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Reprint of: Localization of Cl-35 Nuclei in Biological Solids using Rotational-Echo Double-Resonance Experiments

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ABSTRACT

Chloride ions play important roles in many chemical and biological processes. This paper investigates the possibility of localizing ^{35}Cl nuclei using solid-state NMR. It demonstrates that distances shorter than 3.8 Å, between ^{13}C atoms and ^{35}Cl atoms in 10% uniformly labeled ^{13}C L-tyrosine·HCl and natural abundance Glycine·HCl can be measured using rotational-echo (adiabatic passage) double-resonance (RE(AP)DOR). Furthermore the effect of quadrupolar interaction on the REDOR/REAPDOR experiment is quantified. The dephasing curve is plotted in a three dimensional chart as a function of the dephasing time and of the strength of quadrupolar interaction felt by each orientation. During spinning each orientation feels a quadrupolar interaction that varies in time, and therefore at each moment in time we reorder the crystallite orientations as a function of their contribution to the dephasing curve. In this way the effect of quadrupolar interaction on the dipolar dephasing curve can be fitted with a polynomial function. The numerical investigation performed allows us to generate REDOR/REAPDOR curves which are then used to simulate the experimental data.

1. Introduction

Most NMR spectroscopy focuses on spin 1/2 nuclei due to the relative simplicity of their interactions. However, many important elements have no stable spin 1/2 isotopes. There is a great deal of interest in half-integer quadrupolar nuclei as ^{23}Na, ^{35}Cl, ^{39}K, ^{25}Mg, and ^{17}O, due to their catalytic function in the active sites of proteins. In many cases it is possible to obtain resolved spectra of quadrupolar nuclei by using Magic Angle Spinning (MAS) [1,2], Multiple Quantum MAS (MQMAS) [3], or Satellite Transition MAS (STMAS) [4]. From such spectra, one can extract the quadrupolar coupling constant (C_{qcc}) and the asymmetry parameter (η) for specific sites. The quadrupolar parameters report on the symmetry and the local environment of such nuclei. Pioneering work by Bryce et al. [5] demonstrated that solid-state NMR on ^{35}Cl at high field and fast MAS speeds can give structural information about chlorine in biological solids. The natural abundance of ^{35}Cl is 75%, high enough that isotopic labeling is not required. However, because ^{35}Cl nuclei have a I=3/2 spin, a low gyromagnetic ratio and a relatively large quadrupolar interaction, overall, the NMR sensitivity is poor.

In biological solids, more straightforward structural information can be obtained by measuring inter-nuclear distances between dipolar-coupled spins. Rotational-echo double-resonance (REDOR) is the method of choice for hetero-nuclear distance measurements between two isolated spins in MAS experiments [6]. In the REDOR experiment, the dipole coupling is reintroduced during the mixing period using a train of rotor synchronized π pulses, alternating 90° degrees in phase. The signal intensity of the I spin is recorded as a function of mixing time. The resulting dephasing curve gives a measure of the I-S dipole coupling which is in turn proportional to the distance between the two spins. In biological solids the negative influence of the ^{13}C-^{13}C *J*-coupling on the REDOR dephasing curve was addressed by Frequency Selective REDOR (FS-REDOR) [7]. In this experiment a selective Gaussian pulse is used to refocus only the heteronuclear through-space dipolar interaction.

Although REDOR can also be employed to measure distances between a spin 1/2 nucleus and a quadrupolar nucleus [8,9], in this case the dephasing curve also depends on the quadrupole coupling constant (C_{qcc}) of the quadrupolar nucleus. Even though the I=1/2 spin is usually detected to take advantage of its greater sensitivity, the quadrupolar interaction felt by the S spin nucleus complicates the REDOR experiments as the π pulses do not completely invert the S spin. The effect of π pulses on quadrupolar nuclei depends on the size of the quadrupole coupling constant,

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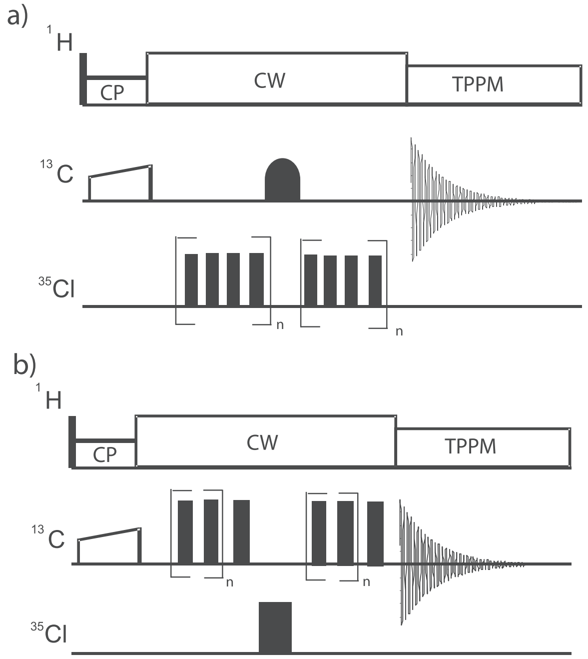

Fig. 1. a) FS-REDOR pulse sequence. Only one selective Gaussian pulse is applied on ^{13}C. b) REDOR/ REPADOR pulse sequence with the refocusing pulses applied on ^{13}C and only one inversion pulse applied to ^{35}Cl. For REDOR this inversion pulse is a strong 180° pulse whereas for REAPDOR the ^{35}Cl pulse is an adiabatic pulse whose length depends on the spinning speed (usually ½ or 2 times rotor period).

the spinning frequency, and the Radio Frequency field strength. Alternatives to the REDOR experiment are: the rotational-echo adiabatic passage double-resonance (REAPDOR) experiment introduced by Chopin et al. [10] and the Rotary Resonance Echo Saturation Pulse Double Resonance (*r*-RESPDOR) experiment introduced by Gan [11]. In this experiment, the π pulses on the S spin are replaced with a constant irradiation. In adiabatic conditions [12], under MAS the constant irradiation induces a nonselective inversion of the spin systems [12–14] and recouples the heteronuclear dipolar interaction. However in most experimental conditions a majority of spins will experience an incomplete inversion.

While the REDOR dephasing curve between two spin-½ nuclei can be fitted analytically, in the case where the S spin nucleus is quadrupolar, one must solve the Schrodinger equation for the two spins in order to extract the dipolar coupling constant. Many recent papers provide an in-depth analysis of the REDOR/REAPDOR experiment when quadrupolar nuclei are involved, just to mention Gullion and Vega [15] and Nimerovsky and Goldbourt [16], or in case the adiabatic pulse is replaced by a saturation pulse S-RESPDOR (Symmetry-based Rotational resonance saturation pulse double resonance) by Gan and Lu et al. [11,17]. For a REAPDOR experiment with a train of refocusing π pulses applied on ^{13}C, Goldbourt et al. [18] suggests an empirical formula specific to spin 5/2, while for RESPDOR experiment Amoreux and coworkers [19] propose a general formula valid for every spin. In addition, for a REDOR experiment between two quadrupolar $I > 1/2$ nuclei, Bertmer et al. [20] demonstrates that the central transition approximation, which assumes that only the central transition of the half-integer quadrupolar nuclei contributes to the dephasing, is adequate for analysis of the initial part of the dephasing curve.

Our goal is to provide further insight into the REDOR/REAPDOR experiment involving a half-integer quadrupolar nucleus, and to address the methodology for precise localization of ^{35}Cl nuclei using solid state NMR. During MAS the quadrupolar interaction felt by each crystallite orientation in the powdered sample changes in time. We perform a numerical investigation in a statistical way looking at the ^{13}C-^{35}Cl dephasing for each crystallite orientation. This allows us to generate and adjust numerical REDOR/REPADOR curves as functions of the quadrupolar interaction and the experiment performed. It is demonstrated that distances between ^{13}C and ^{35}Cl nuclei, shorter that 3.8 Å can be accurately measured in 10% ^{13}C uniformly labeled L-tyrosine · HCl and in ^{13}C natural abundance Glycine · HCl using REDOR/REAPDOR.

2. Re-coupling of dipolar interactions between S=1/2 and I=3/2 spins

Different versions of the REDOR/REAPDOR experiments are available in the literature. The pulse sequence of the REDOR experiment shown on Fig. 1a includes a Gaussian refocusing pulse on ^{13}C to compensate for the ^{13}C-^{13}C *J*-coupling effects and it is recommended for cases where the C_{qcc} of the quadrupolar nucleus is not very large. The pulse sequence shown in Fig. 1b is recommended for natural abundance ^{13}C studies where the influence of the J couplings is reduced and in cases where the C_{qcc} of the quadrupolar nucleus is large. The REDOR pulse sequence shown in Fig. 1b can be used as a REAPDOR experiment in case the quadrupolar nucleus is irradiated for half the rotor period such that it maximizes the spin inversion due to adiabatic energy level crossings.

For a pair of ½ spins (for example ^{13}C-^{15}N) the analytical solution of the REDOR dephasing curve is given [21,22] by:

$$\frac{\Delta S}{S_0} = 1 - \left(J_0\left(\sqrt{2}\,\lambda_n\right)\right)^2 + 2\sum_{k=1}^{\infty}\frac{1}{16k^2-1}\left(J_k(\sqrt{2}\,\lambda_n)\right)^2 \tag{1}$$

where $\lambda_n = nD\tau_r$; τ_r is the MAS spinning period, and D the dipolar constant $\left(D = \frac{\gamma_I\gamma_S\hbar}{2\pi r^3}\right)$; J_k are the Bessel functions $J_k(x) = \sum_{s=0}^{\infty}\frac{(-1)^k}{s\,!\,(k+s)\,!}\left(\frac{x}{2}\right)^{k+2s}$.

Eq. (1) results as a solution of the evolution of the density matrix under the Hamiltonian

$$H = H_{R.F.,I} + H_{R.F.S} + H_D \tag{2}$$

$H_{R.F.,I,S} = \omega_{R.F.,I,S} I_{x,y}$; $H_D = d(t) I_z S_z$; $d(t) = \omega_D(1 - 3\cos^2\theta_D(t))$; $\omega_D = 2\pi D$; θ_D is the angle between the internuclear vector connecting the I and S spins, and the Zeeman field.

When performing a REDOR experiment between $I=1/2$ and $S=3/2$ spins one must take into account the quadrupolar interaction felt by the $S=3/2$ spin. This complicates the Hamiltonian of Eq. (2) by the addition of the quadrupolar term

$$H_Q = \frac{1}{6}\Omega_Q(t)(3I_z - I(I+1)) \tag{3}$$

where

$$\Omega_Q(\theta(t), \varphi(t)) = \frac{1}{2}\omega_Q(3\cos^2\theta(t) - 1 + \eta\sin^2\theta(t)\cos 2\varphi(t)) \tag{4}$$

and $\omega_Q = 2\pi\frac{3C_{qcc}}{2I(2I-1)}$, θ and φ are the polar angles orienting the external magnetic field in the principal axis system of the electric field gradient tensor, while C_{qcc} and η are the quadrupolar coupling constant and quadrupolar asymmetry parameter, respectively.

For a better understanding of the effect of the quadrupolar interactions on the REDOR/REAPDOR experiment we concentrate on the pulse sequence shown on Fig. 1b and investigate by numerical simulations the behavior of the ^{13}C–^{35}Cl spin pair dephasing curve as a function of the quadrupolar interaction felt by