ZnO Marker for a Radio Frequency ESR Spectrometer

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The marker for quantitative determination of free radicals by a radio frequency (RF) electron spin resonance (ESR) spectrometer operating at about 700 MHz was found for the first time. It is zinc(II) oxide (ZnO) with the ESR signal due to oxygen vacancy. The *g*-value was about 1.96 and also the line shape was sharp. When this marker was measured together with TEMPOL aqueous solution by using the RF ESR spectrometer, each signal did not overlap. Furthermore, the signal intensity ratio of TEMPOL to ZnO unchanged even if Q-value of a resonator was decreased.

To investigate the reaction of free radicals in vivo, an electron spin resonance (ESR) spectrometer operating at a radio frequency (RF) of less than 1.2 GHz has been developed.[1,2] For the RF ESR spectroscopy, nitroxyl radicals such as 2,2,6,6-tetramethyl-4-piperidinol-1-oxyl (TEMPOL) have widely been used as spin probe and spin label agents. However, no available marker such as so-called Mn-marker for X-band ESR has been found out for RF ESR spectroscopy, when a quantitative determination of nitroxyl radical concentration is required. In this paper we recommend zinc(II) oxide (ZnO) powder as a novel marker for RF ESR.

The loop-gap resonator (LGR) was used as an ESR resonator.[3] The LGR used in this study is a two-gap-type with electric shields that are located inside the resonator. Its axial length is 10 mm and inner diameter, 41 mm. The resonator was driven at a frequency of approximately 700 MHz. The LGR is magnetically coupled to the external circuits via coupling coil. An RF ESR spectrometer, which was constructed at out laboratory (already described in detail[4,5]), consisted of a main electromagnet (with air-core, water cool, and a two-coil Helmholtz design), a pair of field scan coils, a pair of modulation coils, power supplies, a personal computer, and a 700 MHz-microwave circuit for homodyne detection.

The absolute restriction for a Q-value compensation marker is that the ESR signals of an object and a marker do not overlap and that the signal line shape of the marker is sharp. In addition, the ESR signal must be stable for a long time. When it is assumed that the line width of a solution of TEMPOL and a marker is 0.5 mT, the g-value of a marker is calculated to be $g < 1.847$, $1.917 < g < 1.975$, $2.056 < g < 2.112$ or $g > 2.205$ at 700 MHz resonant frequency. The Mn-marker used in an X-band ESR spectrometer could not be used as a marker of an RF ESR spectrometer, because the ESR spectrum of Mn(II) aqueous solution showed the broad line.[6] It was also confirmed that Mn-marker (Mn(II) in MgO) showed many signal lines in an RF ESR spectrum.[7] These phenomena are theoretically explained on the basis of zero-field splitting.[8] The other paramagnetic metal ions, Cr(III) ($g \cong 1.98$), Cu(II) ($g \cong 2.0$) and Fe(III) ($g \cong 4.2$), were also examined.[9] However, the part of Cr(III) signal overlapped with the central signal of TEMPOL, Cu(II) gave the broad ESR signal overlapping with TEMPOL, and Fe(III) signal appeared around 12 mT which was very far from TEMPOL signal (26 mT). Therefore, these paramagnetic metal ions were not siutable to an RF ESR marker. Diphenylpicrylhydrazyl (DPPH) and lithium tetracyanoquinodimethane (LiTCNQ) could not be used for a Q-value compensation since these radicals overlapped with the central signal of TEMPOL in an RF ESR spectrum. In the meantime, it was found out from literatures[10] that the ESR signal of oxygen vacancy or interstitial zinc atom in ZnO showed $g \cong 1.955$[10] and satisfied the marker condition. Furthermore it is possible to prepare the marker with very high ESR signal intensity, since the spin density of ZnO powder can easily be controlled by changing a heating condition.[9]

Zinc sulfate aqueous solution of 0.5 M (1 M = 1 mol·dm^{-3}) was dropped into sodium carbonate aqueous solution of 1 M at 333 K at pH 10, and the sediment of zinc carbonate was separated. After drying zinc carbonate in the air at 383 K for 1 h, this powder was kept under reduced pressure overnight. Then the powder was heated in air using electric furnace at 873 K for 4 h, and the zinc oxide powder was prepared. An aqueous solution of 0.3 mM TEMPOL was prepared as a typical spin agent solution. Before RF ESR measurement, ESR parameters of ZnO signal were examined using X-band ESR spectrometer. The *g*-value, the line width, and the spin density were determined to be 1.958, 0.35 mT, and 5.64×10^{17} spins·g^{-1}, respectively. In particular, this spin density unchanged at least for six months.

The RF ESR measurements were carried out as follows. The ZnO powder of 0.2 g and TEMPOL solution of 0.3 mL were introduced into glass test tubes (5 mm inner diameter),

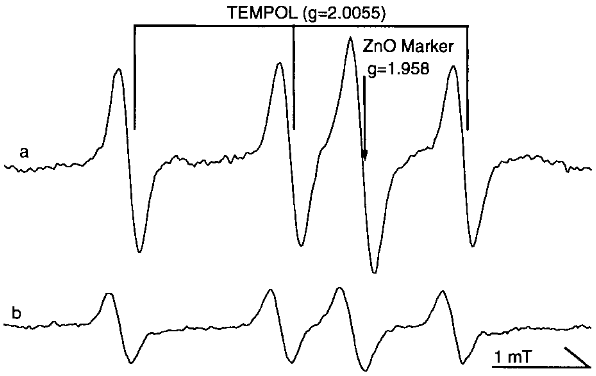

Figure 1. The ESR spectra in measuring both powder of ZnO and 0.3 mL of 0.3 mM TEMPOL aqueous solution. Factors of an external dielectric loss of (a) and (b) were none and 3 mL of 0.3% NaCl aqueous solution, respectively. ESR conditions were: microwave power, 40 mW; microwave frequency, 720 MHz; static magnetic field, 25.5 mT; sweep width, 6 mT; modulation width, 0.2 mT at 100 kHz; scan rate, 15 mT/s and time constant, 1 ms.

respectively. These tubes were placed together at the center of the LGR. The ESR spectrum obtained is shown in Figure 1a. The signal of ZnO powder separated sufficiently from that of TEMPOL. The measurements in which Q-value of the LGR intentionally changed were carried out in order to confirm the quantitative determination. Three milliliters of 0.3% NaCl aqueous solution in the glass test tube (15 mm i.d.) was inserted in the LGR to decrease the Q-value. The ESR spectrum was measured in the same way and is shown in Figure 1b. The ESR signal intensities of TEMPOL solution or powder of ZnO were obtained under the conditions in the absence and the presence of the 0.3% NaCl aqueous solution. The results are given in Table 1. Since the error level of TEMPOL-to-ZnO signal ratio was less than 4%, it is possible to determine quantitatively the radical concentration by using an RF ESR spectrometer with the ZnO marker.

Table 1 Signal intensities[a] of ZnO powder (I_0), 0.3 mM TEMPOL aqueous solution (I_1) and their ratios (I_1/I_0) without (A) and with (B) 0.3% NaCl aqueous solution

	A	B
I_0	$(1.70 \pm 0.04) \times 10^4$	$(6.30 \pm 0.09) \times 10^3$
I_1	$(1.33 \pm 0.03) \times 10^4$	$(4.95 \pm 0.21) \times 10^3$
I_1 / I_0	0.786 ± 0.014	0.785 ± 0.028

[a] Value of analog to digital conversion in computer on-line system

From the results mentioned above, it is concluded that the powder of ZnO is usable as a marker for quantitative determination of free radicals using an RF ESR spectrometer. This marker will be useful for the RF ESR measurement of lossy samples such as biological systems.

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