The Agilent Salt Tolerant Cold Probe

Technical Overview

Introduction

Advantage Statement

The Agilent Salt Tolerant Cold Probe provides the highest sensitivity of any available probe when working with lossy samples such as proteins in aqueous solutions containing high concentrations of buffer or salt. The Agilent Salt Tolerant Cold Probe has shown sensitivity improvements of nearly 75% in triple resonance experiments on protein samples when compared to conventional cryogenically cooled or room temperature probes.

The sensitivity advantage of cold probes resulting from cooling of the receiver coil and it's associated circuitry to cryogenic temperatures is well established. Several hundred cryogenic probes are currently in use for a wide variety of research involving both large and small molecules. It is also well documented that the sensitivity gain associated with cold probes is greatly diminished in lossy samples, for example, samples containing high concentrations of salt or buffer or in solvents having a large dielectric constant [1].

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In such lossy samples such as buffered proteins or urine, the sample strongly couples to the observe coil in the NMR probe. This has two consequences. First, an increase in 90 degree pulse lengths and potential sample heating, since much of the RF power is absorbed by the creation of electric fields (E-Field) in the sample, and second, a loss of sensitivity as the sample itself becomes an additional noise source that is strongly coupled to the observe coil. This is much more an issue with cold probes than room temperature probes.

This interaction between the RF-field created by the observe coil and the sample depends not only on the nature of the sample, for example, its lossiness, but also on the sample's size and geometry. A smaller tube diameter would decrease these losses by the fourth power of the tube radius. However the use of smaller sample tubes also results in reduced sample in the probe to contribute to the NMR signals of interest.

The Agilent Salt Tolerant Cold Probe is the best solution to the reduction in sensitivity associated with lossy samples. This novel product of innovation at Agilent uses a unique sample geometry to maximize the absolute sensitivity for samples such as proteins that are typically available at low concentrations and cannot be concentrated further for use with smaller tubes.

The key to understanding the principle behind the Agilent Salt Tolerant Cold Probe is to realize that not all parts of the traditional cylindrical sample geometry contribute equally to the losses described above. In fact, certain regions of the sample are in E-field hot spots while other regions of the sample experience a minimal E-Field and this region of the sample has minimal effect on E-Field related losses [2] as depicted in Figure 1a.

The innovative sample geometry and RF technology employed in the Agilent Salt Tolerant Cold probe maximizes sensitivity by effectively redistributing the sample into regions of low E-Field thereby minimizing its interaction with areas of the strongest E-Field as shown in Figure 1b.

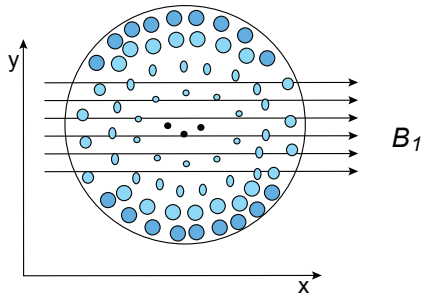

Figure 1a. Traditional cylindrical tube geometry showing E-Field hot spots as the larger blue circles.

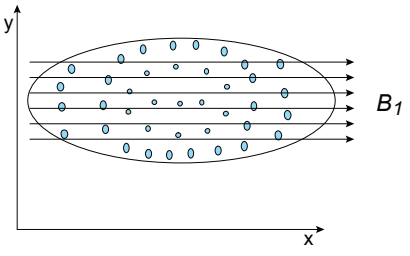

Figure 1b. 3 × 6 S-Tube geometry that effectively concentrates the sample into regions of minimal E-Field.

The geometry of 3 × 6 S-Tube in the Agilent Salt Tolerant Cold Probe thus enables a significant improvement in sensitivity for lossy samples wherein salt concentration might range from 50 mM NaCl to as high as 1 M when compared to the use of either conventional Cold Probes or traditional room temperature probes.

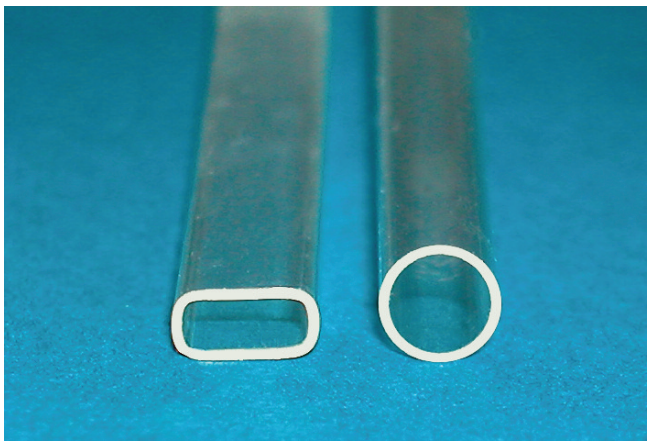

Figure 2. A 3 × 6 S-tube on the left compared to a traditional 5 mm cylindrical NMR tube on the right. A susceptibility matched version of the S-tube is also available from Shigemi. Both S-tube and 5 mm geometries can be used with the Agilent Salt Tolerant Cold Probe.

The use of the Agilent Salt Tolerant Cold Probe is not limited to the 3 × 6 S-tube or a susceptibility-matched Shigemi version of the S-Tube, but does in fact accommodate the traditional 5 mm and 3 mm cylindrical NMR tubes as well. Furthermore, results from the use of a 5 mm tube in the Salt Tolerant Cold Probe are uncompromised when compared to results from a 5 mm tube in a regular cold probe. The Agilent Salt Tolerant Cold Probe thus offers the best possible performance and versatility with both lossy and non-lossy samples.

The powerful performance and versatility of the Agilent Salt Tolerant Cold Probe is demonstrated in Figure 3 where a sensitivity advantage of ~35% is measured for a sample of high salt concentration in the 3 × 6 S-tube in spite of the fact that it contains ~23% less sample than the traditional 5 mm tube by virtue of reduced cross-sectional area in the active region of the coil.

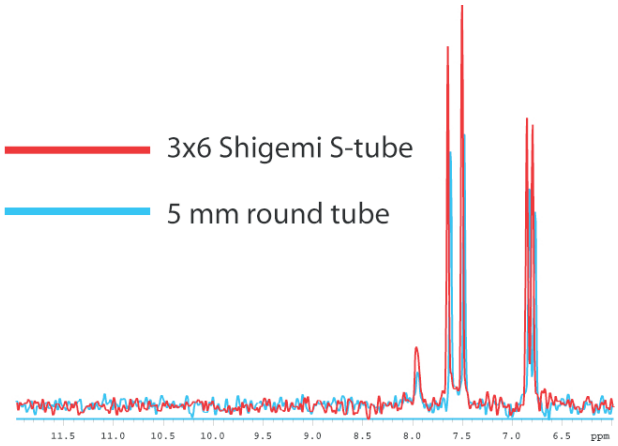

Figure 3. Results comparing traces at 113.7 ppm from ^{15}N-HSQC spectra of a protein sample acquired at 600MHz in a traditional 5 mm cylindrical tube (blue), and a 3 × 6 S-tube (red). The sample contains 300 mM NaCl in 90% H_2O.

Significant gains in sensitivity are also realized in more complex experiments on proteins as can be seen in Figure 4 which compares signal-to-noise from several triple resonance experiments on a protein sample containing 450 mM NaCl. Gains of up to 75% in sensitivity can be achieved using the unique 3 × 6 S-tube over both warm and Cold Probes using traditional 5 mm cylindrical tubes. This data also indicates that traditional Cold Probes do not deliver significant advantage over room temperature probes at the extremely high salt concentrations shown in Figure 4. On the other hand, the unique geometry of the Salt Tolerant Cold Probe offers a significant sensitivity advantage for such lossy samples resulting in remarkable improvements in the quality of the NMR data and/or time savings of more than a factor of two.

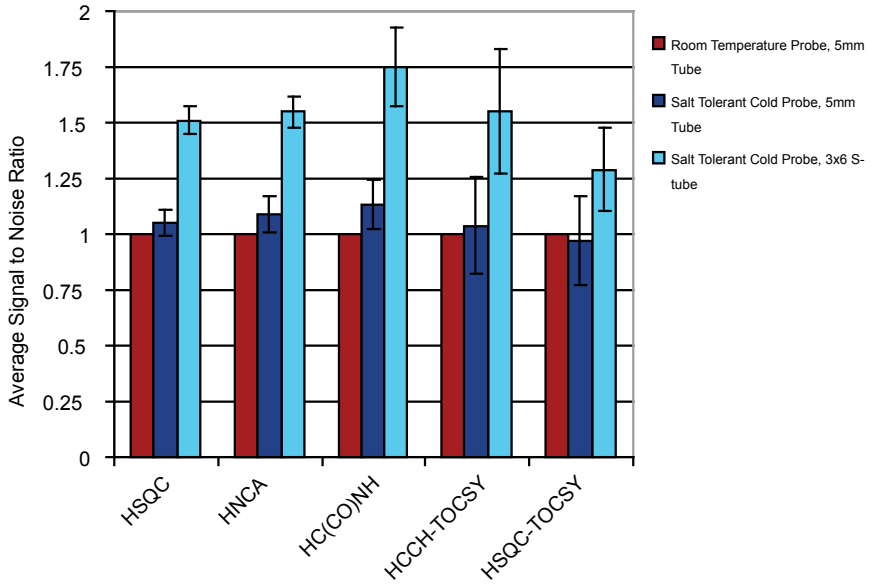

Figure 4. Signal-to-noise of triple resonance experiments on a sample containing {^{15}N, ^{13}C}-labeled GB1 protein in 20 mM Tris buffer and 450 mM NaCl, pH 7.2, in 90% H_2O using an Agilent Salt Tolerant Cold Probe with 5 mm cylindrical and 3 × 6 S-Tube are compared to the signal-to-noise of the same experiments conducted in a Room Temperature Probe. Data courtesy of the Wagner Lab, Harvard Medical School.

When working with protein samples of high salt concentrations, or biofluids such as urine, the Agilent Salt Tolerant Cold Probe offers the highest sensitivity and versatility, making it the probe of choice for such demanding applications in Structural Biology and Metabolomics.

References

1. A. E. Kelly, H. D. Ou, R. Withers and V. Dötsch, "Low-Conductivity Buffers for High-Sensitivity NMR Measurements", J. Am. Chem. Soc. 124, (2002), 12013-12019.
2. T. de Swiet, "Optimal electric fields for different sample shapes in high resolution NMR spectroscopy", J. Magn. Reson. 174, (2005) 331-334

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