# CAUTION CONCERNING THE USE OF SODIUM 2,2-DIMETHYL-2-SILAPENTANE-5-SULFONATE (DSS) AS A REFERENCE FOR PROTON NMR CHEMICAL SHIFT STUDIES

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## 1. Introduction

Sodium 2,2-dimethyl-2-silapentane-5-sulfonate, $(CH_3)_3Si(CH_2)_3SO_3^-Na^+$, or DSS has been used extensively as the internal reference compound for proton chemical shift measurements in aqueous solutions [1–5]. The proton chemical shifts are measured and reported with respect to the singlet methyl resonance. Some problems associated with the choice of reference compounds for NMR chemical shift studies have been discussed previously [6–12], but the results presented in this Letter indicate that DSS is not a suitable reference compound for proton chemical shift studies in aqueous solutions involving aromatic solute molecules. The experiments are carried out as a function of the concentration of the aromatic solute adenosine 5′-triphosphate (ATP) in $D_2O$ (fig.1) which stacks in aqueous solutions. This is an important experimental fact since, for example, DSS has been employed as an internal reference in a study of the chemical shifts of the purine protons as a function of the purine concentration [13], and consequently the results appear to indicate that there is no evidence for purine base stacking.

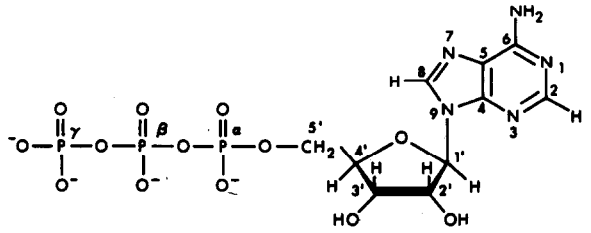

Fig.1. Structure of ATP.

## 2. Materials and methods

The NMR proton chemical shift measurements were carried out at 27°C in the Fourier transform mode (100.0 MHz) using the Varian HA-100-15 NMR spectrometer interfaced with the Digilab FTS/NMR-3 Fourier transform system. The deuterium resonance of the solvent $D_2O$ was used for the lock signal and the shift measurements are accurate to ± 0.005 ppm. The ATP was purified by passing it through a Dowex-50 cation exchange resin and the samples were prepared in $D_2O$ (p*D* of 8.4). The concentration of each of the three internal references, DSS, *t*-butanol and *p*-dioxane in all samples was 2 mM. All samples also contained 2% EDTA (as a mole fraction of the ATP concentration). In all samples, the chemical shift of the *p*-dioxane resonance with respect to the *t*-butanol resonance remained constant and therefore these data are not plotted in fig.2. In addition, experiments were carried out on samples prepared without EDTA and on samples in which the [Reference]/[ATP] ratio was constant (2%) and the results are in agreement with the data in fig.2.

## 3. Results and discussion

In the course of studying the stacking interactions of ATP, we observed that the behavior of the H8, H2 and H1′ proton chemical shifts with respect to the

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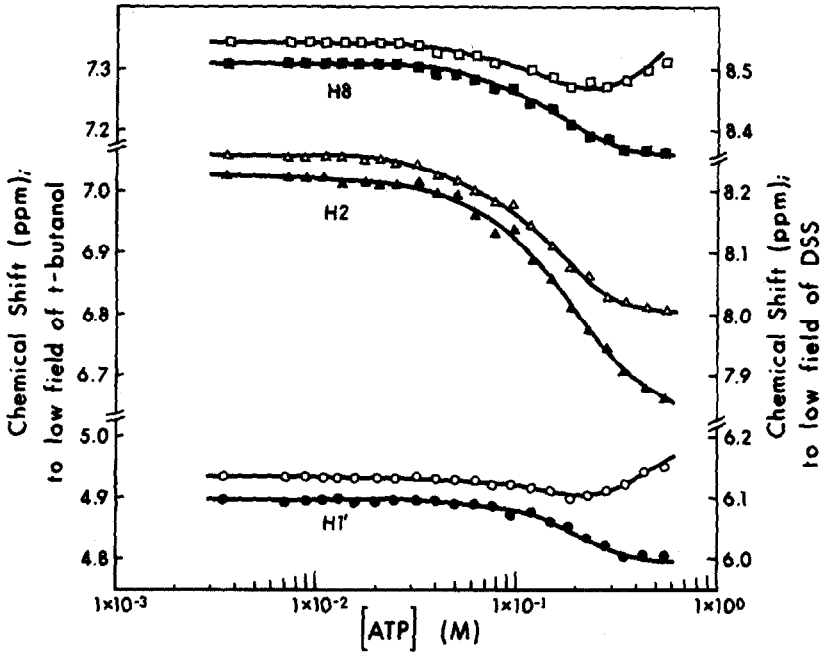


Fig.2. A plot of the proton chemical shifts of ATP as a function of the ATP concentration. The data represented with open symbols (right-hand side scale) were measured with respect to the DSS methyl resonance whereas the data represented by the solid symbols (left-hand side scale) were measured with respect to the *t*-butanol resonance.

DSS methyl resonance was different from that observed when *t*-butanol or *p*-dioxane are used as reference compounds. The proton chemical shifts were measured as a function of the ATP concentration with respect to the three internal references. From the results shown in fig.2, it can be seen that when *t*-butanol is the reference, the three protons, H8, H2 and H1′ experience a shift to high field as the ATP concentration increases, due to ring current shielding effects associated with the stacking of the ATP molecules [14,15]. However, when the proton chemical shifts are measured with respect to the DSS methyl resonance, an anomalous behavior is observed. For example, proton H8 first shifts to high field and then back again to low field as the ATP concentration increases. This has previously also been observed for purine [13]. The third internal reference in these experiments was *p*-dioxane and the *p*-dioxane resonance showed no change with respect to the *t*-butanol resonance for all the samples in fig.2.

These results indicate that the DSS methyl resonance signal is not stable and shifts as the concentration of the ATP molecules is varied. The discrepancies begin to arise at ATP concentrations of 0.07 M and higher. This is very likely due to aromatic ring current effects on the trimethylsilyl group of DSS [6,16].

The methyl resonance of *t*-butanol is influences by aromatic solutes to a smaller extent [9,10]. Consequently, proton resonances calibrated with respect to this reference signal will not be accurate. It has previously been observed [11] that with very high concentrations of aromatic solutes (0.8 M), hydrophobic interactions affect the methyl resonance of DSS. In addition, recent studies [10] on Zn (11) complexes of aromatic ligands forming ternary complexes with DSS have been reported which again indicate hydrophobic interactions and that DSS should also be used with caution in the presence of complexes of labile metal ions.

**Acknowledgements**

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