A Frequency Modulation Detection Scheme of Electron Paramagnetic Resonance Spectroscopy

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Abstract

A frequency modulation (FM) scheme was developed for detecting electron paramagnetic resonance (EPR) spectra in the frequency domain. Using the synchronous tracking method of the resonant frequency of a radio frequency (RF) resonator to the frequency-modulated RF wave, we successfully measured for the first time an experimental EPR signal at 700 MHz. Employment of the FM scheme solved problems of the resonator vibration due to the electromagnetic force caused by the eddy current inherent in magnetic field modulation scheme. The method allowed measurements to be free from the noise caused by resonator vibration called the microphonic noise.

1. INTRODUCTION

For conventional continuous wave (CW) electron paramagnetic resonance (EPR) spectroscopy, the homodyne detection scheme, using magnetic field modulation, has successfully been employed.[1] However, the field modulation scheme faces crucial difficulties such as the passage effect, microphonic noise and side-band effects.[2] For example, when the time-dependent magnetic flux penetrates into the metal surface of the EPR resonator, the magnetic flux induces an eddy current on the surface of the resonator walls. Since the eddy current further interacts with the DC magnetic field through the electromagnetic interaction, i.e. Lorentz force, the resonator walls vibrate with a frequency of the magnetic field modulation (usually 100 kHz). The vibration eventually changes the resonant frequency of the resonator. The impedance matching between the resonator and the microwave circuit of an EPR spectrometer is modified. Consequently, the expected output from the resonator is an amplitude modulated (AM) wave with side-band signals. Such an output causes a sloppy baseline and unstable baseline level. The additional frequency components contribute substantially to the high noise level in the CW-EPR spectroscopy. Since the output is equivalent to the intrinsic EPR signal, the resultant signal from the field modulation scheme disrupts the highly sensitive detection. In particular, the recent trends toward using low-frequency EPR spectrometers for biological systems require a low-noise spectrometer. In principle, the frequency modulation (FM) scheme has been known to be effective in solving the problem of microphonic noise.[3)] The FM scheme has been used in an electron nuclear double resonance (ENDOR) spectrometer,[3)] which conveys a high-resolution measurement of the spectra. However, the ENDOR spectrometers are not necessarily sensitive enough as compared with the conventional CW-EPR.[4)] Using the synchronous tracking method of the resonant frequency of a radio frequency (RF) resonator to the frequency-modulated RF wave, we successfully measured for the first time an experimental EPR signal at 700MHz.[5-6)] And then another continuous-wave EPR spectroscopy, using a frequency modulation (FM) scheme, was developed. Using an electronically tunable resonator and an automatic tuning control (ATC) system, the frequency of the synthesizer changes with the frequency of the resonator, whose resonant frequency is changed by the modulation frequency.[7-8)] Hyde et al. have developed multiquantum EPR (MQEPR) spectroscopy for the detection of the non-saturating spectrum without using the magnetic field modulation.[9-13)]

Magnetic field modulation, which also calls for complex instrumentation, is not necessary in the frequency modulated electron paramagnetic resonance (FMEPR) spectroscopy described here. The present report describes the principle and the instrumental setup for FMEPR, in which the resonator frequency synchronizes to the oscillator frequency. Without using the fixed resonant frequency, we let the resonator frequency track synchronously to the frequency shift of the microwaves through the FM scheme. There by, a new methodology was introduced for the application to the highly sensitive detection of the L-band EPR that still employed the DC magnetic field scan.

2. DESIGN OF THE RADIO FREQUENCY WAVE CIRCUIT

2.1 Concept of FMEPR spectroscopy

In conventional CW-EPR spectroscopy, magnetic field modulation is employed to obtain the first derivative curve of EPR absorption. Since the magnetic field modulation is equivalent to the frequency modulation to record the first derivative signal, the frequency modulation can be used for detecting the EPR absorption in place of the magnetic field modulation. Figure 1 illustrates the concept of the frequency modulated electron paramagnetic resonance (FMEPR) spectroscopy. If radio frequency waves are modulated in the frequency domain, the EPR absorption signals shift in accordance with the FM mechanism. In a given DC magnetic field, one can observe the time-varying absorption amplitude of the EPR signals.

Figure 2 shows a circuit diagram of the FMEPR spectrometer. A basic reference-arm bridge is used. The RF-signal source is frequency-modulated by a low-frequency synthesizer for the phase-sensitive detection (PSD) of EPR absorption. The frequency-modulated output voltage v_o of the RF-signal source is given by the following equation:

$$v_o = A_c \cos(\omega_c t + m_f \sin \omega_m t). \tag{1}$$

Here A_c is the amplitude of the output voltage, ω_c the angular frequency of the carrier waves, ω_m

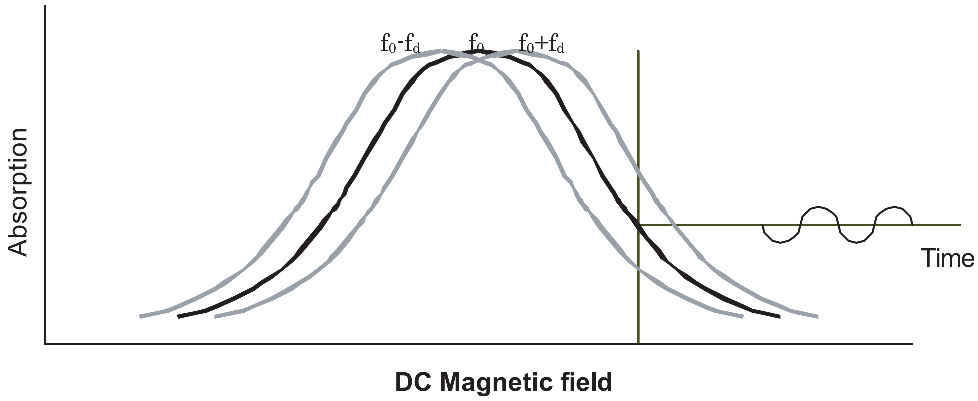

Fig. 1. Concept of the frequency modulated electron paramagnetic resonance (FMEPR) spectroscopy. If radio frequency waves are modulated in the frequency domain, the EPR absorption signals shift in accordance with the FM mechanism, where f_0 is the center frequency of the EPR absorption curve and fd the frequency deviation. Given a certain DC magnetic field, one may observe the time-varying absorption amplitude of the EPR signals.

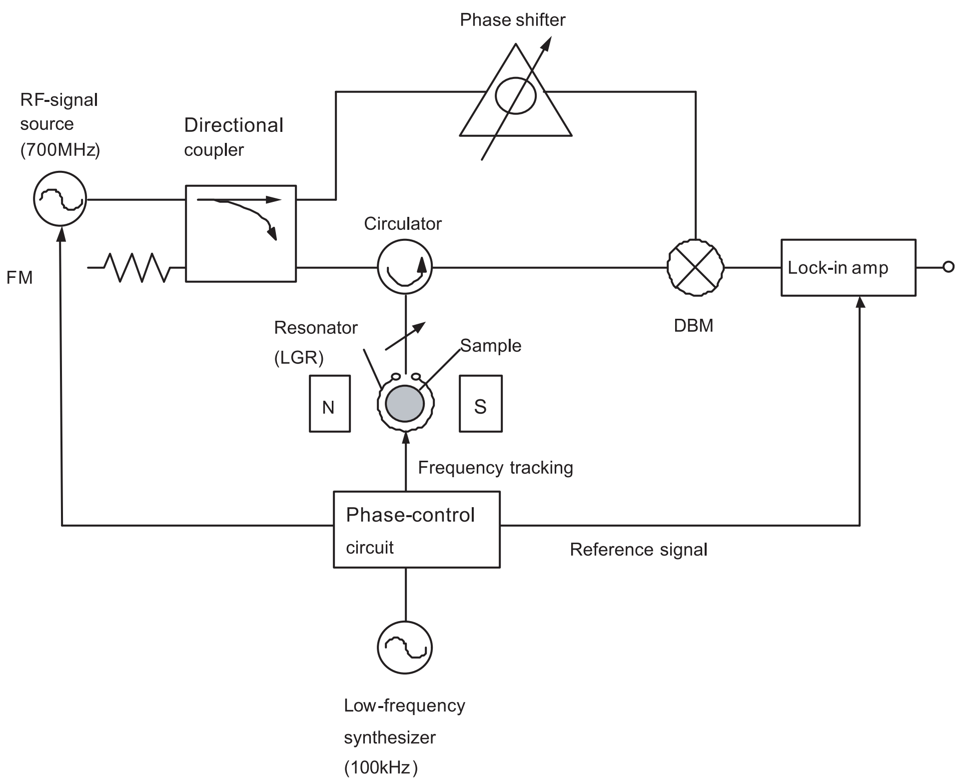

Fig. 2. A circuit diagram of the FMEPR spectrometer. A basic reference-arm bridge is used. The RF-signal source is frequency-modulated (FM) by a low-frequency synthesizer for the phase-sensitive detection of an EPR absorption.

the angular frequency of the modulation frequency, m_f (= δf / f_m) the modulation index, δf the frequency deviation, f_m the modulation frequency. The resonant frequency of the resonator tracks synchronously to the frequency shift of the microwave induced by the frequency modulation.

The reflected voltage v_r from the resonator is given by

$$v_r = A_c\,(1 + m_a \cos\omega_m t)\cos(\omega_c t + m_f \sin\omega_m t), \quad (2)$$

where m_a is the amplitude modulation index. The voltage v_r was amplified by a low noise RF-amplifier, and was mixed with a reference signal of frequency-modulated waves by a double balanced mixer (DBM). DBM output contains components of DC, ω_m, ---, $2\omega_c$-$4\omega_m$, $2\omega_c$-$3\omega_m$, $2\omega_c$-$2\omega_m$, $2\omega_c$-ω_m, $2\omega_c$, $2\omega_c+\omega_m$, $2\omega_c+2\omega_m$, $2\omega_c+3\omega_m$, $2\omega_c+4\omega_m$,---. Components except DC and ω_m are concentrated at the radio frequency region near $2\omega_c$. After filtering the higher components, one amplifies only the ω_m component, and extracts the EPR signal by the lock-in amplifier.

2.2 Resonator

The resonator is one of the most important microwave circuit elements in the present study. We use a one-gap loop-gap resonator (LGR) [14-15] with a varactor diode. The varactor diode located parallel to the gap may vary the resonant frequency when voltage is applied. Figure 3 shows the resonator and its equivalent circuit. The loop gives the inductance L and the resistance R in the radio frequency region. The gap acts as a capacitance C_g. The varactor diode yields the capacitance C_v and the conductance G_v . The quality factor Q of the resonator depends on R and G_v .

In order to compare the sensitivity of FMEPR with that of CW-EPR, we fixed the frequency of the FMEPR at 700 MHz. The size of the loop-gap resonator used here is decided by fine adjusting of the calculation value so that the resonant frequency attains 700 MHz. The size of the resonator is shown in Fig. 3. The resonator is made of gold-plated copper. A Teflon plate is used as the spacer between the resonator main body and the electrodes. To attach the Teflon plate, the electrodes are wound with Teflon tape, although the tape is not shown in the figure. We soldered a varactor diode to the electrodes, and used choke coils to prevent the connection by means of the radio frequency waves between the diode and the control circuit as shown in Fig. 3. The choke coil

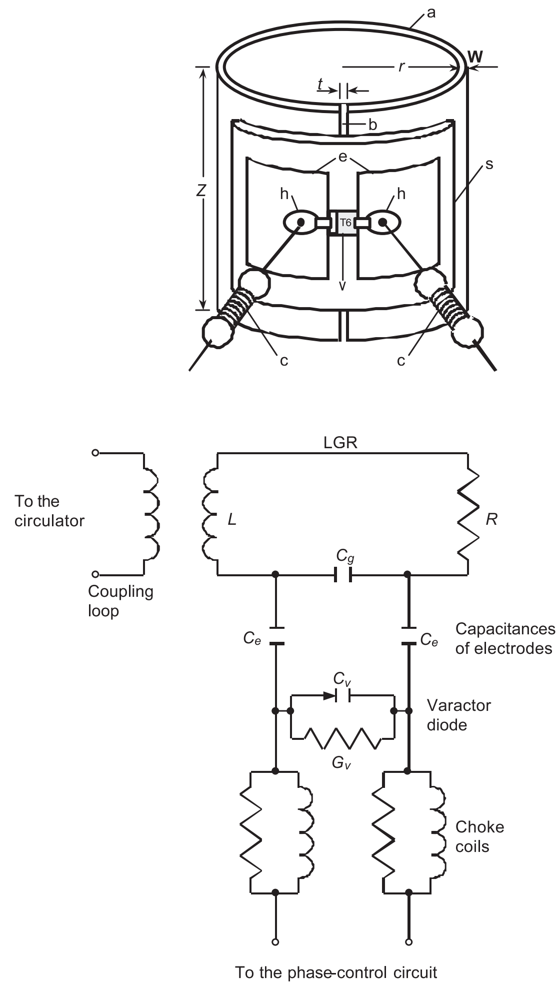

Fig. 3. The resonator design and its equivalent circuit. The loop "a" gives the inductance L (18.5 nH) and the resistance R in the radio frequency range. The inner radius r, thickness W and height Z of the loop are 14.0, 1.0 and 28.0 mm, respectively. The gap b acts as a capacitance Cg (2.80 pF). The length t of the gap is 1.0 mm. The varactor diode v yields the capacitance Cv and the conductance Gv. The gap between the loop and the electrode e (12.5 mm height x 10.0 mm width) functions as the capacitance Ce. Thickness of the Teflon plate s is 0.5 mm. Solder h connects the electrode and the choke coil. The unloaded Q and resonant frequency of the resonator were 200 and 700 MHz, respectively, when voltage was added to the varactor diode.

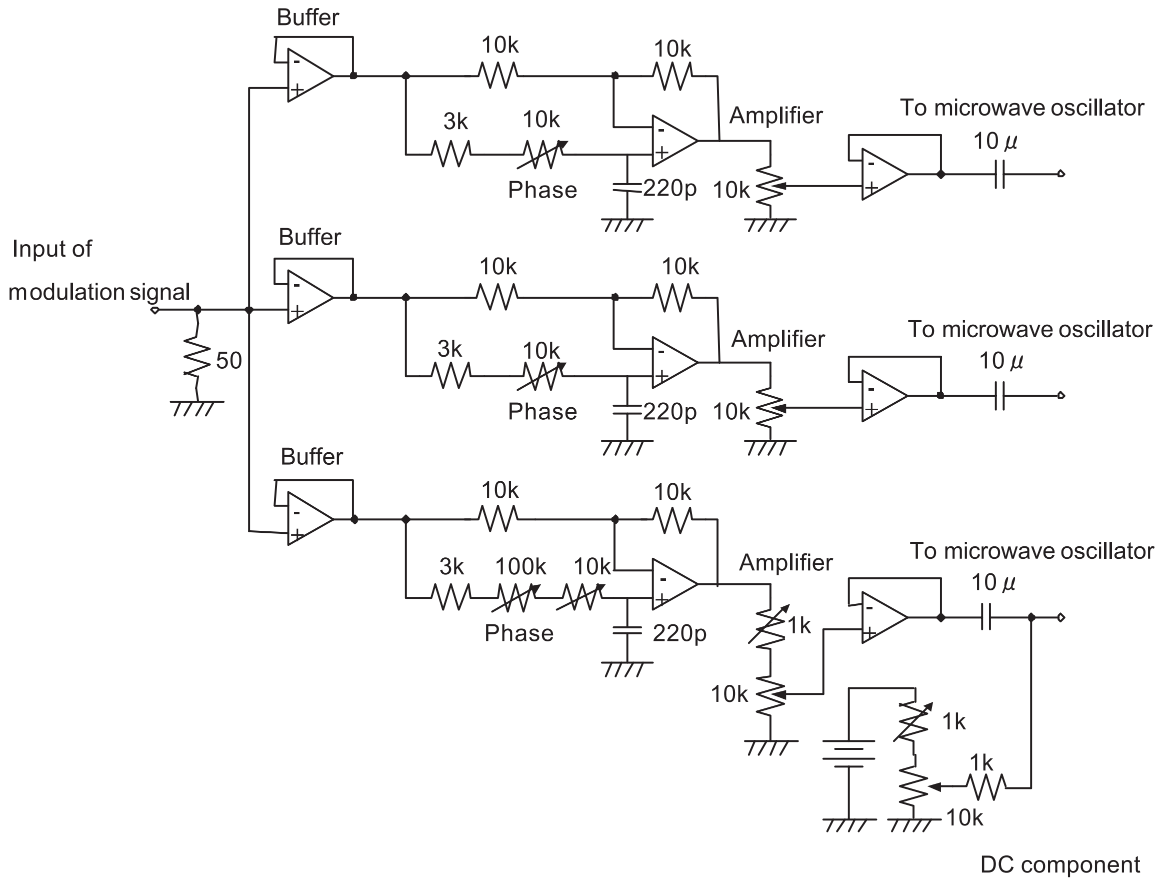

Fig. 4. The phase-control circuit used in the present experiment. The modulation signal through the phase control circuit is used as a reference signal of the phase-sensitive detection, which is performed by a lock-in amplifier.

is wound around a resister. We used the varactor diode (1SV217, Toshiba) for the tunable LGR for the present experiments. To adjust the proposed resonator, we used a vector network analyzer (Anritsu, MS620J).

2.3 Phase-control circuit

A phase-control circuit allows adjusting of the phase of the modulation signal to maintain the minimum reflection from the LGR. Figure 4 shows the phase-control circuit used. The modulation signal through the phase-control circuit is also used as a reference signal of the phase-sensitive detection, which is performed in phase with a lock-in amplifier.

The output voltage of the low-frequency synthesizer used for FM of RF-signal source is about 2.2 V to obtain FM deviation of 1 MHz, and the reverse-bias voltage applied to the diode has to be able to change from 0 V to 30 V in case of above mentioned varactor diode. In order to meet these conditions, we designed the phase-control circuit using a low-pass filter, a buffer, a phase circuit and an amplifier that included an op-amp. We used the operational amplifier OPA2604 (BURR-BROWN) whose characteristics included having two circuits, high speed, low noise and JFET input. Using the control circuit, we were able to cease the deviation of frequency modulation within 1 MHz and converge the frequency of the modulation signal within 200 kHz. The frequency deviation of 1 MHz corresponds to the field modulation of 36 μT at a radio frequency of 700MHz.

2.4 Miscellaneous

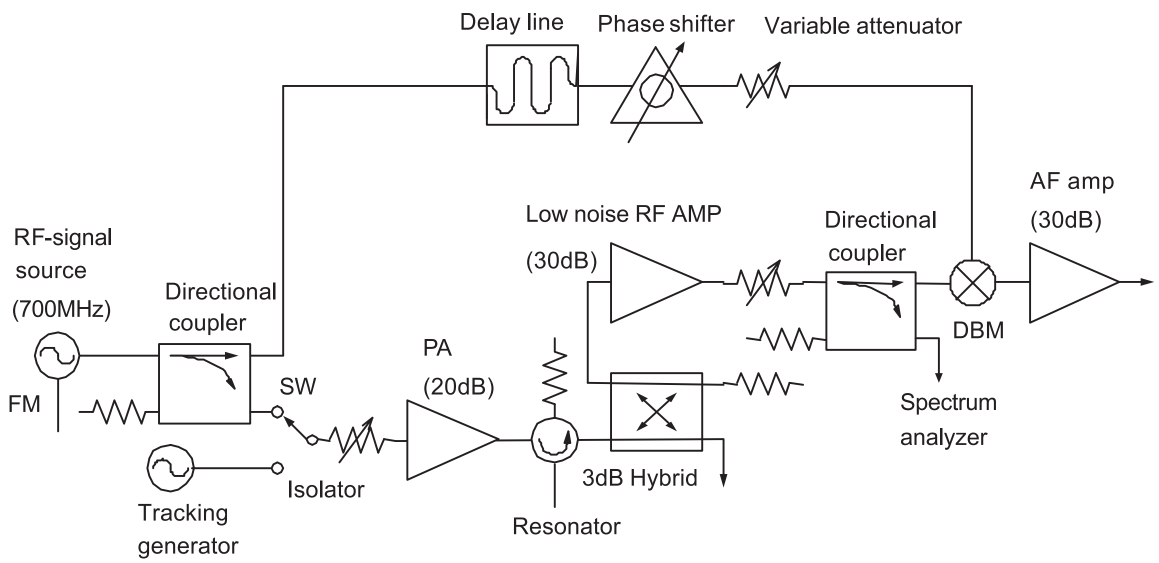

Fig. 5. A RF circuit diagram employed by the experimental set up for FMEPR. We used a RF-signal source (Anritsu, MG3633A), a tracking generator (Anritsu, MH680A), directional couplers (ANAREN, 10014-10, 10 dB), a power amp (Mini-Circuits, ZFL-1000VH, 20 dB), an isolator (TDK, 20 dB), a 3 dB Hybrid (Narda, 4031C), a low noise RF-amp (Watkins-Johnson, A 1031, small signal gain: 28.5 dB, noise figure: 2.7 dB), a spectrum analyzer (Anritsu, MS2601A), a delay line (homemade), a phase shifter (SAGE, 6702-23), a double balanced mixer (R & K, M-2), an AF-amp (TOPNIX, ATN3612, 0-500 kHz, 30 dB) and a lock-in amp (NF Electronic Instruments, 5610).

A DC power supply controlled by a personal computer was used for the sweep of the DC magnetic field. RF elements in the FMEPR spectrometer were diverted from the conventional CW-EPR spectrometer with the magnetic field modulation scheme. Since automatic frequency control (AFC) was not employed in the present spectrometer, a spectrum analyzer was used to measure the resonant frequency. The resonator was connected to either the spectrum analyzer or the spectrometer by a RF coaxial switch.

3. MEASUREMENTS

Figure 5 shows a RF circuit diagram of an experimental setup for FMEPR. A circulator was replaced by a 3dB hybrid because of its good isolation. The measurement procedures of the FMEPR spectroscopy are as follows: (1) swept RF output is applied to the resonator by turning the switch on the tracking generator. The spectrum analyzer checks the resonant frequency and the return loss of the resonator. The resonant frequency of the resonator was synchronized to the measurement frequency by adjusting the reverse-bias voltage applied to the varactor diode. Adjusting the position of the coupling coil achieved a good impedance matching between the resonator and the RF-circuit. Through the FMEPR measurement, it is desirable that the spectrum analyzer always monitors the resonant frequency and the return loss of the resonator. (2) The switch is changed to the RF-signal source, and then non-modulated carrier waves are employed. The frequency of the RF-signal source is set so that the reflected waves from the resonator are at their minimum; namely, the frequency spectra on the display are at their minimum level. Subsequently, the modulation signal (100kHz) is input into the RF-signal source and then the RF-signal is frequency-modulated. Deviation of the frequency modulation is adjusted according to the EPR linewidth (half-width at half-height). (3) The reflection of frequency-modulated waves by the resonator are fixed at the minimum level, namely, the frequency spectra on the display of the spectrum analyzer are set at the minimum level, by adjusting the amplitude and the phase of the modulation signal added to the varactor diode. As a result, the resonant frequency always synchronizes with the oscillator frequency (see Discussion). (4) The DC magnetic field is swept over a frequency range of the EPR absorption. Simultaneously, the lock-in amplifier carries out the data acquisition. The phase shifter is adjusted to obtain the EPR signal.

To demonstrate the feasibility of the FMEPR spectrometer, EPR spectra of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) powder were recorded at 700 MHz both by the FMEPR and the conventional magnetic field modulated CW-EPR. In those measurements, the LGR previously mentioned was used for detecting the EPR phenomenon. The quality factor of the resonator was 200, at a resonant frequency of 700 MHz (3 dB bandwidth is 3.5 MHz). The resonator and other microwave circuit elements yielded the similar values in both FMEPR and CW-EPR. A capillary phantom containing 20 mg of DPPH powder was used as an EPR signal source. The frequency deviation in FMEPR was within 1 MHz that corresponded to the magnetic field modulation of 36 μT at 700 MHz. Measured magnetic flux of density for field modulation in CW-EPR was 36 μT . The frequency deviation is easily spread out over 10MHz using an another RF-signal source.

To meet the best specification of FMEPR scheme, the resonant frequency always synchronizes with the oscillator frequency. In order to reconfirm the synchronicity, we measured the spectra of the reflected waves from the resonator at synchronized resonance and at the fixed resonance. The resultant spectra are shown in Fig. 6 (a) and (b) , for the synchronized resonance and the fixed resonance, respectively. Comparison of these spectra indicates that the reflected waves decrease by about 20 dB due to the impedance matching in the synchronized resonance. We thus concluded that the resonant frequency could always synchronize with the frequency of the RF-signal source.

Figures 7 (a) and (b) show the EPR spectra of DPPH powder as detected by the FMEPR and the CW-EPR spectrometers respectively. A baseline drift was observed in the absence of the AFC. The result confirms that the frequency modulation performs EPR measurements in the same manner as the magnetic field modulation scheme. The spectra were recorded at the first harmonic mode. The linewidth of the spectrum recorded by FMEPR was 0.12 mT, which is identical with that of L-band CW-EPR.

Thus far, it is known that in the AM-EPR scheme the signal-to-noise ratio (SNR) is proportional to the modulation frequency, because of the inverse frequency ($1/f$) law. As in the FMEPR scheme, the reflected wave from the resonator is amplitude-modulated, so that SNR is proportional to the modulation frequency. Figure 8 shows a relationship between the SNR versus the modulation frequency. The relationship reveals that the increment of the modulation frequency from 20 kHz to 140 kHz enhances about twice of the SNR of the spectrometer. This fact suggests that the increment of the modulation frequency to the frequency of ca. 1 MHz enhances the detection sensitivity like in the conventional CW-EPR.

4. DISCUSSION

4.1 Vibration due to field modulation

It has long been noted that the most hazardous factor for the sensitivity enhancement of EPR measurements is the resonator vibration in the vicinity of the modulation frequency, *e.g.*, 100 kHz. In particular, such difficulty becomes more serious in the L-band than in the X-band spectrometer, due to the large dimensions of the resonator and the asymmetry in the resonator shape, such as the loop-gap resonator. This is because the resonant frequency of the resonator is modified and the impedance matching between the resonator and the microwave circuit is disrupted by the vibration originating from the electromagnetic force (I×B).

In contrast, the FMEPR does not have such inherent problems, because it employs the FM scheme in place of magnetic field modulation. Therefore, the highest sensitivity can be achieved by the selection of high-quality components for the FMEPR circuit. Theoretically, it is likely to attain relatively high sensitivity by this detection scheme. In ENDOR experiments, the resonant frequency of a NMR resonator was swept and the spectra were successfully recorded. The frequency bandwidth of the RF-waves was broadened by the FM modulation. To prevent the reflection of the RF-wave, the Q factor of the resonator was suppressed at the low level. Thus, enhancement of the spectrometer sensitivity was not achieved. In the present study, we synchronize the resonator frequency to the shift of the oscillator frequency by the FM modulation, without lowering the

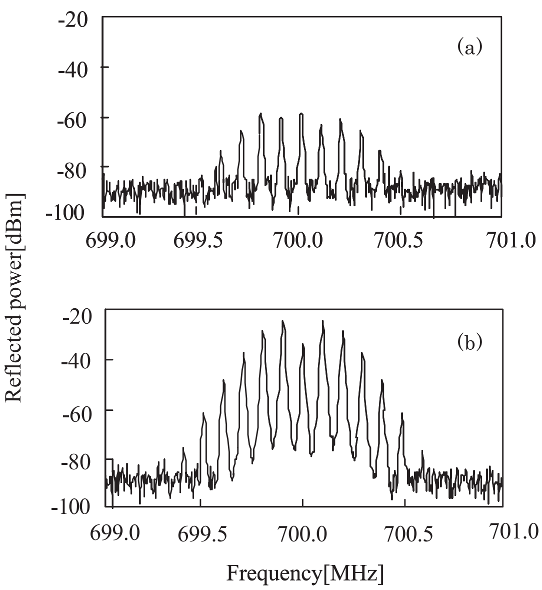

Fig. 6. Recorded spectra of the reflected waves from the resonator (a) at the synchronized resonance and (b) at the fixed resonance. Comparison of these spectra indicates that the reflected waves decrease by about 20 dB due to the impedance matching in the synchronized resonance.

resonator Q factor. In fact, Fig. 6 clearly shows a well-defined synchronicity between the two frequency modes. Therefore, the FM scheme allows highly sensitive detection of EPR signals.

4.2 Baseline drift

The baseline drift is a serious problem for the in vivo EPR spectroscopy. In the present study, we did not employ AFC protocol for either the FMEPR or conventional CW-EPR (vide infra). In principle, the baseline keeps constant for the FMEPR spectroscopy. Nevertheless, the baseline of the spectrum as detected by the FMEPR

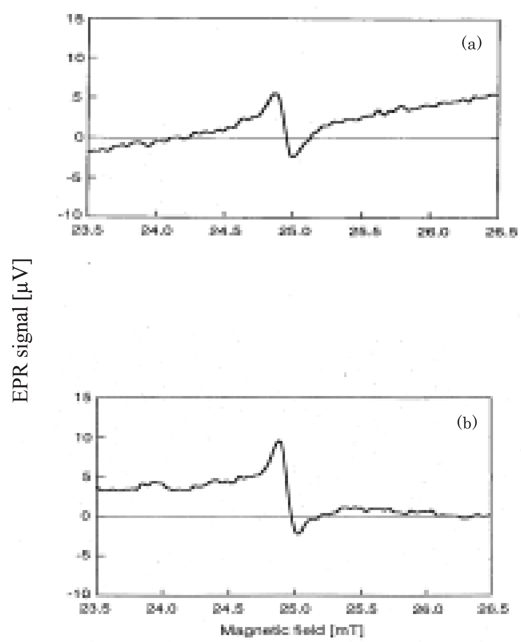

Fig. 7. EPR spectra of DPPH powder (20 mg) detected by (a) the FMEPR and (b) the CW-EPR spectrometers. Carrier and modulation frequencies are 700 MHz and 100 kHz, respectively. The frequency deviation in FMEPR was set at 1 MHz that corresponded to the magnetic field modulation of 36 μT at 700 MHz. Measured magnetic flux of density for field modulation in CW-EPR was 36 μT. A baseline drift was observed in the absence of the automatic frequency control. The result confirms that the frequency modulation can detect EPR spectra in a similar manner as in the magnetic field modulation scheme. The spectra were recorded at the first harmonic mode. The linewidth of the FMEPR spectrum was 0.12 mT, which is identical to that of the L-band CW-EPR.

showed a pronounced drift whose magnitude was ca. 1.0 μV/mT level (see Fig. 7(a)). This is mainly due to the thermal fluctuation during the measurements. On the other hand, the spectrum by the field modulation EPR (Fig. 7(b)) showed a sloppy baseline. The drift is ca. 2.7 μV/mT. This is due to the mechanical vibrations of the resonator, modulation coils and a shield case.

4.3 Radio frequency wave modulation

Unlike the conventional EPR, homogeneity of the modulation field in the FM scheme is equivalent to that of the radio frequency wave field. Thus, with the FMEPR, it is not necessary to provide homogenous magnetic field modulation. This is because the resonator is used both to supply radio frequency waves and to modulate the absorption. In conventional CW-EPR spectroscopy, a resonator (for example, a loop-gap resonator) disturbs the magnetic field modulation. Attempts have been made to solve problems in obtaining good penetration of the magnetic field modulation.[16-17] In the FMEPR, however, the resonator never disturbs the modulation. Furthermore, using a shield case to surround the resonator makes the modulation field more stable.

4.4 Future techniques

Generally, the SNR can be enhanced at the higher frequency domain due to the inverse frequency law. It is well known that sensitivity is proportional to the modulation frequency of the AM mode. Likewise, the present FMEPR experiment confirms in Fig. 8 that the higher modulation frequency enhances the signal sensitivity. In the FM method, the frequency sweep at the fixed magnetic field allows a rapid signal accumulation and thereby enhances the signal sensitivity. If the resonant frequency of the resonator is electronically controlled, an ultra fast frequency scan can be used for the FMEPR detection. When the carrier frequency of the RF-waves is swept synchronously with the resonant frequency, the sweep of the RF-carrier frequency plays the same role in the DC magnetic field scan as in conventional CW-EPR[18]. If the electronic scan of the resonant frequency is rapid enough, the acquisition of the signal by the FMEPR will be done in a short time as compared to the conventional CW-EPR performance. The rapid scan coil technique for the fast acquisition is not as fast as electronic scanning of the RF-frequency. In addition, the FMEPR spectrometer requires no power supply for rapid-scan coils. Consequently, the FMEPR spectroscopy provides the reliable method for a rapid acquisition of EPR signals by means of the swept carrier frequency.

Digital techniques using a DSP (digital signal processor) enable the FMEPR to use the faster AFC and frequency sweep in place of a magnetic field scan. We are going to deal with these problems in future.

We think that measurement sensitivity in the FMEPR is essentially the same as in conventional CW-EPR. But, in practice, sensitivity in the FMEPR will become higher than in the conventional CW-EPR at the high field EPR.

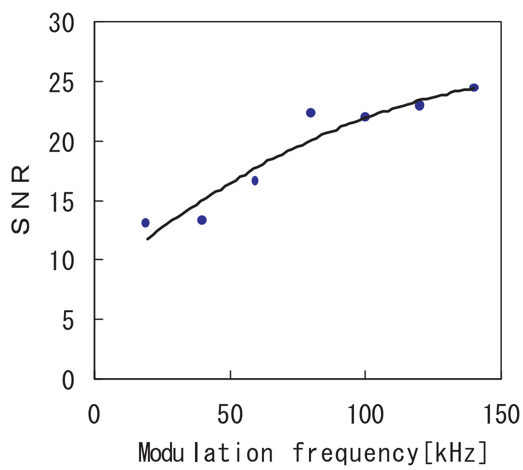

Fig. 8. Relationship between the SNR and the modulation frequency. This reveals that the increment of the modulation frequency from 20 kHz to 140 kHz enhances the SNR of the FMEPR spectrometer by two.

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