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Frequency Dependence of EPR Signal Intensity, 248 MHz to 1.4 GHz

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The electron paramagnetic resonance pulsed free induction decay (FID) of a degassed solution of a triaryl methyl radical, methyl tris(8-carboxy-2,2,6,6-tetramethyl(-d_3)-benzo[1,2-*d*:4,5-*d′*] bis(1,3)dithiol-4-yl) tripotassium salt, 0.2 mM in H_2O, was measured at VHF (247.5 MHz) and L-band (1.40 GHz). The calculated and observed FID signal amplitudes (in millivolts) agreed within 1 and 6%, and the ratio of the normalized FID signals at the two frequencies agreed within 5%. The FID decay time constant was 2.7 μs at both frequencies.

INTRODUCTION

Electron paramagnetic resonance (EPR) began at lower RF/microwave frequencies than subsequently became most common for chemical and biological applications (*1*). The frequency dependence of EPR signal intensity depends on many experimental parameters, but there developed a general pessimism about EPR signal-to-noise ratio (S/N) at low frequency. After some confusion in the literature, predictions can now be made with some confidence if enough is known about the sample and the spectrometer (*2–5*). Our previous papers (*4, 5*) compared S-band and X-band electron spin echo intensities and found good agreement with both the absolute intensities expected and the predicted dependence on frequency.

In this paper we extend the examination of EPR frequency dependence to 250 MHz. EPR at 250 MHz is important for *in vivo* studies. Halpern and co-workers described a 250-MHz CW EPR spectrometer designed for *in vivo* imaging in 1989 (*6*). Beginning in 1993, Krishna and co-workers, in a series of papers, described a pulsed FT EPR spectrometer for spectroscopy and imaging at 300 MHz (*7–11*). A 220-MHz pulsed spectrometer described by the Sotgiu group in L'Aquila (*12, 13*) used orthogonal resonators to excite the spins and detect the free induction decay (FID). As part of the NIH Research Resource "Center for EPR Imaging for In Vivo Physiology," we are constructing a 250-MHz (VHF) EPR spectrometer for pulsed EPR imaging. Here we compare its performance with that of an L-band spectrometer we recently reported (*14*). Key to the performance of the VHF spectrometer is the use of a crossed-loop resonator (CLR) (*15, 16*). This resonator, which provides good isolation between the loop into which the high-power pulse is injected and the loop from which the EPR signal is detected, was first implemented at S-band (*15, 16*) and then at L-band (*17*) and is now demonstrated at 250 MHz. An X-band version is under development. The details of the VHF spectrometer will be described at a later date, after further development. The spectrometer gains and features of the resonators used in the comparison are reported here in support of the conclusion that the previously reported trends in frequency dependence of EPR intensity continue to 250 MHz.

SIGNAL COMPUTATION

By combining Eqs. [10] and [14] of Ref. (*4*) and multiplying by the gain of the spectrometer, G, the voltage at the detector (in volts) for a pulsed EPR signal can be expressed as

$$V_{E\beta} = G\frac{\sqrt{\beta}}{1+\beta}\sqrt{\frac{Z_0}{R}}\omega_0 \int_{\text{sample}} M_0\frac{B_1}{I}\,dV, \qquad [1]$$

where β is the coupling parameter. $\beta = \frac{2Q_H}{Q_{OC}} - 1$, Q_H is the critically coupled Q and Q_{OC} is the overcoupled value under the conditions of the signal measurement. Z_0 is the characteristic impedance, which is 50 ohm for this case. R is the resistance of the resonator, ω_0 is the angular frequency for the experiment $= 2\pi\nu$, M_0 is the spin magnetization per unit volume, and I is the current in the resonator. G is the total gain of the spectrometer from resonator to signal output.

For the resonators and sample geometries used for the signal comparisons it was assumed that B_1 was uniform over the sample and that the sample is homogeneous so that $M_0\frac{B_1}{I}$ can be moved outside the integral in [1]. Equation [1] then becomes

$$V_{E\beta} = G\frac{\sqrt{\beta}}{1+\beta}\sqrt{Z_0}\omega_0 M_v\Lambda, \qquad [2]$$

where Λ is the resonator efficiency parameter (*18*), in Gauss per square root of watt, $M_v = M_0V_s$, and V_s is the volume of the sample in the active region of the resonator.

Each of the parameters in Eq. [2] was measured or calculated from first principles, and the results are summarized in Table 1. To calculate $V_{E\beta}$ using Eq. [2], Λ was calculated from first principles using the geometry of each resonator. The empty, critically coupled Q of each resonator was also calculated from

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TABLE 1
Parameters Used in the Comparison of EPR Signals at L-band and VHF[a]

	Calculated	Measured
VHF		247.5 MHz
$\Lambda(G/\sqrt{W})$[b]	(0.735), 0.728[c]	
Q_{HE} (critically coupled empty)	1035	1135
Q_{HS} (critically coupled with sample)		1115
Q_{OC} for FID		36
β		61
Gain		6.28×10^4
Sample volume (m^3)	2.3×10^{-6}	
$V_{E\beta}$ (measured absolute voltage extrapolated to time $t = 0$) (mV)	333	329[d]
Signal normalized for gain		5.24×10^{-6}
L-band		1.4018 GHz
Λ ($G/\sqrt{W}$)[b]	(0.47), 0.19[c]	
Q_{HE} (critically coupled empty)	2917	2991
Q_{HS} (critically coupled with sample)		485
Q_{OC} for FID		131
β		6.4
Gain		1.95×10^3
Sample volume (m^3)	3.8×10^{-6}	
$V_{E\beta}$ (measured absolute voltage extrapolated to time $t = 0$) (mV)	389	366[d]
Signal normalized for gain		1.88×10^{-6}
Ratio of L-band signal normalized for gain to VHF signal normalized for gain. (includes β, ω, active sample size and Λ)	37.6	35.8
L-band/VHF normalized signals[e]		1.05

[a] The signal is the FID, 0.3 G off resonance, for 0.2 mM Nycomed sym-trityl in deoxygenated aqueous solution.

[b] The value given for Λ is the circularly polarized component.

[c] The value of Λ in parentheses is for a nonlossy sample in the resonator; the second value is reduced by the effect of the lower Q with the aqueous trityl sample in the resonator. Λ varies directly with $\sqrt{Q}$ assuming the frequency does not change. (The small change in frequency with the lossy sample was neglected.)

[d] The FID signal intensity was extrapolated back to time zero by using the measured FID decay time constant of 2.7 μs.

[e] Ratio of calculated and experimental signal intensity ratios.

first principles, but the measured values were used in calculating $V_{E\beta}$.

Λ was calculated as follows. From the definition of inductance, B_1 is given by

$$B_1 = L\frac{I}{A}, \qquad [3]$$

where $L =$ inductance of the resonator loop, $I =$ resonator current, and $A =$ cross-sectional area of the resonator loop. The inductance of the resonator loop is

$$L = \mu_0\frac{A}{z}, \qquad [4]$$

where $z =$ effective length of the resonator loop and $\mu_0 =$ permittivity of free space, $4\pi \times 10^{-7}$ J C^{-2} s^2 m^{-1}. Combining Eqs. [3] and [4], B_1 is given by

$$B_1 = \frac{\mu_0}{z}I. \qquad [5]$$

Therefore, Λ can be written as

$$\Lambda = \frac{B_1}{\sqrt{P}} = \frac{\mu_0}{z\sqrt{R}}, \qquad [6]$$

where $P =$ power delivered to the resonator, and $R =$ resistance of the resonator.

The resistance of the resonator is given by

$$R = R_{SQ}S(r, w, z), \qquad [7]$$

where $R_{SQ} = \sqrt{\omega\frac{\mu_0}{2\sigma_{Cu}}}$, the skin effect resistance per square of the resonator, $\sigma_{Cu} =$ conductivity of copper (the metal used for the resonator), and $S(r, w, z) =$ number of squares, which depends on the geometry of the resonator. $r =$ radius of resonator loop, $w =$ gap width, and $z =$ length of the resonator. A general expression for $S(r, w, z)$ is

$$S(r, w, z) = \frac{2(\pi r + kw)}{z}. \qquad [8]$$

The total series equivalent resistance of the resonator consists of the resistance around the loop plus the equivalent resistance contributed by the resonator gap. The term $2\pi r/z$ in Eq. [8] is the number of squares in the loop.

The term $\frac{2kw}{z}$ in Eq. [8] is the effective number of squares in the gap portion of the resonator. The VHF CLR has a large reentrant loop; therefore, the current in the gap varies from a maximum at the resonator loop to nearly zero at the reentrant loop. If one calculates the equivalent resistance of the gap by integrating Eq. [9], one finds $k = 1/3$.

$$P_{gap} = R_{eq}\int_0^w \left(\frac{I}{w}x\right)^2 dx = R_{eq}I^2\frac{w}{3} \qquad [9]$$

The L-band resonator has a reentrant loop the same size as the resonator loop. In this case one can assume that z is twice the resonator length plus the gap width and that the current is essentially constant from loop to loop. For this case $k = 1/2$.

The loaded $Q_L = \frac{\omega L}{2R}$ was calculated using Eqs. [4] and [7].

EXPERIMENTAL

The 250-MHz CLR had a 25-mm-diameter sample loop. The region that is common to both resonators has an elongated shape, oval on the ends, to maximize the filling factor. Since there was adequate room in the magnet, no attempt was made to minimize the size of the CLR. The 250-MHz spectrometer used a Dressler

Model No. 75A/250 RF amplifier. This amplifier does not have noise blanking, so some signal averaging was needed to decrease the noise from the amplifier during the signal detection time.

The L-band resonator used was a reentrant loop–gap resonator (LGR) analogous to the one reported previously (*14*), but with a 25-mm-diameter sample loop and a 25-mm-diameter reentrant loop.

Calculation of β for use in Eq. [2] requires values of Q. Q was estimated by measuring the ring down following a pulse (*19*, Eqs. [29, 30]). The shape of a reflected pulse was digitized using a Bruker E587 SpecJet transient signal averager, and the decay time constant was fitted using Bruker Xepr software. The critically coupled and overcoupled Q were measured for each resonator with the sample in place. To obtain the data used for the signal intensity comparison, the VHF resonator was overcoupled from Q_H of 1115 to $Q_{OC} = 36$, which corresponds to $\beta = 61$. The L-band resonator was overcoupled from Q_H of 485 to $Q_{OC} = 131$, which corresponds to $\beta = 6.4$.

The triaryl methyl radical used in this study, methyl tris(8-carboxy-2, 2, 6, 6-tetramethyl(-d_3)-benzo[1,2-*d*:4,5-*d'*]-bis(1,3)dithiol-4-yl) tripotassium salt (sym-trityl-CD_3), is depicted in Fig. 1 of Ardenkjaer-Larsen *et al*. (*20*) and was a gift from Dr. Klaus Golman. The sym-trityl-CD_3, 0.2 mM in water, was contained in a 10-mm-od (9-mm-id) Pyrex NMR tube (Wilmad Glass, Buena, NJ, Model No. 513). After the oxygen was removed by bubbling with N_2 gas, the tube was flame-sealed. The aqueous solution was 60 mm long, which filled the active region of the L-band LGR and was longer than needed to fill the active region seen by both sections of the 250-MHz CLR. The 9-mm-diameter aqueous sample was somewhat larger than that which gave the largest ηQ product for the L-band LGR (η is the filling factor). For the VHF resonator a larger sample could have been used. However, by using the same sample in both resonators, any effects of residual O_2 or of concentration (*20*) on relaxation times were kept constant in the comparison. The magnetization (M_v) was calculated using Eq. [6] in Ref. (*4*) and the sample volumes 3.8×10^{-6} m^3 at L-band and 2.3×10^{-6} m^3 at 250 MHz.

The FID for the trityl sample was recorded after a 250-ns pulse. At both frequencies the magnetic field was set 0.3 mG off resonance to give a convenient oscillation frequency of the FID. The FID amplitude was measured as a function of field offset and was reasonably constant out to ca. 0.5 G off resonance, as expected based on the work of Hornak and Freed (*21*). To define the signal intensity, $V_{E\beta}$, the peaks in the oscillation were fitted to a single exponential that was extrapolated back to time zero, which was defined as the end of the pulse.

Calculation of signal intensity using Eq. [2] requires values of Λ, the microwave magnetic field per square root of watt. Use of Eqs. [6] to [8] and the parameters $r = 0.013$, $w = 0.024$, and $z = 0.096$ m gives $\Lambda = 1.47$ G/$\sqrt{\text{W}}$ at critical coupling for the 250-MHz resonator. This corresponds to $\Lambda = 0.74$ G/$\sqrt{\text{W}}$ for the circularly polarized component. The Q for the empty resonator was calculated to be 1135 (Eqs. [4], [7]). To verify this calculated value of Λ the microwave/RF power incident on each resonator was measured at the frequency at which the EPR signal was measured. The 90° pulse length was found by maximizing the trityl FID or the amplitude of the echo from irradiated fused SiO_2. B_1 was calculated from $\theta = \gamma B_1 t_p$. This value of B_1 and the measured power incident on the resonator gave $\Lambda = 0.65$ G/$\sqrt{\text{W}}$. The good agreement between calculated and observed values of Λ provides confidence in the model. The optimum power for the 90° pulse can be estimated to within 1–2 dB, which introduces uncertainty in the experimental value of Λ. The calculated values of Λ were used in predicting signal intensity. Analogous calculations for the L-band resonator gave $\Lambda = 0.47$ G/$\sqrt{\text{W}}$ (using $r = 0.013$, $w = 0.024$, and $z = 0.168$ m) and a Q for the empty resonator of 2917. The value of Λ is proportional to $\sqrt{Q}$ (*19*), so the values input into Eq. [2] were corrected for the decrease in resonator Q caused by the sample. This effect was much larger at L-band than at 250 MHz (Table 1).

The gain of each spectrometer was measured by using a calibrated noise source (NoiseCom, Paramus, NJ) at the input. In this method the noise source was connected to the input of the bridge and the noise from the source is allowed to propagate through the entire detection system. The noise at the output of the bridge was then measured with a true RMS voltmeter (Fluke Model No. 8920A). An equivalent noise bandwidth was calculated by integrating the gain curves of the amplifiers in the bridge and the response curve of the RMS voltmeter. The advantage of this method is that it gives a direct end-to-end gain measurement of the entire signal path and does not depend on multiplying together gains and losses that might be measured separately and which would contain individual measurement errors.

RESULTS AND DISCUSSION

The expected EPR signal intensity was calculated using Eq. [2]. Relevant parameters are given in Table 1. At both 250 MHz and L-band, the calculated values are in good agreement with experiment. The major uncertainty in the comparison is the measurement of the very strongly overcoupled resonator Q. The lower the Q the more difficult it is to measure it accurately.

To check that the pulse settings used to acquire the experimental data were appropriate, the pulse length is compared with Q, and the B_1 is compared with spectral extent. The pulses are roughly rectangular (rise and fall times are <10 ns), so the relation suggested by Mims (*22*) is

$$Q \leq \frac{2\pi \nu t_p}{6.6}. \qquad [10]$$

With our chosen pulse time, t_p, of 250 ns, the Q could be as high as 59 at 250 MHz and 357 at 1.5 GHz and still have the resonator admit the band of frequencies represented in the pulse. The Q values used for the experiments (36 at 250 MHz and 131 at 1.4 GHz) are well below these limits.

Using $\theta = \gamma B_1 t_p$, the 250-ns 90° pulse corresponds to $B_1 = 0.36$ G. The half width of the absorption spectrum of the trityl sample is ca. 0.04 G, so the B_1 is adequate to fully excite the spin system.

Another necessary check was of the FID decay time, which was used to correct from the measured FID intensity to the intensity at time zero. Since the trityl radical EPR linewidth is determined by unresolved hyperfine structure, and not by T_1 and T_2 (*23*), the FID decay rate was expected, and found, to be the same at both frequencies. The FID envelope decayed with a time constant of 2.7 μs, which corresponds to a linewidth (approximated as a Lorentzian line) of 0.042 G, which is consistent with the CW spectrum.

In the current spectrometer configurations the noise in the FID data is dominated by the noise from the high-power pulsed amplifier. In the L-band system there are two switches in series on the output of the CW TWT, which are opened after the pulse to suppress noise during the FID detection time. In the VHF system the isolation of the CLR and noise suppression diodes are used to decrease noise from the CW RF amplifier during FID detection. Eventually, the CW RF amplifier in the VHF spectrometer will be replaced with an amplifier with noise blanking after the pulse, which is expected to be more effective in noise suppression than the current configuration of noise blanking diodes. Because of these differences in spectrometer configuration, we did not attempt a direct comparison of experimental noise levels.

The potential S/N ratio (for comparable noise performance) at VHF and L-band is approximately indicated by the differences in gain required to achieve equal FID signal levels. The VHF gain was 32 times the gain used at L-band, but only 60% of the sample was observed in the VHF resonator, so the corrected gain for equal signals is about 17. This factor of 17 is similar to the factor of $(\omega_L/\omega_{VHF})^{7/4} = 21$ predicted for the ratio of signal intensity at constant sample size, constant resonator size, and constant B_1 for a nonlossy sample (*2*) and provides a realistic estimate of the FID S/N ratio at L-band in comparison with VHF when noise levels are similar.

CONCLUSION

With full accounting for all features of the sample, resonator, and signal detection system, both the absolute EPR FID signal amplitudes and the relative FID signals at 247.5 MHz and 1.40 GHz are in agreement with predictions from first principles. This result extends our prior work from X-band to VHF, a factor of about 36 in frequency, and provides a basis for confident prediction of the performance of a wide variety of EPR spectrometer systems.

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