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The evaluation of different MAS techniques at low spinning rates in aqueous samples and in the presence of magnetic susceptibility gradients

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Abstract

It was recently demonstrated that the nuclear magnetic resonance (NMR) linewidths for stationary biological samples are dictated mainly by magnetic susceptibility gradients, and that phase-altered spinning sideband (PASS) and phase-corrected magic angle turning (PHORMAT) solid-state NMR techniques employing slow and ultra-slow magic angle spinning (MAS) frequencies can be used to overcome the static susceptibility broadening to yield high-resolution, spinning sideband (SSB)-free ^{1}H NMR spectra [Magn. Reson. Med. 46 (2001) 213; 47 (2002) 829]. An additional concern is that molecular diffusion in the presence of the susceptibility gradients may limit the minimum useful MAS frequency by broadening the lines and reducing SSB suppression at low spinning frequencies. In this article the performance of PASS, PHORMAT, total sideband suppression (TOSS), and standard MAS techniques were evaluated as a function of spinning frequency. To this end, 300 MHz (7.05 T) ^{1}H NMR spectra were acquired via PASS, TOSS, PHORMAT, and standard MAS NMR techniques for a 230-μm-diameter spherical glass bead pack saturated with water. The resulting strong magnetic susceptibility gradients result in a static linewidth of about 3.7 kHz that is larger than observed for a natural biological sample, constituting a worst-case scenario for examination of susceptibility broadening effects. *Results*: (I) TOSS produces a distorted centerband and fails in suppressing the SSBs at a spinning rate below ~1 kHz. (II) Standard MAS requires spinning speeds above a few hundred Hz to separate the centerband from the SSBs. (III) PASS produces nearly SSB-free spectra at spinning speeds as low as 30 Hz, and is only limited by T_2-induced signal losses. (IV) With PHORMAT, a SSB-free isotropic projection is obtained at any spinning rate, even at an ultra-slow spinning rate as slow as 1 Hz. (V) It is found empirically that the width of the isotropic peak is proportional to F^{-x}, where F is the spinning frequency, and $x = 2$ for MAS, 0.84 for PASS, and 0.5 for PHORMAT.

Keywords: Magic angle spinning; Magic angle turning; Magnetic susceptibility; 2D-PASS; PHORMAT; TOSS

1. Introduction

A long-standing problem with ^{1}H NMR spectroscopy of intact biological samples such as cells, tissues, and organs is the limited attainable spectral resolution. Spectral resolution is limited mainly by local magnetic field gradients arising from spatial variations in the bulk magnetic susceptibility in cells and tissues, which broaden the NMR lines [1–4]. In principle this broadening can be eliminated by the technique of magic angle spinning (MAS), in which the sample is rotated about an axis with an angle of 54°44′ relative to the external magnetic field direction [5–7]. In a standard MAS experiment, where a single $\pi/2$ pulse is used to observe the signal from the spinning sample (to be called single-pulse MAS or SP-MAS hereafter), the spinning frequency must be larger than the broadening interaction in order to avoid the occurrence of spectral spinning sidebands (SSBs). High-resolution ^{1}H SP-MAS spectroscopy at a spinning frequency, F, varying from several kHz to more than 10 kHz has been used to study metabolites in cells and excised tissues [4,8–13]. However, the large centrifugal forces, F_c, associated with

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these spinning frequencies destroy the tissue structure and even individual cells [4]. F_c is given by $F_c = m\omega^2 r$, where m is the mass, $\omega = 2\pi F$, and r the distance from the rotation axis to the point of interest. For example, a sample configuration with $F = 2\,\text{kHz}$ and $r = 1\,\text{cm}$ yields a maximum estimated F_c of 1.6×10^5 times larger than the gravitational force ($1.6 \times 10^5\,\text{G}$). Therefore the SP-MAS method is not viable for localized spectroscopy or chemical shift imaging of large intact biological samples or in vivo studies. Hence, NMR methods are needed that yield high-resolution, SSB-free spectra at reduced spinning speeds.

The effects of long-term centrifugation upon animals have been previously investigated. For example, beagle dogs that were centrifuged continuously for 3 months at increasing speeds could tolerate 2.5 G for prolonged times without serious impairment of their body structure and function [14]. In another investigation, chronic 3 G centrifugation of rats caused changes in their body mass [15]. If we assume a maximum tolerable centrifugal force of 2 G, then a biological sample in a 2 mm ID cylinder can be spun at maximal 22 Hz, and a mouse contained in a 2 cm ID cylinder can be rotated at maximal 7 Hz. However, in our case centrifugal force gradients exist in the animals rather than the more or less constant centrifugal forces. Also, for our investigation the time the animals have to be exposed to rotation is considerably less, typically up to an hour, rather than months. Moreover, for live animals the spinning frequency may need to be further limited to avoid the possible detrimental effects of eddy current, i.e., the nerve and cardiac stimulations that can be induced in a sample rotating in a magnetic field [16]. Therefore an investigation has been initiated in our laboratory to investigate the tolerance of intact excised organs and of live mice for exposure to MAS. Based upon our preliminary results, MAS frequencies of at least 4 Hz for at least 40 min in a 2 T magnetic field appear to be safe for mice, and for excised tissues and organs (in a 5 mm-ID rotor) MAS frequencies of 30–50 Hz in a 12 T field have been used without causing any apparent structural damage. A comprehensive report on these studies will be published separately.

Recently we reported first results on excised tissues and organs, using modified slow-MAS methodologies originally developed for solid-state NMR. Ref. [1] shows results for excised organs obtained with the 2D-phase-altered spinning sidebands (PASS) [17] technique. Nearly-SSB-free isotropic ^{1}H NMR metabolite spectra were obtained at spinning frequencies as low as 43 Hz and with a spectral resolution comparable to or better than the resolution obtained with fast SP-MAS. However, a disadvantage of PASS is that serious signal attenuation occurs when the spinning frequency approaches a value of $(T_2')^{-1}$ or less, where T_2' is an apparent spin–spin relaxation time, depending on both the intrinsic T_2 and a decay time determined by the molecular diffusion rate and the susceptibility gradients. This issue will be discussed in more detail in Section 3. In biological samples the T_2' values are typically of the order of tens of ms, so the minimum practical spinning frequency with 2D-PASS is 20–30 Hz. In a second recent paper [2] we reported results obtained with a different slow-MAS technique, namely 2D-phase-corrected magic angle turning (PHORMAT) [18]. In this experiment the spinning frequency must be large compared with $(T_1)^{-1}$ rather than $(T_2)^{-1}$ [2]. As $(T_1)^{-1}$ is usually an order of magnitude smaller than $(T_2)^{-1}$ in biological tissues, considerably lower MAS frequencies can be used with PHORMAT than with PASS. Indeed it was demonstrated in [2] that PHORMAT, applied at a spinning speed of 1 Hz, produces spectra of excised rat liver with a resolution approaching that obtained from PASS or fast MAS methods. However, it will be discussed in Section 3 that PHORMAT induces other signal losses, which are inherent to the method itself. So PHORMAT may not always be the method of choice.

The results obtained from PASS [1] and PHORMAT [2] are better than originally expected. It was anticipated that these techniques could not be employed in biological materials at low spinning speeds because the Brownian motion, which causes metabolites to diffuse in the susceptibility gradients, would make it impossible to suppress the spinning sidebands, and would broaden the centerband lines. Leu et al. [19,20] studied the effects of the diffusion of water molecules, which are subjected to strong susceptibility gradients, with SP-MAS and total sideband suppression (TOSS), a 1D technique to eliminate spinning sidebands [21], at lower spinning speeds. For these experiments, samples containing water and glass beads with a diameter of 50 μm were measured. In this sample magnetic gradients arise as a result of the different magnetic susceptibilities of glass and water in the presence of a 11.7 T external magnetic field. With SP-MAS they reported that even at a spinning frequency of 2 kHz the line broadening imposed by the diffusion is still 50 Hz, and that this broadening is inversely proportional to the square of the spinning rate [19]. For the TOSS experiments, the authors mentioned that it was not possible to suppress the spinning sidebands adequately for a MAS frequency of 1 kHz or less, due to amplitude modulation of the magnetization arising from water translational diffusion [20].

In this paper, the performance evaluation for MAS techniques at lower spinning frequencies is extended to PASS and PHORMAT. To this end, NMR spectra obtained with SP-MAS, TOSS, PASS, and PHORMAT were measured as a function of the spinning frequency in a model system of water surrounding densely packed spherical glass beads with diameters of $230 \pm 30\,\mu\text{m}$. It is worth noting that the magnetic susceptibility gradients induced by the beads are much larger than the gradients

occurring in cells and tissues, and that the diffusion rate of free water is considerably larger than the diffusion rates of cellular water and metabolites [22]. Therefore, this sample represents a worst-case scenario for examining the effects of diffusion on the performance of the (slow) MAS methodologies.

2. Experimental

Glass beads with diameters of $230 \pm 30\,\mu m$ were purchased from Polysciences (Warrington, PA, USA) and were used as received. The beads were first loaded into the cylindrical rotor (5 mm ID) then saturated with tap water. The rotor was shaken to make sure that all the beads were completely saturated and that a uniform bead pack was obtained. Hence, the water molecules were free to diffuse in the magnetic susceptibility gradients of the open bead pack. This sample is similar to that used in [19] and [20], the main difference is the diameter of the beads, which was 50 μm in their case. The magnetic susceptibility of the beads used in this work was not provided by the supplier. However, based on the linewidth measured for the stationary sample and the changes in the linewidth as a function of the spinning rate in the PHORMAT experiment (see below), we estimate a magnetic susceptibility gradient of approximately 5 T/m in our sample.

All ^{1}H NMR experiments were performed on a Chemagnetics 300 MHz Infinity spectrometer, with a proton Larmor frequency of 299.982 MHz. A standard Chemagnetics CP/MAS probe with a 7.5-mm OD pencil-type spinner system was used. To spin at low frequencies, the rotor was equipped with a flat drive tip (the drive tip did not contain grooves, which are normally used to drive the rotor) and an airflow restriction was used in the driver channel. The spinning rate was controlled using a commercial Chemagnetics MAS speed controller in the automated-control mode. To this end the rotor was labeled with three evenly spaced marks. It was found that the frequency stability was better than ±0.3 Hz for spinning frequencies of 1–200 Hz, which is sufficient for our experiments. Spinning rates higher than 200 Hz were attained by removing the airflow restriction and replacing the flat drive tip with a standard tip. All experiments were performed at ambi-

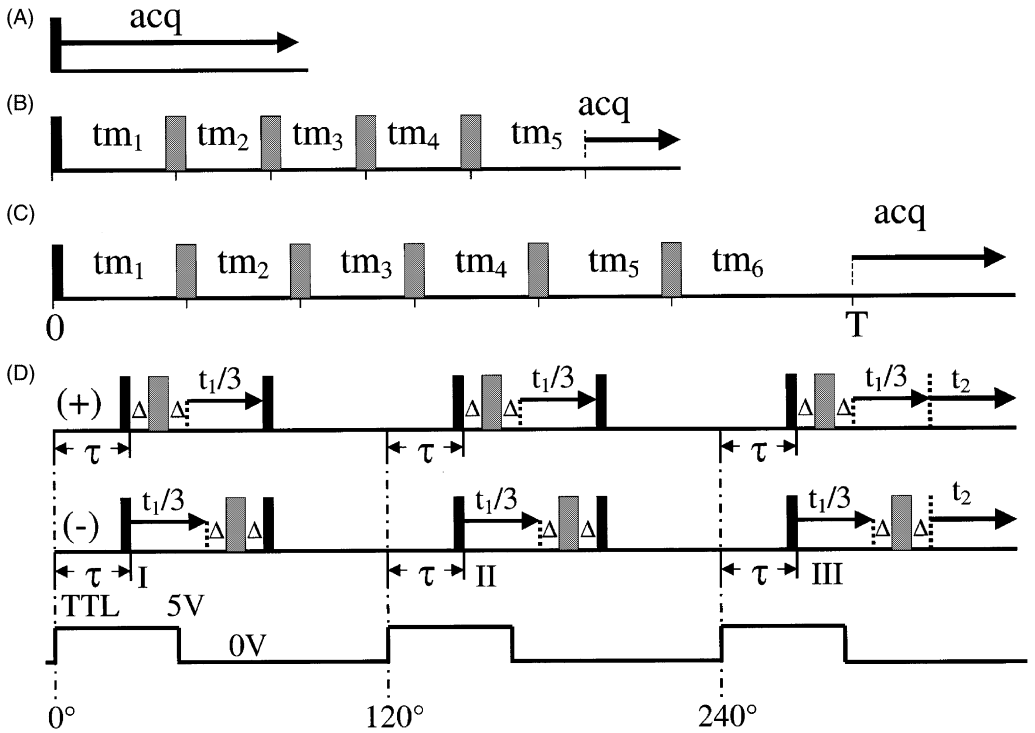

Fig. 1. The various pulse sequences used in this study. The 90° pulses are black while the 180° pulses are gray. The pulse widths were 4.5 and 9.0 μs for the 90° and 180° pulses, respectively. (A) Single pulse sequence used for the ^{1}H SP-MAS experiments. (B) TOSS, where the times between the middle of the pulses measured in unit of rotor period are tm1 = 0.57606, tm2 = 0.2237, tm3 = 0.07744, tm4 = 0.65577, and tm5 = 0.22594, respectively [24]. (C) ^{1}H PASS, where the times tm1–tm6 in unit of rotor period can be found in [17] and the phase cycling of pulses is described in [1,25]. (D) ^{1}H PHORMAT, where the time variables t_1 and t_2 correspond to the evolution and acquisition dimension, respectively. The 90° pulses labeled (I), (II), and (III) are synchronized to 1/3 of the rotor cycle by the TTL pulses generated by marking the rotor 120° apart and an optical detector (the bottom trace). The (+) and (−) pulse sequences are used to produce a pure absorption mode 2D isotropic–anisotropic spectrum and to improve the base plane of the 2D spectrum. The phase cycling of the pulses is detailed in [2,18].

ent temperature, i.e., 25 °C, where the diffusion constant of free water is about $D_0 = 2 \times 10^{-5}\,\mathrm{cm}^2/\mathrm{s}$ [19,23].

Fig. 1 shows the pulse sequences used in the SP-MAS, TOSS, PASS, and PHORMAT experiments. The TOSS sequence is described in [24], for ^{1}H 2D-PASS and PHORMAT similar pulse sequences were used as previously reported [1,2]; the only difference is that in the present experiments no water suppression was applied. Experimental details of the four sequences are given in the figure caption.

3. Results and discussions

Fig. 2 shows the ^{1}H MAS spectra at slow spinning rates obtained by the various methods on the H_2O-in-glass-beads sample. The results for each method are discussed separately.

3.1. SP-MAS

The linewidth of the SP-MAS spectrum acquired at 1 Hz MAS frequency is 3745 Hz (full width at half maximum intensity), the same as that obtained on a stationary sample. Three Gaussian functions with essentially the same isotropic shift values are required to produce a perfect fit of the line shape. The linewidths and the area percentages of these components, determined using the deconvolution program provided by Chemagnetics, are 9604 Hz (16%), 4455 Hz (47%), and 2328 Hz (37%), respectively. The SP-MAS spectrum acquired at a spinning rate of 50 Hz resembles that at 1 Hz with a comb of barely visible SSBs superimposed on the spectral envelope. The SP-MAS spectra at 250 Hz and 1 kHz show well-resolved SSBs. The linewidths of the centerband for the 250 Hz and 1 kHz spectra are 40 and 23 Hz, respectively. This latter value was also ob-

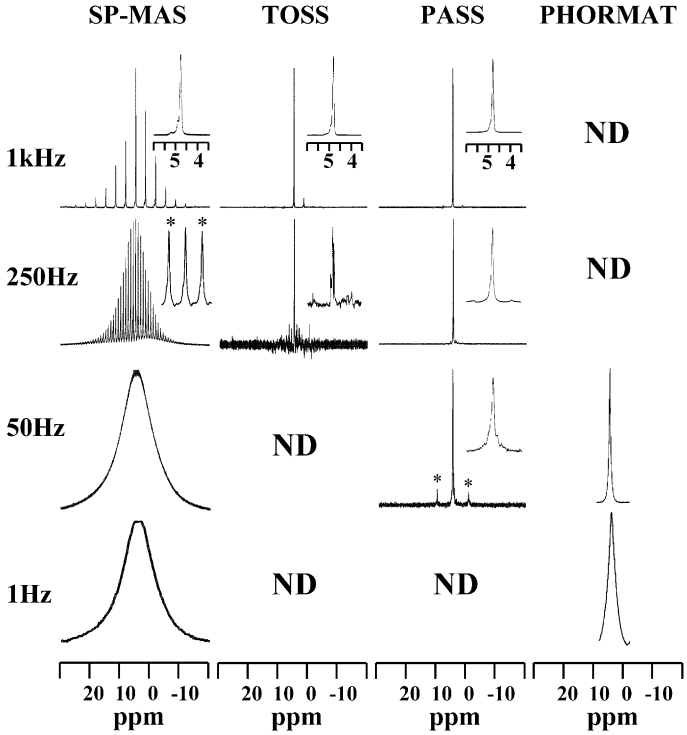

Fig. 2. ^{1}H MAS spectra obtained at slow spinning rates by the various methods on a mixture of H_2O and spherical glass beads with diameters of $230 \pm 20\,\mu$m. SP-MAS: Single pulse experiments at different spinning rates. The number of accumulations is 4000, 1000, 64, and 64 for 1, 50, 250 Hz and 1 kHz, respectively. The recycle delay time is 1 s. The peaks denoted by '*' in the 250 Hz expansion are spinning sidebands. TOSS: Sixty-four scans with a recycle delay time of 1 s were used for each measurement. PASS: The centerband of 2D-PASS spectra at selected spinning rates is shown. The parameters for each PASS experiment are: Sixteen evolution steps with an accumulation number of 16 for each step and a recycle delay time of 1 s were used at the spinning rate of 1 kHz; 32 evolution steps with an accumulation number of 16 for each step and a recycle delay time of 1 s were used at the spinning rate of 250 Hz; 32 evolution steps with an accumulation number of 1632 for each step and a recycle delay time of 1 s were used at the spinning rate of 50 Hz. PHORMAT: The projection spectra along the isotropic dimension of the PHORMAT experiment at selected spinning rates are shown. The experimental parameters for the 50 Hz PHORMAT experiments are as follows: The echo time (Δ) and the recycle delay times were 50 μs and 1 s, respectively. The free-induction decays in the acquisition dimension (t_2) contained 200 complex points and were transformed to spectra with a spectral width of 40 kHz. The 2D data were collected using 60 t_1 steps, incremented by 300 μs, corresponding to an evolution spectral width of 1.333 kHz. 2D data sets were acquired with the (+) and the (−) PHORMAT pulse sequences using a total of 64 scans at each t_1 value. The experimental parameters for the 1 Hz PHORMAT experiment are the same as the 50 Hz PHORMAT except that here a total of 37 t_1 increments were acquired.

served at higher spinning frequencies, indicating that at these frequencies the linewidth is determined only by factors other than molecular diffusion, such as the intrinsic T_2, possible paramagnetic impurities from the glass beads and the residual magnetic field gradients along the MAS spinning axis that are not averaged by MAS. Following [19], we attribute the increasing linewidth with decreasing MAS frequency to the effect of molecular diffusion in the local susceptibility field gradients created by the glass beads.

3.2. TOSS

The TOSS spectra also resemble those reported previously on a sample of water and glass beads with diameters of 50 μm [20]. At a spinning speed of 1 kHz the isotropic peak has a similar width to the centerband obtained by SP-MAS at the same spinning rate. However, the TOSS spectrum at 250 Hz becomes severely distorted. Large residual spinning sidebands are observed, the isotropic peak splits into multiple components (see the expanded insert), and the signal intensity is dramatically reduced; the spectral intensity of TOSS at 250 Hz is only 1.3% that of the TOSS spectrum at 1 kHz. This result indicates that, for the water-in-glass-beads system used in this study, TOSS is restricted to spinning rates of 1 kHz or higher. The failure of TOSS in suppressing the SSBs arising from the magnetic susceptibility broadening in the presence of molecular diffusion has been explained in detail by Liu et al. [20]. They showed that diffusion introduces a periodic modulation and attenuation of the NMR signal amplitude. This periodic modulation introduces extra sidebands that cannot be suppressed by the TOSS sequence. It is also well known that in solid samples where the chemical shift anisotropy (CSA) is the main broadening mechanism, TOSS works poorly when the ratio of the spinning rate versus the width of the anisotropy is low [24]. At low spinning rates the centerbands of the sites with large CSAs have a low intensity and sometimes are inverted in sign [17,24]. As discussed above, in our sample the static spectrum consists of three anisotropically broadened Gaussian lines, with line widths varying by more than a factor of 4. At a spinning frequency as low as 250 Hz, the isotropic peak produced by TOSS for each of the three components may not be in phase even in absence of molecular diffusion, resulting in multiple irregular splittings of the centerband.

3.3. PASS

In contrast to the TOSS results, the results given in Fig. 2 indicate that the PASS method may be used to produce a nearly sideband free isotropic chemical shift spectrum at a frequency as low as 50 Hz. (The peaks marked by the symbol '*' in Fig. 2 are aliased sidebands, arising because only 32 evolution increments were used to acquire the PASS data in this case. This would separate only 32 orders of SSBs without spectral aliasing, whereas at this spinning rate the spectrum contained approximately 90 visible sidebands, requiring at least 90 increments.) It follows from Fig. 2 that the quality of the centerband PASS spectrum at a spinning rate of 250 Hz is as good as that acquired at 1 kHz, albeit with a somewhat larger linewidth, i.e., 26 versus 21 Hz. Moreover, the sensitivity of the PASS experiment per unit experimental time at 250 Hz is still nearly 47% of the sensitivity at 1 kHz. The reason why PASS is better than TOSS is that PASS is a 2D experiment with a constant evolution time of one rotor period. The sidebands are separated by their order (i.e., $0F$ (centerband), $\pm 1F$, $\pm 2F, \ldots$, where F is the MAS frequency) by applying five π pulses at specific time intervals, which are different for each increment, see also Fig. 1. In the first increment the five π pulses are equally spaced, and all the signals, irrespective of their chemical shift values, are in phase. The other increments build the second spectral dimension with the necessary phase evolution so that the order of the sidebands is separated in the 2D plane [17]. In contrast, TOSS is a 1D experiment, where the isotropic peak may be inverted if the ratio of the anisotropy to the spinning rate is large, resulting in positive and negative peaks across the line shape. At a rotation frequency of 50 Hz the shape of the PASS spectrum is still good, and the width of the centerband is only modestly increased to 39 Hz. However, its intensity is only 0.2% of that obtained at 1 kHz. At the lowest spinning rate of 30 Hz used for the PASS experiment on the water/beads system used in this study, the intensity of the centerband was further reduced to about 0.02% of that obtained at the spinning rate of 1 kHz, and the linewidth further increased to 50 Hz. This signal attenuation is caused by the fact that in a PASS experiment the first data point is observed after a delay of one rotor period after the initial excitation pulse [17]. During this rotor period the magnetization decays according to an apparent time constant T_2' resulting in a signal attenuation that increases with decreasing spinning speeds. Moreover, an additional signal attenuation occurs because T_2' decreases at lower spinning frequencies. This can be understood as follows. T_2' is defined as the time constant measured when six π pulses are equally spaced within each rotor period and the number of rotor periods is incremented (the sixth pulse has been inserted at the end of the rotor period to preserve the phase of the signal). Hence the situation is very similar to a CPMG experiment, where the inverse time constant $(T_2')^{-1}$ is given by the sum of the intrinsic spin–spin relaxation rate $(T_2)^{-1}$ and a rate $(T_{\mathrm{diff}})^{-1}$ that increases with increasing diffusion rate, magnetic field gradient, and time between the π pulses [26].

Despite the attractive features of PASS, it should be pointed out that the technique only works when the static spectrum does not depend on the orientation of the sample with respect to the external field [17]. We have indeed confirmed that 2D-PASS fails to separate the spinning sidebands by order for a highly anisotropic sample such as large glass tubes immersed in water. Moreover, a detailed evaluation of the centerband 2D-PASS spectrum at 250 Hz indicates that there exist some small residual sidebands with intensity less than 3% of the center band. An elaborate theoretical analysis, to be published separately, reveals that this is due to the diffusion-induced attenuation of the magnetization amplitude in a PASS experiment, which is different for each evolution increment. However, this effect is much less severe than that of the diffusion-induced distortions in the TOSS spectrum, which is evident by comparing the spectra at, e.g., the 250 Hz spinning rate.

3.4. PHORMAT

The linewidths of the projection spectra (Fig. 2) along the isotropic dimension of the PHORMAT experiment acquired at spinning rates of 50 and 1 Hz are 146 and 917 Hz, respectively. At both frequencies the spectra are free of SSBs, which is an inherent feature of the PHORMAT technique [18]. Also, the signal intensity (sensitivity per unit measuring time) obtained with PHORMAT at 50 Hz is about a factor 15 larger than was measured with PASS at the same MAS rate. However, the sensitivity with PHORMAT is less than that obtained with SP-MAS or PASS at high spinning rates. A detailed discussion of this issue was given in [2]. It was reported [2] that the reduction in the PHORMAT sensitivity is determined by three factors: (a) an intrinsic loss of a factor of 4 due to the use of two projection pulses; (b) an attenuation factor arising from the fact that PHORMAT is a 'true' isotropic/anisotropic 2D experiment [27]; and (c) T_1 loss during the two magnetization storage periods, which have a total time equal to 2/3 of the rotor period. The sensitivity of the PHORMAT experiment relative to that of SP-MAS per unit experimental time at a given spinning frequency F is given by [2]

$$\frac{\text{PHORMAT(2D)}}{\text{SP-MAS(1D)}} \approx \frac{1}{4}\exp\left(-\frac{2}{3FT_1}\right) \times \left\{\frac{T_2}{2t_1^{\max}}\left[1-\exp\left(-\frac{2t_1^{\max}}{T_2}\right)\right]\right\}^{1/2}, \quad (1)$$

where $t_1^{\max}$ is the maximum sampling time corresponding to the evolution dimension (t_1) of the PHORMAT experiment. The ^{1}H NMR T_1 of the water + glass beads system was determined by the conventional inversion-recovery method and found to be 1.6 s. Using $t_1^{\max} = 2T_2$ it follows from Eq. (1) that the ratio PHORMAT(2D)/SP-MAS(1D) is approximately equal to 0.12 for a spinning rate of 50 Hz, close to the experimental value of 0.10. At a 1 Hz spinning rate the sensitivity is further reduced by about 50% due to the T_1-induced attenuation.

Fig. 3 shows the linewidth $\Delta\nu_{1/2}$ (full width at half maximum intensity) of the ^{1}H center line, resulting from the molecular diffusion of the water molecules in the susceptibility gradients, obtained by SP-MAS, PASS, and PHORMAT, as a function of the spinning frequency F. The values of $\Delta\nu_{1/2}$ for SP-MAS and PHORMAT have been determined by subtracting the intrinsic linewidth of 23 Hz, obtained from SP-MAS at high spinning speeds, from the observed values. While the values of $\Delta\nu_{1/2}$ for PASS have been determined by subtracting the linewidth of 21 Hz, obtained from that of PASS at 1 kHz, from the observed values. The results obtained with TOSS have been excluded due to the complex splitting of the resonant line at spinning rates below 1 kHz, see Fig. 2. Different frequency ranges are shown for the different methods: (a) For SP-MAS spinning rates of 100 Hz or larger are required in order to avoid a severe overlap between the centerband and the SSBs. (b) PASS experiment was restricted to spinning frequencies of 30 Hz and larger in order to avoid serious signal losses during the first rotor period. (c) With PHORMAT ultra-low frequencies could be employed without major signal reductions. However, in PHORMAT the spinning frequency cannot be made arbitrarily large, because the longest evolution time t_1 must be smaller than the rotor period $T_r = F^{-1}$ (see Fig. 1D). Hence, for small T_r values the evolution decay becomes truncated, inducing additional broadening in the isotropic dimension. For our sample it was found that the maximum spinning rate that can be used is about 50 Hz. Therefore, with PHORMAT only data are collected between 1 and 50 Hz spinning rates.

It follows from Fig. 3 that the centerband's linewidth dependence on the spinning frequency is different for SP-MAS, PASS, and PHORMAT:

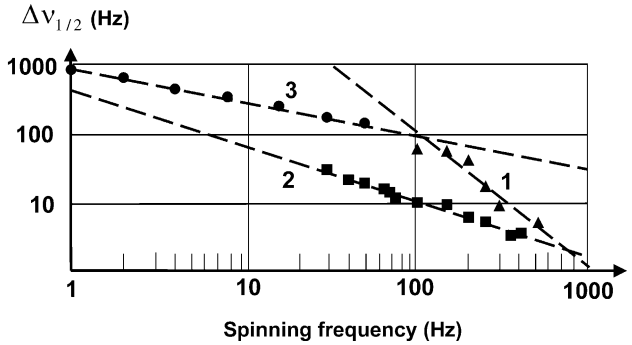

Fig. 3. The diffusion-induced linewidth $\Delta\nu_{1/2}$ (FWHM) of the ^{1}H resonant peak as a function of the sample spinning rate observed with SP-MAS (▲), PASS (■), and the isotropic projection of PHORMAT (●), respectively. Curves 1–3 denote the empirical relationships for SP-MAS, PASS, and PHORMAT, respectively, as given in the text.

3.5. SP-MAS

In SP-MAS the following empirical relation was obtained between the diffusion-induced linewidth $\Delta\nu_{1/2}$ and the spinning frequency F:

$$\Delta\nu_{1/2} = 1.2 \times 10^6/F^2 \ \text{(Hz)}, \quad F > 100 \ \text{Hz}. \tag{2}$$

This observed MAS frequency dependence is in agreement with an elaborate theoretical analysis for the SP-MAS experiment [19] in which diffusion was shown to introduce a periodic modulation and attenuation of the magnetization amplitude. It was found that at higher spinning rates the attenuation exponent at early decay times results in a linewidth that is inversely proportional to F^2, in accordance with our measurements.

3.6. PASS

In PASS the following empirical relationship is found between $\Delta\nu_{1/2}$ and F:

$$\Delta\nu_{1/2} = 490/F^{0.84} \ \text{(Hz)}, \quad F > 50 \ \text{Hz}. \tag{3}$$

PASS produces the narrowest linewidth in the frequency range 30–500 Hz. This is mainly due to the signal attenuation during the first rotor period. As mentioned above, the signal decay obtained in the stationary sample can be simulated with three Gaussian components with different time constants and it is likely that the corresponding T_2' values, which determine the decay during the first rotor period, are also different. The fastest decaying components are more severely attenuated after the first rotor period than the more slowly decaying components. This effect becomes more pronounced at lower spinning frequencies, resulting in an artificial line narrowing at decreasing MAS frequencies. This narrowing counteracts the line broadening at decreasing frequencies due to the diffusion effects. This may explain the fact that compared with SP-MAS the frequency dependence of $\Delta\nu_{1/2}$ is reduced in a PASS experiment. If this explanation is correct, it means that the frequency dependence of $\Delta\nu_{1/2}$ is not generally given by Eq. (3), but will be different for different samples, depending on the actual shape of the decay during the first rotor period. As further evidence of this explanation, SP-MAS provides the same centerband linewidth as PASS when the signal attenuation is the same for both methods. This comparison was performed by deleting the time points corresponding with the first rotor period for SP-MAS NMR experiments with spinning rates higher than 100 Hz before Fourier processing to obtain the spectrum. In this case a similar SP-MAS spectrum was observed as measured with PASS at the same MAS rate and a similar spinning rate dependence of the centerband linewidth as that of PASS was also obtained. Nevertheless, PASS is the method of choice at lower spinning speeds, as it provides an almost perfect separation between the centerband and the spinning sideband spectra.

3.7. PHORMAT

In PHORMAT the empirical relationship between $\Delta\nu_{1/2}$ and F is given by

$$\Delta\nu_{1/2} = 917/F^{0.5} \ \text{(Hz)}, \quad 50 > F > 1 \ \text{Hz}. \tag{4}$$

It follows that a considerably weaker frequency dependence of $\Delta\nu_{1/2}$ is observed in PHORMAT than SP-MAS or PASS. In a theoretical evaluation of the diffusion-induced line broadening in a PHORMAT experiment, which will be published separately, it is shown that for ultra-low MAS the linewidth $\Delta\nu_{1/2}$ can be approximated by

$$\Delta\nu_{1/2} = \frac{2\sqrt{\ln 2}}{3\pi\sqrt{3}}\gamma G_0 D_0^{0.5}/F^{0.5} \ \text{(Hz)}. \tag{5}$$

In Eq. (5) G_0 denotes the magnetic susceptibility gradient, and D_0 is the diffusion coefficient of the molecules under investigation. Hence, Eq. (5) predicts the same frequency dependence of $\Delta\nu_{1/2}$ as found empirically.

It is worth noting that the actual line narrowing factor, i.e., the ratio of the linewidths obtained in a stationary sample and in a spinning sample, that can be achieved with PHORMAT at a specific frequency may depend on the value of the diffusion coefficient D_0. For instance, assume that the system is in the so-called static dephasing regime [28], which means that for a static sample the linewidth induced by the susceptibility gradients in absence of diffusion is much larger than the line broadening resulting from the diffusion of the molecules in the presence of the gradients. Then the static linewidth is proportional to γG_0 and independent of D_0, which means that the line narrowing factor is proportional to $D_0^{-0.5}$, and, henceforth, increases when D_0 decreases. This result is in agreement with some preliminary observations: in the sample considered in this article the line narrowing factor obtained in a 1 Hz PHORMAT experiment is about a factor 4, whereas for metabolites in cell systems, which diffuse much slower than free water [22], this factor was found to be a factor 10–14 in an excised rat liver [2].

4. Conclusions

It is concluded that for slow-MAS experiments both PASS and PHORMAT are the methods of choice for producing high-resolution and spinning sideband-free spectra in aqueous samples containing internal magnetic susceptibility gradients. Both standard SP-MAS and especially TOSS can only be used at high MAS frequencies, i.e., a few hundred Hz or higher, thus these methods cannot be used to study large intact biological

samples. However, in both PHORMAT and PASS the diffusion-induced linewidth is found to decrease with increasing spinning frequency, which means that the frequency should be chosen as high as can be tolerated by the biological sample.

Moreover, for PASS the spinning frequency must be high compared to the apparent spin–spin relaxation rate $(T_2')^{-1}$ in order to avoid signal losses. As $(T_2')^{-1}$ is in part caused by diffusion broadening, this means that $(T_2')^{-1}$, and hence the minimum spinning rate that can be used in PASS, decreases when the diffusion coefficient D_0 decreases. Indeed it was found that for the sample used in this study, where free water is considered, the signal was severely attenuated at a frequency as low as 50 Hz. For the metabolites in cell systems, which have much lower diffusion coefficients [22], spinning rates as low as 30–40 Hz could be used with only minor signal attenuations.

For studies of larger objects such as live animals, PHORMAT and similar magic angle turning techniques, where much lower spinning rates can be used, are the methods of preference, despite its intrinsic signal loss of at least a factor 4 and its relatively long measuring times [2,18]. For small biological samples, with dimensions of a few mm or less, where spinning speeds $\geqslant$30 Hz can be used, PASS is preferred because of its higher sensitivity and relatively short measuring times (a few minutes).

Also, the dependence of the diffusion-induced linewidth $\Delta\nu_{1/2}$ on the spinning frequency was investigated. It was found empirically for our sample consisting of glass beads surrounded by water that $\Delta\nu_{1/2}$ is proportional to F^{-x}, where F is the frequency, and $x = 2$ for SP-MAS with $F > 100$ Hz, $x = 0.84$ for PASS with $F > 30$ Hz, and $x = 0.5$ for PHORMAT with $50 > F > 1$ Hz. The SP-MAS and PHORMAT results agree with theoretical results. In contrast, the frequency dependence observed with PASS is empirical and is probably dependent on the actual shape of the signal decay during the first rotor period, i.e., this frequency dependence may be sample dependent. Also, the actual line-narrowing factors obtained at a specific spinning speed may be sample dependent as well, as these factors may depend on the actual values of the diffusion coefficient and the susceptibility gradient. Studies of phantom samples which mimic tissue structures more realistically [28] and of actual tissues and organs will be undertaken to investigate this matter further.

Finally, the question arises as to what spinning rates are actually needed in biological samples to suppress the diffusion-induced line width $\Delta\nu_{1/2}$ to a value small compared with the intrinsic linewidth $\delta_{1/2}$. As an example we consider the methyl peak, measured in the proton metabolite spectrum of excised fresh, untreated rat liver (see also [2]). In a 7 T magnetic field the static linewidth is about 150 Hz. The intrinsic linewidth, determined from PASS and SP-MAS experiments, is about 10 Hz, whereas the observed overall linewidth in a 1 Hz PHORMAT experiment is about 15 Hz. Hence, even at this low spinning speed $\Delta\nu_{1/2}$ is already reduced to about 5 Hz. It is concluded that in this sample spinning with a few Hz is sufficient to obtain a PHORMAT spectrum with a resolution close to the isotropic resolution. Moreover, for PASS at the lowest possible frequency of typically 30 Hz in a biological sample, the diffusion-induced line broadening is already negligible. These conclusions were confirmed by additional PASS and PHORMAT experiments on other excised organs and tissues.

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References

[1] R.A. Wind, J.Z. Hu, D.N. Rommereim, High resolution ^{1}H NMR spectroscopy in organs and tissues using slow magic angle spinning, Magn. Reson. Med. 46 (2001) 213–218.
[2] J.Z. Hu, D.N. Rommerein, R.A. Wind, High resolution ^{1}H NMR spectroscopy in rat liver using magic angle turning at a 1 Hz spinning rate, Magn. Reson. Med. 47 (2002) 829–836.
[3] F. Adebodun, J. Post, Bulk magnetic susceptibility induced line broadening in the ^{19}F NMR spectra of suspended leukemic cells, NMR Biomed. 6 (1993) 125–129.
[4] P. Weybright, K. Millis, N. Campbell, D.G. Cory, S. Singer, Gradient, high-resolution, magic angle spinning ^{1}H nuclear magnetic resonance spectroscopy of intact cells, Magn. Reson. Med. 39 (1998) 337–345.
[5] E.R. Andrew, R.G. Eades, Removal of dipolar broadening of NMR spectra of solids by specimen rotation, Nature 183 (1959) 1802.
[6] A.N. Garroway, Magic-angle sample spinning of liquids, J. Magn. Reson. 49 (1982) 168–171.
[7] D.L. VanderHart, Magnetic susceptibility and high resolution NMR of liquids and solids, in: D.M. Grant, R.K. Harris (Eds.), Encyclopedia of Nuclear Magnetic Resonance, Wiley, New York, 1996, pp. 2938–2946.
[8] L.L. Cheng, M.J. Ma, L. Becerra, T. Ptak, I. Tracey, A. Lackner, R.G. Gonzalez, Quantitative neuropathology by high resolution magic angle spinning proton magnetic resonance spectroscopy, Proc. Natl. Acad. Sci. USA 94 (1997) 6408–6413.
[9] K.K. Millis, W.E. Maas, D.G. Cory, S. Singer, Gradient, high-resolution, magic-angle spinning NMR spectroscopy of human adipocyte tissue, Magn. Reson. Med. 38 (1997) 399–403.
[10] S. Garrod, E. Humpfer, M. Spraul, S.C. Connor, S. Polley, J. Connelly, J.C. Lindon, J.K. Nicholson, E. Holmes, High-resolu-

tion magic angle spinning ^{1}H NMR spectroscopic studies on intact rat renal cortex and medulla, Magn. Reson. Med. 41 (1999) 1108–1118.

[11] M.E. Bollard, S. Garrod, E. Holmes, J.C. Lindon, E. Humfer, M. Spraul, J.K. Nicholson, High- resolution ^{1}H and ^{1}H –^{13}C magic angle spinning NMR spectroscopy of rat liver, Magn. Reson. Med. 44 (2000) 201–207.

[12] S. Garrod, E. Humpher, S.C. Connor, J.C. Connelly, M. Spraul, J.K. Nicholson, E. Holmes, High-resolution ^{1}H NMR and magic angle spinning NMR spectroscopy investigation of the biochemical effects of 2-bromoethanamine in intact renal and hepatic tissue, Magn. Reson. Med. 45 (2001) 781–790.

[13] J. Chen, B.M. Enloe, C.D. Fletcher, D.G. Cory, S. Singer, Biochemical analysis using high-resolution magic angle spinning NMR spectroscopy distinguishes lipoma-like well-differentiated liposarcoma from normal fat, J. Am. Chem. Soc. 123 (2001) 9200–9201.

[14] A.J. Oyama, Response and adaptation of Beagle dogs to hypergravity, in: Life Sciences and Space Research XIII: Proc. of the 17th Plenary Meeting, Sao Paulo, Brazil, June 17–July 1, 1974, Akademie-Verlag, Berlin, 1975, pp. 11–17.

[15] C.C. Wunder, R.D. Matthes, C.M. Tipton, Knee-ligament loading properties as influenced by gravity: I-junction with bone of 3-G rodents, Aviat. Space Environ. Med. 53 (1982) 1098–1111.

[16] D.J. Schaefer, J.D. Bourland, J.A. Nyenhuis, Review of patients safety in time-varying gradient fields, J. Magn. Res. Imag. 12 (2000) 20–29.

[17] O.N. Antzutkin, S.C. Shekar, M.H. Levitt, Two-dimensional sideband separation in magic-angle-spinning NMR, J. Magn. Reson. A 115 (1995) 7–19.

[18] J.Z. Hu, W. Wang, F. Liu, M.S. Solum, D.W. Alderman, R.J. Pugmire, D.M. Grant, Magic-angle-turning experiments for measuring chemicalshift-tensor principal values in powdered solids, J. Magn. Reson. A 113 (1995) 210–222.

[19] G. Leu, X. Tang, S. Peled, W.E. Maas, S. Singer, D.G. Cory, P.N. Sen, Amplitude modulation and relaxation due to diffusion in NMR experiments with a rotating sample, Chem. Phys. Lett. 332 (2000) 344–350.

[20] Y. Liu, G. Leu, S. Singer, D.G. Cory, P.N. Sen, Manipulation of phase and amplitude modulation of spin magnetization in magic angle spinning nuclear magnetic resonance in the presence of molecular diffusion, J. Chem. Phys. 114 (2001) 5729–5734.

[21] W.T. Dixon, Spinning-sideband-free and spinning-sideband-only spectra in spinning sample, J. Chem. Phys. 77 (1982) 1800–1809.

[22] K.R. Minard, X. Guo, R.A. Wind, Quantitative ^{1}H MRI and MRS microscopy of individual lung tumor spheroids, J. Magn. Reson. 133 (1998) 368–373.

[23] D.C. Chang, H.E. Rorschach, B.L. Nichols, C.F. Hazlewood, Implication of diffusion coefficient measurements for the structure of cellular water, in: C.F. Hazlewood (Ed.), Physicochemical State of Ions and Water in Living Tissues and Model Systems, Annals of the New York Academy of Sciences, vol. 204, New York Academy of Sciences, New York, 1973, pp. 434–443.

[24] D.P. Raleigh, E.T. Olejniczak, R.G. Griffin, Multiple-pulse NMR in inhomogeneously broadened rotating solids: theory of sideband suppression experiments, J. Chem. Phys. 89 (1988) 1333.

[25] D.W. Alderman, G. McGeorge, J.Z. Hu, R.J. Pugmire, D.M. Grant, A sensitive high resolution magic angle turning experiment for measuring chemical shift tensor principal values, Mol. Phys. 95 (1998) 1113–1126.

[26] A. Abragam, in: The Principles of Nuclear Magnetism, Oxford University Press, New York, 1961, p. 61.

[27] R.R. Ernst, G. Bodenhausen, A. Wokaun, in: Principles of Nuclear Magnetic Resonance in One and Two Dimensions, Oxford University Press, New York, 1994, pp. 148 and 349.

[28] D.A. Yablonskiy, Quantitation of intrinsic magnetic susceptibility-related effects in a tissue matrix. Phantom study, MRM 39 (1998) 417–428.