TMS is Superior to Residual C*H*Cl$_3$ for Use as the Internal Reference for Routine ^{1}H NMR Spectra Recorded in CDCl$_3$

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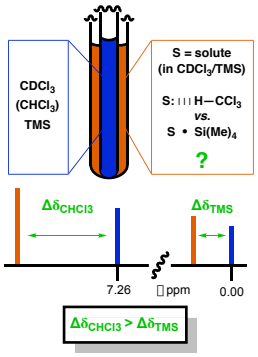
ABSTRACT: Tetramethylsilane was recommended for use as an internal reference compound in proton NMR spectroscopy over 60 years ago. However, it is a common practice that researchers reference the analyte chemical shifts to the residual proton resonance in the deuterated solvent in which the spectrum is recorded. Because CDCl$_3$ is the most commonly used NMR solvent for routine analysis of organic compounds, the effect of various functional groups on the CHCl$_3$ resonance is important. Described here are results that show why referencing spectra to TMS rather than CHCl$_3$ in CDCl$_3$ results in more accurate chemical shifts and should be the recommended practice. Simultaneous measurement of separate compartments of unperturbed CDCl$_3$/TMS vis-à-vis CDCl$_3$/TMS/solute solutions using a concentric tube arrangement was key. This study is reported in this venue because the audience/readership is quite appropriate and is, hopefully, both receptive to and appreciative of the guidance provided.



■ INTRODUCTION

Accurate referencing of the chemical shifts in NMR spectra provides an important means for judging whether different samples do (or do not) contain the same analyte. For proton NMR spectra, the tetramethylsilane (TMS) resonance is often used as an internal reference standard, as first proposed in 1958 by George Tiers of the 3M Company.[1] That suggestion, later endorsed by recommendation of the IUPAC,[2] was made based on i) the assumption that TMS would be relatively non-interactive with other solute molecules (e.g., it has no permanent dipole) and ii) the conveniently non-interfering upfield chemical shift of its methyl protons. The majority of ^{1}H NMR spectra are recorded and reported as solutions in CDCl$_3$. However, it is a common practice to reference the chemical shifts of the solute under study to the shift of the residual proton resonance of CHCl$_3$ (i.e., δ = 7.26[3] ppm) rather than that of added TMS. Although this is an acceptable and sufficient approach for situations in which knowledge of the true chemical shifts of the solute is not critical, it is impossible to anticipate the situations in which future researchers might benefit from having a more accurate set of chemical shifts. The purpose of this focused study is to make the case for routine use of the TMS resonance (δ = 0.00 ppm) rather than CHCl$_3$, an extremely easy practice to implement.

We have often observed some non-trivial changes in the chemical shift difference between residual CHCl$_3$ and TMS (i.e., $\delta_{CHCl3} - \delta_{TMS}$) for certain analytes. That change is concentration dependent for the same analyte. Most often the $\Delta\delta$ is >7.26 ppm, meaning that either one, the other, or both of the chemical shifts of CHCl$_3$ and TMS have been altered by the presence of the solute. The effect of added solute molecules on the chemical shift of CHCl$_3$ alone[4] or on TMS alone[5] have been previously studied, but to our knowledge the work here is the first study in which both have been parsed by simultaneous measurement. It is intuitive that CHCl$_3$ is more likely to interact with solute molecules because it possesses a permanent dipole and is a hydrogen bond donor. Hence, we have always assumed that the shift of the chloroform protons was being perturbed in these situations much more than (exclusively?) that of the TMS protons. Is that the case?

■ RESULTS AND DISCUSSION

We addressed the preceding question through experiments in which a portion of CDCl$_3$ (99.8% D) containing 0.05% TMS but no added solute was compared in a head-to-head fashion

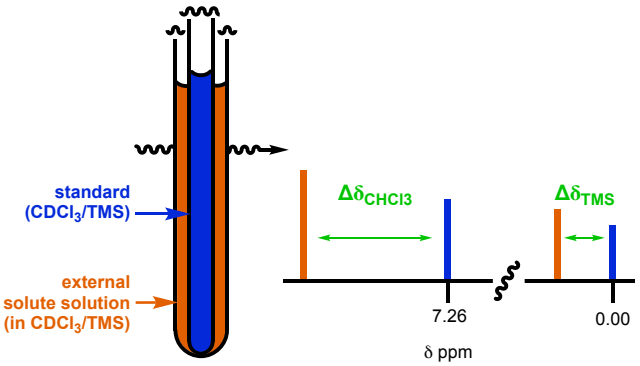


Figure 1. Schematic of the concentric tube arrangement in which the relative chemical shift changes of CHCl$_3$ and TMS induced by the presence of a solute could be simultaneously observed.

with solutions of various solutes in that same NMR solvent. We used a concentric tube arrangement similar to that shown in Fig. 1 to achieve a simultaneous measurement of these two, compartmentalized solutions.[6] The solutes examined are shown in Table 1; each was a small molecule containing a single functional group representing, collectively, the majority of functionalities present in a wide array of organic molecules. The internal capillary portion always contained the same solution of $CDCl_3$/TMS, which we call here the "standard." Using serial dilution, we varied the concentration of solute across a wide range (≤8 M to ≥16 mM). The lowest of these corresponds to a sample size of ca. 4 mg of a solute with a molecular weight of 400 amu in an NMR sample volume of ca. 700 μL.

Shown in Fig. 2 is a representative subset of spectra for one of the solutes, arbitrarily acetone. There it is easy to visualize that the chemical shifts of TMS and $CHCl_3$ resonances in the standard (i.e., inner capillary) are invariant (as is the resonance for trace water). It is also obvious that the chloroform resonance is shifted to a greater extent than that of TMS as the concentration of acetone increases. However, the change in TMS chemical shift, albeit smaller, also demonstrates that TMS is not non-interacting (i.e., not inert). Nonetheless and as presented below, for nearly every solute examined, the effect on $CHCl_3$ was greater than that on TMS.

Table 1. Solutes Examined, Abbreviation, Functional Group, and Molecular Dipole

Solute	Abbrevi-ation	Functional group	dipole (D)
1,8-diazabicyclo[5.4.0]-undec-7-ene	DBU	amidine	3.41[7]
1-fluoronitrobenzene	-	alkyne	2.87[10]
1-pentyne	-	alkyne	0.85[8]
acetone	Me_2CO	ketone	2.9[9]
acetonitrile	MeCN	nitrile	3.2[9]
benzene	PhH	aromatic	0[9]
n-butylamine	$^nBuNH_2$	primary amine	1.00[10]
diethyl ether	Et_2O	ether	1.3[9]
dimethyl sulfoxide	DMSO	sulfoxide	3.96[9]
dimethyl iso-phthalate	DMI	aromatic	2.23[10]
dimethylformamide	DMF	tertiary amide	3.8[9]
ethyl acetate	EtOAc	ester	1.78[8]
hexafluorobenzene	HFB	aromatic	0[10]
hexamethylphosphoramide	HMPA	phosphoramide	5.54[10]
methanol	MeOH	alcohol	1.7[9]
methylene chloride	DCM	chlorocarbon	1.8[9]
pyridine	py	heteroaromatic	2.3[9]
quinuclidine	-	amine	1.20[10]
tetrahydrofuran	THF	ether	1.75[9]
triethylamine	TEA	tertiary amine	0.66[8]
triethyl phosphate	$(EtO)_3PO$	phosphate ester	2.86[10]
trifluoroacetic acid	TFA	carboxylic acid	2.26[10]
triphenylphosphine oxide	TPPO	phosphine oxide	4.4[10]

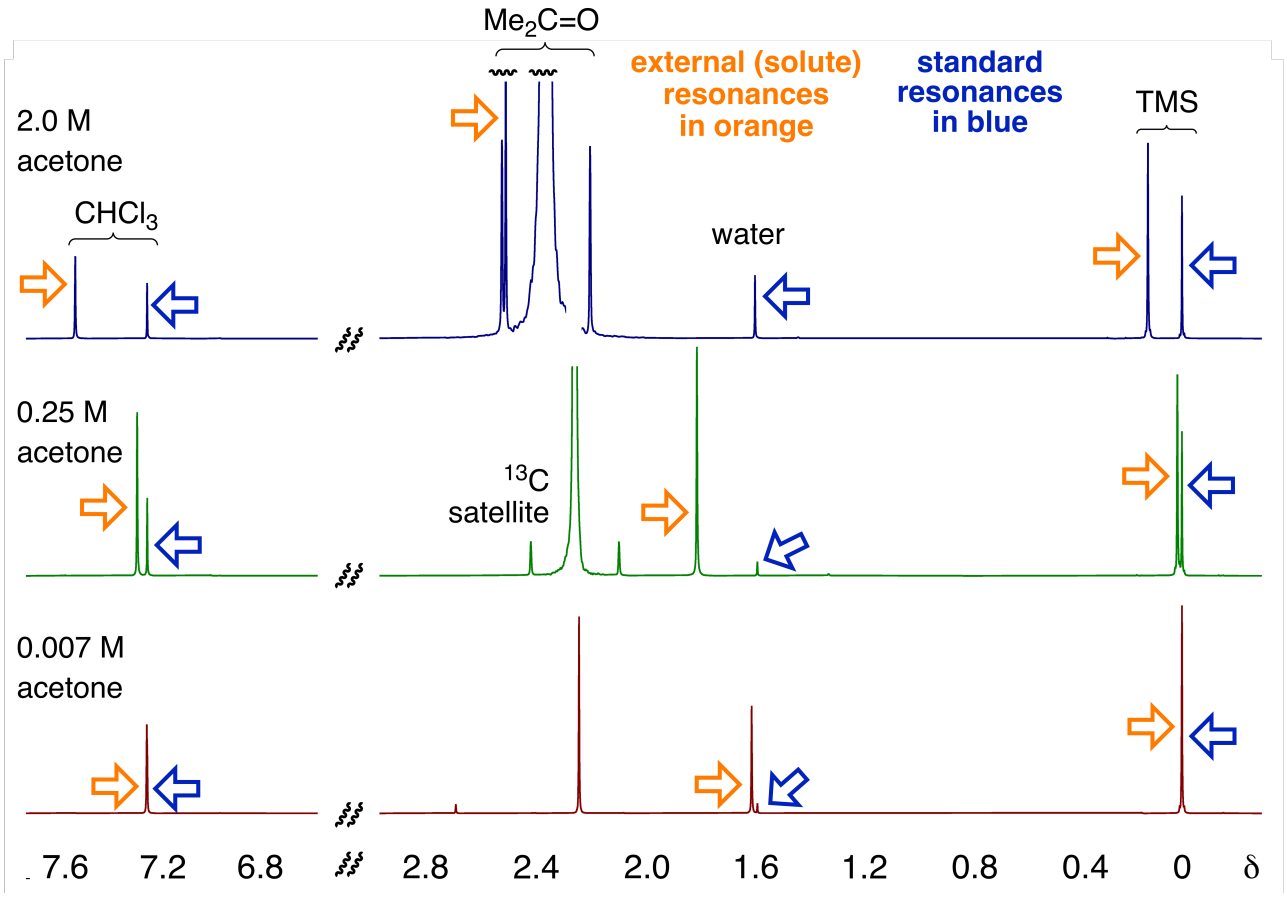


Figure 2. Spectra of various concentrations of acetone in $CDCl_3$ (99.8% deuteration) containing 0.05% TMS. Note: the relative intensities of the TMS to water resonances in the standard differ in the top vs. the bottom two spectra because a different bottle of $CDCl_3$/TMS was used.

Results from a typical full data set, this for CH_3OH, are shown graphically in Fig. 3. Methanol happens to be a relatively weakly interacting solute. The good linearity (viz. R^2) of chemical shift differences across the full range of concentrations is easily seen, as is the similarity of the slopes across the full vs. the low-end range of concentrations (see inset). A single "figure of merit" (hereafter, "FOM") that captures the relative sensitivity (or response) of $CHCl_3$ vs. TMS is the ratio of the slopes for $CHCl_3$ vs. TMS over the full concentration range. This is shown for each solute in columns 4 and 6 in Table 2, discussed further below. The reproducibility of the data using the serial dilution methodology was checked by measuring the shift changes for one of the solutes, arbitrarily benzene, in triplicate, using newly prepared samples for each independent set of measurements. The relative standard deviation of the three slope values for each of the two chemical shifts is ca. 6%. This is perfectly adequate for drawing the overall conclusion that TMS is a more nearly inert substance than is chloroform.

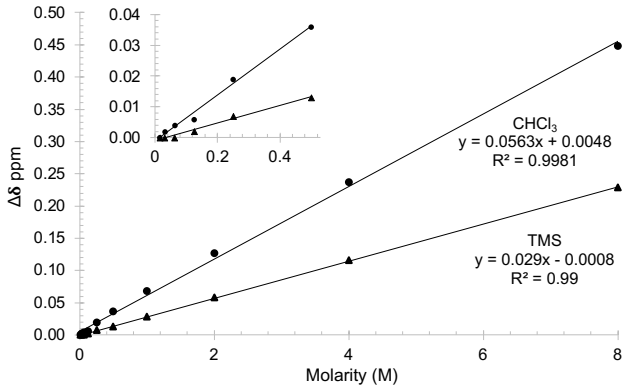


Figure 3. Chemical shift changes of $CHCl_3$ ($\Delta\delta_{CHCl3}$) vs. TMS ($\Delta\delta_{TMS}$) resonances as a function of CH_3OH (solute) concentration.

In Table 2 we have summarized the overall body of results. Columns 1-4 show the solutes (column 1) ordered according to their largest to smallest chemical shift effect on $CHCl_3$ (column 2). Column 3 contains the solute-induced chemical shift changes for TMS. Column 4 shows the FOM for each solute – i.e., the ratio of the chemical shift impact on $CHCl_3$ vs. TMS: |slope of $\Delta\delta_{CHCl3}$| ÷ |slope of $\Delta\delta_{TMS}$|. The data in

columns 5 and 6 of Table 2 are listed in descending order of the magnitude of the FOMs for the solutes.

Perhaps not surprisingly, $CHCl_3$ experiences the largest chemical shift deviations in the presence of functional groups that are good hydrogen bond acceptors.[11] Benzene, hexafluorobenzene, and TPPO are the only solutes examined that induced a net shielding effect on chloroform's proton. In the case of complexes of benzenoid compounds with chloroform, shielding has been attributed to CH-π interactions that position the chloroform proton in the anisotropic shielding region of the aromatic electron density.[12] In the TPPO $CHCl_3$ complex, apparently the anisotropic shielding of the phenyls in TPPO outweighs the deshielding induced by the $Cl_3C–H---O=PPh_3$ hydrogen bond.

Table 2. Slope Values of the Change in the ^1H Chemical Shift vs. Solute Concentration for Chloroform (col 2) and for Tetramethylsilane (col 3) and the Ratio of those Two Slopes [the Figure of Merit (FOM)] as the Indicator of the Relative Sensitivity of $CHCl_3$ vs. TMS to the Presence of Solute (cols 4 and 6).

solutes ordered according to the magnitude of their shift perturbations on $CHCl_3$				solutes ordered according to their relative shift perturbations on $CHCl_3$ vs. TMS	
1	2	3	4	5	6
solute	**slope of Δδ $CHCl_3$ (ppm/M)**	**slope of Δδ TMS (ppm/M)**	**FOM |slope of $\Delta\delta_{CHCl3}$| / |slope of $\Delta\delta_{TMS}$|**	**solute**	**FOM |slope of $\Delta\delta_{CHCl3}$| / |slope of $\Delta\delta_{TMS}$|**
HMPA	0.356	0.019	18.45	pyridine	>100
DBU	0.315	0.017	18.83	DBU	18.83
$(EtO)_3P=O$	0.232	0.045	5.16	HMPA	18.45
TEA	0.205	0.058	3.52	benzene	7.11
n-$BuNH_2$	0.200	0.046	4.32	quinuclidine	6.52
DMSO	0.169	0.030	5.69	DMSO	5.69
DMF	0.154	0.039	3.91	$(EtO)_3P=O$	5.16
acetone	0.142	0.072	1.98	n-$BuNH_2$	4.32
ether	0.134	0.070	1.93	DMF	3.91
pyridine	0.118	[a]	>100	TEA	3.52
EtOAc	0.116	0.055	2.10	THF	3.42
quinuclidine	0.099	0.015	6.52	EtOAc	2.10
THF	0.083	0.024	3.42	acetone	1.98
1-pentyne	0.080	0.062	1.30	MeOH	1.95
MeCN	0.069	0.042	1.63	ether	1.93
MeOH	0.056	0.029	1.95	MeCN	1.63
TFA	0.043	0.043	1.02	1-pentyne	1.30
DMI	0.038	-0.029	1.29	DMI	1.29
4-fluoronitrobenzene	0.010	-0.011	0.96	TFA	1.02
DCM	0.002	-0.002	1.00	DCM	1.00
benzene	-0.107 ± 0.006	0.015 ± 0.0003	7.11	4-fluoronitrobenzene	0.96
TPPO	-0.024	-0.101	0.24	hexafluorobenzene	0.72
hexafluorobenzene	-0.017	-0.023	0.72	TPPO	0.24

[a] $\Delta\delta_{TMS} < 0.01$ ppm even at 8 M.

The nature of the interactions between TMS and the solutes at the molecular level is likely more highly variable for different solutes[5,13] than is the case for the complexes between solutes and $CHCl_3$. The general trend we observed for TMS is that most solutes induce a downfield, but small, shift in the methyl proton resonances. As a side note, we should mention that $CDCl_3$ is notorious for producing and, therefore, containing small amounts of HCl over time. This arises from slow autoxidation to produce phosgene followed by hydrolysis with adventitious water. This perhaps most often has come to light in studies involving low concentrations of basic nitrogenous compounds (e.g., alkaloid natural products).[14]

It is not obvious that the FOMs correlate with any single, simple parameter associated with each of the solutes. For example, we wondered whether the molecular dipole of the solute (cf. Table 1) might map onto the observed trends in FOMs. However, such a correlation is weak at best (see Supporting Information for linear regression plots showing an R^2 of merely 0.01 for FOM vs. dipole moment and 0.21 for the slope of $\Delta\delta_{CHCl3}$ vs dipole moment).

For the great majority of solute functional groups examined, the chemical shift of chloroform was affected to a greater extent than that of TMS. Thus, we conclude that (and recommend) the routine use of TMS as the reference compound when collecting routine spectra in $CDCl_3$. For only TPPO, 4-fluoronitrobenzene, and hexafluorobenzene were the FOMs ≤1.0.

We note that several, more-sophisticated, first principle protocols for referencing spectra to the "absolute" chemical shift of TMS have been developed.[15] While these would be superior to what we are recommending here, none are trivial to implement and would be impractical for adoption on a routine basis.

We also note that there will always exist a (small) subset of instances in which it might be advisable to use a source of $CDCl_3$ that contains no TMS. For example, the interpretation of spectra of organosilane compounds can be convoluted by the presence of Me_4Si. However, there are several relatively straightforward ways of deducing which of an array of upfield singlets in a spectrum is the one due to Me_4Si. For example, the relative intensities of the $CHCl_3$ and TMS singlets will be relatively constant throughout the use of a bottle of TMS-doped $CDCl_3$. The $\Delta\delta$ of the two resonances will likely be ca. 7.26, especially for dilute solutions of the analyte. Finally, if at all in doubt, the sample can be doped with, say, an equal volume of additional NMR solvent, after which only the TMS singlet will have increased in relative intensity to the other silylated CH resonances.

Finally, we wondered whether the trends observed for $CDCl_3$/TMS solutions would carry over to other commonly used NMR solvents. We briefly addressed that question by examining a 2 M solution of, arbitrarily, diethyl ether in acetone-d_6, benzene-d_6, DMSO-d_6, and methanol-d_4 using the concentric tube arrangement (see SI). The resonances for TMS and the residual solvent ^{1}H were perturbed to a very similar extent. Therefore, it appears that the use of TMS as the reference compound is not as critical for these (less frequently used) solvents as it is for $CDCl_3$.

■ CONCLUSIONS

It is true that in some cases having a more accurate set of chemical shifts may not ever matter (e.g., if no other researcher ever records the ^{1}H NMR spectrum of the same substance). But in some cases the improvement afforded by the use of TMS as the reference compound is of value. For example, structurally complex natural products often are reisolated or synthesized (long) after the initial report of their isolation and structure determination. Because it is nearly impossible to know in advance when greater chemical shift accuracy will prove beneficial, why not be in the habit of always collecting more accurate data in the first place, especially since it is trivial to do so?

No internal reference compound will be truly inert, and we certainly are not purporting here that TMS is non-interacting. Nonetheless, referencing chemical shifts to TMS rather than residual $CHCl_3$ is a superior practice, especially given that it is so easy to implement for those not currently doing so.

■ EXPERIMENTAL SECTION

External solute samples were prepared by serial dilutions, starting with relatively high concentrated stock solutions (1–8 M, depending on the molecular volume of the solute) of the solute of interest dissolved in $CDCl_3$ (99.8% D) containing 0.05% TMS. Each external solute sample was then placed into a 5 mm WILMAD 535-PP (PREC 600 MHz) NMR tube. A New Era Enterprises capillary (NE-262-2) was filled with a solution of $CDCl_3$ (99.8% D) containing 0.05% TMS (i.e., the "standard"), capped with a New Era Enterprises Teflon® capillary adapter (NE-325-5/2.5), and carefully inserted into the 5 mm NMR tube (assisted by a NE-341-5 support rod) containing the external solute solution. All NMR spectra were recorded on a Bruker Avance III HD AX-400 instrument (400 MHz). At the outset, the performance of this concentric tube arrangement was checked by using the same, standard solution in both the internal capillary compartment and the outer annulus tube. This produced a spectrum with only a single observable resonance for each of the TMS and residual $CHCl_3$ protons (as well as for the trace of water impurity).

■ ASSOCIATED CONTENT

Supporting Information.

"The Supporting Information (SI) is available free of charge on the ACS Publications website."

FAIR Data (FID for Publication.zip) of the raw data for each NMR spectrum of all solutes listed in Table 1 as well as a master metadata file listing all (260) individual NMR folder names.

A single Excel file (Excel Summary of NMR Shift Data.zip) of all chemical shift differences of $CHCl_3$ and TMS as well as the slope of their concentration dependence for each of the solutes and a metadata file indicating the content of the 25 Excel sheets.

A PDF of the 25 Excel sheets, as requested by a reviewer.

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Notes

The authors have no competing financial interests to declare.

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