INTERNATIONAL HUMIC SUBSTANCES SOCIETY

SERIES

1: Introduction Paper: Solid-State ^{13}C NMR Spectroscopy in NOM Research,

by Heike Knicker, December 2018

What is solid-state CPMAS ^{13}C NMR spectroscopy and what basic information are extractable from such experiments?

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Scope

The invention of nuclear magnetic resonance (NMR) spectroscopy in the 1940s (Rabi et al., 1939; Bloch, 1946) started the development of one of the most powerful experimental methods for the elucidation of molecular level structure. Since then, seven Nobel prizes have been awarded in the field of NMR.

In the field of humic substances research, the first application of this technique concerned solution ^{1}H NMR spectroscopy (Barton and Schnitzer, 1963), but it took about another 20 years until the first solid-state ^{13}C NMR spectra of humic substances were published (Newman et al., 1980). Although a more detailed historical overview of the developments and achievements of NMR spectroscopy in NOM research may certainly be interesting, it is beyond the scope of the present work and the interested reader is referred to Berns and Knicker (2014) and Preston (2015). Nevertheless, the introduction of this non-invasive technique for the characterization of natural organic matter (NOM) opened the door for a more complete understanding of its structural properties by allowing not only the insoluble fractions but also the bulk samples to be subjected to non-degradative characterization without the necessity of extraction.

In spite of the ongoing success story of solid-state ^{13}C NMR spectroscopy in many research fields including NOM research, many researchers not directly involved in analytical chemistry may find NMR to be a mysterious and at times daunting technique. Being aware that potential users of solid-state ^{13}C NMR spectroscopy may not necessarily be deeply familiar with the theoretical background of this technique, the intention of this short introduction is to provide some “relatively simple explanation” for a better understanding of how solid-state ^{13}C NMR spectroscopy works and how routine NMR spectra of NOM can be interpreted. By doing this, I tried to avoid complicated equations and quantum mechanical descriptions. In order to make it simple, I focused on my own experiences (thus using examples of my own work). The reader may forgive me not having presented a complete overview of the state–of-the-art literature in the field of solid-state NMR spectroscopy of NOM. Those desiring more detailed information about the theoretical background of NMR spectroscopy are

referred to several review articles (Conte et al., 2002; Dybowski and Bai, 2006; Simpson et al., 2012; Berns and Knicker, 2014; Preston, 2015; Mao et al., 2017; Simpson et al., 2018) and textbooks (Wilson, 1987; Nanny et al., 1997; Duer, 2005). The ongoing discussion about the reliability of solid-state cross polarization magic angle spinning (CPMAS) nuclear magnetic resonance (NMR) spectroscopy was also not considered. However, a detailed discussion is given in an earlier publication (Knicker, 2011). Aside from a more detailed description of the basic principles of solid-state NMR spectroscopy, this publication provides some explanation about potential sources of non-quantitative NMR results together with practical advice, and relaxation data that are necessary for the correct adjustment of NMR acquisition parameters in solid-state NMR experiments of NOM.

What happens during a basic NMR experiment or what means "resonance condition"?

In principle, NMR spectroscopy is based on the interaction between a nuclear magnetic dipole and an external magnetic field. Here, a magnetic dipole can be looked at as a tiny bar magnet with a north and a south pole, subjected to a stronger magnetic field. Accordingly, it is already evident that NMR only works if the nuclei are nuclear magnetic dipoles. This is only the case if they have a nuclear spin (I). In a simple physical picture, a spin corresponds to a rotation of the nucleus around its axis. Since the nucleus is charged, a magnetic field is produced which then creates the nuclear dipole. Unfortunately, for NOM researchers, the highly abundant ^{12}C nucleus exhibits no nuclear spin and therefore has no magnetic properties; thus, it is unsuitable for NMR spectroscopy. Fortunately, ^{13}C, which has I=1/2 represents an alternative but due to its low natural abundance of 1.1 atomic% (% of all carbon isotopes) we have to cope with its low NMR sensitivity. Other nuclei, which are of interest in NOM research and have appropriate magnetic properties, are ^{1}H, ^{15}N and ^{31}P.

Now, placing the nuclear magnetic dipoles or spins into a strong external magnetic field, they orientate themselves parallel or antiparallel to the field (Fig. 1a) of the external magnet populating two different energy levels (Fig. 1b). Here, the higher one has a less abundant population than the lower energy level (imagine a room with chairs, but slightly more persons than chairs. Accordingly, fewer people will be standing than sitting). Note that the difference between those two energy levels (ΔE) is determined by the strength of the external magnetic field (B_0) and the properties of the

nucleus (i.e. size and charge), commonly expressed as the gyromagnetic ratio, γ, a nucleic constant. Now, energy is supplied to the system in the form of a second oscillating electromagnetic radiofrequency (rf) field (Fig 1b, top).

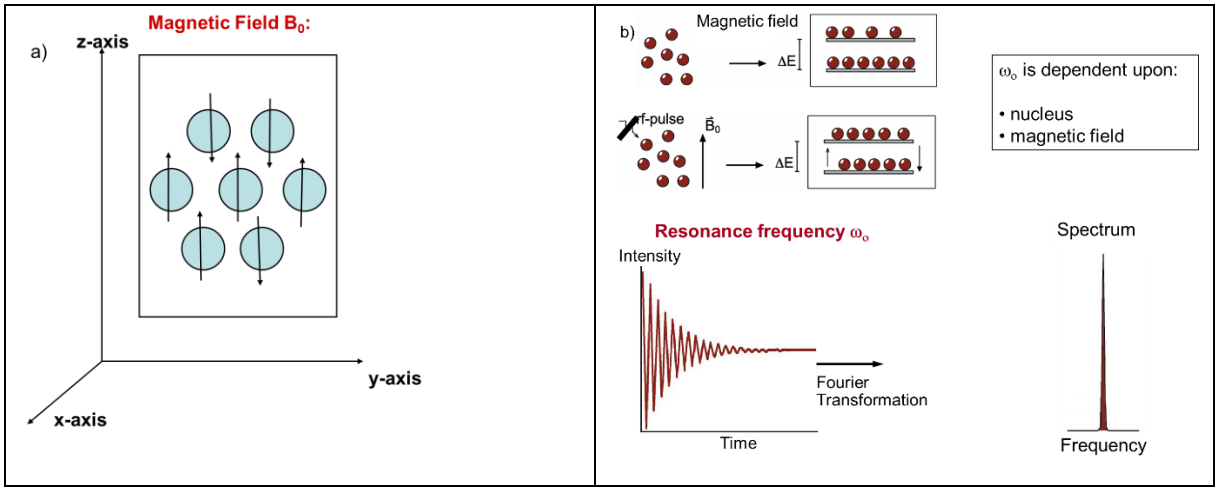

Figure 1: a) Nuclear dipoles in an external magnetic Field. b) the resonance condition (adapted from Knicker (2011))

If this energy corresponds to a multiple of ΔE, the system is in resonance and spin transitions between the energy levels are induced. Staying with our example and being a bit of the old school, this may be visualized as the sitting persons will stand up, possibly due to the fact that somebody important entered into the room. After termination of the rf-pulse, thus the important person leaves the room, the system relaxes to its thermal equilibrium, which represents the initial spin distribution among the energy levels. In our picture, the standing persons become tired and sit down. Because not enough seats are available, however, some have to keep standing, thus they remain in the “higher energy state”. During the relaxation process, energy units (quantum) corresponding to a multiple of ΔE (thus the energy difference between the sitting and the standing positions) are released to the environment. This energy can be detected as a decaying voltage signal (Free induction decay, FID), oscillating with the so-called resonance frequency (ω_0; Fig 1b bottom). With a mathematical transformation, the FID is transformed into a signal that is visible in a spectrum at the frequency ω_0. As we will see later, in a macroscopic sample, not all spins have the same resonance frequency and therefore the FID is composed of overlapping signals. In order to separate those signals into single lines with specific resonance frequencies, the so-called Fourier-Transformation (FT) is applied (fortunately by the instrument software!). The initial intensity of this emission signal (all persons being standing up

and subsequently sitting down) is proportional to the total energy emitted and therefore contains quantitative information about the number of excited spins. The time needed for the complete decay reflects the time needed for the relaxation (named relaxation time) of the system. In our picture, this corresponds to the time needed until all chairs are re-occupied by persons. Now, in summary, we performed one NMR experiment.

In general, NMR spectroscopy is a very insensitive technique and commonly, one pulse is not enough to obtain a signal, which can be distinguished from the noise. In order to increase the signal-to-noise ratio of a NMR spectrum, several single spectra, scans, are acquired and summed up. The number of scans needed for a descent and usable spectrum, however, depends on the abundance or concentration of the studied nucleus in the sample. For ^{13}C in humic extracts, between 2000 and 10,000 scans are usually needed. When analyzing soil samples with C concentration of 1%, between 50,000 and 200,000 scans have to be accumulated to obtain spectra that can be interpreted.

Note that accumulating single scans, it is important that the spin system has returned completely to its thermal equilibrium before a new rf-pulse is applied. If this was prevented by short delay times between the pulses, nuclei that are still in the excited state when the new pulse occurs are not detected. Staying within our picture, this corresponds to the situation that a second important persons enters the room before all chairs are occupied. In this case, the response to the second person, measured by the rising of persons is misinterpreted. The same is true for the spins, and consequently the intensity of the signal is not longer proportional to amount of excitable spins.

How can I distinguish signals from different C-species or what is the chemical shift?

Until now, we have seen that nuclei with different properties, i.e. ^{13}C or ^{15}N, can be distinguished by different resonance frequencies. If that was all this technique could provide, however, it never would had received much attention because cheaper techniques are available to provide this information.

Fortunately, spins are never completely isolated from their environment. They are surrounded by electrons (Fig. 2), which produce a kind of magnetic shield around the spin leading to a reduction in the local magnetic field felt by spin (B_{eff}). This shielding increases with electron density, thus it is higher for C surrounded by protons than for C connected to electron-withdrawing oxygen (Fig. 2). Because the resonance

frequency of the observed C depends on the magnetic field to which it is exposed, two different C-species result in signals with two different resonance frequencies.

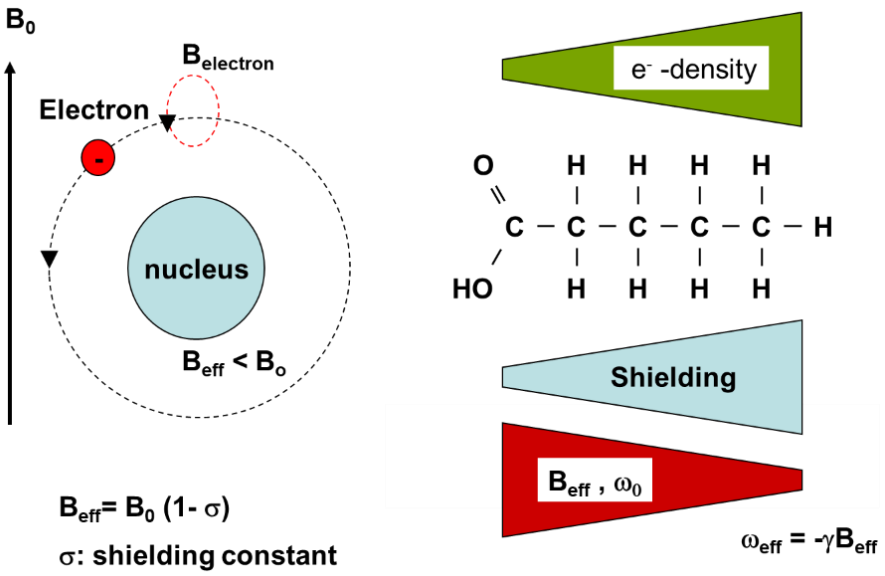

Figure 2: The chemical shift (adapted from Knicker (2011))

The fact that nuclei in different chemical environments resonate at their specific frequencies requires that for each individual situation the respective resonance condition has to be generated. Nowadays, this is achieved by using a very short but intense radiofrequency pulse, which creates a frequency band, allowing the simultaneous excitation of all nuclei of one kind but in different chemical environments. As mentioned above, after termination of the rf-pulse, the sum-FID containing the FIDs of the individual nuclei is Fourier-transformed from the time domain into the frequency domain to result in the spectrum.

Nevertheless, there is still one problem to be solved concerning the use of NMR spectrometers with magnets of different magnetic field strengths. Here, the same sample will lead to different resonance frequencies for the same nuclei. To circumvent this, the location of the signal in the spectrum is compared to a reference and given as chemical shift, δ, in ppm. Note that this unit has nothing to do with concentration. For ^{13}C and ^{1}H nuclei, tetramethylsilane (TMS) represents the commonly used reference. Unfortunately, for nuclei less routinely analyzed by NMR spectroscopy, such as ^{15}N, ^{31}P and some others, various standards are applied. In these cases, comparison of spectra can be achieved with conversion factors.

What is the catch with solid-state NMR?

A big advantage of NMR spectroscopy lies in the fact that it can also be used for studying solids. The independency from solvents turns it into the only alternative that allows concomitantly a qualitative and quantitative characterization of an insoluble bulk sample without prior extraction or chemical alteration. Compared to solution NMR spectroscopy, however, this advantage comes with the price of broader signals. On the other hand, the chemical complexity of NOM and its commonly analyzed fractions leads to resonance lines with close chemical shifts, which are difficult to be resolved even in solution NMR spectra. Thus, also in solution NMR spectroscopy of NOM, broad lines are expected. Considering that the removal of the solvent prevents secondary reactions during the experiment and storage of a dried samples is often much easier than liquids, solid-state NMR spectroscopy may still be a valuable alternative. This is in particular true if one bears in mind that the higher spin density within a given volume of solids compared to dissolved samples increases the sensitivity of the NMR method. Consequently, less single scans have to be accumulated to obtain interpretable spectra.

But what is the reason for the broad lines in solids? It is due to the fact that the spins of a sample are not only interacting with the external magnetic field B_0, but also with each other and each interaction leads to a specific ω_0. In solutions, the movement of the molecules is fast. Thus, the single spin has no time to “feel” the interaction. Consequently, only an average ω_0 can be detected. In solids, on the other hand, the molecular motion is slow and all orientations and locations of the molecules within the magnetic field B_0 create specific environments with specific resonance frequencies. Because the small differences between those resonance frequencies cannot be resolved by the instrument software, the spectrum shows a broad “blub” embracing all possible signals. Although such spectra (powder spectra) can be helpful if spatial information about molecules is wanted, they are commonly useless for the identification of the chemical composition of a sample.

Clever scientists, however, found a solution to this problem (Schaefer and Stejskal, 1976) by looking a little bit deeper into how those interactions are behaving with time. They found that all interactions are averaged to zero, if the **magic angle spinning (MAS)** technique is used. Therefore, the sample is placed relative to the z-axis of the external magnetic field at the magic angle of 54.7°. Subsequently, the sample holder

(the rotor) is rotated with high speed. Nowadays spinning speeds of up to 110 kHz are possible. For common applications in NOM research, however, 12 to 14 kHz are used. The next challenge in solid-state NMR spectroscopy is again related to the low molecular motion in solids leading to slow relaxation (releasing energy to the environment, the so-called lattice, therefore it is called spin-lattice relaxation time, T_1) of the spin systems after termination of the rf-pulse. To avoid signal saturation, hence the proportionality between signal intensity and amount of spins in the sample, long pulse delays have to be applied between the single scans. Low natural abundance of a nuclei leads to a further increase of the T_1, and considerable long relaxation times have to be encountered in ^{13}C NMR spectroscopy of crystalline structures. For example, if cellulose is present in soils, a pulse delay of at least 20 min is required. Because even for C-rich litter layers at least 5000 single scans are necessary, the measurement time sums up to an infeasible 70 days.

Fortunately, the **cross polarization (CP) technique** was invented (Pines et al., 1973). An interesting and nice to read historical description on the invention of the CPMAS technique is provided by Schaefer (2007). With this technique, energy or magnetization is transferred from nuclei with high relative abundance and relatively short T_1 (e.g., ^{1}H) to X nuclei (e.g., ^{13}C or ^{15}N) with low natural abundance and long T_1 (Fig. 3a). Subsequently, the X-nuclei are observed. After the pulse, the excited spins of the ^{1}H-spin system relax. Consequently, no energy is transferred to the ^{13}C spins and thus no ^{13}C signal can be detected (Fig. 3b). It is evident that since the ^{1}H spin system is the “player”, only its spin-lattice relaxation time, T_{1H}, has to be considered to avoid saturation effects. For cellulose the pulse delay (should be 5 times T_1) is reduced to approx. 15 s; for humic extracts delay times of 100 ms are sufficient, (if the electronics of the instrument allows such short pulses). Nevertheless, even with longer pulse delays of 500 ms, the application of this technique enables us to obtain reliable ^{13}C NMR spectra of samples with 1 to 5 % of C within 4 to 24 hours.

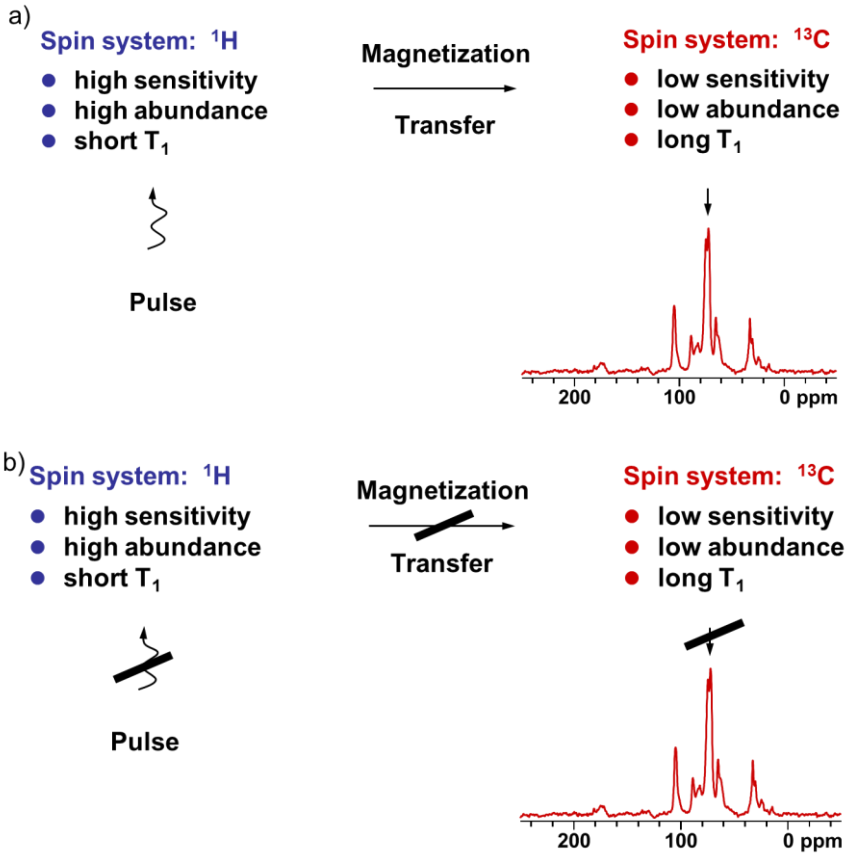

Figure 3: The cross polarization

Can I quantify CPMAS NMR spectra?

Since its introduction into NOM research in the early 1980s (Wilson, 1981), the quantification of CPMAS NMR spectra has been strongly questioned. Indeed, it is true that the quantitative reliability of this technique depends on a number of factors that ask for careful adaptation of the acquisition conditions to the specific requirements of the samples, which is not always an easy task to accomplish. Considering that the intention of this text is to provide a short introduction, I will focus on the main trap related to the cross polarization dynamics. A more detailed discussion of possible sources of biased spectra can be found in Knicker (2011).

A main concern lies in the fact that the efficiency of the magnetization transfer from the ^{1}H to the ^{13}C spin system depends on the distance between the coupling spins and is lower for polycondensed aromatic structures than for peptide chains. Problems, however, are only expected with a distance of more than three bonds between ^{13}C and its nearest ^{1}H (Alemany et al., 1983b). Such carbons can be found in the core of polyaromatic domains composed of more than 10 rings as they occur in the graphenic network of soot (Freitas et al., 1999; Solum et al., 2001), activated coal or anthracite

(Abelmann et al., 2005). Chars produced during natural vegetation fires have atomic C/H ratios > 0.4, which indicates that on average, almost every second aromatic C is protonated, and quantification problems should not be evident.

Another source of material, which can lead to biased spectra of NOM is the presence of paramagnetic material, such as Fe^{3+} oxides in soils, sediments or humic extracts. If bound closely to ^{1}H (i.e. in hydroxyl groups of carbohydrates), its spin may relax before its energy was transferred to the adjunct ^{13}C and its signal intensity will be reduced. To avoid this, such samples are demineralized, i.e. with 10% hydrofluoric acid (HF) (Gonçalves et al., 2003) which removes not only the paramagnetics but also the mineral phase. The enrichment of organic matter after this treatment opens the door for the NMR spectroscopic characterization of C-poor soils (Velasco-Molina et al., 2016) or marine sediments (De la Rosa et al., 2008).

Applying extraction with HF, some C and N losses have to be encountered, since soluble OM, formerly adsorbed to the mineral phase will be released by the destruction of the mineral phase and co-extracted. Unfortunately, in mineral rich samples with very low OM (i.e. in subsoils), such losses can be high and reach values of more than 80% of their organic C (Rumpel et al., 2002; Gonçalves et al., 2003). On the other hand, comparative studies of the samples before and after the treatment together with an in-detail study of the spin dynamics during the CP experiment of both samples confirmed that OM loss induced by the HF-treatment is not selective Thus, a comparable chemical composition of the untreated and HF-treated SOM can be inferred (Knicker, 2011).

How can I interpret a common solid-state NMR spectrum of NOM?

Commonly, the goal of NMR spectroscopy in NOM research is to determine and quantify the different types of chemical species present in the sample. Nevertheless, the large range of organic structures found in NOM and the inherently broad lines associated with solid-state NMR analysis that result in overlapping adjacent signals and make it difficult to obtain detailed information on specific compounds.

On the other hand, dividing the solid-state NMR spectrum into chemical shift regions assigned to the most likely chemical groups causing their signals followed by integration of these regions gives a good overview of the bulk chemical composition of the sample under study. For the analysis of NOM, solid-state ^{13}C NMR spectra are habitually divided into chemical shift regions given in Table 1.

For explaining a possible approach to interpret a solid-state ^{13}C NMR spectra of NOM, we will start with an easy spectrum obtained from wheat straw (Fig. 4c), trying to assign ^{13}C spectral intensities to the contribution of compound classes to total C (Ct) of the sample.

Table 1: Assignments for the peaks in solid-state 13C NMR spectra to typical C groups in geochemical samples (referenced to tetramethylsilane = 0 ppm) (Lüdemann and Nimz, 1973; Kalinowski et al., 1984; Wilson, 1987; Knicker and Lüdemann, 1995) (reproduced from Knicker (2011))

Chemical shift range (ppm)	**Assignment**
220-160	Carboxyl/carbonyl/amide carbons
160 – 140	Aromatic COR or CNR groups, furans
140 – 110	Aromatic C-H carbons, guaiacyl C-2, C-6 in lignin, olefinic carbons, bridging C in polyaromatic units
110 – 90	Anomeric carbon of carbohydrates, C-2, C-6 of syringyl units of lignin
90 – 60	Carbohydrate-derived structures (C-2 to C-6) in hexoses, C-α of some amino acids, higher alcohols
60-45	Methoxyl groups, C-α of most amino acids, N-alkyl C
45 – 25	Methylene groups in aliphatic rings and chains,
25-0	Terminal methyl groups

We must bear in mind that in plant residues most of the N occurs in peptides with minor contributions of amino sugars. Therefore, with an average atomic C/N ratio of 4 for proteins (i.e. in casein, see Table 2) and the wide atomic C/N ratio of 125 of the wheat straw, we can assume that 4 carbons out of 125 are part of peptides (!), therefore approximately 3% of the total C (Ct) of the sample derived from peptides. In our spectrum (Fig. 4c), those carbons contribute 1% to the total ^{13}C intensity to both the amide C (220 to 160 ppm) and the N-alkyl C chemical shift region (60 to 45 ppm) (Table 2). Less than 1% is distributed among the aromatic C and the alkyl C regions. These assignments leave 2% of Ct to carboxyl C (220 to 160 ppm) in uronic acids, which are part of hemicellulose. Approximately 4 % of Ct is attributed to methoxyl C (60 to 45 ppm), mostly of lignin units. The aromatic C of lignin gives resonance lines between

160 to 110 ppm, which accounts for 12% of the total ^{13}C intensity (the contribution of aromatic C in peptides is too low to be considered!). The signals between 160 and 140 ppm correspond to its *O*-aryl C. Considering that in wheat straw a lignin unit contains 6 aryl C, 3 propanyl C and approximately averaged 1 methoxyl C, the total lignin content of the straw sample can be estimated by multiplying the aromatic C content by 1.7. For our sample, this amounts to 21% of Ct.

Bearing in mind that most of the intensity of the propanyl C in lignin gives resonance signals in the *O*-alkyl C region (90 to 60 ppm) with some intensity in the alkyl C region (45-0 ppm), the contribution of carbohydrate C is lower than the intensity observed in the region between 110 and 60 ppm (74%). Thus, for calculating the carbohydrate content of the sample, we have to add to the intensity between 110 and 60 ppm, the intensities derived from the acetyl C (21 ppm) contributing to the alkyl C region (45 to 0 ppm) (6%), and that from carbonyl C in uronic acids (2%, as said before). In total, this sums up to 82% of the total ^{13}C intensity. Note that lipids play a minor role, which is indicated by the absence of a signal at 28 ppm assignable to methylene C. In order to consider the contribution of propanyl C, its calculated intensity (6%) has to be subtracted. Accordingly, the estimated carbohydrate contribution to Ct amounts to 76%.

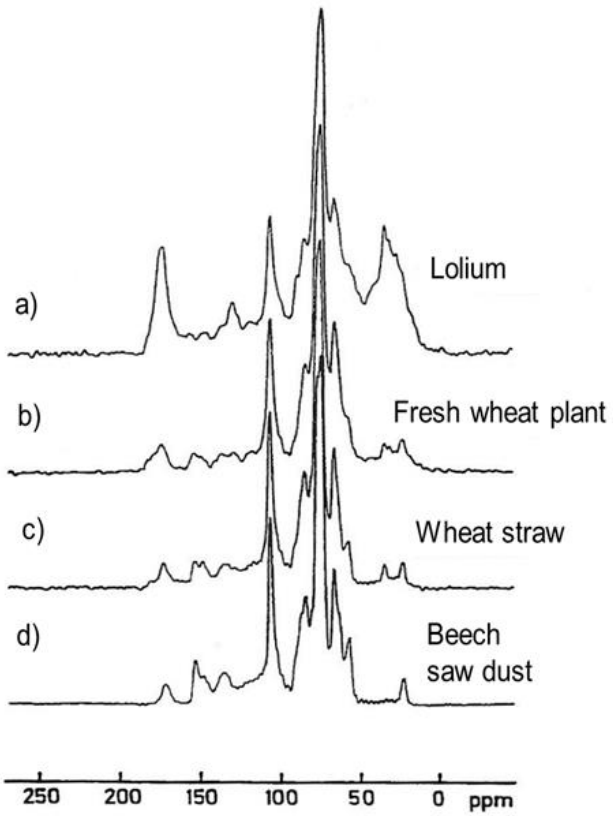

Figure 4: Solid-state ^{13}C NMR spectra of fresh plant residues

Table 2: Intensity distribution (%) of solid-state ^{13}C NMR spectra of plant residues (Knicker, 1993)

	Carbonyl C (220/160 ppm)	Aromatic C (160/110 ppm)	Anomeric C (110/90 ppm)	O-alkyl C 90/60 ppm)	N-Alkyl/ methoxyl C (60/45 ppm)	Alkyl C (45/0 ppm)	Atomic C/N
Lolium	10	11	10	40	9	20	9
Young wheat	4	9	13	57	7	10	41
Wheat straw	3	12	16	58	5	6	125
Beech	2	15	15	59	6	3	537
Casein	**22**	**10**		**8**	**19**	**41**	**4**

Summarizing the results of these calculations, the Ct of the sample is divided into 76% carbohydrate C, 21% lignin C and 3% protein C.

The identification of compound classes in a sample via NMR becomes a bit more complicated if the sample contains a considerable amount of peptides, as it is the case for the spectrum of Lolium (Fig. 4a), algal residues or remains of microbes and fungi. Here, it has to be mentioned that the Lolium used for the presented spectrum was derived from a laboratory incubation experiment in which it was grown with high concentrations of N-fertilizers, leading to material with considerably low atomic C/N ratios. Nevertheless, if high amounts of peptides are present, their contribution to the aromatic C and alkyl C region cannot be neglected. For their consideration, the atomic C/N ratio and the intensity distribution in the solid-state ^{13}C NMR spectrum of casein may be used as a model composition for proteins (Table 2). Accordingly, almost 45 % of Ct of the Lolium residue is assignable to peptides. The C of the latter contribute with approximately 10%, 8% and 18% of Ct to the carboxyl C, N-alkyl C and alkyl C region, respectively. Their aromatic C contribute with approximately 5% to the region between 160 and 110 ppm, leaving 6% of Ct to be assigned to the aromatic core of lignin. Using the calculation proposed before, lignin accounts for approximately 10% of Ct.

Following the intensity distribution in the solid-state ^{13}C NMR spectrum of casein, approximately 4% of the total intensity of the ^{13}C NMR spectrum of Lolium is assignable to peptide C giving resonance lines between 90 and 60 ppm and the propanyl side

chain of lignin contributes with 3% to the same region. With this, *O*-alkyl C in carbohydrates amounts to 43% of Ct. Adding the remaining intensity of the alkyl C region of 2%, which are not assignable to peptide C, the carbohydrate content sums up to 45% of Ct. Thus, Ct of our Lolium is distributed as follows: 45% to peptides, 45% to carbohydrates and 10% to lignin.

Again, the story becomes even more complex if lipids are present to a higher extent as is expected, for example, in algal residues. In order to obtain a first idea about their contribution, one may calculate the ratio between alkyl C and carboxyl C. As can be seen again from the intensity distribution of the casein spectrum, this ratio is approximately 2 for peptides. If this ratio is higher, higher contributions of lipids have to be considered. Starting with the calculation of the peptide contents, however, may help to elucidate, in a step-by-step approach, the remaining main compound classes of the sample. This method may even be applied to other NOM samples, because solid-state ^{15}N NMR spectroscopy has demonstrated that in samples not affected by fire or by input of other pyrogenic materials, almost all of the N is assignable to amide N, most likely of peptide-like compounds. Some samples, however, may contain other biopolymers, such as cutines or tannins that may aggravate the interpretation. Note that a comparable approach has also been discussed by Nelson and Baldock (2005). As is already evident from the above discussion, having an idea of which compound classes could dominate the chemical composition of a sample is extremely useful for a good interpretation of NMR spectra. Identification of marker features in a spectrum represents a further helpful tool for increasing the extractable information of a spectrum. Such marker features may be the presence and absence of signals as well as the occurrence of specific signal combinations. For example, the absence of signal intensity in the chemical shift region assigned to O-aryl C (160 to 140 ppm) may be interpreted as a low contribution of lignin derivatives (and vice versa). Thus, the aromatic C of such samples can be attributed most tentatively to charcoal residues. Comparatively, in bulk soil and sediment samples, aromatic C contents higher than 20% are highly indicative of the presence of charcoal (Knicker et al., 2008).

Aside from the pure determination of the chemical composition of NOM, solid-state ^{13}C NMR spectroscopy is often applied to study the impact of a changing environment on NOM characteristics. Such changing conditions may affect the humification processes in soils, sediments, and aquatic systems. The general rule is that with increasing humification the content of carbohydrates decreases while aliphatic and carboxylic C

contributions increase. To which extent this shift occurs and which carbon groups are mostly affected, however, depends on the biochemical stability of the precursor material as well as on the physical and chemical conditions of the environment. All those parameters strongly affect the microbial activity involved in the degradation process. Thus, this kind of analysis may allow the design of experiments to study the impact of climatic change, acidification, alteration of environmental management practices or the impact of natural events such as fire, storms etc. on the quantity, stability and quality of bulk NOM samples.

Understanding the chemical composition of NOM can also help in the investigation of NOM-mineral interactions, adsorption of metals or organic pollutants or the role of NOM in transport processes. Whereas the first is mainly studied by NMR analysis after physical fractionation of bulk samples according to density or particle size, chemical fractionation according to solubility may be more appropriate for latter.

Final remarks

With this introduction, I hope that I already provided a first access to the technique of ^{13}C solid-state NMR spectroscopy and encouraged some interested colleagues to include this approach in their analytical repertoire. Indeed, the 1D CPMAS technique delivers already sufficient information for many questions in NOM research. This doesn`t mean, however, that progress in solid-state NMR spectroscopy has stopped during the last years. Starting from the basic approaches, new and advanced NMR techniques have been developed and additional molecular level information has become available that was not accessible with other analytical tools. Nevertheless, application of these advanced NMR techniques to NOM is by far more challenging than their use for the study of pure compounds, and due to the fact that most NOM researchers have no direct access to NMR facilities, these approaches may not easily turn into routine analytical approaches for the investigation of NOM. This should not, however, discourage the interested NOM researcher to work together with an NMR specialist and to accept this challenge as a means for obtaining additional exiting scientific news.

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