

This paper describes dissolution DNP-NMR experiments for a variety of reaction schemes using the Bloch-McConnell equations. The topic of the paper is very timely, given the current intense interest in DNP approaches; however, in present form, the main contributions of the paper are difficult to discern.

We thank the reviewer for their supportive words and finding the topic to be of current interest. We also thank the reviewer for their valuable comments, addressed below, and hope that our additional analysis helps to clarify the paper.

1. A great deal of the paper recapitulates the work published as:

Kuchel, P. W., and Shishmarev, D.: Dissolution dynamic nuclear polarization NMR studies of enzyme kinetics: Setting up differential equations for fitting to spectral time courses, *J. Magn. Reson. Open*, 1, 100001, doi.org/10.1016/j.jmro.2020.100001, 2020.

and at least to this reviewer, the novel aspects of the present paper are not clearly distinguished from the earlier work. This could be addressed by an appropriate summary in the conclusion.

We appreciate that our current paper leads on from that previously published. This was commented on in our Abstract. We have added the reference in the abstract to clarify this point. However, our analysis includes a number of aspects not previously considered. Notably, we extend the analysis to include the influence of enzyme cofactors. We use the framework to simulate the kinetics of lactate dehydrogenase, not previously reported using this analysis, and consider the influence of enzyme concentration and the influence of unlabelled lactate on the kinetics of the hyperpolarized pool. Thus, our work is of direct relevance to ongoing clinical trials using this method.

I do not understand the statements at line 260-265:

The equilibrium constant was fixed so that $K = k_1/k_{-1} = 2$; hence the system was not at chemical equilibrium at $t = 0$ s. The simulations highlight an important point: In the absence of exchange the Bloch-McConnell equations predict the recovery of the z magnetizations back to their equilibrium values $M_{z,eq}^A$ and $M_{z,eq}^B$ while under conditions of fast exchange this no longer holds, and a nonequilibrium system will rapidly return to its chemical equilibrium, not to its thermal equilibrium, within the timescale of the NMR experiment; specifically within five T_1 values.

If the system is not at chemical equilibrium, $M_{z,eq}^A$ and $M_{z,eq}^B$ are not constants as represented in Eq. 19. Rather, these quantities become time-dependent, for example:

$$M_{z,eq}^A(t) = M_0 \frac{[A(t)]}{[A(t)] + [B(t)]}$$

$$M_{z,eq}^B(t) = M_0 \frac{[B(t)]}{[A(t)] + [B(t)]}$$

as shown in Eq. 2.4.18 of Ernst et al. (Ernst, R. R., Bodenhausen, G., and Wokaun, A.: Principles of Nuclear Magnetic Resonance in One and Two Dimensions, Clarendon Press, Oxford, 1987). Thus, it is unclear what is shown in Fig. 1 (which the text indicates is based on Eq. 19) and hence what is meant by the above statement.

This is exactly the point that we were making in Fig. 1. Under conditions of exchange the longitudinal magnetization returns to its chemical equilibrium as shown in Eq. 2.4.18 of Ernst et al. not to its initial magnetic equilibrium given by $M_{z,eq}^A$ and $M_{z,eq}^B$. We have modified this statement as follows and hope this adds clarity to the simulations performed:

“The simulations highlight an important point: In the absence of exchange the Bloch-McConnell equations predict the recovery of the z magnetizations back to their magnetic equilibrium values $M_{z,eq}^A$ and $M_{z,eq}^B$ while under conditions of fast exchange this no longer holds, and a nonequilibrium system will rapidly return to its chemical equilibrium, not to its initial thermal equilibrium $M_{z,eq}^A$ and $M_{z,eq}^B$, ...”

Section 4 of the paper expands the dimensionality of the Bloch-McConnell equations to include the molecules that are not hyperpolarized by the DNP. Thus $A^*(t)$ represents hyperpolarized species and $A(t)$ represents the unpolarized species, with $A^*(t) + A(t) = [A(t)]$ is the total concentration of A at time t (and similarly for B). The authors then numerically solve the four coupled differential equations. This is somewhat misleading in my view. Applying a similarity transformation to Eq. 39 with the matrix:

$$S = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 1 & 0 & 1 & 0 \\ 0 & 1 & 0 & 1 \end{bmatrix}$$

separates the four equations to two independent sets of equations. One set is a pair of coupled differential equations for $A^*(t)$ (Eq. 37) and $B^*(t)$ (Eq. 38), describing the evolution of the hyperpolarized magnetization and another set (Eq. 40) describing the evolution of $[A(t)]$ and $[B(t)]$. That is, the hyperpolarized magnetization just evolves according to the normal Bloch-McConnell equations and the chemical system evolves to chemical equilibrium, as expected (note that this situation would be more complicated if time-dependent steady state magnetizations were included as above, but these terms are small and neglected in this section of the paper, given the large DNP enhancements). A four-dimensional set of equations do not need to be solved. I have not checked whether any of the other more complicated schemes presented in the rest of the paper can be similarly decomposed, but it is essential that the authors clarify this issue.

We agree with the Referee that this separation of equations using alternative basis vector is possible for all the situations considered in our manuscript. We have consequently performed this transformation throughout in the revised manuscript to clarify this point.

However, the separation of the equations into independent sets of equations only occurs with first order kinetics. In the first order case, we agree that a four-dimensional set of equations does not need to be solved; for example the situation of an exchange reaction $A^* \leftrightarrow B^*$ reduces to the standard Bloch McConnell equations and the evolution described by the two-dimension DEs is identical to the four-dimensional. Similarly, for the first order situation $A \leftrightarrow B \leftrightarrow C$.

For second order kinetics the transformation can still be performed but the equations are no longer independent. Considering the example of a hyperpolarized reaction $A^* + C \leftrightarrow B^* + D$ and a corresponding non-polarized pool $A + C \leftrightarrow B + D$. The pseudo first order rate constants for A and B are dependent on C and D, and vice versa the pseudo first order rate constants for C and D are dependent on $(A^* + A)$ and $(B^* + B)$. Thus the ‘cold’ pool of A and B will influence the kinetics of A^* and B^* via the involvement of C and D and cannot be separated in the same manner as for first order kinetics. The full system of differential equations must be calculated simultaneously to simulate the kinetics.

We have added a comment highlighting the problem on p25:

“However, we now encounter a problem. The pseudo rate constants for the reactions of $[C(t)]$ and $[D(t)]$ are now given by $k'_1(t) = k_1(A^*(t) + A(t))$ and $k'_{-1}(t) = k_{-1}(B^*(t) + B(t))$, respectively. The time-dependent pseudo first order rate constants are dependent on the concentrations of both ‘hot’ and ‘cold’ pools. In turn the pseudo first order rate constants for $A^*(t)$ and $B^*(t)$ are $k'_1(t) = k_1C(t)$ and $k'_{-1}(t) = k_{-1}D(t)$. Thus, the kinetics of the ‘hot’ pools $A^*(t)$ and $B^*(t)$ become dependent on the kinetics of the ‘cold’ pools $A(t)$ and $B(t)$. This is of particular relevance (as highlighted in Kuchel and Shishmarev, 2019) when extending the equations to describe enzyme kinetics...”

Minor:

Eqs. 19 and 20 seem to be missing a leading minus sign on rhs.

We thank the Referee for spotting this typo, which we have corrected.