

Technical Information

Microsampling in NMR Spectroscopy

No single challenge in NMR has persisted as long as microsampling, in part because NMR is inherently insensitive compared with many other analytical techniques. When an NMR method is developed that works with a variety of samples, NMR spectrometers improve and make yet smaller samples accessible, renewing the need for new and better techniques. Although every method available today has some disadvantages, the vast majority of microsamples will be amenable to at least one of a number of accessible approaches. The cost of the method you choose will vary, from the price of a sample tube to that of a new probe. The method you choose for microsampling will be a compromise between performance and budgetary considerations. So that you'll be better prepared to make such choices, this report discusses many of the sampling methods that can be employed with tiny samples. It concludes with a table that illustrates the use of some of the sampling devices available to meet microsampling requirements.

Standalone Microsample Tubes

A 5mm thin-wall NMR tube holds about 140 μ l in every centimeter of sample height. When your sample size is smaller than 675 μ l, it won't fill the Rf coils of most 5mm probes using 5mm thin wall tubes and the resolution you get will decline. Microsample tubes are the simplest and most cost-effective approach to NMR studies of tiny samples. The first microsample tubes were simple heavy wall Pyrex NMR Tubes, such as WILMAD's 522-PP, which are still available. When the heavy wall tube encloses a spherical or cylindrical microcavity (WILMAD Product Numbers 508-CP or 510-CP, respectively), more of your sample is held in the Rf coil, i.e. you get better filling factor. But the shim systems of high resolution 5mm probes used in superconducting spectrometers are designed to work best with thin wall tubes, not these special '-CP' microsample tubes.

Shimming may be challenging using these tubes in superconducting spectrometers. We recommend their use be restricted to low field spectrometers, where the orientation of the magnetic field and shim systems makes resolution easier to maximize. However, a variation of these tubes is being used in a new microprobe from Varian. When used with this new probe, the tube provides exceptional results with microsamples (see below).

NMR Tube Inserts and Microsamples

NMR Tube Inserts are used for 1) External Reference or Locking and 2) comparing the properties, such as magnetic susceptibility, of two solutions (see Resonance Report NMR-007 for details about these applications). But they also provide a convenient way to study small samples. For example, WILMAD's WGS-5BL, which holds 60 μ l, is used to confine a sample within a smaller volume in the critical lower 50mm of a 5mm tube. You can decrease susceptibility discontinuities by adding solvent to the outer tube, too.

Beware of any difference in the deuterium lock signal inside and outside the insert, though.

This problem occurs when the spectrometer records two superimposed but slightly juxtaposed spectra, one inside and one outside the insert. The spectrometer lock jumps between the two lock signals and your spectra will show peaks that have flat tops. A more flexible insert system is WILMAD's 529 system, which works with standard thin wall NMR tubes, too. The system centers around a PTFE Holder, which holds 1.5mm capillary tubes. Three varieties of inserts are made for use with this holder; a simple 1.5mm OD, 8 μ l/cm capillary . . . a 1.5mm capillary with an 18 μ l spherical bulb on the end . . . and a 1.5mm capillary with a 110 μ l cylindrical bulb on the end. Switching sample volumes and shapes is fast. The microbulbs must be positioned properly in the tube so they're centered in the Rf coils of the probe. If you don't know where the center of the Rf coil is, use water in the spherical bulb to locate it. Just position and shim, reposition and reshim. You'll find resolution, signal intensity, and line shape are maximized when the bulb is in the center. In microsample studies, you can put solvent in the outer tube, too, but beware the lock signal pitfalls outlined above for the WGS-XBL system.

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Other Microsampling Approaches

In 1996, Doty introduced commercially 'Susceptibility Plugs,' used to confine a sample, one plug above and one below, into a small volume in a 3,5, and 8mm sample tubes. Plugs have been prepared to match the Magnetic Susceptibility of many of the most common NMR solvents for microsamples, including D₂O, Chloroform-d, Methanol, and DMSO-d₆. Because of the susceptibility match, you can 'trick' the magnetic field into thinking the sample fills the bottom 50mm of the 5mm sample tube. See WILMAD's Resonance Report NMR-002 for more details about the Doty Susceptibility Plugs.

Nalorac Corporation produces a series of Microsample Probes which provide dramatic improvements in spectrometer throughput of tiny samples. Available in Inverse and Direct Detection or Triple Resonance modes, you use special sample tubes prepared by WILMAD with these Z-Spec probes. The tubes consist of a high quality 5mm tube which tapers to a 3mm OD stem in the lower 50mm. Each tube, WILMAD's 520-1B, holds 230µl of sample, but only 140µl is needed. A version of the tube, 520-1C, is made with an ultra-thin wall stem that holds 160 - 265µl. Using the probe cuts at least 50% from the analysis time of a microsample when compared to analysis in a standard 5mm probe and tube. Bruker has offered a Microprobe in the past that uses the same type of tapered 5mm microsample tube, but is just 2.5mm OD. Today, Bruker, Varian and Nalorac offer flow-through probes, specifically designed for LC-NMR, which can be used with microsamples that must be 'cleaned up' prior to NMR. Minimum sample requirements are listed as 30µg, but 60µg is more commonly used in these probes. Varian has a probe they call a Nano-nmr Probe, which uses a special NMR sample tube made by WILMAD, but available exclusively from Varian. This tube, similar to a shortened 508-CP, provides a 50µl cavity in a heavy wall 4mm OD sample tube, used non-spinning. Samples as small as 400ng have been analyzed with this probe and it takes just a few minutes to analyze routine samples in the 25 - 50µg range (depending on the sample molecular weight). Contact the manufacturers of these special microsampling probes for information about their products.

Product Number	Shape	Volume	Used With
510-CP	Spherical	25µl	Iron Core Magnet NMR Systems
508-CP	Cylindrical	139µl	Iron Core Magnet NMR Systems
WGS-5BL	Cylindrical	60µl	60-400MHz
529-A + 5mm Tube	Spherical	18µl	60-300MHz
529-D + 5mm tube	Capillar	8µl/cm	60-300MHz
529-E + 5mm tube	Spherical	110µl	60-300MHz
520-1	Cylindrical	190µl	Bruker Microprobe
327-PP	Cylindrical	140 - 230µl	Nalorac Z-Spec Microprobe

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