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Increasing sensitivity of pulse EPR experiments using echo train detection schemes

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ABSTRACT

Modern pulse EPR experiments are routinely used to study the structural features of paramagnetic centers. They are usually performed at low temperatures, where relaxation times are long and polarization is high, to achieve a sufficient Signal/Noise Ratio (SNR). However, when working with samples whose amount and/or concentration are limited, sensitivity becomes an issue and therefore measurements may require a significant accumulation time, up to 12 h or more. As the detection scheme of practically all pulse EPR sequences is based on the integration of a spin echo – either primary, stimulated or refocused – a considerable increase in SNR can be obtained by replacing the single echo detection scheme by a train of echoes. All these echoes, generated by Carr–Purcell type sequences, are integrated and summed together to improve the SNR. This scheme is commonly used in NMR and here we demonstrate its applicability to a number of frequently used pulse EPR experiments: Echo-Detected EPR, Davies and Mims ENDOR (Electron-Nuclear Double Resonance), DEER (Electron-Electron Double Resonance) and EDNMR (Electron–Electron Double Resonance (ELDOR)-Detected NMR), which were combined with a Carr–Purcell–Meiboom–Gill (CPMG) type detection scheme at W-band. By collecting the transient signal and integrating a number of refocused echoes, this detection scheme yielded a 1.6–5 folds SNR improvement, depending on the paramagnetic center and the pulse sequence applied. This improvement is achieved while keeping the experimental time constant and it does not introduce signal distortion.

1. Introduction

Pulse EPR experiments are nowadays routinely applied in a great variety of studies ranging from solid state physics to structural biology. In particular, we note pulse Electron–Nuclear Double Resonance (ENDOR) applications to metalloenzymes and the use of Double Electron–Electron Resonance (DEER) to determine distances between selected sites in bio-macromolecules (proteins, DNA). Most of the modern pulse EPR sequences are based on echo detection schemes and experiments are usually performed at low temperatures, where relaxation times are sufficiently long and electron polarization is high. When the samples of interest are limited in amount and concentration, such as biological samples, sensitivity becomes a major issue and common EPR sequences applied to such samples may require, at times, up to 12 h or more of accumulation time to reach sufficient SNR. This in turn translates into low efficiency use of spectrometer time, high cost due to liquid helium consumption, and requirement for very high spectrometer stability. Therefore methods for enhancing SNR are of critical importance. Recent efforts in this direction are the introduction of parallel acquisition schemes [1], the application of chirp [2,3] and shaped pulses based on optimal control [4].

As often in EPR history, NMR is a source of inspiration for EPR sequences and one of the methods used in NMR to increase the SNR is the use of Carr–Purcell (CP) and Carr–Purcell–Meiboom–Gill (CPMG) echo trains to improve sensitivity [5–9]. In its original form these sequences aimed at minimizing the effect of translational diffusion on the Hahn echo decay used to determine the spin–spin relaxation times, T_2 [10]. CPMG, which compensates for pulse imperfection as well, consists of a train of π pulses, applied after a Hahn echo forming sequence according to $\pi/2_x - (\tau - \pi_y - \tau - \text{echo})_k$ [11,12]. As the Hahn echo sequence, the CPMG sequence cannot refocus the time dependent interactions that are responsible for the decay; nevertheless it limits their effects by reducing the time upon which they evolve, hence the echo decay is slowed down. Another sequences that performs similar to CPMG for non-ideal pulses is CPMG-2, $\pi/2_x - (\tau - \pi_x - \tau - \text{echo} - \tau - \pi_{-x} - \tau - \text{echo})_N$ [13].

In solids, the CPMG echo decay is considerably longer than the Hahn-echo decay because the effects of time dependent processes, such as spectral diffusion are minimized when reducing the time τ [14]. It is therefore possible to collect several echoes formed by the π pulse train to increase the SNR of the experiment.

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CP and CPMG have been previously used in EPR as tools to study spin dynamics [14–16] and have been recently applied in spin-based quantum computing experiments for extending decoherence times [17–19] and served as a basis for more advanced refocusing techniques [19–21]. To the best of our knowledge, in 1983 Eliav and Freed were the first to propose the use of this sequence to increase sensitivity in echo detected EPR experiments [22]. Echo trains were applied in two cases to increase sensitivity in EPR. Pannier et al. [23] in the first version of the four pulse DEER sequence used a series of $\pi/2$ pulses to store the magnetization in the z direction to later regenerate the echo; they showed that the refocused echoes present undistorted DEER signal, but they did not demonstrate SNR gain. Later, Twig et al. used CPMG in combination with surface loop-gap micro-resonator to increase SNR in measurements of EPR spectra [24].

In this manuscript we demonstrate that significant SNR improvement can be obtained in a variety of pulse EPR experiments, such as Echo Detected EPR (EDEPR), Davies and Mims ENDOR, ELDOR (Electron-Electron Double Resonance)-Detected NMR (EDNMR) and DEER, by using echo trains as a detection scheme.

2. Materials and methods

All the experiments were performed on a home-built W-band (94.9 GHz) spectrometer with two sets of configurations. The first, similar to that reported earlier [25], features a 1 W amplifier and it is a whole waveguide system. The second configuration is an upgraded version offering higher power (2 W amplifier) and higher sensitivity as the part from the circulator to the cavity is now quasi-optical combined with a corrugated waveguide. This is described in the Supporting information (SI). The implementation of the echo train detection scheme can be easily performed using the SpecMan4EPR software [26] that controls the spectrometer and offers a simple pulse programming language. Measurements performed on a X-band Bruker E580 spectrometer are described in the SI.

We tested the CPMG-2 detection scheme on three different samples: a rigid nitroxide biradical, a TEMPOL solution and a Mn^{2+}:ATP complex in solution. The biradical consists of two TEMPO moieties with a separation of 3.6 nm dissolved in a ortho-terphenyl matrix [27,28]; this sample was used to demonstrate EDEPR, Davies and Mims ^{1}H ENDOR, and DEER experiments. The experiments on the biradical were obtained with spectrometer configuration #1 [25] at 50 K.

The Mn^{2+}:ATP (0.1 mM:1 mM) solution in D_2O/Glycerol (1/1 by volume) was used to test the efficiency of the CPMG-2 detection in ^{31}P Davies ENDOR and ^{2}D Mims ENDOR at 10 K. Finally, application of the CPMG-2 detection to EDNMR was demonstrated on 1 mM TEMPOL in isopropanol at 50 K. The experiments on these two latter samples were carried out using spectrometer configuration #2.

In all the experiments the full transient signal was saved and post processed by a home-written Matlab (The MathWorks, Inc.) program that is available upon request. As each transient signal consists of many echoes, the program allows choosing the number of echoes and the width of the integration window for an individual echo in the construction of the desired spectrum or time domain trace, as well as for evaluating the SNR. The echo integration is realized with a "box-car-like" summation; the width of the integration window was optimized on the first echo so as to have the best SNR. This was then set the same for all the following echoes.

The echo decay was measured for all the samples both by a Hahn echo sequence ($\pi/2 - \tau - \pi - \tau -$ echo) upon increasing the inter-pulse delay τ and by a CPMG-2 sequence with a fixed inter-pulse delay 2τ in the echo train, as noted in Fig. 1a. For both sequences a two-step phase cycling $[+x, -x]$ was applied on the first $\pi/2$ pulse, and the receiver phase was consequently set to $[+, -]$. For the biradical sample the $\pi/2$ and π pulse durations were $t_{\pi/2} = 22.5$ ns, $t_\pi = 45$ ns and the fixed inter-pulse delay was set to $\tau = 800$ ns; the magnetic field was set close to the maximum of the EPR spectrum ($B_0 = 3380$ mT). For Mn^{2+}:ATP $t_{\pi/2} = 100$ ns, $t_\pi = 200$ ns, $\tau = 600$ ns and $B_0 = 3366$ mT, corresponding to the lowest field hyperfine component of the $m_s = -1/2 \rightarrow +1/2$ Mn^{2+} EPR transition. Unless otherwise stated, these field positions were used for all experiments on nitroxides and Mn^{2+} samples. Finally, for the TEMPOL solution the Hahn echo decay and the CPMG-2 decay were measured with $t_{\pi/2} = 100$ ns, $t_\pi = 200$ ns, $\tau = 500$ ns. B_0 was set to 3388 mT, corresponding to the ^{14}N $m_I = -1$ singularity of the g_z

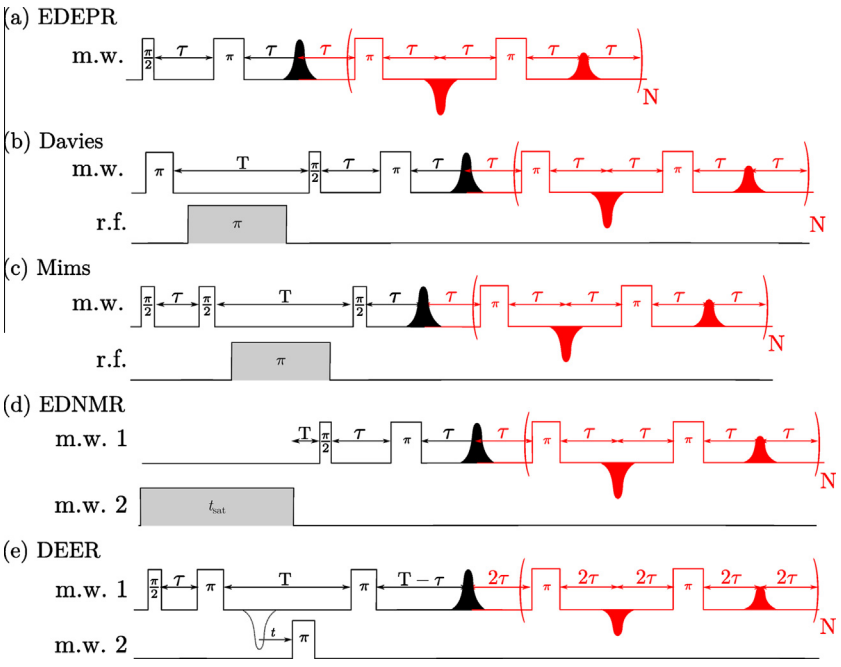

Fig. 1. Pulse sequences used in the experiments: (a) EDEPR, (b) Davies ENDOR, (c) Mims ENDOR, (d) EDNMR and (e) DEER. The echo detection scheme is highlighted in red.