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Efficient dipolar double quantum filtering under magic angle spinning without a ^{1}H decoupling field

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ABSTRACT

We present a systematic study of dipolar double quantum (DQ) filtering in ^{13}C-labeled organic solids over a range of magic-angle spinning rates, using the SPC-n recoupling sequence element with a range of n symmetry values from 3 to 11. We find that efficient recoupling can be achieved for values $n \geqslant 7$, provided that the ^{13}C nutation frequency is on the order of 100 kHz or greater. The decoupling-field dependence was investigated and explicit heteronuclear decoupling interference conditions identified. The major determinant of DQ filtering efficiency is the decoupling interference between ^{13}C and ^{1}H fields. For ^{13}C nutation frequencies greater than 75 kHz, optimal performance is observed without an applied ^{1}H field. At spinning rates exceeding 20 kHz, symmetry conditions as low as $n = 3$ were found to perform adequately.

1. Introduction

Pulse sequences that excite double-quantum (DQ) coherence give the experimenter access to a wealth of structural information and techniques for simplifying the interpretation of NMR spectra of biomolecules. In addition to the ubiquitous application of suppressing natural abundance background signals, DQ coherence can be used to measure vector (torsion) angles in peptide backbones [1–5] and polymers [6], study dynamics [2], and simplify chemical shift assignment [7].

Homonuclear recoupling sequences that rely on zero-quantum mixing—such as dipolar-assisted rotational resonance (DARR) [8], RFDR [9] or CORD–RFDR [10]—provide robust methods for gaining information about spatial proximity but lack selectivity of individual spin pairs and are prone to multi-spin sequential polarization transfers that complicate interpretation. Adiabatic mixing schemes that rely on DQ excitation with low power carbon fields do not permit the isolation of that DQ coherence [11,12]. Mixing schemes traditionally used for the excitation and isolation of homonuclear DQ coherence—such as C7 [13], POST-C7 [14], CMR7 [15], and SPC-5 [16]—have broad bandwidth but require ^{1}H decoupling fields during mixing that are at least three times the ^{13}C field strength [9,17]. The high power ^{1}H decoupling is necessary to avoid the broad heteronuclear interference described in relation to C and R symmetry sequences [18]. This requirement significantly limits the usage of C symmetry sequences not only for the measurement of dipolar couplings, but chemical shift tensors, through the use of sequences like ROCSA [33], which are valuable for developing extremely high resolution protein structures [34].

At higher MAS rates low-power ^{1}H decoupling both during chemical shift evolution and detection are common, and investigations have made clear that at high MAS rates low-power ^{1}H decoupling during recoupling was feasible [17]. While high MAS solves many problems for both resolution and sensitivity for ideal samples, many important systems such as amyloids exhibit inhomogeneous broadening that negates the advantages of long transverse relaxation rates (T_2) at high MAS rates requiring the use of larger capacity rotors to maintain sufficient sensitivity. Additionally, at present all commercially available dynamic nuclear polarization instruments require the use of rotors with maximum MAS frequency of 25 kHz [35]. These experimental limitations motivate the investigation of low-power ^{1}H decoupling at moderate to high MAS rates. While low power proton decoupling during dipolar recoupling has been reported previously [14,18–20,30–32], double quantum filtering (DQF) has not been fully explored in the context of difficult proteins with limited relaxation times and inhomogeneous broadening. Here we present an exploration of the low power decoupling regime for the SPC-n family of recoupling sequences that generalizes the SPC-5 recoupling sequence to

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arbitrary symmetry conditions [16]. We confirm the feasibility of DQ spectroscopy with SPC-*n* double-quantum filters in the absence of decoupling from 13 to 40 kHz MAS rates. We show experimental results for U—^{13}C,^{15}N—N-acetylvaline and U—^{13}C,^{15}N-α-synuclein that demonstrate DQ excitation over the full chemical shift range with high efficiency. These conditions are thus well suited for the study of temperature-sensitive systems and for incorporation into pulse sequences that make use of proton detection and other fast MAS techniques.

2. Materials and methods

2.1. Materials

Uniformly ^{13}C—^{15}N-labeled wild-type α-synuclein was prepared following the method of [21] and fibrils were prepared for solid-state NMR (SSNMR) as described in [22] and packed into a 1.6 mm FastMAS rotor.

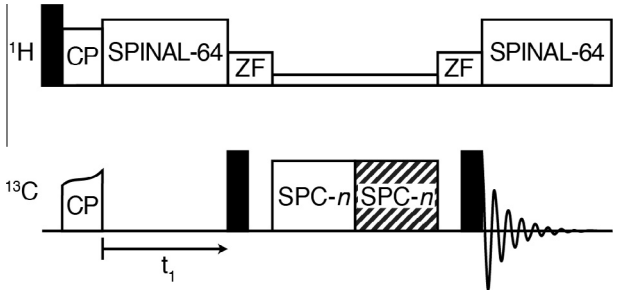

Fig. 1. Pulse sequence utilized for the evaluation of DQ excitation with ^{13}C—^{13}C SPC-*n* homonuclear mixing. The hatching on the second half of the SPC-n period indicates that the phase cycle is shifted by 90° every other scan (with a 180° phase shift of the receiver), according to Ref. [16].

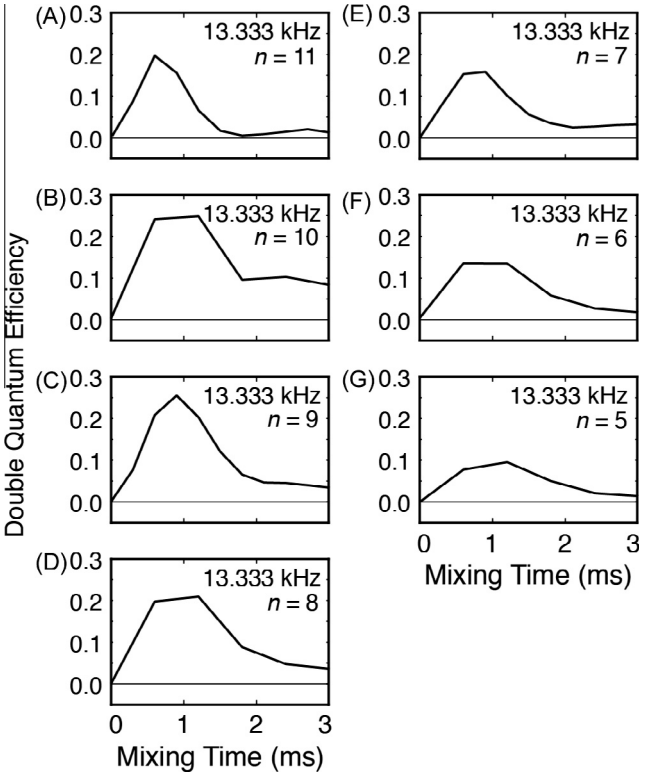

Fig. 2. DQF efficiency as a function of mixing time for α-synuclein at a range of symmetry conditions and a constant MAS rate of 13.333 kHz. The ^{1}H decoupling field is off.

2.2. Solid-state NMR spectroscopy

All experiments were conducted on a 17.6 T (750 MHz) Varian VNMRS spectrometer (Agilent Technologies, Santa Clara, CA) equipped with a FastMAS probe tuned to ^{1}H—^{13}C double resonance. The ^{1}H and ^{13}C $\pi/2$ pulse widths for N-acetyl-L-valine (NAV) were 1.80 and 1.80 μs, respectively, and were 1.75 and 1.70 μs for α-synuclein. MAS frequencies were controlled with a Varian MAS controller to within ±5 Hz at 13.333 kHz MAS and ±15 Hz at 40 kHz. All experiments were carried out with the variable temperature gas at 0 °C, which corresponded to actual sample temperatures of 5 ± 3 °C at 13.333 kHz MAS, 15 ± 5 °C at 40 kHz MAS and intermediate values at moderate spinning rates.

All data were collected using the pulse sequence shown in Fig. 1. In this work the mixing sequence was tested in the context of a standard ^{13}C—^{13}C, 2D experiment with an initial ^{1}H 90° pulse followed by tangent-ramped ^{1}H—^{13}C cross polarization before the indirect evolution period. The carbon magnetization is then placed along the *z*-axis during a short *z*-filter, the SPC-*n* recoupling is

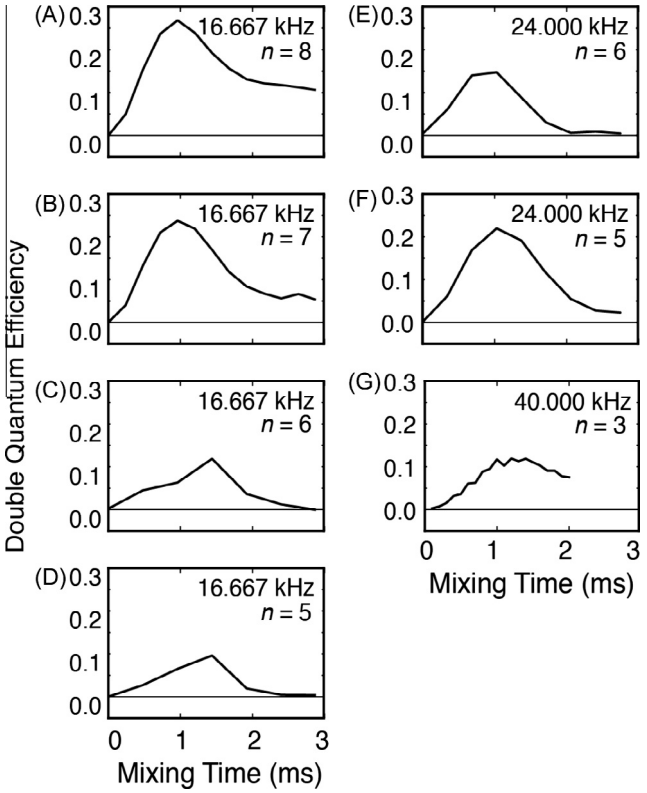

Fig. 3. DQ efficiency as a function of mixing time for α-synuclein MAS rates ranging from 16.667 to 40.000 kHz.

Table 1
Dipolar scaling factors for the symmetry conditions examined. Values calculated using Eq. (2).

SPC symmetry	Dipolar scaling factor
n = 3	0.113
n = 4	0.171
n = 5	0.203
n = 6	0.221
n = 7	0.232
n = 8	0.240
n = 9	0.245
n = 10	0.248
n = 11	0.251