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Enhanced NMR Resolution by Restricting the Effective Sample Volume

Since the very first experiments on the high-resolution NMR of liquid samples there has been widespread interest in the quest for enhanced resolving power to bring out the fine structure due to weak spin–spin interactions and to approach the natural width of the NMR lines. The limiting factors are well known—spatial inhomogeneity of the static field B_0 and instability of B_0 measured with respect to the excitation frequency—but since the pioneering work of Arnold (*1*) and Anderson (*2*) technological improvements in this area have been relatively slow. There has been a steady improvement in field stability, particularly for modern superconducting spectrometers, and pulse excitation reduces the sensitivity to low-frequency instabilities (*3*). In addition there are special techniques such as double irradiation (*4*) and spin-echo experiments (*5–8*) which can circumvent some of the problems of field inhomogeneity.

Enhanced resolution may also be achieved "artificially" by data manipulation after the acquisition of the NMR signal, either by a convolution operation on the frequency-domain spectrum (*9, 10*) or by a weighting function which prolongs the decay of the transient time-domain signal (*10, 11*). Many different prescriptions are in vogue (*12–16*).

A simple and more direct method is to use a very small NMR sample. The resulting loss of sensitivity is seldom a problem for proton NMR, and for less sensitive nuclei it can usually be retrieved by time averaging. Unfortunately this is not quite as easy as it sounds. Suppose that the sample is enclosed within a very small glass container; resolution is significantly degraded because of the field distortions due to discontinuities in magnetic susceptibility between sample, glass, and the surrounding medium. Experiments which employ retaining plugs or small capillary tubes do not in practice achieve the expected resolution.

This communication proposes an alternative method of restricting the *effective* sample volume without the need for the equivalent physical confinement; spinning sample tubes are used with the normal dimensions (5 mm o.d.). The idea is to excite part of the sample by selective irradiation in an imposed field gradient, a concept often used in spin-mapping experiments or zeugmatography (*17*). The field gradient is strong in comparison with the overall width of the multiplet under investigation, and also in comparison with the residual "natural" gradients of the high-resolution magnet. Ideally several gradients might be used, chosen to match the dominant natural gradients, but in practice a single applied gradient is quite effective, since a linear Z gradient, for example, minimizes the extent of excitation in the Z dimension, and automatically takes care of higher-order natural Z gradients. Frequency selectivity is achieved either by means of a "soft" radiofrequency pulse (*18, 19*) or by a regular sequence of very short strong pulses designed to have a cumulative effect in

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only a very narrow frequency band (*20, 21*). Selective irradiation in an applied field gradient limits the physical extent of the sample which is excited; sudden removal of the gradient then permits spins in the chosen region to precess in a particularly uniform magnetic field.

It has been demonstrated that a frequency selectivity of the order of 0.1 Hz can be achieved by a suitable pulse sequence, by using it to burn a hole in an inhomogeneously broadened line (*21*). For the present purpose far less stringent selectivity is acceptable since the excitation is performed in a strong field gradient.

The proposed technique has been tested on a high-resolution spectrometer constructed at the Delft University of Technology (*22*) which utilizes a Varian 300-MHz superconducting solenoid. Selective excitation was achieved with a regular sequence of short intense pulses (*20, 21*), typically 20 pulses of flip angle 3° each (2 μsec), spaced 2.5 msec apart. The applied gradient was a linear Z gradient (the spinning axis and the axis of the solenoid), and was switched under computer control. The subsequent free precession signal was sampled for 16.6 sec (16K data points) and extended with 16K of zero filling, no weighting function being used.

The experimental sample was furan-2-aldehyde (*23*) dissolved in deuterobenzene to give a 0.02 *M* solution. The field was stabilized through the deuterium reference signal. The ring proton at lowest field shows three proton couplings, an eight-line pattern. This is poorly resolved under conventional operating conditions, the instrumental linewidth being about 0.6 Hz (Fig. 1a). For traces b, c, and d the sample was excited selectively in increasingly strong Z gradients, the free precession signals being acquired in the absence of field gradients, and the resolution is clearly improved each time. This enhancement of resolution is achieved at the expense of reduced signal-to-noise ratio as the effective sample volume is restricted. It seems probable that the limiting resolution may be determined by the self-diffusion of the sample during the relatively long acquisition period, increasing the effective volume of the sample. Note that this method should improve resolution even in magnets which are already extremely homogeneous.

This method naturally invites comparison with the widely used artificial method of resolution enhancement, the weighting of the experimental free precession signal to prolong the time constant of the decay. Enhancement of the intensity in the tail of this signal corresponds to emphasizing signal components in relatively homogeneous magnetic fields. In this sense the weighting operation can be thought of as restricting the effective volume of the sample. However, the practical limits of this kind of resolution enhancement are quickly reached if there are noise spikes or distortions of the lineshape, because the weighting process exaggerates such artifacts.

Selective excitation in an imposed gradient restricts the effective sample volume in a more direct way and does not have the same practical limitations as the data-processing method. In principle it should be feasible to carry out a spatial search for the region of optimum homogeneity, either by varying the frequency of the selective excitation in a given gradient or by changing the combination of imposed gradients. In some liquid systems, this resolution enhancement technique could provide a measure of the natural linewidth; this would be particularly useful for individual lines in strongly coupled spin systems. The main limitation of the method appears to be the inevitable loss of sensitivity associated with small samples, but if the imposed

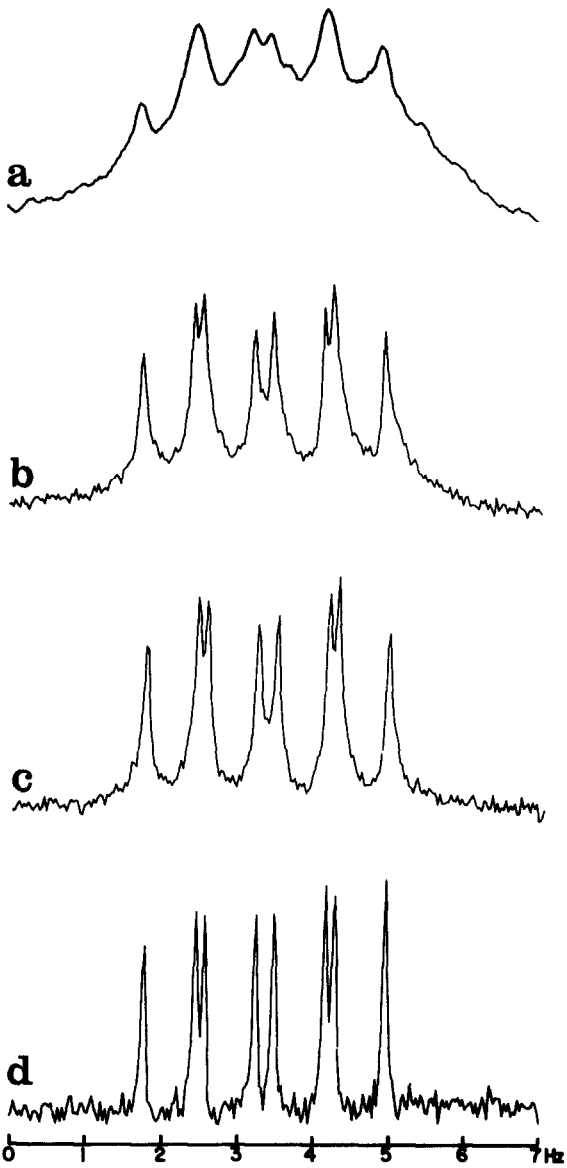

FIG. 1. The low-field ring proton resonance of 0.02 *M* furan-2-aldehyde in deuterobenzene at 300 MHz. Trace (a) was obtained under normal high-resolution conditions, whereas (b), (c), and (d) were obtained after selective excitation in increasingly strong Z gradients which were removed before data acquisition. Note the improvement in instrumental linewidth from approximately 0.6 Hz in (a) to approximately 0.08 Hz in (d), accompanied by a corresponding loss of sensitivity due to the smaller effective sample volume.

gradients are reproducible and the irradiation frequency stable, this loss can be retrieved by time averaging.

Diffusion of nuclei in magnetic field gradients can be a serious limitation on several NMR measurements. For example, spin-echo experiments which use only a single refocusing pulse are particularly sensitive to diffusion; these techniques are being increasingly used in two-dimensional Fourier transform spectroscopy for studying spin–spin coupling (*24*). In the measurement of spin–lattice relaxation times, the diffusion of the inverted spins out of the transmitter coil before the 90° sampling pulse constitutes a source of error for long relaxation times. Diffusion is also a complicating factor in multiple-quantum spectroscopy where "coherence transfer echoes" are employed (*25*). In each of these applications the effects of diffusion may be minimized by ensuring that the preparation stage is restricted to a very small sample volume.

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