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Simulation of Radio-Frequency Field Inhomogeneity Effects. Application to Pulse Trains Aimed at the Determination of csa-dipolar Interference Terms.

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Abstract

The cross-correlation relaxation rate constant between dipolar interaction and chemical shift anisotropy (interference term) is of considerable interest for structural and dynamical determinations. The present study focuses on methods related to its impact on longitudinal nuclear relaxation and especially to a procedure based on the applications of a train of π pulses applied to both nuclei involved in such a process (M.H. Levitt and L. Di Bari, *Bull. Magn. Reson.* **16**, 94-114 (1994)). The resulting steady state leads in principle to a straightforward determination of the relevant interference term. Simulations taking into account inhomogeneity of the radio-frequency (rf) field show that artifacts are responsible for instabilities which in practice preclude such a determination. An alternative method making use of a very limited number of rf pulses, combined with an appropriate filter, proves to be rather robust in view of achieving a proper measurement of the required interference term.

Keywords: NMR, ^{1}H, ^{13}C, relaxation, cross-correlation, simulation, rf inhomogeneity

INTRODUCTION

Besides classical nuclear relaxation rate constants (longitudinal, R_1, and transverse, R_2), cross-relaxation rate constants (depending solely on the dipolar interaction between two nuclei denoted for instance by H and C in such a way that we can use the notation σ_{CH} for the corresponding cross-relaxation rate constant), there exists a variety of other relaxation rate constants called cross-correlation rates (or, alternatively, interference terms) which involve two different relaxation mechanisms (hence the alternative terminology). These quantities have proved to be of considerable interest due to the nature of the information they hold[1]. For instance, we shall be interested in this paper in cross-correlation between the dipolar interaction *CH* and the so-called chemical shift anisotropy (csa) which arises from a shielding tensor (say at nucleus *C*) having non-identical diagonal elements. The corresponding cross-correlation rate, denoted in the following by $\sigma_{csa(C),d(CH)}$, thus involves, in addition to a dynamic part, information about the dipolar interaction (in $1/r_{CH}^3$, r being the internuclear distance) but also about the shielding tensor (usually expressed as $\Delta\sigma_C$, which is the difference between parallel and perpendicular components of the shielding tensor supposed to be of axial symmetry), and ultimately depends on the relative orientation of csa and dipolar tensors. From such interference terms, it is indeed possible to determine the characteristics of shielding tensors in the liquid state[2]. The influence of csa-dipolar interference terms on NMR experiments is well understood[3]. In particular, as far as transverse relaxation[4] is concerned and provided that the two involved nuclei are J coupled, this phenomenon manifests itself by a differential broadening of the two lines in the corresponding doublet. This is the basis of transverse interference term measurements, especially for the amide ^{15}N-^{1}H pair in proteins[5-8], and also of the TROSY experiment[9] which selects the sharpest line of the ^{15}N-^{1}H doublet (its width being governed by the *difference* of two relaxation contributions) thus making feasible NMR investigation of larger proteins. The longitudinal counterpart, although often

considered[10-14] in the past, is not so easily accessible and is less frequently used for dynamical studies in proteins[7]. The subject of this paper is precisely the determination of heteronuclear longitudinal csa-dipolar cross-correlation rates, in particular with regard to an appealing method published some time ago by Levitt and Di Bari[15]. This method rests on the application of π pulse trains which lead to a steady state depending on the considered interference term. After some theoretical developments confirming the validity of the method, it will be shown by appropriate simulations that pulse imperfections have unfortunately a deleterious effect on experimental results. It is then concluded that transient experiments, because they require a limited number of radio-frequency pulses, are to be preferred and we shall propose such an experiment which is based on a gradient filter and yields directly the csa-dipolar cross-correlation rate.

THEORY

Consider a ^{13}C-^{1}H two spin ½ system; its longitudinal magnetization modes are governed by the extended Solomon equations[16] (I_z^H and I_z^C are respectively proton and carbon longitudinal magnetization, while $2I_z^H I_z^C$ is the so-called longitudinal order, zero at equilibrium and which can be created by relaxation or by manipulation of antiphase transverse magnetization):

$$\frac{dI_z^H}{dt} = -R_1^H (I_z^H - I_{eq}^H) - \sigma_{CH} (I_z^C - I_{eq}^C) - \sigma_{csa(H),d}\, 2I_z^H I_z^C \qquad (1)$$

$$\frac{dI_z^C}{dt} = -R_1^C (I_z^C - I_{eq}^C) - \sigma_{CH} (I_z^H - I_{eq}^H) - \sigma_{csa(C),d}\, 2I_z^H I_z^C \qquad (2)$$

$$\frac{d(2I_z^H I_z^C)}{dt} = -R_1^{HC} (2I_z^H I_z^C) - \sigma_{csa(H),d} (I_z^H - I_{eq}^H) - \sigma_{csa(C),d} (I_z^C - I_{eq}^C) \qquad (3)$$

As indicated above, R_1 refer to specific relaxation rate constants, σ_{CH} is the cross-relaxation rate constant and $\sigma_{csa,d}$ are the cross-correlation rates we are interested in. Because $\sigma_{csa(H),d}$ is generally negligibly small, it can be dropped from eq. (1) whereas eq. (3) simplifies as:

$$\frac{d(2I_z^H I_z^C)}{dt} = -R_1^{HC}(2I_z^H I_z^C) - \sigma_{csa(C),d}(I_z^C - I_{eq}^C) \quad (4)$$

Suppose that we have at hand a procedure capable of maintaining I_z^C at zero without affecting the evolution of the longitudinal order $2I_z^H I_z^C$. Eq. (4) becomes

$$\frac{d(2I_z^H I_z^C)}{dt} = -R_1^{HC}(2I_z^H I_z^C) + \sigma_{csa(C),d} I_{eq}^C \quad (5)$$

At long times, a steady state is reached

$$(2I_z^H I_z^C)_{ss} = \frac{\sigma_{csa(C),d}}{R_1^{HC}} I_C^{eq} \quad (6)$$

This result is consistent with the findings of Levitt and Di Bari[15] within the HME (Homogeneous Master Equation) approach; the experimental procedure that they proposed for achieving this goal consists of a train of π pulses applied simultaneously to proton and carbon-13. The steady state can be characterized by taking into account the three (extended) Solomon equations. It will be assumed that the inter-pulse intervals τ are short enough (with respect to relaxation times) so that the relevant differential equations can be solved by means of a first order expansion, i.e. (*G* standing for I_z^H, I_z^C or $2I_z^H I_z^C$)

$$G(\tau) = G(0) + (\frac{dG}{dt})_{t=0} \times \tau \quad (7)$$

The steady state condition can be written as

$$G_{ss} = G(0) = \Gamma\big(G(\tau)\big) \quad (8)$$

where Γ denotes the action of the π pulses on the considered quantity, i.e. $\Gamma\big(I_z^H\big) = -I_z^H$, $\Gamma\big(I_z^C\big) = -I_z^C$, $\Gamma\big(2I_z^H I_z^C\big) = 2I_z^H I_z^C$. It can be noticed that $\Gamma\big(G(\tau)\big)$ can be expressed according to the steady state value of the three quantities involved in this problem. It is then possible to establish an equation such as (8) for these three quantities and to arrive at a set of three

equations yielding their steady state values. We can write (with $\sigma \equiv \sigma_{CH}$, $\sigma' \equiv \sigma_{csa(C),d}$ and with the simplified notations $Z_1 = (I_z^H)_{ss} / I_{eq}^C$, $Z_2 = (I_z^C)_{ss} / I_{eq}^C$, $Z_3 = (2I_z^H I_z^C)_{ss} / I_{eq}^C$).

$$\begin{aligned} Z_1 &= -Z_1 + R_1^H \tau (Z_1 + 4) + \sigma\tau (Z_2 + 1) \\ Z_2 &= -Z_2 + R_1^C \tau (Z_2 + 1) + \sigma\tau (Z_1 + 4) - \sigma' \tau Z_3 \\ Z_3 &= Z_3 - R_1^{HC} \tau Z_3 + \sigma' \tau (Z_2 + 1) \end{aligned} \tag{9}$$

Solving this system of three linear equations leads to

$$(I_z^H)_{ss} / I_{eq}^C = (\frac{4R_1^H + \sigma}{2 - R_1^H \tau})\tau + (\frac{\sigma\tau}{2 - R_1^H \tau})(I_z^C)_{ss} / I_{eq}^C$$

$$(I_z^C)_{ss} / I_{eq}^C = \frac{(R_1^C + 4\sigma)\tau - \frac{\sigma'^2}{R_1^{HC}}\tau + \frac{(4R_1^H + \sigma)}{2 - R_1^H \tau}\sigma\tau^2}{2 - R_1^C \tau - \frac{\sigma'^2}{R_1^{HC}}\tau - \frac{\sigma^2}{2 - R_1^H \tau}\tau^2} \tag{10}$$

$$(2I_z^H I_z^C)_{ss} / I_{eq}^C = \sigma' / R_1^{HC} + (\sigma' / R_1^{HC})(I_z^C)_{ss} / I_{eq}^C$$

It is interesting to go back to the third of eqs. (9) and to notice that Z_2 is negligible (this can be understood from the fact that carbon magnetization is almost entirely saturated, and could be confirmed by a numerical evaluation of the second of eqs. (10)). We thus obtain

$$Z_3 = \frac{\sigma'}{R_1^{HC}}(1 + Z_2) \approx \frac{\sigma'}{R_1^{HC}} \tag{11}$$

This result is entirely analogous to eq. (6) and indicates the possibility of determining $\sigma_{csa(C),d}$ from the measurement of the longitudinal spin order which is better achieved with proton detection so as to take advantage of the highest possible sensitivity. In principle, due to the zeroing of proton longitudinal magnetization (through the π pulse train), main signals arising from protons bonded to carbon-12 should be absent. This should allow for the easy observation of an antiphase doublet (occurring at the position of carbon-13 satellites in the proton spectrum) which would result from the application of a $\pi/2$ proton read pulse (this pulse converts the longitudinal spin order $2I_z^H I_z^C$ into $2I_y^H I_z^C$). Moreover, π pulse

imperfections should not be cumulative as far as the detection of Z_3 is concerned. They just result in a slight modification in Z_3 values in the right-hand side of the third of eqs. (9) and tend to slightly lower the result given by (11). It turns out that this intuitive view is incomplete and that *refocusing of transverse magnetization* will severely alter the final result. This can be assessed by simulations the principle of which is detailed in the next section.

SIMULATIONS OF PULSE SEQUENCES TAKING INTO ACCOUNT RF FIELD INHOMOGENEITY

The principle of such simulations has been published in a previous paper[17] for the case of a single spin. The radio-frequency field (B_1) variation along the sample tube axis is assumed to be the main cause of inhomogeneity. This corresponds to an experimental arrangement where the rf coil is of the saddle shaped design, the static magnetic field being produced by a vertical cryomagnet. B_1 inhomogeneity can be specified either by a series of number related to B_1 values from top to bottom of the sample (measured experimentally) or by a Gaussian curve supposed to represent the B_1 evolution along the tube axis. The sample is decomposed into elementary slices (in practice, between 100 and 500 slices are defined), the flip angle α associated with a given rf pulse being supposed to be perfectly defined for each of them:

$$\alpha_{slice} = \frac{(B_1)_{slice}}{(B_1)_{\max}} \alpha_{\max} \qquad (12)$$

$(B_1)_{slice}$ is extracted from the list of B_1 values, $(B_1)_{\max}$ is the maximum value in this list and $\alpha_{\max}$ is the corresponding flip angle (π in our case). The objective is to determine the evolution of spin quantities in each slice and eventually calculate an average over all slices. This is easily performed for a single spin system because, in that case, this amounts to

rotations of the magnetization components in the rotating frame (around *x* or *y* for rf pulses; around *z* for evolution periods, according to resonance frequency in the rotating frame).

In the case of *J*-coupled spin systems, it is well known that a proper description of the various spin states requires other quantities than the three magnetization components. For example, if I_x^i is appropriate for describing an in-phase doublet (*i* assumed to be *J* coupled with *j*), we must resort to the quantity $2I_x^i I_z^j$ for describing the corresponding antiphase doublet (obtained after an evolution period of duration equal to $1/2J_{ij}$). In fact, any spin state can be described by an expansion over a proper basis of cartesian operator products of the form[16]

$$G_r = N_r \prod_{j=1}^{n} S_j^{(r)} \tag{13}$$

where $S_j^{(r)}$ is E^j (identity), I_x^j, I_y^j or I_z^j (spin operators of spin *j*), *n* being the number of spins in the considered system and $\mathcal{N}_r$ a normalization factor such as the set of G_r operators is normalized and orthogonal (δ_{rs} being the Kronecker symbol)

$$Tr(G_r G_s) = \delta_{rs} \tag{14}$$

As, for a given slice, rf pulses are not pure π pulses, a variety of spin states are created and one has to deal with the evolution of G_r under the application of rf pulses and of evolution periods. Concerning rf pulses, the situation is quite simple as spin operators are simply rotated according to the actual flip angle pertaining to the considered slice (see (12)). For example, under an $(\alpha)_x$ pulse, I_z transforms into $I_z \cos\alpha + I_y \sin\alpha$.

Evolution periods, *i.e.* transformation between rf pulses due to chemical shifts and *J* coupling constants are more difficult to handle. For the sake of simplicity, we have dealt here with a first order hamiltonian

$$H = -\sum_i \nu_i I_z^i + \sum_{i<j} J_{ij} I_z^i I_z^j \tag{15}$$

which can be written as an expansion over the U_r basis

$$H = \sum_r a_r G_r \tag{16}$$

It can be noticed from (15) that all operators in the expansion (16) commute. Now, let us define the density operator at the beginning of the considered evolution period as

$$\sigma(0) = \sum_s b_s G_s \tag{17}$$

We have to establish the form of the density operator $\sigma(t)$ at the end of this period in order to find out which quantities are present and therefore the state of the spin system prior to the next pulse(s). One has

$$\sigma(t) = \exp(-iHt)\sigma(0)\exp(iHt) \tag{18}$$

which can be rewritten as (according to the expansions (16) and (17) and owing to the fact that all operators in (16) commute)

$$\sigma(t) = \sum_s b_s [\prod_r \exp(-it\ a_r G_r)] G_s [\prod_r \exp(it\ a_r G_r)] \tag{19}$$

Two situations can be encountered:

- G_s commutes with each G_r of the hamiltonian; it therefore remains as it was at the beginning of the evolution period and the contribution $b_s G_s$ is unchanged.
- G_s does not commute with one (or more) of the operator products in (16) and some calculations are necessary.

In order to illustrate this latter point, let us denote by G_{r1} and G_{r2} two operators that, supposedly, do not commute with G_s. In (19) G_s is transformed according to the following expression

$$G_s \longrightarrow \exp(-it\ a_{r1} G_{r1})[\exp(-it\ a_{r2} G_{r2}) G_s \exp(it\ a_{r2} G_{r2})]\exp(it\ a_{r1} G_{r1}) \tag{19}$$

Again, the way in which (19) is written is made possible by the fact that G_{r1} and G_{r2} commute: (19) amounts to calculations of the type $\exp(-it\,a_r G_r) G_s \exp(it\,a_r G_r)$ which are easily performed[18] with the help of the two following relations

$$\begin{aligned} \cos(2uI_z) &= (\cos u)E \\ \sin(2uI_z) &= 2(\sin u)I_z \end{aligned} \qquad (20)$$

in addition to the ones below, which are valid only for spins 1/2

$$\begin{aligned} I_x^2 = I_y^2 = I_z^2 &= E/2 \\ I_x I_y = -I_y I_x &= iI_z/2 \end{aligned} \qquad (21)$$

As an example, let us consider that $a_r G_r$ corresponds to $J_{ij} I_z^i I_z^j$ and let us look at the evolution of $G_s = I_x^i, I_y^i, 2I_x^i I_z^i$ and $2I_y^i I_z^i$. With the help of (19) to (21), we obtain

$$\begin{aligned} I_x^i &\longrightarrow I_x^i \cos(\pi J_{ij} t) + 2I_y^i I_z^j \sin(\pi J_{ij} t) \\ I_y^i &\longrightarrow I_y^i \cos(\pi J_{ij} t) - 2I_x^i I_z^j \sin(\pi J_{ij} t) \\ 2I_x^i I_z^j &\longrightarrow 2I_x^i I_z^j \cos(\pi J_{ij} t) + I_y^i \sin(\pi J_{ij} t) \\ 2I_y^i I_z^j &\longrightarrow 2I_y^i I_z^j \cos(\pi J_{ij} t) - I_x^i \sin(\pi J_{ij} t) \end{aligned}$$

Thus it becomes possible to run simulations for any complicated experiment. Calculations are performed *independently* for each slice starting from the equilibrium density operator $\sigma_{eq} = \sum_i \gamma_i I_z^i$ (γ: gyromagnetic ratio). Each pulse in the sequence possibly rotates the operators involved in the expression of the density operator which prevails just before the pulse (denoted σ_-, identical to σ_{eq} at the beginning of the simulation). This results in a new density operator denoted σ_+ which serves as $\sigma(0)$ in equation (18) for the subsequent evolution interval. The procedures outlined above lead to the density operator σ_- pertaining to the next pulse… The process is repeated step by step until the end of the sequence.

RESULTS AND DISCUSSION (THE LEVITT AND DI BARI SEQUENCE)

This sequence can be schematized as follows

$$[\pi(^{13}C)\pi(^{1}H)-\tau]_n-\pi/2(^{1}H)-Acq(^{1}H) \qquad (22)$$

As explained in the theory section, it is rather appealing in the sense that a steady state is normally reached (as far as the number *n* of cycles is sufficient) and should lead to the csa-dipolar term without ambiguity (see (6) and (11)) through the amplitude of the longitudinal two-spin order which is transformed into an antiphase doublet by the $\pi/2(^{1}H)$ read-pulse. However, this statement implies ideal π pulses and this is far from being warranted due to the (more or less) inevitable inhomogeneity of the B_1 field generated by the rf coil of any NMR probe. Actually, this inhomogeneity is responsible for the creation of transverse magnetization just because the flip angle is not 180° in all the regions of the sample; in turn, this transverse magnetization evolves under chemical shifts and coupling constants with the possibility of refocusing by subsequent rf pulses. This process is unpredictable on the basis on simple arguments and, to be fully understood, requires simulations as the ones described in the previous section.

As a matter of fact, although the antiphase doublet originating from the longitudinal two-spin order is generally observable in satisfactory conditions (fig. 1), various experimental observations reveal serious difficulties for reaching the expected steady state (the relevant signal amplitude can indeed be evaluated by means of the values of relaxation parameters, known from conventional experiments). An especially disturbing result is shown in figure 2 where the amplitude of the antiphase doublet (thus of the longitudinal two-spin order created by the sequence) is plotted as a function of the cycles in the pulse train. The experimental result is seen to be quite different of the expected regular build-up such as the one shown at the top of figure 3. The use of composite pulses in place of standard π pulses does not seem to improve this result. The experiment has been repeated showing that reproducibility cannot be

questioned, at least for the asymptotic behavior, which is for a large number of cycles. This seemingly anomalous behavior arises therefore from the experimental conditions and, as explained above, the only way to discover the sensitive parameter(s) is to perform extensive simulations. In the case of perfect B_1 homogeneity, normal results (see the top curve of figure 3) are obtained as expected. This is also true when B_1 inhomogeneity is taken into account provided that the on-resonance condition is respected (*i.e.* identical resonance and transmitter frequencies). However, an offset between these two frequencies, even as low as 1 Hz, produces dramatic changes. This is shown in figure 3 and it can be noticed that the lower trace exhibits the same trend as the experimental results of figure 2. In fact, this is not the only manifestation of instability as revealed by simulations performed for different τ values (see (22)). Although one observes an asymptotic behavior in every case, it is the asymptotic value itself which exhibits a sort of chaotic evolution. This is illustrated by the plots of figure 4 which definitely preclude the use of the method as far as quantitative results are required. Rather than striving to reduce rf field inhomogeneity to an hypothetical low level, we propose in the next section another approach, hopefully and more robust and much less sensitive to such an inhomogeneity.

A NOVEL APPROACH FOR MEASURING THE LONGITUDINAL CSA-DIPOLAR INTERFERENCE TERM (PRELIMINARY)

A simple way for accessing to the csa-dipolar interference term rests on the consideration of extended Solomon equations and more especially on eq. (3) which tells us that, starting from equilibrium, the longitudinal two-spin order can be created *via* relaxation phenomena, based primarily on this interference term. Thus, the latter can be directly deduced from the measurement of the longitudinal two-spin order, at least at short times so as to avoid contributions from carbon longitudinal magnetization, itself coupled to the longitudinal two-

spin order by this interference term and also coupled to proton magnetization by cross-relaxation. For obvious sensitivity reasons, it is better to measure the longitudinal two-spin order from the antiphase doublet which results from the application of a $\pi/2(^1H)$ pulse. This implies however that all other contributions have been filtered out. An easy way to achieve this objective is shown in figures 5 and 6; first, perturb the longitudinal ^{13}C magnetization (for instance by saturation), then let the longitudinal two-spin order to develop under the relevant interference term (mixing interval τ_m), eliminate all contributions but the longitudinal two-spin order by an appropriate filter and finally apply a $\pi/2(^1H)$ read-pulse in order to measure the antiphase doublet. The filter can be made with radio-frequency field gradient pulses according to a methodology previously published[19] (figure 5). Basically, it consists in applying, to both nuclei, rf field gradient (B_1 gradient) pulses originating from the same coil and satisfying the Hartmann-Hahn condition $\gamma_H B_{1H} = \gamma_C B_{1C}$ so that these two nuclei undergo identical nutation angles. Of course, a mismatch of this condition would have deleterious effects since it corresponds to the vital part of the experiment. Because one is dealing with gradient pulses, longitudinal magnetization is destroyed whereas half the longitudinal two-spin order is preserved (by virtue of the identical nutation angles). The efficiency of this filter is assessed by the experimental results shown in figure 6 for two different mixing times. However, the rf field inhomogeneity does not correspond to a true rf field gradient. Thus, it is worthwhile to reconsider this experiment with uniform gradients.

Thanks to the formal equivalence[20] between B_1 gradients and B_0 gradients (static field gradients), it is possible to devise a sequence homologous to the one of figure 5 and which can be run with spectrometers equipped with B_0 gradients, more widespread than those possessing B_1 gradient capabilities. Such a sequence involving a B_0 gradient filter is shown in figure 7 with (optional) refocusing of the antiphase doublet which can then be decoupled

from ^{13}C (for improving the signal-to-noise ratio and simplifying the spectrum). Before the first $\pi/2(^1H)$ pulse there is no proton transverse magnetization; consequently, the gradient acts first on carbon-13 which is subsequently taken back to the z axis (second $\pi/2(^{13}C)$ pulse). Thereafter proton is flipped to the *x,y* plane and is subjected to a gradient four times more intense as the first one. We can thus notice a perfect analogy with the B_1 gradient filter, recognizing that we end up with the desired antiphase doublet which can be possibly refocused. Experimental results obtained with this sequence are reported in figure 8 along with a recalculated build-up curve. The excellent agreement between theory and experiment illustrates the efficiency and the robustness of the present method.

CONCLUSION

In NMR experiments involving pulse trains, inhomogeneity of the radio-frequency field has always been a plague. It can sometimes be compensated for by judicious phase combinations. Concerning the Levitt Di Bari sequence, aimed at determining the csa-dipolar term and which seems at first sight of real interest because it is based on a steady state situation, autocompensated pulses appear to be of little help. This is due to spurious transverse components which evolve in a complicated manner, difficult to rationalize. The only tractable approach is to simulate the fate of the different spin states which build up in the course of the sequence. These simulations have revealed a rather chaotic behavior which makes this experimental method difficult to use on a routine basis. We therefore tried to devised procedures employing a limited number of rf pulses, leaving to a proper filter the task of selecting the relevant information. We have shown that a simple gradient filter (employing B_1 or B_0 gradients) constitute a reliable alternative. The preliminary results presented here are encouraging and should be easily extended to two-dimensional experiments opening the

way to the production of diagrams yielding site by site the value of longitudinal csa-dipolar interference terms.

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Figure Captions

Figure 1. Proton spectrum (400 MHz) of formic acid (non labeled) obtained by a simple read-pulse following the application of a double train of π pulses (acting on both proton and carbon-13, see (22)): 50 cycles separated by 50 ms. Note the antiphase doublet (of splitting J_{CH}) reflecting the amplitude of the longitudinal two-spin order (created by the pulse train) and the relatively satisfactory elimination of the signal corresponding to protons bonded to a carbon-12.

Figure 2. Experimental intensity of the longitudinal two-spin order (in %, relatively to equilibrium magnetization) created by the double train of π pulses, as a function of the number of cycles (50 ms between consecutive pulses).

Figure 3. Simulations corresponding to experimental results such as those of figure 2. The three curves correspond (from top to bottom) to proton offset of 0 Hz, 1 Hz and 5 Hz, respectively. In addition to J_{CH} = 220 Hz, the following relaxation parameters have been used (ambient temperature; measurements at 400 MHz): $R_1^H = 0.17$ s^{-1}, $R_1^C = 0.09$ s^{-1}, $\sigma_{CH} = 0.035$ s^{-1}, $\sigma_{csa(C),d} = 0.018$ s^{-1}.

Figure 4. Evolution of the asymptotic values (such as those in figure 3) as a function of the interval τ between consecutive pulses in the double train of π pulses, considered in figures 1-3. The curves have been obtained for the same offset values as in figure 3.

Figure 5. A pulse sequence for measuring the csa-dipolar interference (cross-correlation) term with rf gradient filters. Gradients arise from the natural inhomogeneity of a standard rf coil

which has the peculiarity to be doubly tuned for both nuclei (^{1}H and ^{13}C). g_1 (1 ms duration) and g_2 (2ms duration) are just used for saturating carbon magnetization with a sufficient irradiation strength to avoid any off-resonance effect. The mixing interval τ_m allows for the construction of longitudinal two-spin order through cross-correlation. The two $\pi/2$ pulses (along with the indicated phase cycle) are such that the sign of the longitudinal two-spin order is changed every second scan in synchronism with the acquisition sign (so as to eliminate spurious components). The filter includes the two rf gradient pulses g_3 (1ms duration) which must satisfy the Hartmann-Hahn condition (see text). The last $\pi/2$ pulse converts the longitudinal two-spin order into an observable antiphase doublet.

Figure 6. Experimental results obtained on pure ^{13}C labeled formic acid (degree of labeling: 99%) with the sequence of figure 5 for two different mixing times: 2 s (top) and 12 s (bottom). Experiments performed at ambient temperature with an home-made spectrometer (^{1}H : 200 MHz); 64 scans were accumulated. The temperature of the sample was kept constant by an air flow.

Figure 7. The homologous sequence of the one in figure 5 with static field gradient pulses. Bipolar gradients are used (for improving the experiment quality) with $g_1/g_2 = \gamma_H/\gamma_C$. The phase cycling has the same purpose as the one indicated in figure 5. At the level of the dotted vertical line, only the antiphase doublet exists. It can be (optionally) refocused by the sub-sequence displayed on the right of the dotted vertical line.

Figure 8. Experimental data (dots) obtained on ^{13}C labeled formic acid (degree of labeling: 99%) at ambient temperature with the sequence of figure 7 (Bruker DRX spectrometer operating at 9.4 T; proton resonance frequency: 400 MHz). These data represent the intensity

of the longitudinal two-spin order as a function of the mixing time. The theoretical curve has been calculated with the parameters given in the legend to figure 3.

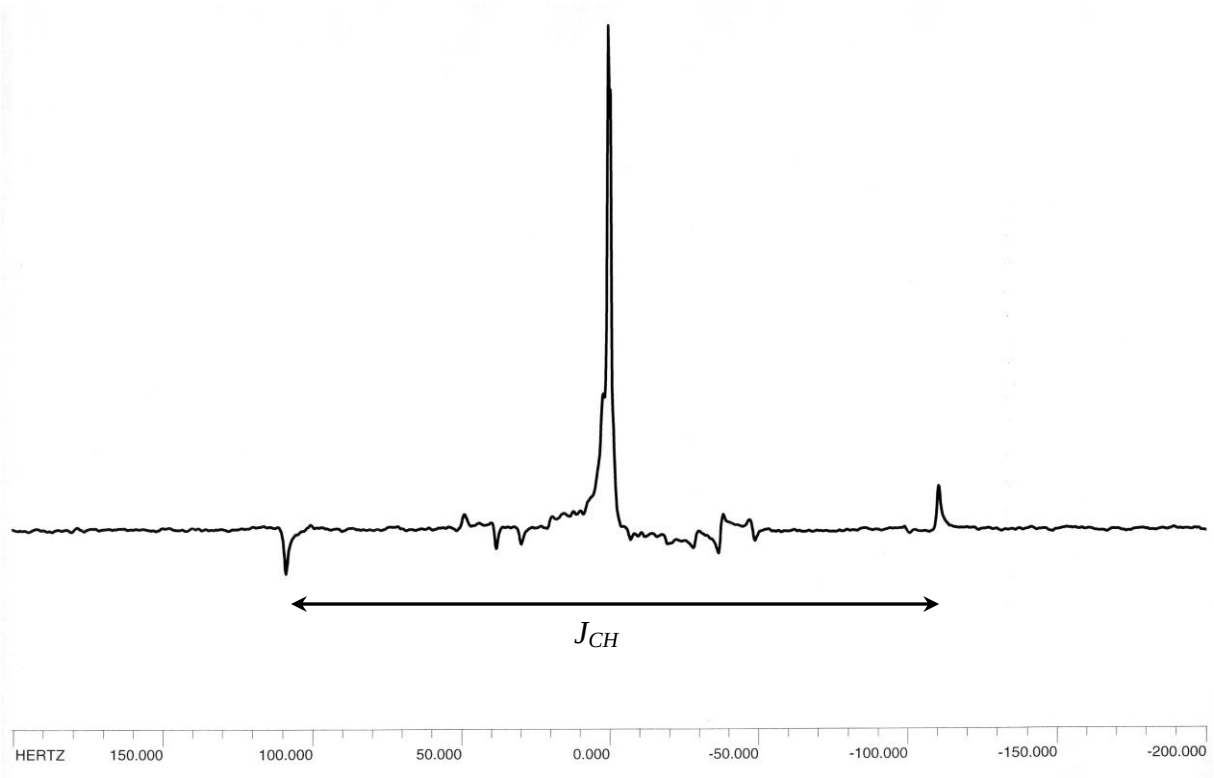
J_{CH}
HERTZ
150.000
100.000
50.000
0.000
-50.000
-100.000
-150.000
-200.000

Figure 1

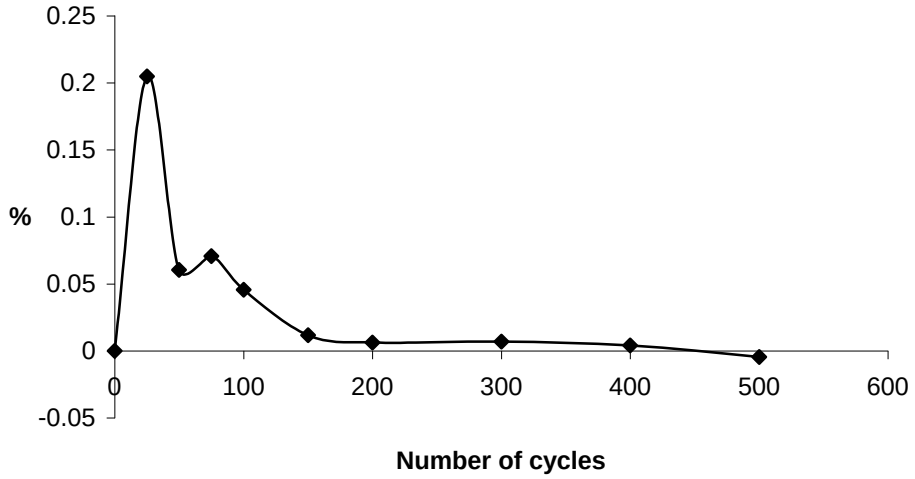
0.25
0.2
0.15
%
0.1
0.05
0
-0.05
0
100
200
300
400
500
600
Number of cycles

Figure 2

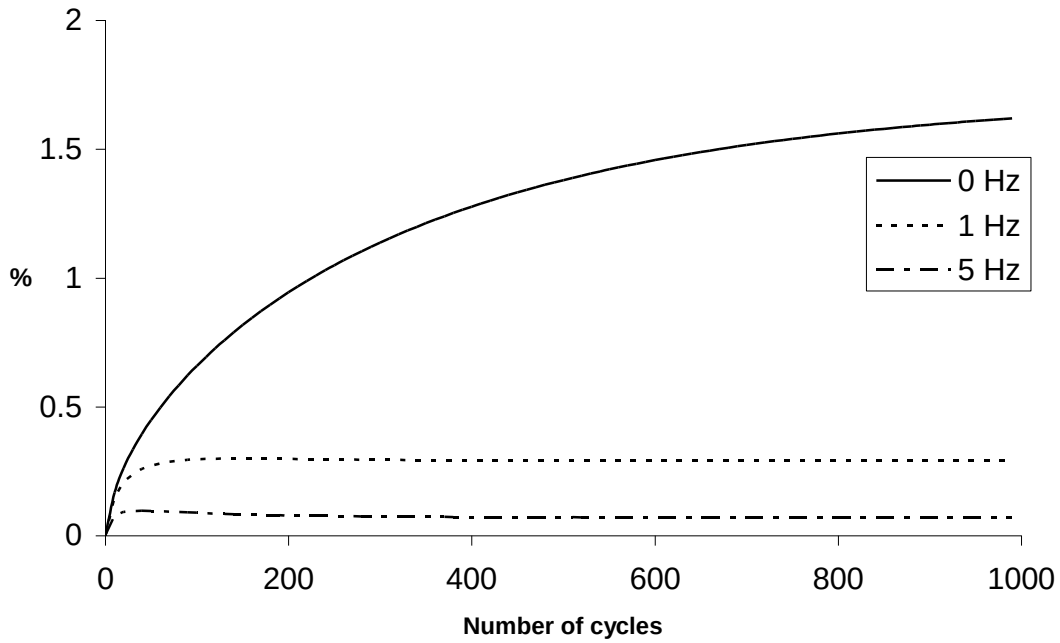

Figure 3

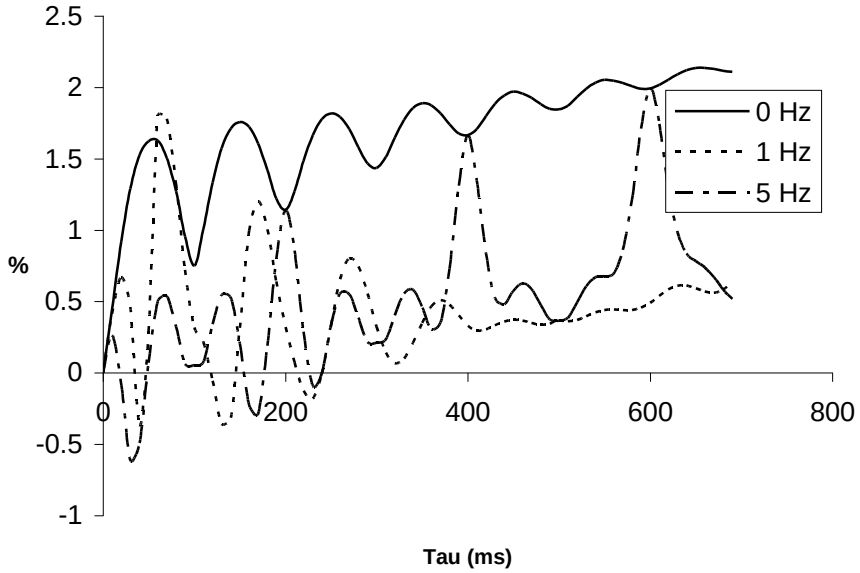

Figure 4

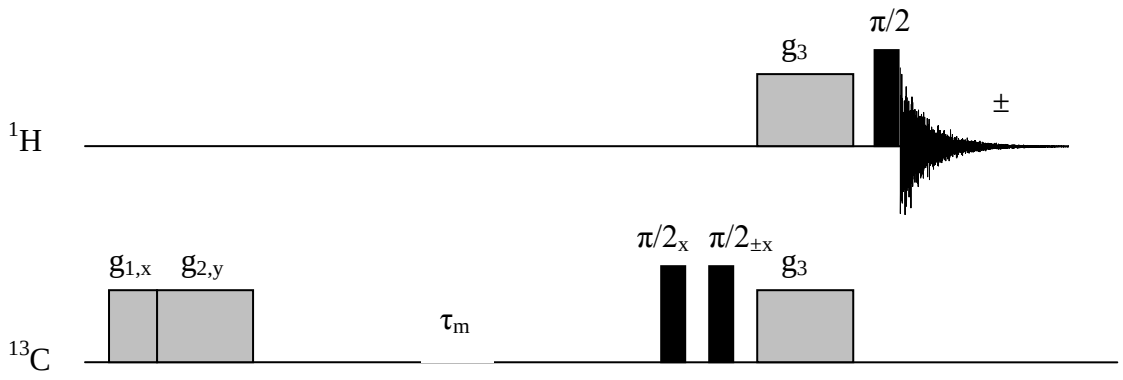

Figure 5

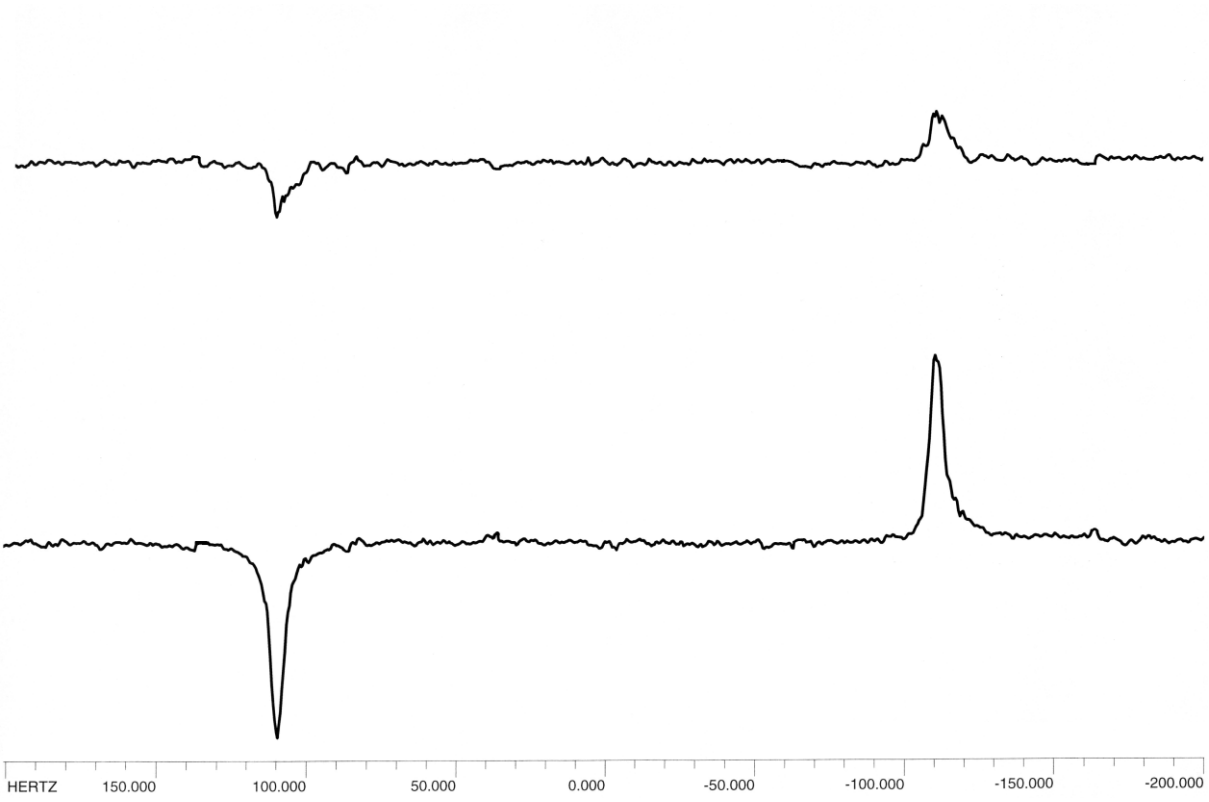

Figure 6

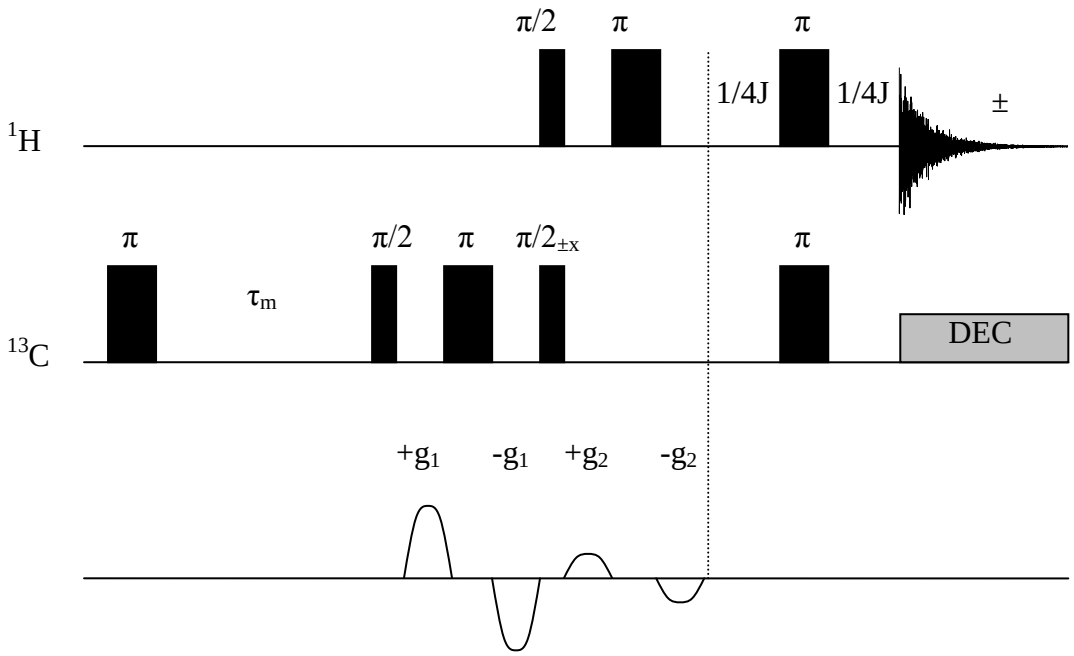

Figure 7

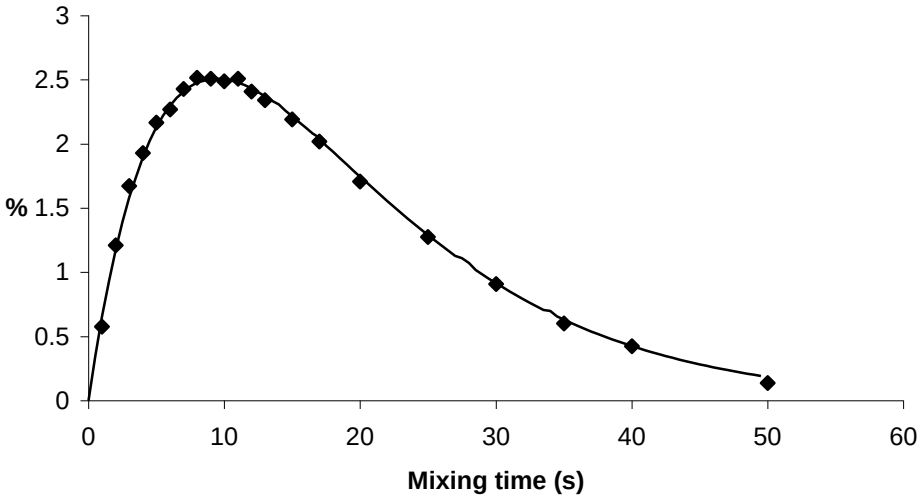

Figure 8