Published in final edited form as:

J Magn Reson. 2015 May ; 254: 86–92. doi:10.1016/j.jmr.2015.03.006.

Multiharmonic Electron Paramagnetic Resonance for Extended Samples with both Narrow and Broad Lines

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Abstract

Multiharmonic electron paramagnetic resonance spectroscopy was demonstrated for two samples with both narrow and broad lines: (i) α,γ -Bisdiphenylene- β -phenylallyl (BDPA) with B_{pp} of 0.85 G plus ultramarine blue with B_{pp} of 17 G, and (ii) a nitroxide radical immobilized in sucrose octaacetate. Modulation amplitudes up to 17 G at 41 kHz were generated with a rapid scan coil driver and Litz wire coils that provide uniform magnetic field sweeps over samples with heights of 5 mm. Data were acquired with a 2-D experiment in the Xepr software through the transient signal path of a Bruker E500T and digitized in quadrature with a Bruker SpecJet II. Signals at the modulation frequency and its harmonics were calculated by digital phase-sensitive detection. The number of harmonics with signal intensity greater than noise increases as the ratio of the modulation amplitude to the narrowest peak increases. Spectra reconstructed by the multiharmonic method from data obtained with modulation amplitudes up to five times the peak-to-peak linewidths of the narrowest features have linewidths that are broadened by up to only about 10% relative to linewidths in spectra obtained at low modulation amplitudes. The signal-to-noise improves with increasing modulation amplitude up to the point where the modulation amplitude is slightly larger than the linewidth of the narrowest features. If this high a modulation amplitude had been used in conventional methodology the linewidth of the narrowest features would have been severely broadened. The multiharmonic reconstruction methodology means that the selection of the modulation amplitude that can be used without spectral distortion is no longer tightly tied to the linewidth of the narrowest line.

Graphical abstract

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1. Introduction

In conventional continuous wave (CW) electron paramagnetic resonance (EPR) the magnetic field is modulated, often at 100 kHz, and the first-derivative of the absorption spectrum is acquired by phase-sensitive detection at the modulation frequency at a series of magnetic fields. In conventional spectroscopy if the ratio of the modulation amplitude to the peak-to-peak linewidth is greater than about 0.25, the spectrum is broadened [1]. Combining information from multiple harmonics of the field modulation frequency has been shown to give the first-derivative spectrum with minimal broadening up to modulation amplitudes that are several times the linewidths [2]. The amplitude at the fundamental and at higher harmonics can be obtained by digital phase-sensitive detection of the signal at the modulation frequency and at its integer multiples [3]. As the modulation amplitude is increased, the intensities at the higher harmonics of the modulation frequency increase, which can be used to improve the signal-to-noise of the spectrum for modulation amplitudes up to about 1.2 times linewidths, provided that sufficient harmonics are included in the data analysis [3]. Accurate reconstruction of the spectrum from the higher harmonics requires that the modulated magnetic field is uniform over the sample. In the initial reports the samples were very small because the magnetic field uniformity that could be obtained with commercial modulation coils was adequate for multiharmonic spectroscopy only over a small region of space and with limited modulation amplitudes [2,3]. The larger scan coils that have been developed for rapid scan EPR improve the magnetic field homogeneity over larger samples [4] relative to what can be achieved with conventional modulation coils. In addition, the rapid scan coil driver can generate larger modulation amplitudes [5] than conventional modulation coil drivers. In the initial reports [2,3] the multiharmonic reconstruction method was applied to samples with single relatively narrow linewidths that could be over-modulated with modest modulation amplitudes. The samples selected for study in this report have more complicated lineshapes that include both narrow and broad lines and require larger modulation amplitudes to achieve overmodulation. These lineshapes are more representative of real-world samples than the previous examples. (i) One sample was composed of ultramarine blue ($B_{pp} = 17$ G) and BDPA ($B_{pp} = 0.85$ G). (ii) The

second sample was a nitroxide immobilized in sucrose octaacetate for which the narrowest feature has $B_{pp} \sim 3$ G, but the spectrum extends over about 150 G. The sample heights were about 5 mm.

2. Experimental section

2.1 Sample preparation

Nitroxide ^{14}N -PDT (perdeuterated 4-oxo-2,2,6,6-tetramethylpiperidiny-N-oxyl) was purchased from CDN isotopes (Quebec, Canada). Solid ^{14}N -PDT and sucrose octaacetate were mixed in a ratio that would result in a 2.50 mM solution. The solids were ground gently in a mortar and pestle to mix the two components. The solid mixture was placed in a 4 mm outer diameter (OD) quartz EPR tube, evacuated overnight to remove oxygen, heated gently above the melting point of sucrose octaacetate, and cooled to form a glass. The tube was flame sealed. The concentration of PDT in the sample was determined by comparison of the double integrated intensity with that for a standard sample of 0.56 mM tempone (4-oxo-2,2,6,6-tetramethylpiperidiny-N-oxyl) in toluene. The number of spins in the sealed sample was 4×10^{16} . The concentration is lower than in the initial mixture due to loss of the volatile nitroxide during the evacuation and melting steps.

Ultramarine blue (UMB) was purchased from Pfaltz and Bauer (Waterbury, CT). α,γ -Bisdiphenylene- β -phenylallyl free radical (BDPA) complex with benzene (1:1), was purchased from Sigma Aldrich (batch #00226KM). Solid UMB and BDPA were each mixed with solid KCl and gently ground to prepare uniform samples with relatively low radical concentrations. The diluted UMB and BDPA samples were placed in separate capillary tubes that were supported in a 4 mm OD quartz EPR tube. The numbers of BDPA and UMB spins in the sample were about 6.6×10^{15} and 2.2×10^{16} , respectively.

2.2 EPR spectroscopy

Conventional and multiharmonic field-modulated X-band spectra were recorded on a Bruker E500T using a Bruker Flexline ER4118X-MD5 dielectric resonator, and the Xepr software. The resonator Q is ~ 9000 for these nonlossy samples and has an efficiency of 3.8 G/W [6]. For conventional spectra the 100 kHz modulation amplitude was 20% of B_{pp} , and the modulated field was generated with the coils that are built into the resonator. The signal was digitized with a Bruker signal processing unit (SPU).

The multiharmonic spectra were obtained using the transient mode of the E500T. In this mode there is a low-noise microwave amplifier prior to the mixer, which is not present in the CW signal path. For the multiharmonic spectra, the field modulation was generated with a resonated scan driver [5]. The scan frequency of about 41 kHz was measured with a Phillips PM680 counter. The scan coils have 200 turns of Litz wire (255 strands of AWG44 wire). The coil constant is 37.7 G/Amp. The modulation ratio, the ratio of the modulation amplitude h_m to B_{pp} for the narrowest line in the spectrum, was varied from 0.2 to 10 for the sample containing UMB and BDPA and from 0.2 to 5.0 for the immobilized nitroxide. The input to the digitizer was filtered with a 4th order low-pass Butterworth filter (Krohn-Hite 3955). The cut-off frequency was 5 MHz, to ensure that all harmonics of interest could be measured. The signal was over-sampled at a rate of 50 MS/s (20 ns timebase). The field-

modulated EPR signal was collected in a 2D experiment with 10 magnetic field points per gauss. At each magnetic field position the EPR signal intensity was digitized in quadrature with a Bruker SpecJet II for multiple cycles of the modulation frequency. Data were acquired in the standard Xepr software. The data acquisition time was calculated as the product of number of cycles, the period of a scan, the number of scans, and the number of field positions. This calculation does not include the overhead in the software, which has not been optimized for these experiments. Data at each magnetic field were transferred to a PC.

2.3 Data Processing Approach

The data analysis for multiharmonic EPR is described in [2,3,7]. This method does not require prior knowledge of the signal lineshape. At each field position the periodic response of the spin system to field modulation is recorded. For modulation ratios substantially less than 1 the signal is a sinusoid, the amplitude of which is proportional to the first derivative at this field point. For higher modulation ratios the signal is the sum of n sinusoids with frequencies at the modulation frequency and its harmonics. The frequency at $n = 1$ is the scan frequency, which is also designated as the fundamental. The frequency at $n = 2$ is the second harmonic. With digital phase-sensitive detection the amplitudes at each of these frequencies can be determined. By sweeping the field and doing phase-sensitive detection at each field position, one obtains a set of n EPR spectra, $s_n(B)$ where $n = 1$ to N_H and N_H is the number of harmonics. The spectrum $s_1(B)$ is the conventional CW spectrum, which has the accurate first-derivative lineshape only if the modulation amplitude is substantially less than the linewidth of the narrowest line. The multiharmonic reconstruction provides the accurate first-derivative lineshape, even if the modulation amplitude is large relative to the linewidth. For the multiharmonic analysis, all of the $s_n(B)$ harmonics were Fourier transformed into the conjugate domain, u .

$$S_n(u) = \int_{-\infty}^{\infty} e^{-juB} s_n(B) dB \quad (1)$$

Reconstruction is done in the Fourier domain in which $F(u)$ is the Fourier transform of the first-derivative EPR spectrum;

$$F(u) = \frac{\sum_{n=1}^{n=N_H} D_n^*(u) S_n(u)}{\sum_{n=1}^{n=N_H} D_n^*(u) D_n(u)} LPF(u) \quad (2)$$

where

$$D_n(u) = \left(\frac{h_m}{4n}\right) j^{(n-1)} \left[J_{n-1}\left(\frac{h_m u}{2}\right) + J_{n+1}\left(\frac{h_m u}{2}\right) \right], \quad n > 0 \quad (3)$$

$J(x)$ in Eq. (3) is the Bessel function of the first kind, h_m is the peak-to-peak modulation amplitude, and $LPF(u)$ is a low-pass filter. To provide an equation that is convenient for the calculations, Eq. (2) was derived by rearrangement of the equations in Ref. [3], with the assumption that noise is sufficiently similar for all harmonics that differential weightings are not required. The details of the rearrangement are given in the Supplementary Information.

Inverse Fourier transformation of $F(u)$ gives the reconstructed first-derivative spectrum. The S/N of the reconstructed spectrum is dependent on the parameters of the low-pass filter [3], which is applied prior to inverse Fourier transformation. The $LPF(u)$ was a convolution of Gaussian function with a square profile with amplitude equal to one between plus and minus the cutoff frequency and zero otherwise. The role of the Gaussian is to smooth the transition in the vicinity of the cutoff and avoid discontinuities in the product function, Eq. (2). The width of the Gaussian as well as the cutoff frequency is optimized for a given spectrum, with tradeoffs between S/N and line broadening. Plots of the filter function overlaid on Fourier transforms of the reconstructed spectra for BDPA + UMB and for immobilized nitroxide are shown in the Supplementary Information. Selection of filter parameters depends on the linewidths in the spectrum and not on the modulation amplitude.

2.4 Processing experimental data

A block diagram for the data processing procedure is shown in Figure 1. At each magnetic field, the output from the quadrature mixer of the spectrometer is digitized by the SpecJet II, producing real arrays $I(t, B)$ and $Q(t, B)$. From the original 8192 data points in the time dimension at each magnetic field, segments corresponding to exactly 6 scan cycles were selected. From these segments of $I(t, B)$ and $Q(t, B)$, the amplitudes for each sinusoidal harmonic up to $n = 100$ were obtained by digital phase-sensitive detection [3] and stored in complex arrays $p_n(B)$ and $p_n'(B)$, respectively. An automatic routine selects the modulation phase by maximizing the amplitude of the real components and minimizing the imaginary components for $n = 1$. For $n = 1$ the phase correction is θ and for higher harmonics it is n times θ . The phase-corrected results are stored in complex arrays $s_n(B)$ and $s_n'(B)$. If the phase correction is greater than about $\pm 90^\circ$, a 180° correction is applied manually prior to iteration. Arrays $s_n(B)$ and $s_n'(B)$ are Fourier transformed as described in Eq. (1), producing $S_n(u)$ and $S_n'(u)$. Reconstruction of $F(u)$ and $F'(u)$ is performed as described by Eq. (2), which includes a low-pass filter. The low-pass filter removes high frequency noise, and was set to a value that depends on the bandwidth of the signal. Setting the value too high in the u domain leaves excess noise in the spectrum. If the cutoff is too low in the u domain, it improves S/N but rejects some high frequency components and may result in signal broadening. After inverse Fourier transformation of complex arrays $F(u)$ and $F'(u)$, the imaginary parts are negligible because of the prior modulation phase correction. The real parts of the inverse Fourier transforms of $F(u)$ and $F'(u)$, respectively, become the real and imaginary parts of $f(B)$. The microwave phase is then set by taking the appropriate combination of the two complex components of $f(B)$. The emphasis in the following discussion is on the absorption signal, although the dispersion is available for other applications.

3 Results

It could be argued that the appropriate way to evaluate the performance of the multiharmonic reconstruction algorithm would be comparison of conventional CW and multiharmonic spectra obtained from the same spectrometer. However, that comparison is complicated by differences in signal path and data processing between the conventional CW path of the E500T and the transient path that was used for the multiharmonic reconstruction.

In the conventional CW signal path the EPR signal is digitized with a Bruker SPU. The user has the option of applying a post-processing smoothing filter. The low-pass filter in the Fourier domain that was used for the multiharmonic reconstruction may impact noise differently than the smoothing option of the SPU. In the transient path there is a low-noise amplifier that is not present in the conventional CW path. At the low microwave B_1 s that were used to acquire the data for the multiharmonic reconstruction, the low noise amplifier can significantly improve the S/N . The impact of these factors on signals recorded at low modulation amplitudes is addressed by the comparisons in Figure 2 for the UMB and BDPA sample.

Spectra for the sample of UMB and BDPA obtained with the same data acquisition time, B_1 , and modulation amplitude, but with different data acquisition pathways and signal processing are shown in Figure 2. The data were acquired with 0.17 G modulation amplitude (a modulation ratio of 0.2), which was selected to be conservative and not broaden the signal obtained using only phase-sensitive detection at the modulation frequency as is done in conventional CW spectroscopy. The spectrum acquired through the conventional signal path and processed with a 3-point smooth is shown in Figure 2A. A 5-point smoothing gives $S/N = 28$. The use of more than 5 points for the smoothing, which corresponds to about half of B_{pp} , improves the S/N , but broadens the BDPA signal. The multiharmonic reconstruction uses a Fourier domain filter with different adjustable parameters than the smoothing function in the Xepr software. Fourier transformation and application of the same low-pass filter in the Fourier domain as was used for the multiharmonic data processing for the UMB and BDPA sample, produces the spectrum shown in Figure 2B. This post-acquisition data processing had little impact on S/N , which demonstrates that the choice of the filter function is not a major contributor to the S/N difference between conventional CW and multiharmonic spectra on the E500T. The $s_I(B)$ spectrum from the multiharmonic data set (Figure 2C) is the conventional first-derivative spectrum and accurately reflects the first-derivative spectral lineshape. The improvement in S/N by about a factor of 2.5 between either Figure 2A or 2B and 2C reflects the differences in the signal detection pathways, which is attributed primarily to the low noise amplifier. A key issue related to multiharmonic reconstruction is whether inclusion of higher harmonics degrades the S/N if there is little intensity at the higher harmonics. At the low modulation amplitude of 0.17 G the lineshape in the spectrum $f(B)$ obtained by multiharmonic reconstruction with $N_H = 100$ (Figure 2D) is essentially the same as in the $s_I(B)$ spectrum (Figure 2C), because there is little or no intensity in the higher harmonics at this low modulation ratio. The S/N is about the same for Figure 2C and Figure 2D which shows that including higher harmonics does not degrade the S/N of the spectrum, and means that large numbers of harmonics can routinely be included in the reconstruction independent of whether these harmonics have significant signal intensity.

To evaluate the performance of the multiharmonic reconstruction method, independent of signal detection pathway or selection of digital filter, spectra are shown in Figures 3 and 4 for $n = 1$ and multiharmonic reconstruction from the same data sets, using the same filter parameters. The data processing for $n = 1$ and for multiharmonic reconstruction are the same (Figure 1) except that the multiplication by the Bessel functions in Eq. (2) is omitted for $n =$

1. The amplitudes of the multiharmonic spectra calculated using Eq. (2) are normalized by dividing by modulation amplitude. For comparison, the amplitudes of the $n = 1$ data in Figures 3 and 4 also are normalized by dividing by modulation amplitude.

Spectra of the sample of UMB and BDPA as a function of modulation ratio are shown in Figure 3 for $n = 1$ and multiharmonic reconstruction. The modulation ratios listed in the figures and in Table 1 are relative to the BDPA linewidth. Since the linewidth for the UMB peak is about 20 times larger than for BDPA, the modulation ratios relative to the UMB peak are all less than 1. The data for modulation ratio = 0.2 are the same as shown in Figure 2. All spectra were obtained with 10 magnetic field points per gauss, which limits the accuracy to which the linewidth of the BDPA signal can be measured to about ± 0.1 G. Linewidths and S/N for the BDPA peak are summarized in Table 1. For spectra obtained with only $n = 1$ the linewidth of the BDPA signal broadens as the modulation ratio is increased above 0.2, as expected for conventional CW spectroscopy. The S/N for the BDPA peak in the multiharmonic spectrum improves from modulation ratio 0.2 to 1.0, but plateaus at a modulation ratio about 1, as reported previously [3]. At higher modulation ratios the broadening of the BDPA peak is very modest compared with the broadening observed for only $n = 1$. Analysis of spectral parameters can be more strongly dependent on accurate lineshapes than on S/N [8], so the accurate lineshapes obtained from multiharmonic reconstruction can improve parameter precision in simulations. For spectra obtained with $n = 1$ the S/N for the UMB peak continues to increase at modulation ratios that are so high that the BDPA peak is severely broadened. The sample of UMB and BDPA was selected for this analysis because the lineshape changes for the two signals could be readily distinguished. For a sample with well resolved broad and sharp lines the S/N for the separate features could be optimized by recording conventional CW spectra at high and low modulation amplitudes. For the more general case, as demonstrated in Fig. 4, broad and sharp features are superimposed. A major advantage of the multiharmonic method is that data can be acquired at relatively high modulation amplitude and decisions made in post-processing that optimize parameters for differing portions of the spectrum.

As the modulation ratio is increased, the number of harmonics with signal intensity greater than noise increases (Table 2). The number of harmonics included in the multiharmonic reconstruction was varied and the linewidth of the BDPA peak was monitored. Including higher harmonics improves the accuracy of the lineshape. Provided that the cutoff frequency in the low pass filter was selected appropriately, inclusion of additional harmonics does not degrade the S/N . Therefore comparisons were based on reconstructions with an arbitrarily large value of $N_H = 100$.

Spectra for the immobilized nitroxide radical sample as a function of modulation ratio are shown in Figure 4 for $n = 1$ and multiharmonic reconstruction. The spectrum contains overlapping broad and narrow features, which is more realistic for real-world samples. The linewidths shown in Table 3 are apparent values measured from the spectra, which are useful monitors of spectral broadening. At low modulation ratio the lineshape for the multiharmonic spectrum matches well with that obtained with $n = 1$. For $n = 1$ the narrowest lines broaden as expected as the modulation ratio is increased to 1.0 or 5.0 (Table 3). For the multiharmonic reconstruction the lineshape is preserved as the modulation ratio

is increased from 0.2 to 1.0 and the narrowest feature is broadened by only about 10% as the modulation ratio is increased further to 5. The S/N improves from modulation ratio 0.2 to 1.0 but then declines slightly at 5.0 due to broadening of the spectrum. Higher modulation ratios increase the scan rate, which could result in rapid scan conditions for ratios larger than 5 [9]. Since the reconstruction algorithm does not apply in the rapid scan regime, modulation ratios higher than 5 were not used for the nitroxide sample.

4. Discussion

The multiharmonic reconstruction method permits use of substantially higher modulation ratios than conventional CW spectroscopy without substantial line broadening, which improves S/N [2,3]. Uncertainty analysis has shown that S/N is not the only criterion for the quality of spectra [8]. The accurate lineshapes obtained by multiharmonic reconstruction are important for extraction of parameters by simulations. In this paper the multiharmonic method was applied to samples with lineshapes that include both narrow and broad lines, thereby demonstrating the generality of the method. The ability to use modulation amplitudes larger than linewidths without substantially broadening the lines is especially useful for these composite lineshapes. This methodology also means that the selection of the modulation amplitude that can be used without spectral distortion is no longer tightly tied to the linewidth of the narrowest line. Multiharmonic reconstruction may be especially important in obtaining accurate projections for EPR imaging because overmodulation can be used to improve the S/N without losing resolution in the projection and the resulting image. The coil driver and Litz wire coils developed for rapid scan EPR [4,5] produce wider scans with a uniform field over a longer sample than is possible with the standard commercial modulation fields. The modulation field uniformity permits the extension of the previously described multiharmonic method to samples with lengths of about 5 mm, which is close to optimal for the B_1 distribution in the Bruker dielectric resonator that was used for the experiments.

When data are acquired digitally the user has options for how to process the information. The user can select multiharmonic reconstruction, which maintains the most accurate lineshape. Alternatively, since the $n = 1$ spectrum from the multiharmonic data set is equivalent to the conventional field modulated signal, the user can decide to work with the $n = 1$ spectrum if that has higher S/N for broad features in the spectrum. For either approach the filter parameters are selected in post-processing and can be adjusted as desired, instead of requiring reacquisition of data as in conventional CW spectrometers. As digital systems become increasingly common, multiharmonic EPR is likely to become more generally available as a tool to improve S/N and to simplify the selection of the modulation amplitude parameter.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Partial support of this work by National Science Foundation grant MRI 1227992 (SSE and GRE), National Institutes of Health CA177744 (GRE and SSE), and K25 EB016040 (MT) is gratefully acknowledged. The rapid scan hardware was developed by Richard W. Quine and George A. Rinard and their suggestions were helpful in implementing the methodology.

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Research Highlights

- Digital phase-sensitive detection measured multiple harmonics of modulation frequency.
- Spectra contained both narrow and broad lines.
- Modulation amplitudes were up to 5 times the narrowest linewidths.
- Spectra reconstructed from multiple harmonics had unbroadened lineshapes.

Figure 1.

Data processing procedure. For each magnetic field step in the spectrum, digital phase-sensitive detection is applied to both quadrature channels from the digitizer, which produces complex arrays $p_n(B)$ and $p'_n(B)$. The modulation phase is adjusted to null the imaginary component of each array. Fourier transformation of both arrays produces $S_n(u)$ and $S'_n(u)$. Reconstruction of the Fourier transform of the spectrum (Eq. (2)) and low pass filtering produces $F(u)$ and $F'(u)$. Inverse Fourier transformation gives the complex spectrum $f(B)$. Microwave phase correction produces absorption and dispersion spectra.

Figure 2.

130 gauss scans of the X-band spectra of the sample containing UMB and BDPA acquired with modulation amplitude of 0.17 G, B_1 of 2.5 mG, and the same 130 s data acquisition time. A) Signal acquired through the SPU, followed by 3-point smoothing, $S/N = 27$, B) Signal in A after Fourier filtering with the same cutoff as used for multiharmonic reconstruction, $S/N = 30$, C) $s_1(B)$ from the multiharmonic data set, $S/N = 80$, and D) $f(B)$ obtained by multiharmonic reconstruction with $N_H = 100$, $S/N = 80$.

Figure 3.

Comparison of 130 gauss scans of the X-band spectra of the sample containing BDPA and UMB obtained with $B_1 = 2.5$ mG and 130 s data acquisition times, reconstructed with $n = 1$ and multiharmonic analysis for modulation ratios of 0.2, 1.0, and 5.0, respectively. Spectral amplitudes were normalized for modulation amplitude and y-axis scales are the same for all portions of the figure.

Figure 4.

Comparison of 150 gauss scans of X-band spectra of ^{14}N -PDT in sucrose octaacetate obtained with $B_1 = 2.5$ mG and 90 s data acquisition time, reconstructed with $n = 1$ and multiharmonic analysis for modulation ratios of 0.2, 1.0, and 5.0, respectively. Spectral amplitudes were normalized for modulation amplitude and y-axis scales are the same for all portions of the figure.

Table 1

Comparison of linewidths and S/N for $n = 1$ and multiharmonic reconstruction of spectrum for BDPA and UMB

BDPA and UMB	fundamental, $n=1$			multiharmonic reconstruction		
modulation ratio ^a	B_{pp} of BDPA ^b	S/N of BDPA ^c	S/N of UMB ^c	B_{pp} of BDPA ^b	S/N of BDPA ^c	S/N of UMB ^c
0.2	0.9	80	20	0.9	80	20
1.0	1.1	3.6×10^2	85	0.9	3.2×10^2	65
2.0	1.3	5.6×10^2	1.6×10^2	0.9	3.8×10^2	75
5.0	3.3	7.1×10^2	3.9×10^2	0.9	3.7×10^2	75
10	7.3	6.3×10^2	7.9×10^2	1.1	3.3×10^2	75

^a Modulation ratio is based on the linewidth of the BDPA signal

^b The peak-to-peak linewidth is in Gauss. The uncertainty for the BDPA linewidth is ± 0.1 G due to data acquisition with 10 points/gauss.

^c Uncertainty in S/N is about 10%.

Table 2

Number of harmonics with signal amplitude greater than noise for spectra of BDPA and UMB with various modulation ratios.

modulation ratio ^a	number of harmonics ^b
0.2	3
1.0	6
2.0	12
5.0	25
10	45

^a Modulation ratio is based on the linewidth of the BDPA signal.

^b The number includes the fundamental of the sweep frequency.

Table 3Comparison of S/N for $n = 1$ and multiharmonic reconstruction for immobilized nitroxide spectrum

^{14}N -PDT in sucrose octaacetate	fundamental $n = 1$		multiharmonic reconstruction	
modulation ratio ^a	S/N ^b	B_{pp} ^d	S/N ^b	B_{pp} ^d
0.2	3.1×10^2	3.1	3.1×10^2	3.1
1.0	$\sim 1.5 \times 10^3$ ^c	4.0	9.6×10^2	3.1
5.0	$\sim 2.5 \times 10^3$ ^c	12.5	8.6×10^2	3.2

^a Modulation ratio is based on the linewidth of the narrowest line^b S/N is based on the narrowest line.^c S/N is too large to measure accurately.^d Apparent peak-to-peak linewidth (G) for central feature.