7.1: Why and how CW EPR spectroscopy is done

Sensitivity advantages of CW EPR spectroscopy

In NMR spectroscopy, CW techniques have been almost completely displaced by Fourier transform (FT) techniques, except for a few niche applications. FT techniques have a sensitivity advantage if the spectrum contains large sections of baseline and the whole spectrum can be excited simultaneously by the pulses. Neither condition is usually fulfilled in EPR spectroscopy. For two reasons, FT techniques lose sensitivity in EPR spectroscopy compared to the CW experiment. First, while typical NMR spectra comfortably fit into the bandwidth of a welldesigned critically coupled radiofrequency resonance circuit, EPR spectra are much broader than the bandwidth of a microwave resonator with high quality factor. Broadening detection bandwidth and proportionally lowering the quality factor *[Math Processing Error]* of the resonator reduces signal-to-noise ratio unless the absorption lineshape is infinitely broad. A quality factor of the order of 10 ’ 000 , which can be achieved with cavity resonators, corresponds to a bandwidth of roughly *[Math Processing Error]* at X-band frequencies around *[Math Processing Error]*. The intrinsic high sensitivity of detection in such a narrow band can be used only in a CW experiment. Second, even if the resonator is overcoupled to a much lower quality factor or resonators with intrinsically lower *[Math Processing Error]* are used (the sensitivity loss can partially be compensated by a higher filling factor of such resonators), residual power from a high-power microwave pulse requires about *[Math Processing Error]* in order to decay below the level of an EPR signal. This dead time is often a significant fraction of the transverse relaxation time of electron spins, which entails signal loss by relaxation. In contrast, in NMR spectroscopy the dead time is usually negligibly short compared to relaxation times. In many cases, the dead time in pulsed EPR spectroscopy even strongly exceeds *[Math Processing Error]*. In this situation FT EPR is impossible, even with echo refocusing, while CW EPR spectra can still be measured. This case usually applies to transition metal complexes at room temperature and to many rare earth metal complexes and high-spin Fe(III) complexes even down to the boiling point of liquid helium at normal pressure (4.2 K). For these reasons, any unknown potentially paramagnetic sample should first be characterized by CW EPR spectroscopy. Pulsed EPR techniques are required if the resolution of CW EPR spectroscopy provides insufficient information to assign a structure. This applies mainly to small hyperfine couplings in organic radicals and of ligand nuclei in transition metal complexes (see Chapter 8) and to the measurement of distances between electron spins in the nanometer range (see Chapter 9). At temperatures where pulse EPR signals can be obtained, measurement of relaxation times is also easier and more precise with pulsed EPR techniques.

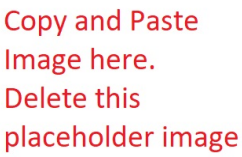

Figure 7.1: Scheme of a CW EPR spectrometer. Microwave from a fixed-frequency source is passed through an attenuator for adjusting its power and then through a circulator to the sample. Microwave that comes back from the sample passes on a different way through the same circulator and is combined with reference microwave of adjustable power (bias) and phase before it is detected by a microwave diode. The output signal of this diode enters a phase-sensitive detector (PSD) where it is demodulated with respect to the field modulation frequency (typically *[Math Processing Error]*) and at the same time amplified. The output signal of the PSD is digitized and further processed in a computer. The spectrum is obtained by sweeping the static magnetic field *[Math Processing Error]* at constant microwave frequency.

The CW EPR experiment

Since the bandwidth of an optimized microwave resonator is much smaller than the typical width of EPR spectra, it is impractical to sweep the frequency at constant magnetic field in order to obtain a spectrum. Instead, the microwave frequency is kept constant and coincides with the resonator frequency at all times. The resonance condition for the spins is established by sweeping the magnetic field *[Math Processing Error]*. Another difficulty arises from the weak magnetic coupling of the spins to the exciting electromagnetic field. Only a very small fraction of the excitation power is therefore observed. This problem is solved as follows. First, direct transmission of excitation power to the detector is prevented by a circulator (Figure 7.1). Power that enters port 1 can only leave to the sample through port 2 . Power that comes from the sample through port 2 can only leave to the detector diode through port 3. Second, the resonator is critically coupled. This means that all microwave power coming from the source that is incident to the resonator enters the resonator and is converted to heat by the impedance (complex resistance) of the resonator. If the sample is off resonant and thus does not absorb microwave, no microwave power leaves the resonator through port 3. If now the magnetic field *[Math Processing Error]* is set to the resonance condition and the sample resonantly absorbs microwave, this means

that the impedance of resonator + sample has changed. The resonator is no longer critically coupled and some of the incoming microwave is reflected. This microwave leaves the circulator through port 3 and is incident on the detector diode.

This reflected power at resonance absorption can be very weak at low sample concentration. It is therefore important to detect it sensitively. A microwave diode is only weakly sensitive to a change in incident power at low power (Fig. 7.2, input voltage is proportional to the square root of power). The diode is most sensitive to amplitude changes near its operating point, marked green in Fig. 7.2. Hence, the diode must be biased to its operating point by adding constant power from a reference arm. The phase of the reference arm must be adjusted so that microwave coming from the resonator and microwave coming from the reference arm interfere constructively.

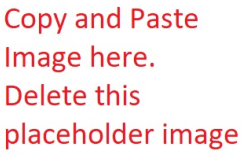

Figure 7.2: Characteristic curve of a microwave detection diode. At small input voltage, the diode is rather insensitive to changes in input voltage. At the operating point (green), dependence of output current on input voltage is linear and has maximum slope. This corresponds to *[Math Processing Error]* output current. If input voltage is too large, the diode is destroyed (red point).

A further problem arises from the fact that microwave diodes are broadband detectors. On the one hand, this is useful, since samples can significantly shift resonator frequency. On the other hand, broadband detectors also collect noise from a broad frequency band. This decreases signal-to-noise ratio and must be countered by limiting the detection bandwidth to the signal bandwidth or even below. Such bandwidth limitation can be realized most easily by effect modulation and phase sensitive detection. By applying a small sinusoidal magnetic field modulation with typical frequency of *[Math Processing Error]* and typical amplitude of *[Math Processing Error]*, the signal component at detector diode output becomes modulated with the same frequency, whereas noise is uncorrelated to the modulation. Demodulation with a reference signal from the field modulation generator (Figure 7.1) by a phase-sensitive detector amplifies the signal and limits bandwidth to the modulation frequency.

Effect modulation with phase-sensitive detection measures the derivative of the absorption lineshape, as long as the modulation amplitude *[Math Processing Error]* is much smaller than the width of the EPR line (Fig. 7.3). Since signal-to-noise ratio is proportional to *[Math Processing Error]*, one usually measures at *[Math Processing Error]*, where lineshape distortion is tolerable for almost all applications. Precise lineshape analysis may require *[Math Processing Error]*, whereas maximum sensitivity at the expense of significant artificial line broadening is obtained at *[Math Processing Error]*. The modulation frequency should not be broader than the linewidth in frequency units. However, with the standard modulation frequency of *[Math Processing Error]* that corresponds on a magnetic field scale to only *[Math Processing Error]* at *[Math Processing Error]*, this is rarely a problem.

Considerations on sample preparation

Since electron spins have a much larger magnetic moment than nuclear spins, electron-electron couplings lead to significant line broadening in concentrated solutions. Concentrations of paramagnetic centers should not usually exceed *[Math Processing Error]* in order to avoid such broadening. For organic radicals in liquid solution it may be necessary to dilute the sample to *[Math Processing Error]* in order to achieve ultimate resolution. For paramagnetic metal dopants in diamagnetic host compounds, at most *[Math Processing Error]* of the diamagnetic sites should be substituted by paramagnetic centers. Such concentrations can be detected easily and with good signal-to-noise ratio. For most samples, good spectra can be obtained down to the *[Math Processing Error]* range in solution and down to the 100 ppm dopant range in solids.

Line broadening in liquid solution can also arise from diffusional collision of paramagnetic

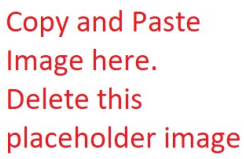

Figure 7.3: Detection of the derivative lineshape by field modulation. The situation is considered at the instantaneous field during a field sweep (vertical dashed line) that is slow compared to the field modulation frequency of *[Math Processing Error]*. Modulation of the magnetic field with amplitude *[Math Processing Error]* (blue) causes a modulation of the output signal *[Math Processing Error]* (red) with the same frequency and an amplitude *[Math Processing Error]*. Phase-sensitive detection measures this amplitude *[Math Processing Error]*, which is proportional to the derivative of the grey absorption lineshape and to *[Math Processing Error]*, as long as *[Math Processing Error]* is much smaller than the peak-to-peak linewidth *[Math Processing Error]* of the line. In practice, *[Math Processing Error]* is usually acceptable. For precise lineshape analysis, *[Math Processing Error]* is recommended.

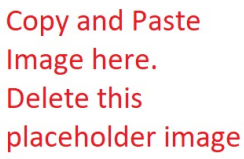

Figure 7.4: Relaxation enhancement by collisional exchange with oxygen in solution. (a) Situation before diffusional encounter. As an example, triplet oxygen is assumed to be in a *[Math Processing Error]*state (red), whereas the spin of a nitroxide radical is assumed to be *[Math Processing Error]* (green). (b) The oxygen molecule and nitroxide radical have collided during diffusional encounter. Their wavefunctions overlap and the three unpaired electrons cannot be distinguished from each other (grey). (c) After separation, the three unpaired electrons have been redistributed arbitrarily to the two molecules. For example, oxygen may now be in the *[Math Processing Error]* state (red) and the nitroxide in the *[Math Processing Error]* state (green). The electron spin of the nitroxide radical has flipped.

species with paramagnetic triplet oxygen (Figure 7.4). During such a collision, wavefunctions of the two molecules overlap and, since electrons are undistinguishable particles, spin states of all unpaired electrons in both molecules are arbitrarily redistributed when the two molecules separate again. The stochastic diffusional encounters thus lead to additional flips of the observed electron spins, which corresponds to relaxation and shortens longitudinal relaxation time *[Math Processing Error]*. Since the linewidth is proportional to *[Math Processing Error]* and *[Math Processing Error]* cannot be longer than *[Math Processing Error]*, frequent collisional encounters of paramagnetic species lead to line broadening. Such line broadening increases with decreasing viscosity (faster diffusion) and increasing oxygen concentration. The effect is stronger in apolar solvents, where oxygen solubility is higher than in polar solvents, but it is often significant even in aqueous solution. Best resolution is obtained if the sample is free of oxygen. The same mechanism leads to line broadening at high concentration of a paramagnetic species in liquid solution. In the solid state, line broadening at high concentration is mainly due to dipole-dipole coupling. Often, the anisotropically broadened EPR spectrum in the solid state is of interest, as it provides information on *[Math Processing Error]* anisotropy and anisotropic hyperfine couplings. This may require freezing of a solution of the species of interest. Usually, the species will precipitate if the solvent crystallizes, which may cause line broadening and, in extreme cases, even collapse of the hyperfine structure and averaging of *[Math Processing Error]* anisotropy by exchange between neighboring paramagnetic species. These problems are prevented if the solvent forms a glass, as is often the case for solvents that have methyl groups or can form hydrogen bonds in very different geometries. Typical glass-forming solvents are toluene, 2-methyltetrahydrofuran, ethanol, ethylene glycol, and glycerol. Aqueous solutions require addition of at least *[Math Processing Error]* glycerol as a cryoprotectant. In most cases, crystallization will still occur on slow cooling. Samples are therefore shock frozen by immersion of the sample tube into liquid nitrogen. Glass tubes would break on direct immersion into liquid nitrogen, but EPR spectra have to be measured in fused silica sample tubes anyway, since glass invariably contains a detectable amount of paramagnetic iron impurities.