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Hybridizing Cross-Polarization With NOE or Refocused-INEPT Enhances the Sensitivity of MAS NMR Spectroscopy

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Abstract

Heteronuclear cross polarization (CP) has been commonly used to enhance the sensitivity of dilute low- γ nuclei in almost all solid-state NMR experiments. However, CP relies on heteronuclear dipolar couplings, and therefore the magnetization transfer efficiency becomes inefficient when the dipolar couplings are weak, as is often the case for mobile components in solids. Here, we demonstrate methods that combine CP with heteronuclear Overhauser effect (referred to as CP-NOE) or with refocused-INEPT (referred to as CP-RINEPT) to overcome the efficiency limitation of CP and enhance the signal-to-noise ratio (S/N) for mobile components. Our experimental results reveal that, compared to the conventional CP, significant S/N ratio enhancement can be achieved for resonances originating from mobile components, whereas the resonance signals associated with rigid groups are not significantly affected due to their long spin-lattice relaxation times. In fact, the S/N enhancement factor is also dependent on the temperature, CP contact time as well as on the system under investigation. Furthermore, we also demonstrate that CP-RINEPT experiment can be successfully employed to independently detect mobile and rigid signals in a single experiment without affecting the data collection time. However, the resolution of CP spectrum obtained from the CP-RINEPT experiment could be slightly compromised by the mandatory use of continuous wave (CW) decoupling during the acquisition of signals from rigid components. In addition, CP-RINEPT experiment can be used for spectral editing utilizing the difference in dynamics of different regions of a molecule and/or different components present in the sample, and could also be useful for the assignment of resonances from mobile components in poorly resolved spectra. Therefore, we believe that the proposed approaches are beneficial for the structural characterization of multiphase and heterogeneous systems, and could be used as a building block in multidimensional solid-state NMR experiments.

Abstract

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Keywords

Cross Polarization; Signal Enhancement; NOE; INEPT; multiphase solids

1. Introduction

Enhancement of signal intensity of dilute nuclei by heteronuclear cross polarization (CP) [1,2] has long been widely employed in nearly all solid-state magic-angle-spinning (MAS) NMR experiments [3,4]. Particularly, one-dimensional proton-enhanced ^{13}C (or ^{15}N) CPMAS NMR spectroscopy has become an indispensable and essential tool for the study of physical and chemical properties of various non-soluble and non-crystalline solids such as bone [5,6], membrane proteins [7–9], amyloid fibrils [10,11], polymers [12–14], pharmaceutical compounds [15,16], etc. Despite that CP leads to a substantial gain in signal sensitivity with an enhancement factor of $\gamma_{\text{H}}/\gamma_{\text{X}}$, the polarization transfer efficiency can be severely limited by weak heteronuclear dipolar couplings, as in the case of dynamic systems or semi-solids where molecular motions suppress dipolar couplings [17]. As a result, poor sensitivity of NMR experiments on low- γ nuclei becomes a serious issue for such systems. Although various CP-based methods [18–30] and other approaches [31–35] have been developed to enhance the sensitivity of low- γ nuclei in MAS NMR experiments, there is still a need for more efficient methods for simultaneous enhancement of signals from both the mobile and rigid components in multiphase and heterogeneous systems. Traditionally, an additional experiment like the single-pulse excitation of dilute nuclei or refocused-INEPT (refocused insensitive nuclei enhanced by polarization transfer) [36–39] is often performed independently of the CP experiment in order to characterize the mobile components in such systems. Recently, a new pulse sequence that combines CP with a single-pulse experiment (referred to as CPSP) [40] was used to analyze various compositions of multiphase polymers with large mobility contrast (Fig.1). Because ^{13}C T_1 of mobile sites are generally shorter than those of rigid ones, the 90° pulse applied before CP on the ^{13}C channel in the CPSP experiment flips the ^{13}C magnetization from mobile groups onto the xy -plane for final detection. As a result, the enhanced signals from mobile groups by CPSP result from the ^{13}C magnetization recovered during the recycle delay rather than from the proton-enhanced CP transfer. Therefore, to fully utilize the proton-enhanced polarization transfer, we propose

methods that rely on transient heteronuclear NOE or refocused-INEPT to further enhance the signals arising from mobile groups.

While NOE is routinely explored in solution NMR experiments for sensitivity enhancement, resonance assignments, structural and dynamics analysis [41], it is still not frequently explored on solids, mostly because of the absence of molecular motions that are fast enough to induce fluctuating local dipolar field for cross-relaxation in solids. Indeed, dipolar cross-relaxation is maximized if the molecular motions induce an oscillating dipolar field with a correlation time $\tau_c < 10^{-10}$ s [41]. Nonetheless, heteronuclear NOE has been explored in a number of solids such as bicelles [42,43], polymers at temperatures above the glass transition temperature [44–48], microcrystalline proteins [49], as well as plastic crystals like hexamethylbenzene and adamantane [45]. Even in rigid solids, the fast C_3 axial rotation of methyl groups could also locally modulate dipolar interactions with a correlation time short enough to induce cross-relaxation [44,50,51]. In heterogeneous systems containing fast-motion chemical groups or components, the heteronuclear NOE could also be used to enhance the intensity of signals from low- γ nuclei.

Unlike heteronuclear NOE, it is difficult to directly concatenate the RINEPT sequence with CP, mostly due to the magnetization loss (for rigid groups) caused by ^{13}C and ^1H T_2 relaxation times during the delay periods of the RINEPT pulse sequence. However, if ^1H signals from the mobile groups can be retained even after the CP signal acquisition, then the residual ^1H polarization could be further transferred to ^{13}C (or any other low- γ nucleus) by the RINEPT sequence, after which the second signal acquisition can be performed before the subsequent recycle delay. As such, one could independently detect signals from both rigid and mobile sites within the same experiment without significantly affecting the overall experimental time.

In light of the above, we firstly demonstrate a combination of CP and heteronuclear NOE (referred to as CP-NOE) for enhancement of S/N ratio from mobile sites that otherwise cannot be enhanced by the conventional CP method. The theoretical details underlying this proposed CP-NOE sequence are presented by revisiting the Solomon equations [52]. We also compare the performance of CP-NOE pulse sequence with that of the CPSP method [40]. Secondly, we demonstrate the feasibility of independent acquisition of signals from rigid and mobile groups within a single experiment by combining RINEPT sequence with CP (referred to as CP-RINEPT) after the CP signal acquisition. The potential benefits and possible drawbacks of the two proposed methods are discussed within the context of their applications on three different systems with considerable molecular mobility contrast.

2. Experimental Details

2.1. Materials

The hydroxyl-terminated 1,4-polybutadiene (PB) oligomers were purchased from Qilu Ethylene Chemical and Engineering Co. Ltd. (China). All other samples were purchased from Aldrich Chemical Co., and used as received without any further purification. The adamantane/glycine mixture was prepared with a 1:1 molar ratio. The PS-PB-PS (polystyrene-polybutadiene-polystyrene), *i.e.* SBS triblock copolymer, has a molecular

weight of 140,000 g/mol and a polydispersity index (PI) of ~ 1.2 . The weight fraction of styrene is ca. 32% as determined from ^1H solution NMR spectroscopy [53]. Molecular weights of poly(methyl methacrylate) (PMMA) and PB are 550,000 g/mol and 4,200 g/mol, respectively. The PMMA/PB blend was prepared by mechanically mixing PMMA and PB in a 5:1 weight ratio.

2.2. Solid-state NMR Experiments

All NMR experiments were performed on an Agilent VNMRS 600 MHz solid-state NMR spectrometer equipped with a triple-resonance 3.2 mm BioMAS probe. The 90° pulse lengths were 3.0 μs for ^1H and 6.0 μs for ^{13}C RF channel, respectively. Ramp-CP [23] with a 18% ramp on the ^{13}C RF channel was used for transferring magnetization from ^1H to ^{13}C . A RF field strength of 89 kHz was used for proton decoupling by TPPM (two-pulse phase modulation) [54] in all the experiments. However, a 93 kHz CW decoupling was used during the first signal acquisition in the CP-RINEPT experiment (Fig. 1d). The ^{13}C chemical shifts in all spectra are referenced with respect to TMS using the low-frequency signal of adamantane ($\delta_{\text{iso}} = 29.4$ ppm) as a secondary reference [55].

2.3. Pulse Sequences

The radiofrequency (RF) pulse sequences used in this study are shown in Fig. 1. Fig. 1a shows the conventional ramp-CP pulse sequence, where only a ^1H 90° pulse is applied before the CP period; whereas the CPSP pulse sequence [40], shown in Fig. 1b, requires the simultaneous irradiation of 90° pulses on ^1H and ^{13}C channels before the CP period. The new pulse sequences proposed in this study, namely the CP-NOE and CP-RINEPT, are shown in Fig. 1c and Fig. 1d, respectively. In the CP-NOE sequence (Fig. 1c), the ^{13}C magnetization is flipped to the $+z$ direction to store the ^{13}C magnetization after the CP period, whereas the ^1H magnetization is flipped to the $-z$ direction to enable the transient $^1\text{H} \rightarrow ^{13}\text{C}$ heteronuclear NOE polarization transfer in the mobile domains. Because heteronuclear NOE relies on molecular mobility, only the signals from mobile groups will be enhanced during this period. In contrast, the signals from rigid groups are nearly unaffected due to their relatively long spin-lattice relaxation times. The NOE mixing time is usually set to about 1 s in order to maximize the NOE polarization transfer and to avoid severe spin-lattice relaxation of signals from rigid groups. It should be emphasized that in the CP-NOE sequence, the ^{13}C 90° pulse applied before the CP period is necessary, otherwise the ^{13}C 90° pulse applied after the CP period will flip the magnetization of mobile components onto the xy -plane, which would result in severe loss of signal as discussed below. In the CP-RINEPT sequence (Fig. 1d), high-power continuous wave (CW) decoupling is applied during the first signal acquisition period in order to improve the spectral resolution, while at the same time retaining the ^1H magnetization (by spin-locking even in the decoupling period) as much as possible. After the CP period, the RINEPT sequence is applied to enable scalar coupling-based $^1\text{H} \rightarrow ^{13}\text{C}$ polarization transfer, and thus the mobile signals are acquired during the second acquisition period. Compared to the conventional ramp-CP method, CP-RINEPT enables the acquisition of signals from both rigid and mobile groups without significantly increasing the total experimental time (generally only ~ 20 ms longer for each CP-RINEPT scan, as compared to a regular ramp-CP or RINEPT).

3. Theoretical Analysis of Heteronuclear NOE

It is well known that heteronuclear NOE is dominated by cross-relaxation, which is induced by the local fluctuating dipolar field between two different spin-1/2 nuclei. Here, we denote I as the abundant nuclear spin (e.g., ^1H), while S denotes the rare or dilute nuclear spin (e.g., ^{13}C). The cross-relaxation behavior between spins I and S can be adequately described by the Solomon equations [52]:

$$\frac{d\langle I_z \rangle(t)}{dt} = -\rho_I [\langle I_z \rangle(t) - \langle I_z \rangle^{eq}] - \sigma_{IS} [\langle S_z \rangle(t) - \langle S_z \rangle^{eq}] \quad (1)$$

$$\frac{d\langle S_z \rangle(t)}{dt} = \sigma_{SI} [\langle I_z \rangle(t) - \langle I_z \rangle^{eq}] - \sigma_S [\langle S_z \rangle(t) - \langle S_z \rangle^{eq}] \quad (2)$$

where $\langle I_z \rangle(t)$ and $\langle S_z \rangle(t)$ are the expectation values for the spin operators I_z and S_z at time t . $\langle I \rangle^{eq}$ and $\langle S \rangle^{eq}$ are the expectation values at equilibrium. ρ_I and ρ_S are the spin-lattice relaxation rates for spins I and S , respectively. σ_{IS} and σ_{SI} are the cross-relaxation rates from spin S to spin I and from spin I to spin S , respectively.

In natural-abundance samples, the cross-relaxation rate σ_{IS} from ^{13}C to ^1H is very small, and thus can be neglected here. Therefore, by solving the above two equations, we obtain the following:

$$\begin{aligned} \langle S_z \rangle(t) = & [\langle S_z \rangle(0) - \langle S_z \rangle^{eq}] e^{-\rho_S t} + \langle S_z \rangle^{eq} \\ & - \frac{\sigma_{SI}}{\rho_S - \rho_I} [\langle I_z \rangle(0) - \langle I_z \rangle^{eq}] (e^{-\rho_I t} - e^{-\rho_S t}) \end{aligned} \quad (3)$$

$$\langle I_z \rangle(t) = [\langle I_z \rangle(0) - \langle I_z \rangle^{eq}] e^{-\rho_I t} + \langle I_z \rangle^{eq} \quad (4)$$

Here, the magnetization of S at time t is closely related to the initial magnetization of I , i.e. $\langle I_z \rangle(0)$, due to the cross-relaxation term [third term in Eq. (3)]. This explains the need to flip the ^1H magnetization to the $-z$ direction after the CP period to maximize the cross-relaxation terms, as shown in Fig. 1c. In fact, if we ignore the CP period, this sequence is similar to the conventional ^1H - ^{13}C heteronuclear transient NOE experiment, which is performed by initially applying a π pulse on the proton channel at an interval τ before the ^{13}C 90° pulse for signal acquisition [41].

For the conventional ^1H - ^{13}C heteronuclear transient NOE experiment, $\langle S_z \rangle(0) = \langle S_z \rangle^{eq}$ and $\langle I_z \rangle(0) = -\langle I_z \rangle^{eq}$

Thus,

$$\langle S_z \rangle(t) = \langle S_z \rangle^{eq} + \frac{2\sigma_{SI}}{\rho_S - \rho_I} \langle I_z \rangle^{eq} (e^{-\rho_I t} - e^{-\rho_S t}) \quad (5)$$

$$\langle I_z \rangle(t) = -2\langle I_z \rangle^{eq} e^{-\rho_I t} + \langle I_z \rangle^{eq} \quad (6)$$

The NOE enhancement factor is given by

$$\begin{aligned} \eta &= \frac{\langle S_z \rangle(t) - \langle S_z \rangle^{eq}}{\langle S_z \rangle^{eq}} = \frac{2\sigma_{SI}}{\rho_S - \rho_I} \frac{\langle I_z \rangle^{eq}}{\langle S_z \rangle^{eq}} (e^{-\rho_I t} - e^{-\rho_S t}) \\ &\approx \frac{2\sigma_{SI}}{\rho_S - \rho_I} \frac{\gamma_I}{\gamma_S} \end{aligned} \quad (7)$$

In most organic solids, ^{13}C spin-lattice relaxation rate is generally slower than the ^1H spin-lattice relaxation rate ($\rho_S \ll \rho_I$). As a result, the NOE enhancement becomes

$$\eta \approx \frac{2\sigma_{SI}}{\rho_I} \frac{\gamma_I}{\gamma_S} e^{-\rho_S t} \quad (8)$$

Therefore, the NOE enhancement will quickly build up, and then decay slowly to zero due to the spin-lattice relaxation (T_I) of spin S .

Under motional narrowing ('extreme narrowing') condition ($\omega\tau_c \ll 1$), the maximum enhancement factor η that can be achieved is $\sim 0.385 \gamma_I/\gamma_S$ (i.e., 1.54 for a ^{13}C - ^1H spin pair) [41].

4. Results and Discussion

Molecular motion plays an essential role in determining the chemical, physical, and functional properties of various classes of materials. For example, the mobile components in polymers could change the topological constraints imposed by the crystallites, improving the stiffness of the material without compromising its toughness [56,57]. Also, the ion transportation capability of proteins is directly affected by their dynamics [58]. However, the signals from mobile components in a typical ^{13}C NMR spectrum obtained with ^1H - ^{13}C CP are generally compromised due to their weak ^1H - ^{13}C heteronuclear dipolar coupling. In fact, when a short contact time is used for the CP transfer, these signals could completely disappear [40]. Therefore, it is necessary to enhance the S/N ratio for signals from mobile groups while simultaneously retaining the signals from rigid groups as obtained with CP.

As a model system, we first examined the performance of the proposed sequences on a mixture of adamantane and glycine powder samples, as shown in Figs. 2 and 3. Glycine is a rigid crystalline solid with restricted molecular motions, whereas adamantane is a plastic solid whose molecules interact through weak van der Waals forces. Therefore, adamantane molecules possess large internal conformational and orientational degrees of freedom, and thus behave as the mobile component in this mixture. Fig. 2 clearly shows that the adamantane signal intensities (peaks at 29.4 and 38.4 ppm) observed in the CP-NOE spectrum are superior to those observed from CPSP and CP experiments. For the peak at 38.4 ppm, the signal intensity in the CP-NOE spectrum is enhanced by 433% in comparison to the CP spectrum, and 53% compared to the CPSP spectrum. Similarly, for the peak at 29.4 ppm, the signal intensity in the CP-NOE spectrum has increased by 289% compared to

the CP spectrum, and 48% in comparison to the CPSP spectrum. These results clearly demonstrate the superior potential of the proposed CP-NOE sequence for enhancing the intensity of signals from mobile groups. It is also evident that the rigid components are not affected by the heteronuclear NOE process because the molecular correlation time is too long to induce cross-relaxation between ^1H and ^{13}C nuclei. However, these signals may suffer from spin-lattice relaxation when the NOE mixing time is sufficiently long, such as the C_α peak of glycine at 43.3ppm shown in Fig. 2d. Fortunately, the T_1 of rigid components are often much longer than those of the mobile ones, and thus the signals from rigid groups are barely affected. The transient heteronuclear NOE signal enhancement could also be well observed by increasing the NOE mixing time (τ), as shown in Fig. 2d. The adamantane signals (peaks at 29.4 and 38.4ppm) were significantly enhanced by increasing the NOE mixing time, where a maximum signal enhancement for adamantane is achieved with about 1 s NOE mixing time.

To confirm that the signal enhancement of the mobile components by the CP-NOE sequence is due to the heteronuclear NOE polarization transfer and not due an effective increase of the recycle delay (with the insertion of a z-filter), we further compared the signals detected in the CP-NOE spectrum with those in the CPSP spectrum acquired with a recycle delay equal to the sum of the recycle delay and NOE mixing time used in the CP-NOE experiment (Figure S1, Supporting Information). As shown in Fig. S1, the adamantane signals still have higher intensities in the CP-NOE spectrum than in the CPSP spectrum. This clearly indicates that the heteronuclear NOE polarization transfer predominates the signal enhancement of the mobile components. Indeed, if the phase of the ^1H 90° pulse after CP is reversed, the adamantane CP-NOE signals would be reduced significantly (data not shown), which further indicates the presence of heteronuclear NOE effect.

The performance of the CP-RINEPT sequence can also be well demonstrated on the adamantane/glycine mixture, as shown in Fig. 3. Because of the plastic crystalline nature of adamantane, the CP transfer is more efficient than the RINEPT transfer for this compound. However, compared to the ramp-CP spectrum, the adamantane signals observed from CP-RINEPT are still more intense, which also demonstrates the efficiency of concatenating the RINEPT sequence right after the CP acquisition period. Indeed, the application of the RINEPT sequence right after the CP signal acquisition also enables the acquisition of mobile signals in the same experiment before the long recycle delay. Consequently, the RINEPT signals could be obtained concurrently with the CP signals, which is highly beneficial for the accurate characterization of heterogeneous systems, as illustrated below.

To further demonstrate the advantage of the proposed schemes, the polystyrene-polybutadiene-polystyrene (SBS) triblock copolymer was also used in this study. SBS triblock copolymers with 60-80% mobile fraction are among the widely used thermoplastic elastomers due to their excellent mechanical performance, such as high tensile strength and elongation at break [59]. As the mobile phase plays an important role in controlling and modulating the mechanical performance of SBS, comprehensive characterization of these mobile components is significant for further understanding their influence on the mechanical properties of SBS copolymers [60]. The molecular structure of SBS is shown in Fig. 4a, where the PS is the rigid component due to its high glass transition temperature ($T_g \sim$

100 °C), and PB constitutes the mobile phase due to its extremely low T_g (~ -90 °C) [53]. Because of the relatively high mobility of the PB component at room temperature, the ramp-CP efficiency is very low for PB as heteronuclear dipolar couplings are almost averaged out, which is clearly shown in Fig. 4b. In fact, the weak PB signals in the CP spectrum most likely arise from the PB components present on the PB-PS interface where PB and PS molecules are intimately mixing with each other. On the other hand, the PB components that are distant enough from the PS components may not be detected due to the inefficiency of ramp-CP, as is well revealed when compared with CPSP and CP-NOE spectra shown in Fig. 4c and 4d, respectively. The three strongest peaks observed in the CPSP and CP-NOE spectra are ascribed to cis/trans CH=CH (peak at 130.5 ppm) and cis/trans CH₂ (1,4-units) (peaks at 28.1 and 33.1 ppm) of 1,4-polybutadiene. Indeed, the intensities of PB signals are greatly enhanced in the CPSP spectrum, where the ¹³C 90° pulse prior to ramp-CP could flip the mobile ¹³C magnetization onto the *xy*-plane for detection due to the short T_1 of the mobile PB. On the other hand, by utilizing heteronuclear NOE following the CP period, the PB signal intensities are further enhanced while the PS signals are retained, as revealed in the CP-NOE spectrum shown in Fig. 4d. The enhancement factors, given by $(I_{\text{CP-NOE}}/I_{\text{CPSP}} - I_{\text{CP}})/I_{\text{CP}}$, of PB signals in the CPSP and CP-NOE spectra relative to the ramp-CP spectrum are summarized in Table 1. For the PB component, more than 18-fold signal enhancement was observed in the CP-NOE spectrum compared to the CP spectrum, and more than 45% enhancement in comparison with the CPSP spectrum.

Notably, the superior performance of CPSP and CP-NOE pulse sequences for signal enhancement from mobile components is also manifested by the appearance of additional peaks in these spectra that were not detected in the conventional ramp-CP spectrum of SBS. Specifically, the peak at ~ 115 ppm as well as four minor peaks (observed around 142.9, 44.0, 39.0 and 30.8 ppm) show up in both the CPSP and CP-NOE spectra, but are absent in the ramp-CP spectrum. These minor peaks can be ascribed to a small fraction of 1,2-polybutadiene components in the block polymers. Overall, the CP-NOE sequence exhibits excellent performance for enhancing signals from mobile components in comparison to CP and CPSP sequences.

It is worth noting that it is difficult to assign the peaks in the 20-50 ppm region in the conventional ramp-CP spectrum (Fig. 4b), which correspond to backbone signal from PS and CH₂ of PB. However, these peaks could be readily assigned in the CP-NOE spectrum due to narrower linewidths and enhanced signal intensity for the PB components. Moreover, resonance assignments could also be further confirmed by the CP-RINEPT experiment, as shown in Fig. 5. Both CP and RINEPT spectra, corresponding respectively to the rigid and mobile components of SBS, could be consecutively obtained from a single CP-RINEPT experiment without compromising the experimental time. As shown, all PB signals were clearly observed in the RINEPT spectrum, including the small fraction of 1,2-polybutadiene components (indicated by the arrows). It is also worth noting that the CP-RINEPT signals of three main PB peaks at 130.5, 33.3, and 28.1 ppm are significantly stronger than those in the ramp-CP spectrum. In fact, these signals are enhanced by ~ 23 , 13 and 21 fold, respectively, as also summarized in Table 1.

The PMMA/PB polymer blend is another system of interest that was also used to demonstrate the performance of each of the proposed pulse sequences. The molecular structures of PMMA and PB are shown in Fig. 6a. PMMA is a semi-crystalline polymer whose glass transition temperature (T_g) and melting temperature (T_m) are both above 100 °C, whereas PB is a liquid with a T_g of ~ -90 °C. In the PMMA/PB blend, PMMA is therefore considered as the rigid component, while PB is the mobile component. As depicted in Fig. 6b, PB signals are evidently undetectable by the conventional ramp-CP sequence, indicating the complete inefficiency of CP for such highly mobile components. On the contrary, all PB signals are clearly observed in the CPSP, CP-NOE and CP-RINEPT spectra (indicated by dashed rectangles). Moreover, the PB signals are all much stronger in the CP-NOE and CP-RINEPT spectra than in the CPSP spectrum. Therefore, both CP-NOE and CP-RINEPT experiments could be well employed to enhance the intensities of mobile signals. Note that a relative reduction in signal intensity observed for the CH₃ groups of PMMA in the CP-NOE spectrum, which could be ascribed to the fast ^{13}C T_1 relaxation of CH₃ groups.

The use of CW decoupling during the CP signal acquisition in the CP-RINEPT experiment might potentially compromise the spectral resolution. Nevertheless, it should be noted that for most multi-component solids, the mobility of rigid components is generally enhanced by the presence of mobile components; thus, CW decoupling with high RF strength can still provide a reasonable spectral resolution. This is clearly demonstrated in Figure S2, where the CP spectrum of rigid PMMA component obtained from CP-RINEPT experiment has basically the same spectral resolution as that obtained from the conventional ramp-CP experiment with TPPM decoupling. Therefore, CW decoupling should not be a major limitation for obtaining high resolution CP spectrum from CP-RINEPT experiment.

The experimental results presented above on three different samples adequately demonstrate the superior performances of CP-NOE and CP-RINEPT pulse sequences for S/N ratio enhancement of signals from mobile components. In the CP-NOE experiment, the enhancement factor for mobile domains is dominated by the heteronuclear NOE cross-relaxation rate, and hence by molecular mobility. Generally a NOE mixing time of ~ 1 s is sufficiently long for heteronuclear Overhauser polarization transfer. Longer NOE mixing times could also be applied depending on the system under investigation; however, care must be taken for the potential loss of rigid signals due to spin-lattice relaxation. It is also worth noting that we did not use steady-state NOE (i.e., a continuous-wave irradiation to saturate all protons), because the transient NOE buildup rate is twice faster than that of steady-state NOE [41]. Moreover, high-power (several kHz) and long-time (several seconds) RF irradiation is required for complete proton saturation in solids, which are not desirable for heat-sensitive samples. In addition, this would also significantly increase the experimental time. In the CP-RINEPT experiment, in order to utilize the ^1H magnetization from mobile groups for further J -based polarization transfer by RINEPT after ramp-CP signal acquisition, the ^1H magnetization must be retained as much as possible during the first signal acquisition period. Hence, only CW decoupling could be applied during the first signal acquisition period, as other composite-pulse decoupling schemes could result in a significant loss of ^1H magnetization. However, under slow-to-moderate spinning frequencies, the CW decoupling method is not as efficient as other decoupling schemes (e.g., TPPM [54] or SPINAL [61]), which might result in a CP spectrum of slightly compromised resolution as obtained from

the CP-RINEPT experiment. Moreover, the ^1H magnetization from mobile groups can suffer from $T_{1\rho}$ relaxation during the CP spin-locking and CW decoupling periods, in which case the mobile signals observed in the CP-RINEPT spectrum may not be stronger in intensity than those observed in CPSP and CP-NOE spectra. Nonetheless, it must be emphasized that the CP-RINEPT pulse sequence has a unique advantage in that signals from both rigid and mobile groups/components can be consecutively detected in a single experiment without increasing the experimental time by making use of a single recycle delay, as compared to the separate application of the conventional CP and RINEPT sequences in two different experiments; therefore, the new approach significantly reduces the data collection time. In addition, the advantages of CP-NOE and CP-RINEPT sequences could be potentially used for resonance assignment in a wide array of multiphase heterogeneous systems, ranging from small nanoparticles to supramolecular assemblies with mobility contrast for the purpose of understanding the interplay of their structure and properties.

5. Conclusions

Heteronuclear CP has already become a routine and indispensable technique in nearly all solid-state NMR experiments for sensitivity enhancement of dilute nuclei. However, CP is largely not suitable for mobile moieties/groups due to molecular-motion-induced averaging of heteronuclear dipolar couplings. To overcome this limitation, we have demonstrated both theoretically and experimentally that heteronuclear NOE could be efficiently combined with CP to further enhance the intensity of signals from mobile components while retaining the merits of CP to enhance the intensity of signals from rigid components in several systems. Furthermore, we have also demonstrated that the RINEPT sequence could be immediately employed right after the CP signal acquisition to enhance the intensities of signals from mobile groups, thereby enabling the consecutive acquisition of rigid and mobile signals in a single experiment. In particular, we have observed significant signal enhancement in the CP-NOE spectrum for the PB components in the SBS triblock copolymer due to the high mobility of PB at room temperature while the rigid PS signals are barely affected. Our proposed schemes are relatively straightforward to set up and very robust for enhancing the mobile signals in multiphase and heterogeneous systems. In particular, when the spectrum is crowded, our proposed methods could also be applied for resonance assignments in multiphase heterogeneous systems. Therefore, we believe that these pulse sequences can be employed as a building block to develop multidimensional solid-state MAS NMR experiments for improving the signal sensitivity of dilute and low- γ nuclei in more complex systems with multiple phases and diverse mobility.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Heteronuclear NOE is used to enhance S/N ratio in CPMAS experiments.
- RINEPT and CP spectra are acquired independently within the same experiment.
- Significant S/N ratio enhancement for mobile components is obtained.
- CP-NOE and CP-RINEPT can be useful for resonance assignment.

Fig. 1.

Variants of ramp cross-polarization (ramp-CP)-based pulse sequences used in this study. (a) conventional ramp-CP; (b) CPSP; (c) CP-NOE; (d) CP-RINEPT. Continuous wave (CW) or TPPM decoupling [54] schemes were applied during ^{13}C signal acquisition periods as indicated. For each pulse sequence, the phase cycling scheme used is also shown.

Fig. 2.

Natural-abundance ^{13}C MAS NMR spectra of adamantane-glycine mixture obtained with (a) conventional ramp-CP, (b) CPSP, and (c) CP-NOE pulse sequences shown in Fig. 1. (d) Adamantane and glycine ^{13}C signals as a function of NOE mixing time (τ). All spectra were acquired by co-adding 8 transients at 15 kHz MAS using a 1 ms CP contact time and a recycle delay of 3 s. The NOE mixing time was 1 s in the CP-NOE experiment.

Fig. 3.

Natural-abundance ^{13}C MAS NMR spectra of adamantane/glycine mixture sample obtained with the CP-RINEPT pulse sequence. The ramp-CP spectrum corresponds to the first signal acquisition, whereas the RINEPT spectrum corresponds to the second signal acquisition in the CP-RINEPT experiment. The CP-RINEPT spectrum was obtained by adding the ramp-CP and RINEPT spectra. The CP-RINEPT spectrum was acquired by co-adding 8 transients at 15 kHz MAS using a 1.5 ms CP contact time and a recycle delay of 3 s. The RINEPT evolution time τ_1 and τ_2 were both set to 1.0 ms.

Fig. 4. Natural-abundance ^{13}C MAS NMR spectra of SBS sample. (a) Molecular structure of SBS (PS-PB-PS) triblock copolymer. Experimental spectra obtained with (b) conventional ramp-CP, (c) CPSP and (d) CP-NOE pulse sequences. The three spectra were acquired by co-adding 1000 transients at 15 kHz MAS using a 2 ms CP contact time and a recycle delay of 3s. For the CP-NOE experiment, the NOE mixing time was 1s.

Fig. 5. Natural-abundance ^{13}C MAS NMR spectra of SBS sample obtained from the CP-RINEPT experiment. The CP spectrum corresponds to the first signal acquisition, whereas the RINEPT spectrum corresponds to the second signal acquisition in the CP-RINEPT experiment. The CP-RINEPT spectrum was obtained by adding the CP and RINEPT spectra. The CP-RINEPT spectrum was acquired by co-adding 1000 transients at 15 kHz MAS using a 2 ms CP contact time and a recycle delay of 3s. The RINEPT evolution times, τ_1 and τ_2 , were set to 1.5 and 1.0 ms, respectively. The pink arrows indicate the signals from a small fraction of 1,2-polybutadiene component in the SBS sample.

Fig. 6. Natural-abundance ^{13}C MAS NMR spectra of PMMA/PB blend obtained from different pulse sequences. (a) Molecular structures of PMMA and PB. (b) Spectra obtained from ramp-CP, CPSP and CP-NOE experiments, where the resonance assignments are indicated. The NOE mixing time was 1 s for the CP-NOE experiment. (c) Spectra obtained from the CP-RINEPT experiment, where the ramp-CP spectrum corresponds to the first signal acquisition, and the RINEPT spectrum corresponds to the second signal acquisition in the CP-RINEPT experiment. The CP-RINEPT spectrum was obtained by adding the ramp-CP and RINEPT spectra. All spectra were acquired by co-adding 200 transients at 20 kHz MAS using a 1.5 ms CP contact time and a recycle delay of 3 s. The RINEPT evolution times, τ_1 and τ_2 , were both set to 1.0 ms. The main PB signals are indicated with the dashed rectangles in cyan, while the purple dashed lines are used for intensity comparison.

Table 1

Enhancement factors observed for PB signals measured from CPSP, CP-NOE and CP-RINEPT spectra of SBS triblock copolymer in comparison to the conventional ramp-CP spectrum. The enhancement factor was determined by $(I_{\text{CP-NOE/CPSP/CP-RINEPT}} - I_{\text{CP}})/I_{\text{CP}}$.

Peak (ppm)	CPSP	CP-NOE	CP-RINEPT
28.1	23 ± 8	40 ± 13	21 ± 3
33.3	12 ± 2	19 ± 3	13 ± 1
130.5	13 ± 2	19 ± 2	23 ± 2