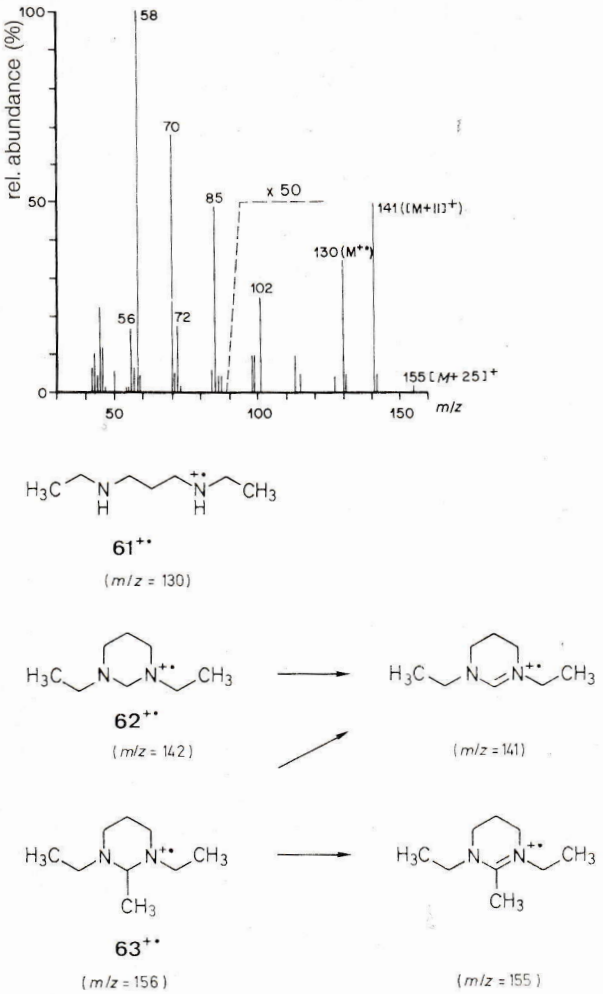


Fig 4.37 The $[M+11]^+$ peak. Mass spectrum of a mixture composed of ca. 99.4% *N,N'*-diethyl-1,3-propanediamine (**61**; M = 130), ca. 0.3% 1,3-diethylperhydropyrimidine (**62**; M = 142) and ca. 0.3% 1,3-diethyl-2-methylperhydropyrimidine (**63**; M = 156), gas inlet, 70 eV. The relative amount of each compound in the mixture was determined by GC

important to point out that the operator stops recording a spectrum when a mass spectrum is obtained which appears to be "sensible". However, if this is only the spectrum of an impurity about which the operator has not been informed, then only time is wasted and the desired information is not forthcoming. For important preliminary information, mass spectra of mixtures (e.g. chromatographic fractions of organic natural materials) can deliver valuable data. The handling of contaminated samples can be facilitated by using a mass spectrometer equipped with a temperature programmed mode. In the following sections, a few frequently encountered contaminants will be discussed[7].

6.1 Solvent

Organic substances are frequently contaminated with solvent residues. This can occur in crystals, lacs and undistilled oils. Usually they vaporise before the actual substance. The spectra of a few solvents are illustrated in Tab. 4.11 (p. 297).

6.2 Foreign Substances in Solvents

For various reasons commercial solvents frequently contain impurities or additives, which can subsequently be found again in samples of substances. To be noted in particular are chloroform (stabilised with ca. 2% ethanol), denatured ethanol (most common additives: benzene or methanol), petroleum ether (contains heavier and therefore less volatile hydrocarbons), diverse ethers (stabilised with 2,6-di(*tert*-butyl)-4-methylphenol, see Tab. 4.12, p. 301) and carbon tetrachloride (upon long standing in the presence of light, various products are formed from dichlorocarbene CCl_2). Because solvents are usually used in a great excess, compared with the amount of a substance, any impurities that are present under these circumstances will become quite significant.

The following example convincingly shows how incorrect conclusions can be made during the evaluation of spectra from contaminated samples.

Nearly all commercially available grades of methanol contain varying amounts of formaldehyde, although usually only in fractions of parts per million. Now there are substances which react quantitatively with even the smallest quantities of formaldehyde, e.g. 1,3-propanediamine. *N,N'*-diethyl-1,3-propanediamine (**61**) reacts with formaldehyde to form 1,3-diethylperhydropyrimidine (**62**). Although the molecular ion of **61** ($m/z = 130$) produces a very weak signal, the $[M-1]^+$ signal of **62** at $m/z = 141$ is extremely strong. (The $M^{+\bullet}$ ion of **62** also produces a very weak signal.) If both compounds are present as a mixture, their mass spectra will be superimposed. Such a case is shown in Fig. 4.37. In this spectrum, the signal abundances for $m/z = 130$ ($M^{+\bullet}$ from **61**) and $m/z = 141$ ($[M-1]^+$ from **62**) are, in terms of their order of magnitude, the same. However, a gas chromatographic measurement determined the ratio of [**61**]:[**62**] to be 99.4:0.3, from which it can be concluded that **62** is present as an impurity with an extremely small concentration. The main fraction of this "mixture" is therefore not in agreement with the result of a superficial mass spectrometric analysis which shows the presence of the straight-chain diamine **61** and the formaldehyde condensation product **62** as a secondary component in an approximately 1:1 ratio, and a third substance (**63**).

Compound **62** can be recognised by an $[M-1]^+$ signal at $m/z=141$. If this signal originated from **61**, it would indicate an $[M+11]^+$ peak. Such signals are almost always observed in the spectra of 1,3-propane- and 1,2-ethanediamine derivatives when methanol is used as the solvent, because methanol is easily oxidised by the oxygen in the air. Formaldehyde reacts equally well with 1,3-propane- and 1,2-ethanediamines, however it does not react in the same way with 1,4-butanediamines.

The contamination of samples can be prevented most easily by employing specially purified solvents (even when this means more work for the chemist!).

6.3 Foreign Substances in Reagents

A few reagents also contain stabilising agents that must be removed carefully from the sample after a successful reaction. These are, among others, kerosene in $LiAlH_4$ and oil in syntheses involving KH and NaH (Tab. 4.12, p. 303). Substances recovered after NMR experiments can still contain tetramethylsilane (TMS).

6.4 Materials from Laboratory Apparatus

Many pieces of laboratory apparatus are made completely or partially from synthetic polymers. These contain various softeners which can be leached out, particularly by solvents (chloroform, among others, is an ideal leachant). The quantity of softeners leached in this way can sometimes be considerable. Of the plastic components, those that should be mentioned in particular are stoppers of flasks, glass bottles, etc., all types of tubing, particularly that plastic tubing which is coloured like rubber tubing, stopcocks, seals, plastic bottles and containers, and incompletely polymerised substances from ion exchangers. (It should be mentioned that even natural rubber is not inert to organic solvents, however the impurities that are leached from it only contribute to a "general background" in the mass spectrometer and do not produce a spectrum of a characteristic and specific compound.) Other sources of contaminants are, among others, isolating liquids in apparatus (e.g. hydrogenation apparatus), stopcock grease and lubricants from fans (e.g. from in-room air conditioners). In addition, filter papers that have not been specially cleaned, adsorbents and chromatographic materials of all kinds should not be neglected as sources of contamination. From the chemical point of view, the principle component in softeners is phthalic acid diester (see Tab. 4.12, p. 302 and Fig. 4.32, p. 244), which has $m/z=149$ as the base peak of its mass spectrum (if such a peak is found in a spectrum, it should be assumed that the sample contains a phthalic acid diester; only when evidence has been produced to show that the sample is pure, can one assume that $m/z = 149$ actually represents a fragment ion signal from the sample being analysed!).

6.5 Contaminants from Thin-layer Chromatography Plates

High activity TLC plates that are produced or kept in the laboratory are also excellent absorbers of substances that are present in the laboratory air. TLC plates can absorb, among other things, oil vapours emanating from oil-filled vacuum pumps which are present in most laboratories. During the subsequent extraction of chromatographed substances from the adsorbent, these materials will also be eluted and will once again contaminate the freshly purified compound.

In a few cases it is difficult to differentiate between mixtures that are present from the beginning or those which have just been thermally produced in the mass spectrometer (see also Sec. 5, p. 246).

If one suspects or knows the possible sources of contaminants, then the chemist is easily able to develop procedures for the prevention or removal of impurities. The problem of impurities in samples has been discussed here because of the high detection sensitivity of the mass spectrometer, however it is not solely a mass spectrometric problem. Contaminants can also be the source of erroneous information from other methods of analysis (IR, NMR, UV, ORD, etc.).

7. Isotopic Labelling Reactions

The specific labelling of functional groups or their environment is an experimental technique that is employed frequently for spectroscopic, kinetic, bioorganic or mechanistic investigations. The employment of ^{2}H (D), ^{13}C, ^{15}N and ^{18}O labelling is particularly favoured. Today, the range of commercially available reagents and compounds which contain these isotopes is very large, so that a multitude of labelling experiments can be conducted. It is the nature of things that, aside from a few examples, the synthesis of ^{13}C- and ^{15}N-labelled compounds usually necessitates a great deal of effort, whereas D-labelling is possible with much less labour.

Labelled compounds are used in order to identify special functional groups or their position within a molecular assemblage, to investigate chemical or biochemical reaction mechanisms, or to clarify the mechanisms of mass spectrometric fragmentation reactions.

Important, constantly recurring reactions are summarised in the following sections. There are a few trivial, but important, comments that should be made at the outset. These are that, under the same conditions, H/D exchange reactions are also D/H exchange reactions, that the air contains a considerable amount of H_2O and that glass and other containers at room temperature are coated with a film of H_2O.

7.1 H/D Exchange Reactions

Acidic Protons

Protons that are bonded to heteroatoms, as in the groups $-NH_2$, $=NH$, $-CO-NH_2$, $-COOH$, $-OH$ and $-SH$, exchange very easily with deuterons. For this purpose, samples are evaporated several times with D_2O, CH_3OD, etc. under high vacuum (not the vacuum from a water siphon!) and finally introduced into the mass spectrometer, whereby D_2O or CH_3OD is introduced simultaneously into the gas inlet system. The number of exchanged deuterons can be determined from the shift in mass of the molecular ion. Under these conditions the exchange is rarely quantitative. Therefore, the chances of obtaining clear proof that more than three protons have been exchanged are normally not very likely.

Aromatic Protons

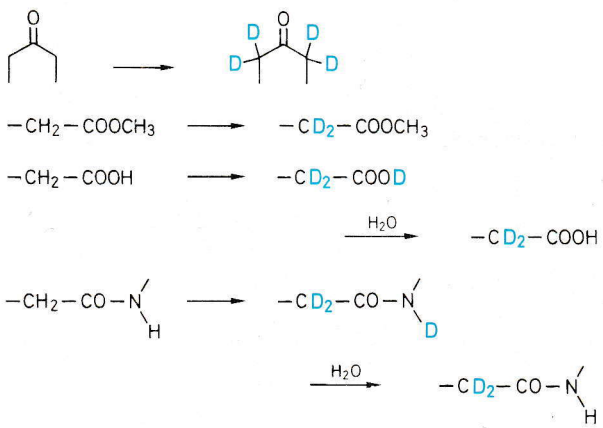
Aromatic protons can be exchanged by an electrophilic aromatic substitution with DCl/D_2O, D_3PO_4 or D_2SO_4.

By employing a final washing with H_2O or CH_3OH, the functional groups with acidic protons (e.g. $-OH$), should any be present in the compound, will be converted back to proton-containing residues.

Example: Evaporate the sample [60 mg (4-phenyl)butylamine] three times (high vacuum or dry N_2) in the reaction vessel, each time with 1 ml CH_3OD (for the removal of H_2O), then add 5 ml 38% DCl/D_2O. Heat at 150 °C for 30 h in a sealed tube, then dilute with 10 ml D_2O. Neutralise with anhydrous sodium carbonate (additionally heated); extract with ether; dry the ether extract with sodium carbonate; evaporate to dryness and distil the residue. After repeating the entire procedure once, a nearly quantitative insertion of 5 *D*-atoms into the aromatic ring is achieved.

Carbonyl Groups With α-Positioned Protons

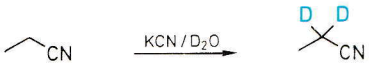
Due to the formation of enols or enolates, the following transformations can be carried out with acids (DCl, D_2SO_4, D_3PO_4, etc.) or bases ($NaOD$, CH_3ONa/CH_3OD, Na_2CO_3/D_2O, etc.):



With all of these reactions, one must be careful to ensure that the neutralisation of the reaction solution is done in the absence of proton donors (H_2O, etc.). In order to remove solvent residues and water, the sample should be evaporated with D_2O or CH_3OD before beginning the exchange reaction.

Example: 100 mg of a ketone was dissolved in a solution of 100 mg sodium in 10 ml CH_3OD; boiled under reflux for 2 h, then neutralised with 20% DCl/D_2O; the resulting precipitate was filtered off, washed with D_2O and the product, after repeating the entire procedure twice, purified by a bulb-to-bulb sublimation (160 °C / 1 Pa).

The exchange of protons that are α-positioned with respect to a nitrile group for deuterons can also be accomplished by using KCN/D_2O or KCN/CH_3OD as the base.

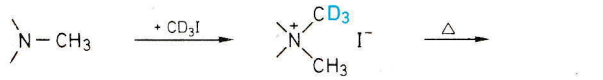


Example: 75 mg 4-phenylbutyronitrile, 81 mg KCN, 4 ml dioxane (all anhydrous) and 3.6 ml D_2O were heated at 165°C under N_2 in a sealed tube for 24 h, then filtered under an inert gas in a bulb; after removal of the solvent, the residue was purified by a bulb-to-bulb distillation (side-product: carbonic acid).

It should also be mentioned that DCl/D_2O has an advantage over many other acidic reagents in that it is readily vaporised and can therefore be separated easily from the compound. The advantage of the exchange reactions just described is that the number of acidic or aromatic protons can be ascertained, which can, from the point of view of a structural analysis, yield important information.

Further Exchange Reactions

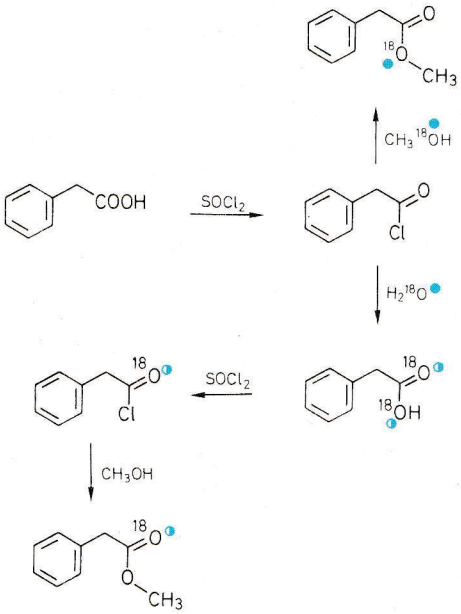
An exchange reaction of a completely different type can be used with tertiary *N*-methyl derivatives.



approx. ratio 1 : 1

Methylation with CD_3I initially yields the quaternary methyl iodide, which, *via* pyrolysis involving the loss of CH_3I and CD_3I, forms the starting material again together with the trideuteromethyl derivative. The ratio of the amounts of each product can depend on steric factors and for achiral products this ratio is 1:1. All of the ions which contain the *N*-methyl group now appear in the mass spectrum as "doublets" (X and X + 3).

The following is an example of an ^{18}O exchange reaction:



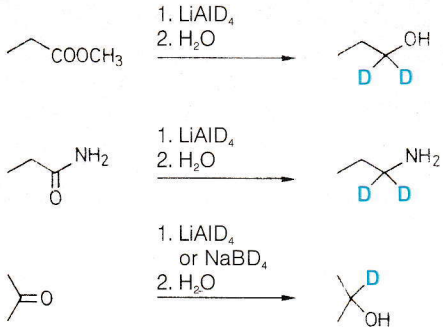
In both of the isotopomeric methyl phenylacetates, the product of the reaction between the acid chloride and $CH_3{}^{18}OH$ has the same ^{18}O content as the reagent. Conversely, the other isotopomer has only half of the isotope content of $H_2{}^{18}O$. Each reaction labels a different *O*-atom.

7.2 Transformations of Functional Groups Under Deuterating Conditions

It is frequently necessary to introduce *D*-atoms at specific positions within a molecule, or to obtain evidence for the existence of a particular functional group in a molecule. A few typical reactions are described below.

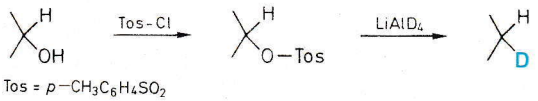
Reduction Reactions

The degree of deuteration of unsaturated compounds with D_2/catalyst is very dependent upon the type and quality, and thus the activity, of the catalyst. In a few cases the correct uptake of 2 *D*-atoms per reduced C=C bond is observed, whereas in other cases a multiple of the theoretically expected number (i.e. without increasing the degree of hydrogenation of the compound) of *D*-atoms is taken up (presumably due to successive dehydrogenation and rehydrogenation reactions). The conversion of functional groups containing *C*-atoms in high oxidation states into functional groups in which these atoms have lower oxidation states can frequently be accomplished with, for example, $LiAlD_4$ and $NaBD_4$.



The reduction of alkoxycarbonyl groups with $LiAlD_4$ and the subsequent work-up in proton-rich solvents frequently results in the inclusion of a somewhat greater number of *D*-atoms than is predicted theoretically. Presumably, before the reduction, additional exchange reactions occur to a small extent at the α-position with respect to the carbonyl group.

The reduction of primary and secondary hydroxy groups can be carried out expediently by the reduction of the tosyloxy derivative with $LiAlD_4$.

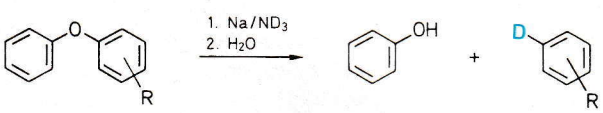


Tos = p–$CH_3C_6H_4SO_2$

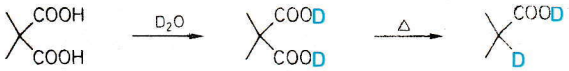
The reduction of ketones with Zn/DCl according to Clemmensen does indeed lead to the formation of the expected dideuteromethylene derivative, but the extent of "over deuteration" (caused by an acid-catalysed enolisation reaction) is too great. Therefore, this reaction is not recommended. An exception is when the carbonyl group has no α-positioned methyl groups or there has already been a complete CH_2/CD_2 exchange.

Benzyl positioned C=O, C–OR and C–N residues can be transformed with D_2/Pd and, in the case of the first two compound types, with $LiAlD_4/AlCl_3$ into di- or monosubstituted derivatives (CD_2, CDH) with good yields and correct D-insertion.

The diphenyl ether cleavage with Na/ND_3 (synthesised from $Mg_3N_2 + D_2O$) is a suitable method for the specific labelling of the sites of aromatic ether linkages.



The decarboxylation of maleic acid derivatives under deuterating conditions produces monodeuterated derivatives[8]:



7.3 Determination of the Degree of Labelling

The aim of this method is the determination of the heavy isotope content (^2H, ^{13}C, ^{15}N, ^{18}O) of the labelled compound, i.e. the establishment of the extent to which the inclusion of these isotopes in the desired compound has been successful. However, before this mass spectrometric determination is carried out, the following analyses should be undertaken:

a) The labelled compound must be chemically homogeneous (melting point, DC, GC) and behave in regard to these properties exactly like the unlabelled compound whose characteristics are to be investigated.

b) Independently from the synthesis, it should be checked spectroscopically (e.g. by IR, ^1H-NMR, ^{13}C-NMR) that the labelled atoms are located at the desired sites in the molecule. Furthermore, a quantitative determination of the D-content can sometimes be carried out by using ^1H-NMR spectra. This can be done when the exact integration of a definite region of resonance is possible. In addition, a combustion analysis where still available, together with an IR spectroscopic determination of the D_2O or HDO concentration, delivers very good results for the total D-content. As a complement to the mass spectrometric determination of the D-content, these procedures give important additional information from which erroneous conclusions are less likely to be drawn.

These analyses are essential in order to obtain the greatest possible value from the information that is available in the mass spectra of labelled compounds.

For the mass spectrometric determination of the degree of labelling of a compound, the molecular ion peak is brought into play. In the ideal case, this peak should have a nicely visible intensity and not be accompanied by the $[M-H]^{+\bullet}$, $[M-2H]^{+\bullet}$ or the $[M+H]^+$ signals. When more abundant accompanying signals are present, overlapping signals will occur for the labelled compound and these signals will make a quantitative evaluation of the spectrum impossible. On the other hand, the margin of error will be too great when evaluating very weak signals that only just appear above the background or general noise of the spectrum. However, if behaviour of this kind is present, the following alternative possibilities are available: the evaluation of low-voltage spectra (in general, in low-

voltage spectra measured at 12 to 15 eV, the $[M-H]^{+\bullet}$ and $[M-2\ H]^{+\bullet}$ signals are weaker and the molecular ion signals are more intense with respect to the rest of the spectrum); the evaluation of the spectra of derivatives, which, under certain circumstances, have a more favourable presentation of the molecular region; or the evaluation of high resolution partial spectra, which have been written down on paper and analysed in conjunction with the elemental composition. In the latter case, a clear separation of the molecular ion signals from the other signals can be obtained. This procedure is limited, however, by the resolving power of the instrument and the abundances of the ions. With this method of analysis, the spectrum of the labelled compound is compared with that of the unlabelled compound. It is therefore important that both spectra are measured under the same recording conditions. (Due to the memory effect, it is essential to take care that the first sample to be measured has passed completely through the mass spectrometer before starting on the second sample.) At least three partial spectra of the molecular ion region are recorded for each substance. Finally, the peak abundances are determined (for practical reasons in mm or %) and separately averaged for each of the isotopomers, so that two sets of spectra are obtained that can be evaluated as described below. [The explanation is given using the example of *N*-(2-phenylethyl)formamide ($C_9H_{11}NO$, M = 149) and its 1-^{13}C-labelled isotopomer. For the synthesis of the latter, a ca. 90% enriched ^{13}C reagent was employed.]

Measurement Results

A) Unlabelled Compound

In the molecular ion region only the signals at $m/z = 149$ and 150 have an abundance greater than 1 rel.%; the average values (from five individual measurements) were $m/z = 149$ (100.00%) and 150 (11.29%).

B) Labelled Compound

The corresponding averaged signal abundances in the molecular ion region are: $m/z = 149$ (10.94%), 150 (100.00%) and 151 (11.11%).

Because there is no signal at $m/z = 148$ for the unlabelled compound, one can presume that $m/z = 149$ in the spectrum of the labelled compound is due to the molecular ion of the unlabelled fraction. The signal at $m/z = 150$ is partially due to the first isotope peak of the unlabelled compound, however the main contribution to this signal comes from the synthetic, singly labelled isotopomer. The ion that produces the first isotope peak of this particle at $m/z = 151$ naturally contains two ^{13}C-atoms and the ratio between the signals at $m/z = 150$ and 151 is proportional to that of the corresponding signals for the unlabelled compound. In this way the averaged spectrum of the unlabelled compound can be subtracted proportionally from that of the labelled compound:

m/z	149	150	151
labelled	10.94	100.00	11.11
unlabelled	10.94 (100)	1.24 (11.29)	
	0	98.76	11.11
		98.76	11.15
		0	−0.04

If the fraction of the unlabelled compound (10.94) and that of the singly labelled isotopomer (98.76) are normalised to 100%, the degree of labelling is obtained as $^{13}C_0$: 10% (9.97), $^{13}C_1$: 90% (90.03). The natural ^{13}C content has not been taken into account in these values. This has been subtracted with the spectrum of the unlabelled compound.

The small "negative" value under $m/z = 151$ can be neglected. Frequently, however, much larger positive or negative values are observed, which make the calculation of the isotope content difficult or even impossible. The causes of such absurdities are frequently impurities or different recording conditions for the two samples (different $[M+H]^+$ and $[M-H]^+$ fractions). It is often possible to reduce or circumvent these difficulties by the evaluation of low-voltage spectra, as described above.

For additional examples and other methods, refer to the literature[9].

In order to establish the degree of labelling of fragment ions, a procedure analogous to that for the evaluation of molecular ions is employed. However, overlapping signals occur more frequently. This problem can sometimes be overcome by the evaluation of high resolution spectra. In low-voltage spectra and the spectra of derivatives, it is possible to find altered ratios of isotope inclusion, which do not correspond with the results from 70 eV spectra, because different fragmentation reactions may occur.

8. Additional Methods and Concepts

Other important concepts and methods of mass spectrometry, which were not discussed in the previous sections, will be explained briefly here. With regard to additional keywords, see the bibliography.

8.1 Chemical Ionisation (CI)

A mass spectrometric ionisation method involving ion/molecule reactions. In principle, a reactant gas (e.g. hydrocarbons, H_2, H_2O, NH_3, alcohols, noble gases) is ionised by electron impact (gas pressure ca. 1 kPa). In the case of methane, the ion $[CH_4]^{+\bullet}$ is formed, which reacts with other methane molecules, e.g.:

$$[CH_4]^{+\bullet} + CH_4 \longrightarrow [CH_5]^+ + CH_3^\bullet$$

(A whole series of additional ions, such as $C_2H_5^+$, $C_3H_5^+$, CH_3^+, etc., are also formed.) At the same time, the molecule to be analysed, M, is also present, although at a much lower concentration. This then reacts with the protonated methane (Brønsted acid), whereby a proton transfer occurs in the gas phase:

$$[CH_5]^+ + M \longrightarrow [M+H]^+ + CH_4$$

$[M+H]^+$ undergoes decomposition reactions and produces a CI spectrum.

If other types of reactant gases are chosen (e.g. noble gases, CO_2, N_2), then a charge exchange occurs (abbr.: CE), instead of the protonation of M:

$$[He]^{+\bullet} + M \longrightarrow [M]^{+\bullet} + He$$

Additional reaction types are electrophilic addition ($M + X^+ \rightarrow MX^+$) and anion extraction ($AB + X^+ \rightarrow B^+ + AX$).

Depending upon the choice of reactant gas, it is possible to control the formation of fragment ions, as shown in Figs. 4.38 and 4.39, which depict spectra obtained from lysine methyl ester (**64**). From this it follows that CI is a soft ionisation method and can therefore be used as an alternative to electron impact ionisation.

Literature review: [10].

An EI and a CI mass spectrum from the same compound can be compared by referring to Figs. 4.56 and 4.57, pp. 271, 272.

64

$C_7H_{16}N_2O_2$, M = 160

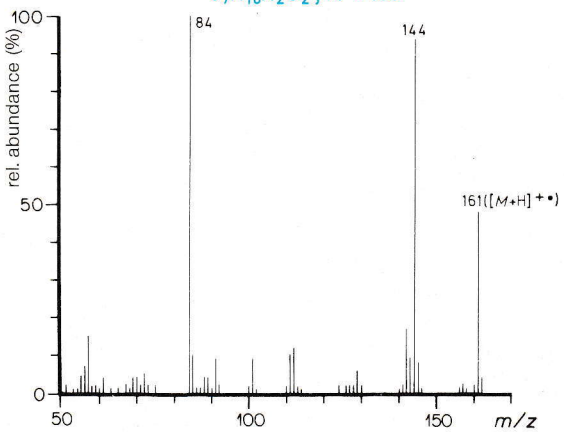
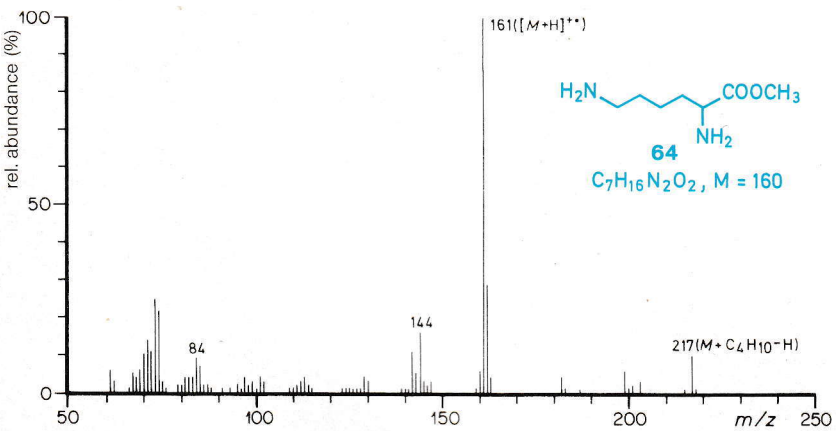


Fig. 4.39 CI mass spectrum of lysine methyl ester (**64**), reactant gas: methane



64

$C_7H_{16}N_2O_2$, M = 160

Fig. 4.38 CI mass spectrum of lysine methyl ester (**64**), reactant gas: 2-methylpropane (isobutane)

8.2 Direct Chemical Ionisation (DCI)

[synonymous terms: in-beam electron ionisation, direct exposure chemical ionisation, flash volatilisation and plasma desorption (not to be confused with the $^{252}_{98}Cf$ plasma desorption technique)]

On the tip of the probe (see p. 221) there is a wire loop (e.g. Pt, Re, W) into which a drop of a dissolved substance is placed, just as is done for field desorption (FD) spectra. The tip is inserted into the mass spectrometer and, after the solvent has been evaporated under vacuum, the thin film of substance on the loop is measured under CI conditions. The spectra resemble both those that are measured under FD conditions (abundant pseudo-molecular ion) and CI spectra (protonation of M, electrophilic addition). The DCI spectrum of glucose (M = 180), with NH_3 as the reactant gas, is shown in Fig. 4.40. To explain the peaks: $198 = [M + NH_4]^+$, $215 = [M + NH_4 + NH_3]^+$. The character of the spectrum is strongly dependent on the temperature of the ion source.

Reference: [11].

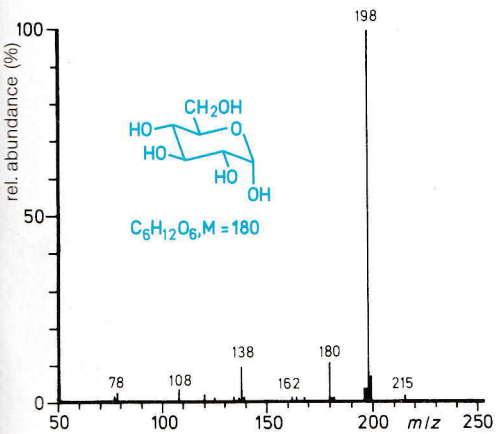


Fig. 4.40 DCI spectrum of glucose (M = 180), reactant gas: ammonia (reproduced with the kind permission of Finnigan MAT, Bremen)

8.3 Electrospray Ionisation (ESI)

In this ionisation method, a solution of a substance is sprayed (flow rate 1 to 20 µl/min) through a capillary into a chamber, cf. Fig. 4.41. A dry gas flows in the opposite direction to this spray mist. A potential of a few kV is applied between the capillary and the chamber wall (cylindrical electrode). Charged droplets are produced which become smaller as the solvent (e.g. CH_3OH/H_2O, CH_3CN/H_2O) vaporises. Driven by the electric

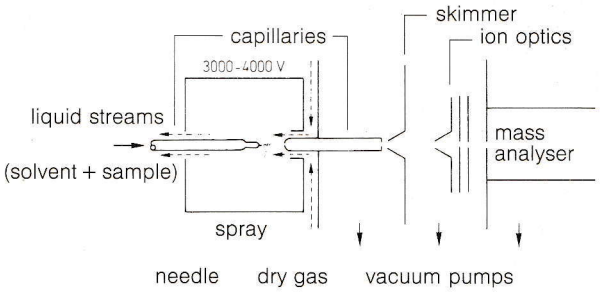


Fig. 4.41 Schematic representation of the electrospray ion source. (Finnigan MAT instrument TSQ-700). The ions, generated by electrospray at atmospheric pressure, are admitted to the mass analyser through a glass capillary, skimmer and ion optics. (Reproduced with the kind permission of Finnigan MAT, Bremen)

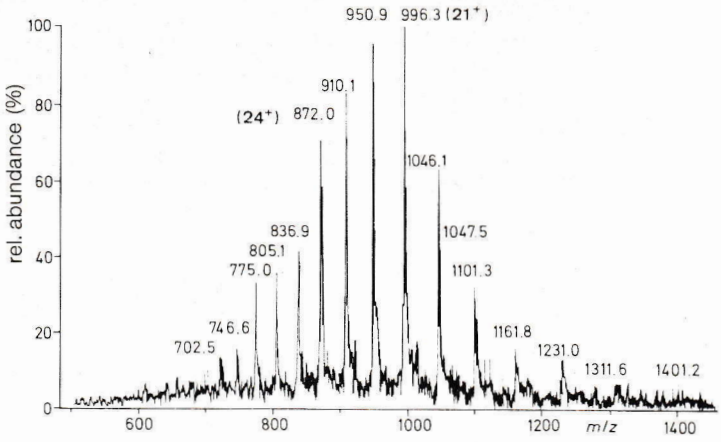
field, the charged droplets move through a glass capillary (ca. 0.5 mm inside diameter) into the pre-analyser region. By focusing the droplets with an electrostatic lens system, they are directed into the analyser of the mass spectrometer (e.g. quadrupole mass spectrometer). The other spray ionisation methods, namely atmospheric pressure ionisation (API)[12] and ion spray[13], are similar.

This procedure results in singly and multiply charged ions, $[M + nH]^{n+}$ and $[M - nH]^{n-}$, where n can be of the order of 100 with suitable molecules. At the same time other molecular ions will also be recorded, which differ successively from each other by one less unit of charge (e). Because m/z ($z = n \cdot e$) is recorded, the instrument's mass scale for displaying the spectra, which is limited to a mass number of ca. 4000, can be used to identify masses higher than 100 000. Neighbouring ion signals, which differ by one charge unit, are used for the calculation of the molecular ions. To illustrate this, the ESI mass spectrum of interleukin 6 (M = 20903) is shown in Figs. 4.42 and 4.43.

The original spectrum contains several differently charged molecular ions (see indicated charges), which, of course, represent only **one** singly charged molecular ion of mass 20903 (Fig. 4.43). The process (relationship between Figs. 4.42 and 4.43) is comparable with the multiple images obtained from **one** person who is standing between two parallel mirrors.

ESI mass spectrometry is a powerful analytical method, because it allows one to analyse the molecular ions of polar and higher molecular compounds in aqueous solution.

Reference: [14].



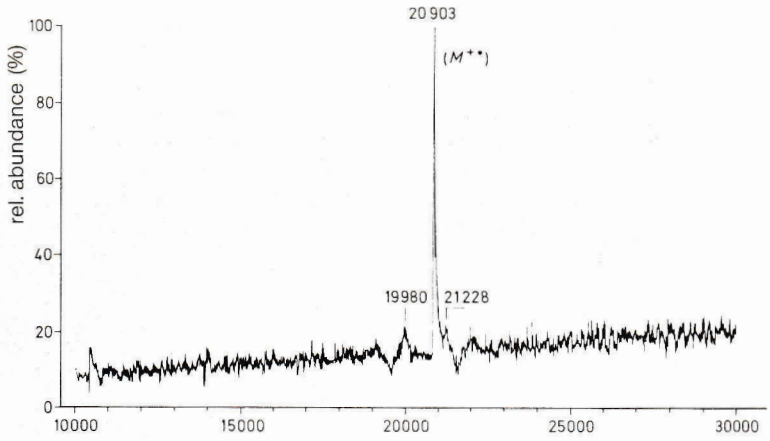


Fig. 4.42 ESI mass spectrum of interleukin 6 (recorded by the Finnigan MAT instrument TSQ-700)

Fig. 4.43 Deconvoluted ESI mass spectrum of interleukin 6 from Fig. 4.42

8.4 Fast Atom Bombardment (FAB)

(also known as liquid secondary ion mass spectrometry, LSIMS)

This is an ionisation method for organic molecules that are either difficult to vaporise or cannot be vaporised. The principle of the method is that fast neutral atoms are shot onto a thin film of the sample in the ion source of a mass spectrometer. The sample ions thereby formed are accelerated, focused and finally analysed with the usual instrumental optics.

The fast neutral atoms (usually argon and less frequently xenon) are generated by a so-called atom gun. Within the gun, $Ar^{+\bullet}$ ions are initially generated by charge separation and then accelerated (5 to 10 keV). They then collide with neutral *Ar*-atoms in a collision chamber, whereby a charge exchange occurs without the loss of very much kinetic energy. This results in a beam of fast $\mathbf{Ar}^0$ atoms. (The fast particles are printed in bold.)

$$Ar^0 \longrightarrow \mathbf{Ar}^{+\bullet} + e^-$$
$$\mathbf{Ar}^{+\bullet} + Ar^0 \longrightarrow \mathbf{Ar}^0 + Ar^{+\bullet}$$

The atom beam is then directed onto the sample film. The sample itself is embedded in a matrix (glycerine is used frequently, although other substances are also suitable, e.g. 3-nitrobenzyl alcohol, thioglycerine), which is placed on a flattened copper tip. The preparation of the sample requires experimental skill and experience.

Upon the arrival of the fast *Ar*-atoms at the surface of the sample, (pseudo)-molecular and fragment ions are formed, which originate from both the substance being analysed and the matrix. (Sometimes pyrolytic processes also occur, which lead to additional ions.) Because the spectrum of the matrix is largely known, this does not disturb the interpretation too severely. However, mutual matrix/sample interactions are also known, which are very dependent upon the nature of the sample. Therefore, it is not possible to correct for these interactions without additional effort. When positive ions are being measured, $[M + H]^+$ and $[M + Na]^+$ ions are usually formed (when measuring negative ions, $[M + H]^-$ ions occur). At the same time, however, [M + glycerine]$^+$ ions also appear. For the determination of the relative molecular weight of an unknown substance, it is advisable, by the addition of sodium chloride or potassium chloride, etc., to generate ions whose masses allow one to home in more easily on the molecular ion of the unknown sample. FAB has been used successfully for the analysis of organic acids (−COOH, $-SO_3H$, $-OPO_3H$) and salts, polypeptides (e.g. the α-amino acid containing peptide, melittin with M=2844.8), oligosaccharides (e.g. γ-cyclodextrin = cyclooctylamylose with M = 1296.4), nucleotides, etc.

The physical explanation of this ionisation process is not completely clear.

Reference: [15].

8.5 Field Desorption (FD)

Under the influence of a strong electric field, positive ions are desorbed from an (activated) wire, onto which a nearly involatile sample has been placed. These ions are then analysed mass spectrometrically. This method is frequently suitable for the determination of the relative molecular weight of polar compounds.

For an example spectrum, see cation addition mass spectrometry (Sec. 8.10, p. 265).

Reference: [16].

8.6 Field Ionisation (FI)

An ionisation method for molecules that employs an extremely high electric field (10^9 to 10^{10} V · m^{-1}). The ionisation occurs at the anode, which is a pointed tip, sharp blade or a very thin wire. Usually the anode is activated before the measurement by surrounding it with a blanket of the finest needles. The method produces, in comparison with electron impact ionisation, more abundant molecular ions and less fragment ions[16].

8.7 Field Ionisation Kinetic (FIK)

A method for the analysis of the kinetic behaviour of ions, which are generated by the field ionisation method (FI). This method allows ions with a lifetime of 10^{-8} to 10^{-11} s to be temporally resolved and studied. It is suitable for the analysis of, among others, the competitive decomposition reactions of radical cations[17].

8.8 Measurement of High Masses

Biologically interesting molecules with high molecular weights, such as peptides, carbohydrates, glycopeptides, glycolipids, nucleic acids, etc., are being isolated in chemical laboratories in increasing numbers. The elucidation of the structures of such substances using spectroscopic methods generally meets with fundamental difficulties. The compounds, which are usually only available in small quantities, frequently contain many structural units that are the same or very similar. These then produce overlapping signals in UV, IR and NMR spectra, which can therefore be difficult to analyse. These structural elements often also exist in several conformations, which further increases the extent to which signals overlap. In these situations the method of mass spectrometric structural analysis comes into its own, in particular for the determination of the molecular weight. In recent times, several methods have become available which permit the molecular ions of these so-called biomolecules to be determined. Electron impact ionisation, for which the vaporisation of the sample is a prerequisite, facilitates the determination of molecular weights up to ca. 1500. This limit is set by the thermal lability of the (bio)molecule. For the measurement of such molecules, it is only possible to employ mild ionisation procedures. The highest molecular weights that have been determined to date are about 25 000 Daltons (FAB), 45 000 Daltons (PD) and 300 000 Daltons (ESI and LDI). Aside from the fact that it is difficult to imagine such enormous gaseous ions, the advance of mass spectrometry into this field has brought with it a series of new problems to be solved. For the mass marking in the high mass region, alkali metal halides, especially CsI, can be used, which form cluster ions of high mass. During such measurements the resolving ability of the instrument should be as high as possible. However, a high resolving power diminishes the ion gain at the detector. Therefore the resolving power must be set as high as is necessary (for peak separation), but as low as possible (to maintain ion abundance).

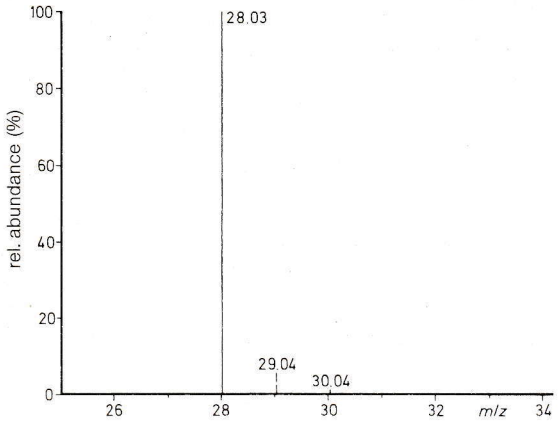


Fig. 4.44 Computer calculation of the isotope pattern of the molecular ion of C_2H_4

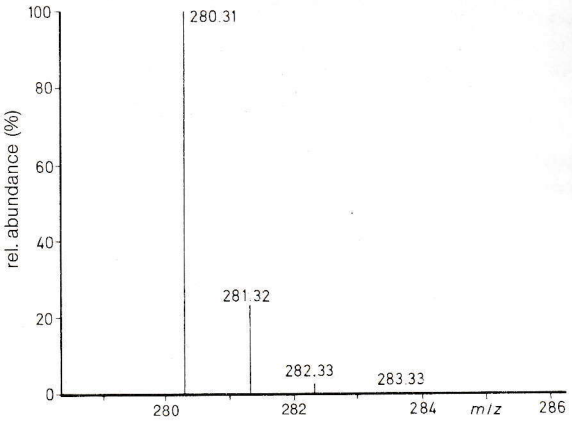


Fig. 4.45 Computer calculation of the isotope pattern of the molecular ion of $C_{20}H_{40}$

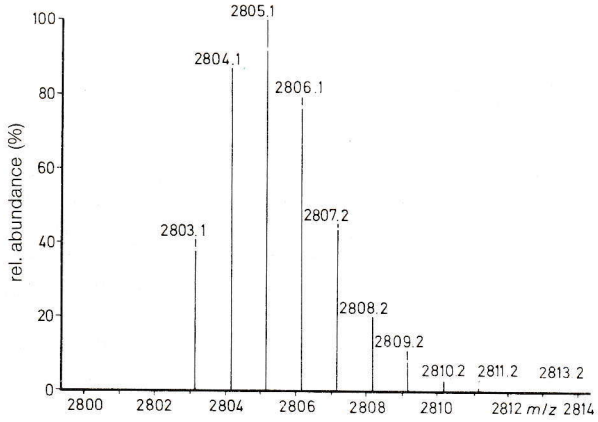


Fig. 4.46 Computer calculation of the isotope pattern of the molecular ion of $C_{200}H_{400}$

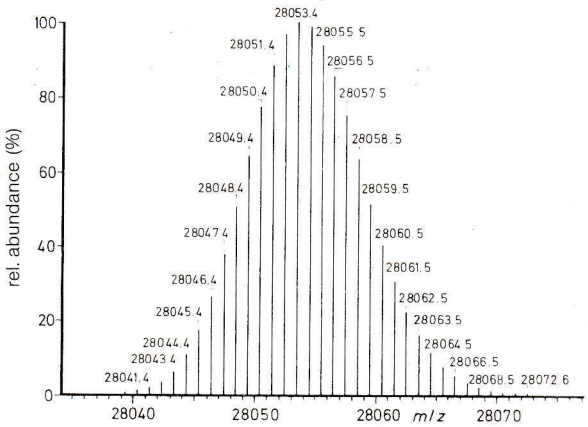


Fig. 4.47 Computer calculation of the isotope pattern of the molecular ion of $C_{2000}H_{4000}$

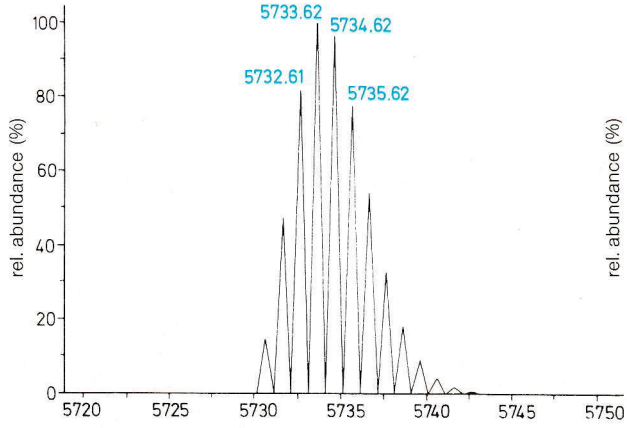


Fig. 4.48 Molecular region of the mass spectrum of bovine insulin ($M^{+\bullet}$=5729.6009); computer calculation of the $[M+H]^+$ signal with a resolving power of 6000

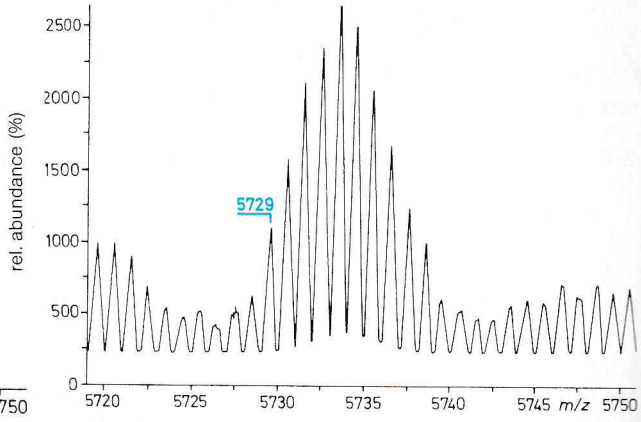


Fig. 4.49 Molecular region of the FAB mass spectrum of bovine insulin; averaged spectrum from 15 individual spectra, computer smoothed (both spectra recorded on MAT 90; reproduced with the kind permission of Finnigan MAT, Bremen)

Tab. 4.5 Comparison of molecular ions from C_2H_4, $C_{20}H_{40}$, $C_{200}H_{400}$ and $C_{2000}H_{4000}$ (from computer calculations)[a]

Composition	Molecular weight[b]	Exact mass of the molecular ion[c]	Absolute abundance[d]	Most abundant signal in the molecular ion region	Fig.
C_2H_4	28.05376	28.031300	97.7337	28	4.44
$C_{20}H_{40}$	280.5376	280.31300	79.5138	280	4.45
$C_{200}H_{400}$	2805.376	2803.1300	10.1023	2805	4.46
$C_{2000}H_{4000}$	28053.76	28031.300	1×10^{-8}	28053	4.47

a) Calculations from Dr. R. Schubert, Finnigan, MAT, Bremen.
b) The calculation of the molecular weights is based on the relative atomic masses, cf. Tab. 4.13 (p. 304 ff.).
c) The exact mass of the molecular ion was calculated from the mass of the principle isotope, see Tab. 4.13 (p. 304 ff.).
d) The value of the absolute abundance was normalised to 100. It refers to the ion for which the exact mass has been given, relative to all molecular ions.

The masses of highly charged ions (e.g. resulting from electrospray ionisation) fall in a lower mass region (up to ca. 2000). Therefore, these masses can be determined very accurately (at 20 000 Daltons to better than 0.02%).

A special problem is the recognition of the exact molecular mass in the spectrum. Using Tab. 4.5 and the schematic spectra in Figs. 4.44-4.47, the nature of this problem is described in terms of the hydrocarbon C_nH_{2n}. Naturally occurring carbon and hydrogen both consist of two isotopes which form the so-called isotope peaks in a spectrum (in both cases at 1 amu heavier than the principle isotope). This was explained in more detail in Sec. 3. The abundances of these isotope peaks from "normal" low molecular weight molecules, which consist of C, H, N, O, are always smaller than that of the molecular ion peak. However, as soon as a certain number of atoms is exceeded (this number is governed by the isotopic abundance; for carbon this is 90 atoms and for chlorine it is 3, cf. Tab. 4.10), the first isotope peak becomes more abundant than the molecular ion signal. If the molecule contains additional atoms that are composed of several isotopes, then the appearance of the relevant molecular ion region in the spectrum becomes very complex and can only be analysed quickly with the aid of a computer. One should also not disregard the fact that, among others, $[M+H]^+$ signals can still occur, which complicates the interpretation further.

As a demonstration example, the molecular region (resolving power 6000) of the FAB spectrum of bovine insulin is reproduced in Figs. 4.48 and 4.49 [$C_{254}H_{377}N_{65}O_{75}S_6$, molecular weight = 5733.5739; $M^{+\bullet}$ (nominal mass): 5727; $M^{+\bullet}$ (exact mass of principle isotope): 5729.6009. The molecular region contains $[M+H]^+$ ions and isotope peaks]. The left-hand spectrum was recorded directly onto photo-sensitive paper, while the right-hand one was smoothed by a computer. The mass spectrometric molecular weight determination and the derivation of the structure from the mass spectrum of an unknown compound with a high relative molecular mass is enormously more difficult than the analogous determination for low molecular weight compounds.

Reference: [18].

8.9 Ionization Methods

Even when mass spectrometry was first used on organic molecules, the thermal lability of many compounds proved to be a hindrance to the determination of the relative molecular mass. Various improvements to the sample injection process for EI mass spectrometers have contributed substantially to the reduction of this disadvantage. However, for fundamental reasons, this problem cannot be eliminated entirely. Whenever the above-mentioned conditions are used for the measurement, the organic sample must be vaporised before it is ionised and thereby rendered accessible for the mass spectrometric analysis. Therefore, there has been no lack of experimental effort to develop ionisation procedures which do not

Tab. 4.6 Alternative ionisation methods

Sample	Ionisation method (Abbreviation)	Explanation, see p.
vaporisable[a]	electron impact (EI)	220
	chemical ionisation (CI)	258
difficult to vaporise or cannot be vaporised	field ionisation (FI)	261
	atmospheric pressure ionisation (API)	259
	electrospray ionisation (ESI)	259
	field desorption (FD)	261
	laser desorption/ionisation (LDI)	268
	secondary ion mass spectrometry (SIMS)	273
	fast atom bombardment (FAB)	260
	direct chemical ionisation(DCI)	259
	thermal desorption (TD)	277
	thermospray ionisation (TSI)	277

[a] including GC analyses

require that organic samples be in the vapour phase before their ionisation. Recently, these endeavours have become particularly topical because the desire of organic and bio-organic chemists to investigate mass spectrometrically the structures of higher molecular and biologically relevant materials is becoming increasingly stronger. Compounds of this kind (such as polypeptides, oligosaccharides, glycosides, nucleotides, etc.) nearly always contain several polar functional groups, which make it impossible to vaporise the compound without pyrolysis occurring.

Another justification of alternative ionisation procedures to electron impact ionisation is that there are various classes of compounds which, under EI conditions, either do not yield a molecular ion or only one whose abundance is too low.

In addition to electron impact ionisation, chemical ionisation is also suitable for vaporisable samples. Because the latter can be operated with many collision gases, which lead to various types of spectra, this method has a very large scope. Furthermore, there is now a very large arsenal available for use with

Tab. 4.7 Frequently employed mass spectrometric ionisation methods

ionisation method (abbreviation)	ionising particle	Types of ions	Possible additional signals	Normal mass region max. up to ca.	Possibility of thermal decomposition
Electron impact ionisation **(EI)**	e^-	$M^{+\bullet}$ and fragment ions	–	3500	yes
Chemical ionisation **(CI)**	charged reactant gas, e.g. CH_5^+, NH_4^+ $Ar^{+\bullet}$	e.g. with NH_4^+: $M^{+\bullet}$, $[M + H]^+$, $[M + NH_4]^+$ and clusters	reactant gas and reactant gas clusters	3500	yes
Fast atom bombardment **(FAB)**	e.g. Ar^0 high kinetic energy	e.g. $[M + H]^+$, $[M + Na]^+$ ($[M + K]^+$) and clusters e.g. $[2\ M + H)^+$	signals from matrix clusters, e.g. [2 glycerine + H]$^+$	3500	very rare
Electrospray ionisation **(ESI)**	none (electrostatic)	$[M + H]^+$, $[M + Na]^+$, ($[M + K]^+$) and clusters	–	100 000	no
Thermospray ionisation **(TSI)**	frequently $CH_3CO_2NH_4$	$[M + H]^+$, $[M + NH_4]^+$	sometimes solvent clusters	3500	no

samples that are either difficult to vaporise or cannot be vaporised. Tab. 4.6 summarises the ionisation methods normally available in organic chemistry departments. The methods given in the table can be supplemented by other procedures, which are not yet as widely used at present as they probably should be. One of these is $^{252}_{98}Cf$ plasma desorption (PD; ionisation by bombardment of a sample situated on a supporting plate with nuclear particles)[19].

It should also be said that each ionisation method has its own special characteristics and requirements with regard to selectivity, speed of the analysis, necessary quantity of a sample and its preparation, etc. However, because of its widespread use, the extensive spectral material available and, finally, because of the problem free recording of the spectra of "simple" organic compounds, electron impact ionisation is still the most important method; cf. summary in Tab. 4.7.

Frequently, the mass spectrometer is arranged so that several ionisation methods can be employed. However, the changeover from one ionisation method to another is not always as simple as just throwing a switch. More often, longer term rebuilds, followed by adjustments, etc., are necessary. As a user, one should understand that it is not possible to perform measurements with every method at all times.

8.10 Cation Addition Mass Spectroscopy

By adding alkali-metal salts to polar organic compounds (M) it is possible, under FD conditions, to obtain so-called cluster ions of the general formula $[M + \text{alkali-metal}]^+$. All alkali-metal cations can be used. The tetraphenylborate anion, $[B(C_6H_5)_4]^-$, is particularly suitable as the counter-ion of the alkali-metal cation. The advantage of the method is that it facilitates the determination of the relative molecular masses of polar or thermally labile compounds. The $[M + \text{alkali-metal}]^+$ signal appears as the most abundant signal in the spectrum. To illustrate this, the spectrum of loroglossin (**65**), a natural product of plant origin, is shown in Fig. 4.50. Although the com-

Possibility for on-line combination with	Advantages	Disadvantages
GC	– fragment ion signals = structural information. – largely correct abundances of the isotope signals.	– $M^{+\bullet}$ is sometimes absent. – (very) polar substances cannot be measured.
GC	– suppression of fragmentation which results in more abundant ions in the M region.	– very polar substances cannot be measured. – in cases of uncertainty it is possible to differentiate between $[M + H]^+$ and e.g. $[M + NH_4]^+$ by changing the reactant gas. – incorrect abundances of the isotope signals.
–	– measurement of polar substances.	– reduced solubility of substances in the matrix (frequently used: glycerine). – fragmentation is rare.
LC or HPLC	– multiply charged ions often produced (structurally dependent). – measurement of high molecular weight substances in solution.	– reduced choices for types of solvents. – big differences in the ionisation of particular classes of substances. – fragmentation very rare.
LC or HPLC	– measurement of polar substances in aqueous solutions. – fragment ions sometimes occur.	– reduced choices for types of solvents. – the presence of a vaporisable electrolyte is necessary.

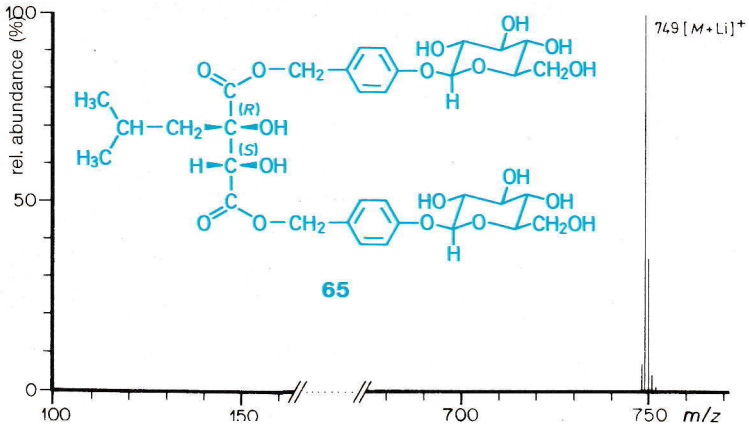


Fig. 4.50 Li^+ ion addition spectrum of loroglossin (**65**)

pound decomposes under EI conditions, an $[M - 2\ H_2O]^{+\bullet}$ signal can be observed under FD conditions. In the Li^+ ion addition spectrum, the only peak to appear is that of the $[M + Li]^+$ ion at $m/z = 749$.

Even without the addition of alkali-metal cations, it is also found that under soft ionisation conditions $[M + Na]^+$ and, less frequently, $[M + K]^+$ ions are recorded. These alkali-metal cations appear to be ubiquitous.

Reference: [20].

8.11 The Coupling of Other Instruments to Mass Spectrometers

Because of the very sensitive detection limit for small amounts of substances, the mass spectrometer is used as a detector for gas chromatographs (GC) and liquid chromatographs (LC). Of course, eluate samples which have been separated by GC and LC can be collected and subsequently analysed individually by mass spectrometry. This latter method can occasionally have certain advantages for the solution of special problems (e.g. identification of the components of fragrances, or the recording of other types of spectra).

Today, the **GC/MS combination** is a standard method of organic chemistry. Both types of column, i.e. packed and capillary columns, can be coupled to mass spectrometers. When a packed column is combined with a mass spectrometer, it is necessary to have a separator at the junction of the instruments in order to reduce the amount of carrier gas.

A discussion of the chromatographic process is not possible within the scope of this book. We shall start with the assumption that a mixture is well separated into its components by a chromatograph (GC or LC). The column of the gas chromatograph is located in a thermostatted chamber (adjustable up to 200°C). The end of the column leads into the ion source of the mass spectrometer, where the succession of components emerging from the column are directly ionised on-line and recorded. The connection, or interface, between the gas chromatograph and the mass spectrometer has a special importance. The substances that have been separated in the chromatograph should not become mixed again while traversing the interface. For this reason, the component connecting the instruments should be as short as possible and should be heated (to ca. 20°C above the GC temperature) in order to avoid partial condensation.

The time required while a single component of a substance emerges from a packed column can be up to ca. 1 min; with a capillary column this time can be of the order of 1 s. The time factor is therefore very important for the recording of the spectra. In order to collect all of the components mass spectrometrically, as many scans as are necessary will be carried out automatically and stored in a computer [magnetic field instrument: scan time 1 s, reset time 2 s, total ca. 3 s for one mass decade (ca. $m/z = 300$ to 30)].

After completion of the measurement, the chromatogram is computed (abscissa: sequence numbers of the mass spectra or the time in minutes; ordinate: concentration of the individual components, measured with the total ion current detector (TIC) instead of the flame ionisation detector (FID) customarily used in GC analyses).

The evaluation of the spectra, i.e. the determination of the structures of the components, is then conducted, preferably by using a computer to compare the spectra with those in a spectral library.

The measurement of a spectrum with the GC/MS technique only requires exceptionally small quantities of the individual components (nano- to femtogram region), cf. Sec. 2.1. Naturally, all of the limitations that apply to gas chromatography also apply to the GC/MS combination: only thermally stable compounds can be measured.

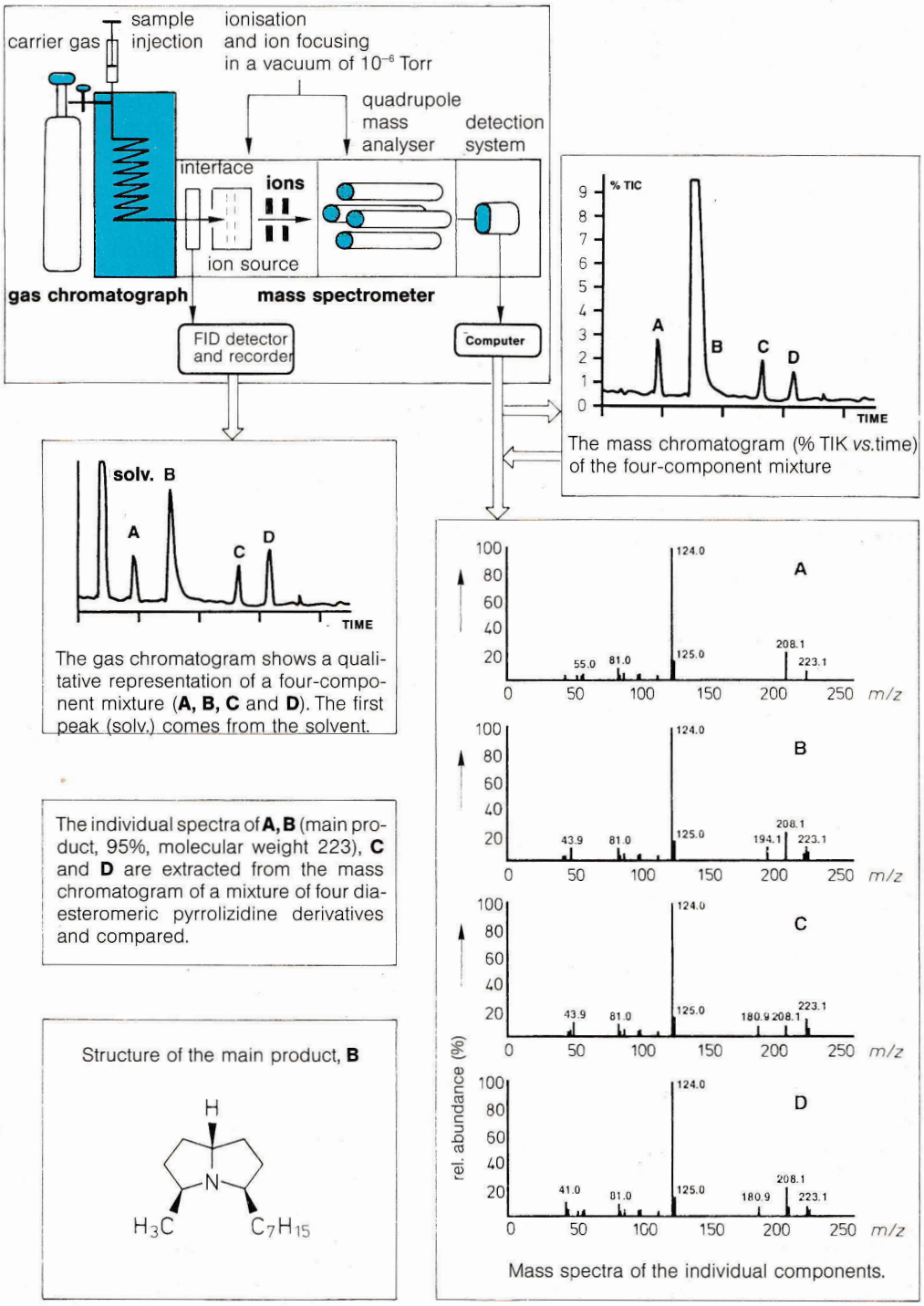


Fig. 4.51 The GC/MS system demonstrated using a four-component mixture

For full details about this method, as well as additional advantages and disadvantages, refer to the specialised literature[21].

The principle of the GC/MS combination is illustrated by the following example (see Fig. 4.51).

Consider a mixture of four compounds, one of which is definitely the synthesised pyrrolizidine alkaloid. The gas chromatogram – detected with a flame ionisation detector (FID) – shows five signals, one of which is the solvent. A mass spectrometric analysis yields the TIC chromatogram (total ion current) which corresponds with the FID chromatogram. The mass spectra were recorded automatically at 1 s intervals, independent of the appearance of any compounds. The best spectrum of each component is illustrated (A, B, C and D). The results show that all four compounds have the same molecular weight (M = 223), however there are small, almost insignificant differences in the abundances of the fragment ions. This evidence, together with the support of other analytical results, identifies the substances as four diastereomers.

A further GC/MS example is given in Chapter 5, exercise 7.

There are special practical difficulties with the **directly coupled LC/MS**. The column chromatography is carried out with pure solvents or mixtures of solvents. However, for the mass spectrometric analysis, these solvents (usually strongly polar, sometimes buffered) must be removed, because, in comparison with the solvent, only a tiny amount of material is of interest. Because the LC method is a very important analytical tool in chemical laboratories, a great deal of effort has recently been put into the development of a usable directly coupled LC/MS. Three methods are available commercially.

The moving belt method. The LC eluate is continuously placed onto an endless rotating ribbon (or wire). After the evaporation of the solvent (IR heater, vacuum), a thin film of the relatively involatile sample remains behind on the ribbon. The ribbon then enters the high vacuum region where the sample is vaporised (IR heater) and introduced into the mass spectrometer. Before the ribbon is reused, it runs through cleaning processes. These are not always completely successful and residues can build up with each cycle of the ribbon. This makes the previously obtained LC separation apparently ineffective.

Polar compounds are readily separated with the aid of LC or high-performance liquid chromatography (HPLC) and they are usually thermally labile. Suitable ionisation methods for these compounds are those which were developed for substances that are either difficult to vaporise or cannot be vaporised, i.e. FAB, FD, LD and SIMS[22].

The thermospray ionisation method (TSI). This second method of analysing products from the directly coupled LC/MS will be explained in more detail in Sec. 8.24, p. 277.

The third procedure is the ionisation of particles by **electrospray** (ESI). This method was presented in Sec. 8.3, p. 259.

Reference: [22].

8.12 Laser Desorption / Ionisation Mass Spectrometry (LDI)

The interaction of a UV laser beam with matter forms positively and negatively charged ions, which desorb from a surface and can then be analysed mass spectrometrically.

The method was developed for the analysis of, among other things, tissue samples and allows the smallest regions to be specifically analysed with the microscopically adjustable laser beam (LAMMA® = laser microprobe mass analyser). (Pseudo)-molecular ions are formed (e.g. $M^{+\bullet}$, $[M + H]^+$ and $[M - H]^-$, as well as M^{2+}, M^{3+} or cluster ions such as $[2M]^+$, $[3M]^{2+}$, $[2M]^{3+}$, etc.). For the mass spectrometric analysis, a time-of-flight (TOF) mass spectrometer is employed with, for example, a 3 keV acceleration potential. The laser is, for example, a Nd-YAG laser with a wavelength of 266 nm and a pulse frequency of 10 ns. The sample being analysed absorbs energy in one of three ways: directly (a chromophore is available) or, as occurs particularly with aliphatic compounds, the metal support underneath the sample absorbs the energy initially and then transfers it to the molecule (e.g. the formation of $[\text{valine} \cdot \text{Ag}]^+$), or the matrix in which the sample to be examined has been embedded initially absorbs the UV radiation (MALDI = matrix assisted laser desorption ionisation). Nicotinic acid or aminobenzoic acid, for example, serve as suitable matrices. LDI is a soft ionisation method. It is possible to determine molecular weights up to ca. 300 000 Daltons and the actual amount of substance required is ca. 10^{-17} mol, i.e. 50 fmol. The remaining unused material can be recovered.

Reference: [23].

8.13 Multiply Charged Ions

As mentioned in Sec. 2 (p. 220), singly charged molecular ions are formed by the electron bombardment of molecules. However, at the same time, even if considerably more rarely, processes do take place in which two and, still less frequently, three electrons are removed from the molecule. This results in the formation of doubly and triply charged particles, respec-

tively. Because the ions are recorded according to the m/z or $m/n \cdot e$ scales (in most cases $n = 1$, so that m/e is valid), doubly and triply charged ions will be registered at $m/2 \cdot e$ and $m/3 \cdot e$, respectively, i.e. they appear at one half and one third of their actual masses, respectively. If the relative molecular masses are even numbers, then, for doubly charged ions, the signals will be recorded at integer m/e values and they cannot be distinguished from those of singly charged fragment ions without additional effort. However, because the first isotopomer of an ion with an even numbered relative molecular mass has an odd numbered mass, the first isotope peak from a doubly charged ion will appear at a half mass number. Hence it is possible to find the position of the doubly charged molecular ions quite easily, because these ions have the same isotope ratios as singly charged molecular ions. The appearance of the doubly charged molecular ion can be used as a good criterion for the checking of correctly counted out mass spectra. The appearance potentials of doubly charged ions lie well above those of singly charged ions (ca. 20 to 30 eV higher). Upon lowering the ionisation potential (low-voltage spectra), the peaks due to multiply charged ions disappear. This is a property which can also be used to identify such ions. One frequently finds doubly charged molecular ions with aromatic compounds and polyolefins.

As well as doubly charged molecular ions, doubly charged fragment ions can also appear, for which the same rules are valid. In a few cases [e.g. bis(benzyltetrahydroisoquinoline) alkaloids from oxyacanthines[24]] the base peak actually results from a doubly charged fragment ion. During the investigation of the mechanism of a fragmentation, it should be remembered that the mass differences must be doubled as well. The calculations for metastable peaks ($m_M^{2+} \rightarrow m_T^{2+}$, $m_M^{2+} \rightarrow m_{T1}^{+} + m_{T2}^{+}$, etc.) can be carried out with the formula given under "metastable peaks" in section 8.25 (p. 277). Multiply charged ions appear during electrospray ionisation and are the principle reason that high masses can be determined by this method (cf. Sec. 8.8, p. 259). Occasionally doubly charged ions are also observed in FD spectra.

8.14 The Memory Effect

If residues of a substance still remain behind in the ion source region of a mass spectrometer after a measurement (e.g. by condensation on cooler parts) and if signals due to these residues reappear during the next measurement, then one speaks of a memory effect (ME). By recording a background spectrum before every measurement, proof of the presence of undesirable sample residues can be obtained. The problem can be eliminated by heating the ion source or by mechanical cleaning.

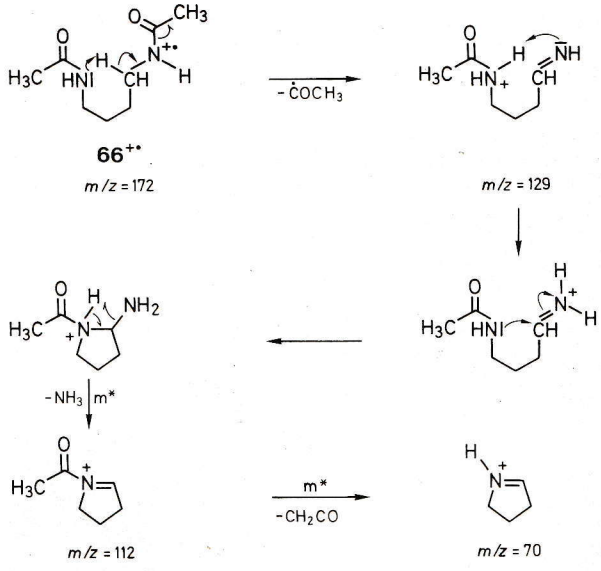
8.15 Neighbouring Group Participation Reactions

Bifunctional Alkanes

The mass spectrometric decomposition of di- and polyfunctional alkanes is essentially characterised by two fundamentally different reactions: on the one hand the structures of fragment ions are explained by the independent fragmentation of every single functional group; on the other hand, however, quite a large number of fragment ions are formed by the mutual interaction of two or more functional groups. Such reactions have been found with a relatively large number of α,ω-disubstituted alkanes, e.g. ω-hydroxycarboxylic acid esters, ω-methoxycarboxylic acid esters, ω-oxocarboxylic acid esters, ω-hydroxyethylene acetals, α,ω-diaminoalkanes, *N*,*N*'-diacyl-α,ω-diaminoalkanes, ω-aminocarboxylic acid esters and ω-aminophenylalkanes. The number of methylene groups between the two functional groups almost always plays a deciding role in the extent to which the fragmentation reaction occurs by neighbouring group participation (cyclic transition states, ring formation reactions).

As an example of this special behaviour, the mass spectrum of 1,4-bis(acetylamino)butane (**66**; M=172) is reproduced in Fig. 4.52 and discussed with the aid of Scheme 4.18.

The fragment ions at $m/z = 129$ ($M-COCH_3$) are typical, as are the two cyclic ions with $m/z = 112$ and 70. Monofunctional systems show a different behaviour (see, e.g., *N*-butylacetamide, **35**, Fig. 4.31).



Scheme 4.18 See Fig. 4.52

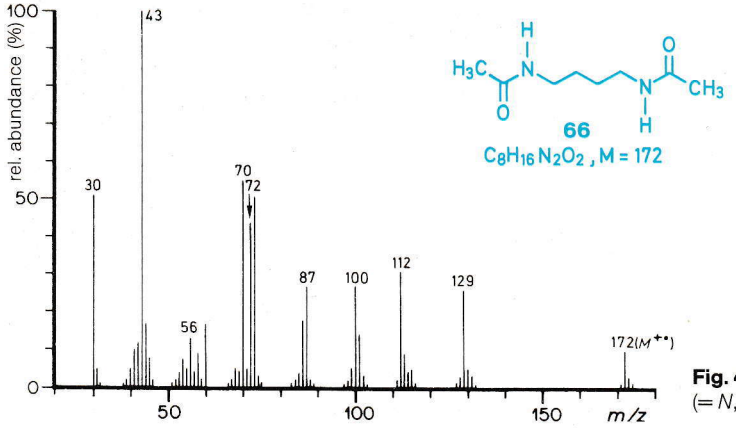


Fig. 4.52 Mass spectrum of 1,4-bis(acetylamino)butane (= *N,N'*-diacetylputrescine, **66**)

Mass spectrometric S_Ni reactions also belong to this class of fragmentation reactions. Literature review: [25].

The *ortho* Effect

This is a special case of mass spectrometric neighbouring group participation reactions which is observed with *ortho*-disubstituted benzene derivatives (or with *peri*-disubstituted naphthalene derivatives). Frequently, a mass spectrometric differentiation between *o*-, *m*- and *p*-isomeric benzene derivatives is impossible. The spectra of the *m*- and *p*-isomers are generally identical, but they can be distinctly different from that of the *o*-isomer. However, a prerequisite for this behaviour is that both of the adjacent substituents undergo, by mutual interaction, reactions that neither of the substituents would be involved in when alone. This indicates that "atypical" fragmentation reactions occur for the special *ortho* arrangement. These reactions are recognisable by the positions of the signals which cause spectra of *o*-isomers to have a different appearance.

A typical example of the similarities (*m*- and *p*-isomers) and differences (*m*- and *p*- versus the *o*-isomer) in the spectra is shown by nitrotoluene: *o*-nitrotoluene (**67**; M = 137, Fig. 4.53), *m*-nitrotoluene (**68**; M = 137, Fig. 4.54) and *p*-nitrotoluene (**69**; M = 137, Fig. 4.55).

The *m*- and *p*-isomers give typical fragment ions for the nitro compounds: $[M - 16]^+$: $m/z = 121$, $[M - 30]^+$: after rearrangement to the nitrite ester $m/z = 107$ and $[M - 46]^+$: $m/z = 91$, Scheme 4.19. For the *o*-compound **67**, these signals are indeed present, but now $m/z = 120$ (**a**) also appears and is the most abundant peak of the spectrum. This corresponds to the loss of $OH^\bullet$ from the molecular ion (Scheme 4.20). Other ions that have a higher abundance in the spectrum of **67** are $m/z = 92$

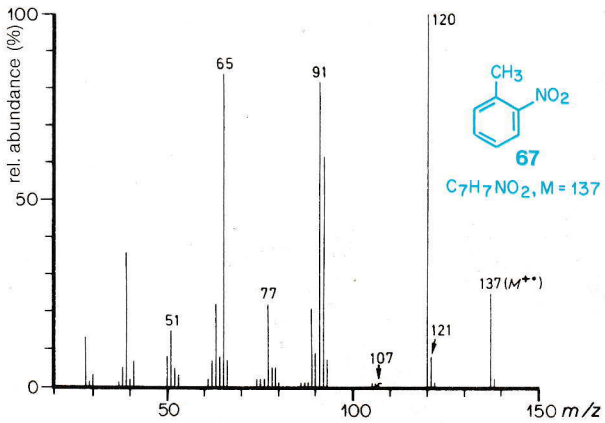


Fig. 4.53 Mass spectrum of *o*-nitrotoluene (**67**)

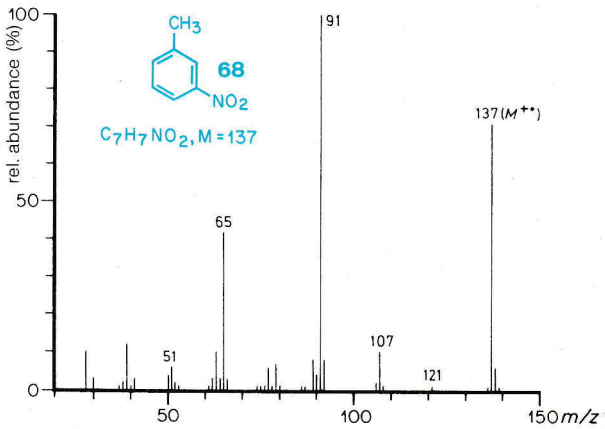
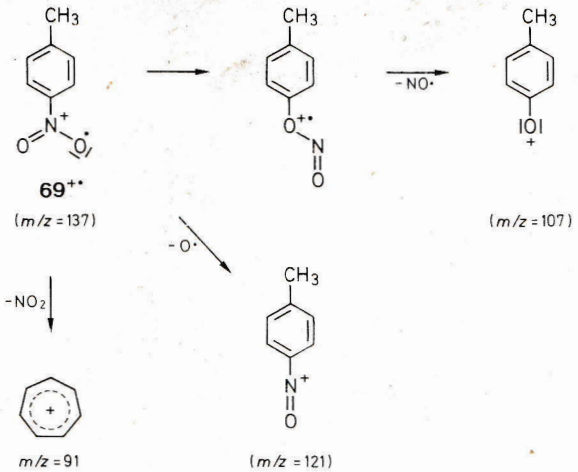


Fig. 4.54 Mass spectrum of *m*-nitrotoluene (**68**)



Scheme 4.19 Cf. Figs. 4.54 and 4.55

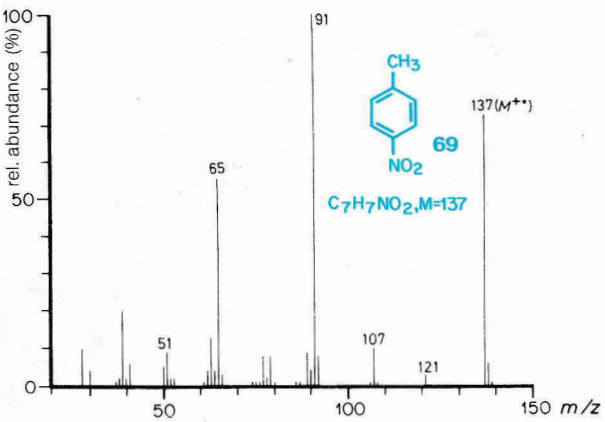
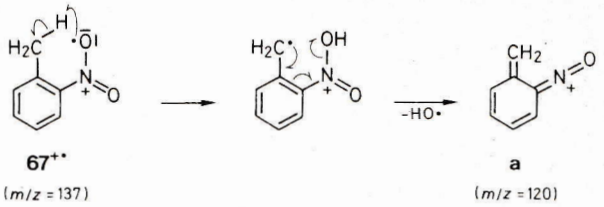


Fig. 4.55 Mass spectrum of *p*-nitrotoluene (**69**)



Scheme 4.20 See Fig. 4.53

and 77. These result from the loss of CO from **a** or the loss of HCN from $m/z = 92$.

The mass spectra of aliphatic nitro compounds rarely contain the molecular ion. Usually the most abundant signals to be recorded are the ions $[M - 30]^+$ and/or $[M - 46]^+$. However, if the spectra are recorded using the chemical ionisation (CI, see Sec. 8.1, p. 258) or fast atom bombardment (FAB, see Sec. 8.4, p. 260) techniques, the (pseudo)-molecular ion will appear. This can be seen by comparing the spectra of 3-(1-nitro-2-oxocyclododecyl)propanal (**70**; M = 283) recorded under EI (Fig. 4.56) and CI conditions (Fig. 4.57).

A benzene derivative, for which the spectra of the *o*-, *m*- and *p*-isomers are the same within the accuracy and reproducibility of the measurements, is xylene. The spectrum of *m*-xylene (**71**; M = 106), which also represents those of the other isomers, is reproduced in Fig. 4.58. The most important fragment ion is $m/z = 91$, which is formed from the molecular ion by the loss of a methyl group (and by a rearrangement).

In connection with this discussion of aromatic compounds, it should also be mentioned that in a few cases the mass spectra

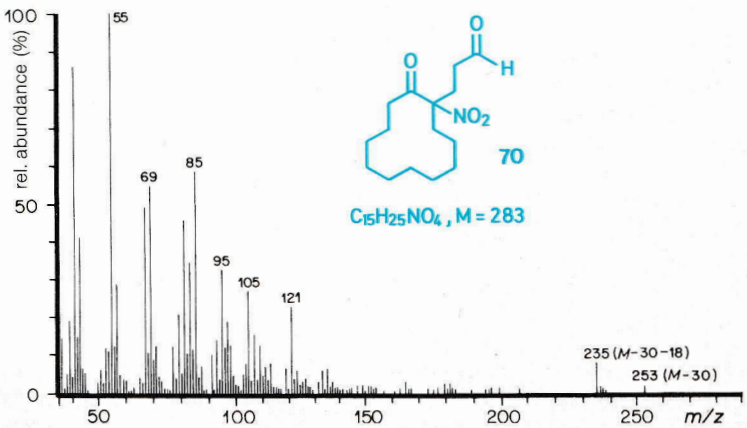


Fig. 4.56 EI mass spectrum of 3-(1-nitro-2-oxocyclododecyl)propanal (**70**)

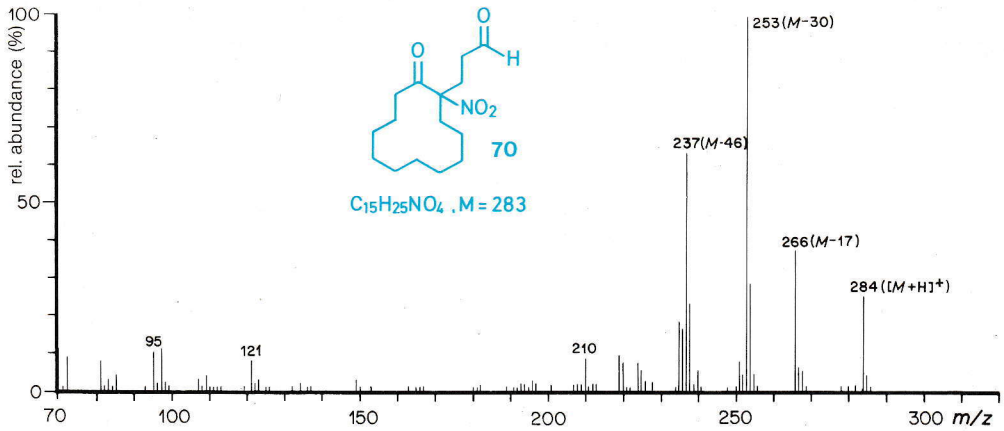


Fig. 4.57 CI mass spectrum of 3-(1-nitro-2-oxocyclododecyl)propanal (**70**); reactant gas: 2-methylpropane (isobutane)

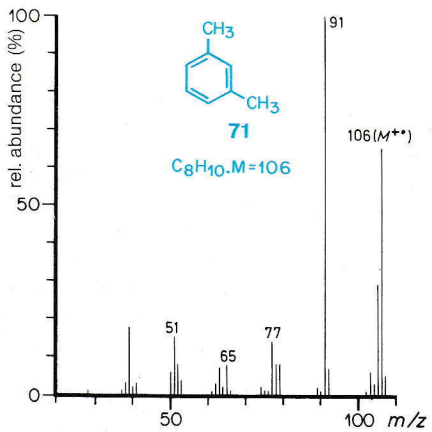


Fig. 4.58 Mass spectrum of *m*-xylene (**71**)

of *p*-substituted compounds can also differ from those of the *m*-isomers. This is because only the *p*-isomers are capable of forming resonance stabilised ions upon the loss of functional groups and the corresponding signals are therefore particularly abundant. *p*-Methoxybenzene derivatives, for example, are capable of such reactions.

Comprehensive literature: [26].

The *peri* Effect

The phenomenon of the mass spectral behaviour of 1,8-disubstituted (*peri*-substituted) naphthalene derivatives is mostly summarised under the discussion of the *ortho* effect.

8.16 Photoionisation (PI)

The ionisation of molecules occurs by irradiation with energetic photons:

$$M + h\nu \rightarrow M^{+\bullet} + e^-$$

The method is particularly suitable for the exact determination of the ionisation potential [also cf. laser desorption / ionisation mass spectrometry (Sec. 8.12, p. 268)].

8.17 Quadrupole Mass Analysers

Quadrupole mass analysers serve to separate ions. The separation is achieved through deflection of the different masses by means of electric fields. Four parallel metal rods, arranged symmetrically about the *z*-axis, form the heart of the device. The rods diametrically opposed to each other are electrically connected. A constant potential U and a modulated radio-frequency potential ($V_0 \cdot \cos \omega t$) are applied (Fig. 4.59) to the rods. The ions, which are injected along the *z*-axis (field axis), oscillate in the *x*- and *y*-directions.

When a particular potential is applied, a specific ion of a certain mass m will execute a stable oscillation and, after passing through the rod system, reach the detector, while under the same conditions, the other masses will be screened out. In this way a mass separation is achieved. A mass spectrum is obtained by varying U and V_0, while maintaining the U/V_0 ratio. The recorded mass m is proportional to V_0.

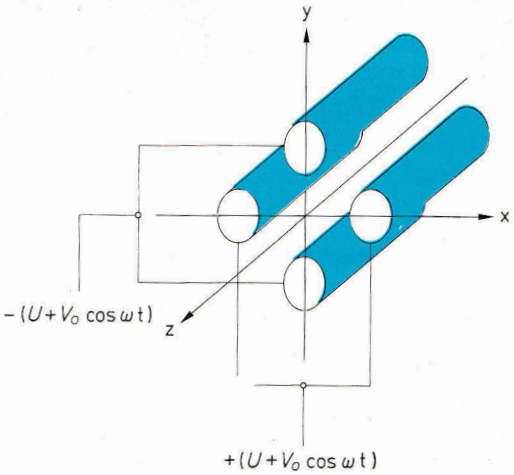


Fig. 4.59 Schematic representation of a quadrupole mass analyser

Although only a maximum *m/z* ratio of 2000 can be achieved with quadrupole analysers and they are only suitable for use as low resolution devices, they are very popular and in widespread use. They are relatively easy to build, relatively inexpensive and can be operated without a lot of experience. It is possible to combine the quadrupole analyser with most of the ionisation procedures and currently it is most frequently used in combination with coupled GC/MS instruments operating under EI or CI conditions.

Reference: [27].

8.18 Secondary Ion Mass Spectrometry (SIMS)

A beam of energetic primary ions (e.g. $Ar^{+\bullet}$ of 2 to 10 keV) is used to generate positively and negatively charged ions from a sample which has been placed on a metal plate (e.g. Ag). The acceleration, focusing, separation and detection of these ions occurs in the mass spectrometer in the usual way. In addition to $M^{+\bullet}$ and $M^{-\bullet}$, the principle ions to be recorded are $[M+H]^+$, $[M-H]^-$, $[M+Na]^+$ (this ion appears even without the special addition of a salt) and $[M+Ag]^+$, as well as fragment ions. The metal ions originate from the metal surface or from impurities. With the help of this method, mass spectra of organic compounds that are either involatile or have only a low volatility (e.g. ammonium salts, peptides, oligosaccharides, glycosides) can be recorded and used for the determination of the relative molecular masses of the compounds, as well as for the elucidation of their structures.

Reference: [28].

8.19 Spectral Libraries

Mass spectra are suitable for digitalisation (mass number *vs.* relative abundance) and storage. In this way spectral libraries can be built up on tapes or disks. It is sensible to store only the mass spectra of structurally known compounds of the highest purity. When spectra of new, structurally unknown samples are recorded, especially those of multicomponent mixtures from GC/MS, a computer can be used to compare these spectra with the spectral library and thereby identify the substances. Spectral libraries with several tens of thousands of spectra can be obtained commercially (see bibliography). Aside from the technical aspects relating to computers, there are a few basic points which should be mentioned. The reproducibility of mass spectra recorded with the same ionisation method is not very great. It is dependent upon the type of instrument, the purity of the sample, crystallinity, etc. Spectral libraries sometimes contain an abundance of fantastic unique structures that are hardly ever measured a second time as an unknown substance. On the other hand, simple compounds are sometimes missing. For the time-saving and effective use of computer comparisons, it is also worth striving to develop one's own spectral library. However, the time-consuming process of building such a library of one's own is only worthwhile if it can also be used, i.e. when this method of substance identification is employed frequently (e.g. in forensic chemistry, the analysis of fragrances, etc.). Of course, special spectral libraries are necessary when different ionisation methods are employed, because even the spectra from the same compound can be different.

8.20 Stereoisomers

Optical Antipodes

The mass spectra of optical isomers are identical and independent of the number of chiral centres (achiral recording conditions of the mass spectra!). The same is true of racemic mixtures.

Geometric Isomers

E,Z-isomers (trans-, cis-isomers). The mass spectra of *E,Z*-isomers can be, but do not have to be, different. This depends primarily on whether or not the fragmentation reaction involves

the double bond or the functional group at the double bond or in its immediate vicinity. If the fragmentation occurs outside the sphere of influence of the double bond, the spectra will be the same, otherwise differences can be observed. These differences are usually evident in distinctly different abundances of individual signals; rarely are completely different mass spectra observed. As examples, the mass spectra of maleic acid (**72**; M = 116) and fumaric acid (**73**; M = 116) are repro- duced in Figs. 4.60 and 4.61.

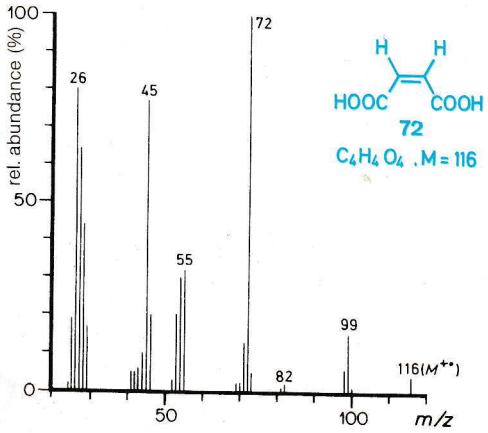


Fig. 4.60 Mass spectrum of maleic acid (**72**)

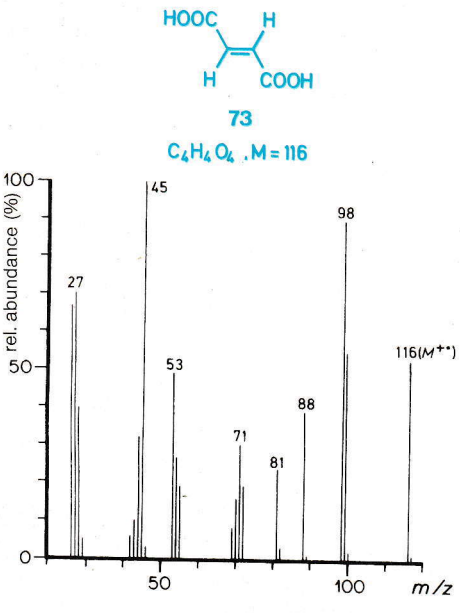
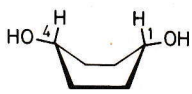


Fig. 4.61 Mass spectrum of fumaric acid (**73**)

Decarboxylation ($m/z = 72$) plays a dominant role in the mass spectrometric decomposition of maleic acid (**72**), whereas in the case of fumaric acid (**73**), the loss of water ($m/z = 98$) and the decarbonylation ($m/z = 88$) of the molecular ion are predominant.

Diastereoisomers

Depending on the distances between two functional groups, diastereoisomers can behave in a mass spectrometer in similar manner to *E,Z*-isomers. According to the type of compound and the fragmentation reaction, the spectra of diastereoisomers can display either almost insignificant or considerable differences. This behaviour is also dependent upon the location of the primary fragmentation. For example, the mass spectra of *cis*- (**74**) and *trans*-1,4-cyclohexanediol (**75**) are very different with regard to the loss of water from the molecular ion. In the spectrum of the *cis*-compound, the loss of water amounts to 1.8% Σ and a subsequent 1,4-elimination (determined by a D-labelling experiment) consumes about half of this (0.9% Σ). In the spectrum of the *trans*-compound, 8.1% Σ is due to the loss of water, but, compared with **74**, an eight times greater proportion (7.3% Σ) is involved in the 1,4-elimination. This find- ing is in good agreement with the geometric arrangement of the hydroxy groups in the boat form. Similar to this, if not quite so pronounced, is the loss of ammonia from *cis*- and *trans*-cyclohexanediamines and their derivatives.



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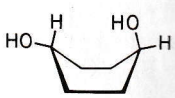

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Review article: [29].

8.21 Collision Activation (CA)

(also known as collision induced dissociation: CID)

This is a method for the analysis of the structures of ions. When ions, which possess a high translational energy (a few hundred eV), impinge upon gaseous neutral atoms or molecules, the ions are electronically excited at the expense of the translational energy. They then undergo decomposition reactions and produce a spectrum which is characteristic of the structure and energy content of the ions (CA spectrum). CA spectra of the same ions (same elemental composition) from

different sources are identical (including the abundances and half-height widths of the lines).

It is particularly useful to record CA spectra in conjunction with those ionisation methods that produce only the molecular ion of a compound (e.g. DCI, ESI, FAB and FD spectra). These spectra then yield fragment ion signals from which structural information may be obtained. For such an apparatus the following sequence is chosen: ion source - magnetic sector - collision chamber with collision gas - electrostatic sector - electron multiplier. Another sequence is shown in Fig. 4.62 (p. 276).

Comprehensive literature: [30].

8.22 Tandem Mass Spectrometry

This refers to two mass spectrometers that are arranged one behind the other, so that the method is also known as mass spectrometer/mass spectrometer (MS/MS). This combination opens up an additional area of mass spectrometric information. The procedure is as follows. A sample is ionised (all types of ionisation are possible) and gives a mass spectrum in the first mass spectrometer (MS 1). If one is now interested in a particular type of ion (fragment or molecular ion), this can be selected and diverted into a collision chamber (cf. Sec. 8.21). As a result of collisions with the gas that exists therein, the kinetic energy of these ions is partially transformed into vibrational energy, which causes the ions to fragment. These fragments then enter the second mass analyser (MS 2) where they are separated and analysed. In this way it is possible to obtain structural information about the type of ion that was selected. The method is suitable for structural analyses and the analysis of mixtures (selection of individual molecular ions), even when the sought-after substance therein is only present in minute quantities (e.g. analysis of biological materials or metabolites). The resulting profusion of data can be processed readily by a computer and this has allowed MS/MS to become one of the most efficient analytical instruments.

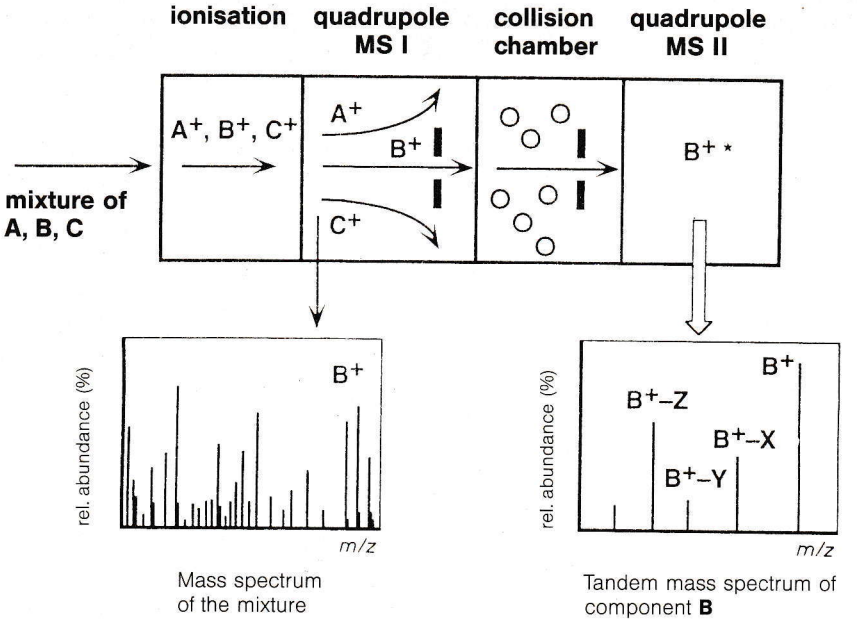
Fig. 4.62 depicts the principle of the method. First of all, a mixture of substances, consisting of three types of molecules, A, B and C, is introduced into the first mass spectrometer (MS 1). This results in the production of a mixed spectrum of all components and their fragments, the signals of which are superimposed on one another. In order to analyse a particular molecular ion more closely, in this case B^+, the instrument is adjusted so that only this type of ion is detected and all of the others are screened out. The selected ions are then directed into a collision chamber where they interact with an inert gas (e.g. xenon). As a result of the collision activation, B^{+*} undergoes a fragmentation into specific particles, $B^+ - X$, $B^+ - Y$, $B^+ - Z$, etc., which are characteristic for a particular structure. B^{+*} means that the ion B^+ possesses additional energy (translational and kinetic energy). This fragmentation is recorded and the mass spectrum MS 2 is produced, from which the compound can be identified, usually by comparing the spectrum with a spectral library.

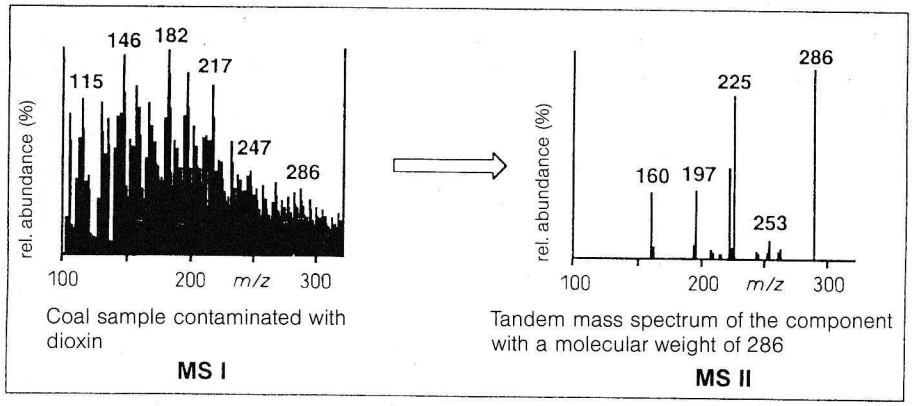
As an example of this method, the proof of the presence of trichlorodibenzodioxin (M=286) in a contaminated coal sample will be demonstrated.

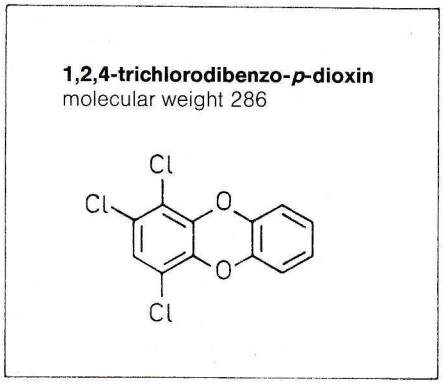
The total spectrum of the mixture, as obtained from MS 1, gives absolutely no information about the presence of the indicated impurity. However, if the desired mass is selected and, after fragmentation in the collision chamber, analysed in MS 2, then a spectrum is obtained (Fig. 4.62), which is in complete agreement with that from an authentic sample of the dioxin. For fundamental reasons, the isotope peaks are absent. When selecting one type of ion, only one mass, $m/z = 286$ (i.e. $^{12}C_{12}{}^{1}H_5{}^{16}O_2{}^{35}Cl_3$), can be taken into account. All other isotope combinations are therefore excluded.

A further illustrative example of the use of the MS/MS combination has been published. The naturally occurring polypeptide, eglin c (M=8092.02), is composed of 70 amino acid units. For reasons of identification, it had to be compared with a preparation that had been synthesised using gene technology. The synthetic product, in spite of almost identical biological, immunological and chromatographic characteristics, had a molecular mass which was 42 amu higher. (All measurements with FAB-MS, matrix: thioglycerine.) What caused the difference (CH_2CO or C_3H_6) and on which atom was the substituent located? In order to answer this question, the enzymatic hydrolase was generated from each of the preparations with trypsin and the mixture (which at any one time contained seven cleaved peptides) was analysed mass spectrometrically without a chromatographic separation having been performed. The cleaved peptide that contained the *N*-terminal amino acid from eglin 3 was 42 amu heavier in the synthetic compound. The molecular ion from this cleaved peptide was then selected from the mixture with MS 1 and treated in a collision chamber with an inert gas. Its mass spectrum was subsequently observed in MS 2. By analysis of the fragmentation pattern, the *N*-terminal amino acid was identified as the carrier of the substituent, which proved to be a CH_3CO-(N) residue (determined by CD_3CO labelling). The quantity of sample required for the experiment was 20 μg.

Reference: [31].







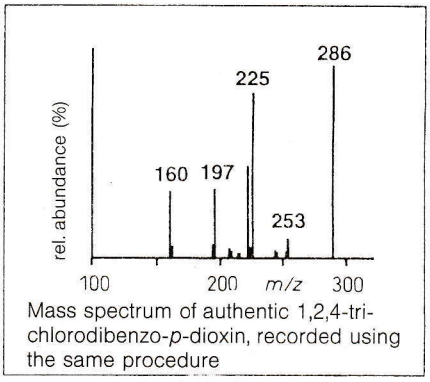


Fig. 4.62 Fundamental outline of a tandem mass spectrometer together with an example

8.23 Thermal Desorption Mass Spectrometry (TD)

(sometimes this process is also known as thermal ionisation)

Organic salts (ammonium, arsonium, oxonium salts) - but also neutral organic molecules when in the presence of Na^+, K^+, etc. - can be brought directly into the gas phase in the ion source of a mass spectrometer if the electron beam is switched off and the temperature is elevated. The acceleration, separation and analysis of the ions that are formed by this method are carried out in the usual manner. The range of applications found for this very new method is not yet reviewable.

Reference: [32].

8.24 The Thermospray Ionisation Procedure[28] (TSI)

A solution (frequently used solvent: CH_3CN/H_2O or CH_3OH/H_2O, where at least 10% should be H_2O), together with an additional vaporisable electrolyte (e.g. 0.1 M CH_3COONH_4), is sprayed under pressure from a hot capillary (inner diameter ca. 0.015 cm, flow rate 0.5-2 cm^3/min, recommended: the end of a liquid chromatography column, LC) into a heated antechamber (1-10 Torr) of the ion source of a mass spectrometer so that a mist of the finest droplets is formed (Fig. 4.63). In contrast to electrospray ionisation, an external electric field is not applied in order to form the ions. The electrolyte causes the formation of an equal number of positively and negatively charged droplets (charge exchange).

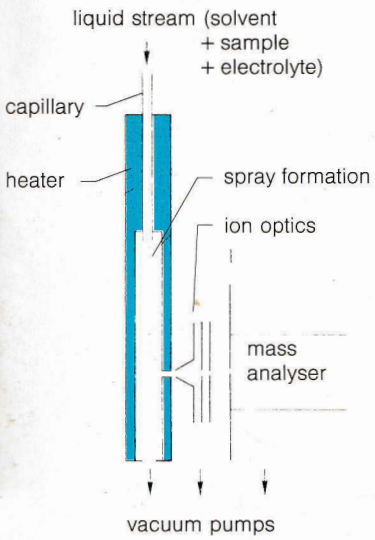


Fig. 4.63 Schematic representation of a thermospray ionisation inlet system

These droplets lose their solvent molecules in the vacuum and the ions thus formed are then analysed by an attached mass spectrometer.

The electrolyte ions can ionise molecules of the substance of interest according to the usual CI processes ($[M+NH_4]^+$, $[M+Na]^+$, also $[M+H]^+$ and sometimes cluster ions with the solvent $[M+CH_3CN+H]^+$).

The advantage of this method lies in its ability to bring polar and thermally labile compounds into the gas phase without having to use a direct vaporisation process.

Reference: [33].

8.25 Metastable Signals

(also known as signals of metastable ions or metastable peaks)

Ions that survive from the ion source to the collector without decomposing must have a lifetime of at least 10^{-5} s (e.g. molecular ions). If they have a significantly shorter lifetime (of the order of 10^{-6} s or shorter), then they decompose while still in the ion source, are correctly accelerated according to their mass and are recorded at the collector as fragment ions. Ions with a lifetime of between 10^{-5} and 10^{-6} s decompose between the ion source and the collector. These ions are known as metastable ions. Of special importance are decompositions which take place in the first field free region (cf. Fig. 4.5, p. 225). These metastable ions have experienced the full acceleration of the mother ion (m_M), but they decompose before they enter the electrostatic analyser. Because the acceleration corresponded to the heavier mass of the mother ion, the speed of the daughter ions (m_D) is slower than that attained by normally accelerated ions of the same mass. Thus they are deflected more severely in the magnetic field and appear in the mass spectrum at masses which are too "small". The signals from metastable ions in the spectrum are readily distinguishable from those of the other ions. They are recorded as broad peaks, which sometimes spread over several mass numbers, and which are usually of a lower abundance. The position of the peak can be calculated as follows [derived from Eqns. (2) and (4), Sec. 2:

$$m^* = \frac{m_D^2}{m_M}$$

As an example, the loss of ethylene (28 amu) from 1,2,3,4-tetrahydrocarbazole (**22**; M=171) produces a metastable peak at $m/z = 119.6$ (cf. p. 237), which can be calculated as follows:

$$m^* = \frac{143^2}{171} = 119.6$$

This signal therefore proves that the ion of mass 143 is formed directly from the molecular ion. In general, it can be said that the appearance of metastable peaks does not exclude other processes which lead to the formation of an ion of the same mass. On the other hand, the absence of m^* peaks does not exclude the existence of a particular fragmentation step. Metastable peaks are important aids for the deduction of fragmentation pathways. Sometimes more metastable ion signals are recorded in the spectrum than can be assigned to consecutive decomposition cascades. Unfortunately metastable peaks will not be registered when the mass spectra are recorded with the aid of a computer, because the programs are designed to suppress broad signals of low intensity.

As an alternative possibility, the so-called "linked scan" should be described. In this procedure, the magnetic field B and the deflection potential V of the electrostatic analyser are scanned simultaneously while a specific relationship is maintained between them. In particular, three procedures should be mentioned:

a) If the scan is conducted with the ratio B/V=const., then all daughter ions of a given mother ion appear in the spectrum. For example, it has been reported that the ions $C7H_7NO^+$ (m/z=121), $C_7H_6NO^+$ (m/z=120), $C_7H_7O^+$ (m/z=107) and $C_7H_7^+$ ($m/z = 91$) are formed from the molecular ion of *o*-nitrotoluene (**67**; $m/z = 137$, cf. Fig. 4.53, p. 270).

b) If the ratio B/V^2=const. is chosen for the scan, then the origin of the daughter ions is recorded. In the present example of *o*-nitrotoluene it can be shown, for example, that the daughter ion at m/z=120 originates from the ion m/z=137.

c) Finally, if the function $B^2/V^2(1 - V)$=const. is used for the scan, then all ions which lose neutral fragments of the same mass are recorded (in the present case, for example, 16 corresponds to O *and* CH_4).

By using the techniques that have been described, it is possible to obtain an insight into the molecular structure of an unknown compound. However, one must constantly bear in mind that a particular fragment ion is not necessarily formed in only one way, but that many decomposition pathways can sometimes be involved (... *many roads lead to Rome* ...).

Reference: [34].