

## Note

# A $^1\text{H}$ -NMR thermometer suitable for cryoprobes

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**Most established NMR thermometers rely on temperature-dependent chemical shift differences measured from samples that are either neat or concentrated solutions (e.g. ethylene glycol, methanol). These are unsuitable for modern cryoprobes on account of strong radiation damping resulting from the high Q of the probe. Using perdeuterated methanol, we established a relationship between the chemical shift difference and temperature, and we show that this relationship is well fitted by a quadratic equation. The actual temperature within a sample tube in the probe was verified using a Pt-100 resistor. Copyright © 2006 John Wiley & Sons, Ltd.**

**KEYWORDS:** NMR; temperature; cryoprobe; shift thermometer; Pt-100 resistor; methanol- $d_4$

## INTRODUCTION

In many NMR measurements one has to know the sample temperature exactly, e.g. during dynamic NMR investigations. Therefore it is convenient to have an NMR sample that has a known temperature-dependent chemical shift, or shift difference, so that the temperature can be determined ('NMR thermometer').<sup>1</sup> Such a thermometer measures the actual temperature of the active sample region. In contrast, the temperature displayed on the spectrometer's temperature control unit shows the temperature recorded at another place in the probe (in most cases at the bottom of the sample insert, below the sample tube), which is seldom the actual temperature. In the past, some standard samples have been used as NMR thermometers and are often delivered by the spectrometer manufacturers, e.g. neat ethylene glycol and neat methanol for  $^1\text{H}$ -NMR measurements<sup>2</sup> or a mixture of deuterated and undeuterated solvents.<sup>3–5</sup> It is the strong temperature dependence of OH protons due to intermolecular hydrogen bonding that makes such samples useful as NMR thermometers.

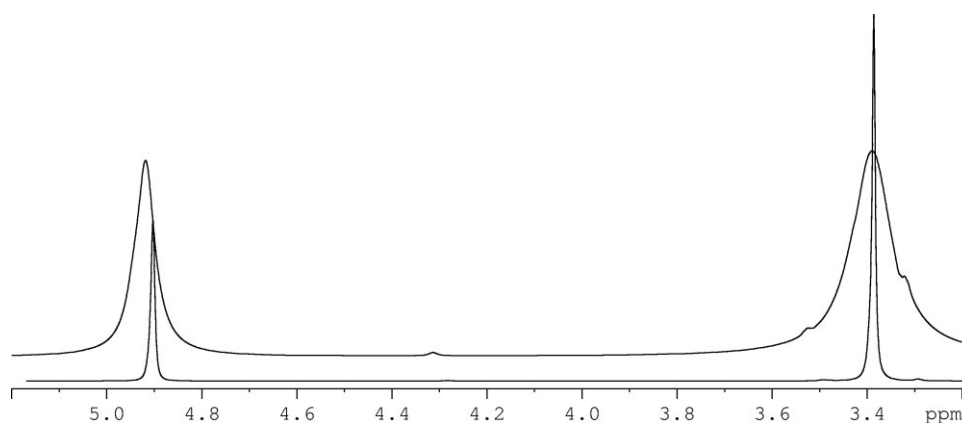
These commonly used NMR thermometers give rise to problems if they are used in a modern cryoprobe. The commonly used sample (4% methanol in methanol- $d_4$  (sample No. WGH-131); Wilmad Labglass, Buena, New Jersey, USA) of 4% protonated methanol in methanol- $d_4$ , which is commercially available, is calibrated in the literature only up to 300 K.<sup>3,4</sup> Furthermore, the lowered boiling point due to sealing under low pressure results in convection, and thereby to line-width and lock instabilities, for temperatures higher than approximately 320 K; this contributes to the limited temperature range over which this sample is useful.

In a cryoprobe, for temperatures higher than 300 K, the commercially available sample of 80% glycol in DMSO- $d_6$  yields unsuitable spectra. Owing to the high quality factor (Q) of such probes, radiation damping is a serious problem. Not only does it result in extremely broad lines, rendering the peak-picking uncertain, but it also has a clear influence on the chemical shift difference of the lines (Fig. 1). In our experiments we obtained, under correct tuning/matching conditions of the probe, line widths of 36 Hz (OH signal) and 69 Hz ( $\text{CH}_2$  signal) at 300 K. Even when using very small flip angles (pulse width 0.1  $\mu\text{s}$ ) the lines remained broad. If the probe was mismatched completely, the line widths were reduced to 7 and 5 Hz, respectively, but then a change in shift difference of 7.5 Hz was observed. Detailed investigations<sup>6</sup> report line shifts of up to 100 Hz depending on the matching/tuning conditions of a cryoprobe. Thus, the use of these common samples is clearly not advisable, and so we sought an alternative, which would be easy to handle within the allowed temperature range of the cryoprobe.

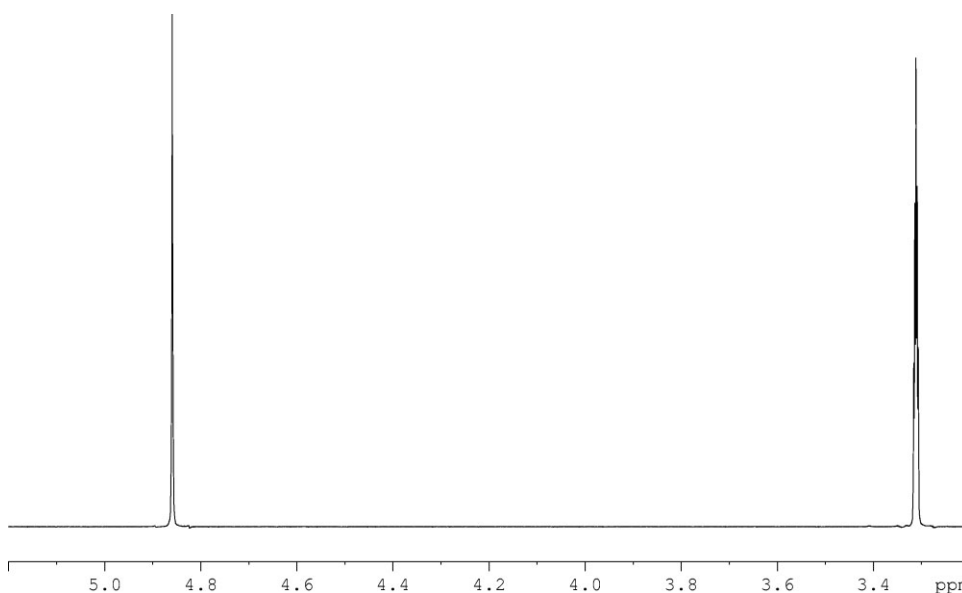
## RESULTS AND DISCUSSION

We first tried to use perdeuterated glycol, but as this is not a commonly available solvent, we switched to perdeuterated methanol, which is readily available. We used a pure methanol- $d_4$  sample and measured the chemical shift difference between the residual OH and  $\text{CHD}_2$  signals over the accessible temperature range of our cryoprobe, 282–330 K. In contrast to the commercial samples delivered by the manufacturer, the tube was not evacuated, but sealed under normal atmospheric pressure to prevent boiling at temperatures below 330 K. The methanol- $d_4$  sample can be locked and shimmed, and yields well-resolved spectra in one scan (Fig. 2).

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**Figure 1.** 700 MHz  $^1\text{H}$  NMR spectra of the commonly used ethylene glycol sample and (80% ethylene glycol in  $\text{DMSO-}d_6$  (sample No. WGH-130); Wilmad Labglass, Buena, New Jersey, USA) at 300 K under different tuning/matching conditions of the cryoprobe (detected with the wobble curve). The spectrum with broad lines was recorded with optimal tuning/matching (line width is about 69 Hz for the  $\text{CH}_2$  signal); the spectrum with the narrow lines was recorded using a mismatched probe. There is clearly a difference in line positions between the two spectra.



**Figure 2.** 700 MHz  $^1\text{H}$  NMR spectrum of neat methanol- $d_4$  at 300 K under optimal tuning/matching conditions of the cryoprobe. The line width of the OH signal is about 2 Hz.

To measure the temperature within the NMR sample we introduced a platinum resistor (Pt-100) into a normal NMR tube filled with silicon oil and placed in the probe. From the measured resistance of the Pt-100 we determined the temperature via the equation of 'DIN EN 60751' (DIN EN 60751:1995 + A2:1995; ( $R = R_0 (1 + At + Bt^2)$ ;  $A = 3.9083 \cdot 10^{-3}/^\circ\text{C}$ ;  $B = -5.775 \cdot 10^{-7}/(^\circ\text{C})^2$ ;  $R_0 = 100 \Omega$ )) and compared this with the temperature displayed by the spectrometer control unit. In our case, the latter displayed a slightly higher temperature than that showed by the Pt-100, the difference ranging from 1.9 K at 282 K to 2.9 K at 330 K.

From the measured relationship between the Pt-100 temperature and the chemical shift difference of the deuterated methanol sample (in the range between 282 K and 330 K, as indicated by the instrument), we obtained the following

quadratic fitting function:

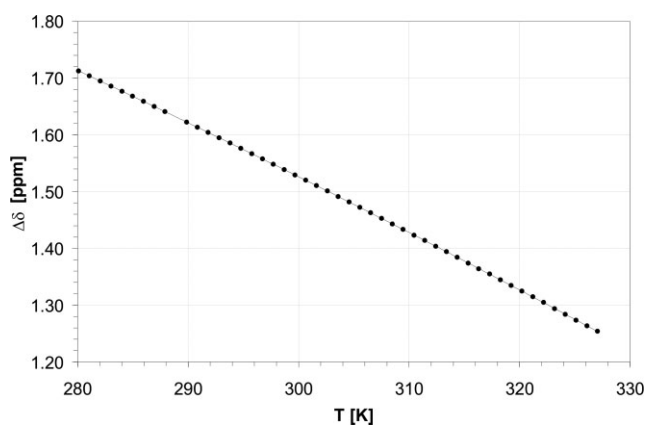
$$\Delta\delta = -1.5660 \times 10^{-5} \times T^2 - 2.6853 \times 10^{-4} \times T + 3.0161 \quad (1)$$

where  $\Delta\delta$  is the chemical shift difference (in ppm), and  $T$  is the absolute temperature of the sample in Kelvin. This equation fits the experimental results very well, yielding a correlation coefficient  $r^2 = 0.99999$  (Fig. 3).

For practical use within the NMR lab, it is more convenient to invert Eqn (1)<sup>7</sup> so as to express the temperature as a function of the shift difference. The required function may be calculated easily, but contains a square root term. However, the square root function may be expanded as a power series. A fit gave the following quadratic function:

$$T = -16.7467 \times (\Delta\delta)^2 - 52.5130 \times \Delta\delta + 419.1381 \quad (2)$$

This equation fits the data very well with a correlation coefficient  $r^2 = 0.99999$ .



**Figure 3.** Graph showing the fit between the temperature (in K), measured by the Pt-100 resistor, and the chemical shift difference (in ppm) for the methanol- $d_4$  sample; see Eqn (1). Note that the indicated instrument temperature is deviating (see text).

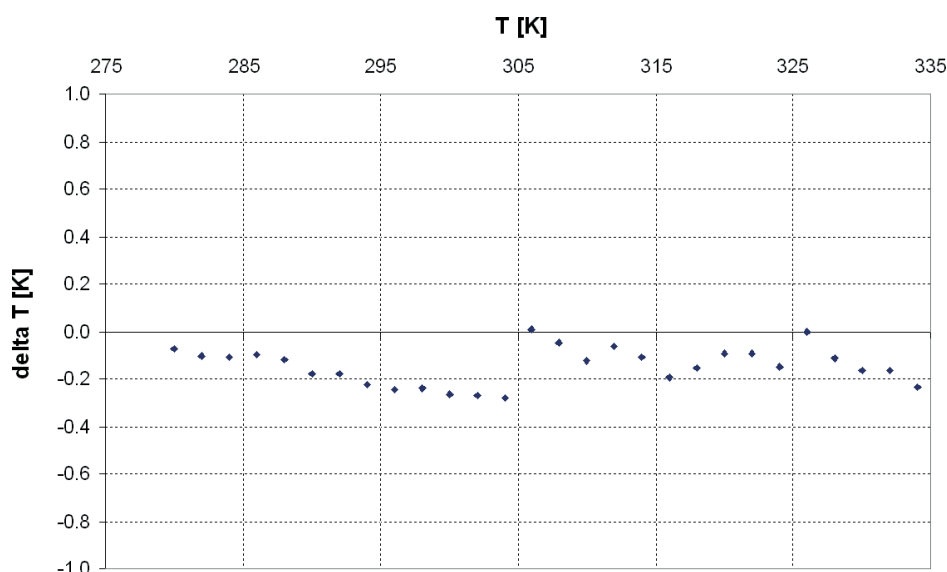
For comparison, we correlated the Pt-100 temperatures to the chemical shift difference of the commercial methanol sample, which was measured in the range from 282 K to 320 K (spectrometer display temperature). We obtained a correlation similar to Eqn (1), but with coefficients of  $-7.5550 \times 10^{-6}$ ,  $-4.9215 \times 10^{-3}$  and 3.6491 for the quadratic, linear and absolute term, respectively. This results in a deviation between the chemical shift differences of both samples in the range 0.020–0.035 ppm.

Of course, the behaviour of the hydrogen bonds, and therefore of the chemical shift difference in the NMR spectra, may show isotope effects, and could also depend upon the degree of deuteration and on the water content.<sup>8</sup> The overall water content of methanol- $d_4$  is low (i.e. comprising  $H_2O$ , HDO and  $D_2O$ ); the latter is not visible in the  $^1H$  NMR spectrum, but may be present owing to the deuterium

enrichment process). It is checked by the manufacturer by means of Karl–Fischer titration and specified by the supplier to be less than 0.025%. Since during the sample preparation some moisture may accidentally be absorbed by the methanol- $d_4$ , we prepared a second methanol- $d_4$  sample under similar conditions from a bottle of methanol- $d_4$ , which had been opened some months earlier (with the same order number but from another batch). Not surprisingly, the OH signal in the  $^1H$  NMR spectrum shows a higher intensity (4.3-fold). The quadratic fit between the Pt-100 temperature and the chemical shift difference (as in Eqn (1)) yields quite similar parameters:  $-1.5243 \times 10^{-5}$ ,  $-5.1576 \times 10^{-4}$  and 3.0528 for the quadratic, linear and absolute term, respectively. This results in shift deviations of less than  $5 \times 10^{-4}$  ppm (corresponding to 0.35 Hz at 700 MHz), which corresponds to a very small temperature difference of less than 0.06 K. Hence, our proposed temperature determination procedure is robust in respect of small increases in moisture content.

To prove the validity of our procedure, we repeated it on another spectrometer with a conventional (non-cryo) probe. We correlated the temperature, determined with our methanol- $d_4$  sample via Eqn (1), to the temperature measured by the Pt-100 temperature with this probe and obtained good agreement. The differences between both temperatures were always less than 0.3 K (Fig. 4). The temperature determined using the NMR thermometer is slightly lower than by the Pt-100. This may be due to a reversed temperature gradient when compared to the cryoprobe.

Our experimental errors can be estimated in the following way. We used a Pt-100 resistor with DIN class '1/3 B' accuracy, which is defined to have a resistance deviation of smaller than  $\pm 0.04\%$  (at  $0^\circ C$ ), corresponding to  $\Delta R_0 = 0.04 \Omega$  in Eqn (3). Our own resistance measurements are estimated to be better than  $0.01 \Omega$ . To estimate the temperature error, one can reduce the quadratic equation in DIN EN



**Figure 4.** Results of the control experiment on a conventional probe head. The figure shows the difference  $\Delta T$  between the temperatures, determined using the suggested methanol- $d_4$  sample from Eqn (1), and measured directly with a Pt-100 resistor in the probe. On the abscissa the destination temperature  $T$  from the spectrometer's control unit is displayed. The difference of the Pt-100 temperature and the temperature derived from  $\Delta\delta$  via Eqn (1) shows a root mean square of 0.17 K.

60751:1995 + A2:1995 to a linear one (at 30 °C the quadratic term is  $5 \times 10^{-4}$  vs  $1 \times 10^{-1}$  of the linear term). Using this approach, the estimation of the largest error (Gauss error propagation) derived from the equation in DIN EN 60751:1995 + A2:1995 is:

$$\Delta T = (AR_0)^{-1} \times \Delta R + R \times (AR_0^2)^{-1} \times \Delta R_0 \quad (3)$$

At 30 °C, the first part of Eqn (3) yields an error contribution of 0.03 °C and the second term 0.12 °C. Accordingly, our temperature measurement should be better than  $\pm 0.15$  °C. Other experimental errors, such as heat dissipation due to heat conduction of the Pt-100 wires or self heating of the Pt-100 resistor, are of course not covered by the equation in DIN EN 60751:1995 + A2:1995. However, we expect these to play only a minor role here, as the maximum temperature difference between the small filament wires and the environment is only 30 K, the Pt-100 has a short time constant of 140 ms and carries a low current of 1 mA and the silicon oil forms a large heat reservoir.

We regard this accuracy as sufficient, because within the NMR tube there is an additional temperature gradient depending on the experimental conditions (e.g. probe design, temperature, gas flow, convection within the sample). We placed the 10-mm long Pt-100 resistor in the centre of the receiver coil to estimate a 'mean sample temperature'. On placing it at the top or bottom of the sample (filling height 5 cm), we found a difference of the Pt-100 temperatures between these positions of 0.2 K (at 282 K) and 0.5 K (at 330 K), with the sample being cooler at the top. The reason for this is that the cryoprobe itself is cold (owing to the cooling by helium gas), but the sample is warmed up by the heating gas, which comes from the bottom of the sample tube. An investigation of temperature gradients in NMR probes is beyond the scope of this paper, but this phenomenon is covered elsewhere.<sup>9</sup> The presence of this temperature gradient should be kept in mind when speaking about 'the temperature' of the sample.

The advantage of our proposed methanol-*d*<sub>4</sub> sample is its applicability over a wider temperature range, since boiling occurs at higher temperatures owing to the atmospheric pressure within the tube. Thus, the whole temperature range of our cryoprobe is covered with only one sample. In addition, the sample is easy to prepare.

## EXPERIMENTAL

### Sample

Methanol-*d*<sub>4</sub> (99.8 at.%, ALDRICH No. 343803-5G), sealed under atmospheric pressure, and NMR tube WILMAD 507-PP with a filling height 5 cm were used in the experiments.

### Spectrometer

The instruments used were a BRUKER AVANCE-700 (with BRUKER BVT-3000 temperature controller and BRUKER

cooling unit BCU, gas flow 670 l/h) with a 5-mm TCI cryoprobe (usable between 276 and 330 K) and a BRUKER AVANCE-600 (with BRUKER BVT-3000 temperature controller and BRUKER cooling unit BCU, gas flow 670 l/h) with a 5-mm TBI probe.

### Spectral conditions

The spectral conditions were as follows: 1 scan; 6.92 s acquisition time; 6.76 ppm spectral width; 128 K processed data points (0.04 Hz/pt). Equilibration time: after temperature change of 1 K steps and after stabilization of displayed temperature at BVT-3000: greater than 6 min for the Pt-100 resistor (checked by the stability of the displayed voltage), and greater than 10 min for the methanol sample. This is in our opinion a good compromise for 1 K steps between practical requirements and the long waiting times used by others.<sup>7</sup>

### Pt-100 ceramic resistor

The resistor used was a Model S1000552 (Telemeter Electronic GmbH, Donauwörth, Germany), length 10.2 mm, diameter 1.5 mm with DIN '1/3 class B' accuracy. Measurement of the voltage drop was done at a constant current of 1 mA with a 4-wire configuration.

### Constant current source

This was a Model '120 Current Source', Lake Shore Cryotronic, Inc., Westerville, Ohio.

### Digital voltmeter

The voltmeter used was a Model 'Multimeter 2000', Keithly Instruments, Inc., Cleveland, Ohio.

### Silicon oil

For heating baths, silicon oil from Sigma-Aldrich No. 85409, CAS 63148-62-9 was used.

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