JOURNAL OF MAGNETIC RESONANCE **132,** 13–18 (1998)
ARTICLE NO. MN981400

Convection Compensation in Gradient Enhanced Nuclear Magnetic Resonance Spectroscopy

Alexej Jerschow[1] and Norbert Müller[2]

Institute of Chemistry, Johannes Kepler University, A-4040 Linz, Austria

Received August 25, 1997; revised February 5, 1998

Compensation of convection artifacts in gradient-enhanced nuclear magnetic resonance spectroscopy is introduced. Natural convection caused by small temperature gradients over the sample volume can lead to significant loss of magnetization in gradient-enhanced nuclear magnetic resonance spectroscopy, sometimes to its complete extinction or partial inversion. Even when the effect is small, it still hampers a quantitative interpretation of spectra, e.g., nuclear Overhauser effect buildup curves. By using modified pulse and gradient sequences it is possible to avoid the interference of convection, which is demonstrated by way of two examples: GOESY and GROESY. The principle of convection compensation is applicable to a wide variety of gradient-enhanced nuclear magnetic resonance experiments, in particular those where the interval between a defocusing and a refocusing pulsed field gradient is relatively long.

Key Words: **pulsed field gradients; convection; GOESY; GROESY; diffusion.**

INTRODUCTION

The use of pulsed field gradients (PFGs) is well established for selection of coherence transfer pathways in NMR (*1*). It bears several advantages over the selection by phase cycles (*2*) such as the suppression of unwanted contributions (*in situ*), before the signal enters the receiver, thus preventing subtraction errors. One disadvantage, however, is that translatory molecular motion can attenuate the desired coherence transfer pathway. Therefore, most gradient sequences are arranged such that the refocusing gradients are as close as possible to the dephasing gradients. In gradient-enhanced high-resolution NMR spectroscopy it has usually been assumed that the only motional process causing signal attenuation is self-diffusion. Recently, however, we have shown that significant convection currents can arise in samples in state-of-the-art NMR spectrometers (*3*) at elevated temperatures, which, under unfavorable conditions, can cause signal attenuation and modulation orders of magnitude stronger than the effect of self-diffusion. In this article we present guidelines for the design of gradient sequences to avoid or compensate convection artifacts in gradient-enhanced high-resolution NMR spectroscopy.

A gradient echo is obtained in an arbitrary pulse sequence if the zeroth moment $\mathbf{m}_0$ of the effective gradient $\mathbf{G}^\star(t)$ vanishes (*4*):

$$\mathbf{m}_0 = \int \mathbf{G}^\star(t)dt = \mathbf{0}. \quad [1]$$

The effective gradient may be written as

$$\mathbf{G}^\star(t) = p(t) \cdot \mathbf{G}(t), \quad [2]$$

with $p(t)$ being the (time-dependent) coherence order. Due to the vector character of $\mathbf{G}^\star(t)$ and $\mathbf{G}(t)$ the echo condition must be achieved for all directions in space. For simplicity, and without loss of generality, we proceed with the corresponding scalar function $G(t)$.

When we have a number of discrete rectangular gradient pulses G_i in the pulse sequence, Eq. [1] can be written as

$$\sum_i p_i G_i \delta_i = 0, \quad [3]$$

which, with δ_i being the gradient durations, is the well-known echo condition for the gradient selection (*1*). When using a gradient shape other than rectangular we can assume that the integral of the shape is taken care of in G_i. The extension of Eq. [3] to heteronuclear systems is straightforward by the introduction of the composite coherence order (*5*).

Not all of the solutions $(G_1, G_2, \ldots, G_n)$ to Eq. [3] are equally good. Many must be discarded because undesired coherence pathways $(p_1, p_2, \ldots, p_n)$ will also solve this equation. This fact has been extensively discussed in (*5*). Here, we would like to emphasize that signal attenuation due to self-diffusion is another condition by which the number of solutions $(G_1, G_2, \ldots, G_n)$ can be restricted. Therefore, it

[1] Present address: Université de Lausanne, Section de Chimie, CH-1015, Lausanne-Dorigny, Switzerland.
[2] To whom correspondence should be addressed.

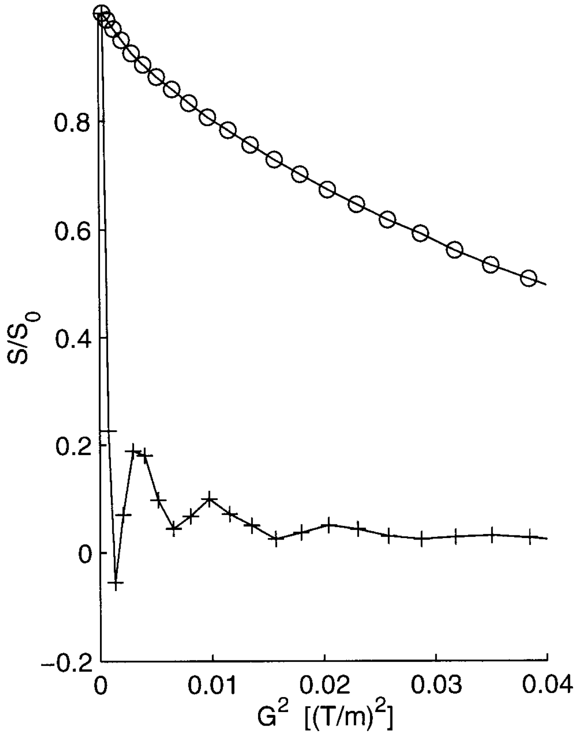

FIG. 1. Echo signal attenuation as a function of G^2 for (+) a simple Stejskal–Tanner diffusion experiment (*25*) on a sample of 0.06% polypropylene (M_w = 261,000; M_w/M_n = 16) in tetrachloroethane-d$_2$ at 320 K, Δ = 150 ms, δ = 3.4 ms, gradient recovery delay = 1 ms, employing z-gradients, and (○) using a convection compensated DSTE experiment (*3*).

is commonly suggested to choose solutions where pairs of defocusing/refocusing gradients are placed closely together. Quantitative estimations of the effect of self-diffusion are given in (*1*) and it can be seen that the restrictions on the intergradient delays are not too severe for medium-sized molecules.

To our knowledge convection artifacts have not been addressed in the context of spectroscopy, but have been known to interfere with NMR self-diffusion measurements (*3, 6*). With this publication we wish to increase awareness of the problem and to present a way to alleviate it. As we have found previously (*3*), relatively strong convection currents can exist along the vertical axis of samples in NMR probes at elevated temperatures. They are caused by inevitable temperature gradients. As an illustration of the effect we show the NMR gradient echo signal as a function of gradient strength in presence of convection and with compensation of convection (*3*) (thus decaying due to diffusion only) in Fig. 1. It is readily seen how troublesome the effects of convection can be.

The relative effects of convection and diffusion depend in a complex way on a number of parameters, such as sample viscosity, temperature, molecular shape and size, sample geometry, probe design, and filling height, which makes predictions unreliable.

Inevitably, these artifacts will decrease the amount of refocused magnetization in gradient echoes. In some cases full extinction or even partial inversion is possible. We present here a simplified model for the explanation of these artifacts. The temperature gradients cause the outer layers of the liquid to move upward and the inner ones to move downward in a laminar flow (the opposite case is also possible in cooled samples).

Translatory motion causes a phase shift of the signal (*4*)

$$\Phi(z) = \gamma \int G^{\star}(t) z(t) dt$$
$$= \gamma\left(z_0 m_0 + \underbrace{\frac{dz}{dt}}_{v} m_1 + \frac{1}{2}\underbrace{\frac{d^2z}{dt^2}}_{a} m_2 + \cdots \right), \quad [4]$$

where m_k is the kth moment of the effective gradient in time (taken over the duration of the experiment, T)

$$m_k = \int_0^T G^{\star}(t) t^k dt. \quad [5]$$

The necessary condition for selection of a particular coherence transfer pathway is $m_0 = 0$, which is the echo condition (Eq. [1]). m_1 at constant velocity (i.e., d^2z/dt^2 and all higher derivatives are zero) generates a constant phase shift $\Phi(v)$ depending on the velocity. Given a distribution of velocities $f(v)$ we have to integrate over the range of velocities giving a signal

$$S = \overline{\exp(-i\Phi(v))} = \int \exp(-i\Phi(v)) f(v) dv. \quad [6]$$

Assuming as a very simple case a uniform velocity distribution between $-v_{\max}$ and $+v_{\max}$,

$$f(v) = \begin{cases} \dfrac{1}{2v_{\max}} & \text{if } |v| \le v_{\max} \\ 0 & \text{if } |v| > v_{\max} \end{cases}, \quad [7]$$

we get

$$S = \frac{1}{2v_{\max}} \int_{-v_{\max}}^{v_{\max}} \exp(-i\Phi(v)) dv = \mathrm{sinc}(v_{\max} m_1), \quad [8]$$

which is a decaying oscillatory function as is also manifest in Fig. 1. It may be necessary to modify $f(v)$ for more complicated types of motion (different integration profiles can be found in (*7*) and profiles for laminar flow have been considered in (*8*)) and/or to include higher terms of the expansion in Eq. [4]. Depending on the experimental conditions (in particular the gradient strengths used) the effect of convection may not be evident as an oscillatory behavior

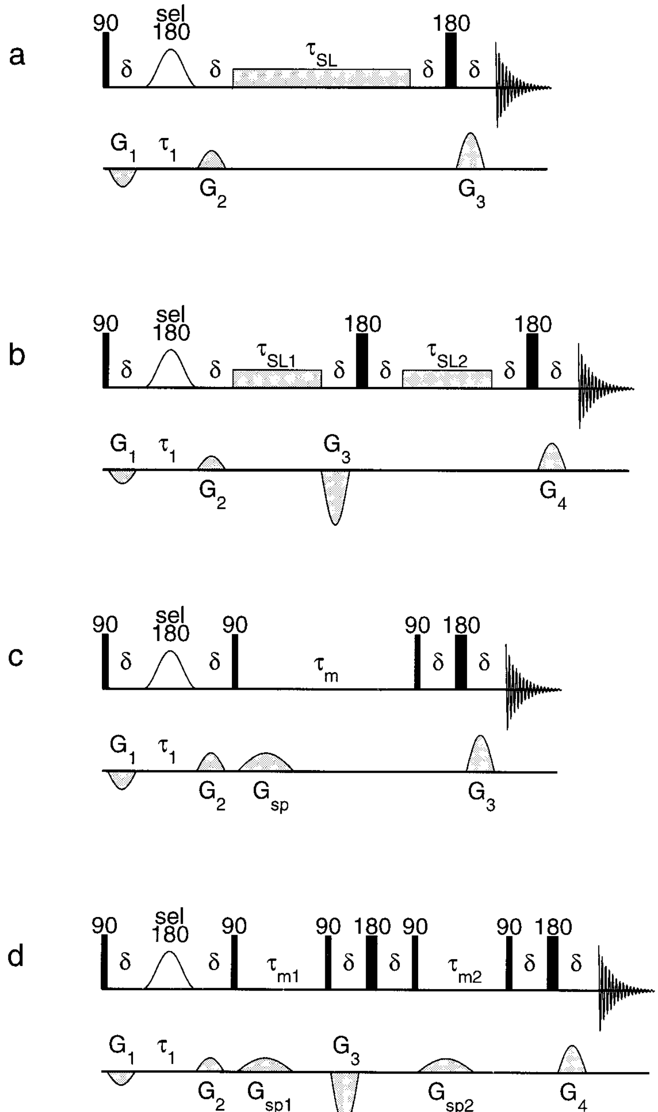

FIG. 2. Pulse sequences: (a) GROESY, $G_1{:}G_2{:}G_3 = -1{:}1{:}2$; (b) convection-compensated GROESY (ccGROESY), $\tau_{SL1} = \tau_{SL}/2 - \tau_1 - 3\delta/4$, $\tau_{SL2} = \tau_{SL} - \tau_{SL1}$, $G_1{:}G_2{:}G_3{:}G_4 = -1{:}1{:}-4{:}2$; (c) GOESY $G_1{:}G_2{:}G_3 = -1{:}1{:}2$; (d) convection-compensated GOESY (ccGOESY), $\tau_{m1} = \tau_m/2 - \tau_1 - 3\delta/4$, $\tau_{m2} = \tau_m - \tau_{m1}$, $G_1{:}G_2{:}G_3{:}G_4 = -1{:}1{:}-4{:}2$. G_{sp}, G_{sp1}, G_{sp2} are transverse, orthogonal spoiler gradients.

but only cause additional damping of the signal, thus leading to erroneous results, e.g., for cross relaxation rates derived from GOESY (*9*) spectra.

The important point for NMR spectroscopy is not so much to describe the observed convection artifacts accurately but to remove them. It is clear that if we can zero m_1, m_2, . . . we will eventually achieve independence of the signal attenuation not only from a constant velocity but also from acceleration, etc. (when the molecules diffuse to neighboring liquid layers exhibiting a significantly different velocity the effect of ''cross-talk'' also has to be considered). According to our experience with convection artifacts in high-resolution NMR instruments it suffices to zero m_1. This condition may be added to the one in Eq. [3] in order to get convection-compensated gradient-enhanced NMR spectra. A general discussion of flow effects and gradient moment nulling within the realm of imaging can be found in (*10, 11*).

RESULTS AND DISCUSSION

We demonstrate the benefits of convection compensation on two pulse sequences, where convection interference is most obvious: GROESY (*12, 13*) and GOESY (*9, 14*). Common implementations of these pulse sequences are depicted in Figs. 2a and 2c. Improved selective excitation schemes (*15*) can be used as discussed in detail recently (*14*). The arguments brought forward here are, however, largely independent of the particular excitation method used.

Since the defocusing and refocusing gradients flank the mixing time (spin lock time), which can be very long, atten-

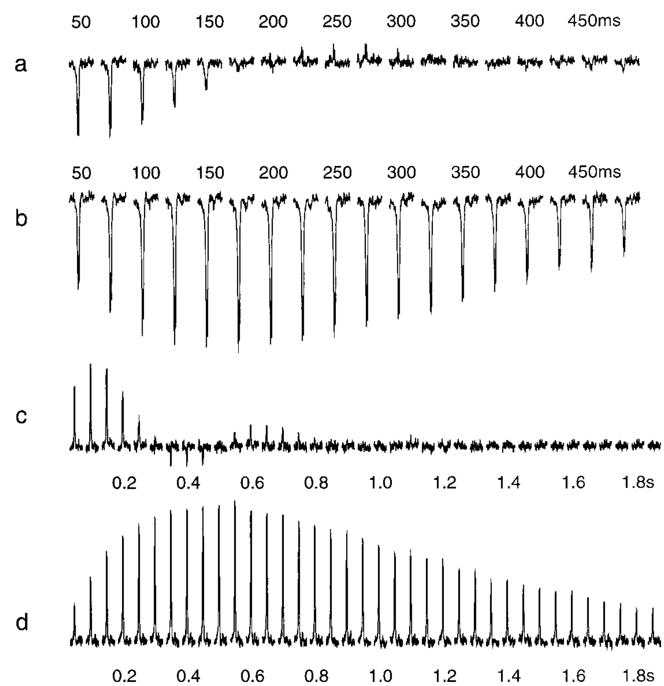

FIG. 3. Mixing time dependence of the GROESY and GOESY experiments on gramicidin S in dmso-d_6 at 320 K. The signal observed is the Leu NH signal at 8.26 ppm after selectively inverting the Orn CαH signal at 4.76 ppm (The signs of the peaks are relative to the Orn CαH signal). The gradient strengths were 0.06 T/m for the last gradient in each sequence and 0.04 T/m for the spoiler gradients, and all were sine shaped with 1.5-ms duration and 100-μs recovery delay; the selective pulse is a 60-ms REBURP (*26*). (a) Ordinary GROESY experiment (Fig. 2a). Convection currents in the sample lead to an oscillatory behavior and eventually to the extinction of the magnetization; (b) GROESY with three gradients weighted for each mixing time to achieve a zero first moment; (c) ordinary GOESY experiment (Fig. 2c)—convection artifacts are present; (d) Convection-compensated GOESY (ccGOESY, Fig. 2d) with a split mixing period.

uation of the magnetization (through self-diffusion and convection) is prominent. The signal attenuation by diffusion in GOESY and GROESY can be calculated from the Bloch–Torrey Equations (*4*):

$$S \propto \exp\left(-D(\gamma G\delta)^2\left(\frac{\tau_1}{4} + \tau_{m,SL} + 2\delta\right)\right). \quad [9]$$

Here it was assumed that the shape of the gradients can be approximated as rectangular. G is equal to G_3 in the pulse sequence (Figs. 2a and 2c). By measuring the amplitudes (S and S') at two different gradient strengths (G and G') the diffusion coefficient may be obtained as

$$D = \frac{\ln(S'/S)}{(\gamma\delta)^2(G^2 - G'^2)(\tau_1/4 + \tau_{m,SL} + 2\delta)} \quad [10]$$

and can be used for the numerical correction of the signal integrals.

In Figs. 3a and 3c the mixing time dependence of the ROE and NOE observed from the Orn CαH to the Leu NH proton in gramicidin S (*16*) is depicted. An oscillatory behavior similar to that in Fig. 1 is observed. Therefore it is clear that convection prevails in this case, which makes impossible both the numerical correction for the diffusion attenuation and the determination of the NOE buildup.

To improve the situation we can use gradient ratios which allow for simultaneous vanishing of the zeroth and the first moments. Considering that τ_1, τ_m, τ_{SL} are usually much longer than the gradient duration δ, we can assume that the gradient shapes are delta functions. In this case the conditions for the zeroth and the first moments read

$$m_0 = \delta(p_1G_1 + p_2G_2 + p_3G_3) = 0 \quad [11]$$

and

$$m_1 = \delta(p_2G_2(\tau_1 + \delta) + p_3G_3(\tau_1 + \tau_{m,SL} + 3\delta)) = 0, \quad [12]$$

from which we get

$$(p_1G_1):(p_2G_2):(p_3G_3) = \frac{\tau_{m,SL} + 2\delta}{\tau_1 + \delta} : -\frac{\tau_1 + \tau_{m,SL} + 3\delta}{\tau_1 + \delta} : 1 \quad [13]$$

and since $p_1 : p_2 : p_3 = 1 : -1 : 1$ we have

$$G_1 : G_2 : G_3 = \frac{\tau_{m,SL} + 2\delta}{\tau_1 + \delta} : \frac{\tau_1 + \tau_{m,SL} + 3\delta}{\tau_1 + \delta} : 1. \quad [14]$$

We have used this gradient ratio to obtain the spectra in Fig. 3b by the ordinary GROESY sequence (Fig. 2a). The modulation due to convection is clearly removed. The diffusion dependence is different from Eq. [9],

$$S \propto \exp\left(-D(\gamma G\delta)^2\left(\alpha_1^2\tau_1 + \tau_{m,SL} + \frac{1}{3}\left(\alpha_1^2 + \frac{1}{4}\alpha_2^2 + 4\right)\delta\right)\right) \quad [15]$$

(here, $\alpha_1 = (\tau_{m,SL} + 2\delta)/(\tau_1 + \delta)$ and $\alpha_2 = (\tau_1 + \tau_{m,SL} + 3\delta)/(\tau_1 + \delta)$, and, again $G = G_3$), but can be compensated for as above by calculating the diffusion coefficient from a second experiment at a different gradient strength:

$$D = \frac{\ln(S'/S)}{(\gamma\delta)^2(G^2 - G'^2)\,(\alpha_1^2\tau_1 + \tau_{m,SL} + (\alpha_1^2 + \alpha_2^2/4 + 4)\delta/3)}. \quad [16]$$

Since this method retains the main advantage of the original GROESY and GOESY pulse sequences of suppressing unwanted coherence transfer pathways in a single scan (with exception of the zero quantum terms), we see it as the preferred implementation of GROESY (and GOESY) as long as diffusion is slow. If diffusion losses dominate, the pulse sequences introduced by Stott *et al.* (*14*) are probably preferable, although they require two transients to cancel the unwanted pathways.

When longer mixing times are used the gradients G_1 and G_2 become very large (Eq. [14]). If this poses experimental problems we suggest a different approach which is analogous to the one taken for the suppression of convection in NMR self-diffusion experiments (*3*), namely doubling of the stimulated echo sequence and placing of an additional gradient in the center (Figs. 2b and 2d). For convenience, we start with a known effective gradient ratio

$$(p_1G_1) : (p_2G_2) : (p_3G_3) : (p_4G_4) = -1 : -1 : 4 : -2 \quad [17]$$

$$(p_1 : p_2 : p_3 : p_4 = -1 : 1 : 1 : -1) \quad [18]$$

and calculate $\tau_{m1,SL1}$ ($\tau_{m2,SL2} = \tau_{m,SL} - \tau_{m1,SL1}$). Since $m_0 = 0$ we only need to ensure

$$m_1 = G_1[-1\cdot(\tau_1 + \delta) + 4\cdot(\tau_{m1,SL1} + \tau_1 + 2\delta) - 2\cdot(\tau_m + \tau_1 + 5\delta)] = 0, \quad [19]$$

from which we have

$$\tau_{m1,SL1} = \frac{\tau_{m,SL}}{2} - \frac{\tau_1 - 3\delta}{4}. \quad [20]$$

Experimental results of a convection-compensated GOESY experiment (ccGOESY) using the sequence of Fig. 2d are displayed in Fig. 3d, where it is seen that convection is well compensated for. The diffusion dependence is

$$S \propto \exp\left(-D(\gamma G\delta)^2\left(\frac{\tau_1}{4} + \tau_{m,SL} + \frac{37\delta}{12}\right)\right), \quad [21]$$

where G is equal to G_4 in the pulse sequence (Figs. 2b and 2d).

The division of the mixing time in two and the additional pulses might introduce new problems such as coherence transfer artifacts in coupled spin systems. This we do not want to contest (at least not with all vigor). We have pointed out one possible approach to the problem. Furthermore, this approach seems appropriate for experiments where the mixing time is split in two anyway, as in QUIET-NOESY (*17, 18*), employing a BIRD pulse in the middle of τ_m.

As in the standard GOESY experiment a single scan per τ_m value is sufficient to suppress unwanted coherence transfer pathways. The sensitivity of the experiment is halved due to the restriction of the coherence transfer by the gradient pulse interrupting the mixing time (but this is well outweighed by the compensation of convection in the cases considered).

CONCLUSIONS

We have described the artifacts convection can generate in gradient enhanced NMR spectroscopy and outlined two approaches to alleviate the problems. Although velocity compensation has been known in the field of MR imaging for some time (*4, 10, 11, 19–21*) and introduced for diffusion measurements recently (*3*), it has not been given sufficient consideration in the context of spectroscopy before. Convection compensation is appropriate whenever the intervals between a pair of defocusing and refocusing PFGs are long (in our experience typically above 20 ms) and measurement conditions require heating (or cooling) of the sample. In particular if the intergradient delay is varied to measure a time dependence such as an NOE buildup, convection compensation is required for a correct quantitative data evaluation.

The first method of convection compensation (modification of the gradient ratios, if three or more gradients are used in the original sequence to generate an echo) is useful when echo delays are relatively short. This has the advantage that it does not introduce any additional pulses and thus does not bear the risk of new coherence transfer artifacts. For longer intergradient delays the second method (splitting and doubling a stimulated echo sequence) should be applied in spite of a signal loss of 50% since the first method may require too strong gradients. Also there are some experiments, like QUIET-NOESY (*17, 18*), where the mixing period is split for other reasons, so a convection compensation scheme of the second type is natural to apply there without a modification of the rf-pulse sequence. The diffusion attenuation is weaker in this case than with three gradients (compare Eqs. [15] and [21]). We emphasize, however, that numerical compensation for diffusion dependence is only meaningful if convection is absent or has been compensated for.

We have demonstrated the principle of convection compensation for GOESY (*9, 14*) and GROESY (*12, 13*) here. Analogous modifications can be applied to any gradient sequence following the procedure given. In particular, pulse sequences like gradient-enhanced NOESY, ROESY, and TOCSY (*22*) and those where an evolution period is flanked by a gradient pair (such as gradient-enhanced HMQC and HMBC (*1, 23, 24*)) will benefit from such a modification, when convection dominates. Also, in any temperature-dependent study involving experiments of the type mentioned above it is probably wise to use convection compensation as a general precaution.

EXPERIMENTAL

All acquisitions in this work were performed on a 500-MHz Bruker Avance DRX spectrometer using a high-resolution TXI probe with actively shielded triple axes gradients with Acustar II gradient amplifier.

ACKNOWLEDGMENTS

This work was supported by the Austrian ''Fonds zur Förderung der Wissenschaftlichen Forschung (FWF),'' project P 10633-ÖCH. We are grateful to one of the reviewers for eliminating a typo in Eq. [19] and pointing us to Ref. (*8*), where the sinc-type behavior of Eq. [8] appears to have been documented for the first time.

REFERENCES

1. J. Keeler, R. T. Clowes, A. L. Davis, and E. D. Laue, *in* ''Methods in Enzymology,'' Vol. 239, p. 145, Academic Press, San Diego (1994).

2. G. Bodenhausen, H. Kogler, and R. R. Ernst, *J. Magn. Reson.* **58,** 370 (1984).

3. A. Jerschow and N. Müller, *J. Magn. Reson.* **125,** 372 (1997).

4. P. T. Callaghan, ''Principles of Nuclear Magnetic Resonance Microscopy,'' Oxford Univ. Press, London, (1993).

5. L. Mitschang, H. Ponstingl, D. Grindrod, and H. Oschkinat, *J. Chem. Phys.* **102,** 3089 (1995).

6. J. Lounila, K. Oikarinen, P. Ingman, and J. Jokisaari, *J. Magn. Reson. A* **118,** 50 (1995).

7. P. B. Kingsley, *J. Magn. Reson. B* **109,** 243 (1995).

8. R. J. Hayward, K. J. Packer, and D. J. Tomlinson, *Mol. Phys.* **23,** 1083 (1972).

9. J. Stonehouse, P. Adell, J. Keeler, and A. J. Shaka, *J. Am. Chem. Soc.* **116,** 6037 (1994).

10. J. M. Pope and S. Yao, *Conc. Magn. Reson.* **5,** 281 (1993).

11. J. L. Duerk and O. P. Simonetti, *Conc. Magn. Reson.* **5,** 105 (1993).

12. P. Adell, T. Parella, F. Sánchez-Ferrando, and A. Virgili, *J. Magn. Reson. B* **108,** 77 (1995).

13. C. Dalvit and G. Bovermann, *Magn. Reson. Chem.* **33,** 156 (1995).

14. K. Stott, J. Keeler, Q. N. Van, and A. J. Shaka, *J. Magn. Reson.* **125,** 302 (1997).

15. T. L. Hwang and A. J. Shaka, *J. Magn. Reson. A* **112,** 275 (1995).

16. N. Nicolai, G. Valensin, C. Rossi, and W. A. Gibbons, *J. Am. Chem. Soc.* **104,** 1534 (1982).

17. S. Vincent, C. Zwahlen, P. H. Bolton, T. Logan, and G. Bodenhausen, *J. Am. Chem. Soc.* **118,** 3531 (1996).

18. C. Zwahlen, S. Vincent, L. Di Bari, M. H. Levitt, and G. Bodenhausen, *J. Am. Chem. Soc.* **116,** 362 (1994).

19. P. T. Callaghan and Y. Xia, *J. Magn. Reson.* **91,** 326 (1991).

20. Y. Xia and P. T. Callaghan, *Macromolecules* **24,** 4777 (1991).

21. B. Manz, J. D. Seymour, and P. T. Callaghan, *J. Magn. Reson.* **125,** 153 (1997).

22. T. Parella, F. Sánchez-Ferrando, and A. Virgili, *J. Magn. Reson.* **125,** 145 (1997).

23. R. E. Hurd and B. K. John, *J. Magn. Reson.* **91,** 648 (1991).

24. A. Bax and M. F. Summers, *J. Am. Chem. Soc.* **108,** 2093 (1986).

25. E. O. Stejskal and J. E. Tanner, *J. Chem. Phys.* **42,** 288 (1965).

26. H. Geen and R. Freeman, *J. Magn. Reson.* **93,** 93 (1991).