NMR Spectroscopy

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Multiple FID Acquisition of Complementary HMBC Data**

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Many advances in the design of high-resolution NMR pulse sequences have been focused on the improvement of sensitivity factors per time unit or on maximizing the amount of information obtained in a given measuring time. Since the first revolutionary developments in fast NMR spectroscopy, such as the combined use of Fourier transformation and signal averaging, the incorporation of pulsed-field gradients for coherence selection, and the large benefits offered by multidimensional NMR experiments, renewed interest is emerging in the acceleration of the acquisition of NMR spectroscopic data even further.[1] One of these fast NMR spectroscopic techniques, time-shared (TS) evolution, makes it possible to obtain a number of different NMR spectra by the simultaneous detection of independent coherence pathways. Typical heteronuclear NMR spectroscopic experiments used for small and medium-sized molecules, such as HSQC or HMBC, can be recorded simultaneously for two different nuclei (for example, ^{13}C and ^{15}N). Thus, considerable savings can be made in the time required to record the spectra when compared to the separate acquisition of individual spectra.[2] The concept of the acquisition of multiple free-induction-decay (FID) signals within the same scan was introduced many years ago as a method for the simultaneous acquisition of complementary NMR spectroscopic data for the same nucleus.[3] Recently, this concept of parallel acquisition (referred to as PANSY) was described for the simultaneous recording of different H–H and H–C correlation spectra in multiple-receiver-coil systems.[4]

The accumulative advantages of combining several fast NMR spectroscopic methods have been described.[5] We propose herein the MATS technique (multiple-FID acquisition–time-shared evolution), the time-effective collection of multiple NMR spectra by combining the individual features associated with the TS evolution of independent coherences and the acquisition of different FID signals within the same scan. We illustrate the major advantages of the MATS principle by applying it to the HMBC pulse sequence,[6] an essential NMR spectroscopic experiment for the structural characterization of chemical compounds. Figure 1 shows the pulse sequence of a multiple-FID-acquisition, time-shared HMBC (MATS-HMBC) experiment, which provides complementary HMBC and HMBC-relayed (first and second FID, respectively, in Figure 1) spectra for several nuclei at the same time. The sequence retains the simplicity and the basic features of the original HMBC and TS-HMBC experiments.[2] The gradients G1–G4 are used for echo coherence selection in the first HMBC data set, whereas the last gradient, G5, is required to refocus a different antiecho coherence for the second HMBC-relayed data set. In principle, an FID-acquisition period can be incorporated into the original pulse sequence with standard spectrometer configurations in a very straightforward way without introducing any alterations in terms of additional pulses and/or delays. Such periods can be understood as simple evolution or relaxation delays and can therefore be implemented in a variety of applications. Under optimum relaxation conditions, multiple FID periods could be incorporated whenever enough longer periods (around 80–100 ms) should be available. Experimentally, it is important to find an optimum balance between FID duration and digital resolution.

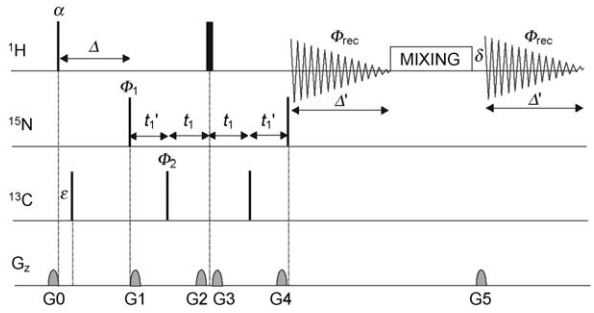

Figure 1. A basic single-shot pulse sequence for recording complementary HMBC spectra simultaneously. The mixing process could be a COSY or a TOCSY process. Thus, multiple FID signals can be collected separately and stored in different memory blocks. The data is then processed and analyzed in the usual way. The phase φ_2 is inverted with respect to φ_1 to discriminate between ^{13}C and ^{15}N cross-peaks. A low-pass $J_{C,H}$ filter (ε) is applied to minimize direct C–H cross-peaks, and gradients are optimized to select independent ^{13}C and ^{15}N coherence pathways. The variable-angle-excitation proton pulse α can be reoptimized if desired when high repetition rates are used. Complete experimental details can be found in the Supporting Information.

Up to four different data sets can be extracted from a single collection of MATS-HMBC data (Figure 2): ^{1}H–^{13}C HMBC, ^{1}H–^{15}N HMBC, ^{1}H–^{13}C HMBC–COSY (TOCSY), and ^{1}H–^{15}N HMBC–COSY (TOCSY). The ability to collect all these data sets simultaneously corresponds to a theoretical reduction in measuring time of 75%. Because of the dependence of the intensity of each cross-peak on the term $\sin(^{n}J_{X,H})$, some expected correlations may be rather weak or even lost in a HMBC spectrum. Fortunately, the analysis of a separate HMBC–COSY spectrum obtained within the same measure-

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

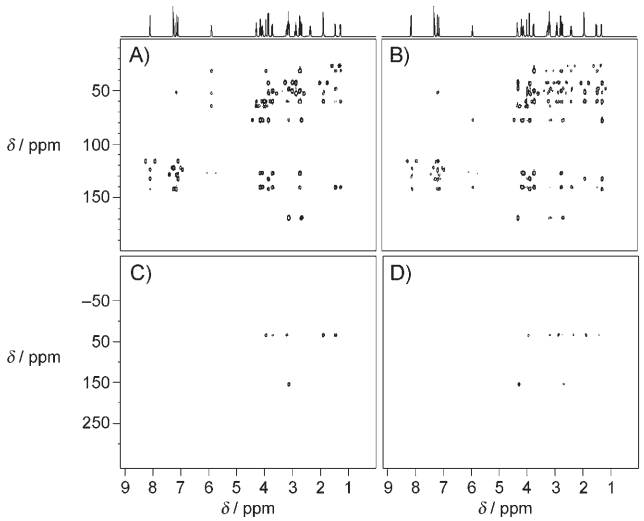

Figure 2. Complementary A) ^{1}H–^{13}C HMBC, B) ^{1}H–^{13}C HMBC–COSY, C) ^{1}H–^{15}N HMBC, and D) ^{1}H–^{15}N HMBC–COSY spectra of strychnine obtained simultaneously by the MATS approach. The measuring time with this method is reduced significantly by 75% relative to that for the separate acquisition of each data set by using traditional single-FID pulse schemes.

ment can uncover missing signals due to two- or three-bond correlations, as well as relayed peaks originating from consecutive $J_{C,H}+J_{H,H}$ pathways (Figure 3). For example, seven four-bond correlations can be detected clearly for the hydrogen atoms 15a-H and 13-H in the ^{1}H–^{13}C HMBC–COSY spectrum of strychnine (Figure 3D). Missing peaks in ^{1}H–^{15}N HMBC spectra are also a common problem in the analysis of many nitrogen-containing compounds, mostly as a result of the small long-range proton–nitrogen coupling constants. The use of the additional information provided by HMBC–COSY (TOCSY) spectra without an extra payment in measuring time can prevent such a lack of significant information (see the Supporting Information). Furthermore, as two-bond heteronuclear correlations can be recognized from H2BC spectra,[7] the concerted analysis of H2BC and MATS-HMBC data could enable the assignment of unambiguous chemical shifts and should facilitate automated structure determination. Interestingly, the proposed MATS strategy is also fully compatible with other HMBC variants that provide better uniform response[8] or use some type of editing, for example, to distinguish between direct and long-range connectivities.[9]

Significant reductions in spectrometer time for the MATS-HMBC experiment under conditions of optimum sensitivity are possible by reducing the duration of the interscan delay and by using an Ernst angle optimized (α) excitation pulse.[10] Experimentally, we found that for short recycle delays, the application of a moderate z gradient (G0 in Figure 1) just before the first proton pulse is essential to remove any residual transverse coherence and to make it possible to obtain high-quality spectra free of undesirable t_1 noise. For optimum fast acquisition, a 120° excitation α pulse should be used for recycle delays below 100 ms (see the Supporting Information).

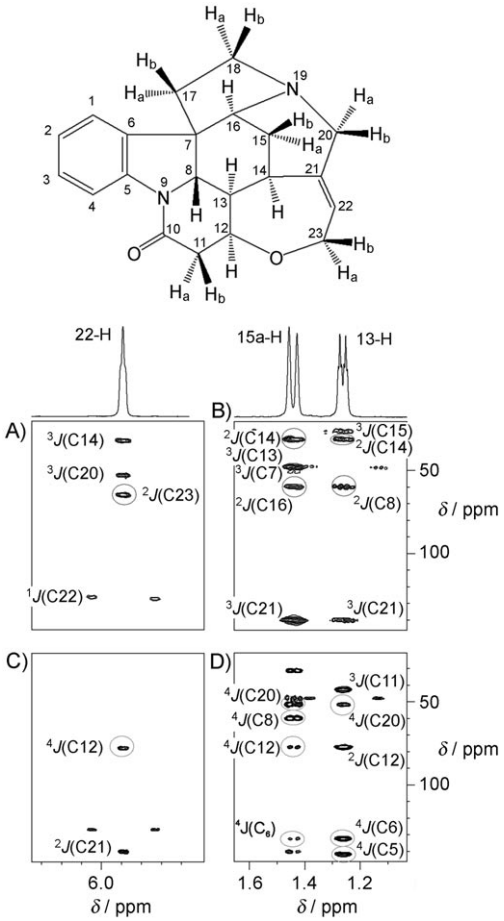

Figure 3. Expanded areas of the HMBC and HMBC–COSY spectra of strychnine (see formula for structure). A,B) HMBC spectra usually provide two-bond (circled) and three-bond heteronuclear correlations. Residual direct correlations can be also observed as large doublets due to $^{1}J_{C,H}$. C,D) Relayed HMBC spectra can provide missing two- and three-bond correlations, as well as longer-range heteronuclear correlations (four-bond correlations are circled).

In summary, we have described the acquisition of multiple NMR spectra in a single experiment. The combined use of multiple FID acquisition (MA) and TS to obtain different sets of NMR spectroscopic data in short measuring times serves as a proof of principle and has potential for application in automated routine NMR spectroscopic experiments. We have already shown that four complementary HMBC spectra can be recorded in a single-shot acquisition, and it should be possible to design other more sophisticated applications of the method, for example, for time-consuming 3D or low-sensitivity NMR spectroscopic experiments. MATS can also be combined with other complementary acquisition schemes that speed up the collection of multidimensional NMR spectroscopic data, such as the recently demonstrated combination of TS and projection-reconstruction principles in a single-shot experiment to provide a ^{13}C–^{15}N correlation map at natural abundance.[5d] It can be anticipated that the future availability of multiple-receiver coils in commercial NMR spectrometers[11] will enable the collection of a number of homo- and/or heteronuclear correlation spectra for multiple

samples in a single-shot experiment.[12] This development will improve significantly the economy of spectrometer time and be of great interest for rapid data collection and high-throughput structure determination. Further investigations into the MATS principle are in progress in our laboratory.

Experimental Section

All spectra were recorded at 298 K on a Bruker Avance-500 NMR spectrometer (at 500.13 MHz for ^{1}H, 125.7 MHz for ^{13}C, and 50.68 MHz for ^{15}N) with a sample of strychnine (50 mM) in $CDCl_3$. The spectrometer was equipped with a triple-resonance inverse TCI cryoprobe (length of the 90° pulse: 7.6 μs for ^{1}H, 15 μs for ^{13}C, 40 μs for ^{15}N). A total of 128 increments $t_1 + t_{1'}$ were accumulated with increments of $\Delta t_1 = 40$ μs (1/SW(C)) and $\Delta t_{1'} = 100$ μs (1/SW(N)–1/SW(C), where SW stands for the spectral width). The maximum times t_1 and $t_{1'}$ were 5.1 and 12.8 ms, respectively, which correspond to spectral widths of 25153 and 7100 Hz for ^{13}C and ^{15}N, respectively. The acquisition time (Δ') was 90.15 ms (spectral width of 10 ppm for ^{1}H) for each FID signal. The data matrix containing 128×900 complex points in $t_1 + t_{1'}$ and t_2, respectively, was zero-filled to 512×2048 complex points. The sine-squared weighting function was applied prior to Fourier transformation in both dimensions, and NMR spectra were processed in magnitude mode to avoid phasing problems.

The interpulse delays for Δ ($1/(2^{n}J)$) and the low-pass J filter ($\varepsilon = 1/(2\,^{1}J_{C,H})$) were set to 62.5 ms ($^{n}J = 8$ Hz) and 3.8 ms ($^{1}J_{C,H} = 135$ Hz), respectively. All gradient lengths (δ) were 1 ms, and the gradient strengths were 15, 60, 50, 30, 60, and 40 for G0–G5, respectively (with 100% corresponding to 53.5 G cm^{-1}). Two data sets were recorded in an interleaved mode by basic two-step phase cycling (data A: $\Phi_1 = x,-x$; $\Phi_2 = x,-x$; Φrec $= x,-x$; data B: $\Phi_1 = x,-x$; $\Phi_2 = -x,x$; Φrec $= x,-x$). If desired, and for longer acquisitions, this phase cycling can be expanded further by inverting the last ^{13}C and ^{15}N 90° pulses along with the receiver, as is usual in conventional HMBC experiments. Data were stored in separate memory blocks, and ^{13}C and ^{15}N data were obtained after time-domain data addition or subtraction (A + B and A–B, respectively).

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[1] For a comprehensive review, see: R. Freeman, E. Kupče, *J. Biomol. NMR* **2003**, *27*, 101–113.
[2] a) M. Sattler, M. Maurer, J. Schleucher, C. Griesinger, *J. Biomol. NMR* **1995**, *5*, 97–102; b) P. Nolis, M. Pérez-Trujillo, T. Parella, *Magn. Reson. Chem.* **2006**, 44, 1031–1036; c) P. Nolis, M. Pérez-Trujillo, T. Parella, *Org. Lett.* **2007**, *9*, 29–32; d) P. Nolis, T. Parella, *J. Biomol. NMR* **2007**, *37*, 65–77; e) P. Nolis, M. Pérez-Trujillo, W. Bermel, T. Parella, *Magn. Reson. Chem.* **2007**, *45*, 325–329.
[3] a) C. A. G. Haasnoot, F. J. M. van de Ven, C. W. Hilbers, *J. Magn. Reson.* **1984**, *56*, 343–349; b) A. Z. Gurevich, I. L. Barsukov, A. S. Arseniev, V. F. Bystrov, *J. Magn. Reson.* **1984**, *56*, 471–478.
[4] a) E. Kupče, R. Freeman, B. K. John, *J. Am. Chem. Soc.* **2006**, *128*, 9606–9607; b) E. Kupče, S. Cheatham, R. Freeman, *Magn. Reson. Chem.* **2007**, *45*, 378–380.
[5] a) P. Schanda, B. Brutscher, *J. Magn. Reson.* **2006**, *178*, 334–339; b) E. Kupče, R. Freeman, *Magn. Reson. Chem.* **2007**, *45*, 2–5; c) Z. J. Sun, S. G. Hyberts, D. Rovnyak, S. Park, A. S. Stern, J. C. Hoch, G. Wagner, *J. Biomol. NMR* **2005**, *32*, 55–60; d) E. Kupče, R. Freeman, *Magn. Reson. Chem.* **2007**, *45*, 103–105.
[6] A. Bax, M. F. Summers, *J. Am. Chem. Soc.* **1986**, *108*, 2093–2094.
[7] N. T. Nyberg, J. Ø. Duus, O. W. Sørensen, *J. Am. Chem. Soc.* **2005**, *127*, 6154–6155.
[8] a) C. E. Hadden, G. E. Martin, V. V. Krishnamurthy, *Magn. Reson. Chem.* **2000**, *38*, 143–147; b) G. E. Martin, C. E. Hadden, *Magn. Reson. Chem.* **2000**, *38*, 251–256; c) M. Kline, S. Cheatham, *Magn. Reson. Chem.* **2003**, *41*, 307–314.
[9] a) A. Meissner, O. W. Sørensen, *Magn. Reson. Chem.* **2000**, *38*, 981–984; b) N. Nyberg, O. W. Sørensen, *Magn. Reson. Chem.* **2006**, *44*, 451–454; c) R. Burger, C. Schorn, P. Bigler, *J. Magn. Reson.* **2001**, *148*, 88–94.
[10] a) A. Ross, M. Salzmann, H. Senn, *J. Biomol. NMR* **1997**, *10*, 389–396; b) P. Schanda, B. Brutscher, *J. Am. Chem. Soc.* **2005**, *127*, 8014–8015.
[11] G. Wang, L. Ciobanu, A. G. Webb, *J. Magn. Reson.* **2005**, *173*, 134–139.
[12] a) G. Fisher, C. Petucci, E. MacNamara, D. Raftery, *J. Magn. Reson.* **1999**, *138*, 160–163; b) X. Zhang, J. V. Sweedler, A. G. Webb, *J. Magn. Reson.* **2001**, *153*, 254–258.

