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Improving solvent suppression in jump-return NOESY experiments

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SUMMARY

The problems associated with solvent suppression in jump-return NOESY spectra and in particular the difficulties experienced with using short mixing times are examined. It is shown that the degree of water suppression depends critically on the extent of radiation damping of the water magnetisation during the mixing time of the NOESY sequence. A new jump-return NOESY sequence is proposed which incorporates field gradients and which achieves good levels of water suppression for all values of the mixing time, and for all increments of the NOESY experiment.

INTRODUCTION

The dynamic-range problem associated with recording NOESY spectra of protein samples dissolved in H_2O is well known and a number of methods have been developed to overcome this problem (Hore, 1989). Presaturation of the water resonance is often the simplest and most effective procedure; however, it is not without significant drawbacks. The irradiation may spill over and saturate the resonances of nearby $C^{\alpha}H$ protons, thereby reducing the intensity of the key H^{α}-NH cross peaks. In addition, if chemical exchange is significant, the NH protons may be partially saturated, and indeed this saturation may be carried to other spins by spin diffusion (Smallcombe, 1993). In cases where presaturation is inappropriate jump-return NOESY spectra are often preferred (Plateau and Guéron, 1982). In this paper we show that in such experiments the combination of radiation damping and the need to achieve F_1 frequency discrimination can result in much poorer water suppression than expected; these effects are particularly troublesome at short mixing times (ca. <100 ms). We go on to show how a simple modification of the jump-return NOESY experiment can eliminate these problems.

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THEORY

Jump-return methods

The basic jump-return sequence is 90_x-Δ-90_{-x}. Like all such sequences, it is designed to be applied in situations where all the magnetisation is aligned along the z-axis. If this is the case, the jump-return sequence produces an excitation profile of the form $\sin(\Omega\Delta)$, where Ω is the offset from the transmitter. The transmitter is placed on resonance with the water which, as a result, is not excited. Transverse magnetisation from spins which are on resonance is unaffected by the jump-return sequence; there is *no* suppression of such signals. These observations have important consequences when a jump-return sequence is used in a NOESY experiment.

The pulse sequence for the jump-return NOESY experiment is shown in Fig. 1A. As it is required that the transmitter be placed on resonance with the water, frequency discrimination in the F_1 dimension is essential. This can be done in a number of ways (States et al., 1982; Marion and Wüthrich, 1983; Marion et al., 1989), all of which turn out, for the purposes of this discussion, to have the same effect on the water magnetisation. We will, therefore, restrict the description to the TPPI method.

In the TPPI method the phase ϕ_1 of the first pulse is incremented by 90° each time the value of t_1 is incremented. When the phase of the first pulse is $(x,y,-x,-y)$ the water magnetisation present at the start of the mixing time is aligned along the $(z,x,-z,-x)$-axes, respectively. As discussed earlier, the jump-return sequence only suppresses the water signal if the corresponding magnetisation is aligned along the z-axis. Clearly this is not the case for the second and fourth increments of the TPPI procedure. For the steps where the water magnetisation is transverse at the start of the mixing time, we would expect no suppression of the water magnetisation.

The question is, then, how is it that the jump-return NOESY experiment is at all usable? We

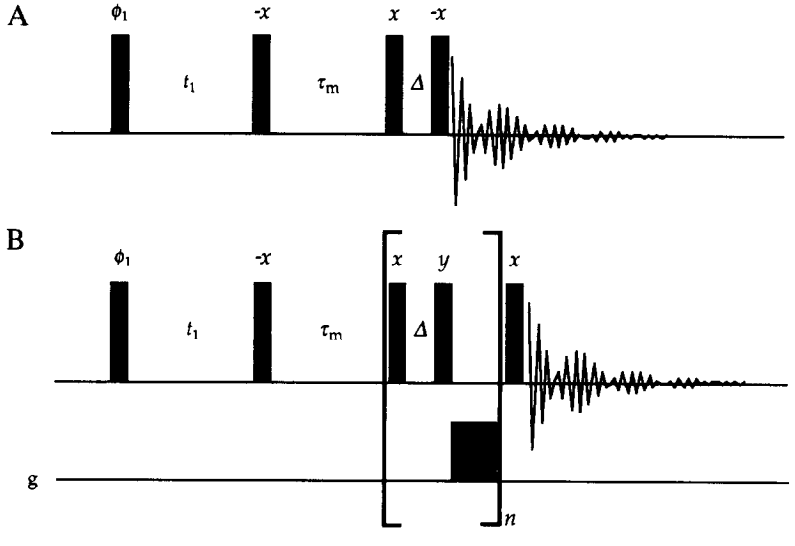

Fig. 1. Pulse sequences used for recording jump-return NOESY spectra. (A) The conventional sequence; and (B) a modified suppression sequence, described in the text. All pulses have flip angles of 90° with phases as shown; the timing of field gradient pulses is shown on the line marked g. The sequence in brackets can be repeated n times.

will show, in the next section, that the presence of radiation damping is crucial to obtaining adequate water suppression in conventional jump-return NOESY spectra.

Radiation damping

The presence of transverse magnetisation in a sample induces an oscillating current in the coil of the NMR probe. In turn, this current generates a transverse magnetic field which, it turns out, has a tendency to rotate the original magnetisation towards the $+z$-axis. This effect, known as radiation damping (Bloembergen and Pound, 1954; Warren et al., 1989), is generally only significant for very intense resonances, such as that from solvent H_2O.

The overall result of radiation damping is to rotate the magnetisation towards the $+z$-axis; in the case of solvent water the time taken for radiation damping to return the magnetisation to the $+z$-axis can be much shorter than that determined by the longitudinal relaxation rate. For example, using a 600 MHz spectrometer we found that following a 90° pulse the water returns from the transverse plane to the $+z$-axis in ca. 50 ms. After a perfect 180° pulse, the magnetisation is along the $-z$-axis; as there is no transverse magnetisation, no radiation damping occurs. However, in practice after a nominal 180° pulse, pulse imperfections and the effect of B_1 inhomogeneity always leave a finite transverse component and thus radiation damping is observed. For example, after a 180° pulse about x, the water damps in the yz-plane; we found the maximum transverse component occurred after ca. 70 ms. If a gradient pulse (of duration 2 ms and approximate strength 10 G cm^{-1}) immediately follows the 180° pulse, the onset of radiation damping is retarded; we found that the water magnetisation passes through the transverse plane after ca. 150 ms.

If the mixing time in a jump-return NOESY is sufficiently long, radiation damping will have returned the water magnetisation to the $+z$-axis, regardless of the alignment of the magnetisation at the start of the mixing time. Thus, the jump-return sequence provides adequate water suppression for all steps of the TPPI procedure. If, however, during the mixing time the water magnetisation has not returned completely to the $+z$-axis, the accompanying transverse component will be unaffected by the jump-return sequence and hence the water suppression will be compromised. Clearly the TPPI increment in which the water magnetisation is placed along the $-z$-axis at the start of the mixing time and in which the return to the $+z$-axis is the slowest, will suffer first. For this TPPI increment the state of the water magnetisation throughout the sequence is illustrated in Fig. 2. Figure 2A shows the case in which the mixing time is long enough for the water magnetisation to have returned to the $+z$-axis by the end of this period. For very short mixing times, Fig. 2B, the magnetisation hardly moves from $-z$; the jump-return sequence leaves this magnetisation along $-z$ and then radiation damping during data acquisition will lead to a large water resonance being recorded. The poor water suppression found when the magnetisation from water is along $-z$ at the start of the mixing time has been noted before (Bax et al., 1987; Sklenář et al., 1987).

It is common practice to use a field gradient or homospoil pulse to remove any transverse water magnetisation present during the mixing time; the question is, in the light of the above discussion, where should such a pulse be placed. If the gradient is placed at the beginning of the mixing time, then the two TPPI scans where the water magnetisation is transverse at this point will benefit; this magnetisation will be dephased and hence not observed. However, the TPPI increment where the water is aligned along the $-z$-axis at the start of the mixing time may suffer as a result of the introduction of a gradient pulse. As explained above, the application of a gradient after an

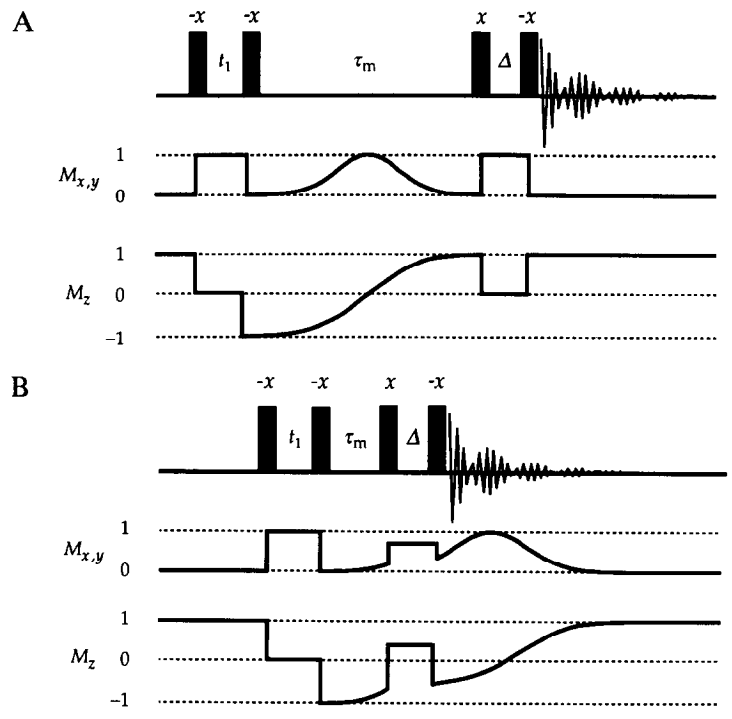

Fig. 2. A schematic representation of the state of the water magnetisation for the TPPI increment in which the magnetisation is aligned along the $-z$-axis at the start of the mixing time; (A) and (B) are appropriate for long and short mixing times, respectively. During a long mixing time radiation damping rotates the water magnetisation through the transverse plane and onto the $+z$-axis; by the end of the mixing time the magnetisation is along $+z$ and is thus not excited by the jump-return sequence. However, when the mixing time is short, the water magnetisation remains close to the $-z$-axis, a situation which is not altered by the jump-return sequence. Subsequent radiation damping during acquisition generates transverse water magnetisation and hence compromises the water suppression. The components of the water magnetisation along the z-axis and in the transverse plane are represented as fractions of the equilibrium magnetisation and are denoted M_z and $M_{x,y}$, respectively.

inversion pulse retards radiation damping and, as a result, the water magnetisation may very well remain close to the $-z$-axis throughout the mixing time. Subsequently, the magnetisation may undergo radiation damping during acquisition, thus compromising water suppression, as illustrated in Fig. 2B.

Placing the gradient pulse at the end of the mixing time is a more effective method for improving the level of water suppression. For the TPPI increment where the water magnetisation is along the $-z$-axis at the start of the mixing time, radiation damping will build up a transverse component which will be eliminated by the gradient pulse, thus improving the water suppression. Indeed, if the damping has proceeded to the point where the magnetisation vector is above the transverse plane, no further difficulties with damping during data acquisition are expected. For the two TPPI increments where the water magnetisation is transverse at the start of the mixing time, radiation damping will return some or all of this magnetisation to the $+z$-axis, depending on the length of the mixing time. Any remaining transverse components will be dephased, enabling the jump-return sequence to achieve a good level of water suppression.

Even with the gradient pulse placed at the end of the mixing time, poor water suppression may still be found for short-mixing-time NOESY experiments. In such cases, or if radiation damping is quite slow, there may not be enough time for the water magnetisation to reach the transverse plane from its initial position along the $-z$-axis. The gradient pulse will destroy the transverse component of the magnetisation, but the component aligned along the $-z$-axis may well, as a result of radiation damping, become observable during acquisition and thus the water suppression will be compromised.

The water magnetisation is also subject to radiation damping during t_1 and as t_1 increases, the radiation damping becomes more significant. As a result of this, the water magnetisation in the third TPPI increment will not be aligned perfectly along the $-z$-axis at the start of the mixing time; there will also be a transverse component. This transverse component increases the initial rate of radiation damping during the mixing time and speeds up the return of the water magnetisation to the $+z$-axis. Thus, the problems outlined above are alleviated as the two-dimensional experiment proceeds.

A new jump-return sequence

In this section we introduce a modified jump-return sequence which avoids *all* of the difficulties noted above, and thus gives water suppression of constant quality, regardless of the mixing time or the TPPI increment. The modified NOESY experiment is shown in Fig. 1B; it contains the suppression sequence 90_x-Δ-90_y-gradient, which may be repeated n times. This sequence takes z-magnetisation and returns it to the z-axis with an amplitude factor $\sin(\Omega\Delta)$; a subsequent 90° pulse turns this z-magnetisation into the transverse plane, giving an excitation profile identical to the jump-return sequence. However, the crucial difference is that y-magnetisation is dephased by this sequence, and for $n \geq 2$ so is x-magnetisation. Thus, unlike the jump-return sequence, the modified method will give good water suppression, regardless of the axis along which the water magnetisation is aligned. The excitation profile for n repetitions is $\sin^n(\Omega\Delta)$.

We saw previously that the TPPI increment in which the phases of the first two pulses are the same (i.e. $-x$, $-x$) gives the poorest water suppression in the conventional jump-return NOESY experiment. At the end of the mixing time the water magnetisation is expected to be somewhere in the yz-plane; the exact position depends on the extent of radiation damping that has occurred during t_1 and the mixing time. A single suppression unit (i.e. $n = 1$) will result in dephasing of *all* the water magnetisation, because this magnetisation will all be transverse when the gradient is applied; thus, none of the aforementioned problems associated with this particular TPPI increment will be experienced. For the two TPPI increments where the phase of the first pulse is y or $-y$, the water magnetisation will be transverse at the start of the mixing time, regardless of the degree of radiation damping during t_1. For these increments one repetition of the suppression sequence is inadequate, as this does not suppress x-magnetisation; a sequence with $n = 2$ is needed. However, for these increments, if the mixing time is long enough for radiation damping to have returned the water magnetisation to the $+z$-axis by the time the suppression sequence is applied, one repetition will suffice.

This suppression sequence has two other advantages. Firstly, it results in a $\sin^n(\Omega\Delta)$ excitation profile in a single scan, enabling good water suppression to be achieved in cases where the water line is inhomogeneously broadened; no additional, and possibly time-consuming, phase cycling is needed. Secondly, in contrast to other jump-return sequences which produce similar excitation

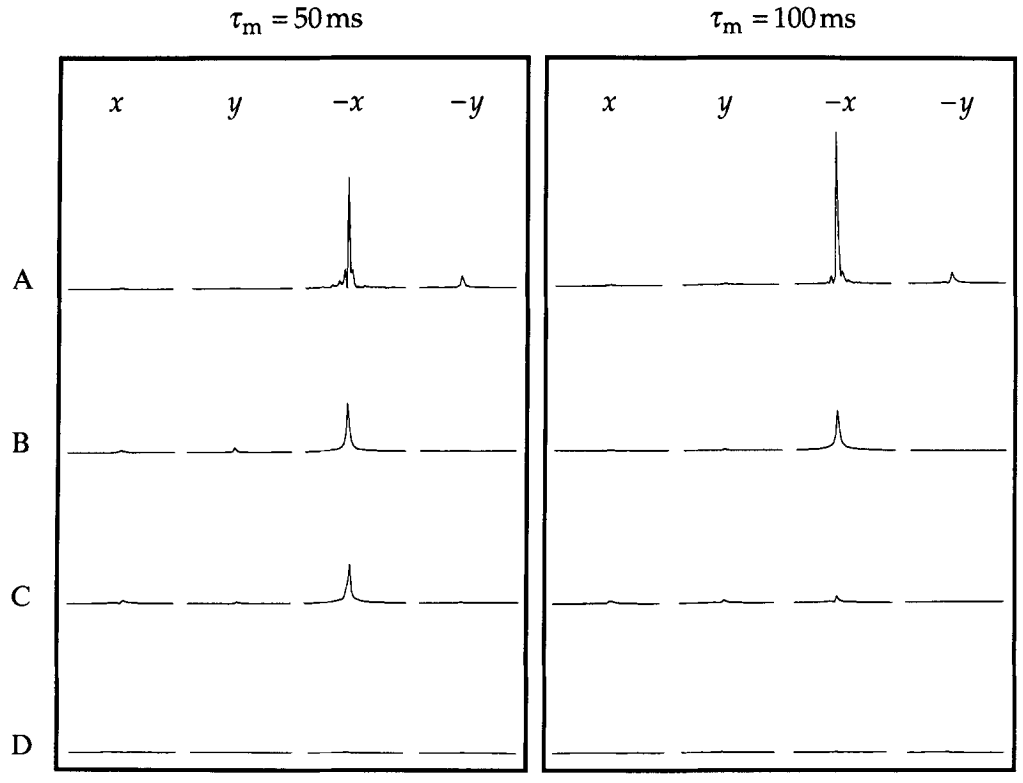

Fig. 3. Absolute-value spectra of the water resonance recorded with t_1 set to zero and using (A) the jump-return NOESY sequence of Sklenář and Bax, (B) and (C) the same sequence, with a gradient pulse placed at the beginning and end, respectively, of the mixing time; and (D) the jump-return NOESY sequence of Fig. 1B with $n = 3$. A spectrum for each TPPI increment, indicated by the phase of the first proton pulse, is shown; two values of the mixing time, 50 and 100 ms, were used as indicated. The three gradient pulses used in the gradient suppression sequence of Fig. 1B were of duration 1.3, 1.7 and 2.0 ms, all shaped to a 5% truncated Gaussian and of peak strength 10 G cm^{-1}; the delay Δ was 138 μs. All spectra were recorded using a 2 mM protein solution at pH 6.5 in 90% H_2O/10% D_2O at 298 K, and are plotted on the same scale. The most intense water resonance in these spectra has approximately the same intensity that would be seen in a simple 90°-acquire spectrum. These spectra were recorded on a Bruker AMX600 spectrometer equipped with a triple-resonance probehead, incorporating a single-shielded gradient coil.

profiles, the resulting spectrum does not require any first-order phase correction. If the unit is repeated a number of times, care must be taken to ensure that subsequent gradients do not refocus the dephasing caused by earlier ones. This is achieved by simply making the strength or length of each gradient different.

RESULTS

Figure 3 shows 1D spectra recorded with different NOESY pulse sequences for the four TPPI increments and with t_1 set to zero; two mixing times of 50 and 100 ms have been used and the phases of the first 90° pulse are as shown. The spectra in Fig. 3A were recorded using the modified jump-return sequence of Sklenář and Bax (1987), which produces a $\sin^3(\Omega\Delta)$ excitation

profile. Note that, as predicted, there is poor suppression of the water resonance in the third TPPI increment. Adding a gradient at the beginning of the mixing time gives the spectra of Fig. 3B; there is some improvement, but the third increment still shows markedly poorer water suppression than any of the others. Adding a gradient at the end of the mixing time results in the spectra of Fig. 3C; for the longer mixing time, the problem is almost solved, but for a mixing time of 50 ms the suppression is still less than ideal. All of these problems are avoided with the new jump-return sequence proposed here, which was used to record the spectra of Fig. 3D. Three repetitions of the suppression unit were used, resulting in even water suppression, regardless of mixing time and TPPI increment.

The choice of the number of repetitions, n, can be made on the basis of the degree of water suppression required and on whether it is desired to observe signals close to the water resonance, in which case a sequence with $n = 1$ or $n = 2$ may be used.

CONCLUSIONS

We have shown that the combination of radiation damping and the need to achieve F_1 frequency discrimination leads to uneven and often unacceptable water suppression in certain increments of jump-return NOESY experiments. These difficulties are most pronounced when short mixing times are used. However, the use of a modified jump-return sequence, which incorporates field gradient pulses, neatly side-steps all of these difficulties and gives even water suppression for all increments of the NOESY experiment. In addition, the modified jump-return sequence gives, in a single scan, an excitation profile of the form $\sin^n(\Omega\Delta)$; no first-order phase correction of the resulting spectra is required.

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