Determination of Fundamental Harmonic-to-secondary Harmonic Ratio of Longitudinal Magnetization Change in Triarylmethyl or Nitroxide Radical Aqueous Solution Caused by ESR at 280 MHz for Nondestructive Oximetry

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In a longitudinally detected ESR (LODESR) method, longitudinal (i.e., parallel to the static magnetic field) magnetization changes caused by on/off modulated ESR irradiation are observed. In this study, LODESR oximetry was newly designed so that a detection frequency was fixed and on/off modulation frequencies were varied. LODESR signal intensities when the modulation frequency was equal to the detecting frequency (fundamental harmonic detection) and when it was half the value of the detection frequency (secondary harmonic detection) were simulated at a constant detection frequency. Fundamental harmonic-to-secondary harmonic ratios (FSRs) were calculated by dividing the former by the latter. This simulation showed that FSR increased with the reciprocal of longitudinal relaxation time. LODESR measurements of phantoms containing triarylmethyl or a nitroxide radical that had been dissolved in 10 cm^3 of a physiological saline solution and various concentrations of oxygen were made at an ESR frequency of 280 MHz. It was found that the FSRs of both phantoms increased as the concentration of oxygen increased.

A longitudinally detected ESR (LODESR) method is one of the techniques designed to observe ESR, where the signal is derived from a longitudinal (i.e., parallel to the static magnetic field) oscillation of magnetization caused by spin flipping under on/off modulated ESR irradiation.[1–5] Because longitudinal magnetization (M_z) changes of electron spins are observed in this method, it is suitable for determining the longitudinal relaxation time (T_1) of electron spins. It was already reported that T_1 could be determined by investigating the LODESR signal intensity as a function of a frequency of on/off modulation of ESR irradiation.[5] In this method, a pickup coil that efficiently detects M_z change must be tuned for multiple detection frequencies (that are equal to modulation frequencies). Furthermore, the pickup coil must be calibrated for each detection frequency because its characteristics (such as Q and electromotive force) vary for each frequency.

In this study, we designed a new method for T_1 determination, in which the detection frequency was fixed and the on/off modulation frequencies were varied. With the detection frequency held constant, LODESR measurement with on/off modulation at the same as and at half the detection frequency were made. The fundamental harmonic of M_z changes was detected in the former, and its secondary harmonic was detected in the latter. The recovery processes of the flipped spins are different in the former and the latter because their frequencies of on/off modulation are different, so it is predicted that ratio of the former to the latter reflects T_1. In this study, simulations of this method for two kinds of free radicals, a narrow single ESR line agent, triarylmethyl (TAM), and a commonly used three ESR line agent, a nitroxide radical, were made. On the basis of the simulation, LODESR measurements of aqueous solutions of these radicals were made for oximetry.

The equations expressing M_z as a function of time (t), which were obtained from Bloch's equations,[6] are

$$M_z = \frac{M_0}{1 + k^2 T_1^2}\left(k^2 T_1^2 e^{\frac{-t}{T_1}} \cos kt + kT_1 e^{\frac{-t}{T_1}} \sin kt + 1\right) \quad (1)$$

at $0 < t < T_{\mathrm{mod}}/2$ where k is the product of magnetogyric ratio and B_1 (RF magnetic field), M_0 is M_z at $t = 0$, and T_{mod} is the reciprocal of the modulation frequency; and

$$M_z = (M_1 - M_0)e^{\frac{-\left(t - \frac{T_{\mathrm{mod}}}{2}\right)}{T_1}} + M_0 \quad (2)$$

at $T_{\mathrm{mod}}/2 < t < T_{\mathrm{mod}}$ where M_1 is M_z when $T_{\mathrm{mod}}/2$ is substituted for t in Eq 1. Under these conditions, the duty factor in the

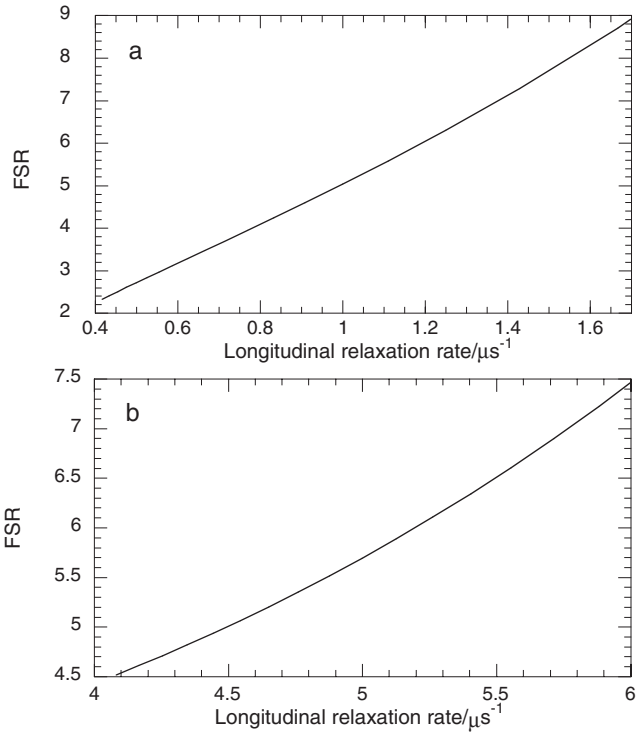

Figure 1. FSRs against longitudinal relaxation rate of TAM (a) and nitroxide radical (b) that were obtained from the simulation.

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on/off modulation was 0.5. M_z was calculated by using these equations, and LODESR signal intensities, which were lock-in detected at a constant detection frequency, when the modulation frequency was equal to, and at half the value of the detection frequency (fundamental and secondary harmonic detection, respectively) were simulated. The fundamental harmonic-to-secondary harmonic ratios (FSRs) were calculated by dividing the former by the latter. As shown in Figure 1, the simulated FSR for both radicals increased as a function of the reciprocal of T_1 (longitudinal relaxation rate).[7] Through the simulation, the detection frequency for TAM or nitroxide radical was set at 145 kHz or 900 kHz, respectively, so that the LODESR signal intensity and the rate of increase of the FSR versus longitudinal relaxation rate were larger.

Ten cm^3 of a 1 mmol dm^{-3} solution of TAM ((8-carboxy-2,2,6,6-tetrahydroxyethylbenzo[1,2-d:4,5-d′]bis(1,3)dithiole-4-yl)methyl sodium salt) or nitroxide radical (3-hydroxymethyl-2,2,5,5-tetramethylpyrrolidine-1-oxyl[8,9]), which had been dissolved in a physiological saline solution (a 0.9% aqueous sodium chloride solution), was poured into a sample tube (inner diameter, 25 mm) for use as a phantom. The phantoms at various oxygen concentrations were prepared by bubbling different oxygen/nitrogen gas mixtures — which were obtained by using a gas blender (GB-3660, Kofloc Co., Japan) — through the solution for 60 min.

A multilayered element resonator (resonant frequency, 280 MHz; inner diameter, 40 mm; axial length, 19 mm)[10] was used as an RF resonator of our LODESR spectrometer. The on/off modulated RF was applied to the phantom in the RF resonator by using an RF oscillator (MG3633A, Anritsu, Japan), a pin switch (ZYSWA, Mini circuit, U. S. A.), and a power amplifier (A1000-1050, R&K, Japan). The modulation frequency can be switched to the same as, or half the detection frequency. A pair of saddle-type pickup coils,[2–4,10] located in the RF resonator, was used to detect the M_z changes. The pickup coils were resonated at a detection frequency. The induced signal in the pickup coils was preamplified and lock-in amplified at the detection frequency. A pair of LODESR spectra that was detected in quadrature (90 degree phase difference) was obtained from the x- and y-channels of a lock-in amplifier (5302, PARC, U. S. A.). The signal intensity was derived from the peak height of an in-phase detected spectrum that was obtained by calculating the vectorial sum of spectra from both channels. To generate static magnetic field, electromagnets of a conventional ESR spectrometer (modified RE-3XL, JEOL, Japan) were used.

LODESR measurements of phantoms (including TAM or nitroxide radical) at various concentrations of oxygen were made.[11] For the detection frequency, the value that was obtained from the simulation was used. LODESR signal intensities of these phantoms by the fundamental and secondary harmonic detections were obtained, and the FSRs were calculated by dividing the former by the latter. As shown in Figure 2, it was found that the FSRs of the phantoms of both radicals increased as the concentration of oxygen increased (Values are mean ± standard error from 6 independent determinations). These results were consistent with the simulation results because T_1 is shortened as the oxygen concentration increases.[12] Thus, we believe that the oxygen concentration of a sample can be non-destructively determined on the basis of the FSR at a constant detection frequency. The rate of increase of the FSR with the oxygen concentration for TAM was greater than that for the nitroxide radical, indicating that the sensitivity of TAM to changes in oxygen concentration was relatively high. On the other hand, the T_1 of a nitroxide radical is independent with the radical concentration while the T_1 of TAM is not.[5,13] This is a disadvantage in using TAM for oximetry. Because both TAM and nitroxide radical can be used for oximetry in the method described here, one can select them according to their characteristics and experimental conditions.

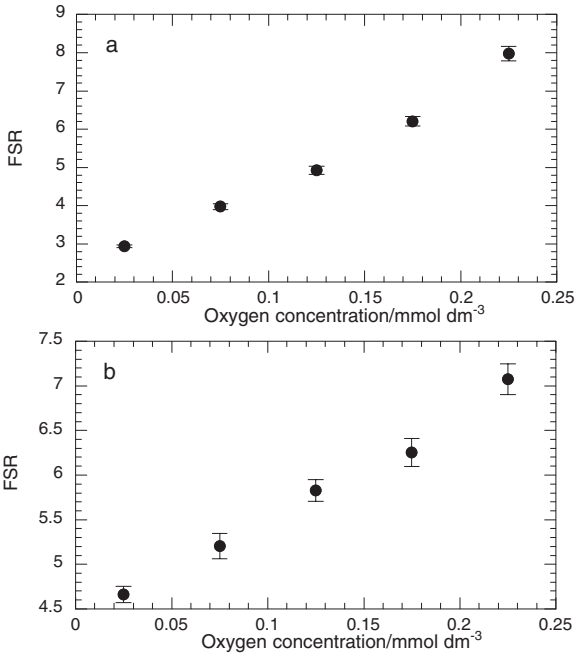

Figure 2. FSRs against oxygen concentration of the phantoms of TAM (a) and nitroxide radical (b) that were obtained from the LODESR measurements.

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References and Notes

1 I. Nicholson, F. J. L. Robb, and D. J. Lurie, *J. Magn. Reson., Ser. B*, **104**, 284 (1994).
2 H. Yokoyama, T. Sato, T. Ogata, H. Ohya-Nishiguchi, and H. Kamada, *J. Magn. Reson., Ser. B*, **129**, 201 (1997).
3 H. Yokoyama, T. Sato, T. Ogata, H. Ohya-Nishiguchi, and H. Kamada, *Magn. Reson. Mater. Phys., Biol. Med.*, **7**, 63 (1998).
4 H. Yokoyama, T. Sato, H. Ohya, and H. Kamada, *J. Magn. Reson.*, **150**, 194 (2001).
5 I. Panagiotelis, I. Nicholson, and J. M. S. Hutchison, *J. Magn. Reson.*, **149**, 74 (2001).
6 F. Bloch, *Phys. Rev.*, **70**, 460 (1946).
7 In this simulation, B_1 was set at 35 or 70 μT for TAM or nitroxide radical, respectively.
8 O. Itoh, H. Obara, M. Aoyama, H. Ohya, and H. Kamada, *Anal. Sci.*, **17**, i1515 (2001).
9 H. Yokoyama, O. Itoh, M. Aoyama, H. Obara, H. Ohya, and H. Kamada, *Magn. Reson. Imaging*, **20**, 277 (2002).
10 T. Sato, H. Yokoyama, H. Ohya, and H. Kamada, *Appl. Magn. Reson.*, **17**, 119 (1999).
11 Applied RF power was controlled so that B_1 was similar to that in the simulation. Other LODESR conditions for the phantom of TAM or nitroxide radical were: loaded Q, 85; scan rate of magnetic field, 2 mT/s at scan width of 2 mT; accumulation number, 16 or 10; and time constant, 1 ms.
12 J. S. Hyde and W. K. Subcynski, in "Biological Magnetic Resonance Vol. 8," Plenum, New York (1989), p 399.
13 It was found that the FSR of TAM solution increased as its concentration increased while the FSRs of the nitroxide radical solutions at different concentrations were almost the same. These findings were not inconsistent with the previous report.[5]

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