Imaging Concentration and Temperature in Cryopreservation Solutions from -80C to Room Temperature

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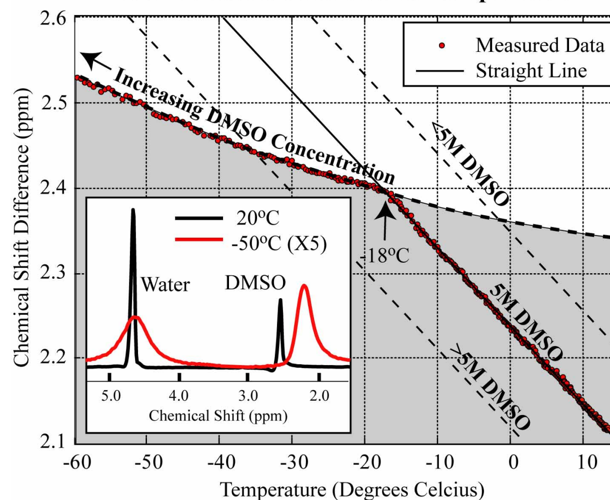
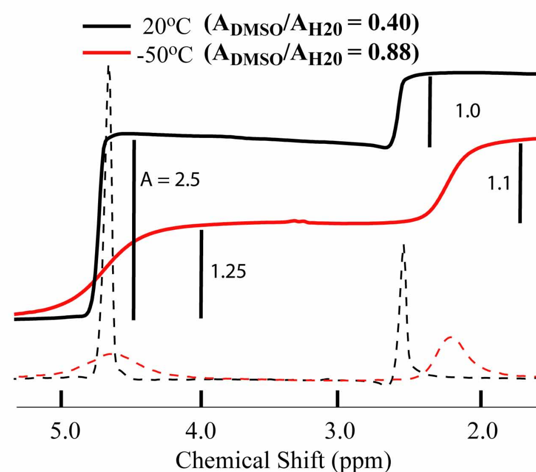
Introduction: Cryopreservation is the process whereby cells or whole tissues are preserved by cooling to low sub-zero temperatures, typically -80°C or colder. Cryoprotective compounds are commonly used for preservation to promote vitrification, the formation of *tissue-friendly* glass-like solids, as opposed to *tissue-damaging* crystalline ice. Low concentrations of cryoprotectant are typically not sufficient to maintain cell viability during freezing, and very high concentrations are toxic, although higher concentrations are tolerated as temperature is reduced. As a result, the design of successful cryopreservation protocols, that maintain cell viability, require control and knowledge of temperature and cryoprotectant concentration throughout cooling (time), and throughout the sample (space). We investigate the use of magnetic resonance spectroscopic imaging for the simultaneous measurement of concentration and temperature. Spectroscopy offers direct evaluation the relative spin density of water and cryopreservative molecules (concentration), and the chemical shift difference between their resonances is examined as an absolute temperature probe.

Methods: Cryopreservatives solutions of 5M dimethyl sulfoxide (DMSO) were pre-cooled to -80°C (1cm diameter test tube). An NMR-compatible platinum resistance thermometer used for recording temperature was frozen in center of the sample. The samples were warmed to room temperature inside a 1.5T Siemens Sonata MRI system over 3 hours and imaged continuously. An echo-planar spectroscopic imaging pulse sequence, designed in house, was used for all experiments. A spin-echo line-scan variation of the pulse sequence was used to allow spectra to be acquired from a line, in a single shot. The line was oriented radially through the vertical-standing tube to allow the tube cross-section to be imaged. Parameters: 300 Hz spectroscopic bandwidth, 2 mm slice thickness, 1 mm spatial resolution, TR = 8 seconds. TE is varied from 8 ms to 200 ms in 16 steps for T_2 measurement, to correct for T_2 signal losses in calculation of DMSO concentration. A 3 cm diameter surface coil was used for signal reception. An automated spectral fitting routine was used to quantify the chemical shift, line width, T_2 and relative spin-density of the water and DMSO peaks. Pixels from the center of the tube were analyzed to match the radial location of the thermometer.

Results: The upper figure shows the variation in water-DMSO chemical shift difference as a function of temperature, with a clear linear relationship from room temperature to -18°C (the known freezing point of 5M DMSO). Sample spectra are shown in the inset. A similar trend in water/DMSO solutions has previously been observed[1]. The deviation from linearity at -18°C most likely reflects the increasing DMSO concentration as water freezes. Our interpretation of this phenomenon is a shift in the linear curve that relates chemical shift difference and temperature, determined by the change in concentration (sample dashed line is shown in Figure). The increased DMSO concentration further reduces the freezing point so that all of the water does not freeze at a single temperature. The lower figure shows that the DMSO spin density *decreases* slightly with warming from -50°C to room temperature, reflecting the reduction in thermal equilibrium magnetization at higher temperatures. The water spin density, on the other hand, increases considerably over the same temperature range as it transitions from solid to liquid phase. The water T_2 increased from 20 ms at -60°C to 200 ms at room temperature, and the DMSO T_2 increased from 40 ms to 240 ms over the same temperature range. (Note that water in the solid phase is not NMR visible for the relatively long TE values used in this study).





The upper panel shows the chemical shift difference between water and DMSO peaks as a function of temperature (red points). The inflection at -18°C is the freezing point of water in 5M DMSO. Sample spectra are shown in the inset at -50° and 20° C. The relative peak areas (spin densities) at these two temperatures are shown in the lower panel (actual peak areas are calculated with spectral fitting).

Conclusion: Spectroscopic imaging of cryoprotectant solutions provides a host of parameters (chemical shifts, line width and visible spin density) in a fast experiment with high spatial resolution. These results confirm the potential for non-invasive temperature mapping and solvent tracking for temperatures as low as -60°C. The presence of DMSO clearly maintains NMR-visibility of water for sub-zero temperatures (i.e. sufficiently long T_2* to resolve the water and DMSO peaks). This approach offers the potential to measure absolute temperatures using chemical shift differences with high spatial resolution and without need for reference information. The equation that describes the observed relationship between chemical shift difference, temperature and concentration is $\Delta\delta = k_1 T + F_1(C_{DMSO})$, where k_1 would be a constant that is specific to the water DMSO system and F_1 would be a function only of the DMSO concentration, C_{DMSO}. This technique will resolve important limitations in current cryobiology research.

References: [1] Tokuhiro T, Menafra L and Szmant HH. Contribution of Relaxation and Chemical-Shift Results to Elucidation of Structure of Water-DMSO Liquid-System. *Journal of Chemical Physics* **61**, 2275-2282 (1974).