

# Effect of RF Field Inhomogeneity and Sample Restriction on Spectral Resolution of CP/MAS-<sup>13</sup>C NMR Spectra of Natural Organic Matter

Anne E. Berns<sup>\*,1</sup> and Pellegrino Conte<sup>2</sup>

<sup>1</sup>Institute of Chemistry and Dynamics of the Geosphere, ICG-4: Agrosphere, Forschungszentrum Jülich GmbH, 52425 Jülich, Germany

<sup>2</sup>Dipartimento di Ingegneria e Tecnologie Agro-Forestali (DITAF), Università degli Studi di Palermo, v.le delle Scienze 13, edificio 4, 90128 Palermo, Italy

**Abstract:** It is well known that the induced  $B_1$  magnetic field in an NMR coil is inhomogeneously distributed. However, this issue has so far received little attention in the field of environmental NMR. As this research field often aims at quantitative results as well as relaxation phenomena, the repercussions of such inhomogeneity on peak integrals and relaxation times need to be taken into account.

The objective of the present study was to test standard recording conditions on different sample positions in an NMR coil in order to determine the effect of the RF field inhomogeneity on the spectrum of a molecularly complex humic material and on some standard molecules of known structure and conformation. To this end, we measured the peak integral and signal half-height width of constant sample amounts at different heights in the rotor. In addition, the effect of sample position in the rotor on  $T_1$ H and  $T_2$ C relaxation times was determined.

We showed that the response profiles of different chemical groups are not necessarily comparable to each other and that spectra of natural organic matter can change when confined to different regions of the coil. Furthermore, the relaxation measurements revealed that  $T_1$ H and  $T_2$ C relaxation times are position-dependent. Finally, the application of sample restriction to the homogeneous region appeared very promising for enhancing the resolution of spectra of complex mixtures.

**Keywords:** CP/MAS <sup>13</sup>C-NMR, RF field inhomogeneity, physical sample restriction, natural organic matter, signal response profile.

## INTRODUCTION

Cross-polarization magic-angle spinning (CP/MAS) <sup>13</sup>C-NMR spectroscopy has become a popular technique for studying natural organic matter (NOM) [1, 2] both in bulk soils and sediments [3] or when NOM is isolated from environmental matrices [4, 5]. However, this technique involves a number of pitfalls.

It is a well-known fact that the magnetic field induced by a radio frequency (RF) in a finite coil rapidly drops at both ends of the coil [6], resulting in an inhomogeneous distribution of the  $B_1$  field in NMR spectroscopy [7]. This inhomogeneity, inherent in all commercial standard solenoid coils, hampers, for example, quantitation of Bloch decay and CP experiments using spin counting [8, 9], where a carefully weighed quantity of an intensity standard [10] is added to a sample and peak integrals are compared. Restricting the sample to the homogeneous region of the solenoid coil is the easiest and most practicable way to avoid an inhomogeneous  $B_1$  field. However, the usable volume might be small and samples containing nuclei with low NMR sensitivity, because of either low magnetogyric ratio values or low natural abundance, may quickly reach the limits of feasibility.

Several optimized winding geometries such as coils with a variable pitch [7, 11, 12] or a variable wire width [13] have been proposed in order to enhance the homogeneous volume of solenoid coils. Furthermore, a number of selective pulse sequences and gradient techniques for spatial localization and sample restriction have been developed [14-20].

In addition to the inherent  $B_1$  inhomogeneity in solenoid coils, RF profiles of different frequencies, e.g. <sup>1</sup>H and <sup>13</sup>C, are not necessarily symmetric about the coil centre as reported by Paulson *et al.* [21]. This phenomenon of wavelength effect, which results in a misalignment of the physical centre of the RF field, was first reported by Stuhlman & Githens in 1932 [22] and becomes experimentally important once the coil wire length reaches about 10% or more of the free space wavelength of the applied RF field [21]. This is especially of concern for the <sup>1</sup>H field as this critical coil length can be easily reached even at lower frequencies. A slight field centre misalignment of the <sup>1</sup>H field is usually counteracted by using a variable amplitude or ramped CP transfer [23], which sweeps through a range of (usually) <sup>1</sup>H RF field amplitudes. For severe misalignments of two or three RF fields in a double or triple resonance probe, Paulson *et al.* [21] developed a balanced series resonant circuit, which splits the capacitance across the inductor instead of using a resonant circuit with only one capacitance. Unfortunately, this hardware change is not practicable in every routine NMR lab.

\*Address correspondence to this author at the Institute of Chemistry and Dynamics of the Geosphere, ICG-4: Agrosphere, Forschungszentrum Jülich GmbH, 52425 Jülich, Germany; Tel: +49 2461 615656; Fax: +49 2461 612518; E-mail: a.berns@fz-juelich.de

Magic-angle spinning in combination with cross-polarization adds to the problem of field inhomogeneity as dipolar interactions become time-dependent. In a non-spinning sample, the most efficient magnetization transfer is reached when the  $B_1$  field mismatch parameter  $\Delta = \omega_{I1} - \omega_{IS}$  between two spin systems I and S is close to zero. In a sample which is spun at a rate  $\omega_r$  the most efficient transfer occurs when  $\Delta = \pm n \omega_r$  (sideband matching condition) [21, 24]. At high spin rates, when the mismatch parameter  $\Delta$  becomes a considerable fraction of the RF field nutation rate,  $\gamma_i B_1$ , the necessity of a homogeneous RF field becomes very important. This requirement is even more stringent at an operating frequency of 800 MHz or more when MAS rates reach or exceed dipolar couplings of  $^1\text{H}$ - $^{13}\text{C}$  or  $^1\text{H}$ - $^{15}\text{N}$  [21].

Spectral resolution depends upon the length (N) of the transform interval:  $N = F/\Delta f$ , where  $\Delta f$  is the smallest frequency difference which can be distinguished at a given sampling frequency F. The frequency difference  $\Delta f$  depends on the signal width, which, in turn, is influenced by the relaxation time of the sampled signal [25, 26]. Relaxation times are affected by the external magnetic field and the applied RF field sent to the sample through the transmitter/receiver coil. As already described above the RF field homogeneity throughout this coil is a major factor affecting the NMR sensitivity and the resolution of the measurements.

The objective of the present study was to check standard recording conditions on different sample positions in an NMR coil in order to determine the effect of the RF field inhomogeneity on the spectrum of a molecularly complex humic material and on compounds of known structure and conformation. To this end, we performed CP/MAS-NMR experiments where constant sample amounts were moved through different heights in the rotor. At each height the peak integral and signal half-height width were measured and used as indicators of sensitivity and spectral resolution. In addition, the effect of sample position on  $T_{1H}$  and  $T_{2C}$  relaxation times was determined.

## MATERIALS AND METHODOLOGY

### Standard Materials

Sodium dodecylsulfate (SDS) was purchased from Sigma-Aldrich® (Steinheim, Germany) in *purum* quality and glycine was purchased from Merck (Darmstadt, Germany) in *pro analysi* quality. The standard materials were used without further purification. Pure quartz was purchased from Merck (Darmstadt, Germany) in *pro analysi* quality and ground in a planetary mill (PM 400, Retsch, Haan, Germany) to fine powder.

### Humic Acid

A humic acid from the A horizon of an andosol from the caldera of Vico (near Rome, Italy) was extracted, purified and characterized as reported in Cozzolino *et al.* [27]. The ash content was below 3 % and the Fe/C ratio  $\ll 1$ , hence the CPMAS  $^{13}\text{C}$ -NMR spectra were not affected by paramagnetic impurities [28].

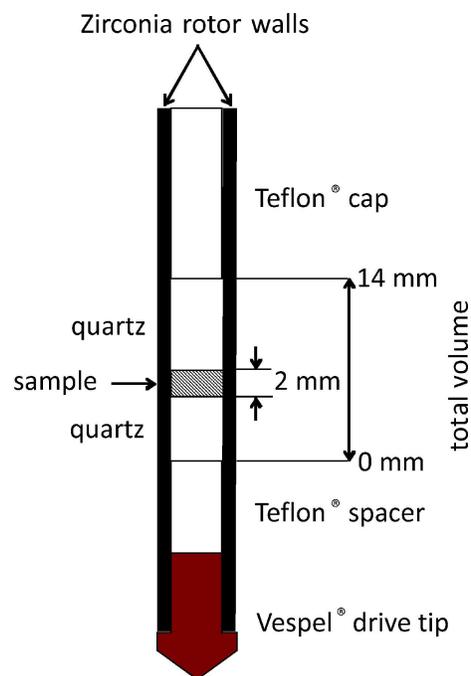
The HA was analysed to determine its elemental composition (C, H, N) in a Fisons Interscience EA1108 elemental analyser. The elemental composition corrected for the ash content ( $< 3\%$ ) was as follows: C 56.8 %, H 4.5 %, N 5.2 %.

The humic acid was also analysed by atomic-absorption spectrometry (AAS) in order to verify the presence of potentially paramagnetic species (Fe, Mn, Cu) in the ash content. The AAS analyses were performed on a Perkin-Elmer Analyst 700 with an instrumental sensitivity of  $0.1 \text{ mg kg}^{-1}$ . Aliquots of the humic acid (50 mg) were boiled in a nitric/perchloric acid solution until complete mineralization of the organic matter and dissolution of the inorganic ashes was obtained. The solutions were transferred to 20-mL volumetric flasks and analysed by AAS. Negligible traces of paramagnetic metals were found.

### General Solid-State NMR Conditions

A 7.05 T Varian UNITY INOVA™ (Varian Inc., Palo Alto, CA, USA), equipped with an Apex HX wide-bore probe operating at a  $^{13}\text{C}$  frequency of 75.4 MHz, was used to acquire the  $^{13}\text{C}$ -NMR spectra. The samples were packed in 6 mm zirconium rotors with Teflon® bottom and top spacers and Vespel® drive tips. The temperature was kept constant at  $25.0 \pm 0.1^\circ\text{C}$ . Magic-angle spinning was carried out at  $7500 \pm 1 \text{ Hz}$ . Repetition times were 3s for glycine and 35 s for SDS. The spectra of glycine and SDS were recorded with a  $^1\text{H}$  RF field strength of 65.3 kHz with a ramp of 7 kHz. CP/MAS spectra of Vico HA were acquired with a  $^1\text{H}$  RF field strength of 40.4 kHz and a ramp of 15.3 kHz to account for inhomogeneities of the Hartmann-Hahn condition [2, 21, 23, 24]. In both cases the matching  $^{13}\text{C}$  and  $^1\text{H}$  RF fields were determined in separate (non-ramped) experiments by fixing the  $^{13}\text{C}$  RF field and arraying the (non-ramped)  $^1\text{H}$  RF field. Decoupling was done using a TPPM sequence with a  $^1\text{H}$  field strength of 54 kHz, a phase of  $8^\circ$  and a pulse length of  $9.3 \mu\text{s}$ .

VNMRJ software (Version 1.1 RevisionD, Varian Inc., Palo Alto, CA, USA) was used to acquire all the free induction decays (FID). Spectra elaboration was performed



**Fig. (1).** Pencil® rotor and dimensions of manufacturer-determined sample volume.

by Mestre-C software (Version 4.9.9.9, Mestrelab Research, Santiago de Compostela, Spain). All the FIDs were transformed by applying first a 4 k zero filling and then an exponential filter function with a line broadening (LB) of 20 Hz. Fully automatic baseline correction using a Bernstein algorithm was applied for baseline corrections [29].

### Sample Positioning in the Rotor

A constant sample amount was incrementally moved in a volume with 2 mm fill height through different heights in the rotor to record the spectra of the two standard substances at different positions in the rotor (Fig. 1). At every height the peak integral and the half-height width of the signals were determined as indicators of sensitivity and spectral resolution. Furthermore, relaxation times of selected volumes were determined. The amount of sample needed to fill a volume of 2 mm fill height was  $32 \pm 1$  mg for glycine (= 0.43 mmol) and  $26 \pm 1$  mg for SDS (= 0.10 mmol). In a first set of experiments with SDS and glycine, the bottom and top parts were filled with fine quartz powder with a constant density of  $26 \pm 1$  mg/mm. This was done to ensure a constant total weight of the rotor ( $3.6 \pm 0.01$  g) and avoid possible height differences due to the lifting of the rotor during spinning. The replacement of a defective preamplifier, although quickly discovered, during the first set of experiments, caused slightly different recording conditions for SDS and glycine so that we decided to verify the reproducibility of these experiments. The repetition of the experiments with SDS and glycine was done with purpose-built boron nitride inlets to facilitate the positioning of the sample and ensure an even better weight constancy of the rotor. The results reported are from this second set of experiments, which confirmed the first set. The correct position of the sample in the rotor was determined with the help of a light table and a template. The total possible sample volume was given by the Teflon® top and bottom spacers provided by the manufacturer matching the coil geometry. The bottom limit of this volume was defined as position 0 mm and the top as 14 mm (Fig. 1). The position of a sample is indicated through the

position of the centre of the sample in question. For example, a sample filling a volume from 2 to 4 mm height is indicated by the position at 3 mm.

After determination of the homogeneous region, experiments were conducted with centre, bottom & top and fully filled rotors. In this case “centre filled” refers to samples where ground quartz powder (or BN inlets) was filled from 0 to 5 mm and from 11 to 14 mm and the sample occupied the volume from a height of 5 to 11 mm. The sample position was checked with the help of a light table and a template. In “bottom & top filled” rotors, the sample occupied the lower and top part of the rotor, while the centre was filled with ground quartz powder (or BN inlet).

### CP/MAS and Relaxation Experiments

$T_1H$  relaxation was determined with the sequence displayed in Fig. (2A) with an array of delay times (d3) from 0 to 2 s for glycine and an array of 0 to 11 s for SDS. A spin echo sequence with an array of tau from 0 to 5333  $\mu$ s was performed to determine the  $T_2C$  relaxation time constant (Fig. 2B). The fitting procedures for the relaxation curves were done with OriginPro 7.5 SR6 (Version 7.5885, OriginLab Corporation, Northampton, MA, USA). The errors reported are obtained from the fitting procedure.

The CP/MAS  $^{13}C$ -spectra were evaluated for their peak integrals and widths at half-height. The determined integrals and widths at half-height were normalized to position 8.

### RESULTS AND DISCUSSION

In the 7.05 T instrument used for the present study, the  $^1H$  radio frequency of 300 MHz corresponds roughly to a free space wavelength of 1 m. Hence, the resulting critical coil wire length, where wavelength effects are to be expected [21, 22], is 10 cm. The Apex Pencil® probe utilized for the present study is equipped with a 5-turn coil with an inner diameter of 6 mm and a wire thickness of 1 mm. Thus, the coil wire length is approximately 11 cm, excluding the lead lengths. The RF of 75 MHz for the  $^{13}C$  nucleus corresponds

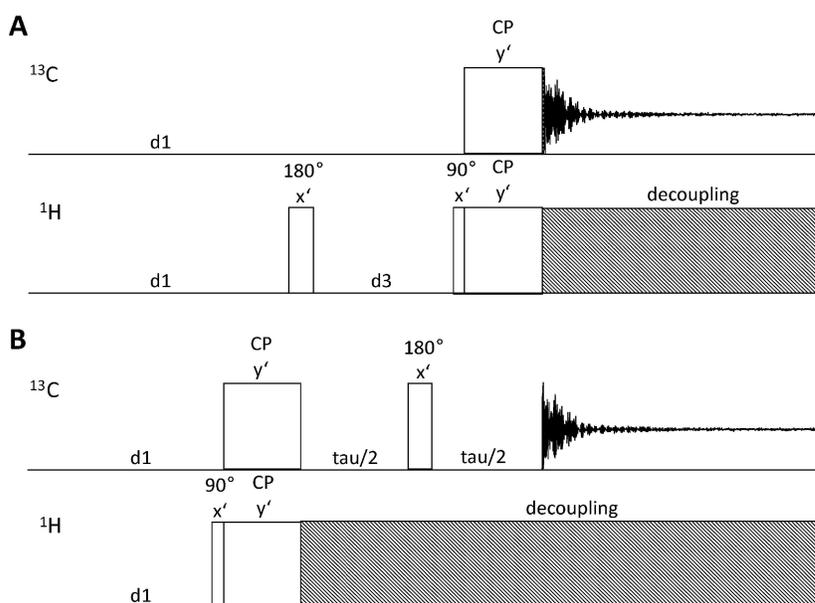


Fig. (2).  $T_1H$  pulse sequence (A) and  $T_2C$  echo pulse sequence (B).

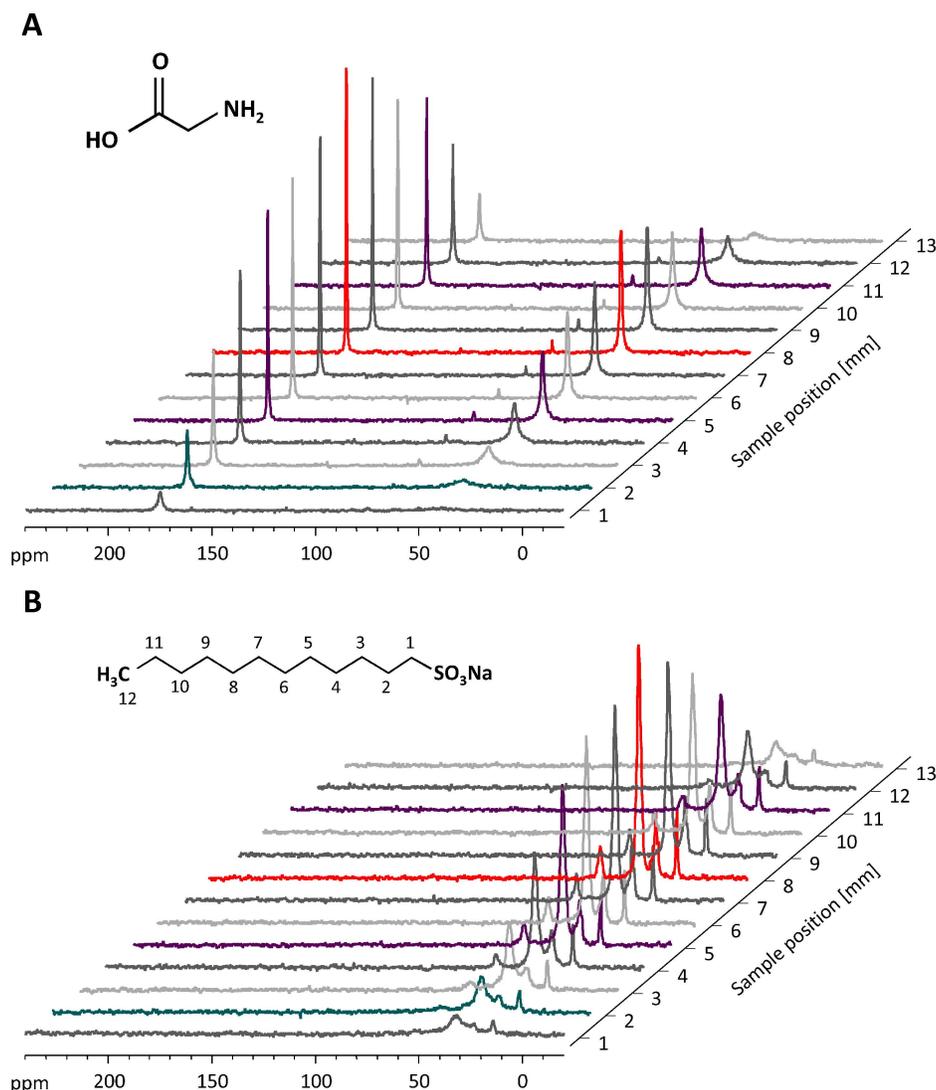
to approximately 4 m, i.e. a critical coil wire length of 40 cm. Hence, for the  $^1\text{H}$  RF field a slight centre misalignment can be expected as the coil wire length amounts to a minimum of 11 % of the applied wavelength. A rapid measurement of  $^1\text{H}$  and  $^{13}\text{C}$ -integrals on a 2-mm-thick adamantane sample confirmed that the centres of both RF profiles were not perfectly aligned (data not shown). Therefore, a ramped  $^1\text{H}$  field to counterbalance this misalignment must be applied.

To avoid RF field nutation rates, which match rates equivalent to the MAS rate,  $\omega_r$ , or twice as great,  $2\omega_r$ , (usually conditions which destroy spin-locked magnetization during cross-polarization through recoupling of chemical shift anisotropy), an RF nutation rate exceeding  $5\omega_r$  is recommended [21, 24]. A spin rate of 7.5 kHz and  $^1\text{H}$  RF fields of 40.4 kHz and 65.3 kHz, as reported in Materials and Methods, were chosen for the present study. These conditions ensured that the RF field strengths exceeded the recommended minimum RF field strength of  $5\omega_r$  (i.e. 37.5 kHz) so that no negative effects on the spin-locked magnetization were to be feared.

It should be noted that pulse widths and matching conditions were set with completely filled rotors and, as a consequence, sensitivity profiles exhibit less dependence than they would if the parameters were optimized on a centre-filled rotor [8].

The CP/MAS spectra of glycine and sodium dodecyl sulfate recorded at different positions within the manufacturer-defined volume are displayed in Fig. (3). The detection-sensitivity profiles clearly show that the signal integrals are strongly dependent on the position of the sample in the rotor. Only in a small region in the centre of the rotor is the signal response reasonably consistent.

Figs. (4A) and (4B) show the normalized signal integrals of these CP/MAS spectra. The signal integrals are normalized to position 8, the centre of the determined homogeneous region. The sensitivity profile of the glycine methylene group displays a smooth curve, while the curve of the COOH group has two outliers in positions 8 and 9. In the profile of SDS, however, only the integrals of signal C2-10 are consistent. Considering the molar amounts present in the rotor, it becomes clear that signal C2-10 corresponds to 0.90 mmol

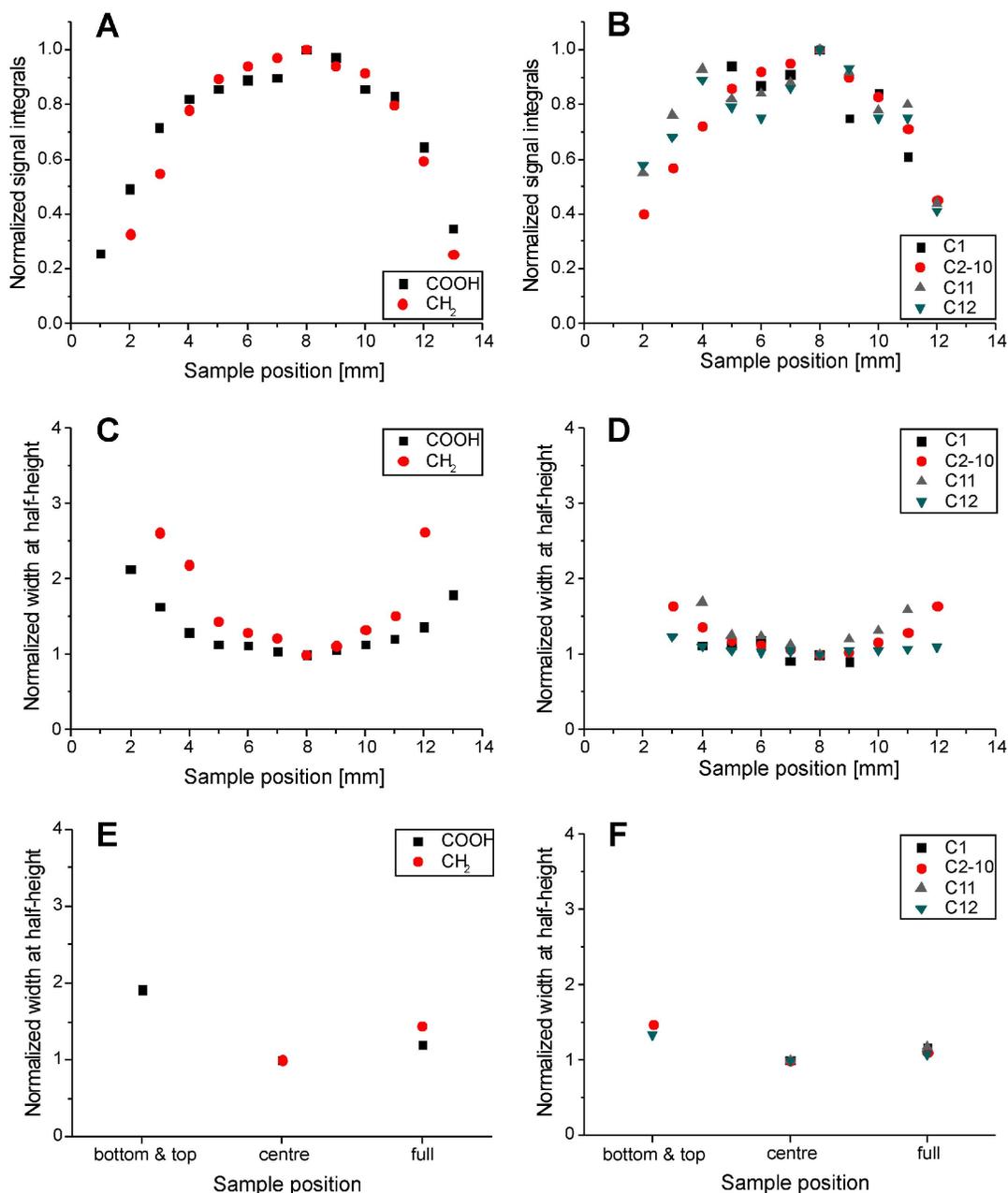


**Fig. (3).** Response profiles of glycine (A) and SDS (B).

of C, the two signals of glycine to 0.43 mmol C each and the remaining signals of SDS correspond to only 0.10 mmol C each. These reduced signals result in a larger error in the evaluation of the spectrum. Nonetheless, the general shape of the profile (as well as the profile of the first experiment, not shown) follows that of glycine with severely reduced signal integrals in the outer regions; the lower positions being worse than the upper regions.

As already stated by Campbell *et al.* [8], sensitivity profiles in cross-polarization experiments (as used in the present study) should be expected to display a much less pronounced drop at the end of the coil. In fact, the inhomogeneous amplitudes of the  $^1\text{H}$  and  $^{13}\text{C}$  RF fields are counterbalanced

through the Hartmann-Hahn signal enhancement as long as the ratio of the  $^1\text{H}/^{13}\text{C}$  RF field levels is maintained (which is given by the use of a ramped  $^1\text{H}$  RF field). Campbell *et al.* [8] mention two factors which need to be considered in such position-dependent sensitivity profiles in Bloch decay and cross-polarization experiments. The first factor is the variation of the RF field magnitude, which results in different pulse flip angles for different regions in the coil. The second factor is the variation of the curvature of the  $B_1$  field, which produces magnetization elements that are directed out of the detection plane. Due to the symmetry of the coil, these non-planar magnetization elements exist in opposing pairs which cancel each other out. Hence, not all of the generated mag-



**Fig. (4).** Sensitivity (A and B) and resolution profiles (C to F) of the Apex Pencil<sup>®</sup> probe for  $^{13}\text{C}$ -CPMAS spectra of glycine (A, C and E) and SDS (B, D and F) (all normalized to position 8 or the central region, respectively).

netization is observed. According to Campbell *et al.* [8], these directional inhomogeneities are the main reason for the spatial dependence of the sensitivity profiles in cross-polarization experiments (as the inhomogeneous amplitudes of the  $^1\text{H}$  and  $^{13}\text{C}$  RF fields should be counterbalanced through the Hartmann-Hahn signal enhancement).

In the case of the present study where the samples were high-rate-spun (Campbell *et al.* [8] studied non-spinning samples), a further factor needs to be considered. As the RF field amplitudes drop at the end of the coil, they may reach a value where the condition RF field strength  $> 5\omega_r$  is no longer met, and they may in contrast approach a condition where the RF field nutation rates  $\gamma_i B_1$  match the MAS rate  $\pm \omega_r$  or  $\pm 2\omega_r$ , thereby destroying the spin-locked magnetization during cross-polarization. Unfortunately, no equipment was at hand for a direct mapping of the RF field strengths. Furthermore, an exhaustive discussion of the effect of MAS on CP is already available in several high-quality papers [21, 24] and would go beyond the scope of the present paper.

For quantitative evaluations it is important to know whether the reduction in signal integral is distributed uniformly all along the NMR signals of each chemical group. In the case of glycine at the lowest positions of the manufacturer-defined region, the response decrease of the methylene group is stronger than that of the carboxyl group. The signal response profiles of the SDS signals are harder to interpret as the signal response is very scattered (even in the homogeneous region determined), but in the lower region it can be seen that the signal of the methylene groups C2-10 tends to be disproportionately reduced as can be seen in the profile of glycine.

The line widths of the signals are also position-dependent as displayed in Figs. (4C) and (4D). The normalized display (to position 8 as in the signal response profiles) shows that the widths at half-height of the different groups broaden to a different extent outside the central homogeneous region. The line width of the glycine methylene group, already broader by nature through its faster relaxation, shows a stronger increase (factors 2.6 at positions 3 and 12) in its line width than the carboxyl group (factors 1.6 and 1.4 at positions 3 and 12). Also in the case of SDS, the methylene signals (C2-10 and C11) are the ones with a larger enhancement of the width at half-height outside the central region, while the line width of the methyl group (C12) is hardly perturbed at all. The width at half-height of the methylene signal closest to the sulfate group is already quite broad in the centre regions, and in most of the outer regions the signal width cannot be measured. The normalized widths at half-height determined on fully, centre and bottom & top filled rotors are shown in Figs. (4E and 4F). The signal widths in the bottom & top filled rotors were elevated similar to the widths in the incremented experiments, whereas the methylene signal of glycine was too broad to be reasonably measured. The widths in the fully filled rotors were in between the widths of the centre and bottom & top filled rotors as expected.

The line width is determined by the overall decay rate of the transverse magnetization  $T_2^*$ . The latter, in turn, is affected by both homogeneous (the transverse relaxation rate  $T_2$ ) and inhomogeneous contributions (called inhomogeneous broadening and often denoted  $T_2^\dagger$ ). The transverse re-

laxation rate  $T_2$  is a fundamental property and is influenced by the type of molecule, its physical environment and its motion in this environment. Inhomogeneous broadening results from non-uniform magnetic fields across the sample. A poorly shimmed probe head, for example, causes spins to experience different Larmor frequencies in different parts of the sample due to an inhomogeneous  $B_0$  field. The homogeneous contribution can be distinguished from the inhomogeneous part through a simple echo sequence. As the latter is due to spins precessing at different frequencies throughout the sample over time they get out of step and eventually cancel each other out, resulting in a decay of the overall magnetization. This simple spin dephasing can be reversed by applying a  $180^\circ$  pulse after an evolution time  $\tau$  and recording the signal after another  $\tau$  period, at which point the phases will have realigned. The homogeneous part cannot be reversed as it is a true relaxation process, i.e. an approach to equilibrium [25, 26]. Measurement of the  $T_2\text{C}$  relaxation times are shown in Figs. (5A and 5B). It can be seen that especially the longer relaxation times of the COOH group of glycine and the methyl group of SDS are strongly influenced by the position of the sample. The application of an echo sequence where decoupling started only with the recording of the signal revealed that dephasing during the echo periods is sensitive to decoupling. Without decoupling being turned on during dephasing the determined relaxation times showed no position dependence and were extremely short by reason of magnetization loss due to coupling (data not shown). It is hence likely that the higher  $T_2\text{C}$  relaxation times determined in the centre of the rotor arise from more efficient decoupling of the sample, due to the increased homogeneity of the RF pulses in this region. Calculation of the overall decay rate of the transverse magnetization  $T_2^*$  from the determined widths at half-height revealed a large discrepancy between the overall decay rate and the  $T_2\text{C}$  relaxation, e.g. a  $T_2\text{C}$  of 25 ms and a  $T_2^*$  of 6.4 ms for the COOH group of glycine. Hence most of the overall decay rate of the transverse magnetization is due to inhomogeneous contributions. As the transverse relaxation takes place after the application of the  $B_1$  field the inhomogeneities influencing this factor must come from the  $B_0$  field. Inhomogeneities in the static magnetic field are counterbalanced as effectively as possible by the shimming procedure. However, the simple presence of the coil in the magnetic field and the pulsed application of the  $B_1$  field lead to disturbances in the  $B_0$  field which are hard to counterbalance completely. Furthermore, the shimming is optimized to the line shape of adamantane, i.e. while the  $B_1$  field is active. The disturbances which the coil produces in the  $B_0$  field are strongest at the edges of the coil and therefore the line widths increase in these regions. In the main, it must be noted that the determination of  $T_2\text{C}$  times in the fully filled rotor obviously underestimates the relaxation times.

Measurement of the longitudinal relaxation  $T_1\text{H}$  shows a longer  $T_1\text{H}$  in the centre position than in the outer regions (Figs. 5C and 5D). This is due to the fact that as the RF field is not homogeneous, the  $90^\circ$  pulse is not perfect throughout the sample. Hence the outer regions of the sample experience a pulse which differs from  $90^\circ$  resulting in a lower magnetization from which the system needs to return to equilibrium. The centre position is closest to the true  $90^\circ$  pulse and hence

has the longest relaxation time. It can be seen that measurements on a fully filled rotor also lead to an underestimation of the  $T_1H$  relaxation times.

In Fig. (6) the influence of physical sample restriction on the resolution of a spectrum of a humic acid is shown. All three spectra were recorded as having a similar signal-to-noise ratio and were graphically normalized to the carboxyl signal at 172 ppm. It can be seen that the relative integrals of some signals in the HA spectrum change, which leads to differences in the peak integrals ranging from 1 to 3 %. The main regions influenced are the aliphatic and O/N-alkyl regions (0-45 and 45-90 ppm). The signal in the aliphatic region of the bottom & top filled rotor (blue spectrum) is shorter and broader than the signal resulting from the central region (red spectrum) and that in the spectrum of the fully filled rotor (black spectrum). Most interesting is the fact that in the O/N-alkyl region the resolution of the signals is enhanced. Between 45 and 65 ppm the blue spectrum of the bottom & top filled rotor shows only one broad signal, whereas in the red spectrum of the centre filled rotor three peaks can be distinguished. The black spectrum of the fully filled rotor merges both features and is less resolved than the red spectrum, but better resolved than the blue spectrum. The more strongly enhanced resolution of the O/N-alkyl region can also be seen in the spectra of glycine (Fig. 4C), where the signal half-height width of the methylene group is reduced by a factor of 2.6 when moving from the outer to the central region. As already stated for the  $T_2C$  measurements,

the increase in resolution probably arises from a more efficient decoupling in the centre of the rotor.

Although the physical reduction of the sample to the central region requires longer measurement times to reach a good signal-to-noise ratio, the resolution enhancement achieved can be of importance when the interactions of humic acids with labelled chemicals [30] or changes in a specific region are monitored [31].

## CONCLUSIONS

Although RF field inhomogeneity is a subject well studied by NMR scientists in chemical and physical research groups, the issue has so far received little attention in the field of environmental NMR. As this research field often also aims at quantitative results as well as relaxation phenomena, the repercussions of the position dependence of the peak integral and of relaxation times on such NMR measurements need to be taken into account.

The present study shows that the response profiles are not necessarily similar for different chemical groups and hence spectra of complex organic matter can change when confined to different regions of the coil. It must be pointed out that the work presented in this paper was done on a 6 mm probe head. The described effects might be smaller in smaller rotors or in coils with different geometry. We therefore recommend that the signal response profile in the coil should be checked, for example by the proposed procedure, and the sample should be restricted to the determined homogeneous region

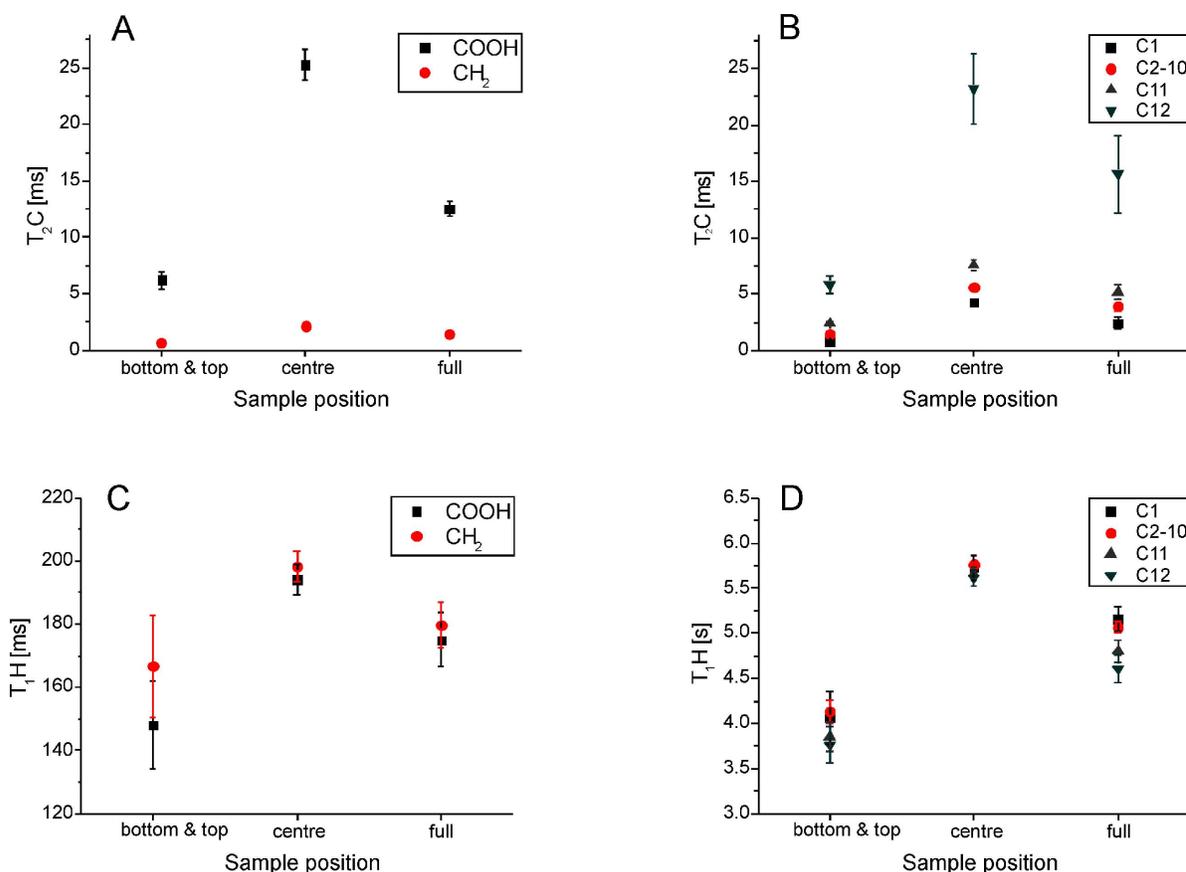
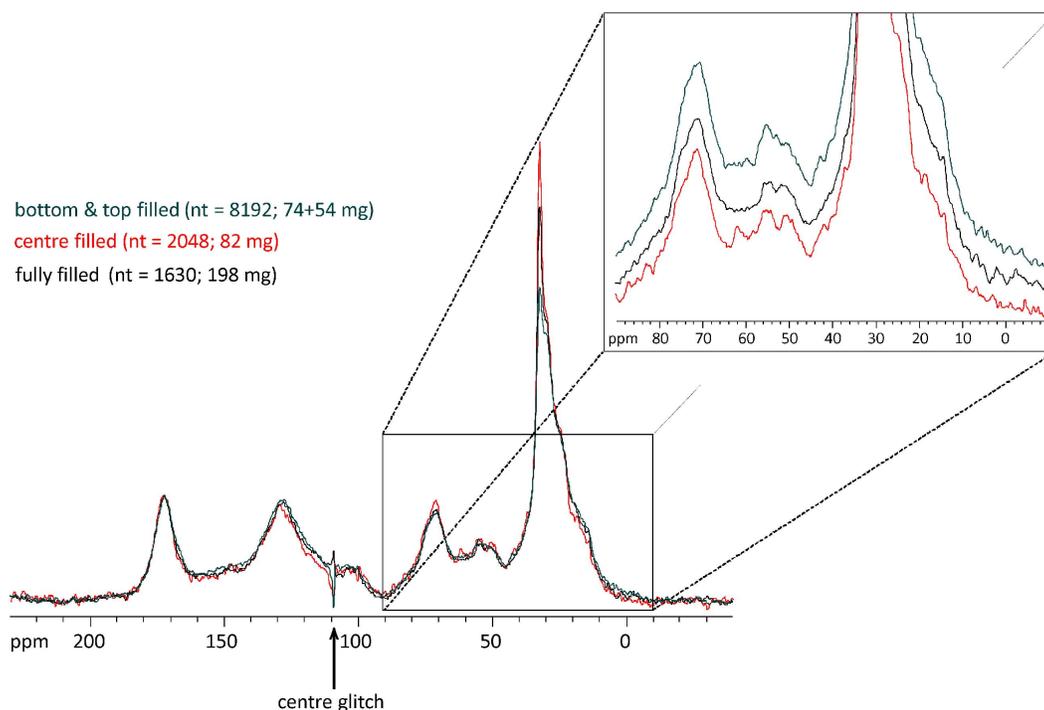


Fig. (5).  $T_2C$  (A and B) and  $T_1H$  (C and D) relaxation rates of glycine (A and C) and SDS (B and D).



**Fig. (6).**  $^{13}\text{C}$ -CP/MAS spectra of Vico humic acid in different positions of the manufacturer-defined sample region.

when quantitation is required as also Bloch decay experiments are sensitive to coil sample position. The same applies to relaxation measurements as relaxation times are also position-dependent. Furthermore, the application of sample restriction to the homogeneous region appears very promising for enhancing the resolution of spectra of complex mixtures.

#### ACKNOWLEDGEMENTS

The authors thank Forschungszentrum Jülich GmbH (Germany) for financing PC as a visiting scientist at the NMR centre of the Institute of Chemistry and Dynamics of the Geosphere, Institute 4: Agrosphere. Many thanks are also due to Dr. Andreas Pohlmeier for helpful discussions on relaxation phenomena. The Language Services of Forschungszentrum Jülich are also gratefully acknowledged for revising the English of the manuscript.

#### REFERENCES

- [1] Cardoza LA, Korir AK, Otto WH, Wurrey CJ, Larive CK. Applications of NMR spectroscopy in environmental science. *Prog Nucl Magn Reson Spectrosc* 2004; 45: 209-38.
- [2] Conte P, Spaccini R, Piccolo A. State of the art of CPMAS C-13 NMR spectroscopy applied to natural organic matter. *Prog Nucl Magn Reson Spectrosc* 2004; 44: 215-23.
- [3] Wilson MA. NMR techniques and applications in geochemistry and soil chemistry. London: Pergamon Press 1987.
- [4] Mao JD, Fang XW, Schmidt-Rohr K, Carmo AM, Hundal LS, Thompson ML. Molecular-scale heterogeneity of humic acid in particle-size fractions of two Iowa soils. *Geoderma* 2007; 140: 17-29.
- [5] Mopper K, Stubbins A, Ritchie JD, Bialk HM, Hatcher PG. Advanced instrumental approaches for characterization of marine dissolved organic matter: Extraction techniques, mass spectrometry, and nuclear magnetic resonance spectroscopy. *Chem Rev* 2007; 107: 419-42.
- [6] Tipler PA. Physik. Spektrum Akademischer Verlag GmbH: Heidelberg 1998.
- [7] Leifer MC. RF Solenoid with extended equirepple field profile. *J Magn Reson Ser A* 1993; 105: 1-6.
- [8] Campbell GC, Galya LG, Beeler AJ, English AD. Effect of RF inhomogeneity upon quantitative solid-state NMR measurements. *J Magn Reson Ser A* 1995; 112: 225-8.
- [9] Smernik RJ, Oades JM. Spin accounting and RESTORE - two new methods to improve quantitation in solid-state C-13 NMR analysis of soil organic matter. *Eur J Soil Sci* 2003; 54: 103-16.
- [10] Hall RA, Jurkiewicz A, Maciel GE. A bicyclic ketone as a solid-state C-13 NMR intensity reference. *Anal Chem* 1993; 65: 534-8.
- [11] Idziak S, Haeberlen U. Design and construction of a high homogeneity RF coil for solid-state multiple-pulse NMR. *J Magn Reson* 1982; 50: 281-8.
- [12] Horne D, Kendrick RD, Yannoni CS. Bond length measurements in amorphous solids by nutation NMR spectroscopy - the role of RF field homogeneity. *J Magn Reson* 1983; 52: 299-304.
- [13] Privalov AF, Dvinskikh SV, Vieth HM. Coil design for large-volume high-B1 homogeneity for solid-state NMR applications. *J Magn Reson Ser A* 1996; 123: 157-60.
- [14] Charmont P, Lesage A, Steuernagel S, Engelke F, Emsley L. Sample restriction using magnetic field gradients in high-resolution solid-state NMR. *J Magn Reson* 2000; 145: 334-9.
- [15] Charmont P, Sakellariou D, Emsley L. Sample restriction using radiofrequency field selective pulses in high-resolution solid-state NMR. *J Magn Reson* 2002; 154: 136-41.
- [16] Tycko R, Pines A. Spatial localization of NMR signals by narrow-band inversion. *J Magn Reson* 1984; 60: 156-60.
- [17] Kemp-Harper R, Styles P, Wimperis S. B1-selective pulses. *J Magn Reson Ser A* 1996; 123: 230-6.
- [18] Shaka AJ, Keeler J, Smith MB, Freeman R. Spatial localization of NMR signals in an inhomogeneous radiofrequency field. *J Magn Reson* 1985; 61: 175-80.
- [19] Hoult DI. Solution of the Bloch equations in the presence of a varying B1 field - Approach to selective pulse analysis. *J Magn Reson* 1979; 35: 69-86.
- [20] Malloy CR, Lange RA, Klein DL, *et al.* Spatial localization of NMR signal with a passive surface gradient. *J Magn Reson* 1988; 80: 364-9.
- [21] Paulson EK, Martin RW, Zilm KW. Cross polarization, radio frequency field homogeneity, and circuit balancing in high field solid state NMR probes. *J Magn Reson* 2004; 171: 314-23.
- [22] Stuhlman O, Githens S. The magnetic field of a solenoid oscillating at radio frequencies. *Rev Sci Instrum* 1932; 3: 561-71.

- [23] Metz G, Wu XL, Smith SO. Ramped-amplitude cross-polarization in magic-angle-spinning NMR. *J Magn Reson Ser A* 1994; 110: 219-27.
- [24] Wu XL, Zilm KW. Cross-polarization with high-speed magic-angle-spinning. *J Magn Reson Ser A* 1993; 104: 154-65.
- [25] Levitt MH. Spin dynamics. Chichester: John Wiley & Sons, Ltd. 2008.
- [26] Keeler J. Understanding NMR spectroscopy. Chichester: John Wiley & Sons, Ltd. 2005.
- [27] Cozzolino A, Conte P, Piccolo A. Conformational changes of humic substances induced by some hydroxy-, keto-, and sulfonic acids. *Soil Biol Biochem* 2001; 33: 563-71.
- [28] Preston CM. Applications of NMR to soil organic matter analysis: history and prospects. *Soil Sci* 1996; 161: 144-66.
- [29] Brown DE. Fully automated base-line correction of 1D and 2D NMR-spectra using Bernstein polynomials. *J Magn Reson Ser A* 1995; 114: 268-70.
- [30] Berns AE, Conte P, Philipp H, Witte EG, Lewandowski H. Interactions between 2-aminobenzothiazole and natural organic matter as evidenced by CPMAS Nitrogen-15 NMR spectroscopy. *Vadose Zone J* 2009; 8: 1-7.
- [31] Golding CJ, Smernik RJ, Birch GF. Investigation of the role of structural domains identified in sedimentary organic matter in the sorption of hydrophobic organic compounds. *Environ Sci Technol* 2005; 39: 3925-32.

---

Received: July 15, 2009

Revised: December 10, 2009

Accepted: December 11, 2009

© Berns and Conte; Licensee *Bentham Open*.

This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.