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Flip-back, an old trick to face highly contrasted relaxation times: Application in the characterization of pharmaceutical mixtures by CPMAS NMR

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ABSTRACT

The ^{13}C–^{1}H CPMAS with flip-back pulse NMR experiment is revisited in view of applications to pharmaceutical mixtures. The analysis of the kinetics of relaxation and CP transfer with and without the flip-back pulse shows that a significant gain in ^{13}C signal can be expected (thus in experimental time) from the flip-back pulse for protons with long T_1. The gain is of the order of T_1 of the protons expressed in seconds. The experiment is applied on samples with highly contrasted spin-lattice relaxation times T_1 for protons, situation encountered in pharmaceutical mixtures. The application of the flip-back increases significantly the relative signal intensity of the component with the longer T_1, making this component detectable even after using short recycle delays. Therefore, this CPMAS with flip-back experiment could be used routinely to get ^{13}C CPMAS NMR spectra of mixtures in constant experimental time and signal-to-noise ratio without the need for optimization of the recycle delays, and for whatever may be the degree of crystallinity of the active principal ingredient (API) and/or excipients.

1. Introduction

Liquid state nuclear magnetic resonance (NMR) is nowadays a technique used routinely for the analysis, characterization or quality control of pharmaceutical, food or other industrial liquids. Magic angle spinning (MAS) solid-state NMR (ssNMR) can also yield invaluable details on the structure of drugs, in particular in the identification and characterization of the polymorphic forms of active principal ingredients (API) and/or excipients [1–4]. Though implemented more and more often, this technique still has not yet fully impacted the pharmaceutical industry, mainly because of the length of the acquisition time (from few minutes up to a couple of days) leading to the high cost of the analysis. Moreover, in this pharmaceutical context, polymorphism is a crucial aspect and the potential difference of magnetic relaxation property between polymorphous forms becomes an obstacle for the popularity of solid-state NMR technique. Therefore, there is a need for procedures yielding ^{13}C NMR spectra in limited or constant amount of time, regardless of the relaxation time constants of the sample or the components of a pharmaceutical mixture, i.e. whatever the polymorph analyzed or the degree of crystallinity of the API or excipients.

There are two main ways to decrease the total experimental time needed to obtain a ^{13}C (in natural abundance) NMR spectrum of a drug: boost the initial ^{13}C magnetization signal (^{13}C labeling, magnetization transfer from ^{1}H, ^{129}Xe [5], DNP with electrons, photons) and/or decrease the waiting time between two successive transients. The ^{13}C signal enhancement is the subject of many studies and developments and the commonly nowadays-used methods involves cross-polarization (CP) [6] magnetization transfer from the ^{1}H spin bath to the ^{13}C. In any NMR experiment, the minimum waiting (recycle) delay between two signal acquisitions is driven by the longitudinal relaxation time T_1. During the recycle delay, the magnetization, which after a given pulse scheme is located in the transverse plane, goes back to its equilibrium orientation, i.e. along the z axis. During the return to equilibrium position, the amount of magnetization along the z axis (M_z) compared to the initial equilibrium magnetization M_0 as a function of time is $M_z = M_0(1-e^{-t/T_1})$, with T_1 the longitudinal relaxation time constant. Experimentally, to ensure a full return of magnetization to equilibrium, the recycle delay between two NMR acquisitions must be longer than five times T_1. The T_1 value depends on various parameters like the molecular structure or the degree of crystallinity of the sample, and is usually much longer for well crystallized than for amorphous materials. It should be mentioned that in the case of ^{13}C fully labeled systems, the use of selective pulses can shorten the apparent T_1 of ^{13}C [7].

The NMR pulse sequence CP with flip-back on I(^{1}H)-magnetization was proposed by Tegenfeldt and Haeberlen [8] in 1979 as a method to solve the problem of samples with long ^{1}H T_1, which require long experimental NMR times. The sequence consists in adding a 90° pulse, a flip-back (FB) pulse, on ^{1}H after the acquisition of the ^{13}C

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signal to force the ^{1}H transverse magnetization back to its equilibrium orientation. For the actual FB pulse to be efficient, the magnetizations of various protons at the end of the ^{13}C signal acquisition period must have an identical phase. This implies that only a ^{1}H decoupling scheme that brings all ^{1}H magnetizations coherently on along the y axis can be implemented in the acquisition period of the ^{13}C NMR spectrum. Today, no effort has been yet made to develop sophisticated decoupling sequences that fulfill this requirement, and a sequence like 64-step small-phase incremental alternation (SPINAL-64) [9], which disperses the ^{1}H magnetization all over the transverse plane, cannot be used in the CP with FB experiment. Therefore, to date, the only possible decoupling scheme that can be combined with a FB pulse is the continuous wave (CW) [10,11] irradiation, with all its limitations on radio-frequency power requirement.

Although very attractive in principle, the limitation in CW decoupling performances probably explains why the notion of CPMAS-FB has been only little used in ssNMR [12,13], and why there is no publication that deals with the CPMAS-FB pulse sequence in itself. However, in the context of ssNMR analysis of industrial products for quality assessment purposes or for counterfeit drugs tracking, where duration to record a ^{13}C NMR spectrum can be as long as a couple of days, proposing much faster identification methods is essential. In this contribution, we revisit, from a practical point of view, the application of CPMAS with FB pulse NMR sequence to small organic molecules. First, an estimation of the gain in signal expected from the addition of the FB pulse is given. The ^{13}C NMR signal gain obtained experimentally with the FB pulse, as well as the limits of the method, are presented through the examples of ibuprofen and caffeine samples, chosen because they have very different T_1 values for the protons. Finally, we show that applied to a mixture of ibuprofen and caffeine, the FB pulse ensures the detection of all components, despite their highly contrasted T_1 values.

2. Experimental

Ibuprofen and caffeine samples were, respectively, purchased from Acros and Merck Chemicals and were used as is. The 2:1 (weight) ibuprofen/caffeine mixture was prepared by grinding appropriate amount of compounds.

All the NMR spectra were acquired on an Avance 500 Bruker spectrometer operating at Larmor frequencies of 500.12 and 125.72 MHz for ^{1}H and ^{13}C, respectively. The samples were packed into 4 or 3.2 mm outer-diameter zirconia rotor closed by Vespel caps and spun at 13 kHz. A continuous wave (nutation frequency 75–100 kHz) or a ^{1}H 64-step SPINAL-64 [3] decoupling scheme (nutation frequency of 100 kHz) was implemented during the acquisition period of all the presented ^{13}C MAS NMR spectra. For ibuprofen, the first 90° proton pulse was set to 2.5 μs (corresponding to a nutation frequency of 100 kHz) and the contact time to 4 ms. For caffeine and the ibuprofen–caffeine mixture, a first 90° proton pulse of 2.95 μs and contact time of 5 ms were employed. For all experiments, a RAMPed-amplitude (RAMP) CP [14] pulse, centered on the $n=+1$ Hartman–Hahn condition and with an amplitude $\Delta\nu_{RF}(^1H)$ of 13 kHz (i.e. $\pm 0.5\nu_{MAS}$, starting from $+0.5\nu_{MAS}$ and decreasing to $-0.5\nu_{MAS}$), was employed. ^{13}C chemical shifts were referenced to the carbon signal versus tetramethylsilane. The data were analyzed with the NMRnotebook [15] software.

3. Results and discussion

3.1. Magnetization transfer and relaxation during CPMAS

A ^{13}C signal enhancement factor $\eta=(\gamma^H/\gamma^C)(M^H/M_0^H)$, with γ_H and γ_C the gyromagnetic ratios of ^{1}H and ^{13}C, respectively, M_0^H the initial proton magnetization and M^H the proton magnetization at time t, can be expected in a CPMAS NMR experiment. However, two major dynamic phenomena (magnetization transfer and proton relaxation), each one characterized by a time constant, occur during a CPMAS NMR experiment and modulate this signal enhancement:

1. T_{CH}: ^{1}H to ^{13}C magnetization transfer occurs during the simultaneous application of the CP contact pulses on the ^{1}H and ^{13}C channels. The amount of resulting ^{13}C magnetization M^C (therefore the efficiency of the CP transfer) as a function of the contact duration $t_{contact}$ depends on the transfer constant rate T_{CH}: $\eta=(\gamma^H/\gamma^C)(M^H/M_0^H)(1-e^{-t_{contact}/T_{CH}})$.
2. $T_{1\rho}^H$: Spin-lattice relaxation in the rotating frame of ^{1}H occurs during the CP contact pulse simultaneously to the magnetization transfer and also during the acquisition time under decoupling pulse. As the spin-lock and decoupling pulses cannot keep the ^{1}H magnetization in the xy plane, the magnetization goes back to the equilibrium state. Therefore, the longer the $T_{1\rho}^H$ the less ^{1}H magnetization is lost during the spin-lock and decoupling steps. The $T_{1\rho}^C$ is usually much longer than that of ^{1}H and its effects can be neglected during CP. The kinetics of the ^{1}H spin-lattice relaxation in the rotating frame is expressed as $M^H=M_0^He^{-t/T_{1\rho}^H}$, with M_0^H and M^H the z axis direction magnetization on ^{1}H at the time 0 and t, respectively. For a given contact time $t_{contact}$, the ^{13}C signal enhancement factor becomes $\eta=(\gamma^H/\gamma^C)e^{-t_{contact}/T_{1\rho,contact}^H}(1-e^{-t_{contact}/T_{CH}})$, with $T_{1\rho,contact}^H$ the proton spin-lattice relaxation in the rotating frame time constant under the ^{1}H CP RF field. In the case of small organic and rigid molecules, the value of $T_{1\rho}^H$ depends on the RF field felt by the nucleus; the higher the RF, the longer the $T_{1\rho}^H$.

Two other phenomena have also to be taken into consideration:

3. T_2^C: ^{13}C transversal relaxation occurs during the acquisition time. During ^{1}H decoupling, the ^{13}C magnetization loses its coherence and gets dispersed with a characteristic decoherence time of T_2. This ^{13}C relaxation decreases the free induction decay (FID) amplitude: $M^C=M_0^Ce^{-t/T_2^C}$, with M_0^C and M^C the ^{13}C magnetization in the transverse plane at the time 0 and t, respectively.
4. T_1^H: The longitudinal relaxation of ^{1}H drives the delay between the two transients of a CPMAS NMR experiment. The ^{1}H magnetization on the z axis after the recycle delay $t_{recycle}$ corresponds to the ^{1}H magnetization source, which will be transferred to ^{13}C by CP in the next transient. The kinetics of the ^{1}H longitudinal relaxation is expressed as $M^H=M_0^H(1-e^{-t_{recycle}/T_1^H})$, with M_0^H and M^H the ^{1}H magnetization along the z axis direction at the time 0 and t, respectively.

3.2. CPMAS with flip-back pulse

In the ^{13}C CPMAS NMR experiment with FB pulse on ^{1}H (Fig. 1), the same relaxation phenomena occur, and therefore the amount of signal gain due to the flip-back pulse on ^{1}H can be estimated using the hereabove equations. An additional aspect must be taken into account, the ^{1}H magnetization (kept in the transverse plane by the CW decoupling) decrease with time constant $T_{1\rho,acq}^H$, possibly different from $T_{1\rho,contact}^H$ during the CP transfer if the RF field employed is different. Thus, at the end of the ^{13}C signal acquisition, the ^{1}H magnetization is $M^H=M_0^He^{-t_{contact}/T_{1\rho,contact}}e^{-t_{acq}/T_{1\rho,acq}^H}$. After the acquisition, the 90° FB pulse brings the M^H back along the z axis, which in turn becomes the M_0^H for the next accumulation.