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Communication

Rapid pulse length determination in high-resolution NMR

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Abstract

The 90°(^{1}H) pulse length can be determined in a single scan using a simple homo-gated decoupling/nutation experiment. We show that the method is fast, accurate and readily amenable to automation.

Keywords: Nutation spectroscopy; 90° pulse; Homo-gated decoupling; Automation

1. Introduction

The sensitivity of ^{1}H NMR experiments depends on the accurate knowledge of the 90° pulse length. On high-field NMR spectrometers, this pulse length critically depends on the chemical properties of the sample, in particular its ionic strength. Traditionally, the 90° pulse length is determined by a series of one-dimensional NMR experiments that aim to measure either the 180° or 360° pulse length [1,2]. Fourier transformation of a series of experiments recorded with systematically incremented pulse length can be used to derive the radiofrequency field strength from peak positions in a frequency spectrum [3]. Although pulse length determinations are easy to perform and take little time, they have to be performed so frequently that automation would be desirable. In the present project, we explored a nutation method that delivers the 90°(^{1}H) pulse length in a single scan. Pulse length determination by nutation spectroscopy is an established method in MR imaging [4,5]. Our results show that the method produces accurate results with an accuracy of about 0.5% under the stringent conditions of high-resolution NMR spectroscopy for different situations of multi-line NMR spectra and is readily amenable to automation.

2. Results and discussion

Nutation spectroscopy is closely related to homo-gated decoupling [6], i.e., the sample is irradiated during the data acquisition by dividing each dwell time into a part for radiofrequency (RF) irradiation and a part for sampling a data point [7]. No excitation pulse precedes the scan. If the sample contains only a single resonance and the carrier frequency is positioned on-resonance, the magnetization nutates around the axis of the RF pulses. The resulting FID records the projection of the magnetization onto the transverse plane. Fourier transformation of the FID yields the nutation spectrum (Fig. 1). In the absence of off-resonance signals, the projection of the magnetization oscillates along a single axis, resulting in a nutation spectrum that is symmetric around zero frequency. The peak separation reflects the average nutation frequency during the scan and its inverse is proportional to the duration of a 360° pulse.

Several points must be observed to obtain the accurate 90° hard pulse, i.e., the length of the 90° pulse at high power. (i) It is recommended to perform the

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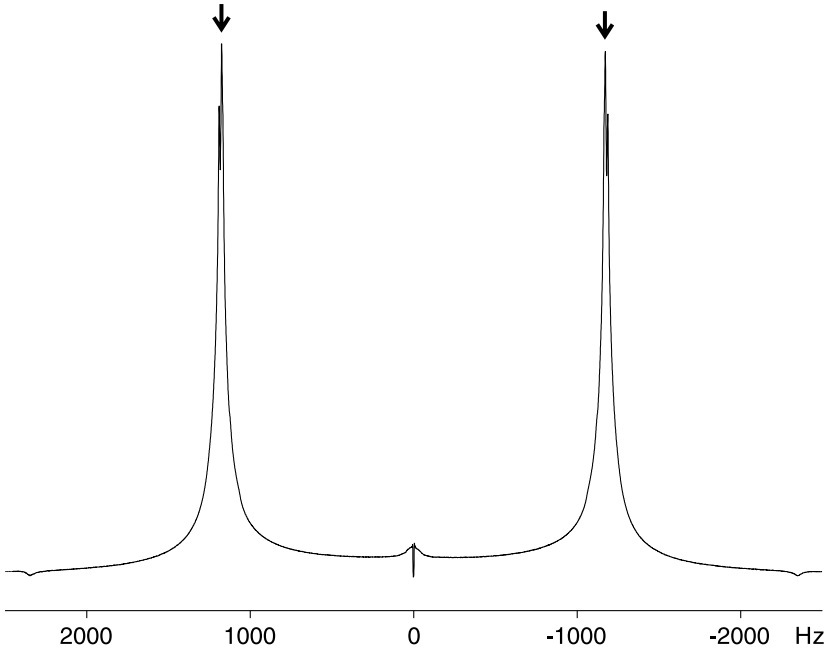

Fig. 1. Nutation spectrum of a 3.6 mM solution of hen egg white lysozyme in 90% H_2O/10% D_2O, pH 7.0 acquired at 25 °C on a Bruker Avance 800 MHz NMR spectrometer. The spectrum was recorded with a power level attenuated 16-fold compared to that of the hard 90° pulse, using a duty cycle of 80%. A magnitude spectrum was calculated after Fourier transformation. A hard 90° pulse of duration $\tau_{90} = 10.65$ μs was derived using $\tau_{90} = d/(32 \times \Delta\nu)$, where d is the duty cycle and $\Delta\nu$ the frequency difference between the peaks identified by arrows. For comparison, a conventional pulse length determination searching for the null point of the water after a 360° pulse yielded $\tau_{90} = 10.63$ μs.

nutation experiment with reduced power to avoid sample heating. In addition, cryogenic probes may not tolerate the application of high power during the full duration of the FID in the case of large duty cycles. (ii) Calculation of the 90° hard pulse from an experiment recorded with attenuated power requires linear amplifiers or, alternatively, a calibration procedure. On our NMR spectrometer, the linearity of the amplifiers is achieved by software correction tables, resulting in predictable and accurate scaling of the RF power. (iii) The duty cycle must be taken into account. We use to record all NMR spectra with a digital filter and oversampling [8], i.e., the dwell times are 32-fold shorter than they would be without oversampling. In our hands, accurate pulse lengths were obtained with an 80% duty cycle (i.e., RF-irradiation during 80% of each dwell time), but not with 20% duty cycle. The duty cycle actually used by the spectrometer (displayed by the status parameter HDDUTY in the file acqus on our Bruker AV800 NMR spectrometer) was about 1% shorter than its nominal value in the parameter file acqu. Consequently, the value calculated for the hard 90° pulse length assuming an 80% duty cycle should have been too long by 1%. This effect was fortuitously compensated by the fact that the nutation peak shows a weak tail towards lower frequencies, corresponding to regions in the sample, where the RF field is both weak and inhomogenous. The magnetization in these regions dephases rapidly during the nutation experiment. Therefore, the maximum of the nutation peak is primarily determined by the most homogeneous region of the RF field distribution and the corresponding pulse length tends to be shorter than that determined from a 180° or 360° rotation, compensating the error made by assuming that the duty cycle was 1% longer than the actually used value. Keeping these deviations in mind, duty cycles other than 80% will work too. To maintain control over the actually used duty cycle, it is recommended to use the same acquisition parameters for all pulse length determinations. (iv) The nutation spectrum must be recorded with the carrier frequency at or close to an NMR signal. If the carrier frequency is off-resonance, the effective rotation frequency ω_{eff} increases according to

$$\omega_{\text{eff}} = \sqrt{\omega_1^2 + \Omega^2}, \tag{1}$$

where ω_1 denotes the RF field strength and Ω the difference between the RF and the Larmor frequency. Small off-resonance effects are well tolerated. For example, for a nutation frequency of 1000 Hz, a 100 Hz off-set creates less than 0.5% error in the pulse length determination. (v) Radiation damping during the nutation experiment reduces the average nutation frequency. However, the magnetization defocuses during the nutation experiment due to RF inhomogeneity [9–11], eliminating radiation damping towards the end of the FID. The positions of the peak maxima in the nutation spec-

trum are thus hardly affected by radiation damping, making pulse length determinations that use the water resonance particularly straightforward and accurate.

The nutation method also yields accurate ^{1}H pulse length determinations for organic compounds in deuterated solvents which often display many ^{1}H NMR peaks of similar intensity without a dominant solvent resonance. In this situation, the nutation spectrum displays several peaks and the 90° pulse must be derived from the symmetric pair of peaks with the lowest frequency (disregarding any peak at zero frequency). The carrier frequency must be placed at the frequency of one of the resonances, since any off-resonance peak will appear at an increased frequency, regardless of whether it is located high-field or low-field from the carrier (see Eq. (1)). An example is shown in Fig. 2. Placing the carrier frequency on the most intense resonance, several signals are obtained. The correct pulse length is derived from the peak shoulder near the peak maximum. Using the peak maxima instead, a 0.5% shorter pulse length would have been derived. This value still is within the error of pulse length determination by the conventional method of minimizing the signals after a 360° pulse, considering that off-resonance effects result in different effective flip-angles at different offsets Eq. (1). For example, a 360° pulse based on the pulse length derived from the inner shoulders in Fig. 2B yielded the spectrum of Fig. 2C, where the resonances near the carrier frequency have nearly disappeared. Shortening the duration of the 360° pulse to minimize the integral of the off-resonance signals required an almost 1% shorter pulse (Fig. 2D). Picking the peak maxima instead of the peak shoulders in the nutation spectrum of Fig. 2B would thus have yielded the 90° pulse length with perfectly adequate accuracy.

The nutation method would be expected to become less accurate when the signal overlap is severe and there is no dominant line in the NMR spectrum. Yet, using a solution of hen egg white lysozyme in D_2O and placing the carrier frequency on the biggest methyl signal (Fig. 3A), the pulse length determined by the nutation method was only 0.3% shorter than that determined by a conventional 360°-pulse determination, because the signals from different methyl groups could not be resolved in the nutation spectrum (Fig. 3B).

It is an important advantage of the nutation method that it is readily amenable to automation. This works

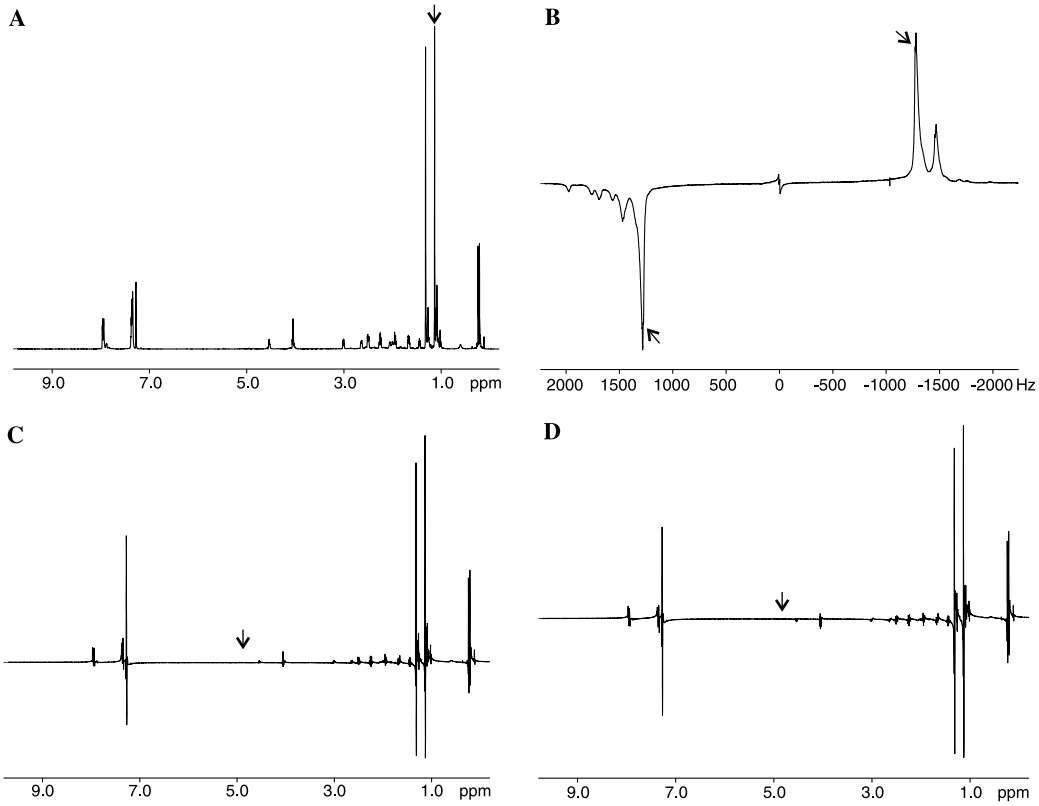

Fig. 2. ^{1}H NMR spectra of an organic compound, $C_{34}H_{53}O_5Si_2$, in deuterobenzene. (A) 1D NMR spectrum acquired after a 90° pulse. The arrow identifies the RF carrier frequency used for the acquisition of the nutation spectrum. (B) Phase-sensitive nutation spectrum. The arrows identify the peak shoulders used for the pulse length calculation, yielding 9.81 μs for the width of the hard 90° pulse. Using the peak maxima would have yielded a hard 90° pulse of 9.76 μs. (C) 1D NMR spectrum recorded after application of a 360° pulse of 4×9.81 μs duration. The carrier frequency is identified by an arrow. (D) 1D NMR spectrum acquired after a pulse of 4×9.73 μs duration. The pulse minimized the residual intensities of the methyl resonances at 1.2 ppm, but was a bit too short for the resonances near the carrier frequency (arrow). Spectra (C and D) were vertically expanded about 25-fold compared to the spectrum in (A).

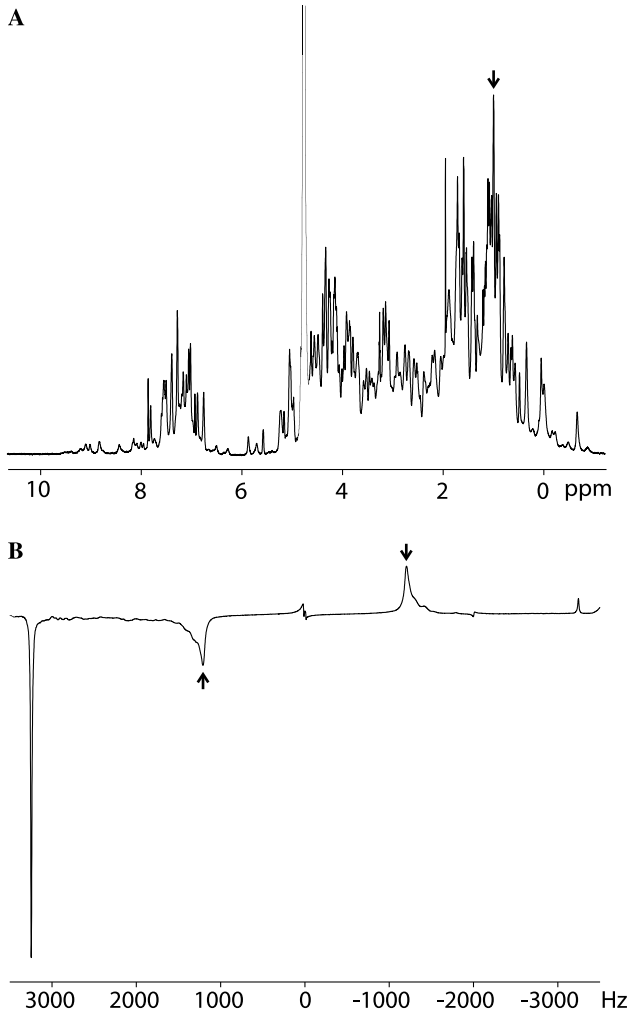

Fig. 3. ^{1}H NMR spectra of a 4 mM solution of hen egg white lysozyme in D_2O. (A) 1D NMR spectrum acquired following a 90° pulse. The arrow identifies the carrier frequency used for the acquisition of the nutation spectrum. (B) Phase sensitive nutation spectrum. Arrows identify the peaks used for the pulse length calculation. The residual HDO signal gives rise to the signals at ±3246 Hz.

particularly well with samples containing large solvent peaks of known chemical shift. A single nutation scan followed by Fourier transformation, magnitude calculation and automatic peak picking of the two biggest signals provides the necessary information to calculate the 90° pulse length (Fig. 1). In the case of NMR spectra with multiple signals, a single-scan 1D ^{1}H NMR spectrum must be recorded first in order to identify the frequency of the biggest signal. In the presence of two or more intense signals, the relevant nutation peaks can be distorted by a magnitude calculation and may not be the most intense signal in the nutation spectrum (Figs. 2B and 3). In this situation we recommend a Fourier transform without magnitude calculation, picking of the peaks with the lowest absolute frequency that can be found in the nutation spectrum in the prospective regions of interest and determination of their frequency with respect to the carrier frequency. If a small potential error in pulse length is acceptable, picking the largest peak in the relevant region of the nutation spectrum and its mirror image on the other side of the carrier frequency will be easier to automate.

Knowledge of the 90°(^{1}H) pulse length is required for the optimal performance of a myriad of NMR experiments. Automation of its determination is important for experiments performed non-interactively with automatic sample changers and in LC–NMR, when different solvent and buffer compositions result in different ionic strength. In addition, automated determination of the 90°(^{1}H) pulse length widens the scope of NMR spectroscopy for non-experts by automating the entire setup of NMR experiments, since parameter sets are usually available that only require an update of the 90°(^{1}H) pulse length. Tuning, matching, and shimming of the probe head is already highly automated on current NMR systems. The nutation method may also be used for the determination of ^{13}C- and ^{15}N-pulses, if a sufficiently intense signal is available. These pulse lengths, however, are much less sensitive with regard to sample properties, and heteronuclear experiments usually can be successfully performed by the use of default values for ^{13}C- and ^{15}N-pulse lengths.

Finally, the exceptional speed with which the 90° pulse can be determined by nutation spectroscopy can be exploited, for example, to minimize the dead time in H/D exchange experiments which are initiated by dissolving a dry compound in D_2O.

3. Conclusions

Although nutation spectroscopy may not be the only way to automate the determination of the 90°(^{1}H) pulse, it is particularly fast, straightforward, and accurate. It accelerates an essential but uninteresting part of the setup of advanced NMR experiments.

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