3.12 ELECTRON SPIN RESONANCE SPECTROSCOPY

Electron spin resonance (ESR) spectroscopy, also called electron paramagnetic resonance (EPR) spectroscopy and electron magnetic resonance (EMR) spectroscopy, is an MR technique for studying species with one or more unpaired electrons. These species include organic and inorganic free radicals and transition metals ions. Unpaired electrons can also exist as defects in materials. Unpaired electrons possess spin, with a spin quantum number $S = 1/2$, and therefore also possess a spin magnetic moment. Just like protons in an NMR experiment, in an applied external magnetic field of B_0, electrons with magnetic moment $m_s = +1/2$ will align antiparallel to the field, while those with $m_s = -1/2$ will align parallel to the field, as shown in Figure 3.77. Just like protons in an NMR experiment, absorption (resonance) will occur under certain combinations of frequency and applied magnetic field. The important point to remember is that we are now talking about electrons, not nuclei. The energy difference or Zeeman splitting seen in ESR is the electronic Zeeman effect.

As shown in Figure 3.78, the energies of the spin states diverge linearly as the magnetic field increases. An unpaired electron can move between the two energy levels by absorbing or emitting electromagnetic radiation such that the energy equals $\Delta E = h\nu$.
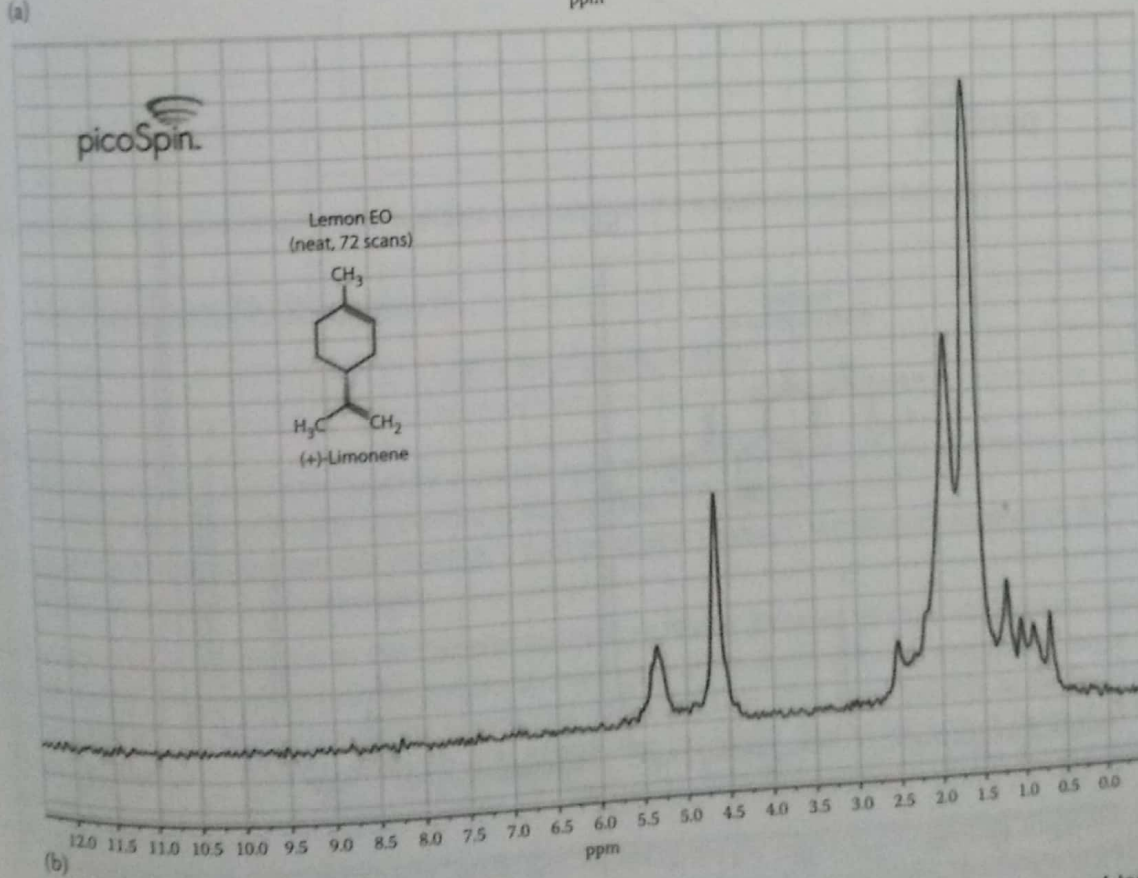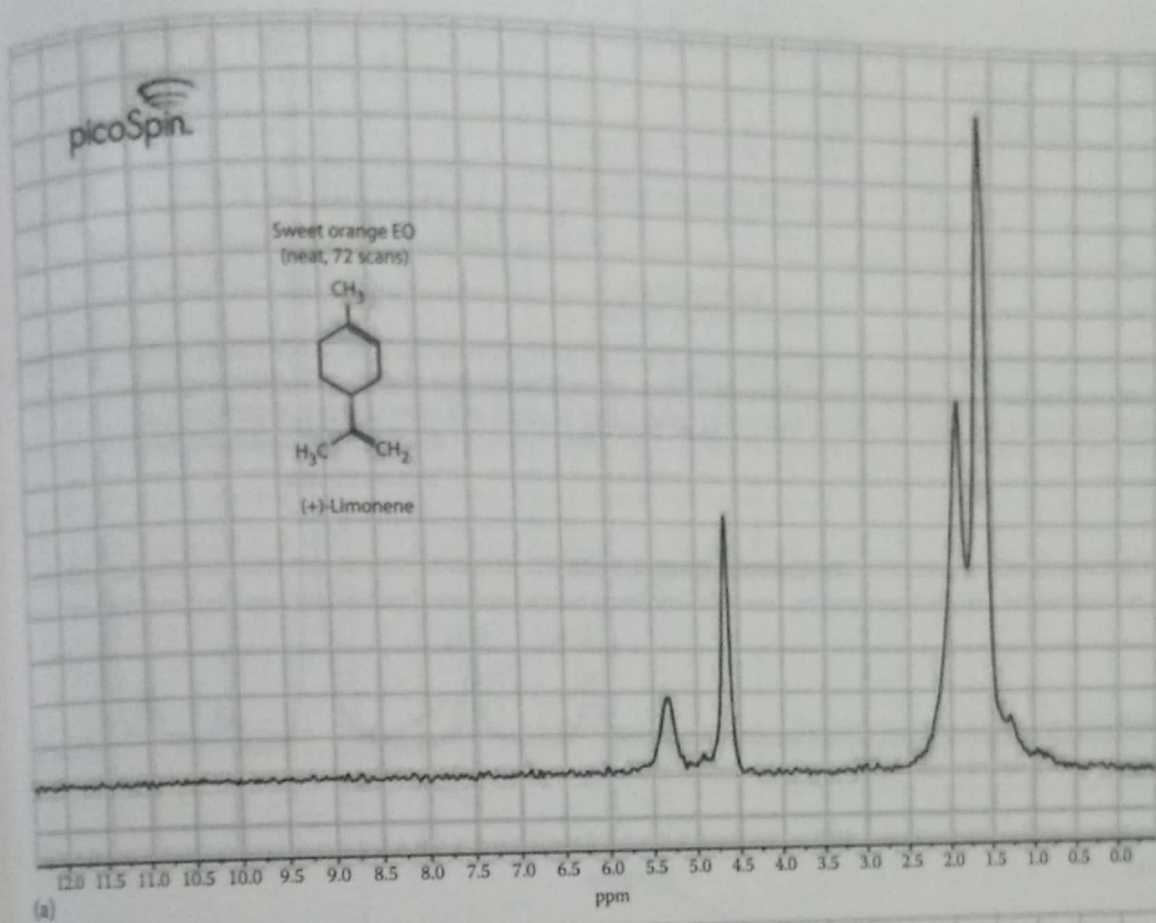
(a)

(b)

Figure 3.75   (a) Proton NMR spectrum of sweet orange essential oil. (b) Proton NMR spectrum of lemon essential oil. Collected neat using the picoSpin-45. The lemon oil shows other trace compounds not found in the orange oil. (Used with permission of picoSpin, LLC, www.picospin.com.)
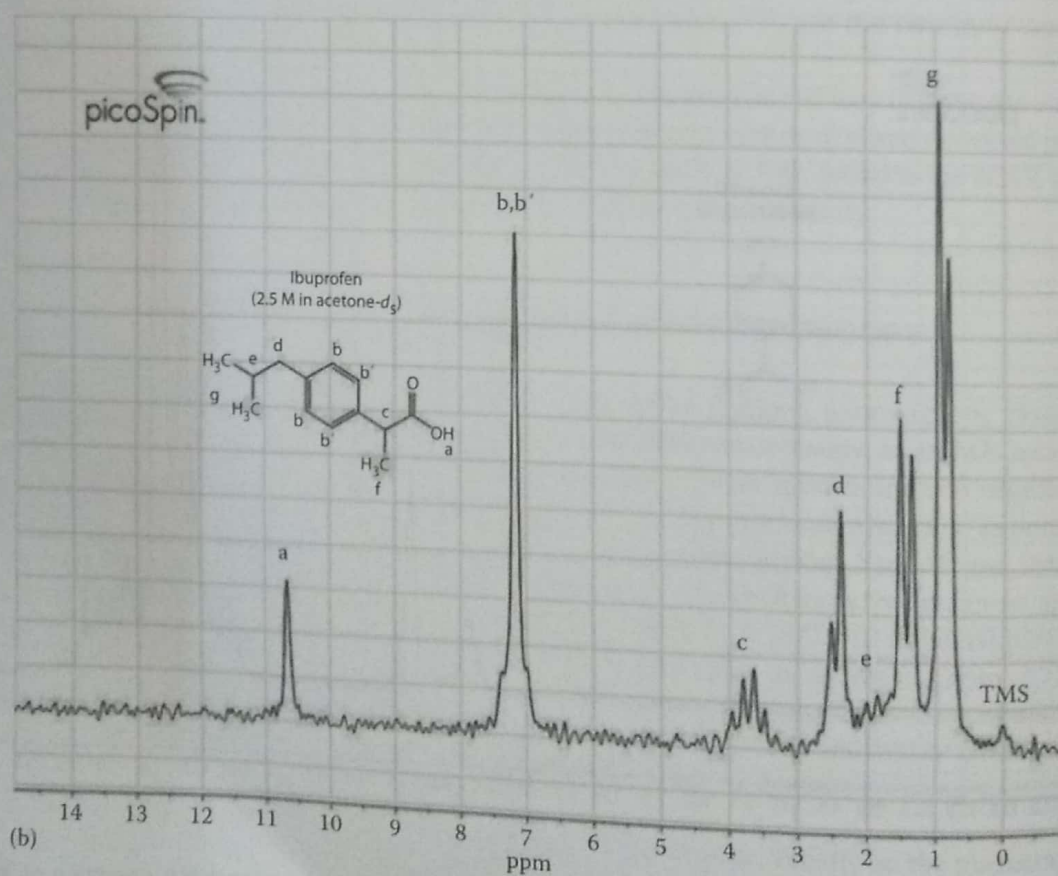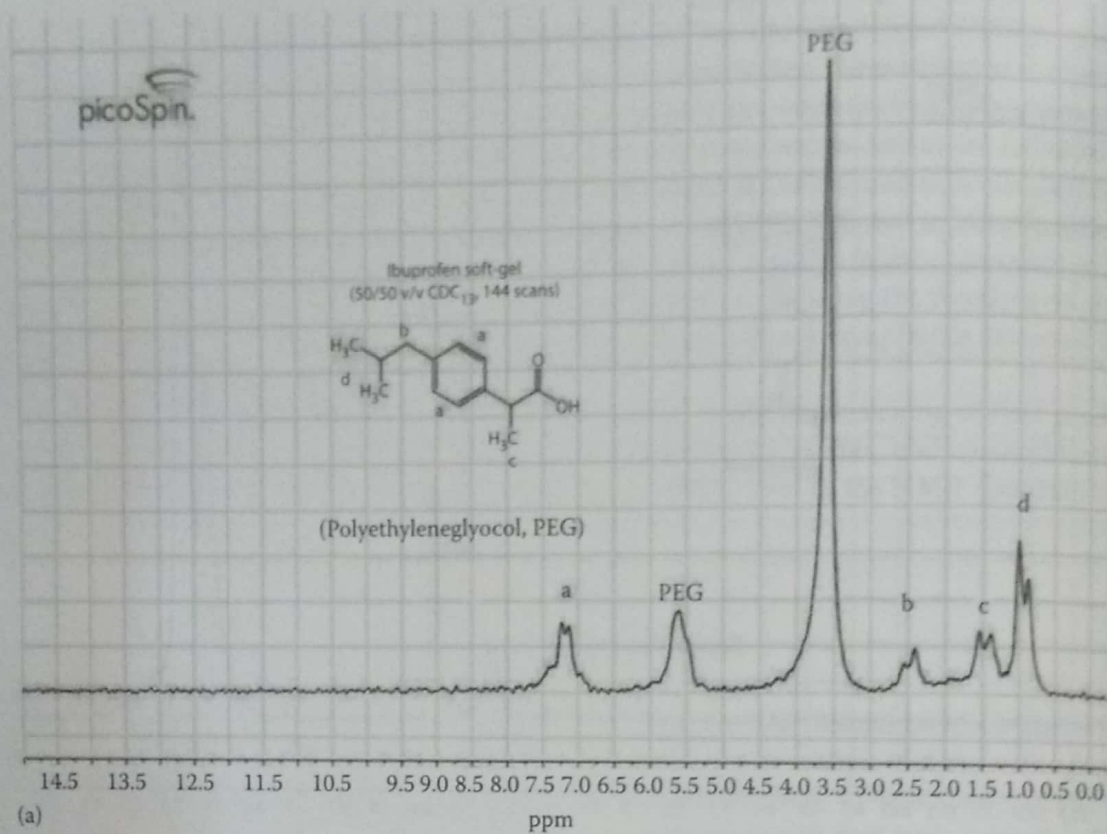
Figure 3.76 (a) Ibuprofen softgel, showing the active ingredient as well as the PEG component. Compare to (b) pure ibuprofen. (Used with permission of picoSpin, LLC, www.picospin.com.)
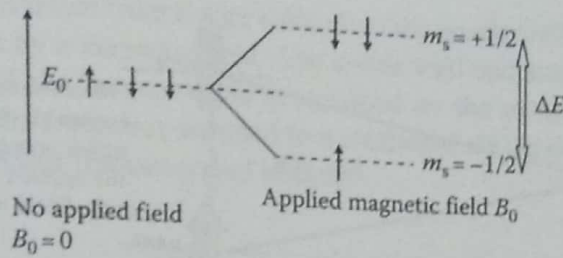
Figure 3.77 In the presence of an applied magnetic field, electrons with $S = 1/2$ can exist in one of two discrete energy levels. The levels are separated by ΔE. The lower energy level ($m_s = -1/2$) has the magnetic moment aligned with the field; in the higher energy state ($m_s = +1/2$), the magnetic moment is aligned against the field.
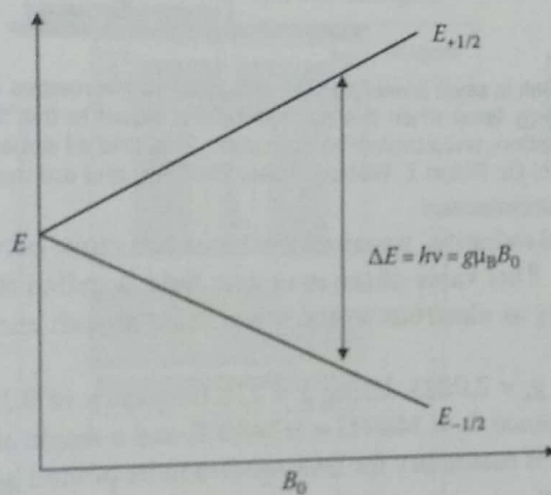

Figure 3.78 Variation of spin state energies as a function of the applied magnetic field, B_0.

The fundamental ESR equation is

$$\Delta E = h\nu = g\mu_B B_0 = g\beta B_0 \tag{3.19}$$

where

g is the g-factor, a proportionality constant approximately equal to 2 for most samples, but which varies depending on the electronic configuration of the radical or ion

μ_B is the Bohr magneton (the electron's spin magnetic moment, sometimes denoted as β)

B_0 is the magnetic field strength

This equation is analogous to the Larmor equation in NMR. Because of mass differences between nuclei and electrons, the electronic magnetic moment is about three orders of magnitude larger than the nuclear magnetic moment. This means that frequencies for ESR are generally in the 9–10 GHz range, much higher than the usual 60–300 MHz range for NMR. The generation of these high GHz frequencies, previously a major challenge for electronics, benefited greatly from the development of radar for World War II.

In principle, an absorption spectrum can be obtained by applying a constant magnetic field and scanning the frequency of the electromagnetic radiation. Alternatively, the frequency of the electromagnetic radiation can be held constant and the magnetic field scanned. Owing to limitations with microwave electronics, the frequency is normally held constant and the magnetic field varied. When the magnetic field tunes the two spin states so that their energy difference matches
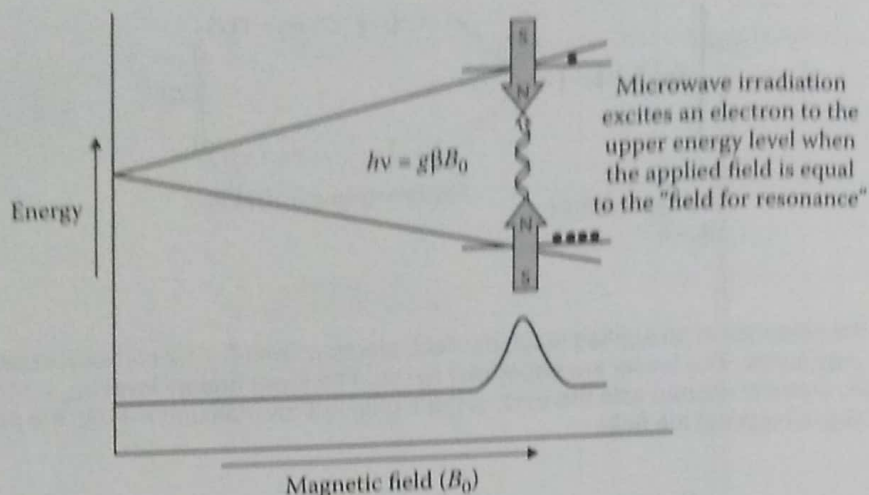
$$hv = g\beta B_0$$

Microwave irradiation excites an electron to the upper energy level when the applied field is equal to the "field for resonance"

Energy

Magnetic field (B_0)

Figure 3.79 An absorption peak is seen (lower portion of figure) as microwave irradiation excites an electron to the upper energy level when the applied field is equal to the field for resonance. (Courtesy of Bruker Corporation, www.bruker-biospin.com. This and all subsequent Bruker EPR schematics are the work of Dr. Ralph T. Weber, Bruker BioSpin, and are used with permission.)

the frequency of the applied radiation, unpaired electrons can move between their two spin states; they are now in resonance. This value of the magnetic field is called the *field for resonance*. An absorption spectrum results as electrons in the lower state absorb energy to move to the upper state, shown in Figure 3.79.

For a free electron, $g = g_e = 2.0023$. Using $g = 2$, a frequency of 9.75 GHz, and Equation 3.19, the predicted field for resonance $B_0 = 3480 \, G = 0.3480 \, T$, and a single absorption peak at this field value would be observed. It is customary for ESR spectra to be plotted as first derivative spectra, as will be seen in the following.

3.12.1 Instrumentation

ESR/EPR spectrometers use a wide variety of magnetic field strengths and microwave radiation sources.

CW ESR, pulsed ESR, and FT ESR systems are available. Table 3.6 provides a sample of frequency and field for resonance commonly used in ESR systems.

Spectrometers in the 1 GHz range are called "L-band" spectrometers; those in the 100 GHz range, "W-band" spectrometers; and so on. A schematic of a commercially available ESR/EPR spectrometer is given in Figure 3.80, along with a photo of a commercial instrument.

Classical sources for microwave generation of GHz and THz frequencies were klystrons or magnetrons. A magnetron microwave source is exactly what is present in your kitchen microwave.

Table 3.6 Field for Resonance, B_{res}, for a $g = 2$ Signal at Selected Frequencies

| Microwave Band	Frequency (GHz)	B_{res} (G)
L	1.1	392
S	3.0	1,070
X	9.75	3,480
Q	34.0	12,000
W	94.0	34,000

Source: Courtesy of Bruker Corporation, www.bruker-biospin.com/cwtheory.html.

In the magnetron, electrons travel from a wire cathode to the anode walls of an evacuated chamber, forced into a circular path by a magnetic field. The anode wall contains hollow resonator cavities. The resonant frequency of the magnetron is determined by the resonator cavity dimensions. In your kitchen microwave, the frequency is tuned to a rotational absorption band in water, so water-containing samples absorb this frequency and heat up.
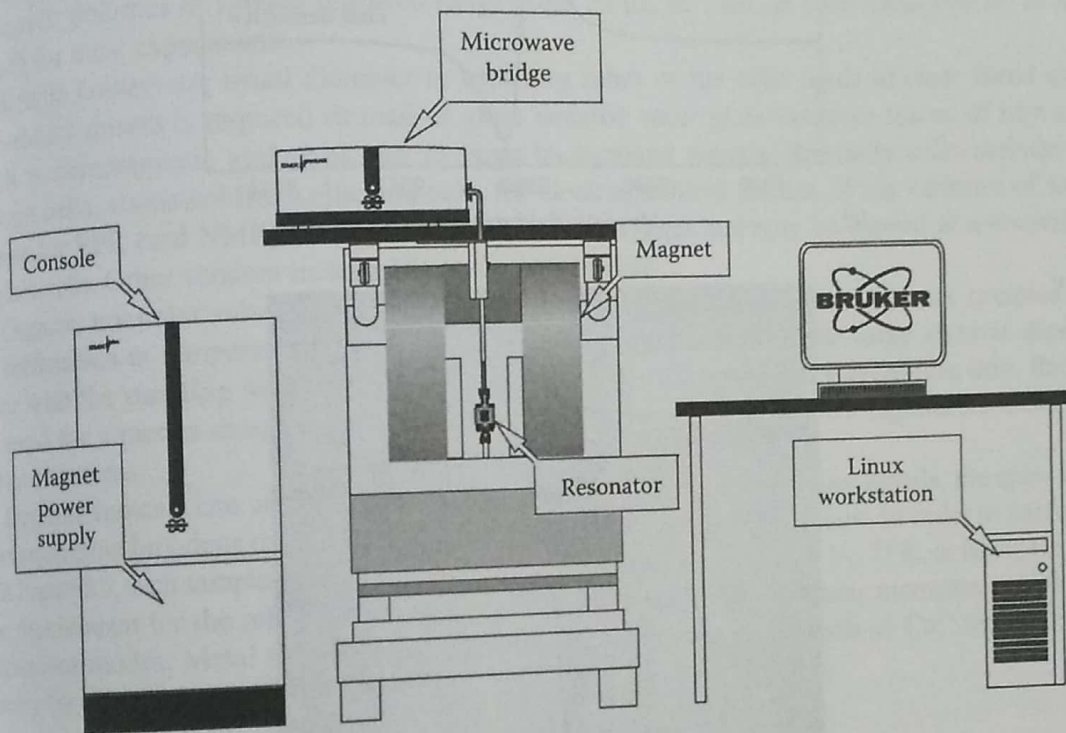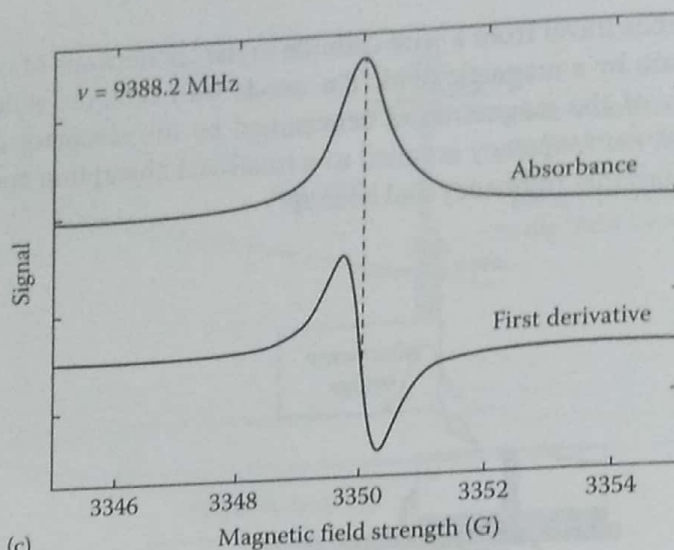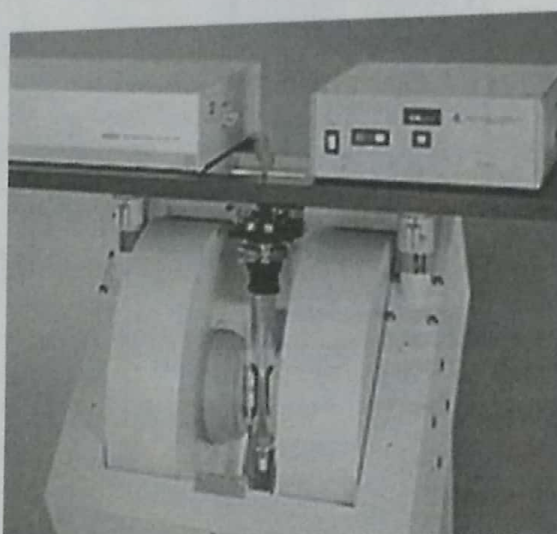

(a)
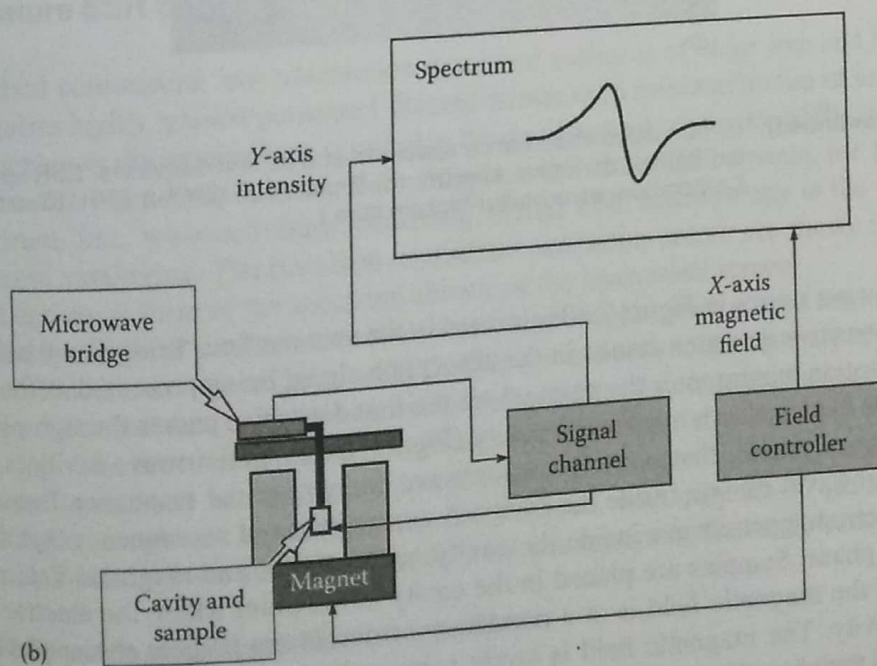
(b)

Figure 3.80 (a) and (b) A schematic ESR/EPR spectrometer. (Courtesy of Bruker Corporation, www. bruker-biospin.com.)

(continued)

(c)

(d)

Figure 3.80 (continued) (c) Simulated absorbance spectrum and its first derivative. ESR spectra are plotted as first derivative spectra. (d) Bruker ELEXSYS-II EPR. (Courtesy of Bruker Corporation, www.bruker-biospin.com.)

The microwave source in Figure 3.80 is located in the area marked "bridge" in the diagram. The use of phase-sensitive detection results in the absorption signal being presented as the first derivative. The absorption maximum is the point where the first derivative passes through zero.

The sample is placed in a microwave cavity (Figure 3.80). A microwave cavity is a metal box or cylinder that resonates, that is, stores, microwave energy. At the resonance frequency of the cavity, all microwaves remain inside the cavity. A consequence of resonance is that there will be a standing electromagnetic wave inside the cavity, with electric and magnetic field components exactly out of phase. Samples are placed in the cavity at the point where the electric field is at a minimum and the magnetic field is at a maximum to obtain the biggest absorption signals and highest sensitivity. The magnetic field is swept using a field controller, as seen in Figure 3.80. When the field matches (Figure 3.79), a spectrum is generated. (For the student interested in the details of instrument control and signal generation, see www.bruker-biospin.com and the EPR theory links.)

3.12.1.1 Samples and Sample Holders

For a sample to be EPR/ESR active, it must have one or more unpaired electrons. Stable free radicals, paramagnetic metal ions, and irradiated materials are some examples of such materials. The amount of sample required depends on the type of spectrometer (what band is used) and on the type of experiment—CW, pulsed, and double-resonance experiments like electron nuclear double resonance (ENDOR) (discussed in the following)—but in general, liquid and solid samples can be measured. Volumes of sample required range from 20 µL to 1 mL at concentrations of 10 nM to 20 mM for most experiments.

Sample holders are small diameter or capillary tubes or flat cells made of clear fused quartz. High-purity quartz is required instead of glass because most glass contains traces of iron oxide, which is paramagnetic and gives rise to large background signals. Specialty cells include flow-through cells, tissue-holding cells, and cells for electrochemical studies. Wide varieties of sample holders for EPR (and NMR) are offered by Wilmad-LabGlass and may be viewed at www.wilmad-labglass.com. Other vendors include Vitrocom and Bruker.

Organic nonpolar solvents can normally be used at all temperatures. Aqueous samples present difficulties at temperatures above 0°C, due to the interaction of the large electric dipole of water with the standing wave in the resonator cavity. To minimize this interaction, thin, flat cells are used for aqueous samples in X-band systems and very narrow quartz capillaries are used in Q-band systems.

Organic radicals can often be measured at room temperature. For many metals, the spin–lattice relaxation time broadens their EPR signals significantly at room temperature. In order to narrow the EPR linewidth, such samples are often run at cryogenic temperatures (liquid N_2, 77 K, or liquid He, 4 K). The mechanism for the relaxation time is interaction of the orbital angular momentum with local vibrational modes. Metal ions with little orbital angular momentum, such as Cu^{2+} and VO^{2+}, can usually be observed at room temperature.

3.12.2 Miniature ESR Spectroscopy

The standard commercial instrumentation described earlier is of large size and high cost and generally requires highly trained personnel. Recent advances in miniaturization of sensor technology and in microwave electronics have resulted in the development of smaller ESR systems. Several companies now offer benchtop systems. For example, a miniature ESR system, the Micro-ESR™ (Active Spectrum, Inc., www.activespectrum.com), brings ESR spectroscopy to the benchtop and to online process monitoring. The benchtop instrument and online sensor are shown in Figure 3.81. Note the first derivative form of the spectrum shown on the instrument screen.

The S-band system uses a samarium cobalt permanent magnet, with a fixed field of 1180 G that is swept by a coil over a narrow range of ±135 G. This corresponds to a frequency of 3.5 GHz. The system (Figure 3.81) includes an integrated touch screen, internal computer, thermoelectric cooler, and power supply. The instrument is available in an x-band version (9.5 GHz; 3480 ± 200 G).

Samples are introduced in capillary tubes, 2.0 mm or 5.8 mm inner diameter tubes. Solid samples and liquid samples in any solvent may be analyzed, with only 25–50 µL sample volume required for the S-band system and 100 µL sample volume minimum for the X-band system.

3.12.3 ESR Spectra and Hyperfine Interactions

The g-factor for a free electron is $g_e = 2.0023$. The excitation frequencies in ESR spectra depend on the total magnetic moment. If we obtain a spectrum at known frequency and magnetic field, we can calculate the g-factor for our sample. The energy levels of a bound, unpaired electron differ
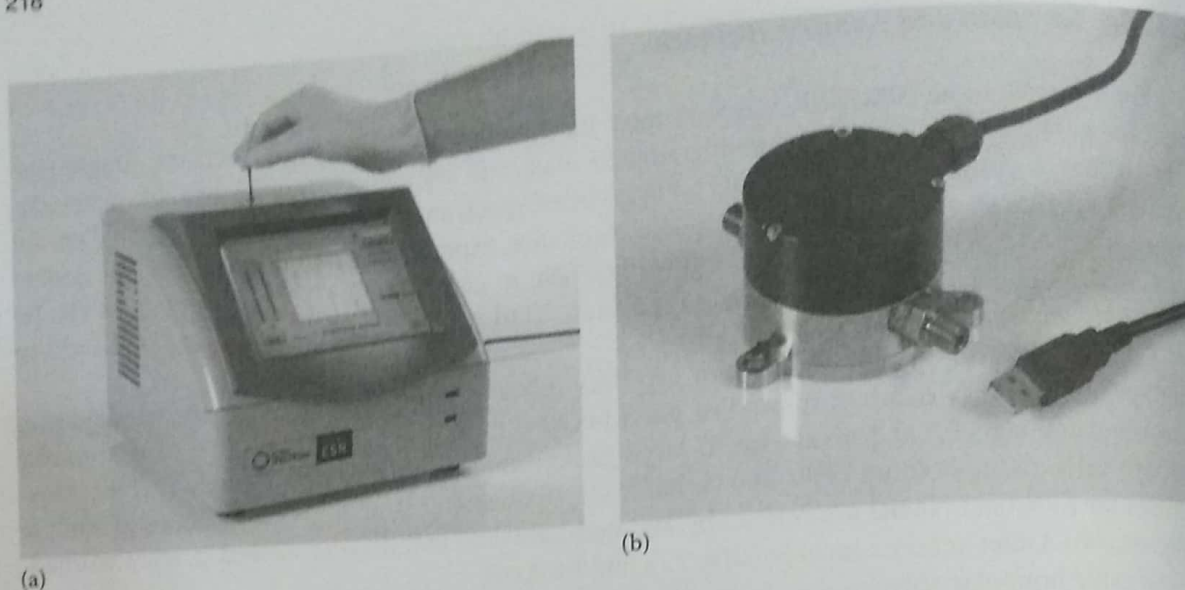
(b)

(a)

Figure 3.81   (a) Benchtop Micro-ESR™ spectrometer with integrated touch screen. The dimensions are 11.25" × 10" × 10". (b) Online Micro-ESR™ sensor with 24V DC power input, USB interface, and 2.5" diameter. (Active Spectrum, Inc., www.activespectrum.com. With permission.)

from those of a free electron due primarily to electron orbital angular momentum. If g does not equal g_e, the electron giving rise to the signal is not a free unpaired electron. The magnitude of the change in the g-factor gives information about the atomic or molecular orbital containing the unpaired electron. Organic radicals have g values from 1.99 to 2.01, while transition metal compounds have a wider range of g values, 1.4–3.0, due to factors like spin–orbit coupling. The g-factor helps us characterize the type of sample we are measuring. It can identify a specific metal ion, its oxidation state, spin state, and coordination environment. For example, cytochrome oxidase, a metalloprotein with more than one metal center, displays an ESR spectrum with multiple g-factors that are used to identify and characterize the different centers.

ESR is a sensitive probe for the local environment of a transition metal. Orbital angular momentum can be very large for the d orbitals of transition metals. As an example, if a transition metal is surrounded by six identical ligands bound symmetrically by the d orbitals, a single transition results (i.e., a single line in the spectrum). As the symmetry is lowered either by substitution of one or more ligands or distortion of the symmetry (Jahn–Teller distortion), anisotropy appears in the ESR signal. ESR can be used to determine the oxidation state and coordination of transition metal centers in compounds.

Since the ESR spectrum results from a change in the electron's spin state (with or against the field), it might be supposed that all ESR spectra would consist of a single transition. Fortunately, as with NMR, this is not the case. Bound unpaired electrons are very sensitive to the nuclei around them, through magnetic moment interaction, shown in Figure 3.82.

The interaction between an unpaired electron and nearby nuclei gives rise to additional allowed energy levels; this interaction is called the hyperfine interaction or hyperfine coupling. Hyperfine coupling can tell us the identity and number of atoms in a radical or complex as well as their distances from the electron. The interactions with neighboring nuclei can be described by Equation 3.20:

$$E = g\mu_B B_0 M_s + a M_s m_I \tag{3.20}$$

where

a is the hyperfine coupling constant
m_I is the nuclear spin quantum number

The value of a is measured as the distance between the centers of two signals (Figure 3.83a).