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SPECTROCHIMICA ACTA PART A

Spectrochimica Acta Part A 70 (2008) 1025-1028

www.elsevier.com/locate/saa

High-resolution NMR spectra under inhomogeneous fields via intermolecular double-quantum coherences

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Received 27 April 2007; received in revised form 29 September 2007; accepted 15 October 2007

Abstract

High-resolution NMR spectroscopy is a powerful tool for analyzing molecular structures and compositions. Line-widths of conventional liquid NMR signals are directly proportional to the overall magnetic field inhomogeneity the sample experiences. In many circumstances, spatial and temporal homogeneity of the magnetic field is degraded. In this paper, a modified pulse sequence based on intermolecular double-quantum coherences (iDQCs) was proposed to obtain 1D high-resolution NMR spectra under inhomogeneous fields using 2D acquisition. Analytical expressions were derived from the intermolecular multiple-quantum coherence (iMQC) treatments. Both experimental and simulated spectra provide high-resolution 1D projection spectra similar to conventional 1D high-resolution spectra. Moreover, the apparent *J* coupling constants are threefold magnified, which allows a more accurate measurement of small *J* coupling constants.

Keywords: NMR; Intermolecular double-quantum coherences; High-resolution spectra; Inhomogeneous fields

1. Introduction

High-resolution NMR spectroscopy is a powerful tool for analyzing molecular structures and dynamics. In conventional high-resolution liquid NMR experiments, the homogeneity of magnetic field exceeding 10^{-8} is typically required. However, there are many chemical and/or biological circumstances where the spatial homogeneities of the magnetic fields are degraded, for instance, by magnetic susceptibilities of various tissues in biomedical in vivo spectroscopy [1,2], with ex situ analysis of rock samples for oil exploration [3], or in studies of porous resin beads in combinatorial chemistry, especially in solid-phase synthesis [4]. Since spatial and temporal variations of magnetic field homogeneity result in spectral line-width broadening, it is therefore difficult to simultaneously obtain useful information of chemical shifts, J coupling constants, multiplet patterns and relative peak areas. Besides magic-angle spinning (MAS) method [5,6], a series of pulse sequences based

on intermolecular dipolar interactions among spins in different molecules have been developed to address the problems related to inhomogeneity of magnetic fields. Intermolecular nuclear Overhauser effect (NOE) produced by intermolecular short-range dipolar interactions has been proposed to obtain high-resolution spectra in inhomogeneous fields [7]. However, the signals are too weak for practical application. Recently, intermolecular multiple-quantum coherences (iMQCs) caused by long-range dipolar interactions have generated tremendous interests [8,9]. In Ref. [8], a pulse sequence, named CRAZED (Cosy Revamped with Asymmetric Z-gradient Echo Detection), was originally introduced as two-dimensional intermolecular double-quantum coherence (iDQC) spectroscopy. Since intermolecular dipolar interactions are effective in the range of $5-500 \,\mu\text{m}$, which is far smaller than typical NMR sample dimension, it is intuitively attractive to apply them to NMR spectroscopy in inhomogeneous fields. Several pulse sequences based on intermolecular zero-quantum coherences (iZQCs) have been proposed, such as HOMOGENIZED (HOMOGeneity ENhancement in Intermolecular ZEro-quantum Detection) [10], composite CPMG-HOMOGENIZED [11], ultrafast iZQC 2D spectroscopy technique [12] by Warren and co-workers, SEL-HOMOGENIZED (SELective HOMOGENIZED) sequence by our group [13], and SEL-HOMOGENIZED-alike sequences

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^{1386-1425/\$ –} see front matter © 2007 Elsevier B.V. All rights reserved. doi:10.1016/j.saa.2007.10.022

by Faber and co-workers [14,15]. Using inherent spectrometer software, 1D high-resolution spectra can be obtained from the direct projection of the 2D spectra acquired by these sequences. Unfortunately, it is difficult to isolate the iZQC signals from the much stronger conventional single-quantum signals. It has been reported that there is no need to use additional phase cycling to obtain pure iDQC signals and their intensities are approximately 30% higher than those from iZQCs [16].

Recently, we proposed the IDEAL (Intermolecular Dipolarinteraction Enhanced All Lines) sequence based on iDQCs [17]. To the best of our knowledge, it is not possible for the original IDEAL sequence to obtain a projection spectrum similar to conventional 1D one by using the inherent programs of NMR spectrometers such as Varian VnmrJ and Bruker Top-Spin. The original experimental data must be exported from spectrometer for signal processing using a special developed program. In this paper, we report a modified sequence to avoid complicated signal processing. It integrates the advantages of pure and strong iDQC signals and simple experimental data manipulation. The 1D high-resolution spectrum can be directly obtained from the projection of the 2D spectrum acquired by the proposed sequence using the inherent spectrometer program, just like the HOMOGENIZED-alike sequences [10,13–15].

2. Theoretical formalism

The modified pulse sequence is shown in Fig. 1. Without losing generality, we consider a homogeneous solution with solute consisting of an AX spin-1/2 system of type S (denoted as S_k and S_l spins with scalar coupling constant J_{kl}), and solvent consisting of a single spin-1/2 system of type I. It is assumed that I spin is abundant and S spins are either abundant or dilute. ω_m is the frequency offset of spin m ($m = I, S_k, S_l$) in the rotating frame in the absence of field inhomogeneity. $\Delta B(\mathbf{r})$ is the inhomogeneous field at position \mathbf{r} . When the spatial position-dependent magnetic field is taken into account, the frequency offset, $\Omega_m(\mathbf{r})$, of the m spin at position \mathbf{r} is given by

$$\Omega_m(\mathbf{r}) = \omega_m + \gamma \Delta B(\mathbf{r}), \tag{1}$$

where γ is the gyromagnetic ratio of proton. Eq. (1) suggests that the magnetic field inhomogeneity causes a shift of angular frequency from the resonance ω_m . In analogy, the precession



Fig. 1. Modified pulse sequence. Full vertical bars stand for non-selective RF pulses, the Gauss-shaped pulse is I-spin selective RF pulse, and dash rectangles represent coherence selection gradients. The phases of the RF pulses are indicated with subscripts.

frequency of iDQCs is given by

$$\Omega_{iDQC}(\boldsymbol{r}, \boldsymbol{r}') = \Omega_m(\boldsymbol{r}) + \Omega_{mn}(\boldsymbol{r}')$$

= $\omega_m + \omega_n + \gamma [\Delta B(\boldsymbol{r}) + \Delta B(\boldsymbol{r}')]$
(m, n = I, S_k, S_l). (2)

Eq. (2) shows that the iDQC component evolves at the sum of the two precession frequencies of the spins involved.

Now we derive the theoretical expressions of final signals from the iMQC treatment. For simplification, the effects of radiation damping, diffusion, relaxation, and intermolecular NOE are ignored, and the correlation gradients are applied along the *z* direction. It is well known that all density operator components except coherence order n = -1 are unobservable and can be disregarded during the detection period [18]. Therefore, only the single-quantum coherence (SQC) terms, which contribute to observable signals, need to be considered during the detection period. Under the effects of chemical shift, scalar coupling, magnetic field gradients, and dipolar coupling, the magnetization density of the S_k component (the same for the S₁ component) attributing to the observable signal during the detection period can be written as

$$\sigma_{\rm eff}(3t_1 + t_2) = \left[\frac{(2t_1 + t_2)i\Delta_s}{16\tau_d^{\rm I}}\right] \\ \times \left(\frac{kT}{\hbar\omega_{\rm I}}\right) M_{\rm S_k}^- \{\exp[-i(\omega_{\rm I} - \omega_{\rm S_k} - 3\pi J_{kl})t_1] \\ \times \exp[i(\omega_{\rm S_k} + \pi J_{kl})t_2] + \exp[-i(\omega_{\rm I} - \omega_{\rm S_k} + 3\pi J_{kl})t_1] \exp[i(\omega_{\rm S_k} - \pi J_{kl})t_2]\}, \quad (3)$$

where $kT/\hbar\omega_{\rm I}$ is the reciprocal of Boltzmann factor; $\tau_{\rm d}^{\rm I} \equiv 1/(\gamma\mu_0 M_0^{\rm I})$ is the dipolar demagnetizing time, in which $M_0^{\rm I}$ is the initial magnetization of spin I, μ_0 is the vacuum permeability; and $\Delta_{\rm s} \equiv [3(\hat{s} \cdot \hat{z})^2 - 1]/2$, in which *s* is the gradient direction. Eq. (3) shows that the intermolecular cross-peaks appear at $(\omega_{\rm I} - \omega_{\rm S_k} - 3\pi J_{kl}, \omega_{\rm S_k} + \pi J_{kl})$ and $(\omega_{\rm I} - \omega_{\rm S_k} + 3\pi J_{kl}, \omega_{\rm S_k} - \pi J_{kl})$, respectively. If the frequency offset of I spin is set to zero, i.e. $\omega_{\rm I} = 0$, the intermolecular cross-peaks between I and S spins will be centered at $(-\omega_{\rm S_k} - 3\pi J_{kl}, \omega_{\rm S_k} + \pi J_{kl})$ and $(-\omega_{\rm S_k} + 3\pi J_{kl}, \omega_{\rm S_k} - \pi J_{kl})$ with separate streaks paralleling to the F2 axis. This indicates that a 1D high-resolution spectrum can be directly obtained through projecting the 2D iDQC spectrum onto the F1 axis. Moreover, the projected spectrum has the same multiplicity pattern as a conventional 1D spectrum with a scale factor 3 of the *J* coupling constants.

Compared to the CRAZED sequence, the main difference of the presented sequence is that the beginning of data acquisition is delayed by twice the evolution period. This ensures that all the streaks in the 2D spectra, just like those produced by HOMOGENIZED-alike sequences, parallel to the F2 axis, which allows a simple F1 projection to extract high-resolution 1D spectrum. In addition, similar to IDEAL sequence, a solvent selective pulse is used to ensure that the intermolecular solute–solvent cross-peaks are obtained while intermolecular solute–solute and solvent–solute cross-peaks are effectively suppressed. Since the magnetization requires a period of the order $\tau_{\rm d}^{\rm l}$ to emerge and the signal decays rapidly in the inhomogeneous field, a hard π pulse is applied to refocus the signal.

3. Experiments, simulations and results

All experiments were carried out at 298 K using a 500 MHz Varian NMR System spectrometer and a 5 mm indirect detection probe. In order to examine the modified pulse sequence shown in Fig. 1, a sample of methyl ethyl ketone (MEK, CH₃COCH₂CH₃) in cyclohexane (C_6H_{12}) solution was used. Acetone- d_6 was added to the sample for field lock and shimming as well as for reducing radiation damping effects. In the 2D experiment, the coherence selection gradient with strength G of 0.08 T/m and duration δ of 1.2 ms was applied. The pulse width of $\pi/2$ RF hard pulse was extended to 53.5 µs by detuning the probe to further suppress the radiation damping effects during the evolution and detection periods. The $\pi/2$ Gaussian pulse had a pulse width of 10.3 ms and was frequency selective on the solvent peak. The spectral widths of F1 and F2 dimension were both 1200 Hz. The pulse repetition time was 3.0 s and the acquisition time t_2 was 0.5 s. To eliminate the influence of the imperfect π pulse, the phase of the π pulse was alternated between 0 and π . The F1 dimension was acquired with 300 increments. The total experimental time for a 2D spectrum was about 40 min. The signal was zero filling to 4096×4096 before regular FFT.

In order to verify the theoretical predictions and experimental observations, the modified Bloch equations were used to simulate 2D iDQC spectra of the same sample based on a simulation algorithm presented preciously [19]. The parameter sets for the simulations were chosen to be as close to the actual ones used in the experiments as possible.

The experimental results are present in Figs. 2 and 3. The 1D spectrum in a homogeneous magnetic field is shown in Fig. 2a. The magnetic field was then intentionally degraded by deshimming the sample to produce a line-width of \sim 50 Hz, and a 1D spectrum was acquired (see Fig. 2b). The 2D iDQC spectrum obtained from the proposed sequence in the same inhomogeneous field is presented in Fig. 3a. It is seen that intermolecular cross-peaks parallel to the F2 axis, which allows us to directly obtain 1D high-resolution projection spectrum using the inherent



Fig. 2. 1D NMR spectra of methyl ethyl ketone in cyclohexane (frequency offset set to 0 Hz) solution. (a) In a homogeneous field, (b) in an inhomogeneous field with line-width of about 50 Hz (phase mode), and (c) accumulated projection of the spectrum in the dotted box of Fig. 3b. Inserts are magnified in both vertical and horizontal directions.

spectrometer program. To optimize the projection, the spectrum of Fig. 3a was dealt with 'p-type' Fourier transformation and rotated counterclockwise by $\pi/4$. These two operations can both be achieved on two kinds of most popular spectrometers: Varian



Fig. 3. 2D iDQC experimental spectra in the inhomogeneous field. (a) Using the pulse sequence shown in Fig. 1 and (b) same as (a) but with 'p-type' Fourier transformation and counterclockwise rotation of $\pi/4$.



Fig. 4. 2D iDQC simulation spectrum in an inhomogeneous field using the pulse sequence shown in Fig. 1 with the flip angles of the hard pulses deviated by a factor of 1/25 and $(0, \pi)$ phase cycling of π pulse.

(e.g. VnmrJ 2.1B) and Bruker (e.g. TopSpin 1.3). The $\pi/4$ rotation transformed the data matrix from (x, y) to (x-y, y), and this is helpful to obtain a "clean" 1D spectrum [13]. The result is shown in Fig. 3b. The projection of the spectrum in the dotted box of Fig. 3b onto the F1 axis yielded a 1D high-resolution spectrum shown in Fig. 2c. The spectrum maintains the information of chemical shifts, relative peak areas, and multiplet patterns which are remarkably similar to the conventional 1D high-resolution spectrum. Moreover, the *J* splitting distances are threefold magnified in Fig. 2c compared to Fig. 2a, which makes it capable to reveal multiplet information in spin systems with small *J* coupling constants.

Since the second RF pulse is a solvent selective pulse, the upper limit of the resolvable field inhomogeneity of the sequence depends on the difference of chemical shifts between solvent and nearest peaks of interest. Although non-selective RF pulses may be more generally useful in large field inhomogeneity, it may result in some unwanted intermolecular cross-peaks as mentioned in the last section, which affect the regeneration of high-quality 1D NMR spectra.

In addition, artifacts may appear in the acquired 2D spectra due to imperfect RF pulses such as flip angle errors of π pulse, which would produce solute dipolar field and result in intermolecular solvent–solute and/or solute–solute cross-peaks. A proper phase cycle scheme would alleviate this situation. The spectrum acquired by phase cycling of π pulse is much "cleaner" than that without phase cycle (data not shown).

The simulation result is present in Fig. 4. The result was obtained under conditions similar to the experimental one with the flip angles of hard pulses deviated by a factor of 1/25 and $(0, \pi)$ phase cycling of π pulse. The simulation and experimen-

tal results are similar and both in excellent agreement with the theoretical prediction.

4. Conclusions

In conclusion, a pulse sequence based on iDQCs was proposed to obtain high-resolution spectra in inhomogeneous fields. Compared to the HOMOGENIZED-alike sequences, strong and pure iDQC signals can be acquired by using the proposed sequence. It is convenient to directly obtain 1D high-resolution projection spectra through inherent spectrometer software. The method would be useful for achieving high-resolution NMR spectra under inhomogeneous fields such as *in situ* analyses of chemical systems.

Acknowledgements

This work was partially supported by the NNSF of China under Grants 20573084 and 10605019, and China Postdoctoral Science Foundation.

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