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Rapid-scan EPR of nitroxide spin labels and semiquinones

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Abstract

Rapid-scan electron paramagnetic resonance is based on continuous direct detection of the spin response as the magnetic field is scanned up-field and down-field through resonance thousands of times per second. The method provides improved signal-to-noise for a wide range of samples, including rapidly tumbling and immobilized radicals. This chapter provides an introduction to the method and practical examples of implementation for organic radicals.

Keywords

direct detection EPR; nitroxide; rapid scan; spin trapping; superoxide

Introduction

An on-going challenge for EPR spectroscopy is the need for improved signal-to-noise ratio (S/N). The rapid-scan direct detection method has been shown to improve S/N relative to continuous wave (CW) EPR for a wide range of samples including rapidly-tumbling nitroxides in fluid solution (D. G. Mitchell, Quine, Tseitlin, Eaton, & Eaton, 2012), spin-trapped $O_2^{\bullet-}$ (D. G. Mitchell, Rosen, et al., 2013), immobilized nitroxides (Yu et al., 2014), images of nitroxides in a phantom at 250 MHz (Biller et al., 2014b), the E' center in irradiated fused quartz (D. G. Mitchell et al., 2011), paramagnetic centers in amorphous hydrogenated silicon (D. G. Mitchell, Tseitlin, et al., 2013), $N@C_{60}$ diluted in C_{60} (D. G. Mitchell, Tseitlin, et al., 2013), and the neutral single substitutional nitrogen centers (N_S^0) in diamond (D. G. Mitchell, Tseitlin, et al., 2013). The historical background and fundamentals of rapid scan are described in (S. S. Eaton et al., 2014). The focus of this chapter is on practical considerations for organic radicals such as nitroxides and semiquinones.

Rapid-Scan Method

In rapid-scan EPR the signal is acquired directly, without using magnetic field modulation and without phase-sensitive detection at the modulation frequency, while the magnetic field is scanned repeatedly through resonance in times that are short relative to electron spin relaxation times. For example, one might scan the magnetic field over the entire width of a 100-Gauss spectrum thousands of times per second, and signal average the response. If the scan time is long relative to the electron spin relaxation times, the resulting signal is indistinguishable from the absorption spectrum that would be obtained by integration of the conventional CW spectrum. If the scan time is short relative to electron spin relaxation times, oscillations are observed on the trailing edge of the signal. The underlying spin physics that causes the oscillations can be mathematically modeled, so the corresponding slow-scan absorption spectrum can be calculated by deconvolution. An example of a rapid-scan signal and deconvolution is shown in Figure 1 for 2,3,4,5-tetramethoxybenzoquinone (TBMSQ) in methanol (Elajaili, Biller, Eaton, & Eaton, 2014). In this experiment a sinusoidal scan was generated with a scan frequency of 41.6 kHz and scan width of 10 G. The signal was recorded as the magnetic field increased and decreased through resonance (Fig. 1A), and rapid-scan oscillations were observed on the trailing edge of the signal. Superficially the oscillations look like an FID response, but closer inspection shows that the spacing between peaks in the oscillations decreases as the field is scanned further from resonance. Although the signal is digitized in quadrature, to simplify the presentation only the absorption channel is shown in Fig. 1A. Detection in quadrature permits phase correction in post processing. The slow-scan absorption spectrum was calculated by deconvolution (M. Tseitlin, Rinard, Quine, Eaton, & Eaton, 2011), resulting in the spectrum shown in Fig. 1B. The characteristic ^{13}C -hyperfine lines are well defined in the spectrum. The more-familiar first-derivative spectrum obtained by numerical differentiation is shown in Fig. 1C and is in excellent agreement with the spectrum obtained by CW spectroscopy.

Why Rapid scan improves S/N

If a CW spectrum is obtained with modulation amplitude that is small enough to provide a first-derivative curve with high fidelity, the modulation amplitude should be less than about 10% of the line width (G. R. Eaton, Eaton, Barr, & Weber, 2010). Thus only a small fraction of the amplitude of the spectrum is measured in each modulation cycle. In the rapid-scan method, the repetitive magnetic field scans are larger than the line width, except for cases of very broad spectra that are discussed below. Thus, in the common cases of spectra that are narrower than the field-scan amplitude, the entire spectrum amplitude is measured. Depending on how conservatively the modulation amplitude is selected in the CW spectrum, this signal amplitude advantage for rapid scan may be as much as a factor of five.

If the relaxation time of the spin system is long enough that the CW spectrum can be power-saturated, it has been shown that higher B_1 can be used in rapid scan than in CW spectroscopy (D. G. Mitchell et al., 2012; D. G. Mitchell, Tseitlin, et al., 2013). For common organic radicals the resulting spectral amplitude can be larger by up to about a factor of 3 in rapid scan relative to CW. The rapid-scan method can be viewed as analogous to pulsed EPR in that the spin is under the influence of the microwave B_1 for a short time. In pulsed EPR the microwave power is turned on and off quickly, forming, for example, 40 ns microwave pulses. In rapid scan, the microwave power is constant, as in CW, but the field is scanned through resonance in times that can be shorter than the spin relaxation time. For example, if the spectral line is 100 mG wide (1 μ T), and the magnetic field is scanned sinusoidally with a scan width of 100 G at 10 kHz, the magnetic field is on resonance for only ~ 32 ns, which is about the same time as for the hypothetical pulse.

Magnetic field modulation, commonly at 100 kHz, is used in CW EPR to move the EPR signal away from base-band to a frequency where the source phase noise is low (G. R. Eaton et al., 2010). Since the time for each cycle in rapid-scan data acquisition is fairly short relative to acquisition capabilities of modern digitizer/averagers, multiple cycles can be acquired and averaged as a single array. Data are acquired continuously as the field is scanned up-field and down-field through resonance for multiple cycles. Averaging of a periodic signal is equivalent to applying a comb filter, with a much narrower filter bandwidth than is used in CW EPR. Noise that is not coherent with the scan frequency is suppressed. This principle was demonstrated in the early days of magnetic resonance for NMR spectra that included ‘wiggles’ and for CW EPR spectra (Klein & Barton, 1963).

By using quadrature detection, and combining signals from up-field and down-field scans, further improvement in S/N in rapid scan is possible (S. S. Eaton et al., 2014).

A disadvantage of rapid scans is that for narrow-line spectra faster scans requires a lower Q (see the following section), which lowers the signal amplitude. In addition, rapid scan requires larger filter bandwidth (proportional to scan rate) in the detection path. However, signal averaging can also be performed more times per second, so the impact of increased filter bandwidth is only a loss of a factor of 2. After deconvolution the filter that can be applied in post processing depends only on the spectral linewidth, so the final filter is the same as would be used for conventional CW.

Experimental data for specific examples showing improvements in S/N are discussed in detail in (S. S. Eaton et al., 2014). For most organic radicals in fluid solution electron spin relaxation times are relatively short, which limits the applicability of pulse experiments. For imaging experiments the gradients decrease T_2^* , which also limits the applicability of pulse measurements. Thus the focus of this chapter is on comparison of rapid scan with CW.

Other advantages of rapid scan

The absorption spectrum is obtained directly

Normal CW EPR spectra are presented as the derivative of the absorption spectra. Rapid-scan spectra are obtained as the absorption spectra. Analyzing the absorption spectrum has several advantages. (i) Uncertainty analysis has shown that when spectral simulation is used to analyze data with the same signal-to-noise, the linewidths can be obtained with the same uncertainties from absorption and first-derivative spectra, and the spin concentrations can be calculated more accurately from the absorption spectrum than from the first derivative (M. Tseitlin, Eaton, & Eaton, 2012). Taking a derivative enhances high-frequency noise, and integration of the first derivative to obtain the absorption signal emphasizes low-frequency noise. Thus, interconverting between absorption and first derivative signal displays changes the noise spectrum of the data. (ii) Spectral-spatial EPR images typically are reconstructed as the absorption spectrum as a function of position in the sample. Particularly when S/N is low there are substantial baseline uncertainties and integration of the first-derivative spectrum can introduce low frequency noise that is difficult to handle in image reconstruction. (iii) The spectral dimension of spectral-spatial images is encoded by recording data as a function of magnetic field gradient (G. R. Eaton & Eaton, 1995). In the presence of a magnetic field gradient the amplitude of the first-derivative spectrum decreases approximately quadratically with gradient, while the amplitude of the absorption spectrum decreases approximately linearly with gradient. The net result is substantially improved S/N at high gradient for rapid-scan spectra than for CW spectra (Biller et al., 2014b). The quality of an image is strongly dependent on the S/N at high gradient, so the improved S/N in the high gradient spectra recorded by rapid scan relative to CW is significant.

Passage effects are explicitly taken into account

For samples with long spin lattice relaxation times, including most organic radicals at cryogenic temperatures, T_1 is long relative to the reciprocal of the modulation frequency which typically is 1/100 kHz. When this is the case, the lineshape in spectra obtained by CW are distorted. These problems can be avoided by using rapid scan, as has been demonstrated for the E' defect in irradiated quartz (D. G. Mitchell et al., 2011) and for defects in diamond (D. G. Mitchell, Tseitlin, et al., 2013).

Hardware Requirements

Uniformity of B_1 and scanned field over the sample

In conventional CW spectra, one can accept variations in B_1 over the sample, so long as one is not attempting to derive relaxation times from the response. For rapid scan, if the experiment is designed to take advantage of the higher B_1 that can be used without

saturating the spin system the sample should be within the uniform B_1 field of the resonator. If not, signal quantitation is compromised, just as it is in CW spectra.

In conventional CW spectroscopy one can accept variations in the modulation field over the sample. Provided that the largest modulation amplitude is small relative to linewidths, variations in the field may contribute to nonuniform detection sensitivity, but do not impact lineshape. However, for rapid-scan EPR the deconvolution algorithms are based on the assumption of uniform scanned field and the accuracy with which the slow-scan lineshape can be calculated by deconvolution is limited by the uniformity of the scanned magnetic field over the sample. These requirements dictate design of the rapid-scan spectrometer. The scan coils should provide a uniform scan field over the entire sample. A consequence of the need for uniform magnetic field scans is that the rapid-scan coils are much larger than the modulation coils used in most CW EPR resonators. Spectra demonstrating the degradation of spectral resolution that occurs when the sample is large enough that the scan field is not uniform over the sample are shown in (S. S. Eaton et al., 2014).

To generate a linear scan, the system has to be designed to produce frequencies at least seven times that of the stated linear scan frequency. When using linear scans only the portion of the scan that is linear to within the specifications of the experiment should be used. At the ends of the scan the “linear” or “triangular” scan becomes rounded, and that portion of the data should be discarded (Joshi et al., 2005). For very fast scans, sinusoidal scans are needed, in which the coil driving circuit is resonated (Quine, Mitchell, Eaton, & Eaton, 2012). The rate varies as a function of position in the scan, but this is taken into account in the deconvolution procedure (M. Tseitlin et al., 2011). Generation of rapid wide scans is facilitated by using scan coils wound from Litz wire (Yu et al., 2014).

Scan coil driver

To accomplish the rapid scans requires a carefully designed scan driver system. The feedback system produces a voltage pattern the purpose of which is to produce a current of the desired shape. If the shape is sinusoidal, capacitors are selected to resonate the scan coils at the desired frequency. Detailed designs of a linear scan driver (Quine, Czechowski, & Eaton, 2009) and of a sinusoidal scan driver (Quine et al., 2012) have been published. More sophisticated designs that include both linear and sinusoidal functions, and have remote control inputs have been built for use in the Denver, Chicago, and Bruker labs.

Resonator design

Rapidly changing magnetic fields induce electrical currents in conductors, and these eddy currents produce magnetic fields that combine with the applied magnetic field, often in a way that reduces the homogeneity of the applied magnetic field. Consequently, conductive parts of the resonator have to be designed to minimize eddy current contributions at the location of the sample. The Bruker X-band dielectric resonator has relatively few metallic parts and has been used extensively for rapid-scan experiments in Denver. At lower frequencies locally designed and built resonators have been used in which metallic components are kept to a minimum.

Magnetic field interaction with the resonator, the transmission line, and the mechanism that couples the transmission line to the resonator results in non-EPR background signals that contribute to the detected signal and may overwhelm weak EPR signals. Since these signals are magnetic-field-dependent, they act as pseudo-EPR signals. However, the major background signal has the frequency of the scan field and its harmonics, so these can be removed post-acquisition (M. Tseitlin, Czechowski, Quine, Eaton, & Eaton, 2009; M. Tseitlin, Mitchell, Eaton, & Eaton, 2012). Mechanical vibrations of the resonator also contribute to the background signal. These can be minimized by empirical design modifications. In practice, one searches for scan frequencies at which the background signals are minimal. Background signals typically are weaker if the resonator shield is inside the scan coils rather than outside.

For experiments at 250 MHz work in the Denver lab has used primarily cross-loop resonators for rapid-scan experiments (Biller et al., 2015; Biller et al., 2014b).

Parameter Selection

Scan width

Unless acquisition of only a segment of the spectrum is desired, the scan width is dictated by the sample. The widest spectrum for which a rapid scan has been acquired in a single segment was an immobilized nitroxide with a spectral width of 155 G (Yu et al., 2014). This width would be sufficient to encompass the fluid solution or immobilized spectrum of most organic radicals. Wider scans typically result in larger non-resonant background signals, so scan widths usually are selected that are just enough to encompass the spectrum.

Linear vs. sinusoidal scans

In a triangular scan, the scan rate is constant across the spectrum and is given by

$$a_t = 2f_s B_m \text{ G s}^{-1} \quad (1)$$

where a_t is the triangular scan rate, f_s is the scan frequency, and B_m is the scan width in gauss.

Wider scans can be obtained with sinusoidal scans and resonated coils than with linear scans because for the same scan width the voltage across the scan coil + capacitor assembly is smaller than for linear scans (Quine et al., 2012). Wider scans encompass broader spectra and also provide faster scan rates for the same scan frequency. For a sinusoidal scan the rate varies across the spectrum. The maximum rate at the center of the scan, a_s , is given by

$$a_s = \pi f_s B_m \text{ G s}^{-1}. \quad (2)$$

Resonator Q

Higher resonator Q results in larger EPR signal (G. R. Eaton et al., 2010) and also results in higher S/N , unless microwave source noise dominates. Resonator Q has a filter function in

rapid-scan EPR that is not encountered in CW EPR. In this context, it is convenient to define resonator Q as

$$Q = \frac{\nu}{\Delta\nu} = \frac{\nu}{BW_{res}} \quad (3)$$

Where BW_{res} denotes the bandwidth of the resonator. The bandwidth of the resonator that is relevant for rapid scan is $\frac{1}{2} BW_{res}$ because the rapid-scan signal is recorded with either increasing or decreasing field/frequency and the signals for each half cycle fall within either the upper or lower half of the resonator bandwidth. Hence, the bandwidth available for rapid scan is

$$BW_{RS} = \frac{\nu}{2Q} \quad (4)$$

The rapid-scan signal bandwidth is related to the spin relaxation time by

$$BW_{sig} = \frac{N\gamma}{2\pi} aT_2^* \quad (5)$$

T_2^* is the time constant for the decay of the oscillations on the trailing edge of the signal. This is the decay time that would be observed for an FID of this spin system. (Note that the frequency bandwidth of the signal depends on T_2^* , not T_2 .) The parameter N is selected to be more or less conservative about the resolution of the signal. $N = 5$ is usually adequate to avoid broadening of the signal by the filter effect of the resonator bandwidth. A practical example may help to clarify the concept. A rapidly tumbling nitroxide such as perdeuterated tempone (PDT) has a narrow 150 mG peak-to-peak line width. An 80 G scan width for PDT

at 40 kHz ($a = 10^7$ g/s) has a signal bandwidth of ca. 62 MHz if $N = 5$. The X-band resonator Q would have to be less than about 78 to avoid broadening the spectrum. Most nitroxides have substantially broader lines so the constraint on Q would not be as severe. If the goal were to maximize S/N and some broadening was acceptable, a Q higher than 78 could be used. Alternatively, if lineshape fidelity was the highest priority, then a slower scan rate would be required if the resonator Q was greater than about 78.

For in vivo experiments at low frequency the lossiness of the animal often lowers the resonator Q sufficiently that signal bandwidth is less of a problem relative to resonator Q than for non-lossy samples at X-band. In imaging experiments the signal bandwidth often is decreased by the gradient.

Scan frequency

The S/N advantage of rapid scan that comes from encompassing the full spectrum in each scan is gained independent of scan frequency. Higher scan frequency also permits more averages per unit time and may push the data acquisition into the rapid-scan regime in which higher B_1 can be used. However the higher the scan frequency, the larger the signal bandwidth (Eq. 5), which may become incompatible with resonator Q .

Examples

Spin trapping

Spectra of nitroxides produced by spin trapping are particularly difficult to measure accurately because of low concentrations and short lifetime. Good S/N for spin-trapped radicals has been obtained by rapid scan under conditions for which the EPR signal was barely detectable using a state-of-the-art CW spectrometer (Biller et al., 2015; D. G. Mitchell, Rosen, et al., 2013). Superoxide was generated by the bacterium *E. faecalis* in the presence of 10 mM glucose and trapped with BMPO (5-*tert*-butoxycarbonyl-5-methyl-1-pyrroline-N-oxide) to form BMPO-OOH. Rapid-scan EPR spectra were obtained on a custom Bruker E500T X-band spectrometer with a dielectric ER4118X-MD5 resonator. The samples lowered the resonator Q to about 850, which corresponds to a resonator bandwidth for rapid scan of about 5.6 MHz (Eq. 4). Sinusoidal scans were generated with a locally-designed and built scan driver (Quine et al., 2012), that includes interchangeable capacitors to resonate the scan-coil circuit. Litz wire coils with 7.6 cm average diameter were mounted outside the resonator, coaxially with the main magnetic field. These coils are large enough to give good scan field uniformity over the active volume of the resonator. The scan frequency

was 51 kHz and the scan widths were 55 G, which produces a scan rate of 8.9 MG/s (Eq. 2) in the center of the spectrum. The unresolved hyperfine splitting broadens the lines in the spectra of the trapped adduct to about 1 G, which is a T_2^* of about 6.6×10^{-8} s. The signal bandwidth is then 4.8 MHz (Eq. 5, $N = 3$). A value of $N = 3$ was used in calculating the signal bandwidth because some broadening of the line was acceptable. Spectra of spin-trapped adducts often are recorded at relatively high modulation amplitudes to improve S/N . Accurate hyperfine splittings are more important for these experiments than precise linewidths. A microwave power of 53 mW ($B_1 = 250$ mG) was selected to maximize signal amplitude with less than 2% line broadening. Data were acquired in segments containing 12 cycles of the sinusoidal scans. These segments were averaged 100k times. Background correction (M. Tseitlin, Mitchell, et al., 2012), sinusoidal deconvolution (M. Tseitlin et al., 2011), combination of signals in real and imaginary channels, and combination of up-field and down-field scans were performed, to obtain the spectrum in Fig. 2B. The resolution of the hyperfine splittings is similar to that obtained by CW EPR with much longer data acquisition times and there is good agreement between hyperfine splitting parameters obtained for BMPO-OOH by CW and rapid-scan EPR. For the same signal acquisition time the signal-to-noise is more than 40 times higher for rapid-scan than CW EPR (D. G. Mitchell, Rosen, et al., 2013).

Immobilized nitroxide

Spectra of immobilized nitroxides are key to many applications with spin-labeled proteins. Rapid-scan spectra of PDT immobilized in sucrose octaacetate (Yu et al., 2014) were obtained with the same spectrometer and resonator as for the spin trapping experiments. For this non-lossy sample the resonator Q is about 9000, which gives a bandwidth for the rapid-scan experiments of about 0.5 MHz. Data were acquired with a sinusoidal scan frequency of 13.4 kHz and scan width of 155 G, which produces a maximum scan rate in the center of the spectrum of 6.5 MG/s (Eq. 2). The narrowest feature in the center of the spectrum was estimated to have a peak-to-peak linewidth about 3 G, which is a T_2^* of about 2×10^{-8} s. Calculation of the signal bandwidth using $N = 3$ gives 1 MHz (Eq. 2), which is larger than the resonator bandwidth. The lineshape of the resulting rapid-scan spectrum (Fig. 3A,B) is in good agreement with the CW spectrum obtained with the same data acquisition time. The small line broadening that results from signal bandwidth larger than resonator bandwidth is not conspicuous for such a broad spectrum. If the spectrum had included more features with narrower linewidths, the scan frequency would need to be decreased to accurately recover the details of the spectrum.

In vivo spectroscopy and imaging

Nitroxide spin labels, spin probes, and radicals produced by spin trapping are very useful for studies of in vivo physiology. Although pulsed EPR imaging of nitroxides is possible under certain circumstances (Hyodo et al., 2009), rapid scan is the method of choice for imaging nitroxides. Efforts in this direction are underway in the H. J. Halpern laboratory in Chicago and the M.C. Krishna laboratory (Subramanian et al., 2007). Published rapid-scan imaging experiments have been performed with phantoms of TCNQ (Subramanian et al., 2007), LiPc (Czechowski et al., 2014), nitroxides (Aminov, Tseitlin, & Salikhov, 1999; Biller et al.,

2015; Biller et al., 2014b; M. Tseitlin et al., 2014) and pH-sensitive amino substituted trityl radicals (Elajaili, Biller, Tseitlin, et al., 2015).

Measurement of redox status is crucial for characterization of tumor physiology (Elajaili, Biller, Rosen, et al., 2015). The interconversion of disulfide bridged nitroxide diradicals and the corresponding monoradicals can be used to monitor redox status. Low-frequency (250 MHz) EPR imaging using rapid-scan methodology has been used to follow reaction of the S-S bond of diradical **I** with glutathione to produce monoradicals. The EPR spectra of disulfide dinitroxides are readily distinguished from those of the corresponding monoradicals that are formed by cleavage of a redox-sensitive disulfide linkage. For example, the spectrum of ^{15}N -substituted ($I = 1/2$) diradical **I** includes a broad peak between the two sharp peaks that are characteristic of the ^{15}N labeled monoradical (Fig. 4B). Spectroscopy and imaging were performed at 250 MHz (9 mT, 90 G) (Quine, Rinard, Eaton, & Eaton, 2010). Rapid sinusoidal scans were generated with a locally-designed coil driver (Quine et al., 2012) and 8.9 cm diameter Litz wire coils to ensure uniformity of the scanning field over the sample. A cross loop resonator (Biller et al., 2014a; Rinard, Quine, Biller, & Eaton, 2010) was used to isolate the detection system from the excitation power. The resonator and scan coils were mounted with vibration isolating Sorbothane polymer (Sorbothane, Inc., Kent, Ohio) in a Nylon bracket. The scan frequency was 2.06 kHz and the scan width was 70 G, which gives a scan rate of 0.45 MG/s (Eq. 2) in the center of the spectrum. The scan was selected to be at a frequency that gives a low rapid-scan background with this resonator assembly. The resonator Q is about 100, which has a band width for rapid scan of 1.3 MHz. The linewidths of the sharpest lines in the absence of gradient are about 0.8 Gauss, which corresponds to T_2^* of 8×10^{-8} s. The signal bandwidth is 0.5 MHz, which is conservative relative to the resonator bandwidth. The maximum magnetic field gradient was 20 G/cm. Images were reconstructed from 20 projections with equally-spaced gradients (M. Tseitlin et al., 2014). The images (Fig. 4) clearly distinguish between the contents of the two tubes of the phantom at the start of the experiments and after partial cleavage of the S-S

bond in only one compartment of the phantom. Work is underway to extend this methodology to mouse tumors.

Wider Scans

Sinusoidal rapid magnetic field scans with widths up to ca. 155 G have been performed. Such scans encompass the full spectra of immobilized nitroxides and most organic radicals, including radiation defect centers in organic solids. However, many species of interest in enzymology have much broader spectra. Two approaches have been taken to extend direct detection and rapid-scan technology to wider scans.

The Hyde lab has implemented a method that they call NARS, in which wide spectra of nitroxides and copper(II) are acquired in segments (Kittell, Camenisch, Ratke, Sidabras, & Hyde, 2011) at a “rate that is sufficiently slow that adiabatic responses are avoided.” For example a series of 28.2 G linear scans was performed at 5.2 kHz, with the B_0 field incremented by 10 G between segments. The segments were combined in software to encompass the full spectrum. About a factor of 5 increase in S/N was achieved relative to CW. A solution of Cu(II) was frozen with a liquid nitrogen flow system and 170 5-Gauss segments were acquired spanning 850 G at 1.9 GHz (Hyde, Bennett, Kittell, Kowalski, & Sidabras, 2013). 45 G triangular scans at 2.6 kHz were averaged after each 5 G step in B_0 . Prior to digitization, a 1–300 kHz filter was applied. Data from the upfield scans of 7 of the resulting 9 segments that contained data from each spectral component were combined. After combining segments, the S/N was about 4 times higher than with conventional CW EPR. Extension of the NARS method to spin-labeled T4-lysozyme (Kittell & Hyde, 2015) included conditions under which the spin label signal was partially saturated and under which adiabatic rapid passage effects were observed. A key characteristic of these experiments was that there was little overlap of successive spectral segments, which caused discontinuities between segments.

A field-stepped direct detection EPR method that is based on rapid-scan technology has been developed with scan widths up to 6200 G (Yu et al., 2015). A scan frequency of 5.12 kHz was generated with a linear scan driver. The field was stepped at intervals of 0.01 to 1 G, depending on the linewidths in the spectra. At each field data for triangular scans with widths up to 11.5 G were acquired. Data from the triangular scans were combined by matching DC offsets for extensively overlapping regions of successive scans. This approach has advantages relative to CW that are similar to rapid scan, even if the scan rate is not fast relative to relaxation rates. A degassed lithium phthalocyanine sample was used to demonstrate that the linear deconvolution procedure can be applied to field-stepped direct detection EPR signals in which rapid scan oscillations are observed (Joshi et al., 2005). Field-stepped direct detection EPR spectra were obtained for PDT in sucrose octaacetate, Mn^{2+} impurity in CaO, Cu^{2+} doped in Ni(diethyldithiocarbamate)₂, Cu^{2+} doped in Zn tetratolylporphyrin, vanadyl ion doped in a parasubstituted Zn tetratolylporphyrin, and an oriented crystal of Mn^{2+} doped in Mg(acetylacetonate)₂(H₂O)₂. This method has the potential to replace conventional CW.

Future applications

This chapter is an interim description of a rapidly-developing new field of EPR spectroscopy. Rapid-scan EPR has already been shown to yield superior S/N in the same data acquisition time relative to standard field-modulated CW EPR and pulsed EPR for a wide range of sample types, from low frequency imaging of nitroxide radicals to defect centers in solids and single crystals doped with transition metals. Accessories to commercial EPR spectrometers are envisioned.

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Figure 1.

Sinusoidal rapid scan of TMBSQ in methanol recorded with 41.6 kHz scan frequency and 10 G scan width. A) Signal acquired for one full sinusoidal scan, showing only one channel of the quadrature detection. B) Absorption spectrum obtained by deconvolution. C) First derivative of the spectrum in part B. Data were acquired at room temperature by H. Elajaili at the University of Denver, 2014.

Figure 2. Comparison of CW and rapid-scan spectra of BMPO-OOH in a suspension of *E. faecalis* with 2×10^6 CFU/mL and a $\text{O}_2^{\bullet -}$ production rate of $0.2 \mu\text{M}/\text{min}$, recorded 10 min after mixing reagents. The concentration of BMPO-OOH is about $0.5 \mu\text{M}$. A) CW spectrum obtained with 55 G sweep width, 0.75 G modulation amplitude, single 30 s scan, 15 ms conversion time, 10 ms time constant, and 20 mW ($B_1 = 170$ mG) microwave power. B) Deconvolved rapid-scan spectrum obtained with 55 G scan width, 51 kHz scan frequency, $B_1 = 250$ mG microwave power, segments consisting of 12 sinusoidal cycles were averaged 100k times, with a total data acquisition time of about 30 seconds. Reproduced with permission from (D. G. Mitchell, Rosen, et al., 2013).

Figure 3.

CW and rapid-scan spectra of 0.15 mM PDT in sucrose octaacetate at 293 K obtained with 10 s acquisition time. (A) Absorption spectrum obtained by rapid scan, (B) first derivative spectrum obtained from (A) by numerical differentiation, and (C) field-modulated CW spectrum obtained with 100 kHz and 0.63 G modulation amplitude, which is 20% of $B_{pp} = 3.2$ G. Reproduced with permission from (Yu et al., 2014)

Figure 4.

2D spectral-spatial images of 0.5 mM ^{15}N -dinitroxide in a two-compartment phantom with a 10 mm spacer between compartments. A) Both compartments contain diradical. B) Slices through the upper (blue) and lower (red) compartments of image in part A. C) Image after reaction of glutathione in the lower compartment. The upper compartment contains diradical and the lower compartment contains the corresponding monoradical. D) Slices through the upper (blue) and lower (red) compartments of image in part C. Data were acquired by H. Elajaili and J. Biller at the University of Denver, 2014.