

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

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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 ¹³C-NMR, RF field inhomogeneity, physical sample restriction, natural organic matter, signal response profile.

INTRODUCTION

Cross-polarization magic-angle spinning (CP/MAS) ¹³C-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. ¹H and ¹³C, 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 ¹H field as this critical coil length can be easily reached even at lower frequencies. A slight field centre misalignment of the ¹H field is usually counteracted by using a variable amplitude or ramped CP transfer [23], which sweeps through a range of (usually) ¹H 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.

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to approximately 4 m, i.e. a critical coil wire length of 40 cm. Hence, for the ^1H 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 ^1H and ^{13}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 ^1H field to counterbalance this misalignment must be applied.

To avoid RF field nutation rates, which match rates equivalent to the MAS rate, ω_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 ^1H 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 ^1H and ^{13}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 ^1H 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-

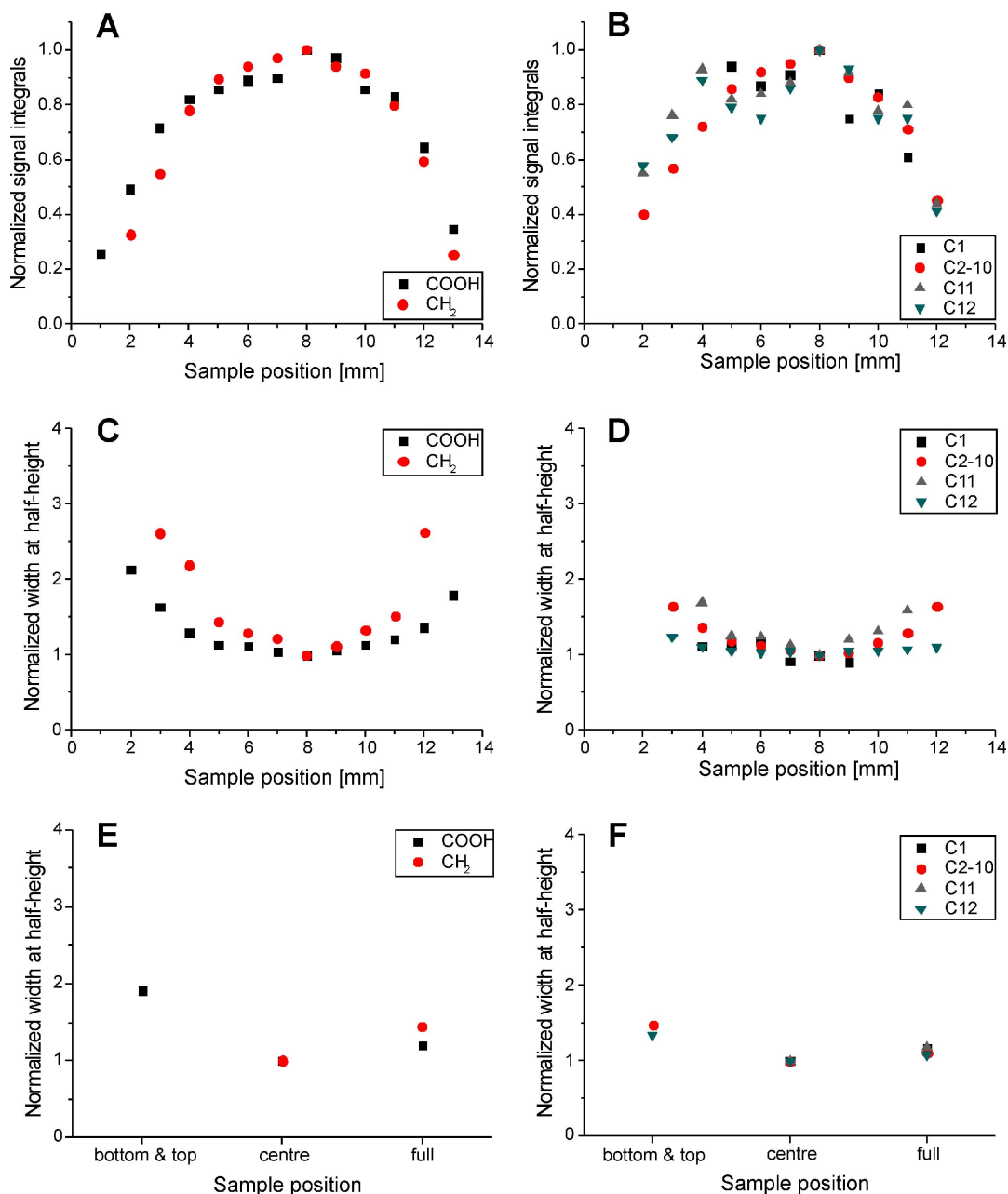


Fig. (4). Sensitivity (A and B) and resolution profiles (C to F) of the Apex Pencil[®] probe for ^{13}C -CPMAS spectra of glycine (A, C and E) and SDS (B, D and F) (all normalized to position 8 or the central region, respectively).

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 ^1H and ^{13}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° pulse after an evolution time τ and recording the signal after another τ 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° pulse is not perfect throughout the sample. Hence the outer regions of the sample experience a pulse which differs from 90° resulting in a lower magnetization from which the system needs to return to equilibrium. The centre position is closest to the true 90° 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

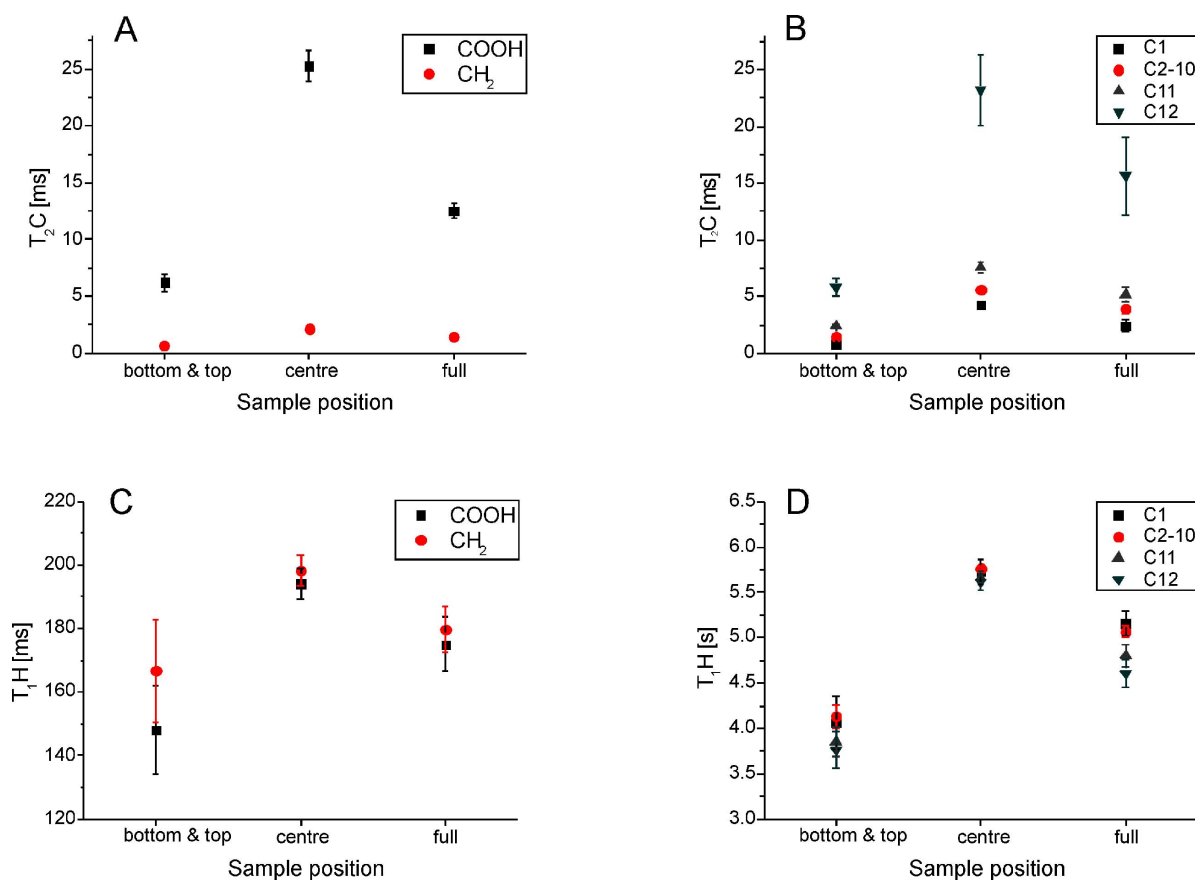


Fig. (5). T_2C (A and B) and T_1H (C and D) relaxation rates of glycine (A and C) and SDS (B and D).

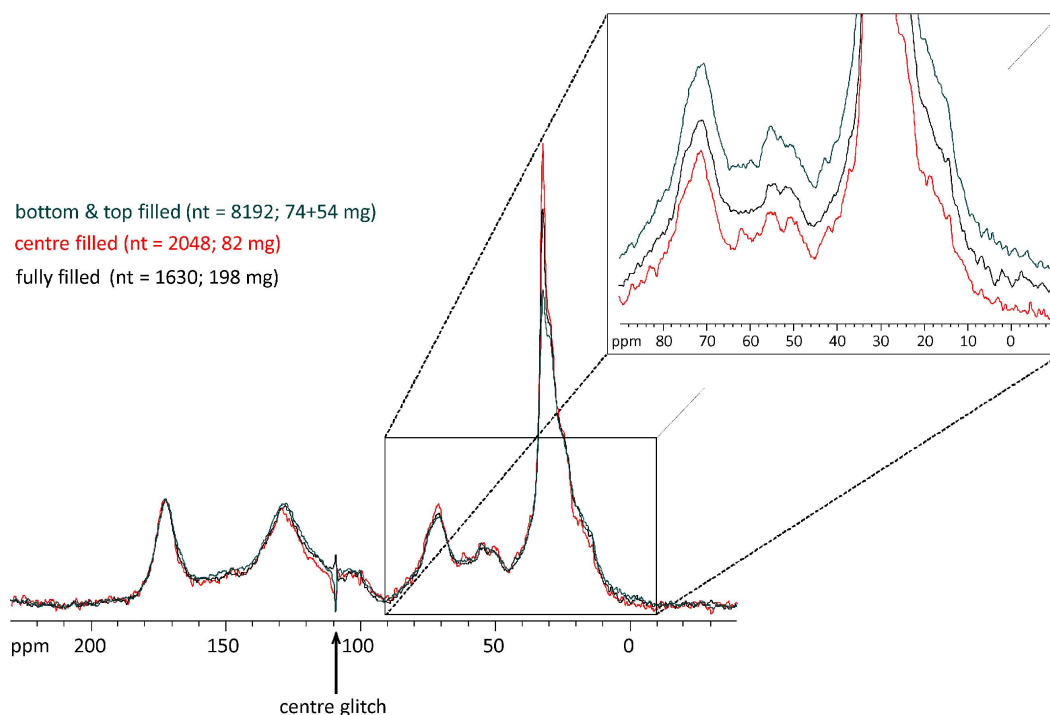


Fig. (6). ^{13}C -CP/MAS spectra of Vico humic acid in different positions of the manufacturer-defined sample region.

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.

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