



Fig. 1 A simple convection-compensated spin echo pulse sequence in which each gradient pulse has a duration δ and a strength G , the total diffusion time is represented by Δ , and the delay imbalance by $\Delta\Delta$.

pathways, to encode spatial position, or to enable diffusion measurement, are those most likely to exhibit anomalies due to convection. When a sample convects, drastic effects on the time evolution of nuclear magnetization during the pulse sequence can sometimes be observed, resulting *e.g.* in spectra containing signals with incorrect phases and amplitudes.^{1,2,5} Signal loss due to convection is unwelcome in any NMR experiment, given the inherently low sensitivity of NMR spectroscopy, but is a particular problem in experiments for the measurement of diffusion. Convection leads to overestimation of the diffusion coefficient, since the coherent convective motion and the random diffusion both contribute to the signal attenuation observed.^{2,3} Even small values of convection velocity (0.05 mm s^{-1}) can lead to significant errors in measured diffusion coefficient values.¹

Various strategies may be used to minimize the effects of convection on NMR experiments. As already noted, choice of solvent is critical; at room temperature, chloroform convects 20 times more readily than D_2O .¹ Sample geometry is also critical; convection in a standard 3 mm OD NMR tube is more than an order of magnitude slower than in a standard 5 mm OD tube.¹ In many experiments the effects of mild convection can be refocused using convection-compensated pulse sequences (it should be noted that in many circumstances it is preferable to suppress convection by using a smaller diameter tube rather than to use a compensated pulse sequence, since the latter normally sacrifices half of the available signal by including a second stimulated echo).^{1,2,6–13} Thermal gradients in probes will also be affected by external sources of heat flow such as shim coils or decoupling, and by the use of air in conventional probes, and water in high gradient probes, to control the probe body (as opposed to sample) temperature. Temperature gradients can if necessary be measured directly, rather than inferred from measurements of convection, by using sensors *in situ*¹ or by imaging the chemical shift of a species with a very temperature-sensitive resonance.³

Convection-compensated pulse sequences^{12,13} for diffusion measurement can be adapted to measure the rate of convection in a sample; here this approach is used to compare convection behavior across a range of instrumental configurations. The convection-compensated diffusion measurement pulse sequence of Fig. 1 with $\Delta\Delta = 0$ divides the diffusion weighting component of the sequence into two equal halves separated by a 180° pulse, thus refocusing the effects of constant-velocity flow while retaining those of diffusion. If the durations of the two halves of the sequence are deliberately unbalanced to an extent $\Delta\Delta$, measurements of the extra signal attenuation as a function of $\Delta\Delta$ allow the form and width of the sample z velocity spectrum to be determined.¹ Experimental signal attenuation data as a function of $\Delta\Delta$ are typically well approximated by a sinc function, corresponding to a rectangular velocity spectrum, so fitting to a sinc function is a simple and effective way to estimate the magnitude of the peak z convection velocity in an NMR sample.

Experimental section

Materials and spectrometers

Measurements of convection velocity were performed using a sample of 5% v/v CHCl_3 in CDCl_3 , doped with 0.2 mg ml^{-1} chromium(III)acetylacetonate ($\text{Cr}(\text{acac})_3$) to reduce the longitudinal relaxation time T_1 and hence allow faster experiments. As summarized in Table 1, experiments were performed using five different NMR spectrometers (Bruker AVANCE III 400, 500 and Ascend 500, 600 and 800, where the figures denote the frequencies for ^1H in MHz).

The 400 MHz spectrometer was equipped with a BBI probe (double resonance broadband probe with inner coil optimized for ^1H observation), and for 500 MHz spectrometers a BBO probe (double resonance broadband probe with the inner coil tunable over the frequency range from ^{31}P to ^{15}N) and a CPPBBO BB probe (“Prodigy” nitrogen-cooled BBO) were used.

For the 600 MHz instrument two different probes were evaluated, TBO (triple resonance broad band probe with the outer coil tuned for ^1H observation or decoupling) and TBI (triple resonance broad band probe with the inner coil tuned for ^1H observation or decoupling), while the 800 MHz spectrometer was equipped with CPTCI probe (proton-optimized triple resonance NMR (TCI) inverse “cryoprobe”/“cold probe” with helium cooling). Three different NMR tube geometries were studied, standard thin-walled 5 mm and 3 mm OD NMR tubes and a standard 5 mm OD NMR tube with a 2 mm OD insert.

Table 1 Spectrometers, probes, gas flow rates, and NMR tube geometries used to acquire convection data

Spectrometer	Probes	Gas flow rates (l h^{-1})	NMR tube geometries
Bruker AVANCE III 400 MHz	BBI S1	670	5 mm; 5 mm with insert; 3 mm
Bruker AVANCE III 500 MHz	BBO S2	400	5 mm; 5 mm with insert; 3 mm
Bruker Ascend AVANCE III 600 MHz	TBI S3	400	5 mm; 5 mm with insert; 3 mm
	TBO S3	400	5 mm; 3 mm
Bruker Ascend 500 MHz	CPPBBO BB	400	5 mm; 3 mm
Bruker Ascend AVANCE III 800 MHz	CPTCI	400	5 mm; 3 mm



NMR experiments

The pulse sequence code used to acquire the convection experiments is given in the ESI.† In each experiment a set of 25 different imbalance delays (Δt) (see Table S1 in ESI†) was used for each temperature, and 36 temperatures were evaluated, from 5 to 40 °C in steps of 1 °C. The duration used for the gradient recovery delay was 0.5 ms; the diffusion encoding gradient pulse duration (δ) was 2 ms, and the gradient strength

used was 80% of the nominal maximum value for each spectrometer (Table S2†).

At each temperature a stabilization delay of 15 min was allowed after the nominal temperature was registered by the temperature controller unit, in order to ensure that the sample was fully equilibrated with its surroundings in the probe. Experimental data were processed in TOPSPIN 3.1 and MATLAB, using a set of AU programs and MATLAB macros (see Fig. S2†).



Fig. 2 Convection velocity versus temperature, from 5 °C to 40 °C, for spectrometers of four different frequencies (400, 500, 600 and 800 MHz), with six different probes (BBI, BBO, CPPBBO, TBI, TBO and CPTCI), and three different NMR tube geometries (5 mm – blue; 5 mm with 2 mm OD insert – green; and 3 mm – red). Each graph includes a tenfold vertical expansion. Graphs (a) to (f) illustrate the data obtained with a 5 mm NMR tube; graphs (g) to (i) a 5 mm NMR tube with 2 mm OD insert; and (j) to (p) with a 3 mm NMR tube. Full experimental data can be downloaded from DOI: 10.15127/1.301247.



Results and discussion

The experimental signal amplitudes were measured using TOPSPIN, and subjected to nonlinear least squares fitting to the sinc function of eqn (1)²

$$F(\Delta\Delta) = S_0 \sin(\gamma\delta G A_G v_{\max} \Delta\Delta) / (\gamma\delta G A_G v_{\max} \Delta\Delta) \quad (1)$$

where S_0 is the maximum signal amplitude, γ is the magnetogyric ratio, G is the gradient amplitude in G cm^{-1} , A_G is the gradient pulse shape factor ($2/\pi$ for half-sine pulses), v_{\max} is the maximum convection velocity sought and $\Delta\Delta$ is the delay imbalance. Fig. 2 shows plots of maximum convection velocity as a function of nominal sample temperature for each combination of spectrometer, probe and NMR tube geometry.

The first thing that is immediately apparent from the graphs in Fig. 2 is that all combinations of spectrometer and probe using 5 mm NMR tubes, with or without the 2 mm OD insert, show convection both above and below T_Q (Fig. 2a–i). In a previous investigation by Swan *et al.*¹ of a range of older instruments, all of the systems studied showed a similar pattern of variation of convection velocity with temperature, a “V” shaped curve approximately centred on the quiescent temperature T_Q , at which the convection velocity was very low. In the more modern instruments investigated here this was not always the case, with some quite different behaviours (Fig. 2b–d, f and i) observed.

The second obvious conclusion is that even in systems showing florid convection in 5 mm tubes, very little convection was observed in 3 mm samples under the experimental conditions used. This reflects both the effect of the confined sample geometry, reducing the rate of convection caused by a given temperature gradient, and the improved freedom of circulation of air around the sample, reducing the magnitudes of the sample temperature gradients. It should however be noted that convection velocities several orders of magnitude smaller than those measured here can be measured if minor changes to the experimental method are made. The most important of these is to use a reporter molecule with a low diffusion coefficient, since diffusional attenuation competes with the signal attenuation caused by convection.

The highest convection velocities recorded (approximately 4 mm s^{-1}) were for the 5 mm tube, in the room temperature BBI S1 probe at 400 MHz (Fig. 2a), at the top end of the temperature range studied; conversely, close to its T_Q of about 21°C this combination showed some of the slowest convection measured for a 5 mm tube. As expected, the nitrogen-cooled (“Prodigy”) and helium-cooled cryoprobes (Fig. 2c and f) showed severe convection in 5 mm tubes over the full range of nominal temperatures studied. No minimum was seen in the curves of v_{\max} versus T , since T_Q in such probes is, as noted earlier, typically below 0°C . Even these probes, however, showed v_{\max} less than 0.1 mm s^{-1} in the 3 mm NMR tube. In ref. 1, all of the probe/spectrometer combinations tested showed a clear minimum in v_{\max} at some temperature. It is not surprising that such a minimum is not seen for the cold probes of Fig. 2c and f studied here, but it is unexpected that some of the room

temperature probes (Fig. 2b and d) failed to show any deep minimum in v_{\max} over the temperature range studied.

Conclusions

The main point of this article is to illustrate the surprisingly wide range of convection behaviours likely to be encountered in modern NMR spectrometers, and in so doing to underline the rather gloomy conclusions of ref. 1. Not only is convection always with us, but the marked differences in behaviour between different instrument configurations, even those with nominal similarities, mean that there is no substitute for direct experimental investigation if convection is a potential problem. The materials presented here and in the ESI† provide the basis for making and interpreting the necessary measurements, and also add to the evidence base for evaluating the relative susceptibility to convection of different probe designs and configurations.

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