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Note

Reference systems for ^{1}H-n.m.r. spectroscopy of carbohydrates

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^{1}H-N.m.r. chemical shifts reported in the literature for compounds dissolved in, for example, deuterium oxide may be given relative to the high-field singlet of dissolved sodium 4,4-dimethyl-4-silapentanesulphonate (DSS)[1], or of external tetramethylsilane (Me_4Si). It is important to observe that the two chemical-shift reference scales differ by ~0.5 p.p.m. when standard, cylindrical cells are used and the Me_4Si (neat liquid) is contained in a coaxial, sealed, glass capillary.

Thus, for a solution of methyl α-D-mannopyranoside in D_2O, the chemical shifts for H-1 are δ(Me_4Si) 5.29 and δ(DSS) 4.80 (Refs. 2 and 3, respectively). Likewise, using a Varian XL-100 spectrometer with cylindrical sample and external reference cells at 70°, we have also shown widely different values for D-glucose, 2-amino-2-deoxy-D-galactose, and numerous oligo- and poly-saccharides. Whereas, for certain solutes[4,5], interaction with DSS may bring about considerable chemical-shift changes for the dissolved DSS, for carbohydrates this is not generally so, and the major differences in chemical shifts are attributable to the DSS (internal)/Me_4Si (external) relationship.

When DSS and Me_4Si are dissolved in a common solvent (*e.g.*, methyl sulphoxide), there is close agreement between the two reference scales[6]. Likewise, the scales for DSS in D_2O and for Me_4Si in $CDCl_3$ are separated by ~0.05 p.p.m. or less[7], implying a shift difference of ~0.45 p.p.m. between Me_4Si in $CDCl_3$ and Me_4Si (neat liquid). Furthermore, for a solution of glycolaldehyde dimer in Me_2SO-d_6, δ(Me_4Si external) values are ~0.4 p.p.m. downfield from those recorded[8] relative to internal Me_4Si in the same solvent.

To resolve these differences, the following factors should be considered: (*a*) the chemical-shift difference of isolated molecules of Me_4Si and DSS, $\Delta\delta_0 = \delta_0(\text{DSS}) - \delta_0(\text{Me}_4\text{Si})$; (*b*) the difference ($\Delta\delta_m$) caused by the various interactions between reference compounds and their respective solvents, given, *e.g.*, by $\Delta\delta_m =$

$\delta_m(\mathrm{DSS/D_2O}) - \delta_m(\mathrm{Me_4Si/CDCl_3})$, such effects being determined experimentally[9]; and (*c*) the difference ($\Delta\delta_\chi$) due to the bulk susceptibilities of the solvents (when an external reference is used) given, *e.g.*, by $\Delta\delta_\chi = \delta_\chi(\mathrm{D_2O}) - \delta_\chi(\mathrm{CDCl_3})$. Thus, Equation *1* gives a chemical-shift difference for the two reference compounds:

$$\delta(\mathrm{DSS}) - \delta(\mathrm{Me_4Si}) = \Delta\delta_0 + \Delta\delta_m + \Delta\delta_\chi. \qquad (1)$$

$\Delta\delta_0$ is difficult to evaluate experimentally. If the same solvent is employed for DSS and $\mathrm{Me_4Si}$, shift differences reflect both $\Delta\delta_0$ and $\Delta\delta_m$, but measurements have shown[6] consistently small values for the combined effects. In view of the structural similarity between the reference compounds, it is reasonable to suppose both $\Delta\delta_0$ and $\Delta\delta_m$ are small—the unlikely alternative is that the terms cancel for each solvent in which measurements have been made.

Bulk-susceptibility corrections (*c*) depend on the geometry of the reference cell, and the relative orientation of the sample tube to the magnetic field. For the usual arrangement of coaxial, cylindrical cells perpendicular to the magnetic field direction, this correction is illustrated in Equation *2*.

$$\Delta\delta_\chi = -\frac{2\pi}{3}[\chi_v(\mathrm{D_2O}) - \chi_v(\mathrm{CDCl_3})], \qquad (2)$$

where χ_v is the volume susceptibility (*cf.* Ref. 9). The correction is different for superconducting magnet systems, and there is no correction for spherical cells. Using standard susceptibility data[10], the $\Delta\delta_\chi$ values relative to neat, external $\mathrm{Me_4Si}$ for solvents commonly used in carbohydrate chemistry are as follows: $\mathrm{CDCl_3}$ +0.40, $\mathrm{D_2O}$ +0.34, $\mathrm{Me_2SO}$ +0.14 (accuracy ±0.02 p.p.m.).

The medium effects (*b*) for a few common internal-reference systems were determined at 38° by measuring the chemical-shift differences between the internal reference signals (for ~1% solutions of $\mathrm{Me_4Si}$ or DSS) and neat, external $\mathrm{Me_4Si}$ in coaxial, cylindrical cells (5-mm diameter n.m.r. tube and a capillary), using a Varian A-60A spectrometer. After correcting for the bulk susceptibility effect, the following values, in Table I, were obtained.

TABLE I

CORRECTIONS FOR MEDIUM EFFECTS

Solvent	*Internal reference*	$\Delta\delta_m$[a]
$\mathrm{CDCl_3}$	$\mathrm{Me_4Si}$	+0.14
$\mathrm{D_2O}$	DSS	+0.12[b]
$\mathrm{Me_2SO}$	$\mathrm{Me_4Si}$	+0.24
$\mathrm{Me_2SO}$	DSS	+0.23[b]

[a]Accuracy ±0.02 p.p.m. [b]Assuming $\Delta\delta_0$ to be negligible.

Using the foregoing corrections for $\Delta\delta_\chi$ and $\Delta\delta_m$, it is possible to explain the differences in reference signals observed in the above examples, or to convert from one reference scale into another. Thus, the difference in chemical shift between internal DSS in D_2O and external Me_4Si (contained in a capillary) is 0.46 p.p.m., which includes the contribution due to the bulk-susceptibility effect of D_2O relative to Me_4Si ($\Delta\delta_\chi$ +0.34 p.p.m.) and the difference in medium effects between D_2O on DSS and neat Me_4Si ($\Delta\delta_m$ +0.12 p.p.m.); experimentally, values of the order 0.50 p.p.m. are found. Similarly, the shift difference between internal Me_4Si in Me_2SO and external Me_4Si (capillary) is 0.38 p.p.m. (0.14+0.24 p.p.m.); the proton chemical shifts of the glycolaldehyde dimer relative to internal Me_4Si[8] in Me_2SO-d_6 differ by 0.39 ±0.02 p.p.m. from those we found relative to external Me_4Si in a capillary, as indicated before. A comparison between DSS and Me_4Si used as internal reference compounds requires corrections for medium effects ($\Delta\delta_m$) only. Table I shows that the medium effects on Me_4Si in Me_2SO (relative to neat Me_4Si), and on DSS in the same solvent, are the same within experimental error; reference scales based on DSS in D_2O ($\Delta\delta_m$ +0.12 p.p.m.) and on Me_4Si in $CDCl_3$ ($\Delta\delta_m$ +0.14 p.p.m.), however, are coincidentally similar.

In view of imprecise usage of the expression "reference to Me_4Si" and impressions from the literature that the main reference signal of internal DSS is at about the same frequency as that of external Me_4Si, it should be emphasized that for normal cylindrical cells the shifts are not necessarily coincident. For the solvent–reference systems commonly used, the differences in reference signals may be up to 0.54 p.p.m.

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