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# Spin echo NMR spectra without J modulation†

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**The spin echo is the single most important building block in modern NMR spectroscopy, but echo modulation by scalar couplings *J* can severely complicate its use. We show for the first time that a general but unacknowledged solution to such complications already exists.**

The spin echo is key to many chemical applications of NMR, being used to suppress the effects of chemical shifts and field inhomogeneity, to measure spin–spin relaxation times $T_2$, and to discriminate between signals with different $T_2$s. Echo modulation was responsible for the original discovery of J couplings,[1] but significantly restricts their use. It is well-known that homonuclear J modulation can be quenched by rapid refocusing.[2] In the Carr–Purcell–Meiboom–Gill (CPMG) experiment[3,4] of Fig. 1a, modulation is suppressed (at the cost of high RF power deposition) if it arises from couplings between spins with chemical shift differences $\Delta\nu \ll 1/\tau$. It has recently been shown[5–10] that the cumulative effect of pulse imperfections can reduce or even suppress modulations in CPMG experiments at favourable resonance offsets, even for interpulse spacings $2\tau$ much longer than $1/\Delta\nu$ (the "SITCOM" effect,[7] 'stabilization by interconversion within a triad of coherences under multiple refocusing'). It is also known[11,12] that J modulation can be refocused in the special case of a weakly-coupled two-spin system AX, in what Takegoshi *et al.* call a "perfect echo"[11] (recently rediscovered[13]), by inserting a 90° pulse at the midpoint of a double spin echo; and that this can reduce J modulation for other spin systems.[12] A zero/double quantum filtration method for two-spin systems has also been proposed recently,[14] but is less general than the perfect echo. J modulation can in favourable cases also be avoided by using multiplet-selective 180° pulses, but only for one multiplet at a time.

The extra 90° pulse in Fig. 1b exchanges coherence between spins and reverses the apparent sense of J modulation, so that the second half of the double spin echo refocuses the modulation caused by the first. The effect is not in fact restricted to AX spin systems; it extends to arbitrary spin systems provided that $\tau J \ll 1$, and has been exploited in *in vivo* measurements of metabolites such as glutamate.[15] (The theoretical basis for the effect, which relies on all coupled spins sharing the same initial state, is described briefly below).

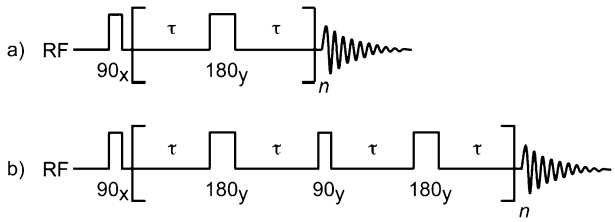


**Fig. 1** Pulse sequences for (a) the CPMG experiment; (b) CPMG with a $90^\circ_y$ refocusing pulse to suppress J modulation ('PROJECT').

It appears, however, to have escaped notice that when J refocusing is integrated into the CPMG experiment it offers a simple *and general* method for suppressing J modulation, even if the interpulse spacings $2\tau$ are relatively long (and the radiofrequency (RF) duty cycle therefore low). Although the results for higher echo numbers were imperfect, the experiment of Fig. 1b is shown explicitly for the AX spin system in the paper of Takegoshi *et al.*,[11] and it is surprising that the extension to other spin systems has not been made, particularly as van Zijl[12] *et al.* give an analysis of the basic perfect echo sequence for $AX_n$ spin systems. Using repeated perfect echoes it becomes possible to measure $T_2$-weighted spectra without J modulation at much lower pulse repetition rates than hitherto (without restriction on resonance offsets), and to suppress signals with short $T_2$s without significant sample heating. As well as offering significant advantages in the many experimental methods that use $T_2$-weighting (*e.g.* NMR metabolomics, drug discovery by saturation transfer difference), it opens up new possibilities in the study of chemical exchange and molecular dynamics, and in diffusion-ordered spectroscopy (DOSY).

Fig. 1 shows the parent CPMG pulse sequence, and the CPMG sequence with suppression of J modulation by $90^\circ_y$ refocusing pulses. The phases of all pulses are shown as fixed, but both phase cycles[16] (between acquisitions) and phase sequences[17] (within a given echo train) may be used to reduce the impact of experimental imperfections. The parent perfect echo sequence is Fig. 1b with $n = 1$; the cyclic analogue $n > 1$ is here distinguished by the name PROJECT (Periodic Refocusing of J Evolution by Coherence Transfer), as it does not form perfect echoes for systems of more than two spins but does still suppress J modulation. Intriguingly, the bracketed cyclic perfect echo component of Fig. 1b has been used previously as a planar mixing sequence for propagating spin waves in linear spin chains,[18] though not for $T_2$ weighting.

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At present, $T_2$ in coupled spin systems is normally measured using very short interpulse spacings $2\tau$, which can cause severe sample heating and suppresses the effects of slow chemical exchange processes. It has recently been shown[8] that measurements can be made at significantly longer spacings (approaching 1 ms) provided that these intervals are carefully chosen with respect to resonance offsets. Here we show, for the first time for arbitrary spin systems, that with the sequence of Fig. 1b, interpulse spacings $2\tau$ can be used that are an order of magnitude greater than this, irrespective of offset.

Fig. 2 compares the results obtained with the sequences of Fig. 1a and b in measuring $T_2$-weighted $^1$H spectra of the macrolide antibiotic clarithromycin in dimethylsulfoxide-$d_6$. Fig. 2a and b show spectra obtained for a total echo time of 128 ms. As expected, the CPMG spectrum (a) suffers from severe J modulation, but spectrum (b) shows none. The effects on individual multiplets are illustrated by (c) and (d), which show expansions of the region around 4.4 ppm containing the sugar 1′ and 4″OH resonances, where again the sequence of Fig. 1b can be seen to suppress J modulation. The 90° pulses may be viewed as spin locking together the multiplet components at a given chemical shift when, as here, $\tau J \ll 1$. The relaxation rates $R_2 = 1/T_2$ obtained here are thus effectively those for in-phase coherences, rather than the weighted average of in-phase and antiphase rates seen in experiments with longer $\tau$. As with any CPMG measurement in a coupled spin system, however, coherence transfer between spins means that the $T_2$ values obtained are not solely associated with any given spin. Just as the CPMG sequence of Fig. 1a causes isotropic mixing of coherences, so the sequence of Fig. 1b causes planar mixing. The practical difference is that the undesirable mixing is a factor of 2 slower with Fig. 1b than with the conventional sequence, Fig. 1a.

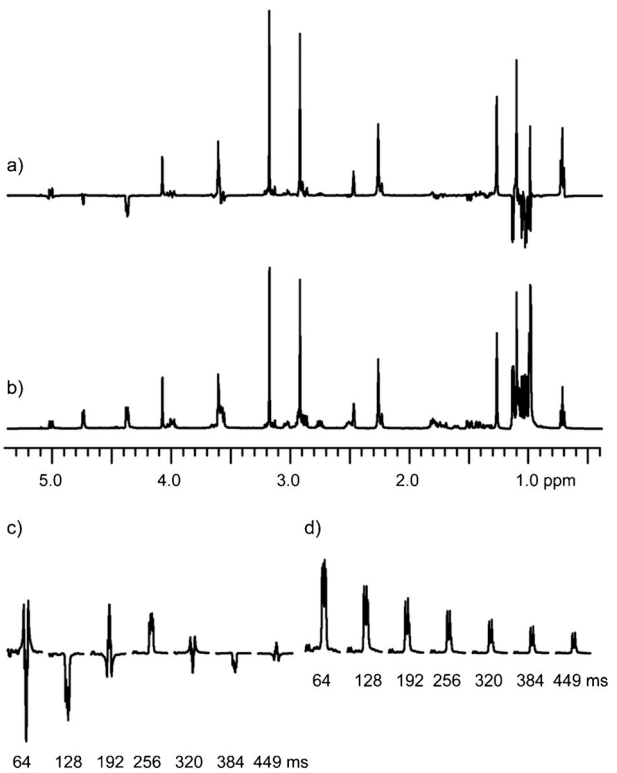


**Fig. 2** 500 MHz $^1$H $T_2$ measurements on 75 mM clarithromycin in dimethylsulfoxide-$d_6$. (a) and (b), spectra obtained using the sequences of Fig. 1a and b respectively with a delay $\tau = 8$ ms and a total echo time of $4n\tau = 128$ ms ($n = 4$). (c) and (d), corresponding results for the two doublets between 4.3 and 4.5 ppm as a function of total echo time $4n\tau$.

$T_2$-weighting is frequently used to suppress interfering signals from high molecular weight, relatively low mobility species, for example in NMR metabolomics[19] and in drug discovery methods such as saturation transfer difference.[20] Long CPMG sequences with high duty cycles are commonly used to attenuate such signals, but cause undesirable sample heating. Fig. 3 shows, for an aqueous sample of beef and yeast extract, that good suppression of broad spectral components can be achieved with very little RF power deposition (here only 25 mW during the echo train). It should be straightforward to adapt such methods for use if strong $T_2$-weighting is required *in vivo*.

The applications of "perfect echo" pulse sequence elements are by no means confined to the above; in particular, the first section of the sequence, $-\tau - 180 - \tau - 90^\circ_y$, may be regarded as a "prefocusing" unit, resulting in J modulation equivalent to a time *minus* $2\tau$, and hence allowing J modulation to be refocused at a time $2\tau$ later. Such a sequence element can for example be used to suppress the troublesome J modulation seen in experiments such as WATERGATE,[21] or as an alternative to a 45° purge pulse in stimulated echo DOSY sequences such as Oneshot.[22]

The theoretical background is as follows. Spin echo J modulation in a system of spins-1/2 arises because the 180° pulse has the double effect of rotating the coherence of one ("active") spin, and of exchanging α and β spin states for its coupling partners ("passive" spins). For a weakly coupled two-spin system IS, the effect of a standard Carr-Purcell spin echo experiment in the product operator formalism[23] is

$$\begin{aligned} I_z + S_z &\xrightarrow{90^\circ(I_x+S_x)} -(I_y + S_y) \\ &\xrightarrow{2\pi J_{IS} I_z S_z 2\tau;\ 180^\circ(I_y+S_y)} -(I_y + S_y)\cos\theta_J \\ &\quad + 2(I_x S_z + I_z S_x)\sin\theta_J \end{aligned} \qquad (1)$$

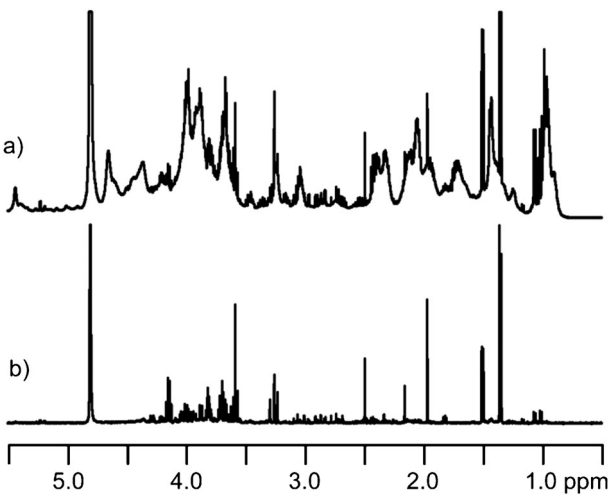


**Fig. 3** 500 MHz (a) normal $^1$H and (b) $T_2$-weighted spectra for a sample of beef and yeast extract ("Bovril", Unilever UK, 5% w/w in $D_2O$). Spectrum (b) used the sequence of Fig. 1b with $n = 44$ and a total refocusing time of 1.76 s; 2 transients were acquired in 8 s.

where the chemical shifts are refocused and hence have been ignored, and $\theta_J = 2\pi J_{IS}\tau$. If a 90° pulse is now applied about the $y$ axis, the effect is to leave the in-phase $y$ terms unchanged but to exchange the $I$ and $S$ antiphase terms, changing their signs so that the net effect of J modulation has been reversed:

$$\begin{aligned} &-(I_y+S_y)\cos\theta_J + 2(I_xS_z+I_zS_x)\sin\theta_J \\ &\xrightarrow{90^\circ(I_y+S_y)} -(I_y+S_y)\cos\theta_J - 2(I_zS_x+I_xS_z)\sin\theta_J \end{aligned} \quad (2)$$

If a second echo is now generated, the J modulation is refocused:

$$\begin{aligned} &-(I_y+S_y)\cos\theta_J - 2(I_zS_x+I_xS_z)\sin\theta_J \\ &\xrightarrow{2\pi J_{IS}I_zS_z2\tau;\ 180^\circ(I_y+S_y)} -(I_y+S_y) \end{aligned} \quad (3)$$

Thus for an AX spin system, as is already known, adding a 90° pulse at the midpoint of a double spin echo completely refocuses the J modulation. The effect does, however, rely on equal initial magnetisations for the two coupled spins, as for example when the spin system is initially at equilibrium.

Consider now the effect of J modulation in such an experiment for a general spin system of $N$ weakly coupled spins-1/2 $I^1\cdots I^N$. If the quantity $\theta_{Jij} = 2\pi J_{ij}\tau \ll 1$ (a much easier condition to fulfill than that for the sequence of Fig. 1a, in which $J$ is replaced by the chemical shift difference), then $\sin\theta_{Jij} \simeq \theta_{Jij} \ll 1$, so that multiply antiphase terms, which are proportional to higher powers of $\sin\theta_{Jij}$, can be neglected, and the effect of J evolution during the first spin echo reduces to

$$\begin{aligned} &\sum_{i=1}^{N} I_z^i \xrightarrow{90^\circ_x} -\sum_{i=1}^{N} I_y^i \\ &\xrightarrow{2\tau;180^\circ_y} -\sum_{i=1}^{N} I_y^i\cos\theta_J + 2\sum_{i=1}^{N}\sum_{j=i+1}^{N}(I_x^iI_z^j + I_z^iI_x^j)\sin\theta_{J_{ij}} \end{aligned} \quad (4)$$

Applying a $90^\circ_y$ pulse at this point once again exchanges and inverts the antiphase terms, and a second echo refocuses the J modulation:

$$\begin{aligned} &-\sum_{i=1}^{N} I_y^i\cos\theta_J + 2\sum_{i=1}^{N}\sum_{j=i+1}^{N}(I_x^iI_z^j + I_z^iI_x^j)\sin\theta_{J_{ij}} \\ &\xrightarrow{90^\circ_y;2\tau;180^\circ_y} -\sum_{i=1}^{N} I_y^i \end{aligned} \quad (5)$$

Thus for short $\tau$ the additional 90° pulse fully refocuses J modulation for arbitrary networks of weakly coupled spins-1/2.

In the case of strong coupling, a different mechanism of modulation suppression comes into play. Because in the strong coupling case $\tau$ is short compared to the inverse of the chemical shift difference $\Delta\delta_{ij}$ as well as to $1/J_{ij}$, the effect of differential precession of the chemical shifts of coupled pairs is small, and as a result the effects of the three terms in the coupling Hamiltonian $I_x^iI_x^j$, $I_y^iI_y^j$ and $I_z^iI_z^j$ cancel: the coupled spins can be approximated as chemically (and indeed magnetically) equivalent over the timescale $\tau$.

All spectra were measured at 25 °C on a 500 MHz Bruker Avance II+ spectrometer equipped with a 5 mm probe. The pulse sequence used is listed in the ESI.

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