Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Magnetic Resonance 180 (2006) 317–320
JMR Journal of Magnetic Resonance
www.elsevier.com/locate/jmr

Communication

New methods for calibration of the RF field strength for indirectly observed nuclei

Andrew J. Benie, Ole W. Sørensen *

Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Valby, Denmark

Received 3 February 2006; revised 6 March 2006
Available online 24 March 2006

Abstract

Two novel pulse sequences, CALIS-1 and CALIS-2, for accurate calibration of the RF field strength for an indirectly observed spin are introduced. CALIS-2 is intended for calibration of e.g., ^{13}C or ^{15}N pulses on natural abundance samples whilst CALIS-1 is recommended primarily for enriched samples. Both experiments can be performed without prior knowledge or guess of the RF field strength and no delays in the pulse sequences are critically dependent on coupling constants.

Keywords: NMR; Pulse calibration; CALIS

1. Introduction

The importance of properly calibrated RF pulses is well known in NMR spectroscopy. Sensitivity-wise, the effect of miscalibrated pulses is usually cumulative, which obviously can be a problem in pulse sequences with many pulses. Another deleterious effect of miscalibrated pulses is generation of spectral artifacts, e.g., when a pulse presumed to be of flip angle π fails to invert spin states or cause refocusing of chemical shifts. Sometimes suitable phase cycling or pulsed field gradients can eliminate the effect of miscalibrated pulses but that is not always the case as in, e.g., low-pass J filters [1] that are widely used in heteronuclear long-range correlation experiments [2,3] for suppression of one-bond correlation peaks.

A simple and widely used method to calibrate the RF field strength for an indirectly observed spin S via a directly observed spin I is [4]:

$$\pi/2(I) - (2J)^{-1} - \theta(S) - \text{acq.}(I), \quad (1)$$

where J usually is the one-bond coupling constant between the spins I and S. The pulse sequence leads to an antiphase doublet that disappears for $\theta = \pi/2$ where the magnetization is transferred into unobservable 2-spin coherence.

This calibration sequence was originally proposed for calibration of the ^{1}H RF field strength via observation of the ^{13}C spectrum. However, for that application there is a considerable sensitivity gain by using the related SEMUT pulse sequence [5] that applies ^{1}H decoupling during acquisition. The sensitivity gain comes from collapsing the ^{13}C multiplet structure by suppressing all heteronuclear couplings.

The configuration in most modern probeheads of being optimized for ^{1}H detection and ^{13}C indirect detection still allows use of the above calibration sequences for calibration of the ^{13}C RF field strength, but there are two points worth mentioning in comparison to the opposite configuration: (1) for natural abundance samples, refocusing of the antiphase doublet is less critical for the sensitivity, as the heteronuclear multiplet structure is only a doublet, but for enriched samples refocusing and decoupling lead to a considerable sensitivity gain; (2) for natural abundance samples the ^{1}H spectrum is dominated by the about 200 times stronger signals from protons attached to ^{12}C isotopes and these are $\pi/2$ out of phase with the antiphase doublets relevant for the calibration, making it practically impossible to calibrate the ^{13}C RF field strength on all

* Corresponding author. Fax: +45 3327 4765.
E-mail address: olews@crc.dk (O.W. Sørensen).

1090-7807/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.jmr.2006.03.006

but the smallest molecules. A modification of the simple sequence in (1) [6] includes a z filter [7] with a gradient to reduce the strong signals from protons attached to ^{12}C isotopes, but a much higher degree of suppression of these signals is possible along with elimination of the needs to be exactly on-resonance for protons, to have ^{1}H pulses being exactly $\pi/2$, and to have an exact match of a delay to $(2J)^{-1}$ (vide infra).

In Fig. 1 are outlined two new pulse sequences that are now applied routinely in our laboratory for calibration of the ^{13}C or ^{15}N RF field strength via observation of the ^{1}H spectrum. Typically, the sequence in Fig. 1A yielding antiphase doublets is used on natural abundance samples whilst the refocused sequence in Fig. 1B is used primarily for enriched samples. Both sequences suppress the ^{1}H magnetization from molecules without ^{13}C or ^{15}N isotopes by formation of heteronuclear gradient echoes and optional phase cycles and are applicable equally well for all ^{13}C multiplicities. We dub these experiments CALIS-2 (Fig. 1A) and CALIS-1 (Fig. 1B) (Calibration for Indirect Spins). Clearly, CALIS-1 also has a sensitivity advantage over CALIS-2 for natural abundance samples but it can occasionally be a problem to determine a zero crossing in the satellite spin system at the same spectral position as a possible residual unsuppressed signal of the ^{12}C-attached proton(s). The carrier frequency for the S spin should be set close to resonance for the spin chosen for calibration to avoid off-resonance effects causing phase distortions.

The key element of the CALIS pulse sequences is the θ–delay–2θ–delay–2θ three-pulse sandwich on the S spin. The gradients and the optional phase cycle are designed to select the coherence transfer pathway that would occur if the sandwich were a perfect $\pi/2$–delay–π–delay–$\pi/2$ element and $\tau = (2J)^{-1}$ were matched exactly.

A feature of the new pulse sequences is in analogy to earlier work [5] a steeper intensity curve around the zero crossing at $\theta = \pi/2$ than for SEMUT and the sequence in (1), which results in excellent accuracy on the calibration. The signal intensity is proportional to $\sin^3\theta \sin 2\theta$. Furthermore, in contrast to the simple sequence in (1) and also to SEMUT, mistuning of the delay $\tau = (2J)^{-1}$ does not affect the accuracy of the calibration.

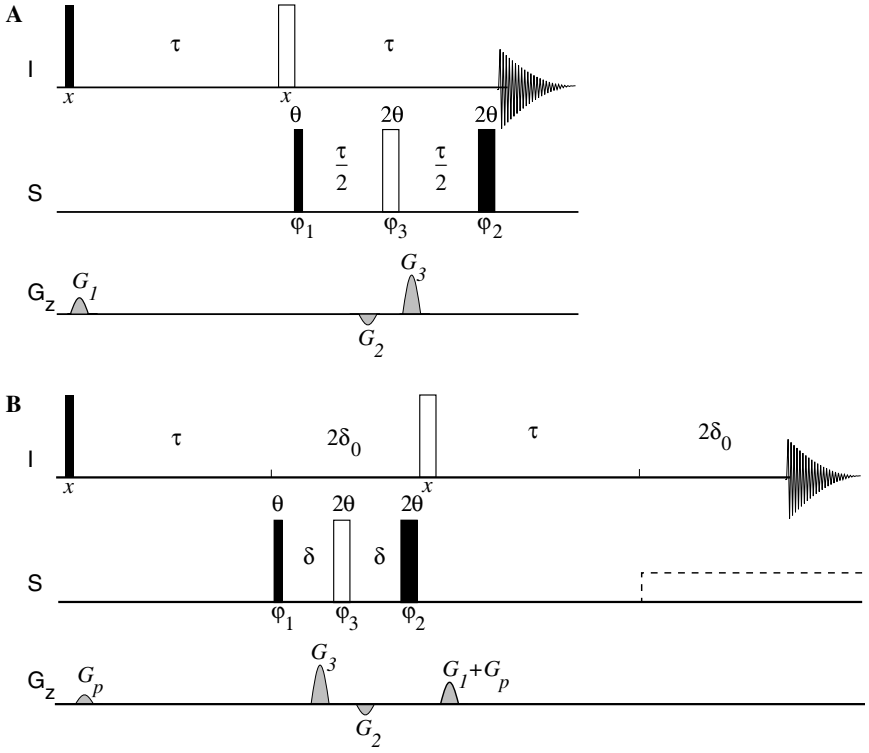

Fig. 1. CALIS pulse sequences for calibration of the S-spin (e.g., ^{13}C) RF field strength via observation of the I-spin (e.g., ^{1}H) spectrum. On the I-spin channel filled and open bars represent $\pi/2$ and π pulses, respectively. A series of spectra with different pulse durations θ is run so as to include what is expected to correspond to $\theta = \pi/2$. The same phasing must be applied to all spectra in the series. The delay τ is set to $(2J)^{-1}$ but is in no way critical. With current Bruker and Varian pulse programming software the pulse programs are conveniently written without simultaneous I- and S-spin pulses. Although the experiments can be performed without a phase cycle, artifact suppression improves upon an even number of phase cycling steps out of $\varphi_1 = \{x, -x, -x, x\}$, $\varphi_2 = \{x, x, 4(-x), x, x\}$, $\varphi_3 = \{4(x), 4(y), 4(-x), 4(-y)\}$ with the receiver phase always alternating between x and $-x$. (A) Pulse sequence CALIS-2 leading to antiphase doublets. The delay τ' is set so that the I-spin chemical shifts for all θ are refocused at the beginning of acquisition, i.e., $\tau' = \tau - 5\theta$ and decremented when θ is incremented, thus resulting in a pulse sequence that has the same length irrespective of the size of θ. (B) Pulse sequence CALIS-1 applying S-spin decoupling leading to no heteronuclear multiplet structure in the I-spin spectrum. The delay δ is set so that the gradients can still be accommodated for the longest θ value. In this sequence, it is the delays δ that are decremented according to $\delta = \delta_0 - 5\theta/2$. The gradient strengths are set so as to fulfill the equation $\gamma_I \times G_1 - (\gamma_I + \gamma_S) \times G_2 - (\gamma_I - \gamma_S) \times G_3 = 0$ or alternatively $\gamma_I \times G_1 - (\gamma_I - \gamma_S) \times G_2 - (\gamma_I + \gamma_S) \times G_3 = 0$. For I and S being ^{1}H and ^{13}C, respectively, the first equation is fulfilled for $G_1 = +5$, $G_2 = -2$, and $G_3 = +10$. The pair of gradients G_p is optional. Pulse programs for CALIS-1 and CALIS-2 can be downloaded from www.crc.dk/nmr.

The CALIS-2 pulse sequence can also be used for calibration via heteronuclear long-range couplings when no one-bond couplings are available. This can be done by replacing the delay τ by a typical HMBC delay Δ and both delays $\tau'/2$ by a δ in the sense of the CALIS-1 pulse sequence.

Fig. 2A shows a series of excerpts from CALIS-2 spectra featuring the anomeric protons of α and β mannose in an equilibrated mixture at natural isotope abundance and calibrating the ^{13}C RF field strength. It is seen that the suppression of ^{12}C-attached ^{1}H magnetization is sufficiently good that also the more sensitive CALIS-1 pulse sequence can be used. Such CALIS-1 spectra are shown in Fig. 2B. The sensitivity enhancement compared to the CALIS-2 experiment is $2\sin(\pi J\tau)$ reduced by additional transverse relaxation during the slightly longer pulse sequence and also modified by the efficiency of the decoupling sequence. In practice, slightly less than a factor of 2 is obtained.

In Figs. 3A and B are shown excerpts from ^{13}C and ^{15}N CALIS-1 spectra, respectively, of the protein CI-2 [8] uniformly labeled with ^{13}C and ^{15}N. In this case, suppression of ^{12}C- or ^{14}N-attached ^{1}H magnetization is obviously not an issue, as these contributions are negligible. The spectra in Fig. 3A represent ^{13}C RF calibration on a methyl group while those in Fig. 3B are for ^{15}N RF calibration on an amide group.

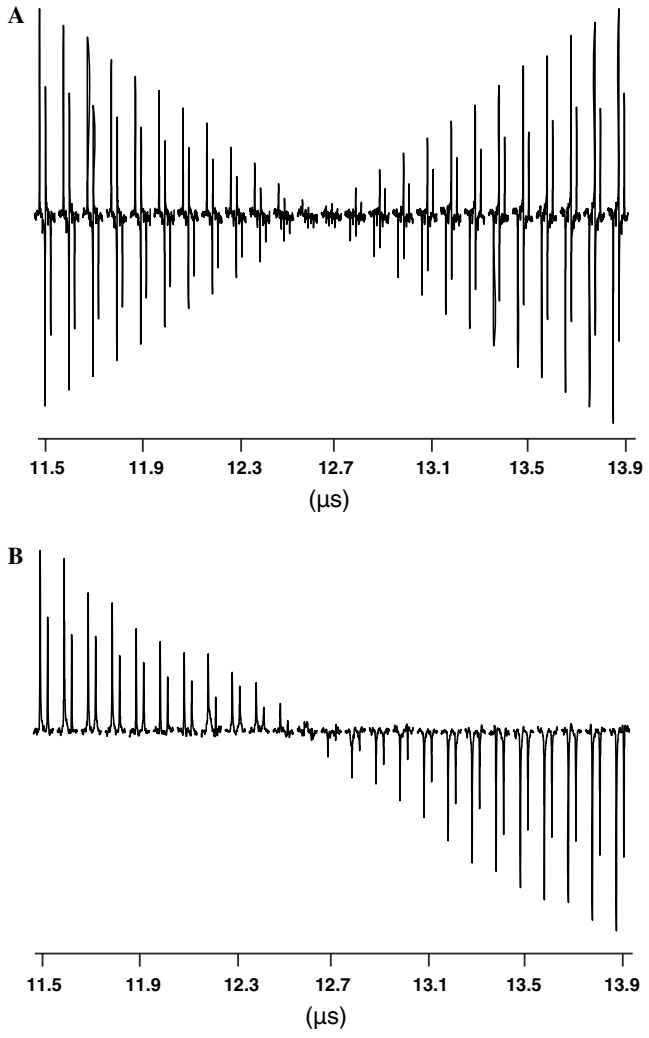

Fig. 2. Natural abundance (^{13}C) CALIS spectra acquired on 50 mM mannose in D_2O on a Bruker DRX 600 spectrometer, showing the resonances corresponding to the α and β anomeric protons, with varying values for θ (^{13}C). The value of τ was optimized for the α anomer ($^{1}J_{CH} = 172$ Hz whereas for the β anomer $^{1}J_{CH} = 158$ Hz) and the number of scans was 64. (A) CALIS-2 spectra for the α and β anomeric protons showing the two antiphase doublets, and excellent suppression of the ^{12}C-attached proton signals. The zero crossing was determined to occur at $t(\pi/2,^{13}C) = 12.6$ μs. (B) CALIS-1 spectra of the same using GARP decoupling, and again showing a zero crossing at $t(\pi/2,^{13}C) = 12.6$ μs.

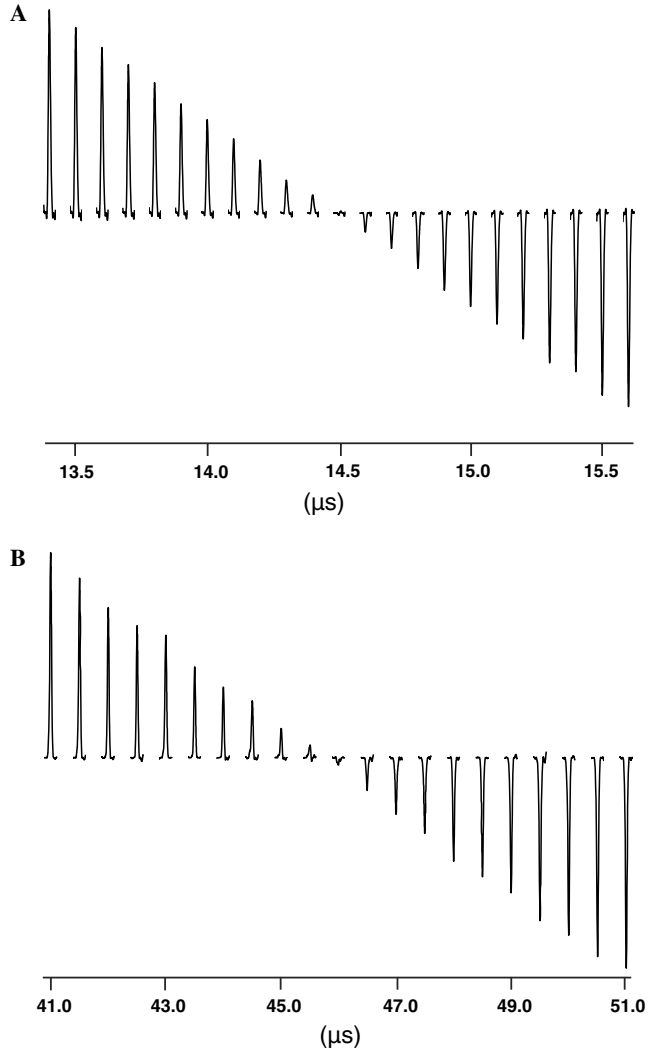

Fig. 3. CALIS-1 spectra acquired on a 0.4 mM $^{13}C/^{15}N$ labeled sample of the protein CI-2 in 90% H_2O/10% D_2O (v/v) on a Varian Unity Inova 800 MHz spectrometer using a cold probe and with 32 scans and adiabatic decoupling. The values of τ utilized were set according to standard $^{1}J_{CH}$ and $^{1}J_{NH}$ values of 140 and 90 Hz, respectively. The carrier frequency was in both cases placed close to the resonance frequency for the attached heteronuclear spin used for calibration. (A) CALIS-1 spectra (^{13}C) of the methyl group with the highest field proton resonance. The zero crossing was determined to occur at $t(\pi/2,^{13}C) = 14.5$ μs. (B) CALIS-1 spectra (^{15}N) of the amide proton with the lowest field proton resonance. The zero crossing was determined to occur at $t(\pi/2,^{15}N) = 45.8$ μs.

In conclusion, we have introduced the CALIS-1 and CALIS-2 pulse sequences for calibration of the RF field strength for indirectly observed nuclei. Apart from a reasonable guess of the pertinent heteronuclear coupling constant to create magnetization suitable for coherence transfer there are no parameters to adjust in these pulse sequences. The RF field strength can be determined with any desired accuracy. The calibration can always be performed on the sample to be investigated no matter whether it is isotopically enriched or at the natural abundance level of the pertinent isotopes.

Acknowledgments

The protein CALIS-1 spectra were recorded on the Varian Unity Inova 800 MHz spectrometer of the Danish Instrument Center for NMR Spectroscopy of Biological Macromolecules.

References

[1] H. Kogler, O.W. Sørensen, G. Bodenhausen, R.R. Ernst, Low-pass *J* filters. Suppression of neighbor peaks in heteronuclear relayed correlation spectra, J. Magn. Reson. 55 (1983) 157–163.

[2] T. Schulte-Herbrüggen, A. Meissner, A. Papanikos, M. Meldal, O.W. Sørensen, Optimizing delays in the MBOB, broadband HMBC, and broadband XLOC NMR pulse sequences, J. Magn. Reson. 156 (2002) 282–294.

[3] N.T. Nyberg, J.Ø. Duus, O.W. Sørensen, Heteronuclear two-bond correlation: suppressing heteronuclear three-bond or higher NMR correlations while enhancing two-bond correlations even for vanishing $^{2}J_{CH}$, J. Am. Chem. Soc. 127 (2005) 6154–6155.

[4] D.M. Thomas, M.R. Bendall, D.T. Pegg, D.M. Doddrell, J. Field, Two-dimensional ^{13}C–^{1}H polarization transfer *J* spectroscopy, J. Magn. Reson. 42 (1981) 298–306;
D.M. Doddrell, D.T. Pegg, M.R. Bendall, P. Caha, in: "Bruker Report" vol. 6, 1980, pp. 18.

[5] N.C. Nielsen, H. Bildsøe, H.J. Jakobsen, O.W. Sørensen, Pulse techniques for calibration of the decoupler radiofrequency field strength, J. Magn. Reson. 66 (1986) 456–469.

[6] G. Esposito, L. Nadalin, G. Verdone, A. Corazza, P. Viglino, M. Fredigo, Improved method for pulse width calibration in indirectly detected experiments, Magn. Reson. Chem. 39 (2001) 249–250.

[7] O.W. Sørensen, M. Rance, R.R. Ernst, *z* filters for purging phase- or multiplet-distorted spectra, J. Magn. Reson. 56 (1984) 527–534.

[8] A.P. Nanzer, F.M. Poulsen, A.E. Torda, W.F. van Gunsteren, A reassessment of the structure of chymotrypsin inhibitor CI2 using time averaged NMR restraints, Biochemistry 33 (1994) 14503–14511.