

RESEARCH ARTICLE

Coherence transfer delay optimisation in PSYCOSY experiments

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Abstract

PSYCOSY is an f1 broadband homonuclear decoupled version of the COSY nuclear magnetic resonance pulse sequence. Here, we investigate by a combination of experimental measurements, spatially distributed spin dynamics simulations, and analytical predictions the coherence evolution delay necessary in PSYCOSY experiments to ensure intensity discrimination in favour of the correlations typically arising from short range (nJ , $n \leq 3$) ^1H - ^1H couplings and show that, in general, a coherence evolution delay of around 35 ms is optimum.

KEYWORDS

^1H , NMR, numerical simulation, pure shift COSY

1 | INTRODUCTION

Signal resolution is one of the primary characteristics of the spectrum resulting from a nuclear magnetic resonance (NMR) spectroscopy experiment. The pursuit of higher resolution has been a major driving force behind the development of magnets operating at higher magnetic fields and greater homogeneity. In the case of proton NMR experiments of small molecules in nonviscous, isotropic solvents, however, a limiting factor in the effective resolution can be the frequency range covered by a particular signal due to the presence of J-coupling (often primarily homonuclear). In cases where the difference in chemical shift between two signals is small compared with the size of the multiplet, signal overlap occurs, and it can be difficult to resolve the two signals. In recent years, a solution to this problem, at least for cases where the overlapping multiplets do not contain significant mutual couplings (“weak coupling”), has been demonstrated using pure shift techniques^[1–3] in which the chemical shift information is retained, but the effects due to homonuclear J-coupling are eliminated so that, in the absence of heteronuclear J-coupling, each chemically distinct nucleus gives rise to a single line. There

are a number of related techniques to achieve this, but they all involve a significant reduction in sensitivity. This is rarely a problem for proton NMR spectroscopy, however. In cases where overlapping or proximate multiplets do contain significant mutual couplings comparable in size to the separation (in Hz) between the chemical shifts (“strong coupling”) broadband homonuclear decoupling is compromised and additional signals may be apparent, typically at the midpoint between the two chemical shifts.

The use of pure shift techniques can be extended to two-dimensional experiments. In the case of homonuclear experiments, it is generally only necessary to introduce the pure shift element into one of the dimensions. If it is desired that the spectrum appear to be pure shift in both dimensions, this can be achieved using covariance processing,^[4,5] although appropriate caution needs to be exercised to avoid the introduction and misinterpretation of potentially misleading artefacts. The synergic use of pure shift techniques and compressive sampling^[1,6–10] allows for an increase in the effective resolution of homonuclear two-dimensional experiments by orders of magnitude without an unacceptable increase in experiment time. This has been demonstrated for proton TOCSY experiments,^[11] but the

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implementation for COSY experiments is more challenging. The reason is that in the TOCSY experiment, the coherence evolution occurs in a period (“mixing time”) separate from the chemical shift evolution (“ t_1 ”), so the digital resolution in the f_1 dimension, which is determined by the longest t_1 value, can be increased independently of coherence evolution. In the classic COSY experiment, coherence evolution and chemical shift evolution both occur in t_1 , so increasing the digital resolution in f_1 also entails increasing the coherence evolution time, leading to possibly undesirable changes in the relative intensities of correlations due to short-range (nJ , $n \leq 3$) and long-range (nJ , $n \geq 4$) couplings. Because discrimination between short-range and long-range couplings is a prerequisite when interpreting the COSY spectrum of an unknown compound, this poses a significant problem.

The classic approach to removing J-coupling in one dimension of a COSY spectrum is the constant evolution time COSY experiment (CT-COSY) first proposed by Freeman and Bax.^[12] In this experiment, the coherence evolution time is the same as the constant evolution period in t_1 . Because the amount of J evolution is the same for each t_1 increment, no effects due to J evolution appear in the f_1 dimension after Fourier transform. Hence, the CT-COSY experiment is effectively broadband homonuclear decoupled in f_1 . However, the fact that the amount of coherence evolution is determined by the fixed evolution period means that as the digital resolution in f_1 increases, the correlation intensity discrimination in favour of longer range couplings also increases, making the problem worse than for the classic COSY experiment discussed above.

Attempts have been made to produce pure shift COSY experiments that do not suffer from the same drawbacks as the CT-COSY experiment, notably pure shift developments of the CLIP-COSY pulse sequence.^[13,14] We recently proposed a COSY sequence (PSYCOSY) in which pure shift evolution in f_1 is achieved using the same technique as used in the one-dimensional PSYCHE experiment.^[15] The PSYCOSY pulse sequence is reproduced in Supporting Information. Because homonuclear J-coupling is refocused by the PSYCHE element in t_1 , there is effectively no coherence evolution during this period as a function of t_1 . Then, a separate period of evolution (t_{ce}) has to be added to the end of the t_1 period to allow the development of the antiphase terms needed to produce cross peaks. Because the amount of both chemical shift and J evolution in this nonincremented period are fixed, neither then show up in f_1 after Fourier transform. However, the amount of coherence evolution now depends solely on the duration of this fixed period and is independent of the chosen digital resolution. By appropriate choice of the duration of this fixed period, it should be possible to ensure that short range couplings are favoured over long range ones.

In this work, we report an investigation of the optimum length of the J evolution period in PSYCOSY using a combination of simulation, experimental measurements, and product operator formalism.

2 | EXPERIMENTAL

Salicylsalicylate (SSA) and 2,3-dibromopropionic acid (DBPA) were purchased from Sigma Aldrich and used without further purification. All spectra of SSA and DBPA were recorded in deuterated 1,1,2,2-tetrachloroethane (TCE) and deuterated chloroform solution, respectively, on a 600 MHz Varian spectrometer equipped with an Agilent OneNMR Probe able to deliver a maximum pulsed field gradient of 62 G cm^{-1} . PSYCOSY spectra were collected using one scan per increment comprising 4,096 complex data points and a spectral width of 1.8 kHz in the case of SSA and 2.4 kHz in the case of DBPA. The repetition time was 2.14 s, of which 1.14 s comprised the acquisition time. The t_1 interval was sampled acquiring 512 increments randomly selected out of 2,048 possibilities (the latter being the number of increments that need to be acquired using Nyquist sampling to produce a digital resolution of 0.9 Hz/point in the case of SSA and 1.2 Hz/point in the case of DBPA). The PSYCHE pulse was created by concatenating two 7 kHz, 30 ms double sweep chirp pulses (“Saltire pulses”^[16]) with $\gamma B_1/2\pi = 44 \text{ Hz}$. A pulsed field gradient (0.8 G cm^{-1}) was applied for the duration of the PSYCHE pulse. The total experimental time was 1 hr and 22 min. Spectra were reconstructed using the Iterative Soft Thresholding algorithm implemented in MestreNova Version 12. A matched \sin^2 window function was applied in both dimensions and the final Fourier transform size was $4,096 \times 4,096$ complex data points. For the spectra of SSA, the intensities of cross peaks were measured relative to the intensity of the residual solvent peak (TCE) that, other than by relaxation, does not evolve as a function of the coherence evolution delay (t_{ce}). For the spectra of DBPA, where the residual solvent peak lies well outside the spectral window, the intensities of cross peaks were measured as a fraction of the total intensity for all peaks in the spectral window.

Analytical estimates were obtained using the product operator formalism methods suggested by Rance et al.^[17] The numerical simulations were performed using the Fokker–Planck theory module of Spinach^[18] as previously described^[15] and included explicit time-domain propagation through saltire pulses^[16] for spin systems with explicit spatial discretisation to enable accurate treatment of spin dynamics in the presence of magnetic field gradients. Efficient parallelisation was achieved by simulating different indirect dimension slices of a two-

dimensional free induction decay on different workers of the Matlab parallel pool. All relevant source code for the Spinach simulations is available in the example set of Spinach Versions 2.4 and later (<http://spindynamics.org/Spinach.php>). An example Spinach input file is also included in the Supporting Information. The results from the Spinach simulations were output as time domain data in JSON format for subsequent processing and analysis using MestreNova Version 12.

3 | RESULTS AND DISCUSSION

In order to define an appropriate value for the coherence evolution delay in the PSYCOSY experiment, we looked at the behaviour of coherences as a function of the evolution delay in a number of systems. Because running large numbers of PSYCOSY experiments with different values of coherence evolution delay is very demanding of spectrometer time, we used a combination of numerical simulations, experimental measurements, and analytical estimates. The least costly of these in terms of time is the theoretical predictions based on the method suggested by Rance et al.^[17] for the COSY experiment. Although these have been well verified for the classic CT-COSY experiment, their validity for the PSYCOSY experiment was not certain, due to the presence of chirp pulses and artefacts in $f1$ due to strong coupling. Numerical simulations using Spinach are demanding of computer time but not of spectrometer time and are therefore an attractive option when the spatial grid is small enough for the simulation to run in a reasonable time. Spinach also accurately reproduces the strong coupling artefacts observed experimentally. Finally, experimental measurements are demanding of spectrometer time but provide the ultimate test of the validity of theoretical predictions and simulations. We have therefore used all three techniques for a small number of model systems and then used numerical simulation and analytical estimates only on other systems.

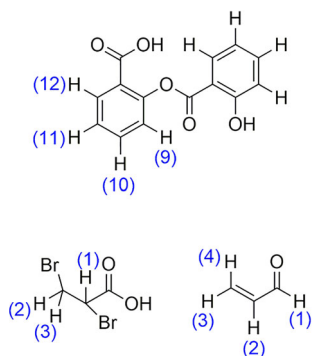


FIGURE 1 Molecular structures of salicylsalicylate (upper), 2,3-dibromopropionic acid (lower left), and acrolein (lower right)

Our initial work focused on small spin systems chosen to represent a range of proton environments (alkyl, vinyl, and aryl). Specifically, we looked at DBPA, acrolein, and one of the aromatic rings in SSA (Figure 1). In all these cases, the coupling was weak at the relevant magnetic field (14.1T), and no significant second-order coupling effects were observed. Spinach computations were carried out using the methodology described previously,^[15] using values for chemical shifts and coupling constants measured from spectra.

In evaluating the results from simulations and experiments, we focused on the intensities of off-diagonal peaks as a function of the coherence evolution time. When we look at the behaviour of the off-diagonal peaks linking two diagonal signals, A and M, we recognise that the off-diagonal signal at the same frequency in $f1$ as the diagonal Signal A represents magnetisation that originates on A and is transferred to M in the mixing step, whereas the off-diagonal signal at the same frequency in $f1$ as the diagonal signal M represents magnetisation that originates on M and is transferred to A in the mixing step. This distinction becomes important in extended spin systems where passive couplings need to be considered. As an example, we consider the three spin system AMX whose CT-COSY spectrum is represented schematically in Figure 2. J_{AM} and J_{MX} are both finite, whereas J_{AX} is zero. According to the theory developed for CT-COSY,^[17] the intensity of the off-diagonal signal at the same frequency in $f1$ as the diagonal Signal A (coloured red in Figure 2) is proportional to $\sin(\pi \cdot J_{AM} \cdot t)$ where t is the fixed coherence evolution time in the CT-COSY experiment, whereas the behaviour of the off-diagonal signal

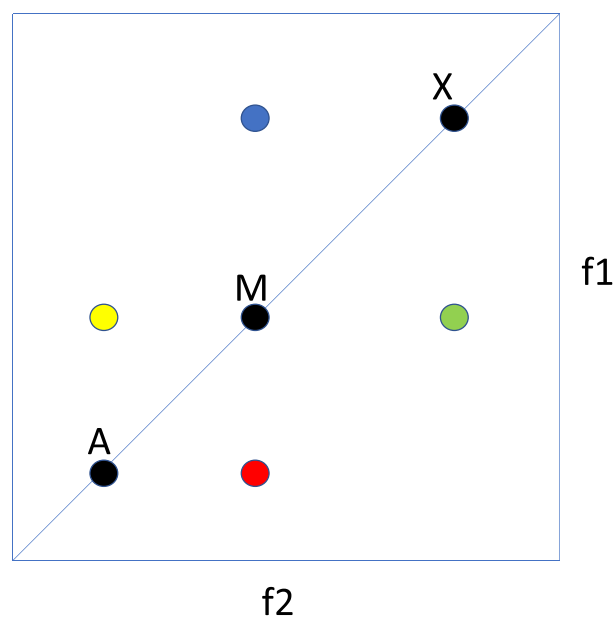


FIGURE 2 Schematic representation of the CT-COSY spectrum of the AMX spin system

at the same frequency in f_1 as the diagonal Signal M (coloured yellow in Figure 2) depends sinusoidally on the active coupling at M (J_{AM}) and cosinusoidally on the passive coupling at M (J_{MX}) so is proportional to $\sin(\pi * J_{AM} * t) * \cos(\pi * J_{MX} * t)$. Thus, eq. 5 in the paper by Rance et al. can be used to predict the expected intensity of the off-diagonal signals as a function of the coherence evolution time in a CT-COSY spectrum. It is worth emphasising that for a given value of coherence evolution time, the red and yellow off-diagonal elements in Figure 2 would not be expected to have the same intensity. This effect is often masked in the classic COSY spectrum where each increment in t_1 has a different coherence evolution time, so the effects due to different combinations of active and passive couplings tend to get averaged out. In the PSYCOSY experiment, we have a fixed coherence evolution time and therefore do not anticipate averaging of these effects. We therefore wanted to check, *inter alia*, whether the observed intensities as a function of t_{ce} followed the behaviour predicted by Rance et al.

When interpreting the proton COSY spectrum of an unknown, we work on the assumption that the majority of observed intense correlations arise from comparatively large J-couplings (mostly 2J and 3J plus and occasionally 4J). Our previous work^[15] showed that for the PSYCOSY sequence (as for the CT-COSY sequence), this is not necessarily the case at all values of the coherence evolution time, t_{ce} . We therefore wish to determine what value(s) of t_{ce} will give PSYCOSY spectra strongly favouring correlations from comparatively large J-couplings over small ones. We have simulated the PSYCOSY spectra of the structures shown in Figure 1 as a function of t_{ce} . Figure 3 shows the simulated evolution of the cross peak intensity for one correlation in SSA and compares the simulation with

experimental data and with the prediction outlined by Rance et al.^[17] Comparisons between simulation and experimental outcomes for other correlations in SSA and for other mentioned molecules are given in Supporting Information. In general the agreement between the three methods is good except at long values of t_{ce} (>100 ms) where there is some divergence. We have made no attempt to take accurate account of the effects of relaxation on the observed intensities, which may contribute to the discrepancies at long value of t_{ce} . These results show that the

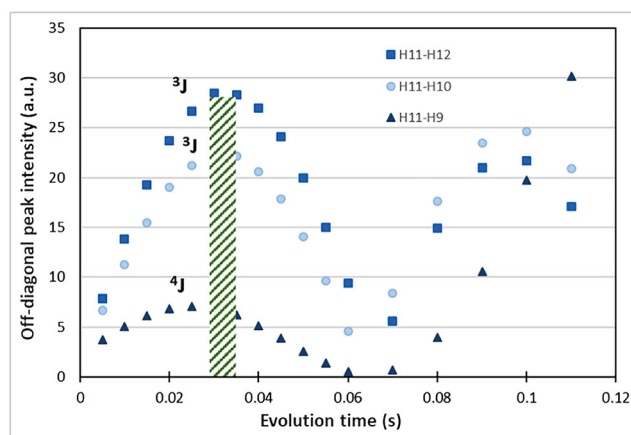


FIGURE 4 Off-diagonal peak intensity (arbitrary units) as a function of evolution time from Spinach simulations for salicylsalicylate (numbering as in Figure 3) showing the discrimination in favour of 3J -couplings— 3J (H11–H12) = 7.9 Hz, 3J (H11–H10) = 7.5 Hz, and 4J (H11–H9) = 1.2 Hz—at around 35 ms (green hatched area). Note that such discrimination is not present at all evolution times (e.g., 0.11 s). The simulations used a magnetic field strength of 14.1T (corresponding to a 1H Larmor frequency of 600 MHz), and the chemical shift values (δ /ppm) for the relevant protons were 8.14 (H12), 7.71, (H10), 7.44 (H11), and 7.28 (H9). All couplings were therefore in the “weak” regime.

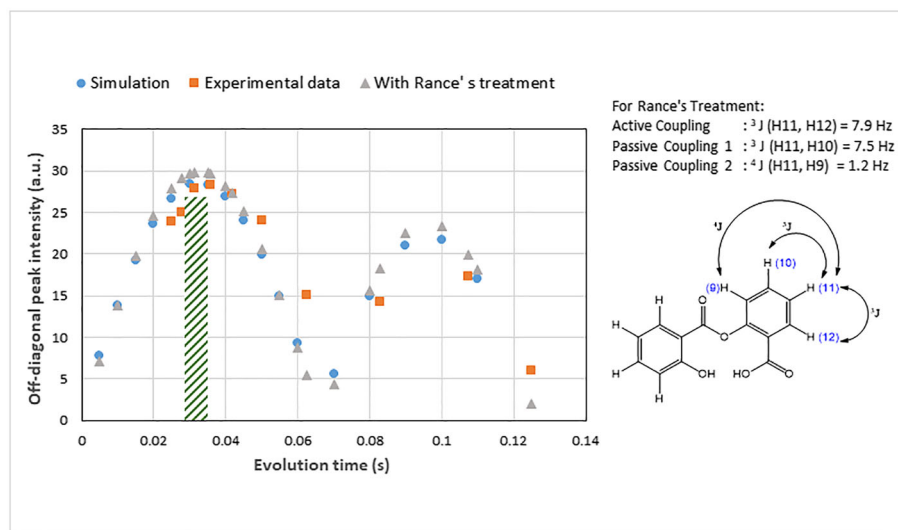


FIGURE 3 Comparison of simulated evolution of PSYCOSY off-diagonal peak intensity (arbitrary units) with experimental data and with the predictions outlined by Rance et al. for one coupling (H11–H12) in SSA

predictions of Rance et al. reproduce the experimental observations and do not diverge significantly from the Spinach simulations at relevant values of t_{ce} .

Whereas the size of 3J proton–proton couplings can vary between zero and 10 Hz, the majority tend to be in region of 6 to 8 Hz. Therefore, discrimination in favour of couplings in that range is desirable. In the case of a single active coupling, the first maximum for coherence transfer occurs at $t_{ce} = 1/2J$. So for isolated active couplings between 6 and 8 Hz, coherence intensity reaches a first maximum at between 62 and 84 ms. However, when passive couplings of about the same size are present, the first maximum is reached at about half of that value. Simulations of the various structures shown in Figure 1 demonstrate that all show discrimination in favour of short range couplings at values of t_{ce} around 35 ms (Figure 4 and Supporting Information). The observed discrimination is largely independent of the number of passive couplings. However, it is worth noting that having a single, defined value for the coherence evolution time eliminates the averaging effects commonly encountered in a standard COSY experiment, so that the intensities of the two off-diagonal peaks linking a pair of diagonal peaks will not necessarily be the same and can differ significantly if the number of passive couplings involved is not the same for each.

4 | CONCLUSIONS

Numerical simulations of the PSYCOSY experiment as a function of the coherence evolution time (t_{ce}) show excellent agreement with experimental data in a number of cases, and both simulations and experimental data show good agreement with the predictions of Rance et al. at relevant values of t_{ce} (<80 ms). Good discrimination in favour of short-range proton–proton couplings (2J and 3J) is obtained in the PSYCOSY experiment using a t_{ce} value of 35 ms for a range of chemical structures regardless of the number of passive couplings.

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DATA ACCESSIBILITY

The raw NMR data used to generate the figures in this paper can be downloaded from: <https://collections.durham.ac.uk/files/r1qf85nb30g#.XKchXWzPUJ> (doi: 10.15128/r1qf85nb30g).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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