



Communication

Quantitative cross-polarization at magic-angle spinning frequency of about 20 kHz

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ABSTRACT

We study the QUantitative Cross-Polarization (QU-CP) method proposed by Hou et al. (Chem. Phys. Lett. 421 (2006) 356) under the moderate MAS speed of 23 kHz, re-examining its two building blocks, namely, the CP polarization transfer from ¹H to ¹³C, and the thermal equalization of the ¹³C magnetizations. We show that the nuclear-integrated cross-polarization (NI-CP) scheme is conveniently used for ¹H–¹³C polarization transfer, because of its simplicity, robustness to rf-mismatch, and compatibility with fast sample spinning. In the mixing part, in addition to dipolar-assisted rotational-resonance (DARR) recoupling, we examine the Phase-Alternated Recoupling Irradiation Schemes (PARIS and PARIS_{xy}), and Second-order Hamiltonian among Analogous Nuclei Generated by Hetero-nuclear Assistance Irradiation (SHANGHAI) sequences, and show that SHANGHAI gives the best performances in equalizing the ¹³C magnetizations.

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1. Introduction

Cross-polarization (CP) [1] is a standard technique in solid-state nuclear magnetic resonance (NMR) to enhance the magnetization, and thereby the sensitivity, of nuclei with low gyromagnetic ratios, such as ¹³C or ¹⁵N, and/or long longitudinal relaxation times. The main problem of CP is that the resulting peak intensities do not reflect the molar fractions of the spins. One solution is to use Nuclear Overhauser Polarization, as proposed by Takegoshi et al. [2,3] which is applicable to uniformly ¹³C-labeled molecules that include methyl groups. Another way is to realize a QUantitative CP (QU-CP) experiment, as proposed by Hou et al. [4,5]. In this method, the conventional CP transfer was followed by a Dipolar-Assisted Rotary-Resonance (DARR) [6,7] thermal equalization of the ¹³C magnetizations of the various groups, including carbonyl, aromatic, and aliphatic carbons. The DARR recoupling consists of proton irradiation with a constant rf-field whose amplitude is usually equal to the spinning speed, $\nu_{\text{mix},1\text{H}} = \nu_{\text{R}}$, to benefit from the rotary resonance recoupling (R³) process [8]. This experiment demonstrated uniform enhancement of the ¹³C spectrum, independent of the experimental parameters for CP, and irrespective of whether the initial ¹H magnetization is in thermal equilibrium [9].

The original QU-CP experiments were demonstrated under magic angle spinning (MAS) at $\nu_{\text{R}} = 12\text{--}14$ kHz. Meanwhile, progress in the probe technology has pushed up the attainable spinning speeds. High MAS frequencies increase spectral resolu-

tion, spectral width in rotor-synchronized experiments, and sensitivity per sample volume, as well as they decreased losses and spinning sideband amplitudes at high magnetic fields. However, it must be reminded that all sequences usable for thermal equilibration are second-order methods, which means that their efficiencies are inversely proportional to the spinning speed. In fact, the DARR efficiency was reported to be poor under ultra-fast MAS ($\nu_{\text{R}} \geq 50$ kHz) [10]. The use of thermal equilibration can thus be better performed with moderate to fast speeds ranging from $\nu_{\text{R}} = 20$ to 35 kHz.

However, at high MAS frequencies, 'classical' CP becomes less robust against rf-amplitude mismatches. Indeed, the Hartmann–Hahn matching profile splits into five narrow bands separated by the spinning frequency. As a consequence, small deviations in the two rf amplitudes from their optimum values degrade the efficiency of ¹H → ¹³C magnetization transfer. In addition, it is not clear if DARR is efficient under moderate to fast MAS from the viewpoint of homogenizing the ¹³C magnetizations.

In this work, we examine the efficiencies of the two building blocks of the QU-CP sequence, namely, the CP and equalization parts, at the moderate MAS frequency of $\nu_{\text{R}} = 23$ kHz. We show that the nuclear-integrated cross-polarization (NI-CP) [11,12] sequence gives good performance of polarization transfer from ¹H to ¹³C. NI-CP employs an adiabatic frequency sweep on the ¹H channel from far off-resonance toward on-resonance, and was proven to be robust against the deviation in the rf-amplitude. Thus, NI-CP is expected to be compatible with fast MAS speeds.

Regarding the equalization of the ¹³C magnetizations, several variations of DARR have been proposed recently to improve the

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performance of ^{13}C mixing under fast MAS. They include Phase-Alternated Recoupling Irradiation Schemes (PARIS [13–15] and PARIS_{xy} [16,17]) and Second-order Hamiltonian among Analogous Nuclei Generated by Hetero-nuclear Assistance Irradiation (SHANGHAI) [18]. In the following, we compare these methods by examining the intensities of the ^{13}C peaks for various mixing times.

2. Experimental

All NMR experiments were performed at room temperature in a magnetic field of 9.4 T with a home-built NMR spectrometer [19–21], and a Varian 3.2 mm T3 probe. The spinning speed was set at $\nu_R = 23$ kHz. The carrier frequencies for the ^1H and the ^{13}C channels were 400.2 MHz and 100.6 MHz, respectively. We have used two polycrystalline samples of *L*-histidine: one uniformly ^{13}C - ^{15}N labeled and the second without any isotopic labeling. During acquisition of the ^{13}C signals, TPPM decoupling was applied to the ^1H spins with the rf intensity, the pulse width, and the phases of 100 kHz, 5 μs , and $\pm 7.5^\circ$.

Fig. 1 shows a single pulse ^{13}C spectrum of the labeled compound, without any initial cross-polarization from the protons. The atomic sites are labeled with the IUPAC nomenclature [22], and the peaks are assigned according to Refs. [23–25]. In order to estimate the spin-lattice relaxation time of the ^{13}C spins, we carried out a ^{13}C saturation recovery experiment, and found that the longest relaxation time was $T_{1,^{13}\text{C}} = 34$ s for peak C4. Accordingly, the spectrum in Fig. 1 was recorded with a recycle delay of 600 s, which is much longer than the longest $5T_{1,^{13}\text{C}}$ value, so that the spectrum reflects the ^{13}C magnetization at thermal equilibrium.

3. CP transfer

Fig. 2a depicts the NI-CP pulse sequence. Unlike the conventional CP scheme (Fig. 2b), NI-CP does not employ an initial $\pi/2$ -pulse in the ^1H channel just before the contact time. Instead, the frequency of ^1H irradiation is adiabatically swept from far off-resonance toward on-resonance during the contact time τ_{CP} . The ^1H irradiation was tangentially turned on at the beginning of the frequency sweep. This adiabatic sweep serves for locking the ^1H magnetization along the effective field, which is initially parallel to the

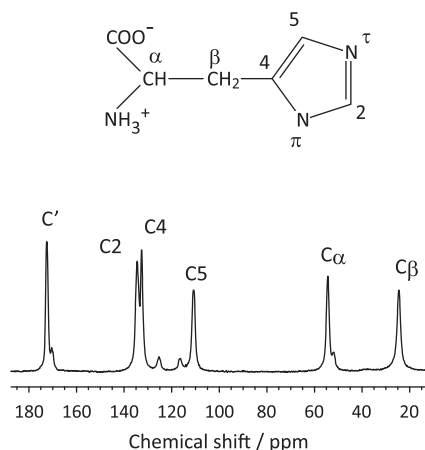


Fig. 1. ^{13}C MAS spectrum of a powder sample of uniformly ^{13}C , ^{15}N labeled *L*-histidine. The static field and the spinning frequency were 9.4 T and 23 kHz, respectively. TPPM decoupling was performed during acquisition. The ^{13}C signals were accumulated over 100 times with a delay interval of 600 s. The carbon and the nitrogen sites are numbered with the IUPAC nomenclature [22]. The peaks are assigned according to Refs. [23–25]. The relatively small, unassigned peaks are due to impurities.

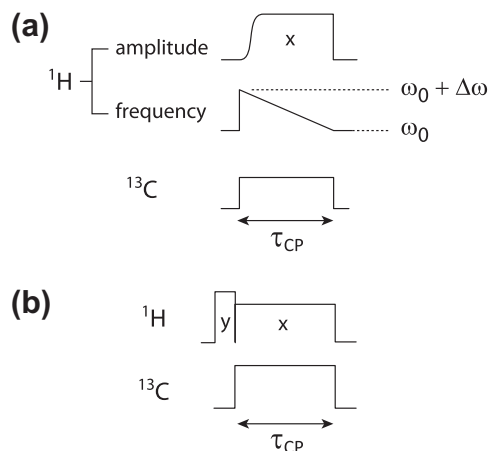


Fig. 2. Pulse sequences for (a) NI-CP and (b) the conventional CP.

static field and then gradually tilted as the frequency approaches the ^1H Larmor frequency. In NI-CP, the spin-lock is efficient even in the presence of considerable spectral distribution of the spin packets compared to the rf intensity. Indeed, since the effective field magnitude varies considerably during the sweep, all spin packets experience the Hartmann–Hahn matching and participate in the process of polarization transfer for a wide range of rf amplitudes. Thus, NI-CP is expected to be robust against errors in rf-amplitude mismatch.

In order to optimize the NI-CP sequence, we examined the dependence of the ^{13}C magnetizations on the contact time, τ_{CP} . The result is shown in Fig. 3. The intensities of the peaks are normalized to those obtained with the single-pulse experiment with a sufficiently long recycling interval (Fig. 1). Each ^{13}C peak has its own optimal contact time (see Fig. 3a). For carbons that are covalently bonded to protons, the maximum CP enhancement factor was attained with contact times ranging from $\tau_{\text{CP}} = 0.75$ to 1.25 ms, while the others showed much slower build-up behavior. In Fig. 3b is plotted the net ^{13}C magnetization versus the contact time (the first point in FID), which indicates that its overall best value was $\tau_{\text{CP,opt}} \approx 1$ ms.

Next, we examined the robustness of the conventional CP and NI-CP sequences against mismatch in the rf intensity. Fig. 4 shows the measured enhancement factors of the ^{13}C magnetizations for various rf amplitudes ranging from 40 to 100 kHz for the ^1H spins, and from 20 to 80 kHz for the ^{13}C spins. In the case of the conventional CP, we found that the Hartmann–Hahn matching profiles split into five sidebands that were separated by the spinning frequency, exhibiting diagonal ridges in the contour plots for all ^{13}C peaks (Fig. 4a–d). The data indicate that the two rf amplitudes have to be adjusted within an error of ca. 10%. In addition, care should be taken to make sure that the rf powers are stable over the experimental time. In NI-CP, on the other hand, polarization transfer was efficient for a wider range of the rf amplitudes, as demonstrated in Fig. 4e–h. This is due to the fact the amplitude of the ^1H effective field varies over a considerable range during the frequency sweep. Thus, one can expect that deviation in the rf intensity would not affect the performance of polarization transfer very much. Regarding to variation of the effective field, ramp-CP may be an alternative to NI-CP, as it employs ramping of the rf intensity in either channel, which would broaden the profile of Hartmann–Hahn matching in the conventional CP plotted in Fig. 4a–d. Usually, the ramping is performed over 10–20% of the nominal rf intensity. On the other hand, the ^1H effective field varied over an order of magnitude wider range in the present NI-CP experiment, so that the requirement for the accuracy in the rf amplitudes is much less demanding.

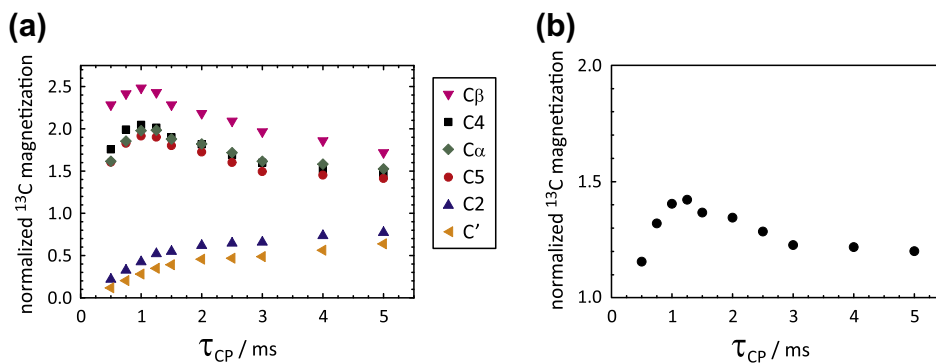


Fig. 3. (a) Evolution with the contact time τ_{CP} of the ^{13}C CP enhancement factors in uniformly ^{13}C labeled L-histidine obtained using the NI-CP sequence. These factors are calculated as the ratios of peak heights in NI-CP spectra to those in the single-pulse spectrum of Fig. 1. The global average CP enhancement factor is plotted in (b) as function of τ_{CP} . During the contact time, adiabatic frequency sweep was performed at the ^1H spins from +400 kHz off-resonance to on-resonance. The frequency was updated every 1 μs with a linear decrement, so that the frequency is on-resonance at the end of the sweep. The rf amplitudes were set at $\nu_{\text{CP},1\text{H}} = 55$ kHz and $\nu_{\text{CP},13\text{C}} = 74$ kHz in the ^1H and the ^{13}C channels, respectively. The signals were accumulated over 100 times with a recycle delay of 3 s.

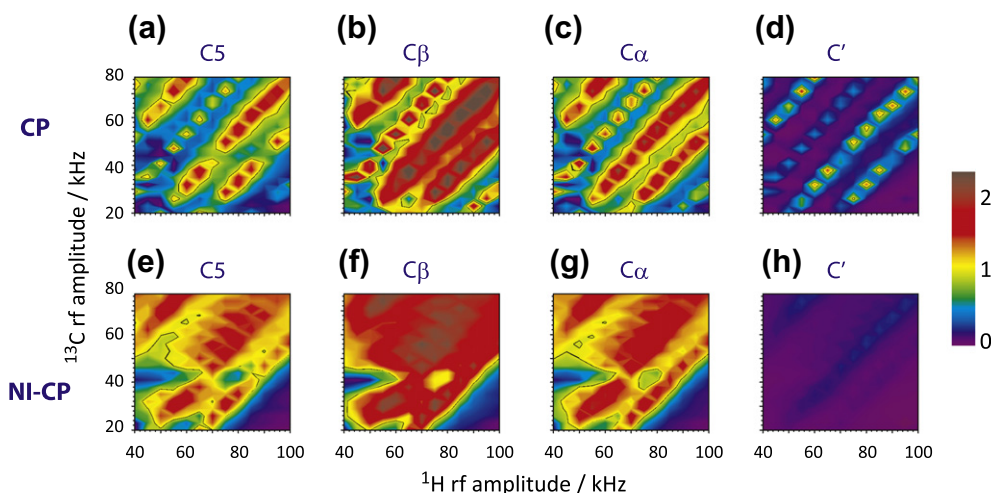


Fig. 4. Experimentally measured normalized enhancement factors of the ^{13}C magnetizations obtained in labeled L-histidine by: (a–d) conventional CP and (e–h) NI-CP. In the ^1H channel, the rf intensity was varied from 40 to 100 kHz with an increment of 5 kHz, whereas it was incremented from 20 to 80 kHz with a 3 kHz step in the ^{13}C channel. (a and e) $\text{C}\beta$, (b and f) $\text{C}\alpha$, (c and g) $\text{C}\gamma$, and (d and h) C' . The contact time was 1 ms for both conventional CP and NI-CP. The other experimental parameters are identical to those indicated in the caption of Fig. 3.

Another notable feature of NI-CP is that the sequence does not use an initial $\pi/2$ -pulse, which makes the implementation of the sequence simpler. As long as the frequency is adiabatically swept from off-resonance to on-resonance over a range wide enough to lock the entire ^1H spin packets, one has only to vary the contact time to optimize polarization transfer. Such frequency sweep that is required for NI-CP is easily implemented with modern spectrometers. It should be noted that the performances of both the conventional CP and NI-CP transfers were not very good for the carboxyl carbon (Fig. 4d and h), which is relatively far away from the ^1H nuclei. Nevertheless, one can expect obtaining the quantitative ^{13}C spectrum by mixing the ^{13}C magnetizations, as described below.

It is worth noting here that adiabatic frequency sweeps can be applied simultaneously to the ^1H and ^{13}C spins. This strategy, known as Simultaneous ADiabatic Spin-locking CP (SADIS-CP) [12], is advantageous when the spectral width of the target spin is large, and would be of interest for, e.g., $^1\text{H} \rightarrow ^{14}\text{N}$ transfer. In the present case, however, the ^{13}C spin packets distribute by no more than ca. 15 kHz, and one cannot expect much superiority of SADIS-CP over NI-CP.

Fig. 5 compares measured Hartmann–Hahn matching profiles versus $\nu_{\text{CP},1\text{H}}$, with $\nu_{\text{CP},13\text{C}} = 80$ kHz, of the various CP schemes,

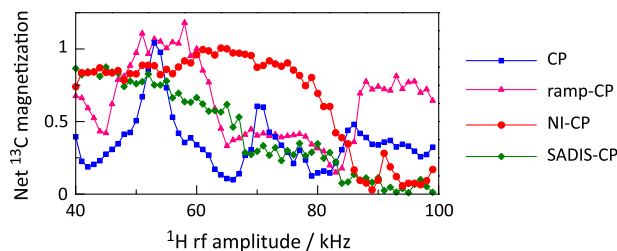


Fig. 5. Net ^{13}C magnetizations obtained experimentally in uniformly ^{13}C labeled L-histidine by conventional CP (squares), ramp-CP (triangles), NI-CP (circles), and SADIS-CP (diamonds). The intensity of the first point of the ^{13}C FID is plotted as a function of the ^1H rf intensity. The spinning speed was 23 kHz, and the contact time was 1 ms. In ramp-CP, the ^{13}C rf intensity was linearly varied from 68 to 92 kHz, whereas it was set to 80 kHz in other schemes. In NI-CP, the ^1H irradiation frequency was swept over 200 kHz from off-resonance toward on-resonance. In SADIS-CP, frequency sweep was also performed on the ^{13}C channel over 50 kHz. The recycle delay and the number of scans were 3 s and 40. The other experimental parameters are identical to those indicated in the caption of Fig. 3.

including conventional CP, ramp-CP, NI-CP, and SADIS-CP. Here, the first points of the ^{13}C FID are plotted, so that the data reflect the net ^{13}C magnetization in labeled L-histidine. In conventional

CP, the efficient polarization transfer was attained for a relatively narrow range around $\nu_{CP,1H} = \nu_{CP,13C} - \nu_R = 57$ kHz. As expected, the matching profile was broadened by employing ramp-CP. In the case of NI-CP, we found a notably flatter profile over a wide range of rf-amplitude, up to $\nu_{CP,1H} = 80$ kHz, above which the CP efficiencies were poor because the Hartmann–Hahn condition could not be fulfilled during the adiabatic sweep. Even though the optimal ^{13}C magnetizations obtained in CP and ramp-CP were larger than that in NI-CP, the robust feature of NI-CP against deviation in the rf intensity would find interest in many circumstances.

4. Thermal equalization

Fig. 6a depicts the pulse sequence leading to uniform enhancement of the ^{13}C magnetization. After polarization transfer from ^1H to ^{13}C by NI-CP, the ^{13}C magnetization is stored along B_0 . The ^{13}C spins are then allowed to exchange their magnetizations during the mixing time τ_{mix} . Here, the dipolar recoupling scheme was applied to the ^1H spins by one of the four options: DARR, PARIS, PARIS_{xy} , and SHANGHAI (Fig. 6b). Finally, the resultant ^{13}C magnetization is de-stored and observed under ^1H decoupling. In the following, we will analyze how these different schemes equilibrate the magnetization of the different ^{13}C sites and allow recording quantitative CP spectra. The criteria for this analysis are the required rf field, and the mixing time required to equilibrate the ^{13}C magnetizations of the different sites (the shortest τ_{mix} , the highest the sensitivity).

These four schemes use ^1H irradiation with a constant amplitude, $\nu_{\text{mix},1H}$, only the phases of this irradiation differ between the various sequences. In DARR, a steady phase is used, whereas the phase is changed every half rotor period in other schemes. In PARIS scheme, the phase is inverted in between x and $-x$. PARIS_{xy} employs a phase inverted irradiation cycled over mT_R followed by another cycle with the overall phase shifted by 90° . In this work, we used the most efficient PARIS_{xy} version with $m = 2$. In SHANGHAI, the four phases are super-cycled according to the sequence described in Fig. 6b. All recoupling schemes broaden the ^{13}C resonance lines during the mixing time, and the spectral overlap among the ^{13}C peaks drives exchange of their magnetizations.

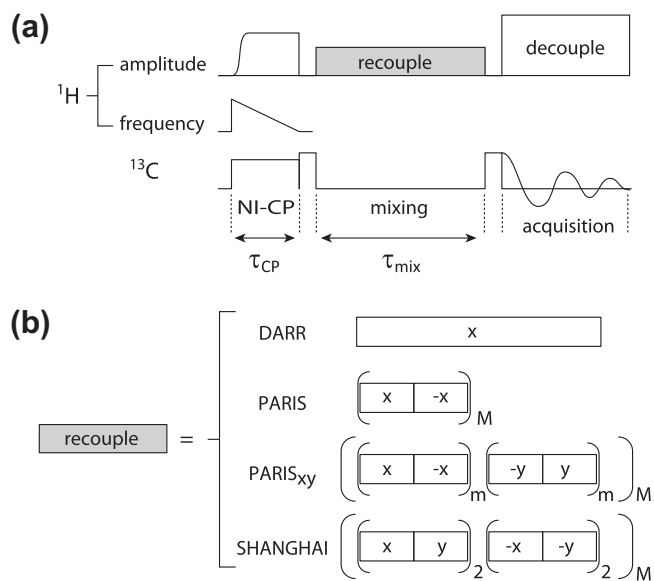


Fig. 6. (a) Pulse sequence for the proposed quantitative CP experiment. (b) Four recoupling schemes, DARR, PARIS, PARIS_{xy} , and SHANGHAI. The recoupling cycle is repeated M times for optimum ^{13}C thermal equilibration.

Fig. 7 shows ^{13}C spectra of L-histidine obtained using the sequence in Fig. 6 with $\tau_{CP,opt} = 1$ ms, and mixing times ranging from $\tau_{\text{mix}} = 0.1$ to 2 s. The rf intensity was set to $\nu_{\text{mix},1H} = \nu_R = 23$ kHz, which corresponds to the R^3 condition usually used for DARR. For all four recoupling schemes, the spectra were found to approach that observed in thermal equilibrium shown in Fig. 1 when τ_{mix} was long. For SHANGHAI (Fig. 7d), changes in the spectra were not discernible between $\tau_{\text{mix}} = 1$ and 2 s. This indicates that the ^{13}C spin system had already reached its equilibrium within a time interval of the order of one second. On the other hand, for the other three schemes (Fig. 7a–c), slight changes in the intensity of the carboxyl peak were appreciable between the spectra with $\tau_{\text{mix}} = 1$ and 2 s. These results suggest that, under the moderate MAS speed and rf-amplitude of $\nu_R = \nu_{\text{mix},1H} = 23$ kHz, which satisfy the R^3 condition, the required mixing time to achieve quantitative CP increases in the following order: SHANGHAI, DARR, PARIS_{xy} , and PARIS.

For comparison, we also examined thermal mixing by SHANGHAI in non-labeled L-histidine. We found little change in the peak intensities over the mixing times of up to 2 s, as shown in Fig. 7e. This result indicates that QU-CP would be impractical without ^{13}C labeling.

Fig. 8 compares the four schemes in terms of the evolution of the ^{13}C peak intensities for rf amplitudes lower than, and equal to, the R^3 condition: $\nu_{\text{mix},1H} = 15$ and 23 kHz. In all cases, the initial non-uniform ^{13}C magnetizations created by NI-CP evolved toward a common value. Here, we performed curve fitting of the build-up behavior of the C' magnetization, which was the smallest one just after NI-CP transfer. The obtained time constants, also indicated in Fig. 8, serve as a measure of the thermal equilibration efficiency; the shorter the build-up time is, the better the mixing scheme works. For the two rf amplitudes, we found that the overall ^{13}C exchange was faster in DARR and SHANGHAI compared to PARIS and PARIS_{xy} .

Very recently, the ^{13}C – ^{13}C recoupling efficiency versus $\nu_{\text{mix},1H}$ and the carbon frequency difference, $\Delta\nu_{\text{iso}}$, has been studied in detail [18]. It was shown that in the $(\Delta\nu_{\text{iso}}, \nu_{\text{mix},1H})$ plane there exist 'recoupling bands' leading to efficient ^{13}C exchanges, and that these bands are very different according to the sequence used. In DARR, the recoupling arising from the rotational resonance band (R^2B : $\Delta\nu_{\text{iso}} \approx \nu_R$) and that due to the band at $\Delta\nu_{\text{iso}} \approx \nu_{\text{mix},1H}$ cross with each other when $\Delta\nu_{\text{iso}} \approx \nu_{\text{mix},1H} \approx \nu_R$ leading to the broad recoupling R^3 zone [18]. PARIS, on the other hand, excites simultaneously two bands, one at $\Delta\nu_{\text{iso}} \approx 0$ (Zero Band: ZB) and one at $\Delta\nu_{\text{iso}} \approx \nu_R$ (R^2B), which are independent from the $\nu_{\text{mix},1H}$ rf value. With respect to PARIS, PARIS_{xy} presents additional recoupling zones also independent from the $\nu_{\text{mix},1H}$ value: at $\Delta\nu_{\text{iso}} \approx \nu_R/2$ if $m = 1$, and at $\Delta\nu_{\text{iso}} \approx \nu_R/4$ and $3\nu_R/4$ if $m = 2$. SHANGHAI yields even more (seven altogether) recoupling bands that are effective for any sufficient rf amplitude: $\nu_{\text{mix},1H} \geq 10$ kHz.

With an rf-amplitude of 23 kHz, the R^3 recoupling zone ($\nu_{\text{mix},1H} \approx \nu_R = 23$ kHz) of DARR correctly covers most of the ^{13}C spectrum of L-histidine, especially when taking account the broadening of this zone related to the numerous protons in the molecule [18]. Indeed, in this sample the $\Delta\nu_{\text{iso}}$ range covers c.a. 150 ppm, corresponding to c.a. 15 kHz for the static field of 9.4 T. For this reason, DARR, which was proven to homogenize the ^{13}C magnetizations at low spinning speeds [4,5], still works well in the present case of moderate spinning at 23 kHz and the medium static field of 9.4 T. As increasing the static field, the DARR recoupling zone would no longer be able to cover the whole ^{13}C spectral range. In this sense, the other broadband mixing schemes may be of interest.

The process of ^{13}C mixing was found to be relatively inefficient with PARIS, as demonstrated in Fig. 8b. Indeed, the main R^2B recoupling band does not contribute to ^{13}C exchange in the present case, because the spinning frequency ($\nu_R = 23$ kHz) was larger than the ^{13}C spectral distribution (c.a. 15 kHz). At this spinning speed, the efficiency of PARIS is hence expected to increase for higher static

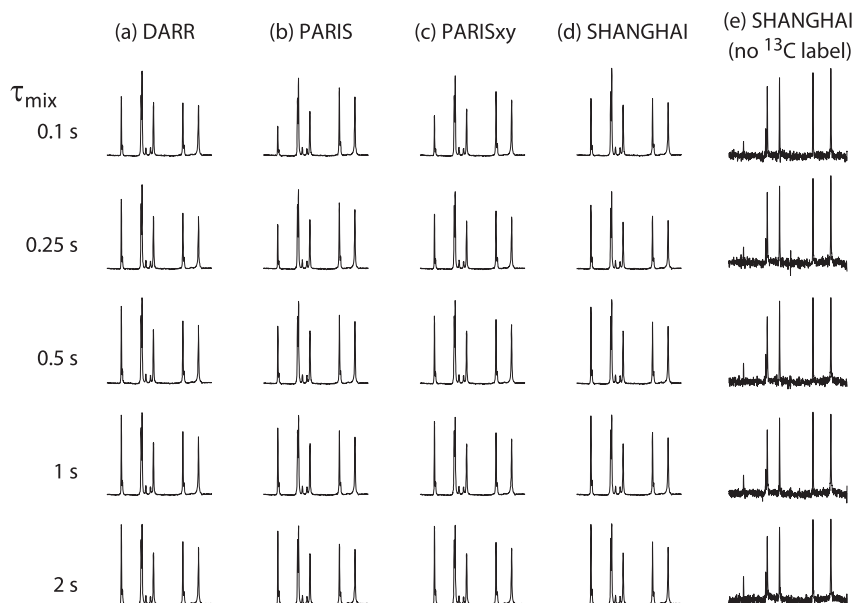


Fig. 7. L-histidine ^{13}C spectra recorded for five τ_{mix} values with the pulse sequence in Fig. 6 using (a) DARR, (b) PARIS, (c) PARIS_{xy} ($m = 2$), and (d) SHANGHAI recoupling schemes. Both the spinning frequency and the ^1H rf intensity during the mixing time were 23 kHz. The vertical scales are common for all the spectra in (a–d), which were obtained for labeled L-histidine. Spectra in (e) were obtained in non-labeled L-histidine with the same experimental conditions as those in (d). The numbers of accumulations were 40 for (a–d), and 1000 for (e). The recycle delay was 3 s for all the data.

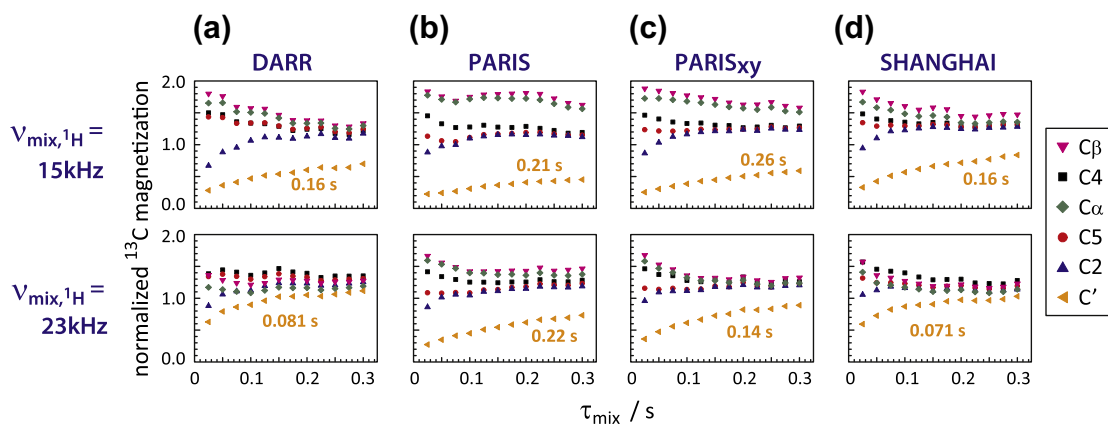


Fig. 8. Mixing time dependence of the labeled L-histidine ^{13}C peak intensities with the pulse sequence in Fig. 6. For mixing, DARR was used in (a), while PARIS, PARIS_{xy} ($m = 2$) and SHANGHAI were employed in (b–d), respectively. The spinning speed was 23 kHz, and the rf amplitudes used for ^1H irradiation were 15 kHz and 23 kHz for the top and bottom rows. The signals were accumulated over 40 times with a recycle delay of 3 s. The value indicated in each graph is the time constant of the build-up curve of the C' magnetization, which was obtained by exponential curve fitting.

fields. However, due to the ZB recoupling band, exchanges are expected to be efficient between carbon spins with a small difference of chemical shifts. In fact, we have indeed observed a relatively faster mixing between C2 and C4 (Fig. 8b), whose isotropic shifts are close to each other (see Fig. 1).

The situation was improved in PARIS_{xy} ($m = 2$), where additional recoupling bands were introduced at $\nu_{\text{R}}/4$ and $3\nu_{\text{R}}/4$, namely, $\Delta\nu_{\text{iso}} \approx 6$ and 17 kHz. For this reason, the magnetization of the carboxyl carbon (C') built up faster in PARIS_{xy} ($m = 2$) compared to PARIS (Fig. 8c), since PARIS_{xy} recouples C' with C4, C5 and $\text{C}\beta$.

In SHANGHAI, the mixing was much more efficient, as demonstrated in Fig. 8d. This is ascribed to the five recoupling bands introduced between the ZB and the R^2B . Although SHANGHAI and DARR were found to give nearly the same ^{13}C mixing performance with the former being slightly better than the latter for $\nu_{\text{mix},1\text{H}} \approx \nu_{\text{R}} = 23$ kHz, they are based on different mechanisms. The recoupling bands of SHANGHAI participating in the ^{13}C exchange are always active whatever the $\nu_{\text{mix},1\text{H}}$ value, whereas

the R^3 zone of DARR only works if $\nu_{\text{mix},1\text{H}} \approx \nu_{\text{R}}$. Thus, SHANGHAI is expected to be more robust than DARR against rf mismatch or rf inhomogeneity at higher MAS frequencies.

In practice, minimizing the rf-power is always desirable. We have thus also compared the thermal equalization with the weaker rf-amplitude of $\nu_{\text{mix},1\text{H}} \approx 15$ kHz. Again, the best results were obtained with DARR and SHANGHAI (first row in Fig. 8), but the fastest global ^{13}C mixing was observed with SHANGHAI. This is due to the fact $\nu_{\text{mix},1\text{H}} \approx 15$ kHz is considerably below the R^3 condition, whereas SHANGHAI is relatively robust against changes in rf-amplitude (Fig. 8d). It is anticipated that the advantage of SHANGHAI would be prominent with experiments performed in higher static fields than that used in this work, because of the large number of the recoupling bands that give the broadband recoupling capability to SHANGHAI.

One should note that the profile of the recoupling bands is completely dependent on the spinning speed and the recoupling method used, and that in between the recoupling bands the efficiency

can be weak [18]. None of the four recoupling sequences introduced in this article provides a complete broad-band recoupling profile. Nevertheless, one may take advantage of the complementary nature of the recoupling bands of PARIS_{xy} ($m = 2$) and SHANGHAI, by implementing them successively during the mixing time. Such a situation can be relevant when the spinning frequency is increased from the present case of 23 kHz to, say, 30–35 kHz.

5. Conclusions

In summary, we studied the efficiency of ¹³C quantitative-CP (QU-CP) in the magnetic field of 9.4 T and under the moderate MAS speed of $\nu_R = 23$ kHz. Enhancement of ¹³C polarization by CP may be preferably replaced by the NI-CP scheme. It was demonstrated that the efficiency of ¹H–¹³C polarization transfer versus the rf amplitudes showed a broad profile. The robustness of NI-CP against rf mismatch and rf inhomogeneity is advantageous for spinning frequency faster than that (12–14 kHz) used in the original QU-CP works. In the second part of QU-CP, namely, the broadband ¹³C mixing via ¹H dipolar recoupling, DARR and SHANGHAI were found to exhibit better performances compared to PARIS and PARIS_{xy} at $\nu_R = 23$ kHz. In ¹³C-labeled L-histidine, SHANGHAI resulted in slightly faster ¹³C mixing than DARR with the rf intensity at the R³ condition: $\nu_{\text{mix},1\text{H}} \approx \nu_R$. For lower rf powers, SHANGHAI showed the best mixing performances. In addition, the advantage of SHANGHAI would become prominent for QU-CP experiments in higher static fields and/or faster spinning, due to its large number of recoupling bands. Since the recoupling bands of SHANGHAI and PARIS_{xy} ($m = 2$) mixing schemes complement with each other, their successive implementation during the mixing time can also be advantageous. Such experiments and QU-CP studies in the fast MAS regime are under investigation. However, thermal equilibration at faster MAS will require new equalization methods.

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