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Quantitative cross-polarization NMR spectroscopy in uniformly ^{13}C-labeled solids

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Abstract

A novel *QU*antitative *C*ross *P*olarization (QUCP) scheme is proposed, which can be applicable for quantitative measurement of cross-polarization magic angle spinning (CP/MAS) spectroscopy in solid-state NMR. In this QUCP experiment, the combination of CP and broadband homonuclear recoupling technique (DARR) 'homogenizes' the non-uniform CP-prepared polarizations of dilute spins so that quantitative CP/MAS spectra can be obtained. Not only are all magnetizations enhanced uniformly by QUCP scheme, but also is the experimental time reduced greatly. In addition, the uniform enhancement is independent of the experimental parameters of cross-polarization. The present scheme is applicable for systems containing carbonyl, aromatic, and aliphatic carbons or some of these groups.

1. Introduction

The CP/MAS method plays an instrumental role in extending the analytical capabilities of solid-state NMR to the studies of 'rare' nuclei, such as ^{13}C and ^{15}N [1–3]. Nuclear polarization is usually transferred from abundant spins I with a large gyromagnetic ratio γ_I and short longitudinal relaxation time T_{1I} to diluted spins S with a small γ_S and long T_{1S}, increasing the magnetization of S and the repetition rate of the experiment. Nowadays, CP/MAS has been applied widely into a variety of fields including materials science, biology, medicine and polymer [4], as a prerequisite technique in solid-state NMR. However, one of the major drawbacks of CP is that the present technique cannot be applied to quantitative measurement or analysis [5–7].

The study on quantitative analyses from CP spectra has been reported over the past decades [8–11]. The common approach is to use variable amplitude or variable contact time in CP period. Recently, Fu et al. [12] proposed a new approach of quantitative measurement in CP/MAS NMR, where the I spin magnetization is spin-locked at the magic angle by the Lee–Goldburg sequence and the carrier frequency offset of the S spins is modulated sinusoidally during the CP contact time. However, in addition to the MAS reduction of heteronuclear dipolar coupling strength and local motion, the numbers of I spins around S spin is also a decisive factor determining the final enhancement factor of S spin, especially for labeled samples. Consequently, it may be difficult to perform authentically quantitative analysis by above approaches. Nuclear Overhauser polarization (NOP) [13,14], introduced by Takegoshi et al. [15–17], is another quantitative analysis technique that is based on nuclear Overhauser effect (NOE) and a recoupling scheme of ^{13}C–^{1}H dipolar-assisted rotational resonance (DARR), a technique incorporating rotational resonance and rotary resonance [18]. The NOP is applicable for uniformly ^{13}C-labeled systems, but the fast

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rotating methyl groups are required in systems for ^{13}C NOE enhancement.

In the present work, an improved CP scheme is proposed, which is an application of rotary resonance technique in solid-state NMR. The combination of CP/MAS and broadband homonuclear recoupling technique redistributes the non-uniformly enhanced magnetizations of dilute spins with a small gyromagnetic ratio, finally the uniform enhancement can be obtained. It is applicable for quantitative measurement. We refer to this method of uniform enhancement as *QU*antitative *C*ross *P*olarization (QUCP).

2. Experiments

The QUCP scheme is shown in Fig. 1. At first, a standard cross-polarization is used to obtain the magnetizations of various S spins with different enhancements. They are then flipped to the direction of the longitudinal magnetic field by a 90° pulse following CP. In the mixing period, the S–S dipolar interaction is recovered by the DARR homonuclear recoupling technique, causing the initial enhanced but non-uniform polarizations of S spins to reach a quasi-equilibrium [13,14,19,20]. Consequently, an overall uniform enhancement of S nuclear magnetization is realized.

Uniformly 99% ^{13}C, ^{15}N-labeled L-tyrosine (Tyr) and uniformly 10% ^{13}C-labeled DL-alanine (Ala) were purchased from Sigma–Aldrich Inc., and used in powder solid without further purification or recrystallization. All spectra presented here were recorded at a Varian Infinityplus-300 NMR spectrometer with a magnetic field of 7.1 T at room temperature. A $^{1}H/^{13}C$ double resonance magic angle spinning probe with a 4.0 mm o.d. rotor was used, which was tuned to ^{1}H and ^{13}C frequencies at 299.8 and 75.4 MHz, respectively. The MAS spinning frequency was 12 kHz, and the variation in spinning frequency was controlled to within ±2 Hz. The proton continuous wave (CW) decoupling intensity was 96 kHz during acquisition time. The 90° pulse widths were 2.15 and 2.60 μs for ^{1}H and ^{13}C, respectively. The matching condition of CP satisfied Hartmann–Hahn sidebands matching condition, $\omega_{1C} = \omega_{1H} \pm n\omega_{MAS}$, which was optimized experimentally by varying the ^{13}C RF intensity. For the QUCP experiments, the above CP conditions were employed and the DARR irradiation strength on protons in the mixing time was 12 kHz. The relaxation intervals were optimized in all NNR experiments. Eight scans were used to accumulate the ^{13}C signals of tyrosine, and 32 scans for alanine.

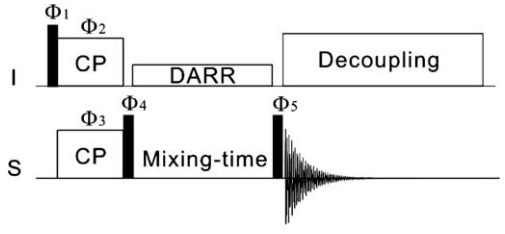

Fig. 1. The QUCP pulse sequence. The DARR irradiation with intensity of $\omega_{1H} = \nu_{MAS}$ is applied on I channel during the mixing time. Solid bars denote π/2 pulses. Phase cycles: $\Phi_1 = x, -x$; $\Phi_2 = y$; $\Phi_3 = y, -y, y, -y, x, -x, x, -x$; $\Phi_4 = -x, x, -x, \quad x, y, -y, y, -y$; $\Phi_5 = x, -x, -y, y, x, -x, -y, y$; receiver $= y, -y, x, -x, y, -y, x, -x$.

3. Results and discussion

Rotational resonance (R^2) [15,16] reintroduces the homonuclear dipolar couplings when the rotational frequency of the sample matches a R^2 condition, $\Delta = n\nu_{MAS}$, where Δ denotes the resonance frequency difference between two coupled spins. However, it is difficult for the R^2 condition to be satisfied for all spin pairs in a multi-spin system. A ^{1}H radio frequency (RF) irradiation is applied with amplitude of ν_{MAS} or $2\nu_{MAS}$ in the mixing time of QUCP experiments [21–23]. Under the irradiation of DARR on protons, the ^{13}C–^{13}C recoupling is modified by a recoupled ^{13}C–^{1}H dipolar interaction. Spectral overlap is necessary to conserve energy for ^{13}C–^{13}C polarization transfer, and it is achieved by the ^{13}C–^{1}H dipolar line broadening. The DARR irradiation on protons, with $\omega_{1H} = \nu_{MAS} = 12$ kHz, gives a larger dipolar broadening, and leads to broadband recoupling and polarization transfer among carbons. Therefore, it does not have the limitation that polarization transfer occurs only between a specific spin pair with a fixed chemical shift difference, a feature more flexible than the conventional R^2. It is found that this approach is applicable for broadband recoupling between methyl/methylene/methyne carbons and carbonyl/aromatic carbons.

Fig. 2 shows the ^{13}C MAS (a), CP-MAS (b), QUCP (c) and NOP (d, e) spectra of uniformly ^{13}C, ^{15}N-labeled tyrosine spun at 12 kHz. The enhancement factors from the ^{13}C NMR spectra recorded using different experimental schemes are calculated, with respect to the corresponding intensity of ^{13}C MAS spectrum (Fig. 2a). The obtained enhancement factors in CP (Fig. 2b) are 0.83, 0.85, 1.63, 0.89, 1.80, 1.82, 2.08 for C_o, C_4, $C_{2,6}$, C_1, $C_{3,5}$, C_α, and C_β, respectively. It is obvious that the enhancement factors for different carbon sites in CP are significantly different, i.e., not uniform. The efficiency of magnetization transfer is sensitively dependent on the I–S heteronuclear dipolar interaction, deteriorated by molecular or atomic group motion. These effects make it difficult to obtain uniform enhancement by conventional CP scheme. For QUCP experimental scheme, however, the enhancement factors for different carbon sites are 1.37, 1.35, 1.38, 1.36, 1.37, 1.39 and 1.38, which are in range of 1.37 ± 0.02 and almost uniform. This demonstrates that the QUCP experimental scheme can be applied to quantitative measurement or quantitative analysis in uniformly ^{13}C-labeled system. Moreover, to obtain the quantitative NMR spectroscopy in the same intensity, the experimental time by QUCP sequence is drastically reduced to about 1/40 ($500 \times 1.37/(16 + 1) = 40$) of that in the experiment using a single 90°

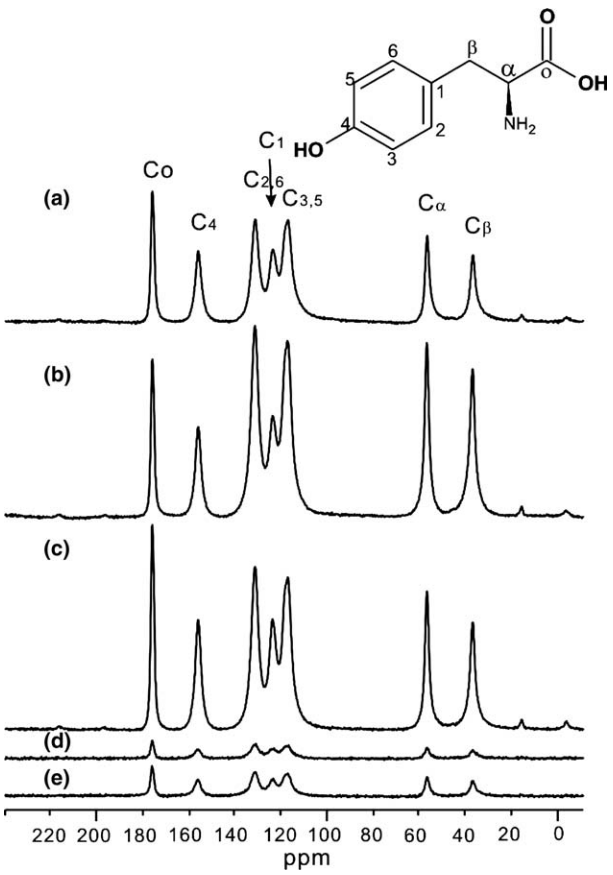

Fig. 2. ^{13}C MAS spectra of uniformly ^{13}C, ^{15}N-labeled tyrosine acquired with a single 90° pulse with proton decoupling (a), CP (b), QUCP (c) and NOP (d, e). Eight FIDs were accumulated for each experiment, and the spectra were plotted on the same amplitude scale. The relaxation interval was 500 s for (a), 16 s for (b–e). The contact time was 0.5 ms for (b) and (c). For QUCP experiment, the DARR irradiation time was 1 s. The mixing times are 10 and 20 s for (d) and (e), respectively.

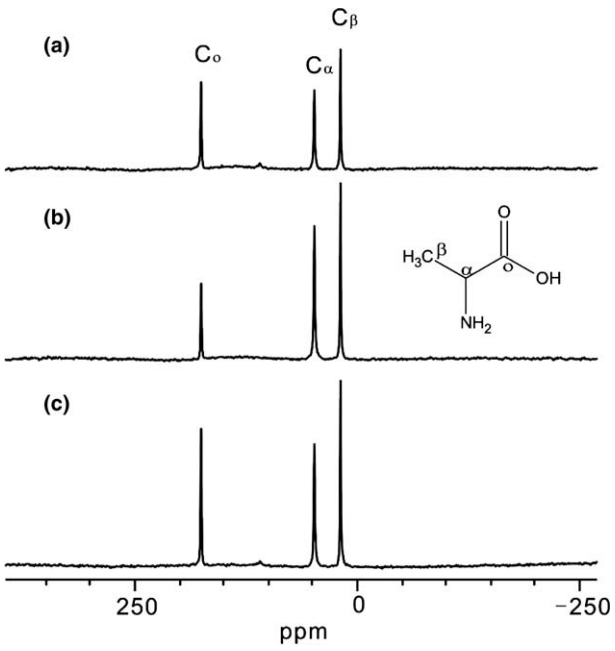

Fig. 3. ^{13}C MAS spectra obtained with 10% uniformly ^{13}C-labeled alanine using a single 90° pulse (a), CP (b), and QUCP (c) under rotational frequency of 12 kHz. Thirty-two FIDs were accumulated for each experiment, and the spectra were plotted on the same amplitude scale. The relaxation interval was 50 s for (a), 4 s for (b) and (c). The contact time was 0.6 ms for (b) and (c). For QUCP experiment, the DARR irradiation time was 1 s.

pulse, which is significant for quantitative measurement in solid-state NMR experiment, especially for the interested spins with longer spin-lattice relaxation time. For comparison, the NOP experiments were also carried out, and the ^{13}C spectra of tyrosine are shown in Fig. 2d and e, corresponding to the NOP experiments with mixing time of 10 and 20 s, respectively. It is evident that the intensities of all the individual peaks in the NOP spectra are considerably reduced even though long mixing time is used. The absence of methyl groups in tyrosine results in NOP much less effective for the signal enhancement. However, since the signal enhancement by QUCP arises from cross-polarization, the efficiency of uniform enhancement is independent on the presence of methyl groups.

We note that this technique is equally applicable for systems containing fast rotating methyl groups, such as alanine. Fig. 3 shows ^{13}C MAS spectra of 10% uniformly ^{13}C-labeled alanine using a single 90° pulse (a), CP (b), and QUCP (c) under rotational frequency of 12 kHz. The enhancement factors in ^{13}C CP and QUCP spectra were obtained, relative to the corresponding peak in Fig. 3a. The obtained enhancement factors in CP are $\eta_{CP} = 0.91$, 1.72, and 1.50 for carboxylic, methyne and methyl carbon, respectively, which are significantly different. The enhancement factors in QUCP are found to be 1.53, 1.54, and 1.54 for carboxylic, methyne and methyl carbon, respectively. The three ^{13}C signals are almost uniformly enhanced in QUCP, but not in CP. It is shown that the QUCP scheme is also applicable for the uniform enhancement of the systems containing fast rotating methyl groups.

The effect of contact time in CP and QUCP experiments were compared. We performed a series of CP and QUCP experiments (spectra not shown) with different contact times, varied from 0.02 to 10.0 ms. The enhancement factors were obtained. The dependence of ^{13}C signal enhancement factor in CP and QUCP experiments on contact time (τ_{CP}) was studied. The CP magnetization build-up curves in CP experiment were plotted in the scale of enhancement factor, as shown in Fig. 4a. It shows clearly that the CP dynamics curves of different ^{13}C resonances are distinct, due to the different ^{13}C–^{1}H dipolar couplings and molecular motion. In addition, it is found that the optimal contact times for different chemical groups are not uniform. For instance, the optimal contact time is about 4.0 ms for *Co* and C_4, but 0.6 ms for C_α and C_β. Moreover, even when the optimal contact times for all ^{13}C resonances are uniform, the enhancement factors for different ^{13}C resonances are not uniform [14], which is illuminated from the respective maximum value of magnetization build-up curves. It

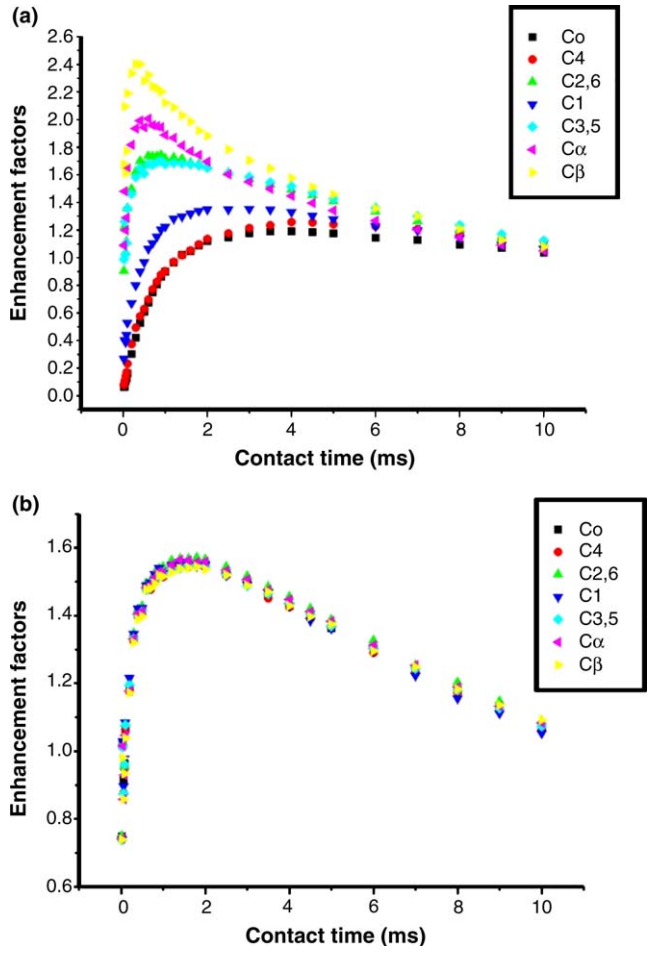

Fig. 4. The CP magnetization build-up curves for L-tyrosine with different experimental schemes. The enhancement factors of ^{13}C signal intensities were recorded as a function of contact time for: (a) CP and (b) QUCP experiments. The enhancement factors were obtained from the corresponding ^{13}C NMR spectra, relative to the single pulse spectrum in Fig. 2a. The MAS frequency was 12 kHz. The mixing-time in QUCP experiment was 1 s.

can be seen obviously that the maximum enhancement factor of C_α is about 2.4, but less than 1.2 for C_o. However, the dependence of ^{13}C magnetization on contact time in the QUCP experiments in Fig. 4b shows that the CP magnetization build-up curves of different ^{13}C resonances exhibit a common trend and reach a common value. It is principally owing to the ^{13}C–^{13}C polarization transfer in the mixing time. Though the magnetizations for different ^{13}C resonances built up by CP are different, the broadband homonuclear recoupling under the DARR irradiation promotes the ^{13}C–^{13}C polarization transfer, leading to a uniform enhancement. Therefore, viewing from the dependence of enhancement factor on contact time, the ^{13}C signals of different chemical groups are enhanced uniformly, irrespective of contact time and local environment. The enhancement factor in QUCP increases with contact time initially, and then decreases after passing the maximum. The enhancement factors reach the maximum (1.55 ± 0.02) when the contact time is about 1.5 ms. This type of

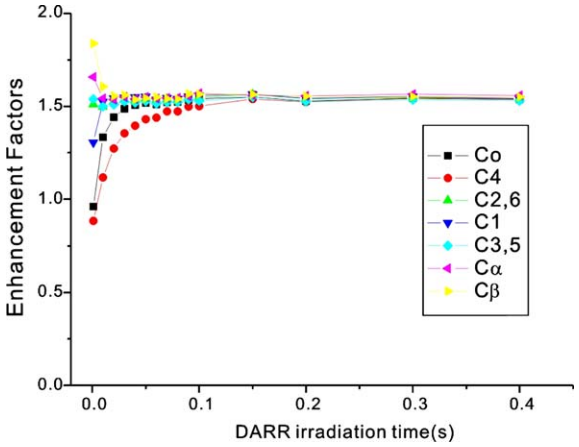

Fig. 5. Mixing time dependence of the signal enhancement factors of ^{13}C resonance for uniformly ^{13}C, ^{15}N-labeled L-tyrosine spun at 12 kHz.

trend reflects CP dynamics, in the sense of averaged process. As a result, the final enhancement factor in QUCP is dependent on the efficiency of the preceding CP, but the character of uniform enhancement is not affected.

Fig. 5 shows the mixing-time dependence of the signal enhancement factors for the seven ^{13}C resonances of L-tyrosine. The contact time was 1.5 ms and the amplitude of DARR irradiation was 12 kHz for all QUCP experiments. The enhancement factors were obtained from the ^{13}C QUCP spectra (data not shown) with different mixing times, relative to the ^{13}C single pulse spectrum in Fig. 2a. It is observed that the magnetization is transferred from the carbons with larger initial values (e.g., C_α and C_β) to the carbons with smaller initial values (e.g., C_o and C_4). The system reaches quasi-equilibrium within 0.4 s, much less than the spin-lattice relaxation, T_1. The enhancement factors of the different carbons approach a uniform value, as the mixing time is set sufficiently long.

4. Conclusions

In conclusion, we present an experimental scheme of quantitative measurement and analysis of CPMAS spectroscopy in solid-state NMR. In this QUCP experiment, the broadband homonuclear recoupling technique (DARR) in the mixing time 'homogenizes' the non-uniform CP-prepared polarizations of S spins so that quantitative CP/MAS spectra can be obtained. It can be seen that not only are all magnetizations enhanced uniformly, but also is the experimental time reduced greatly. While the final enhancement factor in QUCP is influenced by the total CP efficiency during contact time, the character of uniform enhancement is independent of the experimental parameters of cross-polarization. It is applicable for systems containing carbonyl, aromatic, aliphatic carbons or some of these groups. For systems without fast rotating methyl carbons, where the NOP scheme is of limited use, the QUCP scheme is also applicable. Uniform enhancement in the QUCP experiments can always be achieved,

so long as the DARR irradiation time is sufficiently long. The polarization transfer in uniformly labeled systems rapidly reaches quasi-equilibrium within less than 1.0 s, and the uniform enhancement is obtained. The approach could be expected to be applicable to other nuclear spins (such as ^{31}P and ^{15}N) or complicated systems (such as membrane protein and polymer). Further developments of the approach could involve the combination with other experimental schemes, e.g., heteronuclear or homonuclear correlation techniques. These works are still in progress.

Acknowledgments

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