J. Am. Chem. Soc. **2001,** *123,* 7467−7468 7467

An Experimental Strategy to Dramatically Reduce the RF Power Used in Cross Polarization Solid-State NMR Spectroscopy

Srinivasan C. Shekar, Dong-Kuk Lee, and
Ayyalusamy Ramamoorthy*

Biophysics Research Division
Department of Chemistry
Macromolecular Science and Engineering
The University of Michigan, Ann Arbor, Michigan 48109

Received May 16, 2001
Revised Manuscript Received June 8, 2001

Cross polarization (CP)[1−5] is the most extensively applied solid-state NMR technique to enhance the sensitivity of low gyromagnetic ratio and low natural abundance nuclei in chemical, biological, and polymer systems. A stringent requirement for CP is to achieve the Hartmann−Hahn (HH)[6] match, $\omega_1^I = \omega_1^S$, where $\omega_1^X = \gamma_X B_1^X$. In an experiment, it is achieved by adjusting the ratio of RF field (in Gauss) generated in the sample coil, B_1^S/B_1^I, equal to the ratio of gyromagnetic ratios of *I* and *S* nuclei, γ_I/γ_S. However, since RF power is proportional to $(B_1^X)^2$, any power loss due to the nature of the sample demands more RF power to generate the same RF field strength. In addition to being technically demanding, the use of high RF power leads to significant heat dissipation in the sample. In particular, a CP experiment on a hydrated phospholipid bilayer sample containing a membrane-associated protein is power lossy due to the high water content, and at the same time, the dissipated heat dehydrates the bilayer and can denature the sample.[7] These concerns are especially important for long-duration solid-state NMR experiments (typically a few days)[8] as most biological samples are not abundantly available and extensive signal averaging is unavoidable. Also, the benefit of higher magnetic fields to increase the signal-to-noise ratio (S/N) and resolution comes with the price of requiring higher RF power for solid-state NMR experiments. Therefore, there is a critical need for techniques that employ low RF power pulses for solid-state NMR applications. In this communication, we demonstrate the use of resonance-offset to significantly reduce the required RF power in CP experiments.

The proposed pulse sequence is given in Figure 1A. A spin−echo sequence,[9] $\tau-180°-\tau$, is used to circumvent the dead time problem of the receiver; only the second-half of the *S* spin−echo is acquired. In Figure 1, offset, Δ, is the difference between the resonance frequency of *S* nuclei and the RF carrier frequency on the *S* channel, and θ is the angle between the effective field (B_{eff}) and the direction of the RF field (assumed to be *x*-axis). A standard CP[1,2] experiment has $\Delta = 0$ kHz and $\theta = 0°$. For simplicity, we consider offset on only one of the RF channels (*S*-channel). To compensate a HH-mismatch, $B_{\text{RF}}^S < B_{\text{RF}}^I$, the RF carrier frequency on the *S*-channel during the CP is offset by Δ to satisfy the condition $B_{\text{eff}}^S = B_{\text{RF}}^I$. Since the effective fields during the CP period on both the RF channels are the same, the transverse magnetization of *I* nuclei is transferred to *S* nuclei via the *I*−*S* dipolar interaction. Following the CP period, a post-pulse, θ_y, tips the *S* magnetization locked along the B_{eff}^S direction to the transverse plane (*x*-axis). Thus, the HH-mismatch deliberately generated by reducing the RF power in one of the channels can be compensated with the offset to achieve efficient CP.

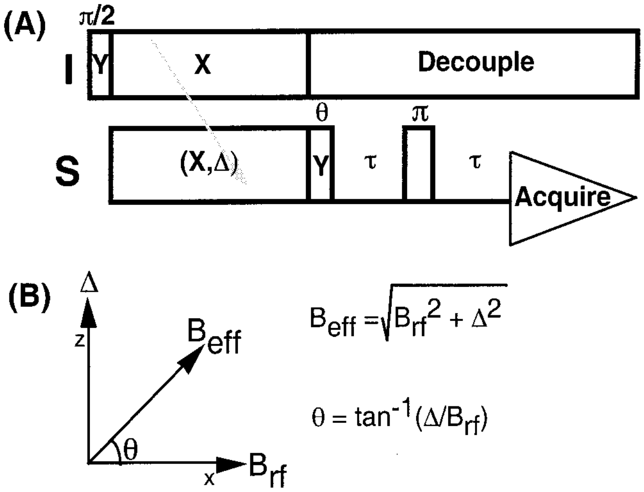

Figure 1. (A) A pulse sequence to reduce the required RF power in cross polarization experiments. An RF offset, △, generated during the contact time on the *S* channel effectively reduces the magnitude of the RF power required to achieve the Hartmann−Hahn condition to transfer the magnetization from *I* to *S* nuclei. (B) An effective field, B_{eff}, generated by an RF pulse applied at an offset Δ.

All spectra were acquired using a *Chemagnetics/Varian Infinity-400* spectrometer and a double-resonance *Chemagnetics* MAS probe operating at 400.139 MHz (9.4 T) for ^{1}H and 40.551 MHz for ^{15}N at room temperature. Proton-decoupled ^{15}N chemical shift spectrum of a powder sample of *N*-acetyl-L-^{15}N-valine (NAV) is shown in Figure 2. The spectrum shown in Figure 2A was obtained using the standard CP sequence[1] with $\Delta = 0$ kHz and $\theta = 0°$ in the pulse sequence shown in Figure 1A. An RF field strength of 41.7 kHz was used in ^{1}H and ^{15}N channels during CP. Figure 2B represents the ^{15}N chemical shift spectrum that was obtained by deliberately missetting the ^{15}N RF field strength during CP to 20.8 kHz from the HH-match (HH-mismatch of 20.9 kHz); all other parameters were the same as Figure 2A. It is obvious that the S/N in Figure 2B is close to zero and it is solely due to the HH-mismatch.

The reduction of 20.9 kHz RF power, from the HH-match, on the ^{15}N channel can be compensated by setting $\Delta = 36.1$ kHz during the CP as illustrated in Figure 1B. This procedure generates a 41.7 kHz RF field along a vector that makes 60° degrees from the *x*-axis. The ^{15}N chemical shift spectrum acquired under this condition is shown in Figure 2C; all other conditions were the same as used to obtain Figure 2B. The S/N has significantly improved in Figure 2C, as compared to Figure 2B, despite 20.9 kHz HH-mismatch. This is because the magnitudes of ^{15}N effective field (see Figure 1B) and ^{1}H RF field during CP are the same, and thus the transfer of ^{1}H magnetization to ^{15}N increases the S/N of ^{15}N spectrum. However, the S/N in Figure 2C is lower than that in Figure 2A. This is due to the fact that the transferred

---

(1) Pines, A.; Gibby, M. G.; Waugh, J. S. *J. Chem. Phys.* **1972**, *56*, 1776-1777.

(2) Slichter, C. P. *Principles of Magnetic Resonance*, 3rd ed.; Springer: Berlin, Heidelberg, New York, 1996; Chapter 7.

(3) Mehring, M. *Principles of High-Resolution NMR in Solids,* 2nd ed.; Springer: Berlin, Heidelberg, New York, 1983; Chapter 4.

(4) Fyfe, C. A. *Solid State NMR Chemists*; C.F.C. Press: Guelph, Ontario, 1983; Chapters 5−8.

(5) Schmidt-Rohr, K.; Spiess, H. W. *Multidimensional Solid-State NMR and Polymers*; Academic Press: New York; 1994, Chapter 3.

(6) Hartmann, S. R.; Hahn, E. L. *Phys. Rev. Lett.* **1962**, *128*, 2042−2053.

(7) Lee, D. K.; Ramamoorthy, A. *J. Magn. Reson.* **1998**, *133*, 204−206.

(8) Ramamoorthy, A.; Gierasch, L. M.; Opella, S. J. *J. Magn. Reson.* **1996**, *B111*, 81−84; Marassi, F. M.; Ramamoorthy, A.; Opella, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 8551−8556; Ramamoorthy, A.; Wu, C. H.; Opella, S. J. *J. Magn. Reson.* **1999**, *140*, 131−140; Hong, M. *J. Biomol. NMR* **1999**, *15*, 1−14; Ishii, Y.; Tycko, R. *J. Am. Chem. Soc.* **2000**, *122*, 1443−1455; Marassi, F. M.; Opella, S. J. *J. Magn. Reson.* **2000**, *144*, 150−155; McDermott, A.; Polenova, T.; Bockmann, A.; Zilm, K. W.; Paulsen, E. K.; Martin, R. W.; Montelione, G. T. *J. Biomol. NMR* **2000**, *16*, 209−219; Rienstra, C. M.; Hohwy, M.; Hong, M.; Griffin, R. G. *J. Am. Chem. Soc.* **2000**, *122*, 10979−10990.

(9) Hahn, E. L. *Phys. Rev.* **1950**, *80*, 580−594.

10.1021/ja016212t CCC: $20.00

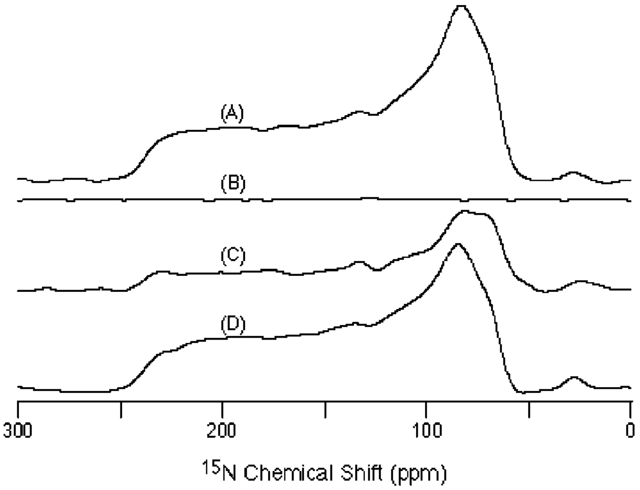

Figure 2. ^{15}N spectra of a powder sample of *N*-acetyl-L-^{15}N-valine obtained using the pulse sequence given in Figure 1A. (A) At the Hartmann−Hahn match with 41.7 kHz ^{15}N RF during CP, $\Delta = 0$ kHz and $\theta = 0°$. (B) At a Hartmann−Hahn mismatch condition with 20.8 kHz ^{15}N RF during CP, $\Delta = 0$ kHz and $\theta = 0°$. (C) All the conditions were the same as in (B) but with $\Delta = 36$ kHz and $\theta = 0°$. (D) All the conditions were the same as in (B) but with $\Delta = 36$ kHz and $\theta = 60°$. It is important to note that an RF power of 418 W was used to generate 41.7 kHz in A, whereas only 82 W was used to generate 20.8 kHz RF field in C and D on the ^{15}N channel during the CP. Other experimental parameters used to obtain these spectra are 41.7 kHz ^{1}H RF during CP and 77 kHz during ^{1}H decoupling, 3 ms contact time, $\tau = 60$ μs, 560 scans, and 5s recycle delay.

^{15}N magnetization is along the effective field direction, and therefore only the projection of the magnetization on to the *x*-axis ($M \cos \theta$) contributes to the ^{15}N S/N in Figure 2C unlike in Figure 2A. As explained earlier, the S/N can be further enhanced by setting the flip angle of the postpulse, θ. The spectrum in Figure 2D was obtained with $\theta = 60°$, and all other parameters were the same as those used to obtain Figure 2C. It is gratifying to note that the line shapes in Figure 2, A and D, are well preserved and 86% of the S/N is recovered in Figure 2D. This experimental result suggests that a deliberate mismatch of 20.9 kHz on the ^{15}N RF channel was successfully compensated, using the pulse sequence in Figure 1A with 36.1 kHz of offset on the ^{15}N RF channel during the CP. Most importantly, this procedure reduces the required ^{15}N RF power by a factor of 5.1. Our experimental results suggest that the pulse sequence also performs well for various magnitudes of HH-mismatch. It was observed that the use of 30.7 kHz RF, $\Delta = 28.2$ kHz, and $\theta = 42.6°$, instead of the required 41.7 kHz ^{15}N RF during the CP, reduces the RF power by a factor of *two*.

The S/N for powder samples, in general, increases with the contact time until it reaches a steady state.[5] Since the offset during CP in the pulse sequence scales the ^{1}H−^{15}N dipolar coupling, a relatively longer contact time is needed to reach this steady state in powder samples. On the other hand, in the case of uniaxially oriented samples, the contact time needed to obtain the maximum S/N depends on the sample orientation relative to the external magnetic field.[10] It is important to note that the usage of offset on the ^{1}H channel can also reduce the required RF power during CP. This would be of great advantage, especially at higher magnetic fields, as the ^{1}H RF frequency approaching the microwave region, at higher RF power, leads to considerable sample heating. However, care must be taken if the large offset creates an effective field at the magic angle as it suppresses the ^{1}H−^{1}H dipolar interaction and causing slower spin diffusion.[5,7]

We have also successfully performed this experiment on single-crystal samples of peptides and other powder samples under static and MAS conditions. The proposed experiment is very efficient in compensating the HH-mismatch. The extent of HH-mismatch compensation depends on the strength of the RF field used on the ^{1}H RF channel during CP and also on $T_{1\rho}$ of the sample. The pulse sequence shown in Figure 1 is easy to set up on most solid-state NMR spectrometers, home-built or commercial, but it is important to have a frequency switch capability during the pulse sequence. The offset can be set at the beginning or during the recycle delay, and can be switched to zero (or on-resonance) before the post-pulse in Figure 1A. The idea of using offset to reduce the power of an RF pulse can be employed in the development of new solid-state NMR methods as only a fraction of a microsecond is needed to switch the frequency. Therefore, we believe that this method can be used for various applications of NMR spectroscopy to investigate molecules in the solid state.

Acknowledgment. This research was supported by NSF through Grant MCB-9875756 (CAREER Development Award to A.R.). We thank Kevin Hallock for useful discussions.

JA016212T

---

[10] Taylor, D. M.; Ramamoorthy, A. *J. Magn. Reson.* **1999**, *141*, 18−28.