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Two-dimensional Hadamard spectroscopy

Ēriks Kupče[a] and Ray Freeman[b,*]

[a] *Varian, Inc., Eynsham, Oxford, UK*
[b] *Jesus College, Cambridge University, Jesus Lane, Cambridge CB5 8BL, UK*

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Abstract

Direct frequency-domain excitation of NMR with an array of different radiofrequencies has been used to speed up two-dimensional NMR experiments by a large factor. Multiplex excitation in the F_1 frequency dimension is restricted to the signal-bearing regions and is encoded according to a Hadamard matrix of dimension N by N, where N is a relatively small number. The detected signals are decoded by reference to the same Hadamard matrix. Alternatively a phase-encoding scheme can be employed. Two-dimensional correlation experiments (COSY and TOCSY) and cross-relaxation measurements (NOESY) implemented on proton systems can be completed in less than a minute in cases where the intrinsic sensitivity is sufficiently high that prolonged multiscan averaging is not required. The results are presented in the form of a high-resolution contour diagram similar to the familiar two-dimensional spectra obtained by Fourier transform methods. Experiments on strychnine demonstrate more than two orders of magnitude improvement in speed compared with the traditional methods.

Keywords: Hadamard spectroscopy; Fast two-dimensional spectra; Multichannel excitation; Phase-encoding; COSY; NOESY; TOCSY

1. Introduction

Two-dimensional NMR spectroscopy is widely used in chemical and biochemical laboratories world-wide. As predicted by Jeener [1] the inherent sensitivity of these experiments is broadly equivalent to traditional one-dimensional NMR spectroscopy because the rate of data collection is near optimum. However, when two-dimensional techniques are applied to reasonably strong samples in high-field spectrometers, the minimum *duration* of the experiment is often much longer than that dictated purely by signal-to-noise considerations. This is because a large number of t_1 increments must be examined in order to achieve satisfactory definition and spectral width in the F_1 dimension, and some phase cycling is usually required. Yet many applications require only a relatively small number of correlations or cross-relaxation measurements to solve a particular molecular structural problem, and much of the detailed information is essentially redundant. There is much to be gained by speeding up these experiments.

Frequency-domain Hadamard spectroscopy [2] offers a solution to this dilemma without impairing sensitivity per unit time. Instead of conventional hard-pulse excitation followed by free precession, the F_1 dimension is addressed directly with an array of frequency-selective radiofrequency pulses. These multiple excitation "channels" need not be regularly spaced across the spectrum but can be restricted to the chemical sites that give rise to the interesting correlations. An exploratory one-dimensional low-resolution Fourier transform spectrum quickly identifies the signal-bearing regions and a peak-picking routine finds the required irradiation frequencies; alternatively they may be designated by the operator. Only a relatively small number of excitation channels are needed. This speeds up the experiment by eliminating the usual requirement that the entire F_1 frequency dimension be explored uniformly and in detail. This advantage is particularly marked in high-field

* Corresponding author. Fax: +44-1223-336362.
E-mail address: rf110@cam.ac.uk (R. Freeman).

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spectrometers and for nuclei such as fluorine-19, carbon-13 or phosphorus-31, where very wide spectral ranges are involved.

2. Encoding methods

A single frequency-selective pulse can be implemented by means of a radiofrequency waveform generator, roughly equivalent to—the DANTE scheme [3]. Selectivity is governed by the duration of the sequence, typically of the order of 100 ms in the present experiments. The effective irradiation frequency is offset from the carrier by incrementing the radiofrequency phase in small equal steps along the sequence [4]. An array of M different irradiation frequencies can be devised by combining M sequences with the same pulse repetition rate but different rates of phase ramping. At each step of the sequence, M individual radiofrequency pulses are combined by vector addition. The result is a "polychromatic" pulse sequence [5,6] which serves as the excitation stage for the two-dimensional experiment.

2.1. Hadamard encoding

As described in an earlier publication [2] this method of frequency-domain excitation involves encoding the radiofrequency channels according to the signs in an N by N Hadamard matrix [7]. Mathematicians confidently believe that Hadamard matrices exist for all orders $N = 4k$, where k is an integer; the lowest-order matrix not yet discovered has $N = 268$ [8]. Perhaps the best-known Hadamard matrices are those for $N = 2^m$ where m is an integer. If B is a Hadamard matrix and $-B$ is the same matrix with inverted signs, then

$$\begin{matrix} +B & +B \\ +B & -B \end{matrix}$$

is also a Hadamard matrix. In the applications described below, the Hadamard matrix is quite small and can be quickly calculated, and only a few selective radiofrequency pulses are needed, so they can be programmed quite rapidly. This is all implemented before the two-dimensional measurements are started.

Suppose there are M chemical sites of interest. A Hadamard matrix is selected such that $N > M$ and the program allocates M irradiation channels to the appropriate chemical shift frequencies. Before the individual soft pulse sequences are combined into a polychromatic sequence, the radiofrequency pulses are coded (+ or −, or in some cases on or off) according to the rows of the Hadamard matrix. For example, for $N = 8$, the matrix would be

Channel:	1	2	3	4	5	6	7	8
Scan (1)	+	+	+	+	+	+	+	+
Scan (2)	+	+	+	+	−	−	−	−
Scan (3)	+	+	−	−	+	+	−	−
Scan (4)	+	+	−	−	−	−	+	+
Scan (5)	+	−	+	−	+	−	+	−
Scan (6)	+	−	+	−	−	+	−	+
Scan (7)	+	−	−	+	+	−	−	+
Scan (8)	+	−	−	+	−	+	+	−

The measurement requires eight successive scans with the corresponding eight signal acquisitions. Each scan generates a composite response made up of NMR signal components from all M active channels (the "all-plus" channel is not normally used). Because a different coding pattern is employed for each scan, this provides a method for extracting the NMR signal in a particular frequency channel while rejecting signals in all other channels. This "magic" property of Hadamard matrices can be appreciated by an example from the above matrix—if the sum of the composite signals from even-numbered scans is subtracted from the sum of the responses from odd-numbered scans, then only the signal from channel five is retained. All the rest vanish.

Fig. 1 is a schematic diagram outlining the procedure for two-dimensional Hadamard spectroscopy. The method is described in terms of homonuclear systems, although the same principles apply to heteronuclear experiments. After direct frequency-domain excitation the conventional COSY, TOCSY or NOESY pulse sequences take over, but without the usual evolution period. The output from the receiver is a set of N different composite free induction decays, each one a superposition of responses from all the M chemical sites. These are decoded by reference to the appropriate columns of the Hadamard matrix. A total of N scans is required because all N composite signals are needed for decoding, but since M is less than N, some of the channels are not used. This highlights the importance of choosing the smallest Hadamard matrix to accomplish the task in hand, in order to keep the overall duration of the measurement as short as possible. In all cases N is much less than the number of t_1 increments required in the corresponding conventional two-dimensional experiment; this is the main reason why the Hadamard mode is so much faster.

The decoding stage separates the individual free induction decays, one from each site, and Fourier transformation as a function of t_2 converts them into traces running in the F_2 dimension. These can be used directly to illustrate correlations or nuclear Overhauser effects. Whereas conventional correlation spectra are limited in resolution in the F_2 dimension by practical restrictions on the total size of the two-dimensional data matrix, there are so few Hadamard F_2 traces that such limitations do not apply. Consequently, direct frequency-do-

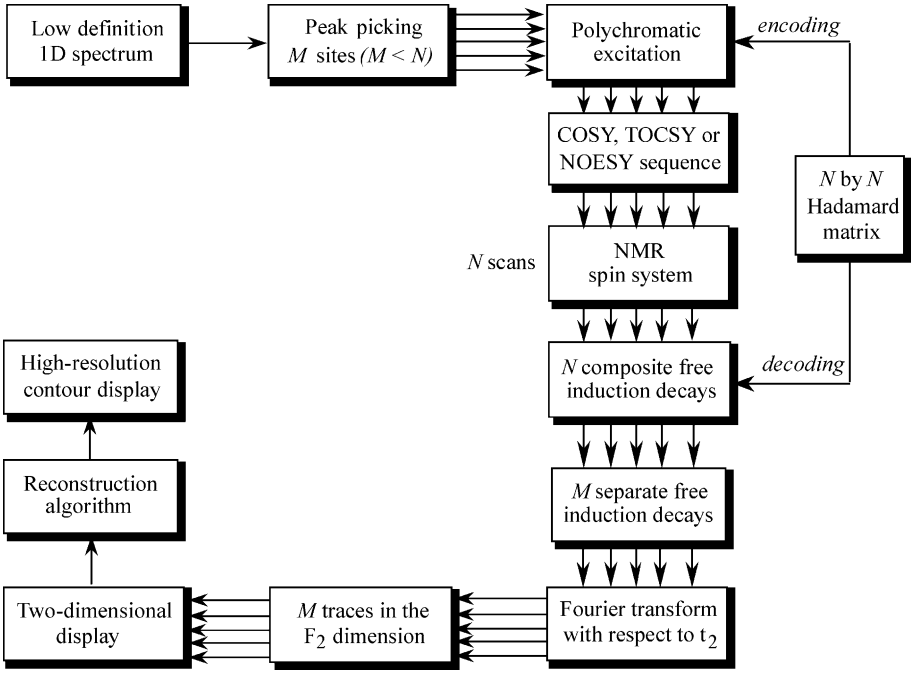

Fig. 1. Schematic diagram for two-dimensional Hadamard spectroscopy. The final result is a contour map very similar to that obtained by conventional two-dimensional methods, but completed in a much shorter time.

main excitation has much to offer for the investigation of fine structure.

The F_2 traces can be displayed in the form of a two-dimensional spectrum by locating them at the appropriate F_1 ordinates and converting the result into a contour map showing all the correlations but without fine structure in the F_1 dimension. This form of Hadamard spectrum should be well-suited to the creation of a library of two-dimensional spectra, and the rapid acquisition of these spectra would facilitate the identification of unknown compounds by reference to that library. In situations where not all the correlations are needed, more spectrometer time can be saved by limiting the excitation to the important sites, with a corresponding reduction in the size of the Hadamard matrix.

Apart from the lack of fine structure in the F_1 dimension, this form of Hadamard spectrum is virtually equivalent to a classic two-dimensional Fourier transform spectrum recorded with a large number of increments in the t_1 dimension, and yet the Hadamard scheme uses only a very small number of scans and is completed in a much shorter time. By concentrating attention on selected signal-bearing regions, direct excitation in the frequency domain offers a flexibility that is denied to the traditional "evolution time" methodology. The sensitivity per unit time is essentially the same as in the conventional mode, since both techniques benefit from the multiplex advantage [9].

Traditional two-dimensional spectroscopy is known to be susceptible to certain "noisy" artifacts generated by spectrometer imperfections, usually grouped together under the general term t_1 noise [10]. Hadamard spectroscopy is similarly sensitive to small changes in spectrometer conditions between one scan and the next, but these may be reduced to some extent by the sign alternation in the columns of the Hadamard matrix. Some baseplane noise is suppressed in the two-dimensional Hadamard spectrum because the regions between the active chemical sites are not excited, but this is *not* a sensitivity advantage.

Hadamard encoding acts in much the same way as the phase cycles that are widely employed in conventional spectroscopy, suppressing several undesirable artifacts. This is why the "all plus" column of the matrix is not normally used. Additional phase cycling is usually unnecessary, thus shortening the overall duration of the measurements. Furthermore, the usual quadrature detection in the F_1 dimension is not employed in the Hadamard mode because the signs of the irradiation frequencies are defined by the senses of the phase ramps. These two factors contribute to a further increase in speed compared with the traditional method. There is another advantage of the Hadamard technique—it is easy to exclude the strong solvent peaks [2] that often cause problems in two-dimensional spectroscopy.

There is one potential drawback of direct frequency-domain excitation. It has been shown [11–16] that during simultaneous irradiation of two coupled sites a new phenomenon comes into play called the double-resonance two-spin effect (TSETSE). In contrast to hard radiofrequency pulses, soft pulses allow evolution of

J-coupling, and because of the longer pulse duration, further conversions occur. A key parameter is the product of the pulse duration and the spin coupling constant. The first manifestation is a build-up of anti-phase dispersion terms, and on a longer time scale, longitudinal magnetization and multiple-quantum terms make their appearance. For studies of coherence transfer, TSETSE constitutes an interference phenomenon that may slightly alter the cross-peak intensities. In cross-relaxation studies it can give rise to undesirable cross-peaks similar to those caused by coherence transfer in conventional NOESY spectra. Both types of artifact are significantly reduced by the application of pulsed magnetic field gradients. Relatively short spin inversion pulses (45 ms) are used in Hadamard-NOESY experiments in order to minimize TSETSE effects. Although some weak TSETSE responses could be detected in the Hadamard spectra, they are no worse than coherence transfer artifacts in the conventional mode, and appear to pose no significant problems.

2.2. Phase-encoding

There is an alternative to Hadamard encoding that has the practical advantage that N may be any integer greater than two. The soft excitation pulses are phase-encoded [17] in steps of $2\pi jk/N$, where j and k are integers less than N. This is repeated in N successive scans with new codes derived from a progressive phase matrix. The linear phase ramp in each irradiation channel essentially defines a "virtual" frequency that acts as a unique label for that channel. The N composite responses are decoded by reference to the same phase matrix. For example, when seven different chemical sites (A–G) are involved, the radiofrequency pulse phase is incremented in steps of $2\pi/7$ in the second scan, $4\pi/7$ in the third scan, and so on until all seven scans are completed:

	A	B	C	D	E	F	G
Scan (1)	0	0	0	0	0	0	0
Scan (2)	0	$2\pi/7$	$4\pi/7$	$6\pi/7$	$8\pi/7$	$10\pi/7$	$12\pi/7$
Scan (3)	0	$4\pi/7$	$8\pi/7$	$12\pi/7$	$2\pi/7$	$6\pi/7$	$10\pi/7$
Scan (4)	0	$6\pi/7$	$12\pi/7$	$4\pi/7$	$10\pi/7$	$2\pi/7$	$8\pi/7$
Scan (5)	0	$8\pi/7$	$2\pi/7$	$10\pi/7$	$4\pi/7$	$12\pi/7$	$6\pi/7$
Scan (6)	0	$10\pi/7$	$6\pi/7$	$2\pi/7$	$12\pi/7$	$8\pi/7$	$4\pi/7$
Scan (7)	0	$12\pi/7$	$10\pi/7$	$8\pi/7$	$6\pi/7$	$4\pi/7$	$2\pi/7$

Note that the cyclic property makes$16\pi/7$ equivalent to $2\pi/7$, and so on. Seven composite free induction decays (real and imaginary components) are acquired and stored in separate memory locations.

In the original description of this technique [17] decoding was implemented by a phasing program "*phfid*" which takes the appropriate combination of real and imaginary parts of the free induction decays, acting as a variable receiver reference phase. The composite signals were separated into "pure" responses derived from individual chemical sites by combining suitably phase-shifted versions according to the angles in the columns of the phase matrix. In practice an unconventional but effective alternative procedure was adopted here. Because each irradiation channel is labeled with its unique phase ramp, Fourier transformation of the composite time-domain responses with respect to the scan index separates them into M pure responses, spreading them out at *regular* intervals in a "virtual" frequency dimension. This is not the "true" F_1 frequency axis, for that is defined by the actual irradiation frequencies used for each chemical site, but the virtual spectrum can be

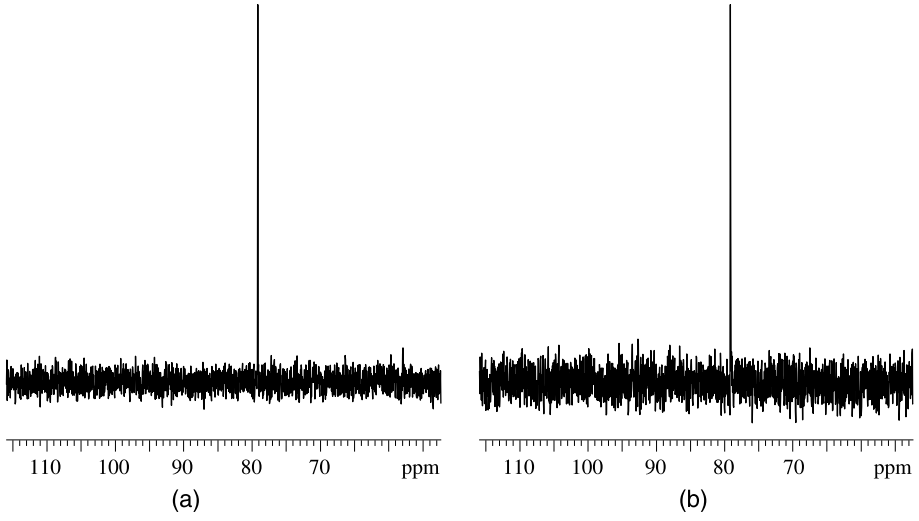

Fig. 2. Comparison of a carbon-13 response measured by frequency-domain multiplex excitation (a) with Hadamard encoding and (b) with phase-encoding. Because the Hadamard technique monitors the full nuclear magnetization vector whereas the phase-encoding technique has to detect a rotating vector, in principle the Hadamard method is $\sqrt{2}$ times more sensitive. The measured ratio of signal-to-noise coefficients is 1.47.

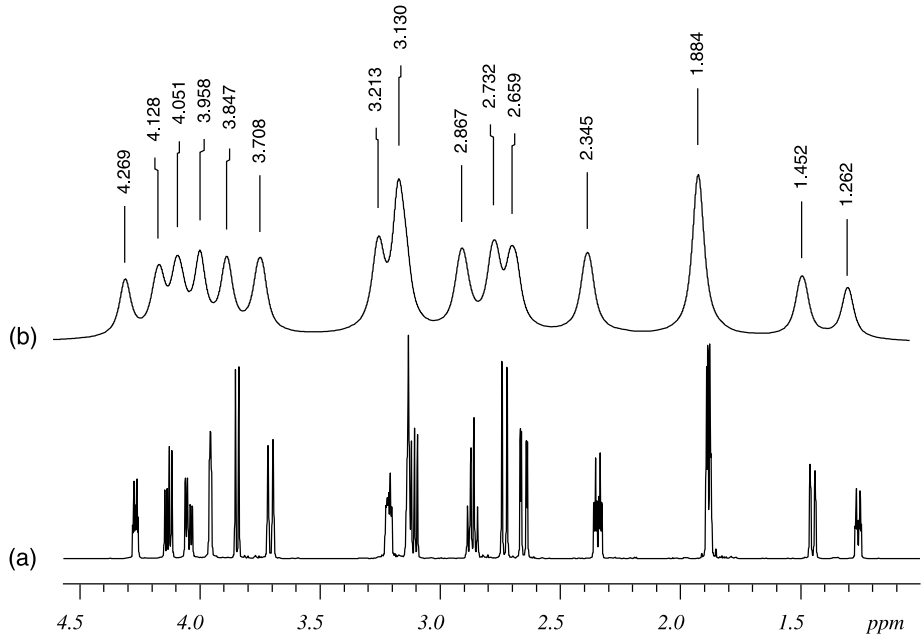

Fig. 3. (a) Part of the 700 MHz proton spectrum of strychnine. (b) The low-resolution spectrum (45 Hz linewidth) used to enable an automatic peak-picking routine to select suitable irradiation frequencies for the different chemical sites.

converted into the conventional format by reintroducing the appropriate gaps between the excitation frequencies.

The analogy with the Hadamard scheme is quite close, except that the order of the phase matrix N can now be set equal to the number of sites M so that no columns are wasted unnecessarily, allowing the minimum number of scans to be used. However, in order to detect the full amplitude of the rotating magnetization vector the phase-encoding method requires the acquisition of both real and imaginary signals, which doubles the duration of the experiment. Consequently, the sensitivity of the phase-encoding scheme is $\sqrt{2}$ times lower than that of the Hadamard method, reflecting the difference between the detection of a rotating vector and one that alternates plus and minus. This sensitivity difference has been observed experimentally for a one-dimensional carbon-13 spectrum (Fig. 2). It is interesting to note that conventional two-dimensional Fourier transform spectroscopy also relies on phase-encoding in the indirect frequency dimension, so in principle it is less sensitive than Hadamard spectroscopy by a factor $\sqrt{2}$, unless special techniques [18] are employed to enhance the sensitivity of the conventional mode.

3. Experimental

Illustrative two-dimensional spectra were recorded on the extensively studied proton spectrum of strychnine on a Varian INOVA 700 spectrometer. Attention was focused on the crowded region between 1.0 and 4.5 ppm containing 17 distinct proton sites. The exploratory one-dimensional Fourier transform spectrum used 45 Hz line-broadening (Fig. 3b), which had the effect that the close resonances from two protons near 1.88 ppm coalesced into a single response, as did the overlapping resonances from two protons near 3.13 ppm. Consequently, the automatic peak-picking routine found only 15 distinguishable sites. This required encoding with a

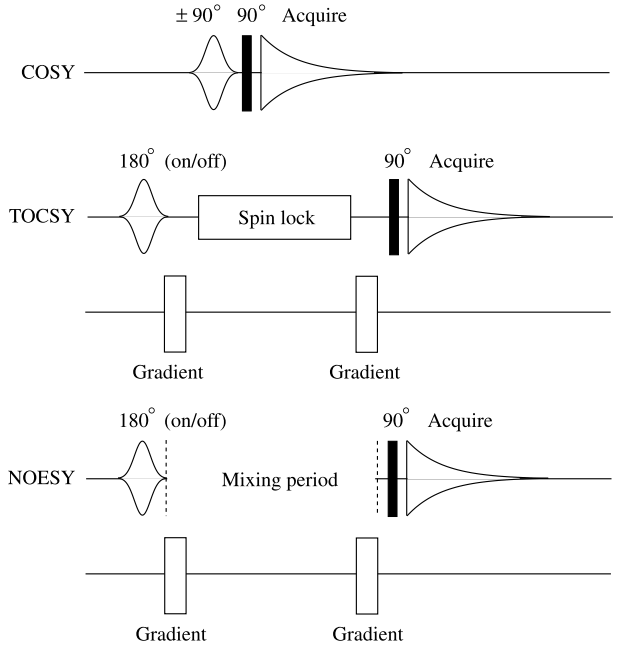

Fig. 4. Pulse sequences used for frequency-domain Hadamard spectroscopy. The initial soft Gaussian pulses consist of 15 separate irradiation frequencies encoded ±90° for COSY or 180° (on/off) for NOESY and TOCSY, according to the signs in the appropriate row of the Hadamard matrix.

Hadamard matrix of order 16, with 15 separate selective irradiations and 16 successive scans.

The standard COSY technique generates antiphase transverse magnetization by J-evolution during t_1. In the new Hadamard method J-evolution takes place during the soft polychromatic excitation pulse of fixed duration (Fig. 4). A Gaussian pulse shape is employed for this purpose [19]. Its duration contributes little to the length of the experiment and relaxation losses during the pulse are negligible. The radiofrequency pulses in the 15 separate irradiation channels are set to +90° or −90° according to the signs in the appropriate row of the Hadamard matrix. A final hard 90° pulse implements the coherence transfer ($2I_XS_Z \rightarrow -2I_ZS_X$ in the product operator formalism), generating a free induction decay made up of 15 different interfering responses with relative signs that match the encoding sequence. After 16 scans have been acquired, each with its unique code, the decoding stage separates out 15 "pure" free induction decays. The standard Fourier transform program converts these into 15 high-resolution F_2 traces. These have better digital definition than traces from the conventional COSY spectrum because more data points can be assigned to the free induction decays without creating a large two-dimensional data array. This is an advantage when the fine structure is of interest. A typical COSY trace is illustrated in Fig. 5.

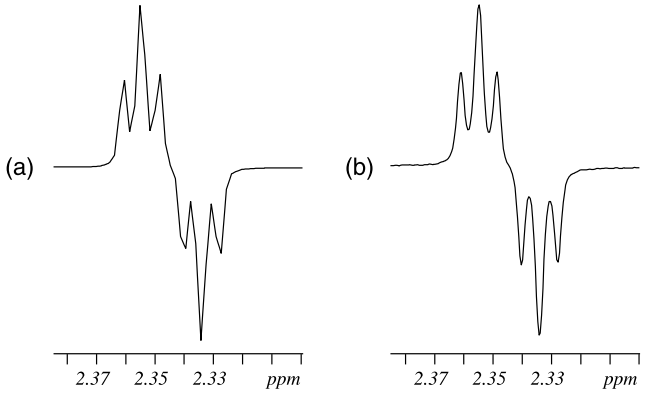

Fig. 5. A typical F_2 trace from the COSY spectrum of strychnine (a) in the conventional mode where the digital definition is limited by practical restrictions of the size of the two-dimensional data array and (b) in the Hadamard mode where fine digitization is readily achieved because only 15 traces are recorded.

Fig. 6 shows the 700 MHz high-resolution COSY spectrum obtained by the Hadamard method compared with the spectrum obtained by the conventional methodology. The latter is slightly marred by a false cross-peak attributed to folding in the F_1 dimension. Otherwise the two spectra are largely comparable. Long-duration soft excitation pulses (125 ms in this case) generate a relatively high level of antiphase magnetization from the smaller coupling constants, emphasizing the intensities of these cross-peaks relative to those from the large coupling constants. This method of enhancing the weak cross-peaks is similar to the "pseudo-echo" technique sometimes employed in conventional correlation spectroscopy [20].

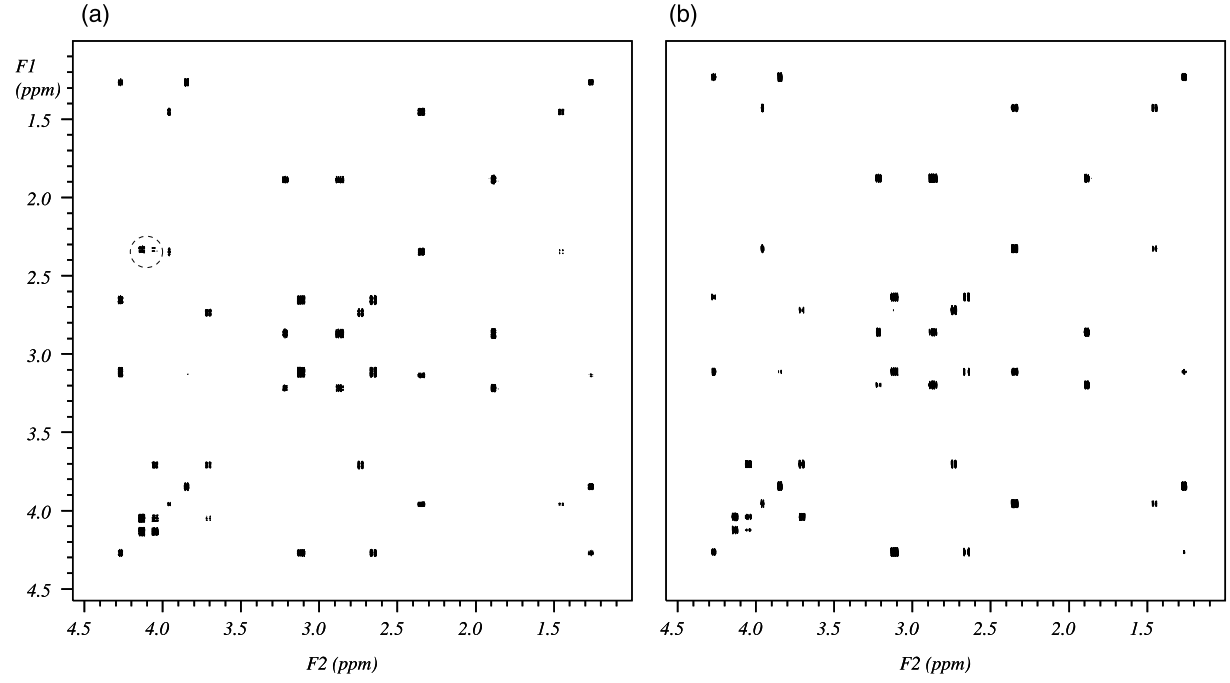

Fig. 6. (a) Part of the conventional 700 MHz proton COSY spectrum of strychnine obtained in 2 h 46 min. The dashed circle indicates a response folded in F_1. (b) The corresponding Hadamard COSY spectrum derived from 15 F_2 traces and obtained in only 44 s.

It is instructive to examine how much each experimental parameter contributes to the time-saving in the Hadamard mode. The most important factor is the ratio of the number of t_1 increments in the standard COSY experiment (512) compared with only 16 Hadamard scans. Quadrature detection in the conventional mode doubles the number of required scans and phase cycling increases this fourfold. The mean value of the evolution time in the conventional mode (102 ms) and the soft pulse duration in the Hadamard mode (125 ms) have only a minor effect on the timing. In both modes the relaxation intervals were set at 1.5 s and the acquisition time was 0.82 s. Two dummy scans were used to establish a steady-state; this was considered important to ensure accurate subtraction in the Hadamard mode. The conventional COSY spectrum used a total of 4098 scans (4096 active scans) whereas the Hadamard experiment, with no quadrature detection or phase cycling, employed only 18 scans (16 active scans). Consequently, the Hadamard experiment was completed in only 44 s. This is to be compared with 2 h 46 min for the conventional COSY spectrum, a time saving factor of 225. The much longer accumulation time means that the signal-to-noise ratio is higher in the traditional COSY mode, but this is of little consequence in cases like this where the sensitivity is already more than adequate.

Total correlation spectroscopy (TOCSY or HOHAHA) relies on preparing a differential perturbation at two interacting sites prior to isotropic mixing. In the conventional mode this is achieved by chemical shift evolution during t_1, but in the Hadamard mode a quite different preparation scheme is used. Selective 180° pulses are applied at the sites designated by "+" in the first row of the Hadamard matrix, while the sites represented by "−" are not perturbed. Consequently, isotropic mixing only takes place between those pairs of interacting sites that have experienced a differential perturbation. In the remaining pairs, the populations at both sites are inverted or both remain unperturbed, so there is no TOCSY effect. The coding is changed for every subsequent scan until all 16 have been completed, by which time all possible interactions have been probed eight times.

The pulse sequence is set out in Fig. 4. After two dummy scans to establish steady-state conditions, a 45 ms 180° Gaussian polychromatic pulse initiates the experiment and a pulsed field gradient dissipates any transverse magnetization. This is followed by a 120 ms isotropic mixing period employing WURST-2 adiabatic pulses [21], an acquisition time of 0.82 s, and a relaxation delay of 1.5 s. The overall experimental duration is 42 s, compared with 2 h 47 min for the conventional TOCSY spectrum which uses 512 increments in the evolution period, phase cycling and quadrature detection. The time-saving factor is 239. Fig. 7 compares the two-dimensional Hadamard-TOCSY spectrum of strychnine with the conventional TOCSY result. Apart from the folded response in the conventional spectrum and the different form of the diagonal peaks, the two results are broadly equivalent.

Hadamard cross-relaxation spectroscopy (NOESY) requires a differential preparation of the spin populations

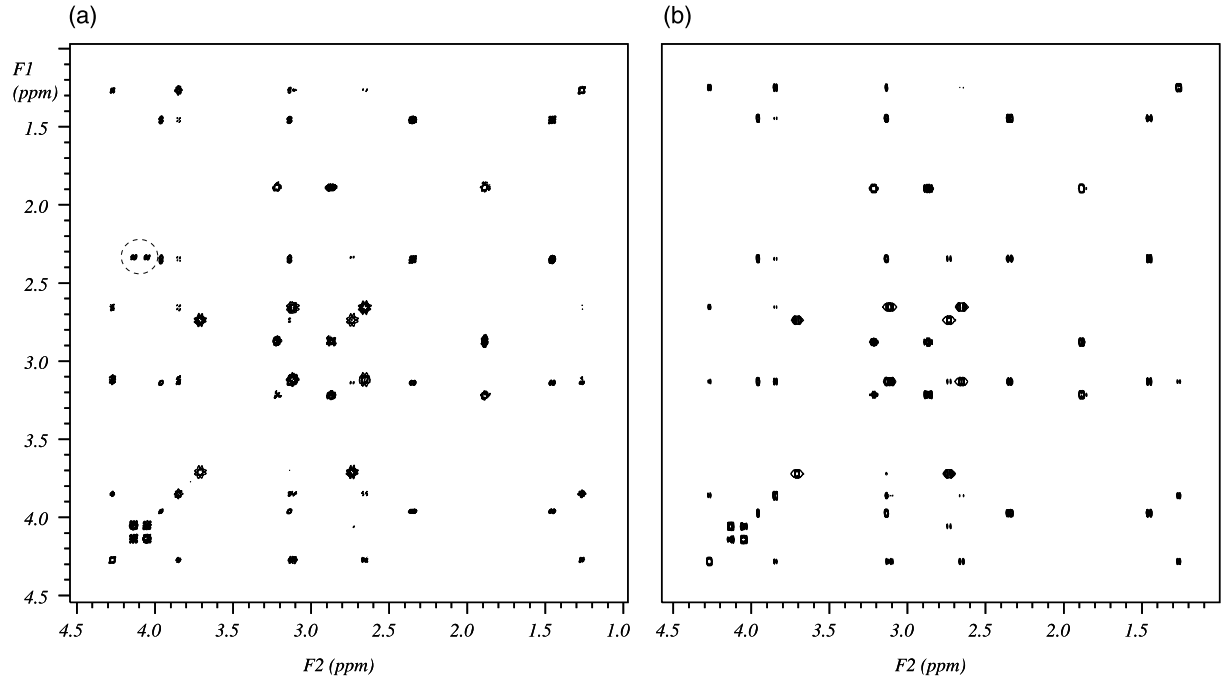

Fig. 7. (a) Part of the conventional 700 MHz proton TOCSY spectrum of strychnine obtained in 2 h 47 min. The dashed circle indicates a response folded in F_1. (b) The Hadamard TOCSY spectrum derived from 15 F_2 traces and obtained in only 42 s.

at each pair of interacting sites, so the pulse sequence is similar to that employed for the TOCSY experiment (Fig. 4). It is initiated with a 45 ms 180° Gaussian polychromatic pulse that inverts populations at those sites represented by "+" in the appropriate row of the Hadamard matrix, leaving the rest unaffected. A 400 ms mixing period allows the build-up of intensity in cross-peaks through the transient nuclear Overhauser effect for those pairs of sites prepared differentially. The coding is changed for each subsequent scan and after16 active scans, all possible cross-relaxation effects have been investigated eight times. The resulting F_2 traces contain all the required cross-relaxation and fine-structure information. A typical Hadamard F_2 trace is compared with the corresponding conventional NOESY trace in Fig. 8, where it can be seen that although the signal-to-noise ratio is somewhat lower in the Hadamard mode (as expected) it is still more than adequate for the purpose.

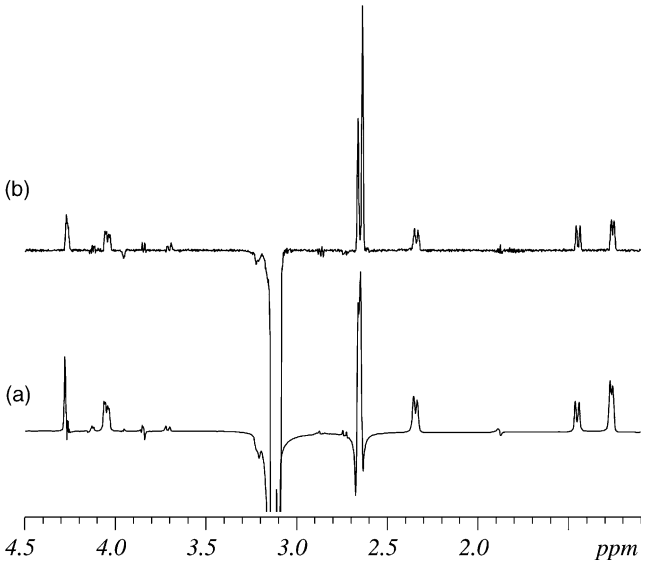

Fig. 8. The F_2 trace at $F_1 = 3.12$ ppm taken from the 700 MHz NOESY spectra of strychnine (a) in the conventional mode and (b) by direct frequency-domain excitation and Hadamard encoding. Note that the conventional method, which accumulates data for a much longer time, has a better signal-to-noise ratio. The response at 2.65 ppm shows evidence of undesirable coherence transfer that is absent in the Hadamard mode.

A two-dimensional Hadamard-NOESY spectrum is compared with the conventional NOESY spectrum in Fig. 9. Both the conventional and Hadamard techniques employ pulsed field gradients to reduce the intensity of TSETSE [16] and coherence transfer peaks, respectively. The short duration of the selective inversion pulses (45 ms) helps minimize TSETSE effects. Note that both spectra show evidence of artifactual cross-peaks arising from incompletely suppressed coherence transfer and TSETSE effects (the circled responses). The diagonal peaks are less obtrusive in the Hadamard mode. Whereas the Hadamard experiment requires only 49 s to complete, the equivalent conventional NOESY measurements take 3 h and 13 min. The time saving factor is therefore 236.

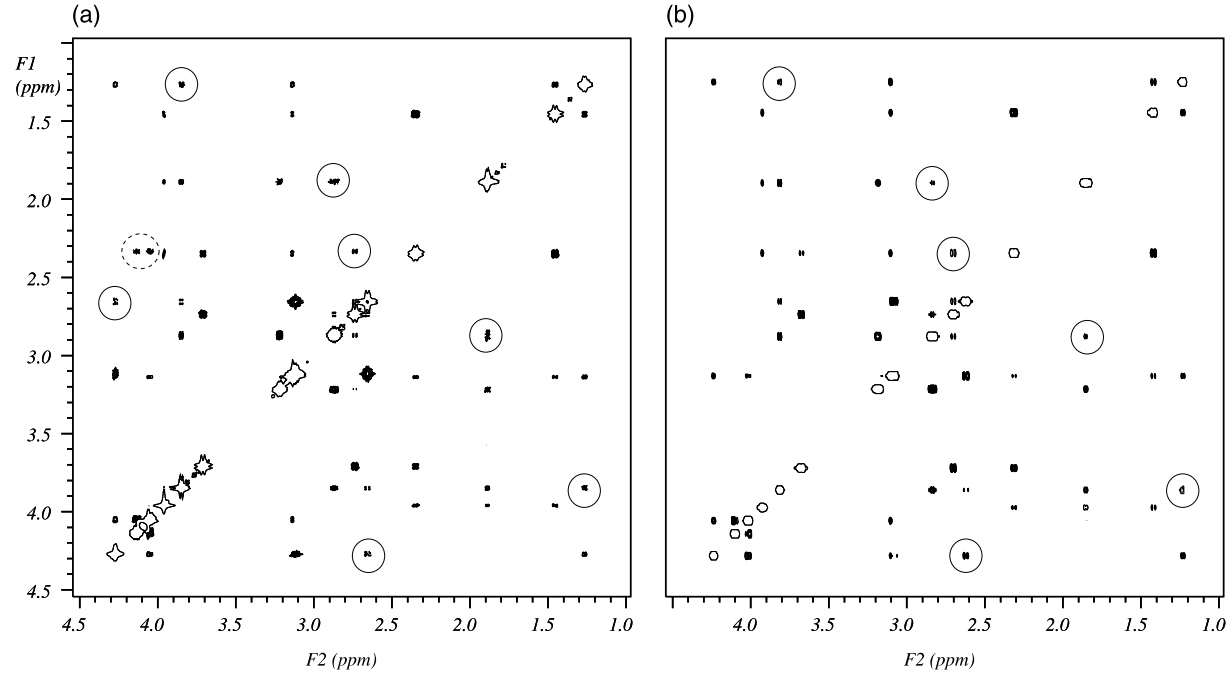

Fig. 9. (a) The conventional 700 MHz proton NOESY spectrum of strychnine obtained in 3 h 13 min. The dashed circle indicates a response folded in F_1. (b) The corresponding Hadamard NOESY spectrum derived from 15 F_2 traces and obtained in only 49 s. Diagonal peaks are negative and are denoted by a single open contour. Both spectra show artifact peaks (circled) from residual coherence transfer (a) and the TSETSE effect (b).

3.1. High-resolution two-dimensional spectra

Chemists have become accustomed to two-dimensional NMR spectra that have fine structure in both frequency dimensions. Although the Hadamard experiment only generates traces running in the F_2 dimension, these can be used to reconstruct the entire *high-resolution* contour display by exploiting the intrinsic symmetry properties of two-dimensional spectra. Cross-peaks occur in matched pairs, representing $I \rightarrow S$ and $S \rightarrow I$ transfer, where I and S are coupled or related by cross-relaxation. They lie in symmetrically related positions on opposite sides of the principal diagonal and their basic structures, neglecting line-broadening effects, are related by a 90° rotation. Consequently, in the Hadamard experiment, the $I \rightarrow S$ trace running in the F_2 dimension has the same form as a *hypothetical* $I \rightarrow S$ trace running in the F_1 dimension. The geometric form of a cross-peak is such that it can be reconstructed from correlation information carried in a related pair of $I \rightarrow S$ and $S \rightarrow I$ traces that both run in the F_2 dimension [22,23]. All that is needed to create the analogue of a conventional spectrum is a processing scheme to reorganize this information, and an existing symmetrization program does just this.

Reconstruction is a global operation that symmetrizes the entire two-dimensional spectrum as if it had been folded about the diagonal, retaining only those signals that are exactly superimposed by the folding process. Intensities of pixels that are symmetrically related with respect to the diagonal are compared and replaced with the lower of the two values. To illustrate this process, consider just the two F_2 traces that carry the information about a given cross-peak. In an extension of the earlier technique [22,23] the appropriate sections of these F_2 traces are convoluted in the F_1 dimension with a rectangular function of width equal to the excitation bandwidth (45 Hz in this case). This

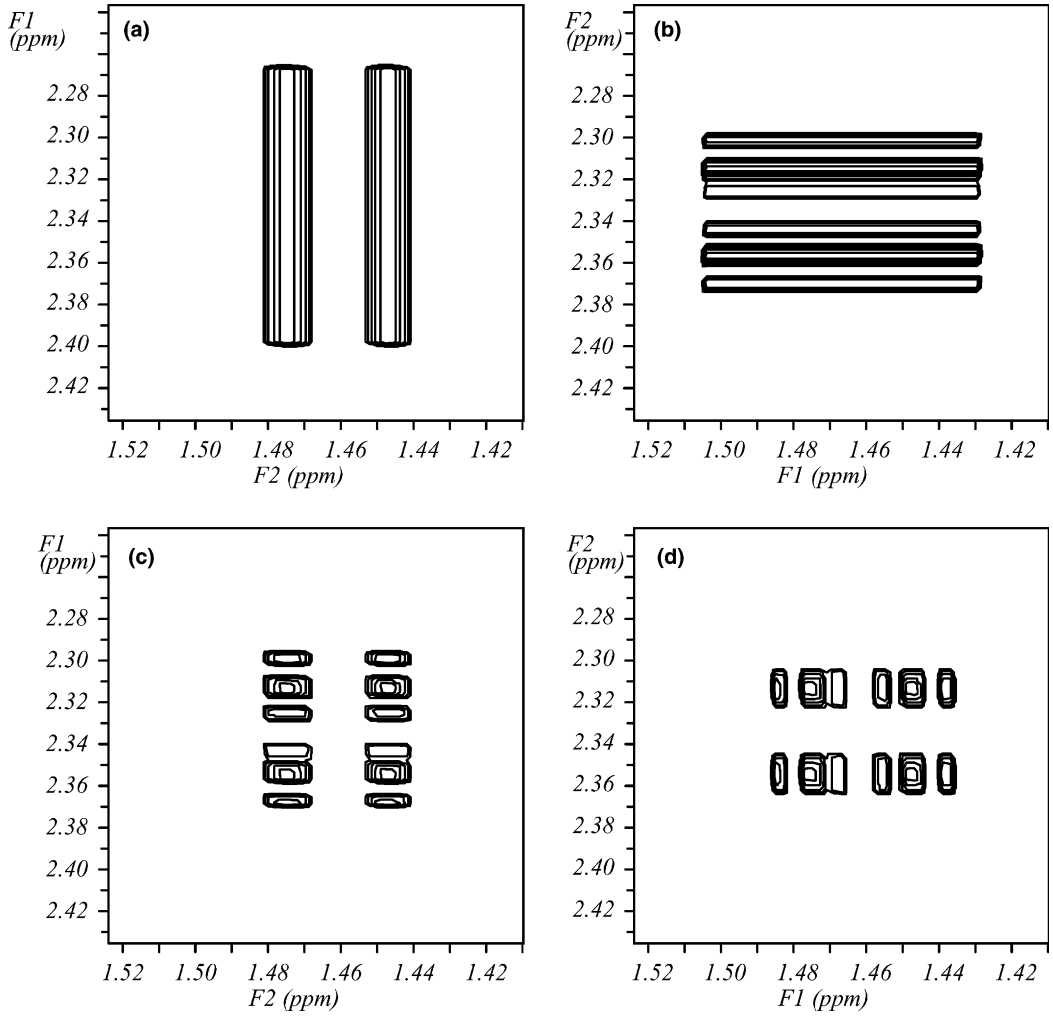

Fig. 10. Illustration of the cross-peak reconstruction method. (a) A Hadamard-TOCSY F_2 trace is convoluted with a rectangular function in the F_1 dimension, creating elongated contours. (b) The same procedure is applied to another F_2 trace that carries the related correlation information. Folding about the diagonal causes the 90° rotation. (c) The data arrays (a) and (b) are superimposed and the intensities at each location are compared and replaced by the lower value, leaving only well-resolved fine structure. (d) The mirror-image cross-peak is derived by a 90° rotation.

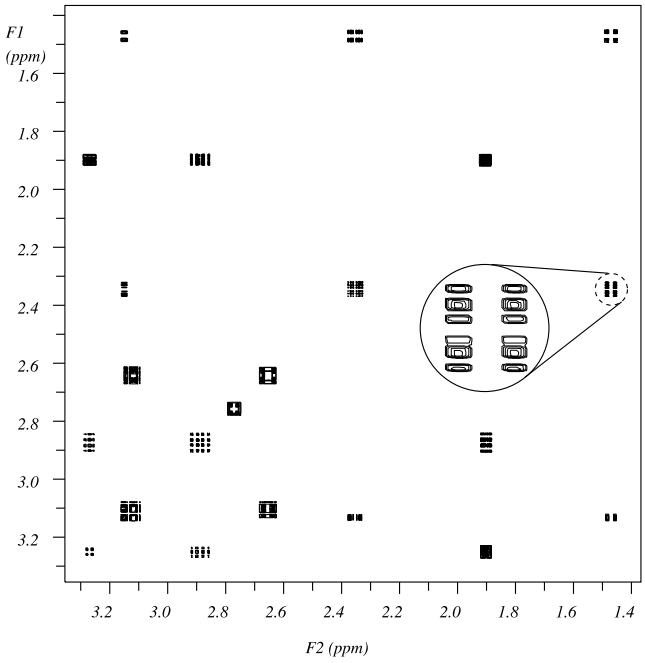

Fig. 11. High-resolution Hadamard-TOCSY spectrum of strychnine in the crowded region between 1.4 and 3.3 ppm. The symmetrization program has been used to reconstruct the fine structure of the cross-peaks, one of which is shown on expanded frequency scales.

generates the elongated contours shown in the two data arrays of Figs. 10a and b. The folding operation superimposes the pixels of Fig. 10a onto those in Fig. 10b and the lower-value algorithm suppresses pixels where there is no overlap of intensity, leaving only a well-resolved cross-peak (Fig. 10c). The corresponding cross-peak on the opposite side of the diagonal is obtained simply by a 90° rotation (Fig. 10d). When this symmetrization routine is applied to all the Hadamard traces the resulting two-dimensional spectrum has well-resolved fine structure in both dimensions. There is always the danger that a very weak correlation could be overlooked unless *both* $I \rightarrow S$ and $S \rightarrow I$ traces are recorded and carry detectable responses, but weak cross-peaks are also lost in conventional correlation spectra if they fall below the lowest contour level.

Fig. 11 shows how this reconstruction procedure alters the presentation of one region of the Hadamard-TOCSY spectrum of strychnine. As can be appreciated from the expansion of one of the cross-peaks, the multiplet structure is at least as detailed as that expected in the conventional TOCSY mode, although all the NMR information was collected in only 42 s.

4. Discussion

Hadamard methods have previously been used for stochastic irradiation with a pseudo-random binary sequence of radiofrequency pulses, in order to achieve broadband excitation with a relatively low radiofrequency power [24–27]. These were intrinsically time-domain measurements that used the Hadamard transform to derive free induction decays, and they necessarily required Fourier transformation before a spectrum could be recorded. The entire range of NMR frequencies was excited in an essentially uniform manner; only the initiative of Tomlinson and Hill [28] attempted to "tailor" the excitation envelope. The intent behind all these experiments was to acquire transient *time-domain* signals. In contrast, the present Hadamard method replaces the usual evolution period of two-dimensional spectroscopy with direct excitation in the frequency domain. Fourier transformation is only employed in the F_2 dimension, where the rate of data gathering is already near optimum.

The present fast two-dimensional Hadamard scheme is a generalization of the principle exploited in earlier experiments [17,29–37] that used a small number of simultaneous selective radiofrequency pulses with Hadamard encoding. It demonstrates that the entire process can be implemented automatically, generating a complete two-dimensional contour display, virtually identical to the corresponding traditional two-dimensional spectrum. The savings in spectrometer time are impressive and there is no penalty in sensitivity per unit time.

Two-dimensional spectroscopy has always been handicapped by the protracted nature of the data gathering and the necessary phase-cycling. Hadamard spectroscopy drastically limits the extent of sampling in the indirect dimension and dispenses with phase cycling and quadrature detection. The considerable time saving achieved in the experiments reported here relates in fact to a rather unfavourable case—a highly congested region of the strychnine spectrum. In a more common situation, where the entire proton chemical shift range needs to be investigated and where the sites of interest are not so densely packed, the number of scans required in the conventional method compared with those in the Hadamard mode could easily exceed three orders of magnitude. Still higher gains are anticipated for the sparse spectra of carbon-13, fluorine-19 or phosphorus-31. Direct frequency-domain excitation promises even more impressive improvements in three-dimensional spectroscopy, which normally requires very long periods of data accumulation, sometimes running into days. In principle the time savings factors in each indirect dimension are multiplicative. Investigations along these lines are in progress.

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