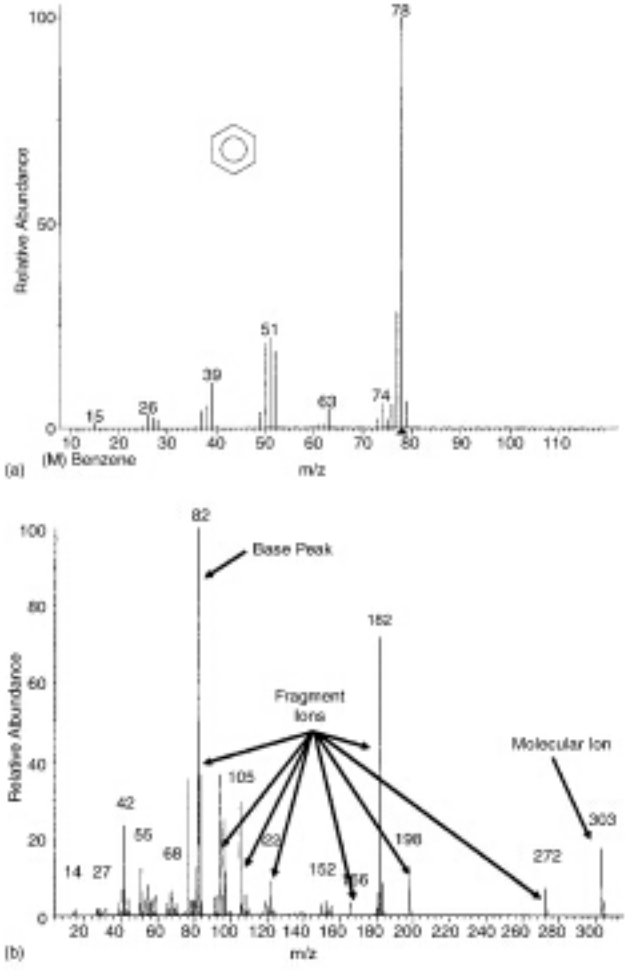

Figure 9.2 (a) A mass spectrum of benzene, C_6H_6, (MW = 78), plotted as m/z vs. relative abundance, with the most abundant peak set to a relative abundance of 100. In this spectrum, the most abundant peak (or base peak) is the molecular ion peak at m/z 78, marked by the dark triangle on the x-axis. Some fragment ion m/z values are also marked on the spectrum. (b) A mass spectrum of cocaine. In this spectrum the molecular ion peak is not the base peak; the fragment ion at m/z 82 is the most abundant ion. (The cocaine spectrum is courtesy of Dr. Robert Kobelski, CDC, Atlanta, GA.)

accurate mass), which has been determined accurately by MS. MS separates compounds containing ^{12}C atoms from compounds with ^{13}C atoms precisely because of the difference in mass between the isotopes. Table 9.1 gives a few examples of the atomic weights of some of the elements found in organic compounds as well as the measured accurate mass and abundance of the isotopes of the element. In a mass spectrum, a given ion is monoisotopic, not a weighted average. For example, the molecular weight of acetone, C_3H_6O, is calculated from the atomic weights of the elements to be $(3 \times 12.011) + (6 \times 1.00797) + (1 \times 15.9994) = 58.0795$ g/mol. The molecular ion of acetone as measured by MS has a mass that consists of only contributions from the one most abundant isotope of each element, that is, H, ^{12}C, and ^{16}O; its mass can be calculated from the formula $^{12}C_3^1H_6^{16}O$ to be $(3 \times 12.000) + (6 \times 1.00783) + (1 \times 15.9949) = 58.0419$ u or 58.0419 g/mol if we had a mole of the monoisotopic compound.

Table 9.2 Mass Spectral Data for Benzene

m/z	Relative abundance	m/z	Relative abundance
37	4	53	0.80
37.5	1.2	63	2.9
38	5.4	64	0.17
38.5	0.35	73	1.5
39	13	74	4.3
39.5	0.19	75	1.7
40	0.37	76	6.0
48	0.29	77	14
49	2.7	78	100
50	16	79	6.4
51	18	80	0.18
52	19		

The term m/z is the correct term to use for the mass-to-charge ratio of an ion. Older literature used the term m/e; however, the symbol e is used for the charge on the electron in coulombs and is *not* what goes into the divisor when the mass is given in unified atomic mass units, u. Two terms used in the older literature that are no longer acceptable for use in MS are "parent ion" for molecular ion and "daughter ion" for fragment ions. Ions do not have gender. The terms molecular ion and fragment ion should be used; "precursor ion" and "product ion" are used for tandem MS-MS experiments described later in the chapter.

9.1.1. Resolving Power and Resolution of a Mass Spectrometer

The **resolving power** of a mass spectrometer is defined as its ability to separate ions of two different m/z values. Numerically, the resolution is equal to the mass of one singly charged ion, M, divided by the difference in mass between M and the next m/z value that can be separated. For example, to separate two singly charged ions of 999 and 1001 Da requires a resolving power of $999/(1001 - 999) = 500$. That is:

$$\text{resolving power} = \frac{M}{\Delta M} \tag{9.9}$$

In practice, it is found that if we wish to distinguish between ions of 600 and 599 Da, the resolving power required is 600, or 1 Da in 600 Da. As a rule of thumb, if we wish to distinguish between ions differing by 1 Da in the 600 mass range, we need a resolving power of 600. If we need to distinguish between ions differing by 1 Da in the 1200 Da range, we need a resolving power of at least 1200. This is not a very high resolution. Some isotopes of *different* elements or molecular fragment ions composed of different combinations of atomic isotopes adding up to close to the same mass may produce ions differing by *much less* than 1 Da. To distinguish between these types of species, high-resolution mass spectrometry, with resolution in the 20,000–100,000 or higher range, is required.

The resolving power is determined by actual measurement of the mass spectral peaks obtained. The method for calculating ΔM must also be specified. Two methods are commonly used to indicate the separation between peaks and these are shown in Fig. 9.3. One definition is that the overlap between the peaks is 10% or less for two peaks of equal height; that is, the height of the overlap should not be more than 10% of

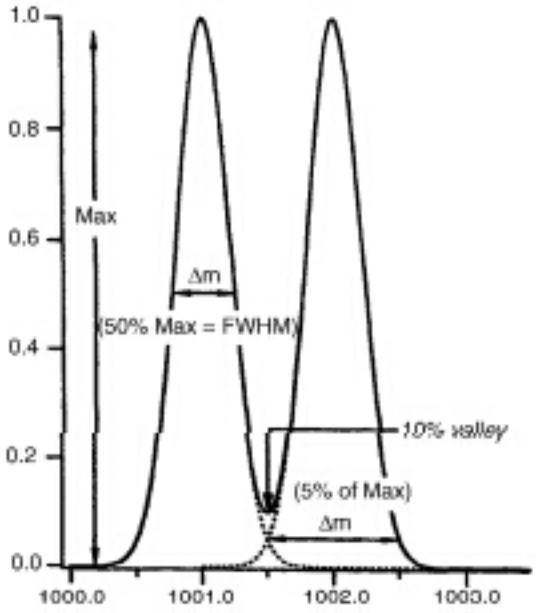

Figure 9.3 Illustration of the peaks used to calculate the resolving power of a mass spectrometer showing the location of the FWHM, the 10% valley, and the 5% valley. (From Sparkman, used with permission of the publisher.)

the peak height. A second method is to use the full width at half maximum (FWHM) as a measure of ΔM. The FWHM method results in a resolving power twice that of the 10% overlap method, so it is important to state how the calculation was performed.

Resolving power for commercial mass spectrometers depends on the instrument design, and can range from 500 to more than 1×10^6. In general, the higher the resolving power, the greater the complexity and cost of the MS instrument.

Resolution is the value of ΔM at a given M and is often expressed in ppm. For the spectrometer with a resolving power of 600 earlier described, the resolution would be 1 part in 600 parts. To distinguish between $^{14}N_2^+$, with an exact mass of 28.0061 Da and $^{12}C^{16}O^+$, with an exact mass of 27.9949 Da, we would need a resolution of (28.0061 − 27.9949) ≡ 0.0112 Da in 28 Da. To convert this resolution to ppm, divide 0.0112 by 28 and multiply by 1×10^6; a resolution of 400 ppm is required. The resolving power needed would be 27.9949/0.0112 = 2500.

9.2. INSTRUMENTATION

All mass spectrometers require a sample input system, an ionization source, a mass analyzer, and a detector. All of the components with the exception of some sample input systems or ion source volumes are under vacuum (10^{-6}–10^{-8} torr for that portion where ions are separated by mass, i.e., the **analyzer**, or 10^{-4}–10^{-5} torr in some **ion sources**, where the ions are initially formed), so vacuum pumps of various types are required. Modern mass spectrometers have all of the components under computer control, with a computer-based data acquisition and processing system. A block diagram of a typical mass spectrometer is shown in Fig. 9.4.

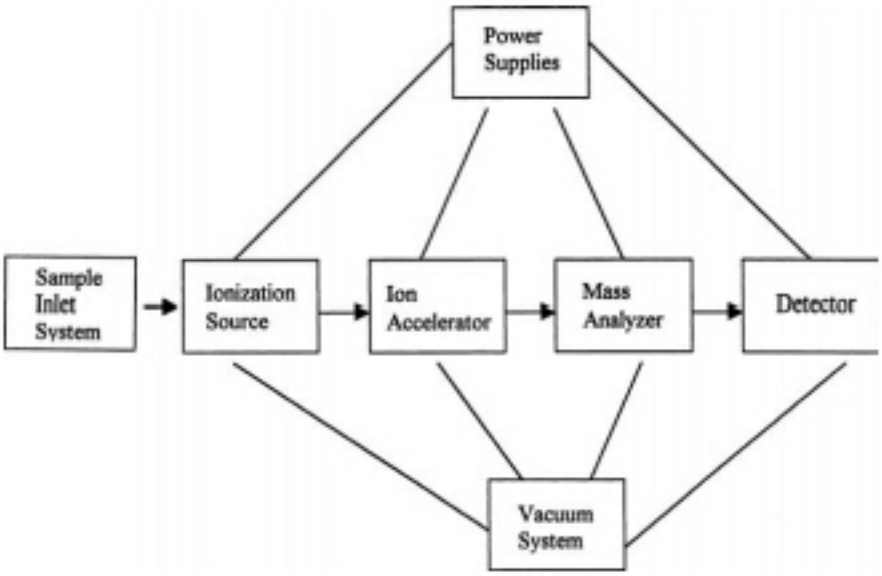

Figure 9.4 Block diagram of a mass spectrometer.

9.2.1. Sample Input Systems

9.2.1.1. Gas Expansion

This method of sample introduction is useful for gases and for liquids with sufficiently high vapor pressures. The gas or vapor is allowed to expand into an evacuated, heated vessel. The sample is then "leaked" into the ionization source through pin holes in a gold foil seal. This is sometimes termed a **"molecular leak" inlet.** Vacuum pumps control the system so that the pressure in the ionization source is at the required 10^{-6}–10^{-8} torr.

9.2.1.2. Direct Insertion and Direct Exposure Probes

A **direct insertion probe** is used for introduction of liquids with high boiling points and solids with sufficiently high vapor pressure. The sample is put into a glass capillary that fits into the tip of the probe shown in Fig. 9.5. The probe is inserted into the ionization source of the mass spectrometer and is heated electrically, vaporizing sample into the electron beam where ionization occurs. A problem with this type of sample introduction is that the mass spectrometer can be contaminated because of the volume of sample ionized.

A **direct exposure probe** usually has a rounded glass tip. The sample is dissolved in solvent, a drop of the solution is placed on the end of the probe and the solvent is allowed to evaporate. A thin film of sample is left on the glass tip. The tip is inserted into the ion source and heated in the same manner as the direct insertion probe. Much less sample is introduced into the ion source and the spectrometer is less likely to be contaminated as a result.

9.2.1.3. Chromatography and Electrophoresis Systems

The appropriate chromatographic instrument can separate mixtures of gases and liquids and the separated components are then introduced sequentially into a mass spectrometer for detection. Mass spectrometrists consider the chromatograph to be a "sample inlet" for their spectrometers while chromatographers consider the mass spectrometer to be "a detector" for their chromatographs. The truth is that these hyphenated (or coupled)

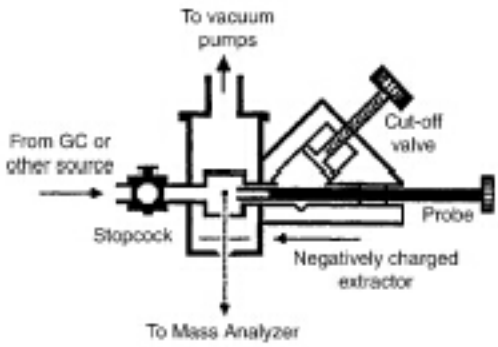

Figure 9.5 Schematic diagram of a direct insertion probe for solids and high boiling liquids. The sample is placed into the cavity in the tip of the probe. When not in use, the cutoff valve is closed and gas samples can be introduced by opening the stopcock shown. The black dot at the center of the square shows the point at which the electron beam impacts the sample. (From Ewing, used with permission.)

chromatography-mass spectrometry systems are extremely powerful analytical techniques, much more powerful than either instrument alone. The major problem to be overcome in coupling these two types of instruments is that the chromatography systems run at atmospheric pressure with large amounts of "carrier gas" or solvent. The mass spectrometer operates at very low pressures as noted above. The carrier gas or solvent must be removed without losing the analyte before the analyte can be introduced into the evacuated ionization source or analyzer regions. Alternatively one may use an ionization source which can ionize the target analytes under the conditions of higher pressure produced by the accompanying carrier fluid, and then selectively extract the ions into a lower-pressure region while diverting and discarding the vast majority of that vaporized fluid. Interfaces for these systems have been developed and are described in the chapters on the individual chromatographic techniques.

GC coupled with MS is known as GC-MS. It is a well-established method for separating gases or volatile compounds; the mass spectrum of each component in a mixture can be obtained and the components measured quantitatively. The interfacing, operation and applications of GC-MS are discussed in Chapter 12.

Several types of LC and one nonchromatographic separation system for liquids have been interfaced with MS. HPLC is widely used to separate nonvolatile organic compounds of all polarities and molecular weights. Coupled to a mass spectrometer, the technique is called LC-MS. Supercritical fluid chromatography (SFC) and the nonchromatographic separation technique of capillary electrophoresis (CE) are also used with mass spectrometric detection. The interfacing, ionization sources, operation, and applications of these hyphenated methods are covered in Chapter 13.

9.2.2. Ionization Sources

9.2.2.1. Electron Ionization (EI)

The EI source is a commonly used source for organic MS. Electrons are emitted from a heated tungsten or other metal filament. The electrons are accelerated by a potential of 50–100 V toward the anode (Fig. 9.6). As shown in this figure, the paths of the electrons and sample molecules meet at right angles. Ionization of the sample molecules and fragmentation into smaller ions occurs as a result of interaction with the high-energy electrons.