

Reviewer 2

Thank you for your positive evaluation and constructive comments. Below we copied our evaluation in black and we present our response in red.

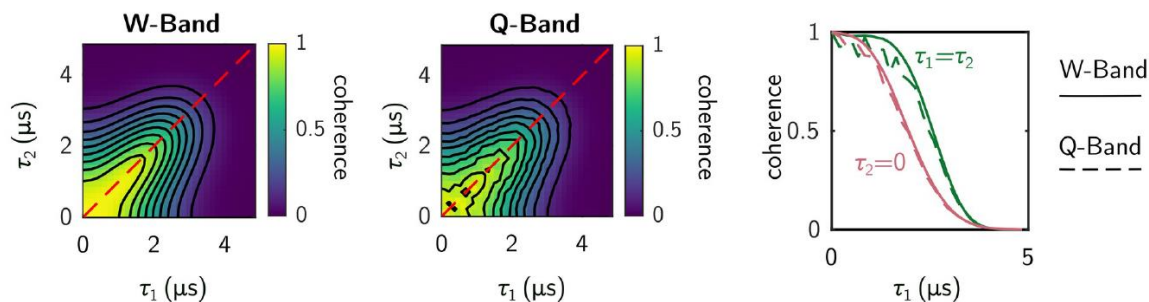
The authors describe in this manuscript the optimization of pulse settings (the original Hahn echo time) of a 4-pulse DEER experiment. Experiments are shown for nitroxide, trityl and Gd paramagnetic centers at W-band frequencies for deuterated and protonated water solvent. They demonstrate that the time interval t_1 optimally is optimally set as t_2 for short times of t_2 and somewhat shorter for long t_2 values. These experimental findings could be quantitatively reproduced by computer simulation of the interaction between the radical with 2-5 nuclear spins - similar to an earlier publications where such calculations have been demonstrated for experiments at more common Q- and X-band frequencies. Overall this work demonstrates very impressively that quantitative simulation of the experimental data can be achieved with coupled spin clusters (of at least 3).

I have some questions and remarks which might be helpful also for other readers in the final version of the paper:

1) The data are all recorded at W-band. This has of course the advantage that the classical ESEEM effects are reduced. On the other side the authors describe the decoherence of the EPR signal as a nuclear-spin-cluster electron spin decoherence arising from the interference of nuclear coherences arising from the different hyperfine coupling of two nuclear spins. This is an interesting model but would probably also imply a field dependence. It would be interesting to give a statement in this direction.

In our earlier work (Canarie et al., J.Phys.Chem.Lett., 2020), we have shown that the effect is field independent (no change between X and Q band). Also, theoretical considerations of a simple system of one electron spin and two spin-1/2 nuclei (see Lenz et al, ChemComm, 2017) shows that the effect is independent of the field and depends only on the ratio of the nucleus–nucleus coupling to the difference in the two hyperfine couplings.

Below are simulations for W-band versus Q-band. They show that the only difference is in the ESEEM modulations, and that the nuclear-spin-cluster driven dephasing is field independent:



We will add a statement that refers to the field independence of the decoherence effect and add this figure as S2 to the SI.

Also, the term $SzAzxIx$ is omitted in this calculations (different from the earlier publication for data at Q-band and X-band). It would be good to also explain this in more detail.

The $SzIx$ is in fact included in the numerical simulations in Fig. 9 that reproduce the experimental data, and also in the simulations in Fig.10, with the single exception of the top left panel (the $\langle V2 \rangle$ LCE term).

We will clarify this better in the Methods section.

The experiments are performed at the maximum of the EPR spectra. T_m for nitroxides at high field is known to be orientation dependent. Again the authors should comment on this aspect.

We carried out similar measurements at a different field position (g_{zz}) and did not see any differences. We will mention this on p. 7 and add a Figure in the SI. On line 168 we will add “We also checked this behavior by setting the magnetic field to the region of g_{zz} and saw the same behavior (see Fig. S1).”

2) The authors demonstrate that for samples with 25% 50% 75% and 100% protonation this dephasing is efficient but claim that this is not the case for fully deuterated samples. In the cited work by Soetbeer et al. it is shown that dynamic decoupling is also effective for 100% deuterated samples. It would be nice to discuss this point more carefully.

The relevant comparison is with H-NO in D_2O /glycerol. We did not find DD data on this sample in the ChemRxiv preprint by Soetbeer et al, 2021. There are data on the D-mNOPEG, where the spin labeled is attached to a polymer with a significant number of protons. Similarly, in Soetbeer et al 2018, they looked at nitroxides attached to a molecule with many protons. These will contribute to dephasing which can be refocused by DD. We discuss this in lines 180-184.

Also in the above mentioned work Tm was analysed by two different components with stretched exponential with a 50/50 ratio. It would again be important to discuss this differences to the treatment here which is relying only on one mechanism.

Although in this work we focused on the decay of the refocused echo as a function of the time intervals τ_1 and τ_2 , it is interesting to compare our Hahn echo decay shapes with those reported earlier by Jeschke and co-workers, where the data were analysed in terms of a sum of two stretched exponential, one with a fast decay and another with a slow decay. We do not observe the fast decay, which was particularly prominent in the protonated and deuterated nitroxides in D₂O/glycerol-d₈ at low temperatures (10-50 K). Similarly, this was not observed in our earlier report on trityl, nitroxide and Gd(III) spin labels, free and attached to a protein. The difference could be due to the field (Q-band vs W-band) or the different type of nitroxide used.

We will add this to the discussion (line 365). Although we do not see in the echo decays the fast relaxation, the small cusp observed for short tau2 may be an indication for a small contribution of another mechanism, but we did not investigate further because it did not affect the position of the maximum.

3) The red maxima shown in the 2D datasets should also be presented in 4 6b and 7b for consistency.

These mentioned figures refer to deuterated solvents. In these, the red maxima would be coinciding with the coordinate axis, since the effect observed in protonated solvents is absent. Therefore, we have omitted these lines.