra in terms of isolated species is more satisfactory. For practical purposes, these low concentrations of ions impose severe restraints on methods of investigating them. The concentrations of ions are so low that other common physical methods such as ultraviolet, infrared and nuclear magnetic resonance spectroscopy cannot be used to elucidate the structures and internal energies of the ions. At best, postulated ion-structures can only be described as consistent with indirect evidence based on isotopic labelling, thermochemical arguments and reasoning by analogy. As its name implies, a mass spectrometer measures mass (or strictly mass-to-charge ratio) and gives no direct information on ion structures. Because of this lack of knowledge of structures, fundamental investigation of the mechanisms of ion decomposition has been severely hampered. It is stressed that even without any sound, basic

understanding of ion structures and mechanisms of mass spectrometric fragmentations, a great deal of analytical information may be extracted by use of empirical rules and concepts. At this stage, it is worth reminding the reader that mass spectrometry alone seldom gives a unique solution to a problem and it is best used in conjunction with information obtained by other means.

Following the construction of the first mass spectrometers many years ago, there followed a period of modest interest by physicists and chemists until it was realized by the organic chemist that here was an instrument which could be of immense value to his work. When *Mass Spectrometry for Organic Chemists* first appeared (1972), the technique already held a prime position in chemistry, and particularly in organic chemistry, for elucidation of molecular structures. Both the applications and development of mass spectrometry were, and still are, increasing rapidly. In the intervening ten years, mass spectrometry has been applied with advantage in many other, diverse fields. Notably, its use has spread to biochemistry, medicine and toxicology. The National Gallery in London has taken receipt of a mass spectrometer to aid its investigations concerning conservation, history and authenticity of works of art. Mass spectrometers have travelled as far as, if not further than, any other analytical apparatus since several space probes have carried miniaturized mass spectrometers for chemical analysis of extra-terrestrial atmospheres and soils. Many of these wider applications are due to improved instrumentation. In particular, many recent advances can be attributed to three interconnected factors: (*a*) the linking of mass spectrometers to computers, (*b*) improvements permitting ready combination of mass spectrometers with gas and liquid chromatographs and (*c*) development of sophisticated means of producing ions from neutral species. These three topics are accorded separate chapters in this book. Included in category (*c*) is the production of negative ions. In mass spectrometry, positive ions have been investigated more thoroughly than negative ones but studies of negative ions are increasing owing to improvements in the efficiency of their generation and to the complementary analytical information gained. Therefore, the majority of the text concerns positive ions but discussion of negative ions is included where appropriate.

The present text was conceived as a simple up-dating of *Mass Spectrometry for Organic Chemists*, but the increasing applications of the technique begged a wider scope for the book and the newer developments necessitated considerable rewriting and expansion. Therefore, it is appropriate that the title of the text be amended to accommodate these changes and *Mass Spectrometry for Chemists and Biochemists* was selected. To write a simple introductory text to this subject is a somewhat uncomfortable task since it becomes necessary to make statements which may be arguable, but the authors are of the opinion that simplicity and accuracy are not mutually

exclusive in this context. After an introductory chapter, instrumentation for mass spectrometry is described briefly, then there follows a new chapter about the application of computers to mass spectrometry. Combined gas chromatography/mass spectrometry, being a routine and important tool in many different fields, is described in some detail in chapter 4. Technologically, combined liquid chromatography/mass spectrometry is less advanced but its use and worth are growing rapidly with developments in methodology. Hence, it is covered in the same chapter with emphasis on technique. A separate chapter was considered preferable to scattered references to the important topic of derivatization as an adjunct to mass spectrometric investigations. This subject is discussed in chapter 5, which contains a section on derivatization of inorganic compounds. One trend discernible in mass spectrometry is away from simple identification of unknown substances and towards a quantification of known compounds and more fundamental mechanistic studies. Chapter 6 is devoted to the first of these aspects and will be of most use to those interested in life sciences and analytical chemistry. The following three chapters are in part concerned with more fundamental topics such as elucidation of fragmentation mechanisms and ion structures, and measurement of thermochemical and kinetic parameters. In chapter 7, some additional methods of generating ions are discussed (with otherwise intractible substances in mind) whilst chapter 8 covers the special case of metastable ions. These ions are at the heart of several methods of analysis and will play an expanding role within mass spectrometry in the future because they can be used to probe not only the products of mass spectrometric reactions but also the reactions themselves. In this introductory text, it has been felt necessary to include some theoretical aspects of mass spectrometry as well as its empirical applications, because, by understanding fundamentals, an empiricist may make better use of the method. Such an approach is embodied in chapter 9. The next two chapters (10 and 11) deal with empirical application of mass spectrometry to structural elucidation, which forms a large part of routine mass spectrometry for the typical user. The examples of structural elucidation were chosen to illustrate many of the modern methods of mass spectrometric analysis. Throughout, the various aspects of mass spectrometry are introduced and explained briefly, but when more detailed explanations are desirable, these have been included in chapter 12 (Further discussion of selected topics). Miscellaneous topics which would not be in context elsewhere in the book are also included in this chapter.

The book is suitable for those without any prior knowledge of mass spectrometry and, whilst being an introductory text, does provide an approach to the more advanced topics for the interested reader. As part of this approach, an extensive bibliography is included, facilitating consultation of further, more specialized literature. For the convenience of the reader, a simple table of quantitities (conversion factors) appears at the front of the book.

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Table of quantities

1 kcal = 4.184 kilojoules (kJ)
1 electron volt (eV) = 1.602×10^{-22} kJ
1 torr = 133.3 newtons per square metre (Nm^{-2})
1 microgramme (μg) = 10^{-6} g
1 nanogramme (ng) = 10^{-9} g
1 picogramme (pg) = 10^{-12} g
1 femtogramme (fg) = 10^{-15} g

List of abbreviations

ADC, analogue-to-digital converter
CI, chemical ionization
DAC, digital-to-analogue converter
EI, electron impact
FD, field desorption
FI, field ionization
GC/MS, combined gas chromatography/mass spectrometry
IKES, ion kinetic energy spectroscopy
LC/MS, combined liquid chromatography/mass spectrometry
MIKES, mass-analysed ion kinetic energy spectroscopy
PFK, perfluorokerosene
QET, quasi-equilibrium theory
%RA, percentage relative abundance
%RIC, percentage reconstructed ion current
%TIC, percentage total ion current
TMS, trimethylsilyl

CONTENTS

1 The mass spectrum

1.1. Formation

Most research into the mass spectrometry of organic and inorganic compounds has been carried out on positive ions and these are discussed in detail. Negative ions and ion-pair formation are mentioned as the occasion arises.

When a molecule is ionized, a *molecular ion* ($M^{+\bullet}$) is produced and this may contain sufficient internal energy to fragment by ejection of a neutral particle (N) with the formation of a *fragment ion* ($A^{+\bullet}$ or A^{+}). A neutral molecule gives a radical-cation as the molecular ion, and the fragment ion may be a cation or a radical-cation. The ejected neutral particle (N) may be a radical or neutral molecule.

$$M \xrightarrow{-e} M^{+\bullet} \longrightarrow A^{+} + N^{\bullet}$$

or

$$M \xrightarrow{-e} M^{+\bullet} \longrightarrow A^{+\bullet} + N$$

If the fragment ion (e.g. A^{+}) has sufficient internal energy, then further decomposition may occur with the formation of new fragment ions (B^{+}, C^{+}, etc.) until there is insufficient internal energy in any one ion for further reaction.

$$M^{+\bullet} \xrightarrow{-N^{\bullet}} A^{+} \xrightarrow{-N_a} B^{+} \xrightarrow{-N_b} C^{+} \xrightarrow{-N_c} \text{etc.}$$

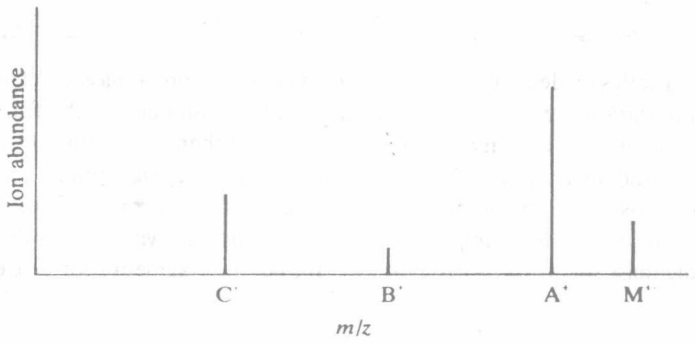
Such a series of decompositions when elucidated from a mass spectrum is a *fragmentation pathway*. The molecular ion ($M^{+\bullet}$) and any of the fragment ions (A^{+}, B^{+}, C^{+}, etc.) may decompose by more than one pathway. The various fragmentation pathways together compose a *fragmentation pattern* characteristic of the compound under investigation. At one extreme, the fragmentation pattern might consist of only one pathway and result in a very simple mass spectrum. At the other extreme, the fragmentation pattern

contains many, often interlocking pathways producing a complex spectrum. The extent to which fragmentation takes place along the individual pathways is determined by the amount of internal energy originally imparted to the molecular ion ($M^{+\cdot}$), its structure, and by the time allowed between ion formation and detection. Hence, the *mass spectrum* is not simply the fragmentation pattern but is the appearance of the fragmentation pattern at specified energies and times.

A mass spectrometer is designed to separate and measure the masses of ions by making use of their mass-to-charge (m/z) ratios. An ion is usually formed with a single positive charge ($z = 1$) so that m/z is then equivalent to m and gives the mass of the ion directly. Until recently, e and not z has been used to represent the charge on the ion. Accordingly, many existing publications represent the mass-to-charge ratio as m/e. The term m/z is used here to conform with recent recommendations (section 12.5).

During any one interval of time, molecular ions will be produced with various internal energies. Each molecular ion will fragment at a rate determined by its initial energy because, at the low pressures normally obtaining in an ion source, ion/ion and ion/molecule collisions are rare so that collisional equilibration of internal energies does not occur (except in chemical ionization - see later, sections 2.3 and 7.2). Some molecular ions may have insufficient energy to fragment whereas others have so much energy that decomposition proceeds right through a fragmentation pathway. Because of the initial range of internal energies in the molecular ions, a short period of time after ionization would see the presence of ions $M^{+\cdot}$, A^+, B^+, C^+, etc. in amounts determined by their individual rates of formation and decomposition and the initial energy imparted to M. If a sample of these ions in the ion source is obtained and their relative amounts measured, the results can be displayed with m/z values as abscissae and ion abundances as ordinates (figure 1.1). Such a picture of a sample of the ions in the ion source is the basis of the

Figure 1.1. Abundances of ions $M^{+\cdot}$, A^+, B^+ and C^+ in sample from ion source.



mass spectrum which may be recorded on a cathode ray tube, paper chart, photographic plate or via a computer. An example of part of a mass spectrum recorded as peaks on a paper chart is shown in figure 1.2. As a standard practice, it is usual to make a record of a mass spectrum in either a *normalized* (or *percentage relative abundance*, % RA) form or as a *percentage of total ion current* (% TIC). In a normalized record, the biggest peak in the spectrum is called the *base peak* and its height is put equal to 100 units; the relative heights of all other peaks are referred to this base peak and lie between 0 and 100 units (note that the base peak is not necessarily the molecular ion peak although it may be). The height of a peak represents the abundance of ions at that particular *m/z* value. Table 1.1 illustrates part of the mass spectrum of 1, 3-dimethylbenzene recorded in this way and figure 1.3 shows the same spectrum as a normalized line diagram. Very often not all the peaks in a mass spectrum appear in the normalized line diagram since peaks of less than one per cent of the size of the base peak are frequently arbitrarily omitted as unimportant. Care must be exercised in this respect because, even though ions may not be abundant, they can be important for elucidating structures from mass spectra. For example, the molecular ions of a compound undergoing extensive fragmentation will have low abundance but are still a very important feature of the spectrum. Fragment ions may decompose at about the same rate as they are formed when again their abundance would be low but they could be important for unravelling a fragmentation pathway. For compounds of high molecular weight, the low mass ions (e.g. below *m/z* 50) may be numerous and abundant but also may have little value for the interpretation of a spectrum; such low mass ions are sometimes omitted from the normalized line diagram.

To obtain a record of a mass spectrum by the percentage of total ion current method, abundances of all ions giving peaks of significant size, from the molecular ion down to a suitably chosen low mass (often around *m/z* 40, except in chemical ionization – see later, section 7.2), are added together as a

Table 1.1. *Normalized ion abundances*[a] *in the mass spectrum of 1,3-dimethylbenzene*

m/z	Abundance	*m/z*	Abundance	*m/z*	Abundance
38	1.2	65	5.5	102	1.1
39	7.1	74	1.1	103	6.1
50	2.8	77	11.3	104	2.6
51	9.1	78	5.1	105	28.7
52	5.0	79	6.5	106	61.7
53	2.2	89	1.9	107	5.4
62	1.6	91	100.0		
63	4.2	92	7.8		

[a] Ion abundances less than one per cent of the base peak have been omitted.

Figure 1.2. Part of a mass spectrum recorded on photographic paper (the three traces record the spectrum at increasing sensitivities from the lower to the upper trace).

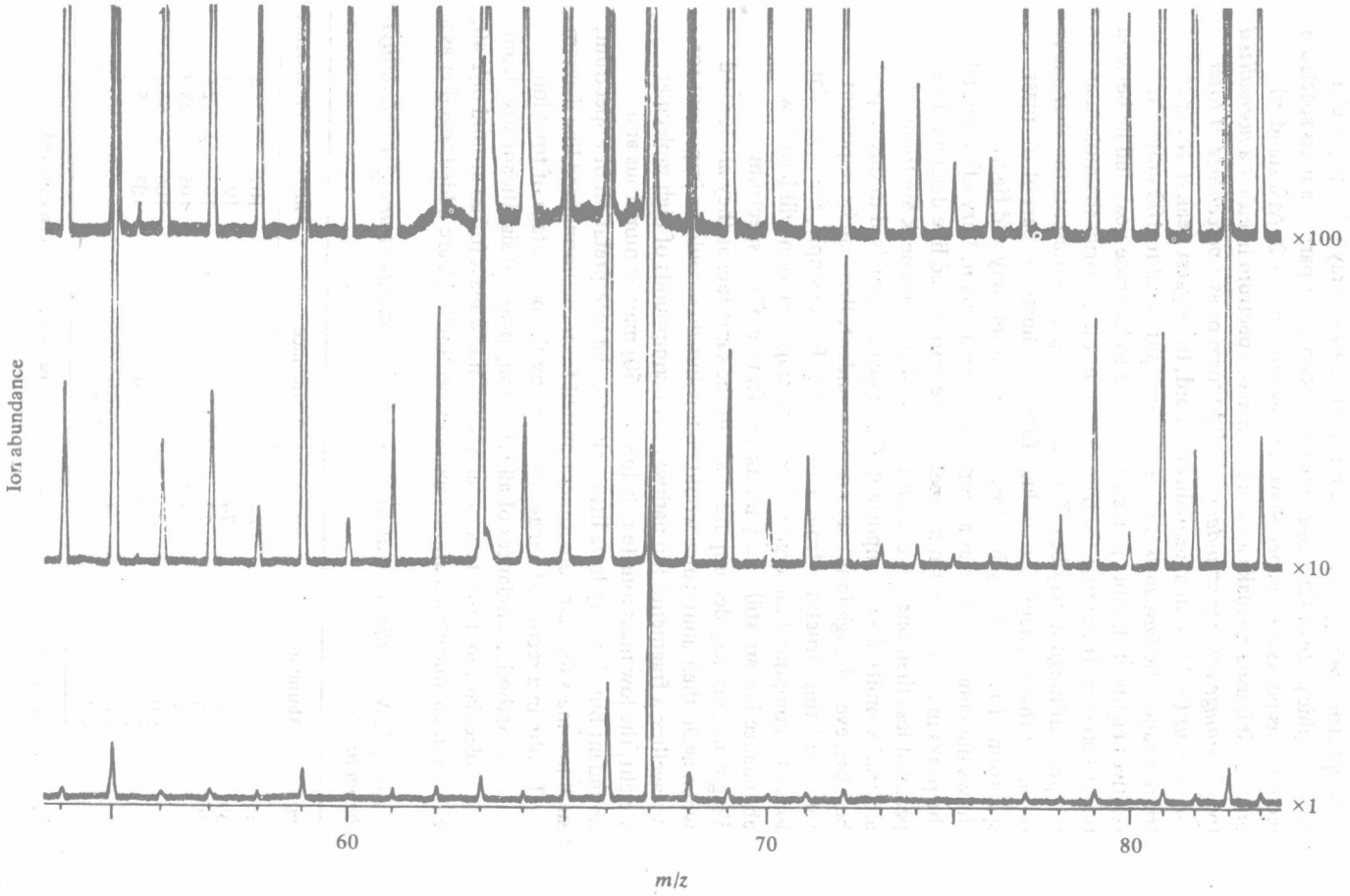


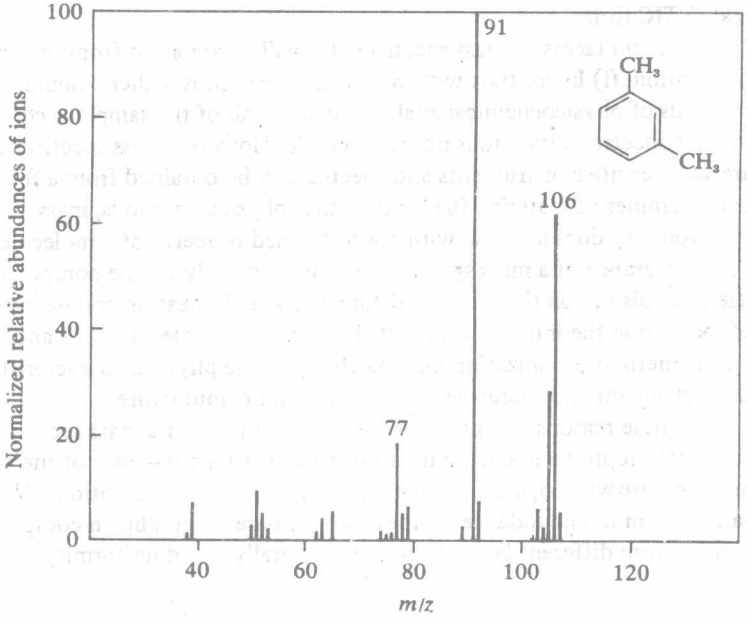
Table 1.2. *Ion abundances*[a] *in the mass spectrum of 1, 3-dimethylbenzene as % TIC*

m/z	Abundance	m/z	Abundance	m/z	Abundance
39	2.5	65	1.9	92	2.7
50	1.0	77	3.9	103	2.1
51	3.2	78	1.8	105	10.0
52	1.7	79	2.2	106	21.4
63	1.5	91	34.8	107	1.9

[a]Ion abundances of less than one per cent are omitted.

measure of the total ion current (TIC). The relative contribution of each ion to this total is then calculated as a percentage (% TIC). Table 1.2 records part of the mass spectrum of 1,3-dimethylbenzene from *m/z* 38 to the molecular ion with abundances of ions as % TIC. This last method of recording a mass spectrum is used less frequently than the normalization method but has some advantages in emphasizing the relative importance of an ion in the whole spectrum. It is important to know this relative importance when the mass spectrometer is used not to record a whole mass spectrum but to monitor the masses of only one ion or a small number of ions in a mass spectrum (see later, chapter 6). Also, the percentage ion current method finds some favour in comparing the spectra of isomers when changes in the relative

Figure 1.3. Line diagram showing normalized relative abundances of ions in the mass spectrum of 1,3-dimethylbenzene; *m/z* 91 = 100 units.



contributions of certain ions to the total ion current may be sufficient to distinguish between the isomers. The difference between the two ways of recording spectra in tabular form is more apparent than real since the relative abundance of a peak in a normalized spectrum is simply related numerically to its value as a percentage of total ion current. However, if the spectrum has been processed to remove 'background' ions of constant, unwanted impurities such as chemical ionization reactant gases (sections 2.3 and 7.2) or decomposition products of the stationary phases of gas chromatographic columns in combined gas chromatography/mass spectrometry systems (sections 2.2 and 4.2), this simple relationship may no longer exist. If such processing has been performed then the % TIC method is often replaced by an equivalent method: the percentage reconstructed ion current (% RIC). This latter sums the ion current from all consequential ions remaining after subtraction of 'background' ions. It should be noted that the normalization method is likely to be the less accurate because a small irregularity in recording or measuring the base peak will markedly affect all the other ion abundances normalized against it. The measurement of height of the base peak is prone to error, as caused for example by the recorder going off-scale. Usually, the percentage of total ion current method is virtually unaffected by such anomalies and is to be preferred, particularly if the mass spectral data are acquired and processed by a computer when the greater numeric difficulty of the method is unimportant. Most data systems allow either or both methods to be selected, with presentation of the spectrum in tabular or diagrammatic form. Table 1.3 shows a computer output listing of the mass spectrum of decahydronaphthalene in both % RA and % TIC forms.

Two main facets of mass spectrometry will be apparent from the above description. (i) In contrast with what happens in most other common methods of physicochemical analysis, some or all of the sample is consumed in mass spectrometry and is not recoverable. However, mass spectrometers are very sensitive instruments and spectra may be obtained from a few nanogrammes of material. (ii) Unlike other physical methods, mass spectrometry does not deal with a well-defined property of a molecule. The appearance of a mass spectrum depends not only on the compound itself but also upon the interval of time between ionization and detection of ions, upon the initial energy distribution in the molecular ions and hence on the method of ionization, and partly upon the physical characteristics of the instrument and therefore on its design and manufacture.

For these reasons it is not possible to guarantee that a mass spectrum is accurately reproducible from instrument to instrument – even of the same manufacture when operating under apparently identical conditions. Normally, variations in ion abundance of a few per cent are acceptable in comparing spectra from different laboratories, but generally, good uniformity is found

Table 1.3. *Ion abundances*[a] *in the mass spectrum of decahydronaphthalene*

Mass	% RA	% TIC
41	64.02	5.25
42	9.79	0.80
43	7.19	0.59
50	0.67	0.06
51	3.02	0.25
52	1.82	0.15
53	12.99	1.07
54	21.23	1.74
55	46.84	3.84
56	25.85	2.12
57	1.94	0.16
63	0.46	0.04
65	3.73	0.31
66	8.24	0.68
67	100.00	8.20
68	59.97	4.92
69	35.17	2.89
70	3.19	0.26
71	0.45	0.04
77	5.02	0.41
78	1.08	0.09
79	9.43	0.77
80	5.99	0.49
81	86.55	7.10
82	78.53	6.44
83	15.70	1.29
84	17.16	1.41
85	0.82	0.07
91	2.14	0.18
93	2.87	0.24
94	3.50	0.29
95	60.05	4.93
96	99.19	8.14
97	11.35	0.93
108	0.81	0.07
109	30.31	2.49
110	9.07	0.74
111	0.42	0.03
123	0.89	0.07
137	0.91	0.07
138	99.35	8.15
139	10.37	0.85
140	0.31	0.03

[a] All peaks over *m/z* 40 of greater than 0.3 percentage of relative abundance are included.

and not too much difficulty is experienced from small inconsistencies. The popular method of identifying compounds by matching sample spectra against standard mass spectra stored in a computer library (section 3.4) is a testament to this.