

Communication

Arrayed acquisition of 2D exchange NMR spectra within a single scan experiment

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Abstract

We have recently demonstrated that magnetic field gradients in combination with frequency selective pulses, can be employed to collect a complete multi-dimensional NMR spectrum within a single scan. Following similar guidelines, field gradients could also be exploited to parallelize other types of NMR experiments where the final results arise from the collection and analysis of a series of time-incremented spectra. The present Communication exemplifies this concept by showing how a combination of gradients can be employed to monitor within a single continuous acquisition, a slow dynamic process which is in turn followed by systematic increments in the duration of a magnetization transfer time. Further, since 2D exchange NMR spectra can nowadays be themselves collected within one scan, the acquisition of a complete set of mixing-incremented 2D exchange patterns could be achieved within a single experiment entailing a total time of ≈ 1 s.

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One of the unique characteristics of NMR is its ability to rely on the systematic changes that spin systems may undergo as a function of a parametric time incrementation, in order to quantify both coherent and incoherent dynamic processes [1,2]. This principle was initially exploited in the development of spin relaxation measurement techniques [3–6], and subsequently extended to quantify chemical exchange, NOE, and diffusion processes occurring slowly in the NMR timescale [7–10]. Arguably, however, this strategy found its most widespread application in the characterization of coherent forms of spin evolution, particularly with the advent of 2D NMR spectroscopy [11,12]. Signals $S(t_1, t_2)$ in these experiments are directly monitored solely as a function of one of the two variables involved, the physical acquisition time t_2 , whereas the spins' behavior along the remaining time axis involved is indirectly monitored by a systematic incrementation of its associated t_1 parameter. A by-product of this mode of operation is that 2D NMR acquisitions are inherently

more time consuming than their unidimensional counterparts, a feature which is also generally shared by parametric relaxation and/or magnetization transfer measurements. Higher-dimensional acquisitions will require the collection of an incremented set of lower-dimensional data sets, leading in turn to an exponential increase in the duration of an NMR experiment as the number of time parameters that is to be independently monitored increases.

Very recently, we proposed and demonstrated an alternative method for carrying out 2D NMR experiments whereby it is in principle possible to complete the collection of complete spectral data sets within a single scan [13,14]. This “ultrafast” NMR methodology is based on the application of magnetic field gradients, which in combination with radiofrequency (rf) pulses possessing properly chosen frequency offsets enable the effective partitioning of the sample into a series of sub-ensembles. Manipulations of the magnetic field gradients make it possible to remove any position-dependent encoding from the spins' evolution, leading to signals that only reflect the internal coupling frequencies affecting spins of chemically inequivalent sites. As inequivalent

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sub-ensembles can be endowed with different effective t_1 evolution times, this process can transform the serial collection of time-domain signals usually involved in 2D NMR into a parallelized version (Fig. 1) which can be completed within one continuous acquisition. Furthermore, the application of multiple linearly independent gradients allows one to extend this protocol to encode what could in principle amount to an arbitrary number of indirect time-domains, enabling the collection of arbitrary N -dimensional NMR spectra within a single scan [15].

The application of frequency-selective pulses in combination with properly designed gradient field distributions (followed if needed by the discriminated acquisition of signals originating from different portions of the sample), could be exploited for accelerating other kinds of NMR experiments that normally rely on monitoring multiple parametric increments of a time variable [5–10]. Such guidelines could be used for instance for collecting complete sets of inversion-recovery or magnetization-transfer data, within a single continuous NMR acquisition. This approach would differ from the single-scan NMR method proposed by Kaptein et al. [16], which speeds up the collection of a time-incremented data set by setting the final observe pulse to a small value, in that it would treat the sample inhomogeneously. Implementing a gradient-assisted partitioning of the sample would then keep the nature of the pulse sequence that is seen by spins within independent sub-ensembles essentially unchanged, enabling its application even when the time parameter that is incremented forms part of a more complex one- or multi-dimensional NMR scheme. The goal of the present Communication is to demonstrate this potential, as it applies to the estimation of a kinetic rate from the analysis of a complete set of mixing-incremented 2D exchange NMR experiments collected within a ca. 1 s timescale.

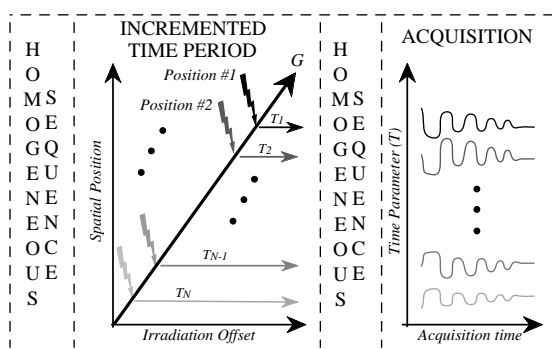


Fig. 1. Principles involved in the acquisition of time-incremented NMR data sets within a single-scan, based on the application of a train of frequency-shifted pulses (jagged arrows) in the presence of a field gradient (G).

Fig. 2 presents the pulse sequence chosen to test this principle. This scheme is based on the well-known 2D exchange NMR sequence proposed by Jeener et al. [17], modified for the sake of its acceleration in two main aspects. One of these involves replacing the conventional homogeneous excitation triggering the beginning of the experiment, with an incremented train of spatially selective excitation pulses. In the particular example under consideration such pulses are assumed applied while in the presence of a longitudinal magnetic field gradient, endowing spins at different z coordinates within the sample with incremented values of their indirect t_1 evolution times. In such manner, the initial evolution frequencies of the spins prior to the mixing period become spatially encoded and susceptible to a single-scan identification [13,14]. A homogeneous storage pulse and a mixing period τ_m follows, where a slow dynamic process is allowed to act on the chemical system and eventually give origin to cross-peaks whose relative intensities will depend on the product between τ_m and the exchange rate k of the dynamics [17,18]. Finally, in order to monitor the cross-peaks' build-up as a function of the mixing time, a second modification is introduced onto the experiment and the homogeneous rf pulse normally placed at the conclusion of the mixing period is replaced with a series of spatially heterogeneous recall pulses. The spatial heterogeneity imposed by such pulses is arranged along a transverse (e.g., the x) axis so as not to interfere with the z encoding used in speeding up the 2D experiment, and it effectively partitions the sample into an additional set of sub-ensembles characterized by different τ_m mixing intervals. Following each of these selective recall pulses a short refocusing gradient is applied, and the corresponding signal is recorded in combination with an echo-planar spectroscopy protocol capable of

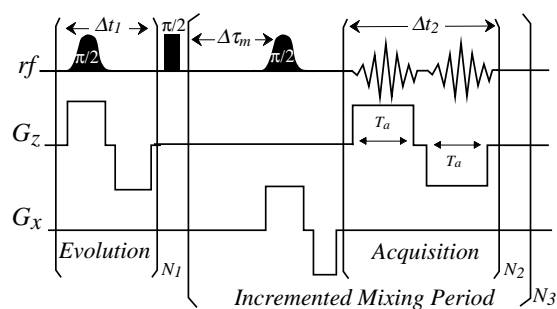


Fig. 2. Pulse sequence assayed to collect a series of 2D exchange NMR experiments with incremented mixing periods, within a single continuous experiment. The pulse sequence concatenates two trains of spatially selective events of the kind illustrated in Fig. 1: a first one (involving a z gradient) which triggers the t_1 encoding of spin evolution frequencies, and a second one (along the x axis) which endows spins located at different positions within the sample with incremented values of their mixing times. Though selective rf pulses are illustrated in the Figure as Gaussians, their actual shape is not of particular relevance.

unraveling the frequencies that have been active during the time t_1 [13]. This recall/acquisition procedure is repeated over all different transverse slices, so as to characterize an array of effective mixing periods.

To explore the kind of results afforded by such a pulse sequence a well-characterized dynamic system, *N,N*-dimethylacetamide (DMAc), was chosen as a test case. This molecule exhibits a ^1H NMR spectrum possessing three inequivalent peaks [19,20]: one at 2.08 ppm arising

from the carbonyl-bonded methyl ($\text{C}=\text{O}-\text{CH}_3$), and two

at 2.90 and 3.08 ppm arising from the pair of methyl groups which are bonded to the nitrogen ($-\text{N}(\text{CH}_3)_2$).

Though unequal in their chemical environments such methyls may undergo a mutual interconversion, given the rotation that the compound's C–N bond may undergo in spite of its partial double-bond character. This dynamics proceeds at room temperature with a rate k that is slow in the NMR timescale, yet which can still be studied by monitoring the mutual build-up of methyl cross-peaks revealed by the compound's 2D ^1H exchange NMR spectra. The ultrafast pulse sequence in Fig. 2 was thus attempted on a 10% w/w DMAc/D₂O solution, using a Bruker Avance 800 MHz NMR spectrometer equipped with a triple-axis gradient probehead as acquisition platform. Experiments were carried out slightly above room temperature, where a relatively long spin-lattice relaxation time (≈ 3 s) and an exchange rate $k \approx 1$ –10 Hz enabled us to insert a number of acquisition windows within relevant increments of the mixing time's duration. Two kinds of experiments were then assayed: one in which series of single-scan 2D exchange NMR spectra were collected as a function of τ_m , and another involving a single NMR experiment where the complete series of 2D NMR spectra was continuously collected following the guidelines introduced in Fig. 2. Fig. 3 compares the line shapes afforded by both kind of schemes. Despite some artificial peak wiggles arising due to the limitations of the hardware employed, both progressions are very similar and clearly show the expected patterns and build-up of cross-peaks between amide methyls with increasing mixing time.

In view of the readiness with which Fig. 2 enables the acquisition of a variable- τ_m series of 2D exchange NMR spectra, such ultrafast experiments were repeated as a function of temperature for the sake of a kinetic characterization. Unfortunately the range of temperatures that could actually be explored in our system was fairly limited, as at room temperature the exchange rate of the dynamic process significantly exceeded the longitudinal rate of spin relaxation (which in turn puts an upper bound to the achievable τ_m 's), while at higher temperatures our gradient setup shut down and ceased to operate. Still, Fig. 4 summarizes data arising from a series

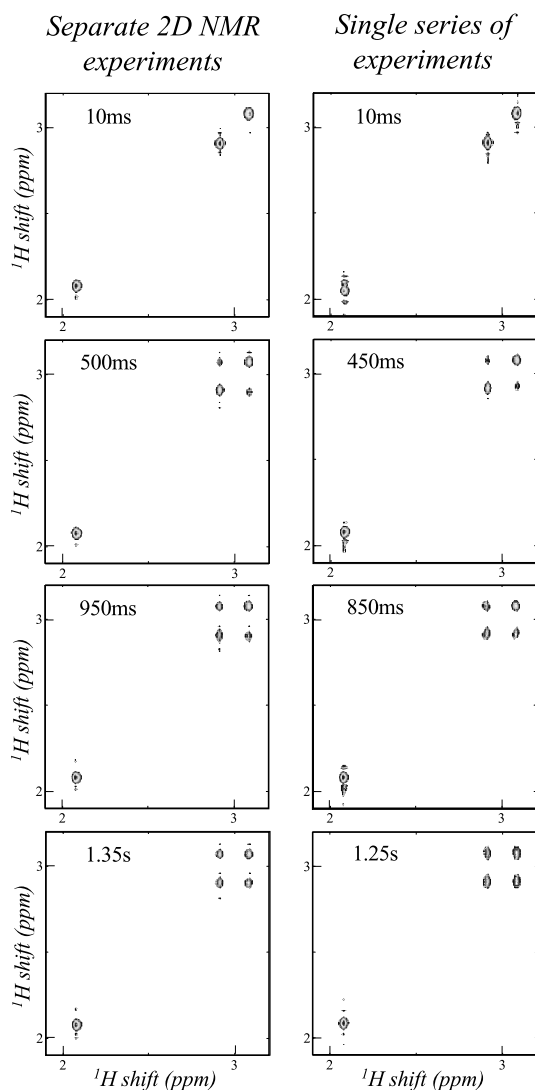


Fig. 3. Comparison between a series of single-scan 2D exchange NMR spectra collected on a DMAc/D₂O solution as a function of the indicated mixing periods (left-hand column), and an analogous series collected within a single experiment utilizing the sequence in Fig. 2 (right). Indicated within each spectrum is the effective mixing time over which the exchange was allowed to take place. Both sets of spectra were acquired with certain common parameters: $T = 318$ K (uncalibrated), $N_1 = 66$ square excitation pulses that were $167 \mu\text{s}$ long and applied at 6 kHz offset increments while in the presence of a $\gamma_e G_e = 180$ kHz/cm z gradient, acquisitions involving $N_2 = 64$ gradient echoes with a $2T_a$ dwell time along t_2 of $560 \mu\text{s}$, an acquisition gradient $\gamma_a G_a = \pm 68$ kHz/cm incorporating $20 \mu\text{s}$ gradient switching times, and a $2 \mu\text{s}$ physical dwell time. For the single-scan experiment a 118 kHz/cm gradient was also applied along the x direction, which in combination with $N_3 = 6$ recall pulses ($200 \mu\text{s}$ long each, 10 kHz offset increments) partitioned the diameter of the 5 mm sample into ≈ 0.51 mm thick slices. The time required to collect each of the 2D NMR experiments shown on the left-hand column was 66 ms plus the indicated duration of the mixing τ_m ; the acquisition of the full series of 2D NMR spectra illustrated on the right-hand column required a total of 1.499 s (including all the nested mixing delays).

of single-scan experiments that could be collected within the limits of our thermal window. Such 2D NMR spectra clearly evidence the emergence of exchange

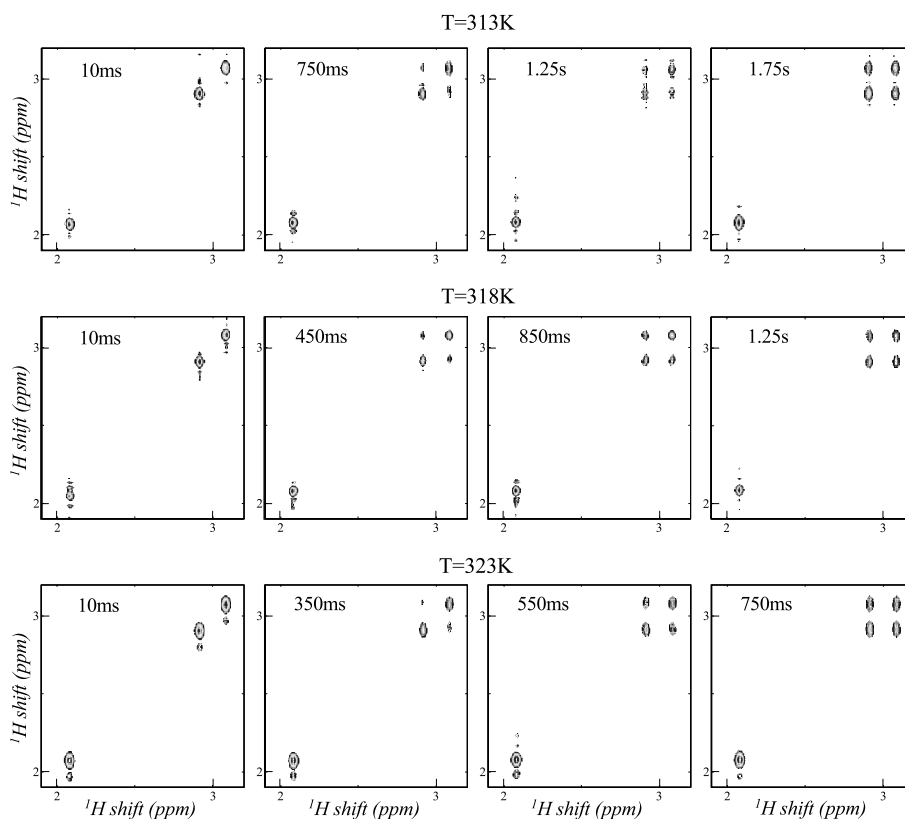


Fig. 4. Series of variable- τ_m ultrafast 2D exchange NMR spectra, recorded on DMAc/D₂O as a function of the sample's temperature. Each of these three sets was collected within a single continuous experiment, using acquisition parameters similar to those detailed in Fig. 3. The duration of each of these experiments—involving the acquisition of six 2D exchange NMR spectra, only four of which are shown—was 2.05, 1.50, and 0.90 s for data collected at 313, 318, and 323 K, respectively.

cross-peaks as a function of mixing, as well as the speeding up of the dynamics with increasing temperatures. The well-known equation that relates the relative cross- and diagonal-peaks intensities with τ_m in this type of experiments [17]

$$\frac{I_{\text{dia}}}{I_{\text{cross}}} = \frac{1 + \exp(-2k\tau_m)}{1 - \exp(-2k\tau_m)}, \quad (1)$$

could then be used to estimate the exchange rate k , and from here conclusions about the process' thermal activation parameters. The plots in Fig. 5 summarize the predictions that the ultrafast NMR experiments in Fig. 4 make for the exchange rates of the amide inversion process. The activation energy resulting from fitting these data, $E_a = 24$ kcal/mol, is comparable to the one reported in the literature for the amide bond inversion of a 10% solution of DMAc in DMSO ($E_a = 22$ kcal/mol, [21]).

The main aim of the present paper was to present an additional example of the potential brought about by the gradient-assisted partitioning of a sample, towards the acceleration of well-established NMR experiments. This time the focus was on a magnetization transfer process, of the kind that is usually monitored by the

systematic incrementation of a parametric recovery or transfer delay. The approach demonstrated for speeding up this acquisition presents evident similarities with the gradient-based methodology recently introduced for speeding up the collection of multi-dimensional NMR data; still, the two schemes also presents a number of significant differences. One of them relates to the fact that in the new experiment hereby discussed, each frequency selective pulse is directly followed by a period of signal acquisition. This implies that the time increments to be employed for monitoring the incoherent magnetization transfer have to exceed the duration of the acquisition, a timing limitation which did not affect the ultrafast 2D NMR acquisitions. The sequence in Fig. 2 also implies that each of the signals observed throughout the multiple acquisitions following the mixing periods will arise from just a portion of the sample rather than, as in the case of the 2D NMR experiments, from the constructive interference of all spins that were initially excited. Both of these limitations could be alleviated if the acquisition along the direct domain were to be endowed with spatially resolving characteristics along the transverse (x) dimension used for monitoring the mixing process. Yet these advantages would come at significant costs both in terms of pulse sequence

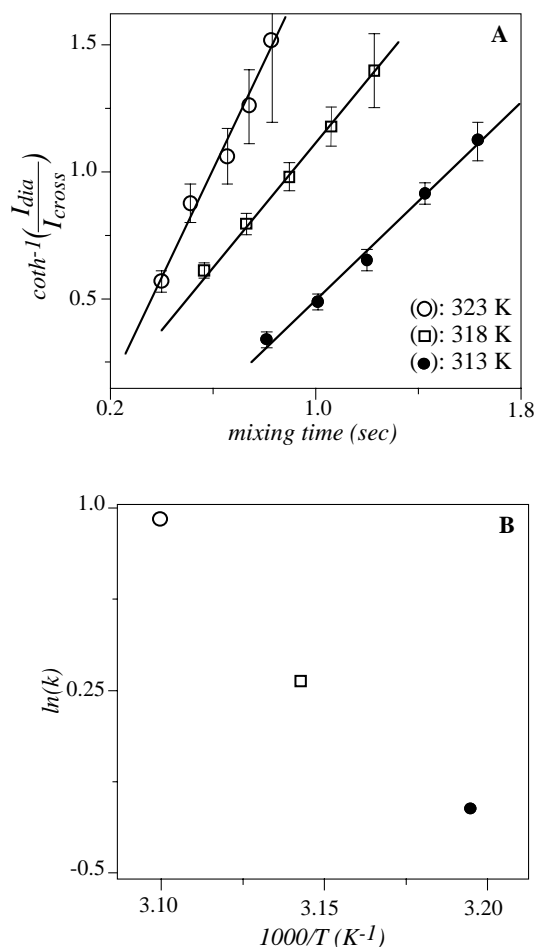


Fig. 5. (A) Fitting of the relative peak intensities arising from the variable-temperature spectra in Fig. 4 to Eq. (1), after recasting the latter into the alternative form $\coth^{-1}(I_{\text{dia}}/I_{\text{cross}}) = k \cdot \tau_m$. The slopes of these linear fits yield the exchange rate k ; error bars are estimates arising from independent data acquisition and processing events. (B) Arrhenius graph resulting for DMAC's amide inversion process in D_2O , from the linear fits plotted in (A).

complexity, and of the subsequent processing of the data [15]. We believe that several applications of interest including analyses of non-equilibrium dynamic systems, quantitative NOE characterizations, or studies on large arrays of samples or on unstable NMR spectrometers, could find useful the simpler methodology introduced in the present work.

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Note added in proof

While this paper was in press, an additional group of single-scan T1 measurement methods has also been discussed: N.M. Loening, M.J. Thrippleton, J. Keeller, R.G. Griffin, *J. Magn. Reson.* 164 (2003) 321.

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