497. *Internal Reference Standards for Proton Magnetic Resonance Spectroscopy in Aqueous Solution.*

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Acetonitrile and dioxan are suitable for use as internal reference standards for proton nuclear magnetic resonance in aqueous solution. t-Butyl alcohol is less acceptable because of interaction with aromatic solutes.

The use of tetramethylsilane as an internal reference standard for proton magnetic resonance has become standard since its introduction by Tiers [1] in 1958. Its only serious drawback is its insolubility in water. Tiers has recently suggested that sodium 3-trimethylsilylpropanesulphonate ($Me_3Si{\cdot}[CH_2]_3{\cdot}SO_3Na$) can be used as an internal reference in aqueous solution,[2] but this compound is at present not easy to obtain. We wished to find some simpler compounds which might conveniently be used as secondary standards.

A primary requirement for such a standard is that the position of its resonance shall not vary greatly with changing concentration of its aqueous solution. To examine this we measured a series of aqueous solutions of compounds which might possibly be of use as reference standards (t-butyl and neopentyl alcohol, acetone, acetonitrile, acetic acid, dioxan, and tetrahydrofuran) at concentrations varying between pure water and pure compound, with tetramethylsilane as an external standard. The intention was to calculate how much of the observed shift was due to the different magnetic susceptibilities and thus by subtraction to find how much of the shift could be assigned to interaction between water and the reference compound.

This preliminary work indicated that three of the compounds, t-butyl alcohol, acetonitrile, and dioxan, were less affected by changing concentration than the others; the shift between 1% and 10% solutions was not more than about 0·02 p.p.m. and this may have been mainly a susceptibility effect. However, a combination of errors in the measured susceptibilities and of slight uncertainties in the shape factor prevents us from making a susceptibility correction to this accuracy. As the signals for these three compounds fall in different parts of the spectrum (τ values ~8·8, 8·0, and 6·3, respectively) we felt that they might provide a satisfactory set of secondary standards.

It seemed that the best way to check their suitability might be the empirical one of comparing the spectra of a number of simple compounds in aqueous solution measured against the three possible standards. The solutes we chose were methyl acetate, trimethyl phosphate, methyl methanesulphonate, methylal, dimethylformamide, triethylamine,

[1] Tiers, *J. Phys. Chem.*, 1958, **62**, 1151.
[2] Tiers, personal communication; Tiers and Coon, *J. Org. Chem.*, 1961, **26**, 2097.

4-picoline, quinol, sodium *p*-tolyl oxide, and *p*-toluidine hydrochloride. In each case the solution contained 10% w/v of the solute (except for quinol where a saturated solution contained about 7%) and 2—3% of one of the standards, a quantity which gives a conveniently sized signal.

We measured the chemical shifts between each solute peak and the standard peaks. For example, the centre of the doublet of trimethyl phosphate was 0·07 p.p.m. below the dioxan peak, 1·75 below that of acetonitrile, and 2·57 below that of t-butyl alcohol. The differences between the dioxan and acetonitrile values and the acetonitrile and t-butyl alcohol values are therefore 1·68 and 0·82, respectively. These differences are identical with those obtained from dilute aqueous solutions of the standards when measured against a tetramethylsilane external standard. The inference is that, as expected, the interaction between the standard and trimethyl phosphate is too small to cause a significant shift of the resonance positions of either.

In the same manner we calculated the differences between the standards for all the peaks of the various solutes. The dioxan–acetonitrile figure was very constant (mean of seventeen values 1·68, standard deviation 0·01) but the acetonitrile–t-butyl alcohol difference depended on whether the solute was aliphatic (0·82 $\pm$ 0·01) or aromatic (0·77 $\pm$ 0·01). This must be due to a (not unexpected) strong specific interaction between the t-butyl alcohol and the aromatic solutes causing a downfield shift of the butyl peak by 0·05 p.p.m. It was shown that this was the correct explanation by measuring the spectra of trimethyl phosphate and quinol in solutions containing all three standards together and using an external standard of tetramethylsilane. The positions of the dioxan and acetonitrile peaks in both solutions and of the t-butyl alcohol peak in the trimethyl phosphate solution were all identical with the positions in plain aqueous solution, but in the quinol solution the t-butyl alcohol peak was lowered by 0·05 p.p.m. The results of these experiments are shown schematically below.

Dioxan		MeCN	Bu^tOH		$SiMe_4$
	1·68		0·82	1·68	external
	1·68		0·77	1·73	

Separation of peaks (in p.p.m.)

Above: in the absence of aromatic compounds. Below: with about 10% of aromatic solute.

Thus both dioxan and acetonitrile may be adequate reference standards in aqueous solution but while t-butyl alcohol may be satisfactory for use with aliphatic solutes, caution must be exercised if it is to be used with aromatic ones.

We now require effective τ values for use with the three standards in dilute aqueous solution in order to correlate results obtained by using our secondary standards with those obtained in other solvents by using tetramethylsilane. These cannot be obtained directly; tetramethylsilane cannot be used as an internal standard in water and the use of an external standard is too inaccurate. Nor is it satisfactory to use a value obtained directly in some other solvent because of the different interactions of the standard with the different solvents.

Again an empirical approach was used. We measured the same six aliphatic compounds as before, using the three standards, but this time in chloroform solution. Again the difference between the value for a peak measured relative to dioxan and that measured relative to acetonitrile was 1·68 $\pm$ 0·01 but this time the acetonitrile–t-butyl alcohol difference had fallen to 0·73 $\pm$ 0·01 because of strong interaction between the alcohol and chloroform. By using an internal standard of tetramethylsilane we obtained τ values for dilute chloroform solutions of dioxan, acetonitrile, and t-butyl alcohol of 6·32, 8·00, and 8·73, respectively.

Chemical shift in chloroform less chemical shift in water.

Compound and group measured		Chemical shifts measured relative to: Dioxan	MeCN	Bu^tOH
Me acetate	CH_3O	−0·04	—	+0·06
	$CH_3{\cdot}CO$	−0·02	—	+0·07
Me methanesulphonate	CH_3O	+0·01	+0·03	+0·08
	$CH_3{\cdot}SO$	+0·10	+0·12	+0·18
Me_3 phosphate	CH_3O	−0·01	−0·01	+0·04
Methylal	CH_3O	−0·03	−0·03	+0·05
Triethylamine	CH_3	−0·07	−0·08	−0·01
	CH_2	−0·09	−0·11	−0·02
Dimethylformamide	HCO	−0·13	−0·14	−0·02
	NMe_2 (mean)	−0·04	−0·04	+0·03

We then compared the spectra obtained in the two different solvents (see Table). The spread of differences is fairly large. If aromatic solutes are used the shifts become larger still; for the α-H, β-H, and CH_3 groups of 4-picoline, with dioxan as the standard, the corresponding values are −0·31, −0·10, and −0·17 p.p.m. These large differences are due to the interactions of the solutes with the solvents. It might be supposed that the interactions of chloroform with triethylamine and with the formyl-hydrogen of dimethylformamide would be fairly large and it is not unreasonable that the interaction with the methanesulphonyl group should also be considerable. The shifts relative to acetonitrile and dioxan for the other groups average about −0·02. Thus, bearing in mind the magnitude of the errors which might be involved, we may correct the τ values for dioxan and acetonitrile obtained in chloroform to values to be used in water by subtracting 0·02, giving 6·30 and 7·98, respectively. In aqueous solutions which do not contain aromatic solutes the t-butyl alcohol peak is found at 0·82 p.p.m. above the acetonitrile peak so that its τ value will be 8·80. Under the conditions used in our experiments its τ value in the presence of aromatic solutes may be about 8·75.

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