Communication

Enhanced resolution in proton solid-state NMR with very-fast MAS experiments

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ABSTRACT

We present a new smooth amplitude-modulated (SAM) method that allows to observe highly resolved ^{1}H spectra in solid-state NMR. The method, which works mainly at fast or ultra-fast MAS speed ($v_R > 25$ kHz) is complementary to previous methods, such as DUMBO, FSLG/PMLG or symmetry-based sequences. The method is very robust and efficient and does not present line-shape distortions or fake peaks. The main limitation of the method is that it requires a modern console with fast electronics that must be able to define the cosine line-shape in a smooth way, without any transient. However, this limitation mainly occurs at ultra-fast MAS where the rotation period is very short.

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Proton nuclear magnetic resonance (^{1}H NMR) spectroscopy is widely used to characterize the structure and dynamics of molecules in the liquid state. Due to its high NMR receptivity, the proton is potentially an attractive nucleus for probing the molecular structure of solid materials. Unfortunately, strong homo-nuclear dipolar interactions typically result in a broad featureless ^{1}H NMR peak void of chemical shift and *J*-coupling information. Thus, one of the critical remaining challenges in NMR is to develop techniques to narrow ^{1}H spectra of solids and recover chemical shift and *J*-coupling information. There are currently three distinct ways to approach high-resolution ^{1}H spectra of solids: (i) isotopic dilution with deuterium [1], (ii) fast magic angle spinning (MAS) [2] and (iii) radio-frequency (rf) multi-pulse irradiation [3]. The first method is tedious and expensive. MAS alone does not generally achieve enough resolution due to the homogeneous character of ^{1}H–^{1}H interactions. Multi-pulse spin-space narrowing is effective in averaging homo-nuclear dipolar interactions, but does not average broadening resulting from chemical shift anisotropy (CSA ≈ 10–15 ppm). An obvious remedy is to apply the last two strategies simultaneously, which leads to the combined rotation and multiple pulse spectroscopy (CRAMPS) [4]. Almost all CRAMPS sequences use a quasi-static approximation, in which low spinning frequencies ($v_R = 2$–5 kHz) ensure an approximately static sample on the cycle time (τ_c) of the rf pulse sequence [5–9]. However, due to the development of very large B_0 magnetic fields, these slow spinning speeds are nowadays unable to average the ^{1}H CSA. To overcome this problem, two CRAMPS methods: DUMBO and PMLG, have been developed recently. Both methods use a continuous irradiation of fixed amplitude (v_1), only the phase being changed during τ_c. Two different DUMBO sequences exist, in which the phase is

described by a Fourier series of either 12 (DUMBO$_1$) [10], or 6 (eDUMBO) [11] coefficients. In the PMLG sequence [12], the ramped phase of the original FSLG [3,13–15] is replaced by 3, 5, 7 or 9 discrete phase steps. Another method has also been proposed for fast spinning speeds, which uses a very short semi-windowless CRAMPS scheme (swWWH$_4$) [16]. One of the successes of these schemes is their short cycle times: $\tau_c v_1 = 3, \sqrt{8/3}$, and 1.25 for DUMBO, FSLG/PMLG and swWHH$_4$, respectively. However, these pulse sequences have been developed in the quasi-static approximation and MAS interferes with the averaging scheme if the rotor period T_R is not much larger than τ_c. Only one method has been proposed up to now, that is not based on a quasi-static approximation [17]. This method uses rotor-synchronized pulse sequences (CN_v^p and RN_v^p) that exploit selection rules generated by appropriate synchronization of the rf pulses and the sample rotation [18]. All these previous methods, which are limited to c.a. $v_R = 25$ kHz, are also submitted to several distortions: image and zero-frequency peaks, non-constant scale factor λ over the spectra, off-resonance frequency irradiation that must be optimized for optimal resolution and rotor and radio-frequency (RRF) [19] extra lines for DUMBO, FSLG/PMLG and swWWH$_4$. All these distortions, except the RRF lines, are related to pulse transients [19] and the fact the effective Hamiltonian of the sequences does not correspond to an Oz rotation [9]. Indeed, all four methods are submitted to large sudden changes in rf phase and amplitude (for swWHH$_4$). Two very detailed analysis of the pulse transient effects have been published [20,21], which have lead to a very elegant new PMLG sequence with Oz rotation, which is freed from pulse transient distortions, but that nevertheless still presents RRF lines [22].

An important challenge of modern solid-state NMR methods is to take benefit of indirect proton detection at high MAS rates and/ or magnetic fields, especially in biology. Recently, such kind of

techniques were successfully applied in the case of biopolymers and proteins, which were either ^{1}H spin diluted [23,24] or fully protonated [25,26]. For isotopically unmodified samples, proton resolution and signal enhancement were then achieved from the spinning speed alone (up to 40 kHz [26]). However, even at ultra-fast MAS speeds currently commercially available (up to v_R = 70 kHz), strong ^{1}H–^{1}H interactions are only partially cancelled leading to ^{1}H spectra with a resolution lower than that obtained with swWHH$_4$, PMLG or DUMBO at much slower speed. Very fast spinning speeds present many advantages, especially at large magnetic fields. Indeed, (1) it increases the rotor-synchronized indirect dimension of most experiments, (2) it allows an efficient proton decoupling with weak ^{1}H rf-field, (3) it increases the T'_2 constant times and (4) it allows using strong rf-fields due to the small rotor diameter used. Therefore, we have designed our sequence with the following point in mind: we focus on improving resolution at fast spinning speeds as below such speeds, efficient techniques already exist. Our design can not rely on a quasi-static approximation with such short rotor periods (14–33 µs for 30 kHz $\leqslant v_R \leqslant$ 70 kHz). To avoid any risk of interference between MAS decoupling and rf effect, only rotor-synchronized sequences are envisaged here. It has been shown that CN_v^p sequences described in Ref. [17] with the basic C element corresponding to a 2π rotation, lead to a null scale factor ($\lambda = 0$), and thus can not be used for our purpose [27]. We were thus left with the rotor-synchronized RN_v^p sequences of this article [17]. Unfortunately, these sequences correspond to an Ox rotation, hence presenting all previous distortions but the RRF lines. We have therefore developed a new class of symmetry-adapted sequences (ZN_v^p), which correspond to an Oz rotation [28]. These sequences are not based on 2π or π rotation elements, like the usual CN_v^p and RN_v^p sequences, but they use an amplitude-modulated basic element that is made of two identical subsequent pulses with opposite phases ($\pm x$) [28]. Among these new possible sequences, we have selected those that decouple the homo-nuclear dipolar interaction, but recouple the chemical shift. However, these sequences are composed of numerous pulses every rotor period, which may be difficult to realize at ultra-fast MAS. Experimentally, this would introduce a large proportion of pulse transients, which would lead to very large spectral distortions.

In order to avoid these phase transients, we have 'smoothed' the ZN_v^p pulses, and our recoupling sequence thus consists in a cosine amplitude modulation, rotor-synchronized with n rf periods fitting in one rotor period T_R. This approach has already been successful in the case of hetero-nuclear dipolar re-introduction under MAS using SFAM$_{1,2}$ [29,30]. We name this sequence SAMn after smooth amplitude modulation.

SAM$_1$ and SAM$_2$ re-introduce CSA, homo- and hetero-nuclear dipolar interactions and thus cannot be used to get ^{1}H improved resolution, while sequences with $n > 2$ decouple CSA and homo- and hetero-nuclear dipolar interactions. One can thus expect improved resolution with SAMn methods, if n is larger than 2. In this communication we present preliminary results obtained with integer n values.

Simulations and experiments have shown an interesting behavior: one observes an increasing decoupling effect as the peak value of applied rf field (v_{1peak}) increases. Unfortunately, the chemical shift is also scaled down by a factor λ that depends on v_{1peak}. One then observes a competition between the two processes that leads to an optimum rf value (v_{1peak}^{opt}) maximizing the resolution.

Spectra presented below have been obtained by ^{1}H–X (X = ^{13}C or ^{31}P) CP-MAS HETCOR (Fig. 1) with standard Bruker AVANCE II console, preamplifiers and commercial 1.3 and 2.5 mm probes at 14 and 9.4 T, respectively. First, we have acquired at 9.4 T ^{1}H–^{31}P spectra of NaH$_2$PO$_4$, which contains three ^{1}H and two ^{31}P different species, using SAMn decoupling sequences with various n during t_1. Only those corresponding to small integer n values (n = 3 and
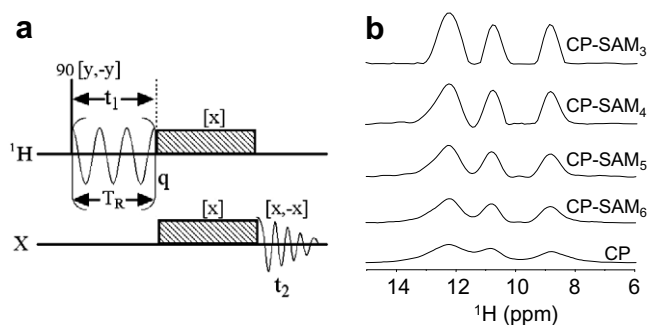

Fig. 1. (a) Pulse scheme of ^{1}H–X CP-MAS HETCOR with SAM$_3$ decoupling during t_1. (b) ^{1}H projection of the ^{1}H–^{31}P CP-MAS 2D spectrum of NaH$_2$PO$_4$. B_0 = 9.4 T, v_R = 30 kHz, v_{1peak} = 85 kHz. Phase cycling, shown in brackets, follows the normal CP with States method for 2D hyper-complex acquisition, while the SAM$_3$ shape-pulse keeps a constant phase throughout the experiments.

4) gave the best results, except those with n = 1 and 2 that re-introduce the CSA, homo- and hetero-nuclear dipolar interactions [28]. This was confirmed with simulations. The best resolution was always observed for the largest rf-field (v_{1peak} = 85 kHz) available on our old 2.5 mm probe, and with the SAM$_3$ sequence which provided peaks resolved to the baseline (Fig. 1). With this rf-field, the experimental scale factor was λ_{exp} = 0.72, 0.83, 0.90 and 0.94, for n = 3, 4, 5, 6, respectively. The better decoupling efficiency of SAM$_3$, with respect to other SAMn sequences (n integer), was confirmed by recording ^{1}H–^{13}C spectra of histidine·HCl·H$_2$O, at B_0 = 9.4 T, v_R = 30 kHz and v_{1peak} = 85 kHz (Fig. 2a and b). We also did experiments on a 1.3 mm probe at ultra-fast MAS recording ^{1}H–^{13}C spectra of histidine·HCl·H$_2$O at B_0 = 14 T, v_R = 65 kHz and v_{1peak} = 210 kHz. We observed a ^{1}H resolution enhancement mainly on the CH$_2$ resonance submitted to the largest ^{1}H–^{1}H broadening (Fig. 2c and d). Other ^{1}H resonances such as the peak at 17 ppm observed on ^{13}C COOH slice (173 ppm), for which homo-nuclear dipolar interaction was already fully removed by MAS alone, only slightly suffered from SAM irradiation as they exhibited 70% of the intensity of regular CP-HETCOR. Moreover, it must be noted that experiments at v_R = 65 kHz were affected by phase transients
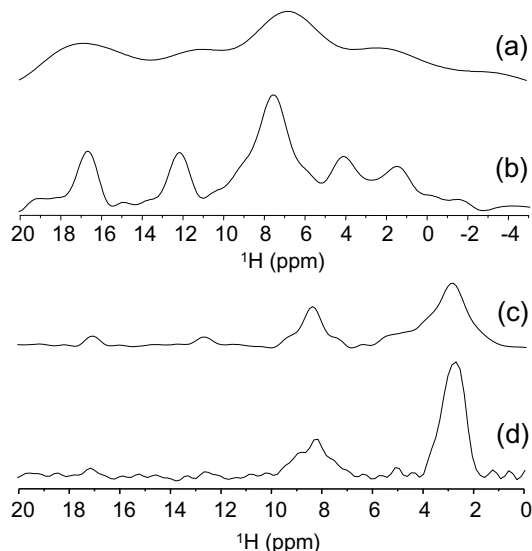

Fig. 2. Slices of ^{1}H–^{13}C CP-MAS spectra of histidine·HCl·H$_2$O. (a and b) B_0 = 9.4 T, v_R = 30 kHz, v_{1peak} = 85 kHz, δ_{13C} = 173 ppm (COOH site). (c and d) B_0 = 14 T, v_R = 65 kHz, v_{1peak} = 210 kHz, δ_{13C} = 57 ppm (CH$_2$ site). Spectra (b and d) and (a and c) are acquired, respectively, with and without SAM$_3$ decoupling during t_1.

as 350 ns steps were used to define the cosine line-shape, meaning less than four discrete steps to alter rf between 0 and v_{1peak}.

It is important to mention that observed SAMn spectra could be more resolved with larger rf-fields. Actually, the optimum rf-field for SAMn methods is equal to $v_{1peak}^{opt} = 1.15nv_R$ and the optimum scaling factor is then $\lambda_{opt} = 0.70$ [28]. As v_{1peak}^{opt} is proportional to n, with fast or ultra-fast MAS ($v_R > 25$ kHz), this leads to rf-field strengths hardly accessible for larger n during the long decoupling time (several ms), especially with double or triple resonance probes. For this reason SAM$_3$ is the best SAMn decoupling sequence with integer n.

It is possible that the decoupling efficiency of SAM$_3$ will not surpass that of well adjusted swWHH$_4$, DUMBO, FSLG, PMLG or RN$_v^p$ experiments. Actually, SAM$_3$ should be considered only at fast or ultra-fast MAS ($v_R > 25$ kHz) where other previous methods do not work. It is remarkable that SAMn optimization is straightforward. This is related to the fact these sequences are: (i) rotor-synchronized, (ii) that they correspond to an Oz rotation and (iii) that they do not introduce any pulse transients (if rf-steps are small enough). As examples of SAM$_3$ robustness, let us mention: (1) the spinning speed does not have to be perfectly stabilized; (2) the demand with respect to spectrometer hardware and tuning is acceptable; (3) the rf-inhomogeneity does not prevent at least a 2-fold resolution improvement and thus full rotor samples can be used, which is important in case of few sensitive experiments; (4) the rf-field strength is easy to optimize; (5) the scale factor is constant over the proton spectra; (6) there are no image or zero-frequency peaks, which allows phase-sensitive methods such as States; (7) the sequence is rotor-synchronized, which prevents RRF lines and finally (8) SAMn can be used with conventional double or triple resonance probes, as the dissipated rf power is only two thirds ($1.15^2/\sqrt{2}$) that with equivalent RN$_v^p$ or ZN$_v^p$ sequences (for identical resolution). The main limitation of the method is that it requires a modern console with fast electronics that must be able to define the cosine line-shape without transients for spinning speeds faster than 30 kHz.

This scheme may also be used to increase ^{1}H coherence lifetimes in correlation spectra [11]. Other rotor-synchronized smooth line-shapes and other non-integer n values may be envisaged; their analysis is underway. The price one must pay for the detection of high-resolution proton spectra with SAM$_3$ is the increased measuring time required because of the use of indirect detection. For some experiments, however, such as indirect detection of insensitive nuclei (^{1}H–X–^{1}H HMQC/HSQC), direct detection of highly resolved ^{1}H spectra is much desirable. Preliminary experiments showed that this is feasible, and this will be described elsewhere [31].

Acknowledgments

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