

Single-Shot Experiments for the Acquisition of Coherence-Transfer Functions in Real Time

Burkhard Luy and Steffen J. Glaser¹

Institut für Organische Chemie der J. W. Goethe-Universität, Marie-Curie-Str. 11, D-60439 Frankfurt, Germany

Received November 3, 1998; revised January 26, 1999

Simple pulse sequences are introduced that make it possible to acquire experimental Hartmann–Hahn transfer functions for arbitrary multiple-pulse sequences in a single shot. With this approach it is possible to study the detailed dependence of coherence-transfer functions on experimental parameters in real time. © 1999 Academic Press

Key Words: single-shot experiment; Hartmann–Hahn transfer; HEHAHA; TOCSY; coherence-transfer functions.

INTRODUCTION

Homonuclear and heteronuclear Hartmann–Hahn transfer has become one of the most important techniques for the transfer of polarization and coherence in high-resolution NMR spectroscopy (1–6). The mixing time dependence of the integrated intensities of cross peaks and diagonal peaks is given by the corresponding coherence-transfer functions. The transfer functions depend on a number of parameters, such as the coupling topology, coupling constants, relaxation rates, and the mixing sequence used. The acquisition of experimental coherence-transfer functions can provide information about the optimal duration of Hartmann–Hahn periods in multidimensional experiments in the presence of experimental imperfections and relaxation. Furthermore, experimental coherence-transfer functions contain information about the size and relative sign of coupling constants. Coherence-transfer functions can be analyzed directly by comparing them to theoretical transfer functions that can be calculated analytically (1–5, 7–11) or numerically (1, 12–16). For the determination of coupling constants it can be more convenient to analyze so-called coherence-transfer spectra (15, 17–19), which are obtained from the coherence-transfer functions by a Fourier transformation. However, the acquisition of experimental coherence-transfer functions can be rather time-consuming. In high-resolution NMR, transfer functions have been extracted from a series of two-dimensional experiments with a range of different mixing times (with a typical total measuring time of days) (1, 14, 20, 21), from a two-dimensional experiment where the Hartmann–Hahn mix-

ing period is incremented in concert with the evolution period t_1 (22) or from a series of one-dimensional experiments with a range of different mixing times using selective excitation (with a typical total measuring time of several hours) (1, 17, 23, 24). However, in solid-state NMR it has been demonstrated that the dynamic variation of a given spin system can be observed almost instantly in Hartmann–Hahn-type experiments based on a string of short pulses with delays (25). Here, we present a simple extension of this approach that makes it possible to acquire experimental coherence transfer functions in a single shot for arbitrary multiple-pulse sequences (with a typical total measuring time of seconds). Single-shot experiments have previously been successfully used for a number of applications, such as the sampling of zero-quantum coherence (26), monitoring the motion of a spin during a shaped pulse (27), and diffusion measurements (28–31). The method is also closely related to homonuclear decoupling experiments in liquids (32).

EXPERIMENTAL

Figure 1 shows the simplest pulse sequences for the direct acquisition of heteronuclear Hartmann–Hahn coherence-transfer functions. These sequences can be applied if both spins (I and S) are on resonance in the doubly rotating frame and if no further signals are present in the spectrum of the detected spin I . The detection period follows immediately after the excitation of spin I (Fig. 1A) or spin S (Fig. 1B) and consists of N alternating periods τ and Δ . Heteronuclear Hartmann–Hahn transfer takes place during the period τ which is an integer multiple of the cycle time τ_c of the mixing sequence. Δ is the minimum delay that is needed to acquire a single data point a_k without perturbation by the preceding RF irradiation. The amplitude of the points a_k with $1 \leq k \leq N$ represents the integrated amplitude of the spin I spectrum after the accumulated mixing time $\tau_{\text{mix}} = k\tau$. Hence, the acquired signal directly represents the desired coherence-transfer function. If $\Delta \ll \tau \ll J_{IS}^{-1}$, the effect of the heteronuclear coupling can be ignored during Δ .

Experimental single-shot coherence transfer functions were acquired for the I_3S spin system ($I = {}^{19}\text{F}$, $S = {}^1\text{H}$) of halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) with a het-

¹ To whom correspondence should be addressed. Fax: +49 69 7982 9128; E-mail: sg@org.chemie.uni-frankfurt.de.

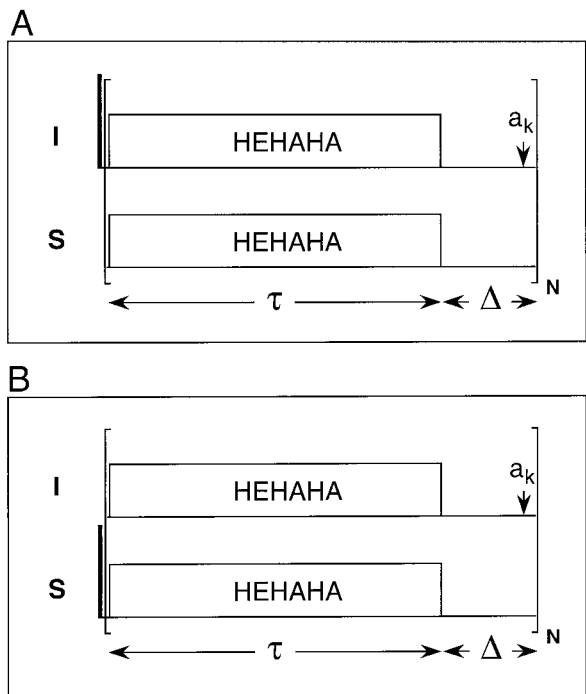


FIG. 1. Basic pulse sequences for the single-shot acquisition of heteronuclear Hartmann–Hahn transfer functions ($I_x \rightarrow I_x$ (A) and $S_x \rightarrow I_x$ (B)). Hartmann–Hahn transfer during τ and the acquisition of a data point a_k is repeated N times. The phase of the 90° excitation pulse is y .

eronuclear coupling constant J_{IS} of 5 Hz. The sequence DIPSI-2⁺ (33, 34) was irradiated simultaneously at the resonance frequencies of spins I and S (with an RF amplitude of 5 kHz and an uncompensated 30_x° spin-lock pulse) during the period $\tau = \tau_c = 5.76$ ms. In the spectrometer used (Bruker DRX-600 with a QXI probe), the minimum delay between a period of RF irradiation and the point of data acquisition was on the order of 50 μ s. Including a sampling time of 3 μ s, a delay $\Delta = 54$ μ s was used in our experiments. The experimental transfer functions T_{II} for the transfer $F_x \rightarrow F_x$ (Fig. 2A) and T_{SI} for the transfer $S_x \rightarrow F_x$ (Fig. 2B) with $F_x = \sum_{k=1}^3 I_{kx}$ were acquired with the sequences shown in Fig. 1A and 1B, respectively. For comparison, theoretical coherence-transfer functions (dashed curves) are also shown in Fig. 2 for an I_3S system under planar mixing (I). In the absence of relaxation, the transfer functions T_{II} and T_{SI} are given by (4, 17)

$$T_{II}(\tau_{\text{mix}}) = \frac{1}{24} \{19 + 2 \cos(\alpha) + 2 \cos(\sqrt{3}\alpha) + \cos(2\alpha)\} \quad [1]$$

and

$$T_{SI}(\tau_{\text{mix}}) = \frac{1}{24} \{5 - 2 \cos(\alpha) - 2 \cos(\sqrt{3}\alpha) - \cos(2\alpha)\}, \quad [2]$$

with $\alpha = \pi J_{IS} \tau_{\text{mix}}$. In order to fit the experimental data and to mimic the effect of relaxation, these transfer functions were multiplied with an exponential damping function of the form $\exp\{-\tau_{\text{mix}}/T_{\text{damp}}\}$. The dashed curves correspond to a heteronuclear coupling constant $J_{IS} = 5$ Hz and an exponential damping constant $T_{\text{damp}} = 700$ ms.

Figure 3 shows a slightly modified single-shot sequence with two additional 180° pulses during the period Δ . This simple modification eliminates offset effects of spin S during the delays Δ . In addition, J_{IS} is effectively decoupled during Δ and hence the condition $\Delta \ll \tau \ll J_{IS}^{-1}$ can be relaxed. With this improved sequence it is possible to measure the offset dependence of transfer functions for windowless Hartmann–Hahn sequences. This is demonstrated in Fig. 4A, which shows experimental transfer spectra for the transfer $F_x \rightarrow F_x$ with $\nu_I = 0$ and -1.5 kHz $\leq \nu_S \leq 1.5$ kHz. The complete set of 61 transfer functions was acquired in a total measurement time of 13 minutes. In order to reduce offset effects induced by the 180° pulses that are applied during Δ , they were implemented using composite pulses ($90_x 240_y 90_x$) with an RF amplitude of 7 kHz and an additional 60° phase shift, yielding an effective

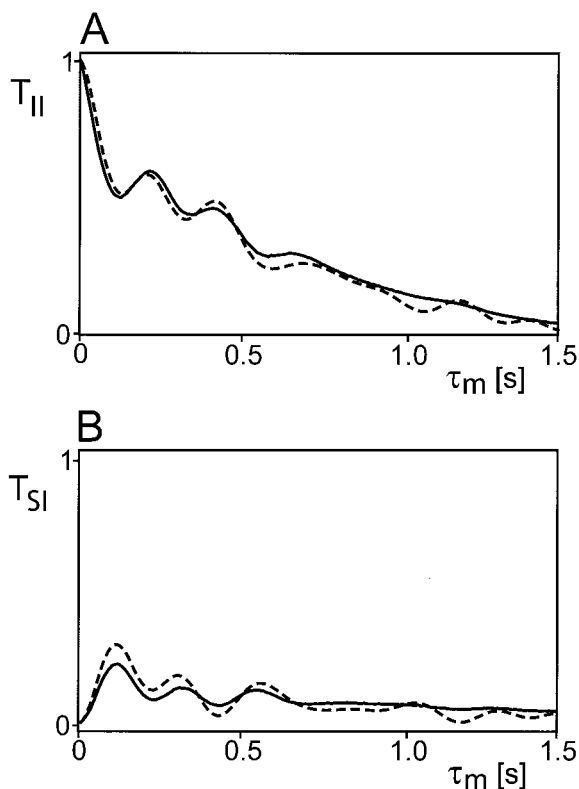


FIG. 2. Experimental (solid) and simulated (dashed) coherence-transfer functions of the I_3S spin system ($I = {}^{19}\text{F}$, $S = {}^1\text{H}$) of halothane with $J_{IS} = 5$ Hz. The experimental transfer functions $T_{II}(F_x \rightarrow F_x)$ (A) and $T_{SI}(S_x \rightarrow F_x)$ (B) with $F_x = \sum_{k=1}^3 I_{kx}$ were acquired with the sequences shown in Fig. 1A and 1B, respectively. The Hartmann–Hahn sequence was DIPSI-2⁺ with an RF amplitude of 5 kHz with a cycle time $\tau_c = 5.8$ ms. $N = 256$ data points a_k were acquired in a single shot.

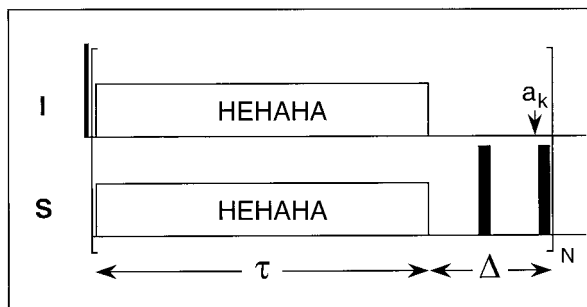


FIG. 3. Modified single-shot sequence for the detection of the offset dependence of heteronuclear coherence-transfer functions. The hard (or composite) 180° pulses eliminate the effects of an offset ν_s and of the heteronuclear coupling J_{IS} during Δ .

bandwidth of ± 2.1 kHz (35). The duration of Δ was $300 \mu\text{s}$ and 256 data points a_k were acquired. The resolution of the coherence-transfer spectra was increased using linear prediction to 2048 points and by multiplying the resulting transfer functions by an exponential damping function with $T_{\text{damp}} = 850$ ms.

For comparison, coherence transfer functions were simulated using the program SIMONE (15) for the same range of offsets and the DIPSI-2⁺ sequence with an RF amplitude of 5 kHz. An exponential damping function with a longer T_{damp} of 1.2 s was applied in order to resolve the individual coherence-

transfer frequencies. The corresponding coherence transfer spectra are shown in Fig. 4B. For $\nu_I = \nu_S = 0$, the expected transfer frequencies of $J_{IS}/2 = \pm 2.5$ Hz, $\sqrt{3} J_{IS}/2 = \pm 4.33$ Hz, and $J_{IS} = \pm 5$ Hz are found with an intensity ratio of 2:2:1, as predicted by Eq. [1]. The remaining discrepancies between experimental and simulated coherence transfer spectra can be attributed to the different linewidths and the effects of RF inhomogeneity, which were not included in the simulation.

CONCLUSIONS

In summary, we demonstrated the acquisition of Hartmann–Hahn coherence-transfer functions in a single shot by introducing delays in windowless multiple-pulse sequences. In favorable spin systems this leads to a drastic reduction of the total measurement time and makes it possible to investigate the detailed dependence of coherence-transfer functions on experimental parameters. The method relies on the selective excitation of the source spin and the selective acquisition of the target spins. This can be readily accomplished for heteronuclear Hartmann–Hahn transfer as demonstrated for the I_3S spin system of halothane. However, the method may also be applicable to homonuclear spin systems if selective pulses are used for excitation and to align all spins except for the target spin along the z axis during the acquisition window Δ .

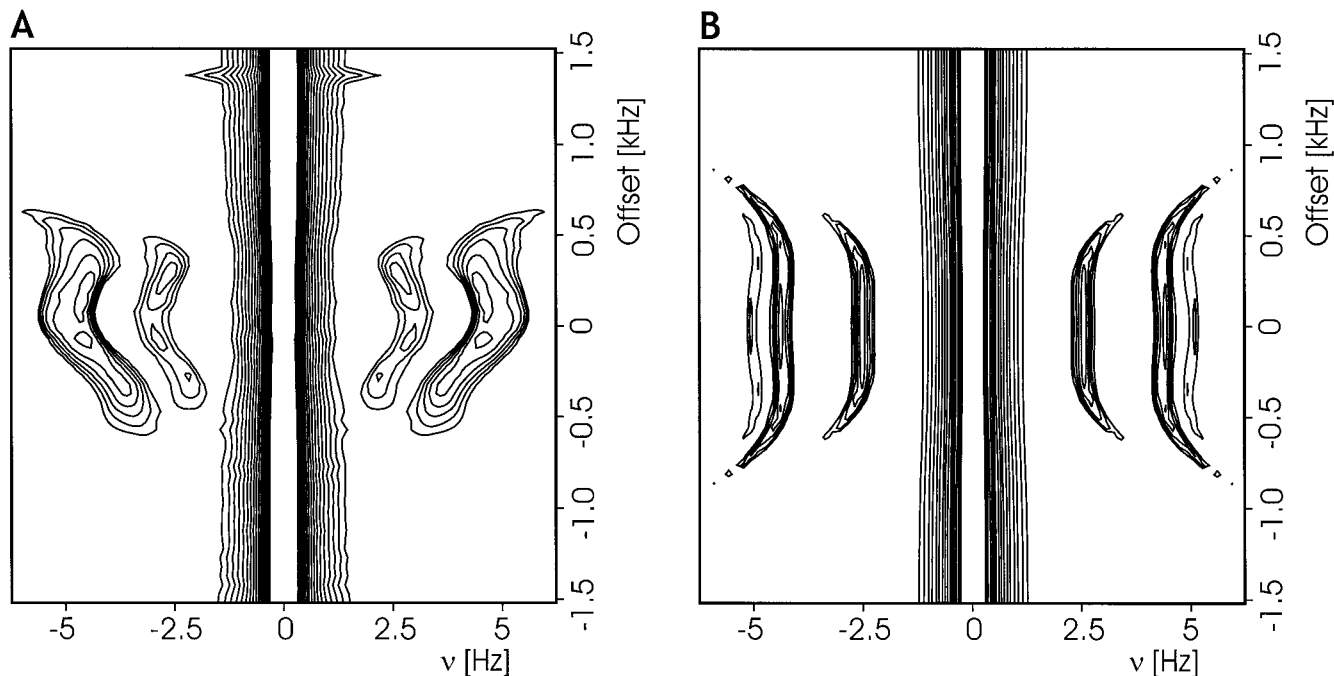


FIG. 4. Experimental (A) and simulated (B) offset dependence of the coherence-transfer spectra $F_x \rightarrow F_x$ for the I_3S system of halothane. The Hartmann–Hahn sequence was DIPSI-2⁺ (same experimental parameters as in Fig. 2); 61 single-shot experiments were acquired with the sequence shown in Fig. 3. The spins I were on resonance, whereas the offset of spin S was incremented in steps of 50 Hz in the range between ± 1.5 kHz. The total experiment time was about 13 minutes.

ACKNOWLEDGMENTS

We thank W. Bermel for discussions and technical support. This work was supported by the DFG under grant Gl 203/1-5 and 1-6. B.L. acknowledges a scholarship of the Fonds der Chemischen Industrie. S.J.G. thanks the DFG for a Heisenberg Stipendium (Gl 203/2-2). The experiments were performed at the "Large Scale Facility for Biomolecular NMR" (ERB CT 950034). We thank one of the referees for pointing out Ref. (25) to us.

REFERENCES

1. S. J. Glaser and J. J. Quant, Homonuclear and heteronuclear Hartmann-Hahn transfer in isotropic liquids, in "Advances in Magnetic and Optical Resonance" (W. S. Warren, Ed.), Vol. 19, pp. 59-252, Academic Press, San Diego (1996).
2. S. R. Hartmann and E. L. Hahn, Nuclear double resonance in the rotating frame, *Phys. Rev.* **128**, 2042-2053 (1962).
3. L. Müller and R. R. Ernst, Coherence transfer in the rotating frame. Application to heteronuclear cross-correlation spectroscopy, *Mol. Phys.* **38**, 963-992 (1979).
4. G. C. Chingas, A. N. Garroway, R. D. Bertrand, and W. B. Moniz, Zero quantum NMR in the rotating frame: J cross polarization in AX_N systems, *J. Chem. Phys.* **74**, 127-156 (1981).
5. L. Braunschweiler and R. R. Ernst, Coherence transfer by isotropic mixing: Application to proton correlation spectroscopy, *J. Magn. Reson.* **53**, 521 (1983).
6. A. Bax and D. G. Davis, MLEV-17-based two-dimensional homonuclear magnetization transfer spectroscopy, *J. Magn. Reson.* **65**, 355 (1985).
7. N. Chandrakumar, G. V. Visalakshi, D. Ramaswamy, and S. Subramanian, Analysis of collective modes in some A_nX_N systems, *J. Magn. Reson.* **67**, 307-318 (1986).
8. G. V. Visalakshi and N. Chandrakumar, Automated generation of the commutator algebra for NMR problems, *J. Magn. Reson.* **75**, 1-8 (1987).
9. O. Schedletsky and S. J. Glaser, Analytical coherence-transfer functions for the general AMX spin system under isotropic mixing, *J. Magn. Reson. A* **123**, 174-180 (1996).
10. A. Majumdar, Analytical expressions for isotropic mixing in three- and four-spin topologies in ^{13}C systems, *J. Magn. Reson. A* **121**, 121-126 (1996).
11. Oliver Schedletsky, Burkhard Luy, and Steffen J. Glaser, Analytical polarization and coherence transfer functions for three coupled spins $\frac{1}{2}$ under planar mixing conditions, *J. Magn. Reson.* **130**, 27-33 (1998).
12. M. L. Remerowski, S. J. Glaser, and G. P. Drobny, A theoretical study of coherence transfer by isotropic mixing: Calculation of pulse sequence performance for systems of biological interest, *Mol. Phys.* **68**, 1191-1218 (1989).
13. J. Cavanagh, W. J. Chazin, and M. Rance, The time dependence of coherence transfer in homonuclear isotropic mixing experiments, *J. Magn. Reson.* **87**, 110-131 (1990).
14. H. L. Eaton, S. W. Fesik, S. J. Glaser, and G. P. Drobny, Time dependence of ^{13}C - ^{13}C magnetization transfer in isotropic mixing experiments involving amino acid spin systems, *J. Magn. Reson.* **90**, 425-463 (1990).
15. S. J. Glaser and G. P. Drobny, Assessment and optimization of pulse sequences for homonuclear isotropic mixing, in "Advances in Magnetic Resonance" (W. S. Warren, Ed.), Vol. 14, pp. 35-58, Academic Press, San Diego (1990).
16. S. S. Wijmenga, H. A. Heus, B. Werten, G. A. van der Marel, J. H. van Boom, and C. W. Hilbers, Assignment strategies and analysis of cross-peak pattern and intensities in the three-dimensional homonuclear TOCSY-NOESY of RNA, *J. Magn. Reson. B* **103**, 134-141 (1994).
17. R. D. Bertrand, W. B. Moniz, A. N. Garroway, and G. C. Chingas, ^{13}C - 1H cross-polarization in liquids, *J. Am. Chem. Soc.* **100**, 5227-5229 (1978).
18. N. Chandrakumar and A. Ramamoorthy, Zero-field-in-high-field zero-quantum NMR spectra under isotropic mixing, *J. Magn. Reson.* **99**, 372-376 (1992).
19. J. S. Waugh, Broadband homonuclear cross-polarization using a heteronuclear decoupling sequence, *J. Magn. Reson.* **68**, 189-192 (1986).
20. P. F. Flynn, A. Kintanar, B. R. Reid, and G. P. Drobny, Coherence transfer in deoxyribose sugars produced by isotropic mixing: An improved intraresidue assignment strategy for the two-dimensional NMR spectra of DNA, *Biochemistry* **27**, 1191-1197 (1988).
21. J. P. M. van Duynhoven, J. Goudriaan, C. W. Hilbers, and S. S. Wijmenga, Quantitative evaluation of TOCSY data. Application to sugar ring conformational analysis, *J. Am. Chem. Soc.* **114**, 10055-10056 (1992).
22. G. Kontaxis and J. Keeler, The accordion approach for "Tailored" TOCSY, *J. Magn. Reson. A* **115**, 35-41 (1995).
23. F. Inagaki, C. Kodama, M. Suzuki, and A. Suzuki, Analysis of NMR spectra of sugar chains of glycolipids by 1D homonuclear Hartmann-Hahn and NOE experiments, *FEBS Lett.* **219**, 45-50 (1987).
24. S. J. Glaser and G. P. Drobny, Controlled coherence transfer by a multiple-step tailored TOCSY experiment, *Chem. Phys. Lett.* **184**, 553-559 (1991).
25. W.-K. Rhim, D. P. Burum, and D. D. Elleman, Multiple-pulse spin locking in dipolar solids, *Phys. Rev. Lett.* **37**, 1764-1766 (1976).
26. A. Bax, T. Mehlkopf, J. Smidt, and R. Freeman, A simple method for detection of zero-quantum transitions in NMR, *J. Magn. Reson.* **41**, 502-506 (1980).
27. H. Sengstschmid and R. Freeman, A window on the motion of the nuclear spins, *J. Magn. Reson. A* **121**, 212-216 (1996).
28. L. Li and C. H. Sotak, Diffusion measurement by pulsed field-gradient multiple spin echoes, *J. Magn. Reson.* **92**, 411-420 (1991).
29. P. van Gelderen, A. Olson, and C. T. W. Moonen, A single-shot diffusion experiment, *J. Magn. Reson. A* **103**, 105-108 (1993).
30. S. J. Doran and M. Décorps, A robust, single-shot method for measuring diffusion coefficients using the "burst" sequence, *J. Magn. Reson. A* **117**, 311-316 (1995).
31. S. Sendhil Velan and N. Chandrakumar, High-resolution NMR measurement of the molecular self-diffusion by fast multi-spin-echo diffusion sequences, *J. Magn. Reson. A* **123**, 122-125 (1996).
32. J. P. Jesson, P. Meakin, and G. Kneissel, Homonuclear decoupling and peak elimination in Fourier transform nuclear magnetic resonance, *J. Am. Chem. Soc.* **95**, 618-620 (1973).
33. M. Ernst, C. Griesinger, R. R. Ernst, and W. Bermel, Optimized heteronuclear cross polarization in liquids, *Mol. Phys.* **74**, 219-252 (1991).
34. A. J. Shaka, C. J. Lee, and A. Pines, Iterative schemes for bilinear operators; application to spin decoupling, *J. Magn. Reson.* **77**, 274-293 (1988).
35. R. Freeman, S. P. Kempell, and M. Levitt, Radiofrequency pulse sequences which compensate their own imperfections, *J. Magn. Reson.* **38**, 453-479 (1980).