Geoderma 226–227 (2014) 405–414

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

Cross polarization-single pulse/magic angle spinning (CPSP/MAS): A robust technique for routine soil analysis by solid-state NMR

Denis Courtier-Murias [a,b], Hashim Farooq [a], James G. Longstaffe [a], Brian P. Kelleher [c,d], Kris M. Hart [c,d], Myrna J. Simpson [a], André J. Simpson [a,*]

[a] Department of Chemistry, University of Toronto, 1265 Military Trail, Toronto, ON M1C 1A4, Canada
[b] Université Paris-Est, Laboratoire Navier (ENPC–IFSTTAR–CNRS), France
[c] School of Chemical Sciences, Dublin City University, Glasnevin, Dublin 9, Ireland
[d] Irish Separation Science Cluster, Dublin City University, Glasnevin, Dublin 9, Ireland

ARTICLE INFO

Article history:
Received 31 May 2013
Received in revised form 4 March 2014
Accepted 5 March 2014
Available online 13 April 2014

Keywords:
Organic carbon
NMR spectra
C-13
Solid phase
Signal-to-noise ratio
Quantitative methods

ABSTRACT

Soils are amongst the largest organic carbon reservoirs on earth containing approximately three times the carbon contained in all living systems and roughly double the amount present in the atmosphere. Soil organic matter is central to agriculture, carbon cycling, and contaminant sequestration, but due to its extreme heterogeneity it is challenging to study with most modern analytical approaches. As such ^{13}C NMR spectroscopy has emerged as an indispensable technique for the characterization of soil organic matter in the solid-state. Single pulse (SP) ^{13}C NMR approaches theoretically provide the highest level of quantitation for soil organic matter, however, due to its relative insensitivity, sample analysis can take a prohibitively long time. Consequently, for routine studies the more sensitive approach of cross-polarization under magic angle spinning conditions (CP/MAS) is more commonly utilized. In particular, ^{13}C CP is extremely useful when low organic carbon content samples are compared and is normally used to reveal the nature, transformations and fate of organic matter in soils. Here, the performance of a novel NMR scheme, which so far has not yet been applied to soils samples, is investigated. The ramp-CPSP scheme adds a SP block to a ramp-CP scheme, taking advantage of both techniques without extending the experimental time. This method shows enhancements higher than 100% for key regions of the ^{13}C spectra and also provides ^{13}C profiles that are closer to quantitative when compared to the widely used ramp-CP scheme. In addition, a critical analysis of these enhancements is presented. Even under the worst case scenario, when the SP element adds little additional signal, the result still reflects the conventional ramp-CP experiment. As such the ramp-CPSP approach can be implemented in a routine fashion without drawback. The results shown here suggest replacing conventional ramp-CP with the ramp-CPSP sequence for routine soil analysis in the solid-state since it can save considerable experimental time and provide a more representative ^{13}C spectrum of the soil in general.

1. Introduction

Soil quality, structure, composition and chemistry all play a role in various key issues linked to human health, such as agricultural production, ecosystem sustainability, fate of contaminants and global warming (Batjes, 1996; Bronick and Lal, 2005; Lupwayi et al., 2001; Meggo and Schnoor, 2011; Munyanziza et al., 1997; van der Oost et al., 2003). Over time the importance of soils and their impact on human health are becoming increasingly evident (De Rosa et al., 1998; Hough, 2007). As a result of its multifaceted function and crucial importance a wide range of analytical approaches are used for characterization of soils and soil organic matter (Kogel-Knabner, 2000). However, the complexity and heterogeneity of soils hamper a complete understanding of several key features such as soil structure, composition and chemistry. As such soil has appropriately been deemed the most complex biomaterial on the planet (Young and Crawford, 2004).

^{13}C nuclear magnetic resonance (NMR) spectroscopy in the solid-state using cross-polarization (CP) and under magic angle spinning (MAS) conditions arguably represents the most powerful experimental approach to collect direct information on the structural and conformational characteristics of natural organic matter (Conte et al., 2004). Since solid samples are rigid, strong dipole interactions arise between nuclei, leading to considerable line broadening and poor chemical shift resolution. This is particularly prominent in ^{1}H NMR of solids due to strong interactions between nearby protons. These interactions are less prominent in the case of ^{13}C nuclei at natural abundance and the effect of line broadening can be easily reduced under MAS conditions (Knicker, 2012). Consequently, solid-state NMR studies remain predominately ^{13}C detection under proton decoupling (Simpson et al., 2011) and MAS conditions.

---

* Corresponding author. Tel.: +1 416 287 7547.
E-mail address: andre.simpson@utoronto.ca (A.J. Simpson).

http://dx.doi.org/10.1016/j.geoderma.2014.03.006
0016-7061/© 2014 Elsevier B.V. All rights reserved.

Due to the relatively low gyromagnetic ratio and natural abundance of ^{13}C nuclei, CP is often used to increase its sensitivity for detection. Single pulse (SP) experiments (also termed Bloch decay or direct polarization experiments) provide quantitative spectra if they are run with a sufficiently long recycle delay to allow complete relaxation of the ^{13}C magnetization. However, this may involve a delay of many seconds or even minutes (Conte et al., 2004) to satisfy this requirement. It should be noted that correct quantification of ^{13}C NMR spectra of soils may be achieved using various advanced approaches but these procedures become excessively time-consuming, specifically if a large number of spectra are to be compared (Smejkalova et al., 2008). Moreover, it has been shown that ^{13}C SP experiments cannot always provide quantitative data, since some geopolymers (e.g. cellulose) relax extremely slowly (Knicker, 2012), making acquiring spectra with adequate signal unfeasible.

As previously mentioned, CP based experiments are powerful in terms of sensitivity but are inherently less quantitative. This can be discerned from the simplified dynamics that describe signal behavior by CP (Wilson, 1987). The signal enhancement in the ^{13}C nucleus depends on the polarization transfer time (T_{HX}), and both ^{1}H and ^{13}C rotating-frame spin–lattice ($T_{1\rho}$) constants. These values are dependent on by the distance of ^{13}C from ^{1}H nuclei and molecular motions. Therefore, ^{13}C nuclei from C, CH, CH$_2$ and CH$_3$ groups should experience dissimilar enhancements. In addition, the variety of different components in soil likely has a range of different molecular mobilities that further exacerbates the issue. Moreover, sensitivity to the Hartman–Hahn (HH) match is much greater at the higher MAS rate required for high magnetic fields (Smernik, 2005). For instance, it has been reported that CP can be unsuccessful at completely exciting ^{13}C in various soil systems (Preston, 2001; Smernik et al., 2002a,2002b). In general, C atoms that are remote from protons and those in moieties with high molecular mobility tend to be underestimated due to the weak H–C couplings (Johnson et al., 2005; Smernik and Oades, 2000b). Even though these limitations exist, the CP experiment prevails over the single pulse (SP) experiment for the routine analysis of soil samples because of its faster acquisition time, which can be used to characterize several dynamic processes in soils ranging from vegetation succession to long term cultivation (Lima et al., 2009; Nierop et al., 2001; Plaza et al., 2013; Schnitzer et al., 2006).

The central aim of this work is to assess the applicability of a novel NMR scheme to soils research, which has been recently introduced in material sciences (Shu et al., 2011). This approach can provide higher ^{13}C signal-to-noise ratio (S/N) values for selected regions without the need of extra experimental time compared to commonly used CP experiments. For instance, similar approaches have been previously implemented for the studies of peptides, reporting overall intensity enhancements of 10–15% when compared to CP in multidimensional NMR experiments (Rienstra et al., 2000).

2. Theory

Here, we used a ramped amplitude CP single pulse (ramp-CPSP) scheme (Fig. 1), which combines magnetization from both CP and SP sequences in one single experiment and also uses the ramped amplitude approach during the CP block. This sequence begins with 90° pulses in both ^{1}H and ^{13}C nuclei, which flip the magnetizations to the XY plane. This is where the CPSP pulse sequence differs from the usual CP sequence, in which only the ^{1}H nuclei are brought into the XY plane with a 90° pulse in the ^{1}H channel alone. The ^{1}H and ^{13}C magnetizations are then spin-locked during the subsequent contact time pulse. This is done using ^{1}H and ^{13}C spin lock pulses that fulfill the HH condition. During this time the purpose of the ^{13}C spin lock pulse is two-fold: one is to hold the magnetization from the more mobile components so that it will not dephase during the contact time and the other is to transfer polarization from ^{1}H nuclei, which was previously rotated to the XY plane, to ^{13}C nuclei for the rigid moieties.

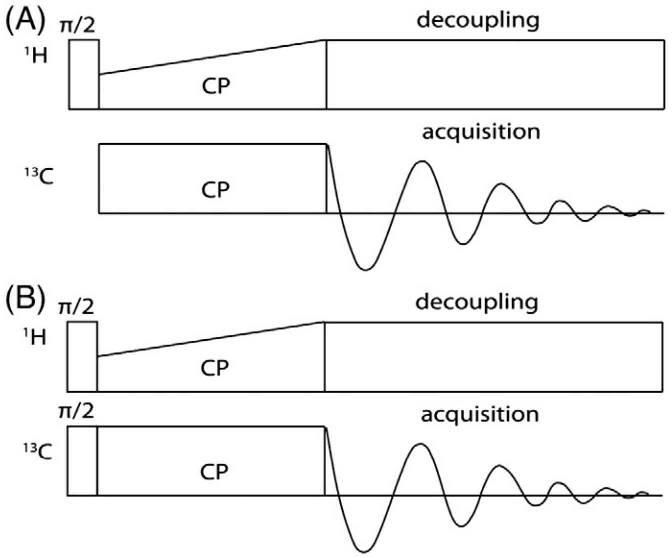

Fig. 1. Schematic representations of the ramp-CP based sequences used in this work; A) conventional ramped-CP commonly used to study soils in the solid phase and B) ramp-CPSP scheme applied to soils for the first time in this work. The ramp-CPSP sequence utilizes the following phase cycles: y, −y (^{1}H 90°); x (^{1}H contact time); y, −y, −y, y, −x, x, x, −x (^{13}C 90°); x, x, −x, −x, y, y,−y,−y (^{13}C contact time); and x, −x, −x, x, y, −y, −y, y (receiver phase) (Shu et al., 2011). The authors will be pleased to supply the Bruker version of the ramp-CPSP sequence on request.

In the CP sequence, transfer of ^{13}C magnetization to the XY plane relies solely on transfer from ^{1}H nuclei. The advantage of the CPSP sequence is that it ensures a "baseline" of such magnetization via the direct route of the 90° pulse in the ^{13}C channel. The cross polarization process is best understood in terms of a transfer of spin "temperature" in which a large pool of "cool" or highly coherent ^{1}H nuclei are brought into thermal contact with a small pool of "hot" or incoherent ^{13}C nuclei under the Hartman–Hahn condition. Under ideal conditions, the ^{13}C nuclei reach the same "cool" spin temperature as the ^{1}H nuclei; this explains the maximum CP enhancement of around four, equal to the ratio of ^{1}H to ^{13}C gyromagnetic ratios. Thus, the CPSP sequence ensures that all ^{13}C nuclei begin with one quarter of that optimal coherence (notwithstanding saturation effects - see below) before the onset of cross polarization. Further increases are not precluded during the contact time for ^{13}C nuclei that cross polarize efficiently (hence there is no disadvantage in the CPSP sequence). For ^{13}C nuclei for which cross polarization is inefficient, the coherence provided by the direct ^{13}C pulse might represent the majority of total coherence. Relaxation effects provide a further complication, since for the CP experiment the return to equilibrium is controlled by the more rapid ^{1}H T_1 relaxation time (T_{1H}), whereas for the SP experiment the return to equilibrium is controlled by the slower ^{13}C T_1 relaxation time (T_{13C}). Spectral signal is decreased through saturation when the repetition rate (time between consecutive scans) is less than 5 times the relevant T_1 value. In the CPSP sequence, the relevant T_1 value is T_{1H} for ^{13}C nuclei that cross polarize quickly, but T_{13C} for ^{13}C nuclei that cross polarize slowly. Thus the greatest benefit for CPSP over CP is for ^{13}C nuclei that cross polarize inefficiently and have a relatively short T_{13C} values. For soil organic matter this is likely to include highly mobile moieties and ^{13}C nuclei relatively close to organic or inorganic paramagnetic centers (Smernik and Oades, 1999).

As previously discussed (Shu et al., 2011), the ramp-CPSP scheme is suitable for systems with marked difference in mobility and possessing short T_1 relaxation values. This study was performed using a NMR instrument at 7.04 T, however, the applicability of this technique using higher magnetic fields was only theoretically assessed. Since T_1 relaxation values increase with magnetic field strength, it is important to test this scheme empirically at higher magnetic fields. Here, the