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Chemical shift referencing in MAS solid state NMR

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Abstract

Solid state ^{13}C magic angle spinning (MAS) NMR spectra are typically referenced externally using a probe which does not incorporate a field frequency lock. Solution NMR shifts on the other hand are more often determined with respect to an internal reference and using a deuterium based field frequency lock. Further differences arise in solution NMR of proteins and nucleic acids where both ^{13}C and ^{1}H shifts are referenced by recording the frequency of the ^{1}H resonance of DSS (sodium salt of 2,2-dimethyl-2-silapentane-5-sulphonic acid) instead of TMS (tetramethylsilane). In this note we investigate the difficulties in relating shifts measured relative to TMS and DSS by these various approaches in solution and solids NMR, and calibrate adamantane as an external ^{13}C standard for solids NMR. We find that external chemical shift referencing of magic angle spinning spectra is typically quite reproducible and accurate, with better than ± 0.03 ppm accuracy being straight forward to achieve. Solid state and liquid phase NMR shifts obtained by magic angle spinning with external referencing agree with those measured using typical solution NMR hardware with the sample tube aligned with the applied field as long as magnetic susceptibility corrections and solvent shifts are taken into account. The DSS and TMS reference scales for ^{13}C and ^{1}H are related accurately using MAS NMR. Large solvent shifts for the ^{13}C resonance in TMS in either deuterochloroform or methanol are observed, being +0.71 ppm and −0.74 ppm from external TMS, respectively. The ratio of the ^{13}C resonance frequencies for the two carbons in solid adamantane to the ^{1}H resonance of TMS is reported.
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1. Introduction

Referencing of chemical shifts in liquid phase NMR (lpNMR) is simplified by the use of internal standards [1], the best of which have shifts that are largely independent of concentration and solvent. This approach makes it possible for the NMR spectroscopist to largely ignore complications that would otherwise arise in comparing shifts for samples having differing bulk magnetic susceptibilities. In solid state NMR (ssNMR) internal referencing is less widely used. At present no good internal reference compound for biological ssNMR has been developed. Such samples are often only semi-solid, containing significant amounts of water, lipids, and other fluid components that could dissolve an otherwise solid small reference molecule. The desire not to contaminate or further complicate the preparation of such samples then makes external referencing a much more attractive proposition. Fortunately for ssNMR, shifts recorded using magic angle spinning (MAS) are independent of the isotropic bulk magnetic susceptibility of the sample, and therefore external referencing should be quite accurate.

As pointed out by Garroway [2] the demagnetizing fields arising from isotropic diamagnetism of an infinitely long cylindrical sample [3,4] vanish when the sample is oriented at the magic angle $\theta_M = \cos^{-1}(1/\sqrt{3})$. This is readily appreciated by considering the z-component of the field $\vec{B}$ experienced by a nucleus in an infinite cylinder of volume susceptibility χ_v, while immersed in a static magnetic field H_0 placed along z:

$$\begin{aligned} B_z &= \mu_0 H_0 \left[(1-\sigma) + \frac{\chi_v}{3}\left(\frac{3\cos^2\theta - 1}{2}\right)\right] \quad \text{(SI units)} \\ &= H_0 \left[(1-\sigma) + \frac{4\pi\chi_v}{3}\left(\frac{3\cos^2\theta - 1}{2}\right)\right] \quad \text{(cgs units)} \end{aligned} \tag{1}$$

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The inclination of the cylinder to H_0 is given by the angle θ, and σ is the chemical shielding. As further elaborated upon by others [5,6], such χ_v and sample shape dependent contributions to the resonance frequency and lineshape [7] vanish even for a finite length sample if the sample is also spun while oriented at the magic angle. External referencing in MAS ssNMR is therefore not subject to the shifts that appear in lpNMR in the conventional case that the sample tube is placed along $\theta = 0°$. In the zero angle spinning (ZAS) instance chemical shifts δ measured relative to an external reference must be corrected [8] for the difference between the sample volume susceptibility, $\chi_{v,sample}$, and that of the external reference, $\chi_{v,reference}$. For an infinite length cylindrical sample the correction is given by

$$\begin{aligned}\delta_{sample,observed} - \delta_{reference,external} &= \delta_{sample,true} - \delta_{reference,external} \\ &\quad + \frac{1}{3}\left(\chi_{v,sample} - \chi_{v,reference}\right) \quad \text{(SI units)} \\ &= \delta_{sample,true} - \delta_{reference,external} \\ &\quad + \frac{4\pi}{3}\left(\chi_{v,sample} - \chi_{v,reference}\right) \quad \text{(cgs units)}\end{aligned} \tag{2}$$

As long as the sample length is several times its diameter the demagnetizing field shift calculated from this equation is also a good approximation for a finite length cylinder. When an internal reference is used, $\Delta\chi = \chi_{v,sample} - \chi_{v,reference} = 0$, removing any problems with the demagnetizing field correction or the concentration dependence of $\chi_{v,sample}$. Any remaining concentration dependence of the chemical shift difference between the sample and reference resonance will be due to actual differential concentration dependence in chemical shielding. Such solvent shifts provide an interesting measure of intermolecular interactions in the liquid state as long as they can be differentiated from susceptibility effects [9].

The difficulties associated with accurately performing such corrections for external referencing in ZAS lpNMR are numerous [10–13]. The inaccuracy of the infinite sample length approximation, the concentration dependence of $\chi_{v,sample}$, the need to not shim the magnetic field between sample exchanges, and the likelihood that $\chi_{v,sample}$ is unknown, make external referencing a bad choice in conventional ZAS lpNMR. Although external referencing in ssNMR should not be subject to such difficulties, there are good reasons to distrust any referencing scheme that involves exchange of samples, especially if the NMR probe is removed and reinserted into the magnet in the process. Other approaches, such as placing an internal capillary reference tube within a MAS sample [5] are by and large impractical on a routine basis. Typical experimental practice in ssNMR is then to quote a generous margin of error in chemical shifts and thereby avoid the potential quagmire of evaluating the various small contributing shifts that are not of chemical origin. Related complications arise when referencing ssNMR chemical shifts to the tetramethylsilane (TMS) and DSS lpNMR shift scales, often by their relation to a more convenient secondary external standard sample for ^{13}C ssNMR such as adamantane or glycine. As a volatile liquid TMS is inconvenient to include in a solid sample, and DSS must be dissolved in water to be used as a reference compound. One then either uses external referencing of these liquid samples with MAS NMR, or alternatively will relate the desired solid compound's shift to the TMS or DSS scale by making a solution sample containing the primary and secondary references together. The attraction of the latter approach is that the measurement can be done conveniently using a standard lpNMR probe.

All of the above methods work to varying degrees of accuracy, and agree to within about 1 ppm. Solid state MAS NMR does however have much higher inherent precision, and there are applications where more accurate referencing is desirable. Although aspects of this problem have been investigated in the context of MAS NMR of both solids [14–16] and liquids [17], there remain inconsistencies between many of the various shift scales in use for ^{13}C NMR. These details can be especially troublesome when comparing shifts acquired in aqueous and organic solvents. To address this problem we have systematically investigated the reproducibility of external referencing in MAS NMR for solids, and have compared the results to standard ZAS lpNMR.

An additional complication that should be mentioned for solid samples is the possibility that the bulk magnetic susceptibility is anisotropic (ABMS). Demagnetizing fields from ABMS do not vanish under MAS, and for a single crystal sample a shape dependent ABMS shift remains [5,16]. For powders the situation is fairly complicated, with each crystallite experiencing a somewhat different residual demagnetizing field shift. The principal result is ABMS broadening of the lineshape. Our experimental experience indicates that ABMS broadening for powders is symmetrical about the resonance center of gravity, and does not lead to an overall shift of the MAS lineshape. Since all of the samples used in this study have isotropic magnetic susceptibilities this effect is not an issue with the data reported upon here. That said, the potential for an ABMS shift in solid samples must be considered when solid state and solution phase chemical shifts are to be compared.

2. Description of experiments

Samples used in this study included solid adamantane, TMS at 2% to 3% by volume in $CDCl_3$, TMS saturated with adamantane, adamantane in $CDCl_3$ with added TMS for referencing, neat TMS, DSS dissolved in

Table 1
^{13}C Chemical shifts referenced to external neat TMS

Sample	Experiment	Resonance	$\delta_{observed}$ from external TMS (ppm)	s[a]	$\delta_{corrected}$ for χ_v shift (ppm)[b]	$\Delta\delta = \delta_{corrected} - \delta_{MAS}$ (ppm)
Neat TMS	ZAS	TMS	0	—	0	0
DSS 5% in D_2O[c]	ZAS	DSS	-2.57_6[g]	—	-1.88_5	0.04_2
DSS 0.5% in D_2O[c]	ZAS	DSS	-2.67_1	—	-1.98_0	0.02_6
TMS in $CDCl_3$[d]	ZAS	TMS	-0.05_2	—	0.74_8	0.04_1
Adamantane in $CDCl_3$ + TMS[d]	ZAS	TMS	-0.07_0	—	0.73_0	0.04_8
Adamantane in $CDCl_3$ + TMS[d]	ZAS	CH	28.31_3	—	29.11_3	0.04_9
Adamantane in $CDCl_3$ + TMS[d]	ZAS	CH_2	37.72_2	—	38.52_2	0.04_6
Adamantane in TMS[e]	ZAS	CH	28.80_7	—	28.80_7	-0.02_5
Adamantane in TMS[e]	ZAS	CH_2	38.23_8	—	38.23_8	-0.02_6
TMS in methanol[f]	ZAS	TMS	-0.63_0	—	-0.72_6	0.01_1
Neat TMS	MAS	TMS	0	0.00_2		
DSS 5% in D_2O	MAS	DSS	-1.92_7	0.00_4		
DSS 0.5% in D_2O	MAS	DSS	-2.00_6	0.00_8		
TMS in $CDCl_3$	MAS	TMS	0.70_7	0.01_0		
Adamantane in $CDCl_3$ + TMS	MAS	TMS	0.68_2	0.01_0		
Adamantane in $CDCl_3$ + TMS	MAS	CH	29.06_4	0.02_6		
Adamantane in $CDCl_3$ + TMS	MAS	CH_2	38.47_6	0.02_2		
Solid adamantane TMS slurry	MAS	TMS	0.02_6	0.00_5		
Solid adamantane TMS slurry	MAS	Solid CH	29.45_6	0.01_8		
Solid adamantane TMS slurry	MAS	Solid CH_2	38.48_4	0.01_5		
Solid adamantane TMS slurry	MAS	Solution CH	28.83_2	0.00_8		
Solid adamantane TMS slurry	MAS	Solution CH_2	38.26_4	0.00_5		
TMS in methanol	MAS	TMS	-0.73_6	0.00_4		

[a] Sample standard deviation $s = (\sum_{i=1}^{N}((\delta_i - \bar{\delta})^2/(N-1)))^{1/2}$.
[b] Corrected using $\delta_{corrected} = \delta_{observed} - (4\pi/3)(\chi_{v,sample} - \chi_{v,TMS})$.
[c] $\chi_{v,sample} = \chi_{v,D_2O} = -0.714$ ppm (cgs).
[d] $\chi_{v,sample} = \chi_{v,CDCl_3} = \chi_{v,CHCl_3} = -0.740$ ppm (cgs).
[e] $\chi_{v,sample} = \chi_{v,TMS} = -0.549$ ppm (cgs).
[f] $\chi_{v,sample} = \chi_{v,methanol} = -0.526$ ppm (cgs).
[g] First uncertain guard digit indicated as a subscript in table entries.

D_2O at various concentrations, and TMS in CH_3OD. All compounds were used as received. All experiments were performed at ambient room temperature, approximately 25 °C. In the case of solids NMR experiments, a healthy flow of 2–4 °C gas was passed over the spinning sample to minimize sample heating when high rotation rates were used.

Liquid phase ZAS NMR experiments were performed on a Varian INOVA NMR instrument operating at 500 MHz for the ^1H resonance frequency. After the initial adjustment of the room temperature (RT) shims, no further shimming was done to eliminate the possibility that the average field over the sample would be shifted as a result. The field frequency lock was also not employed. At least five independent measurements of the resonant frequencies were done for the samples under investigation, interleaving them in time. Bloch decays of a few scans were acquired using a direct detect ^{13}C probe with ^1H decoupling, and using acquisition times of over 1 s. Spectra were zero-filled once to help define the peak maxima, which were recorded as the observed resonance position. Line fitting was not used as the lineshapes, while narrow (full-width at half maximum (FWHM) ~ 8 Hz), are not well described by any standard profile without additional shimming. As the drift was determined to not be experimentally observable on the time scale of the experiments performed, data from multiple observations are simply reported as averages.

Measurements were repeated using MAS NMR on a Varian INOVA NMR instrument operating at 799.6 MHz for the ^1H resonance frequency. A ^{13}C direct detect Varian nanoprobe was used for MAS NMR of solution samples as well as solid adamantane. Solid adamantane shifts were also measured for comparison using a home built cross-polarization MAS (CPMAS) probe of our own construction. Spinning of the samples at 3 kHz in the nanoprobe makes it feasible to study small solution samples as well as motionally narrowed solids such as adamantane. The experimental procedures followed were the same as described above for the lpNMR data. After initial adjustment the currents to the RT shim coils were not altered. Under MAS conditions the lineshape is less sensitive to shimming, and as such lines in the nanoprobe and CPMAS data were more consistently Lorentzian in shape from experiment to experiment (FWHM ~ 5 Hz for liquids). Changing samples in either the nanoprobe or the CPMAS probe involves removal of the probe from the magnet bore as with most MAS spectrometers. Care was taken to insert

and remove the NMR probe slowly, and attention paid to reproducibly position the probe in the magnet bore without excessive movement of the magnet on its vibration isolation support legs. Drift compensation of the 18.78 T Oxford Instruments 64 mm RT bore magnet (base drift ~ 14 ^{1}H Hz/hr^{-1}) is accomplished by linearly incrementing the current to the RT shim Z_o coil at a calibrated rate under software control. The drift compensation was determined to be constant and set so accurately that additional compensation in analyzing the data was not required. No additional special precautions were taken, and the data was reduced as described above.

To further cross-check the referencing of the ^{13}C spectrum of solid adamantane to TMS, a nanoprobe sample was constructed to contain both. After packing the glass nanoprobe sample tube with powdered adamantane, an aliquot of TMS was added. The TMS dissolved a small portion of the adamantane, resulting in a slurry of solid adamantane wetted with an adamantane saturated TMS liquid phase. Spectra of this sample afforded simultaneous measurement of the ^{13}C resonance frequencies for solid adamantane, adamantane dissolved in TMS, and TMS itself, with all the foregoing measurements related to external neat TMS.

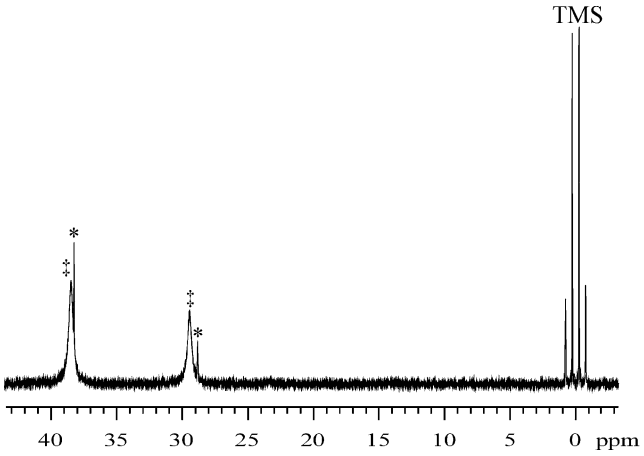

Fig. 1. MAS ^{13}C NMR spectrum of sample of solid adamantane suspended in liquid TMS as described in the text. Acquired at 201 MHz and an MAS rate of ~3 kHz. Some adamantane dissolves in the TMS and appears as the narrower lines (*) shifted from the solid state resonances (‡).

3. Results and discussion

Table 1 contains the bulk of the reported results, all referenced to external neat TMS in the respective experimental arrangements. With ordinary experimental care the repeat to repeat variability in the MAS results for any particular sample were typically better than ± 0.01 ppm, or $\sim \pm 2$ Hz, essentially at the digital resolution used. The same observation was made in the ZAS lpNMR results. It should be noted that in our experimental setup the MAS probe is pushed firmly against the lpNMR spinner or upper barrel. The arrangement positions the MAS sample accurately in the homogeneous center of the static field, precisely where the field value is the least sensitive to minor adjustments in probe position.

The ^{13}C spectrum in Fig. 1 provides the rationale behind the samples chosen. The two broad peaks are for the ^{13}C resonances in solid adamantane, while the pair of narrower resonances are for adamantane dissolved in TMS. There is an obvious and fairly large solvent shift of the adamantane resonances, being ~ -0.62 ppm for the methine carbon. The TMS apparently does not experience a significant solvent shift, as the resonances in both this sample and the externally referenced neat liquid are within 0.02_6 ppm of each other, just outside of our observed reproducibility on a single sample.

The accuracy of the solid adamantane ^{13}C shifts in this nanoprobe spectrum are somewhat compromised by the width of the solid phase lines and the overlap with the solution resonances. In this particular spectrum low level continuous wave (CW) decoupling was applied on resonance with the adamantane ^{1}Hs. The power level was adjusted to minimize the solid adamantane ^{13}C linewidths in concert with the 3 kHz MAS. Notice that this low power level is insufficient to decouple the TMS resonance. Higher power ^{1}H decoupling results in broader lines as the MAS and CW decoupling interfere. Since the nanoprobe is not designed to take high enough power to overcome the MAS interference problem, these line positions were also independently determined using a solid adamantane sample in a CPMAS probe. The same 3 kHz MAS rate and low power decoupling were used to reproduce the spectrum for solid adamantane shown. The MAS rate was then increased to 20 kHz and the low power decoupling once more optimized to produce the narrowest adamantane ^{13}C resonances (FWHM ~ 4 Hz). While the ^{13}C resonances in the CPMAS experiments are much narrower, the shifts measured with respect to external TMS are identical within experimental error. Application of conventional high power CW or TPPM decoupling was not observed to result in any shift of the adamantane ^{13}C resonance positions, at least within the limits of accuracy imposed by the concomitantly shorter acquisition time. In this particular probe the sample temperature is known to rise between 10 and 15 °C when spun at 20 kHz. The constancy of the shifts measured indicate they are insensitive to small variations in temperature.

The solvent shifts of the adamantane lines when dissolved in $CDCl_3$ are less than those observed when TMS is the solvent, but they are still significant and outside the inherent experimental error. Again the methine carbon experiences the larger solvent shift. While the

relative shifts of the methylene and methine carbons are quite similar in both TMS or $CDCl_3$ at 9.43 and 9.41 ppm, respectively, the relative shift in the solid state is much less being 9.03 ppm. These observations account for the some of the differences in shifts that have been used by different laboratories for adamantane as a secondary ^{13}C chemical shift standard (vide infra).

MAS lpNMR is the sizeable 0.71 ppm solvent shift for TMS upon dissolution in $CDCl_3$. The observation that TMS can experience a significant intrinsic solvent shift was investigated early on by Bacon and Maciel in 50 different solvents [9]. Their work, predating the MAS lpNMR approach, compared shifts measured in both the parallel and perpendicular geometries to separate the solvent shifts from demagnetizing field shifts. These early measurements utilized 20% TMS solutions, and measured a comparable -0.67 ppm shift for the ^{13}C resonance of TMS in $CHCl_3$. We also find that when methanol is the solvent an even larger shift in the opposite direction of -0.74 ppm is observed. This sensitivity of the ^{13}C shift in TMS to solvent environment adds further potential for confusion in relating different referencing schemes when TMS is used for the primary reference.

Cognizant of these problems, the shift for DSS was measured as a function of concentration in D_2O. By MAS NMR the shift was found to weakly depend upon concentration from 0.5–5% by weight (0.023–0.23 M). A small but reproducible shift to lower frequency by $\sim$0.1 ppm was observed over the entire concentration range, the slope being +0.02 ppm/wt%. External ^{13}C referencing of the DSS solution to neat TMS by ZAS lpNMR gave statistically identical results for the concentration shift. The effect of ionic strength on the DSS shift was also investigated. A series of four samples were prepared with the DSS concentration fixed at 2.5 wt% and an increasing concentration of NaCl. The total salt concentration (DSS + NaCl) ranged from 0.12 to 0.42 M, and the observed DSS shift appeared between -1.97 and -1.94 ppm from external TMS. Although the experimental trend is unidirectional, the shift is on the order of our uncertainties and not judged statistically significant.

Having identified and characterized the solvent and concentration dependence of the measured shifts and magnetic susceptibilities, it should be possible to reconcile the shifts measured by MAS and ZAS NMR relative to the same external reference. Using literature values for the volume susceptibilities, one can predict the shift that should be observed in the MAS lpNMR experiments on the basis of the ZAS lpNMR results. The infinite length sample correction factor is simply to subtract $(4\pi/3)(\chi_{v,sample} - \chi_{v,TMS})$ (cgs) from the ZAS numbers to predict the MAS shift results [8]. Using this approximate correction we see that the shifts are satisfactorily accounted for in Table 1. Differences $\Delta\delta$ between corrected ZAS and observed MAS shifts are small, the largest $\Delta\delta$ being no more than $\sim|0.05|$ ppm, or about 50% more than our estimate of the inherent experimental error.

These differences tend to be systematically one sided for a particular solvent system, suggesting that some of the difficulty may be in the χ_v values used. The latter were computed using densities [18] at 20 °C as compiled in [18], except for TMS where the reported density is at 19 °C, and for CH_3OD where the density [19] is taken from [19]. Using these densities and literature cgs values for the molar diamagnetic susceptibilities [20–22], the χ_v values used in the calculations reported in Table 1, were computed as $\chi_{v,D_2O} = -0.714$ ppm [20], $\chi_{v,CHCl_3} = -0.740$ ppm [21], $\chi_{v,methanol} = -0.526$ ppm [21], and $\chi_{v,TMS} = -0.549$ ppm [22]. It is possible to relegate the differences between observed and predicted shifts to the third decimal place if the susceptibilities are all slightly adjusted, in all cases by less than 2%. As they stand, the $\Delta\delta$ values are statistically insignificant given our experimental errors. Furthermore, these differences are just as likely to be due to the uncharacterized finite sample length corrections, variability in sample tube susceptibility or the departure of the sample temperature from 20 °C that have not been taken into account.

From these data we see that the experimental errors in external referencing of solids MAS NMR spectra produces shifts which can be conservatively reported to an accuracy of ± 0.03 ppm. Larger discrepancies between shielding values measured by MAS ssNMR and ZAS lpNMR are likely to be due to solvent shifts. Especially problematic is the 0.71 ppm solvent shift we observe between neat TMS and TMS in $CDCl_3$. Prior work has principally used neat TMS as the primary reference [5] for referencing ^{13}C MAS ssNMR spectra, and we will choose to continue with this as our primary standard as well. Using the collected results herein, we can relate this scale to other widely used shift scales. For ZAS lpNMR these would be internal referencing to TMS in $CDCl_3$, $\delta_{internal}^{TMS\ in\ CDCl_3}$, or internal referencing to DSS in D_2O, $\delta_{internal}^{DSS\ in\ D_2O}$. For MAS ssNMR this would be external referencing to neat TMS, $\delta_{MAS}^{neat\ TMS}$, or external referencing to adamantane, $\delta_{MAS}^{adamantane}$. One would also like to be able to accurately relate the scales used for ssNMR to the common solution NMR scales. Note that the chosen notation is to place the reference compound (that which is designated to have a chemical shift of 0 ppm) in the superscript, and to place any experimental descriptors in the subscript. Keeping neat TMS in a MAS lpNMR experiment as the base scale, we can calculate the shifts desired on this scale from those measured using the other standards as

$$\begin{aligned}\delta_{MAS}^{neat\ TMS} &= \delta_{internal}^{TMS\ in\ CDCl_3} + 0.71 = \delta_{internal}^{DSS\ in\ D_2O} - 2.01 \\ &= \delta_{MAS}^{solid\ adamantane} + 38.48. \end{aligned} \quad (3)$$

In this relation $\delta_{\mathrm{MAS}}^{\text{solid adamantane}}$ is taken as the shift from the methylene carbon, and $\delta_{\text{internal}}^{\text{DSS in } D_2O}$ is for a concentration of 0.5 wt%.

These results explain what had appeared to be discrepancies in referencing of ^{13}C in solids, with the methylene of adamantane being taken as $38.5_6\pm$ 0.1 ppm [5] or 37.6 ppm [23] from TMS. On the basis of our results we infer that the latter value is most likely for TMS in $CDCl_3$ as the external reference. We note that our values for the ^{13}C shifts of the two lines in adamantane at 38.48 and 29.46 ppm from external TMS agree well with Earl and VanderHart's [5] reported and widely accepted number of $38.5_6 \pm 0.1$ and $29.5_0 \pm 0.1$ ppm.

It is also worth pointing out that the apparent constancy of the TMS ^{13}C shift in ZAS lpNMR, whether measured as a neat liquid or in $CDCl_3$, is a very misleading observation. This is not an indication that the TMS shift is insensitive to solvent, but that the solvent shifts and the bulk susceptibility corrections are balanced so as to almost completely cancel one another. These results reported also clarify the relationship of the DSS and TMS ^{13}C shift scales [24,25]. We find 0.5 wt% DSS conveniently resonates at -2.01 ppm with respect to external neat TMS. Without the susceptibility and solvent shift corrections, 0.5 wt% DSS would appear to resonate at -2.67 ppm with respect to external TMS, and at -2.62 ppm with respect to TMS in $CDCl_3$. Again the apparent constancy of this result is misleading, having its origins in the TMS solvent shift nearly matching the differential susceptibility shift between neat TMS and $CDCl_3$. When internal referencing in ZAS lpNMR is used the susceptibility shift is eliminated, but the solvent shift remains and is significant at 0.71 ppm. Further confusion is possible as TMS in methanol has been reported [24] to resonate at +1.7 ppm with respect to 10 mM DSS in D_2O in a ZAS lpNMR experiment using a coaxial sample cell. Repeating this measurement we obtain a +1.95 ppm shift difference. MAS NMR finds a solvent shift for TMS in methanol in the opposite direction of ~ -0.74 ppm. Dry methanol has a χ_v of -0.526 ppm (cgs), close to that of TMS itself, and this largely reconciles our MAS and ZAS lpNMR results. We expect the difference between our value and the previous report is due to the expected sensitivity of the methanol χ_v to water content.

Given the widespread use of heteronuclear correlation methods, it is convenient to know the resonance frequency ratios of ^1H and ^{13}C for referencing one set of nuclei via the other [25]. Following the IUPAC recommendations [1], these are reported as ratios, which we have determined using MAS lpNMR. For our two preferred reference samples, the average of eight determinations gives

$\Xi^{\text{neat TMS}}$ % for ^{13}C in neat TMS $= 25.145003_8$,

Ξ^{DSS} % for ^{13}C in 5% DSS in $D_2O = 25.144954_8$

with standard deviations of 0.5×10^{-7}. In each case the ratio quoted is for the ^{13}C resonance frequency to the corresponding ^1H resonance frequency of the same reference compound $\times 100$. The latter value has been reported previously [25] as 25.144952_8, which is the average of three values (25.144952_8, 25.144953_7, and 25.144951_9) for a set of slightly different samples. The difference between our measurement and among these three is of the same order of magnitude, but does however seem to be outside our estimated experimental errors.

These ratios can be used as in solution NMR to set the ^{13}C reference scale on the basis of a ^1H resonance reference frequency, or perhaps more important for solids samples, reference the ^1H dimension on the basis of a ^{13}C reference standard such as adamantane. If adamantane is used the following ratios are convenient:

$\Xi^{\text{neat TMS}}$ % for the methylene ^{13}C in solid adamantane $= 25.145972_7$,

$\Xi^{\text{neat TMS}}$ % for the methine ^{13}C in solid adamantane $= 25.145745_7$.

The ratios reported are for the corresponding adamantane ^{13}C resonance frequency over the ^1H resonance frequency of external neat TMS $\times 100$. In each case five determinations were made and the standard deviation is 0.5×10^{-7}. For setting the ^1H decoupling frequency ν_{decouple} at a desired ^1H chemical shift δ_H^{TMS} (in ppm relative to external TMS), the following equation referenced to the adamantane methylene ^{13}C resonance frequency $\nu_{\text{adamantane CH}_2}^{\text{C-13}}$ in Hz is useful

$$\nu_{\text{decouple}}\left(\delta_H^{\text{TMS}}\right) = 3.9767799_4 \times \nu_{\text{adamantane CH}_2}^{\text{C-13}} \times \left(1 + \left(\delta_H^{\text{TMS}} \times 10^{-6}\right)\right), \quad (4)$$

where the frequencies are entered in units of Hz.

Another secondary standard for MAS ssNMR that we have considered is glycine, as it is commonly used in setting the magic angle and other parameters in ^{13}C ssNMR experiments. We have however not chosen to investigate glycine as a reference compound since the ^{13}C spectrum of glycine in our experience is quite variable depending upon the mixture of crystal polymorphs present.

Using Eq. (3), as well as the known susceptibility correction factor, it is now possible to compare most sets of carbon or proton chemical shifts, regardless of the reference chosen. As an example, consider a case where a sample is added to $CDCl_3$ with 1% TMS used as a proton reference, as per IUPAC recommendations. The IUPAC defined $\Xi = \Xi^{1\%\ \text{TMS}}$ of 25.145020 allows calculation of the carbon reference frequency of 1% TMS in $CDCl_3$. It is then straightforward to compare data referenced in such a manner to that referenced to any of

the carbon references we have described, as the scales are simply related by fixed solvent or chemical shifts. Thus a ssNMR shift referred to the methylene in solid adamantane can be converted to the IUPAC scale by adding 37.77 ppm.

4. Conclusions

External referencing of MAS ssNMR and lpNMR spectra is greatly simplified by the removal of demagnetizing field shifts. Repeatability of sample exchanges can be quite good, especially when slow steady magnet drift is carefully accounted for. The precision of externally referenced shifts for MAS ssNMR spectra can easily be ± 0.03 ppm, and arguably made better with experimental care. The primary uncertainty in the precision of these measurements lies in the lineshape. Refinement of MAS probe hardware to achieve better static lineshape and careful sample packing so as to avoid sample shape dependent lineshape factors are both steps that can be taken to increase the precision of the shift measurement. Improvement in the accuracy of the measured shifts requires a more careful treatment of the temperature and composition dependence of absolute shifts. This must be considered both for the sample to be measured and for the reference system to be employed.

Difficulties principally arise when comparing externally referenced ssNMR shifts to different solution based shift scales. Although the shift of DSS in D_2O mixtures is slightly concentration dependent, the range that would be encountered at practical concentrations of DSS is well under the typical experimental uncertainty in determining line positions. TMS based scales for ^{13}C NMR however are unfortunately problematic. The IUPAC recommendations have adopted a scale using 1% TMS in $CDCl_3$, and as such include the large solvent shift of +0.71 ppm relative to neat TMS. We have chosen to report our results instead with respect to neat TMS. This has been utilized more often as an external ^{13}C reference in ssNMR simply because of sensitivity considerations. Regardless of one's choice of reference scale, care should be taken to ascertain the conditions under which TMS has been used as a ^{13}C shift standard before comparisons of independently collected data are made. Conversion to the IUPAC scale is straightforward as the two scales are simply offset by the solvent shift.

While not required in the present work, the solvent shift [9] for the ^1H resonance of 1% TMS in $CDCl_3$ relative to neat TMS would be useful to measure by MAS lpNMR so that ^1H shifts recorded on these two scales can be accurately related. Given the unique insights that solvent shifts provide in probing basic intermolecular interactions in liquids, it should prove profitable to reinvestigate the systems studied in the pioneering work of Bacon and Maciel using modern high field NMR instrumentation and MAS lpNMR techniques.

In spite of the complications explored in this work, the comparison of internally referenced ZAS results for liquids and externally referenced MAS NMR results for solids can be made with a great deal of confidence. The two approaches eliminate isotropic demagnetizing field shifts from the relative shifts, leaving only solvent shifts to be accounted for. If the solid sample has an ABMS, the possibility for a residual demagnetizing field shift under MAS remains. While the experimentalist should be cognizant of this caveat, in the vast majority of cases it is expected that an ABMS will primarily lead to a broadening but not a shift of the lines in MAS spectra of finely divided powder samples. Experimental factors in MAS ssNMR, such as the absence of a field frequency lock, or the need to remove the probe for sample changes, are not the precision limiting factors that one might suspect. More problematic would be field shifts associated with RT shimming between sample changes, although this is not typically done in MAS ssNMR, as MAS removes much of the sample to sample variability due to isotropic magnetic susceptibility effects.

In conclusion, the measurement of ^{13}C chemical shifts to within ± 0.03 ppm in MAS ssNMR spectra should be routinely achieved with careful but not exceptional experimental practice. Given the need, a factor of three or more in accuracy seems readily achievable with careful attention to details not pursued here.

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References

[1] R.K. Harris, E.D. Becker, S.M.C. De Menezes, R. Goodfellow, P. Granger, NMR nomenclature. Nuclear spin properties and conventions for chemical shifts—(IUPAC recommendations 2001), Pure Appl. Chem. 73 (2001) 1795–1818.
[2] A.N. Garroway, Magic-angle sample spinning of liquids, J. Magn. Reson. 49 (1982) 168–171.
[3] W.C. Dickinson, The time average magnetic field at the nucleus in nuclear magnetic resonance experiments, Phys. Rev. 81 (1951) 717–731.
[4] F.D. Doty, G. Entzminger, Y.A. Yang, Magnetism in high-resolution NMR probe design. I: General methods, Concepts Magn. Resonance 10 (1998) 133–156.
[5] W.L. Earl, D.L. VanderHart, Measurement of C-13 chemical-shifts in solids, J. Magn. Reson. 48 (1982) 35–54.

[6] T.M. Barbara, Cylindrical demagnetization fields and microprobe design in high-resolution NMR, J. Magn. Reson. Ser. A 109 (1994) 265–269.
[7] D. Doskocilova, D.D. Tao, B. Schneider, Effects of macroscopic spinning upon linewidth of NMR signals of liquid in magnetically inhomogeneous systems, Czech. J. Phys. B 25 (1975) 202–209.
[8] D.H. Live, S.I. Chan, Bulk susceptibility corrections in nuclear magnetic resonance experiments using superconducting solenoids, Anal. Chem. 42 (1970) 791–792.
[9] M.R. Bacon, G.E. Maciel, Solvent effects on the five shielding constants in tetramethylsilane and cyclohexane, J. Am. Chem. Soc. 95 (1973) 2413–2426.
[10] J.W. Emsley, J. Feeney, L.H. Sutcliffe, in: High Resolution Nuclear Magnetic Resonance Spectroscopy, vol. 1, Permagon Press, Oxford, 1965, pp. 256–279.
[11] A.A. Bothnerby, R.E. Glick, Medium effects in nuclear magnetic resonance spectra of liquids. 2. Simple aliphatic molecules, J. Chem. Phys. 26 (1957) 1647–1650.
[12] A.A. Bothnerby, R.E. Glick, Medium effects in the nuclear magnetic resonance spectra of liquids. 3. Aromatics, J. Chem. Phys. 26 (1957) 1651–1654.
[13] A.A. Bothnerby, Medium effects in the nuclear magnetic resonance spectra of liquids. 4. Nature of the effects, J. Mol. Spectrosc. 5 (1960) 52–61.
[14] S. Hayashi, K. Hayamizu, Shift references in high-resolution solid-state NMR, Bull. Chem. Soc. Jpn. 62 (1989) 2429–2430.
[15] S. Hayashi, K. Hayamizu, Chemical-shift standards in high-resolution solid-state NMR (1) C-13, Si-29 and H-1 nuclei, Bull. Chem. Soc. Jpn. 64 (1991) 685–687.
[16] D.L. VanderHart, Magnetic, susceptibility & high resolution NMR of liquids & solids, in: D.M. Grant, R.K. Harris (Eds.), Encyclopedia of Nuclear Magnetic Resonance, Wiley, Chichester, 1996.
[17] S. Hayashi, M. Yanagisawa, K. Hayamizu, Nuclear-magnetic-resonance chemical-shifts of pure organic-solvents determined by magic angle spinning, Anal. Sci. 7 (1991) 955–957.
[18] D.R. Lide (Ed.), CRC Handbook of Chemistry and Physics, 78th ed., CRC Press, Boca Raton, FL, 1997.
[19] D.W. Davidson, A dilatometric study of solid methanol-D, Can. J. Chem. 34 (1956) 1243–1248.
[20] F.E. Hoare, Diamagnetic susceptibility of heavy water, Nature 137 (1936) 497–498.
[21] S. Broersma, The Magnetic susceptibility of organic compounds, J. Chem. Phys. 17 (1949) 873–882.
[22] M.W. Lister, R. Marson, Magnetic susceptibilities of some compounds of silicon, Can. J. Chem. 42 (1964) 2101–2106.
[23] P.T.F. Williamson, G. Grobner, P.J.R. Spooner, K.W. Miller, A. Watts, Probing the agonist binding pocket in the nicotinic acetylcholine receptor: a high-resolution solid-state NMR approach, Biochemistry 37 (1998) 10854–10859.
[24] D.S. Wishart, C.G. Bigam, J. Yao, F. Abildgaard, H.J. Dyson, E. Oldfield, J.L. Markley, B.D. Sykes, H-1, C-13 and N-15 chemical-shift referencing in biomolecular NMR, J. Biomol. NMR 6 (1995) 135–140.
[25] J.L. Markley, A. Bax, Y. Arata, C.W. Hilbers, R. Kaptein, B.D. Sykes, P.E. Wright, K. Wuthrich, Recommendations for the presentation of NMR structures of proteins and nucleic acids—(IUPAC Recommendations 1998), Pure Appl. Chem. 70 (1998) 117–142.