

# Discrimination of $^{13}\text{C}$ NMR signals in solid material with liquid-like behavior presenting residual dipolar proton–proton homonuclear interactions: application on seeds

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## Abstract

In this communication, we propose, a modified spin echo fourier transform (SEFT) experiment run under magic angle spinning (MAS) to obtain structural informations of the liquid-like domains inside complex organic materials. It includes a proton–proton dipolar decoupling such as BLEW12 or Lee–Goldburg sequence just after the  $180^\circ$   $^{13}\text{C}$  refocusing pulse and short echo delays are used in order to overcome  $T_2$  relaxation. This very easy implemented sequence allows a clear discrimination among fast relaxing  $^{13}\text{C}$  signals between those with a pure liquid-like behavior and those presenting residual proton–proton dipolar coupling. The interests of the sequence, combined with other classical NMR experiments, are illustrated on whole vegetable seeds that represent an example of a complex material.

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## 1. Introduction

Recently we have shown that by combining, under magic angle spinning (MAS), single pulse excitation (SP) and cross polarization (CP) experiments that both liquid and solid domains of the seeds of *Lactuca sativa* can be selectively characterized [1]. Similar approaches have been reported by several groups in the last years [2–7]. Such experiments are easily set up with the CP/MAS probes devoted to solid materials that are currently commercially available provided that they have been optimized to give minimum residual background  $^{13}\text{C}$  signal under SP excitation. The main idea behind the above strategy is to selectively edit the  $^{13}\text{C}$  spectra of these components in a complex material using their residual heteronuclear dipolar interactions between protons and carbon [8–10]. Molecular mobility of the different parts of seeds can be

defined by NMR spectroscopy as its consequence of the more or less complete averaging to zero of the proton–carbon dipolar interactions. The commonly used CP/MAS experiment leads to record signals of carbons belonging to rigid domains, cell walls for instance, while the carbons belonging to liquid domains, such as vacuoles and oil bodies [11], can be selectively edited with the SP/MAS experiment. It is worth noting that the quality of the edited spectra, considering selectivity and signal-to-noise ratio, relies on the use of short recycling delays. For vegetable seeds recycling delays in the 5–15 s range represent a good compromise.

Using such experimental conditions on the seeds of *Pisum sativum*, spectra a and b, in Fig. 1, were recorded and were easily assigned to lignocellulosic material and to the triacylglycerol (TAG) moiety, respectively, on the bases of previous works [12,13]. Compared to the spectra of *L. sativa*, previously recorded under identical conditions [1], the SP/MAS spectrum of the seeds of *P. sativum*, in Fig. 1b, leads to record two additional broad signals noted 13' and 14'

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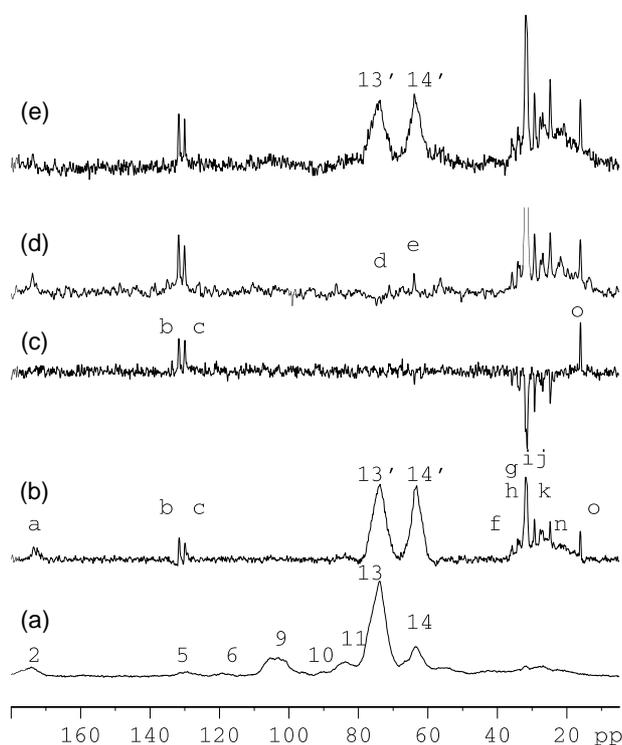


Fig. 1. High resolution solid-state  $^{13}\text{C}$  NMR spectra of seeds of *Lactuca sativa* acquired on a Bruker Avance spectrometer operating at 100.6 MHz. Intact seeds were placed in a 4mm-double-bearing rotor. The spinning rate was set in the range of 5000 Hz. The  $^1\text{H}$  radio-frequency field strength was set to give a  $90^\circ$ -pulse duration in the order of  $4.5\ \mu\text{s}$ ; the same value was used for the dipolar decoupling process. The  $^{13}\text{C}$  radio frequency field strength was obtained by matching the Hartman–Hahn condition. The  $^{13}\text{C}$  and  $^1\text{H}$  field strengths set for CP/MAS experiments can be directly used for the other sequences. (a) CP/MAS spectrum acquired with contact time and recycle delay, of 1 ms and 5 s, respectively. (b) Direct polarization  $^{13}\text{C}$  NMR spectrum obtained with MAS by applying a single pulse (SP) with high power decoupling during the acquisition. The chemical shift values were obtained via the glycine carbonyl signal, set at 176.03 ppm relative to TMS. (c) DEPT spectrum was recorded sequence used for liquid state NMR with a polarization transfer pulse set to  $3\pi/2$  in order to record CH and  $\text{CH}_3$  with phase opposite to  $\text{CH}_2$ . (d) SEFT spectrum was recorded with a sequence used for liquid state NMR with a  $\tau$  delay of 1 ms (such a delay does allow to assign the signals). (e) BLEW12-SEFT spectrum recorded with identical conditions than (d) but a BLEW12 proton decoupling sequence was introduced during the delay placed after the  $180^\circ$  pulse. The  $^{13}\text{C}$  and  $^1\text{H}$  field strengths were identical to those used in the CP experiment.

at 72 and 65 ppm, respectively, which might be assigned to sugars. A tentative explanation concerning the presence of these signals on SP/MAS spectra may be, either the presence of sugars in the liquid domains, as observed for the TAG molecules contained in the oil bodies, or a partial polarization of carbons of sugar moiety belonging to solid domains. This latter point may give an explanation since such signals are quite intense (see spectrum A) and could be partially polarized during the SP sequence.

In previous works [6,14] it has been shown by several groups that when residual dipolar coupling was low enough the multipulse experiments used for liquid phase NMR could be directly transposed on such complex material. For instance, SEFT [15], DEPT [16], and different two-dimensional proton–carbon correlations have been successfully applied for structural studies. In the present study spectra of seeds of *L. sativa* were recorded with two different experiments designed to liquid phase NMR: first the SEFT sequence using a delay of 6.6 ms (spectrum not shown) and second the DEPT sequence using a  $3\pi/4$  polarization transfer pulse (spectrum C). In both experiments the two broad signals 13' and 14' previously mentioned disappear and only the TAG signals remain visible and behave as expected in a solution. These two experiments are based on spin echo experiments with additional proton to carbon coherence transfer in the case of DEPT. In the SEFT and related experiments the  $^{13}\text{C}$  magnetizations are expected to evolve under indirect proton–carbon coupling,  $^1J_{\text{CH}}$ , during appropriate delays in which the proton decoupling is gated off. These spin properties have been widely used, for instance, to selectively detect  $^{13}\text{C}$  magnetization according to the number of protons bound to their carbons.

As signals 13' and 14' are much broader than the TAG signals their disappearance could be explained by carbon  $T_2$  relaxation during the different delays in those sequences. In order to check this hypothesis the delay, in the SEFT experiment was progressively reduced to 1 ms (spectrum D): the two broad signals 13' and 14' were still not recovered under these conditions. If one considers them as homogeneous lines, their linewidths,  $\Delta\nu$ , are in the order of 500 Hz. Therefore  $T_2$  can be estimated to 0.639 ms using the classical relation  $\Delta\nu = 1/2\pi T_2$ . Such values cannot justify that the corresponding signals vanish out during the SEFT sequence.

The origin of their disappearance has to be addressed. If the corresponding signals are not any longer detected at the end of the sequence it means that they evolved under additional coherent interactions that are not quenched during the sequence. To check this hypothesis proton–proton dipolar decoupling was introduced during these delays in order to quench this interaction. BLEW24 or Lee–Goldburg decoupling was therefore applied during the appropriate delay in the sequences [17–20]. Fig. 2 shows the modified SEFT experiment.

Spectrum E was recorded with the modified SEFT experiment in which, as suggested above, a BLEW12 homonuclear dipolar decoupling was introduced during the second evolution  $\tau$  period. The result was unambiguous since signals 13' and 14' were recovered without any problem. This experiment demonstrates that these signals can be definitively attributed to carbons belonging to sugars oligomers in which the proton–proton dipolar interactions are not completely averaged to zero

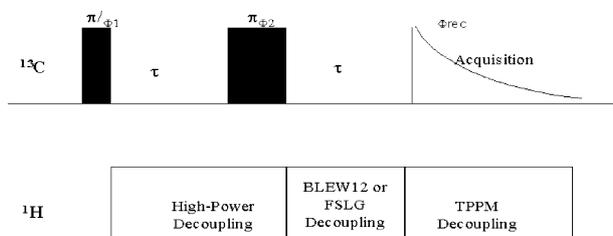


Fig. 2. The pulse sequence suitable to combine BLEW12 or Lee–Goldburg proton–proton dipolar decoupling and spin echo fourier transform experiment (SEFT). The efficiency of the decoupling conditions was tested on adamantane sample by acquiring CP/MAS spectra of adamantane with such decoupling scheme during the acquisition: the quality of the multiplet pattern, arising from the residual indirect proton–carbon coupling is an excellent test to set up the experiment. A sixteen-step phase cycle is used for the pulses on carbon channel:  $\phi_1 = (x)_4 (y)_4 (-x)_4 (-y)_4$ ,  $\phi_2 = x - xy - yy - y - xy - y - xx - xy - y$ . On receiver,  $\phi_{\text{rec}} = (x)_2 (-x)_2 (y)_2 (-y)_2$ .

either through molecular motion or under MAS conditions. Note, however, that the overall molecular tumbling of these products remains fast enough to lower their  $T_{1C}$  in a range that allows their detection through direct  $^{13}\text{C}$  excitation under magic angle spinning (SP/MAS) with a recycling delay in the range of 5 s. Polysaccharides are known to form multi-stranded arrangements such as triple helix in their solid state as well as in water soluble solution [21]. In such molecular arrangements, the intramolecular proton–proton dipolar interactions are certainly not averaged to zero through molecular motions.

This simple experiment, easily to set up on modern spectrometers, can be considered as an efficient tool among all the classical solid state NMR experiments to characterize  $^{13}\text{C}$  NMR signals of mobile products that exhibit residual proton–proton dipolar interactions. In such cases, relaxation properties very often lead to shorten  $T_1$  making direct polarization efficient. Therefore the sequence we propose allows a clear separation between the signals of products presenting a classical liquid behavior alone and those presenting also residual proton–proton dipolar interactions. It can be very complementary to editing methods based only on relaxation properties of materials [22,23]. The potential applications of such sequences appear to be promising in polymer sciences to study swollen polymers or gels. The interest of such sequences is obvious when working with lignocellulosic material for which the morphological features can drastically change with moisture content [24]. For biological studies such an assignment may be very informative since it indicates the presence or not of tri-dimensional organizations as encountered in biological membranes [25]. We are currently running these experiments on DNA polymers with different moisture contents, and we are carrying out theoretical studies to quantify these residual interactions.

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