

Polarization Transfer

Many interesting nuclei suffer from poor inherent sensitivity as a result of low natural isotopic abundance or their low gyromagnetic ratio. Notable among these are the nuclei carbon-13 and nitrogen-15, now extensively used for the simplification of proton spectra of biologically important macromolecules. Since the magnetic moment, the Boltzmann populations, and the nuclear precession frequency (which determines the induced NMR voltage), all increase in proportion to the gyromagnetic ratio γ , the signal intensity increases as γ^3 , give or take some minor corrections for radiofrequency losses in the probe. As noise increases as the square root of the frequency, sensitivity is roughly proportional to $\gamma^{5/2}$. Thus, apart from the special case of radioactive tritium, protons have by far the highest intrinsic sensitivity of all nuclear species.

From the earliest days of NMR it was realized that considerable improvements in sensitivity could be achieved by artificially increasing the Boltzmann factors. The possible techniques include prepolarization in a very intense magnetic field, the electron–nuclear Overhauser effect (1), Hartmann–Hahn cross-polarization (2), optical pumping experiments, chemically induced nuclear polarization, and the exploitation of the special properties of parahydrogen (3). However, for the most part, these methods are insufficiently general to be important for high-resolution liquid-phase spectroscopy.

The nuclear Overhauser effect* provides a signal enhancement given by

$$E = 1 + \frac{1}{2} \left(\frac{\gamma_I}{\gamma_S} \right) \quad [1]$$

in situations where the spin–lattice relaxation of the low-sensitivity nuclei (the S spins) is dominated by their dipole–dipole interaction with the protons (the I spins). Consequently, carbon-13 spectra can benefit from a signal-to-noise improvement as high as three-fold when the attached protons are saturated. Unfortunately, since the gyromagnetic ratio of nitrogen-15 is negative, the maximum enhancement $E = -4$, and if the dipolar relaxation has to compete with other mechanisms (leakage), the observed nitrogen-15 signal can be near zero.

Polarization transfer experiments offer a bigger sensitivity advantage for two reasons. First, by transferring the benefits of proton polarization to the S spins, they augment the signal-to-noise by the population factor (γ_I/γ_S) which is larger than E in eqn [1]. Second, they permit multiple acquisitions of the S-spin signal at a rate limited only by the spin–lattice relaxation of the protons, which is usually more

efficient than that of the S spins. No penalty is incurred by a negative gyromagnetic ratio. Thus nitrogen-15 signals are enhanced by a factor of ten.

THE 'INEPT' TECHNIQUE

Insensitive nuclei enhanced by polarization transfer (INEPT) is now used as a module in a large number of pulse sequences designed to improve sensitivity. First it is helpful to consider an earlier experiment called selective population transfer (4) in which a selective 180° spin inversion pulse is applied to one proton line of the doublet generated by the heteronuclear coupling J_{IS} . The appropriate energy-level

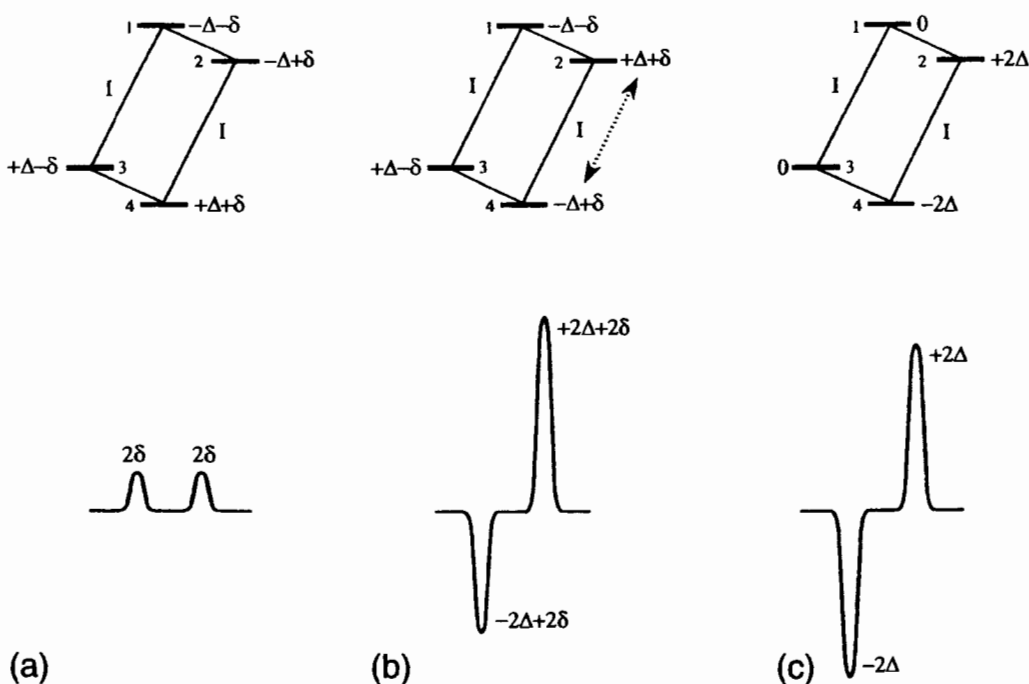


Fig. 1. Energy-level diagram for a two-spin IS system illustrating how a selective 180° pulse inverts the populations across one of the I-spin transitions (2–4). (a) Boltzmann populations and intensities. (b) Perturbed populations and intensities. (c) Changes from Boltzmann populations, indicating a sensitivity advantage of $\Delta/\delta = \gamma_I/\gamma_S$.

diagram is set out in Fig. 1. At Boltzmann equilibrium the spin populations on these levels can be written as

- (1) $-\Delta - \delta$
- (2) $-\Delta + \delta$
- (3) $+\Delta - \delta$
- (4) $+\Delta + \delta$

where 2Δ represents the equilibrium population difference across the proton (I) transitions and 2δ corresponds to the population difference across the S-spin transitions; thus $\Delta/\delta = \gamma_I/\gamma_S$. Selective inversion of (say) the (2–4) proton transition, leaving the (1–3) transition unaffected, rearranges the spin populations into a non-equilibrium distribution:

$$\begin{aligned} (1) & -\Delta - \delta \\ (2) & +\Delta + \delta \\ (3) & +\Delta - \delta \\ (4) & -\Delta + \delta \end{aligned}$$

If we now evaluate the population differences across the S-spin transitions (1–2) and (3–4), we see that they are increased to $+2\Delta + 2\delta$ and $-2\Delta + 2\delta$, instead of their normal values 2δ . The *changes* are therefore $\pm 2\Delta$, one line being increased in a positive sense and the other inverted (Fig. 1). We shall see below that this ‘up–down’ pattern of intensities is characteristic of many polarization transfer experiments.

Although selective population transfer is hardly a general method of signal enhancement, because the appropriate proton transition has to be located by a tedious trial-and-error search (it is one of the weak satellites of the main proton resonance) it anticipates the mechanism of the INEPT experiment. Here the effect of selective population inversion is achieved by a hard-pulse technique that is effective over the entire band of proton chemical shifts. It may be analysed either by the vector model* or by the product operator formalism*.

After the initial 90° excitation pulse, we represent the proton (I-spin) transverse magnetization by two vectors, α and β , precessing freely at frequencies $(\nu_I + \frac{1}{2}J_{IS})$ and $(\nu_I - \frac{1}{2}J_{IS})$ in the XY plane. After an interval $\tau_1 = 1/(4J)$ they will have precessed to positions that depend on both ν_I and J_{IS} , but the angle between the two vectors will be just 90° (Fig. 2(b)). Then we apply simultaneous hard 180° pulses to both spin species. The I-spin inversion pulse flips these vectors into mirror image positions with respect to the XZ plane of the rotating frame (Fig. 2(c)). This serves to refocus the I-spin chemical shift after a further period τ_1 of free precession, but the 180° pulse applied to S spins (Fig. 2(d)) interchanges the I-spin labels α and β , so that the I-spin vector that initially precessed at a frequency $(\nu_I + \frac{1}{2}J_{IS})$ now precesses at $(\nu_I - \frac{1}{2}J_{IS})$, and vice versa. Consequently the divergence due to J-coupling persists, and the two vectors reach the $\pm X$ axes at time $2\tau_1$ (Fig. 2(e)). At this point a 90° pulse applied about the +Y axis (that is to say, a phase-shifted pulse) turns the two vectors into the $\pm Z$ axes (Fig. 2(f)). One of the two proton transitions has suffered a population inversion while the other is at Boltzmann equilibrium. This is just the configuration that would have been reached by a selective population transfer experiment, but it has been achieved for all the I spins, irrespective of chemical shift. A final 90° read pulse applied to the S spins generates ‘up–down’ doublets for all the S-spin sites. Usually the natural (unenhanced) signals from the S spins are cancelled by difference spectroscopy by applying alternating $90^\circ(\pm Y)$ pulses on the I spins at the stage shown in Fig. 2(f).

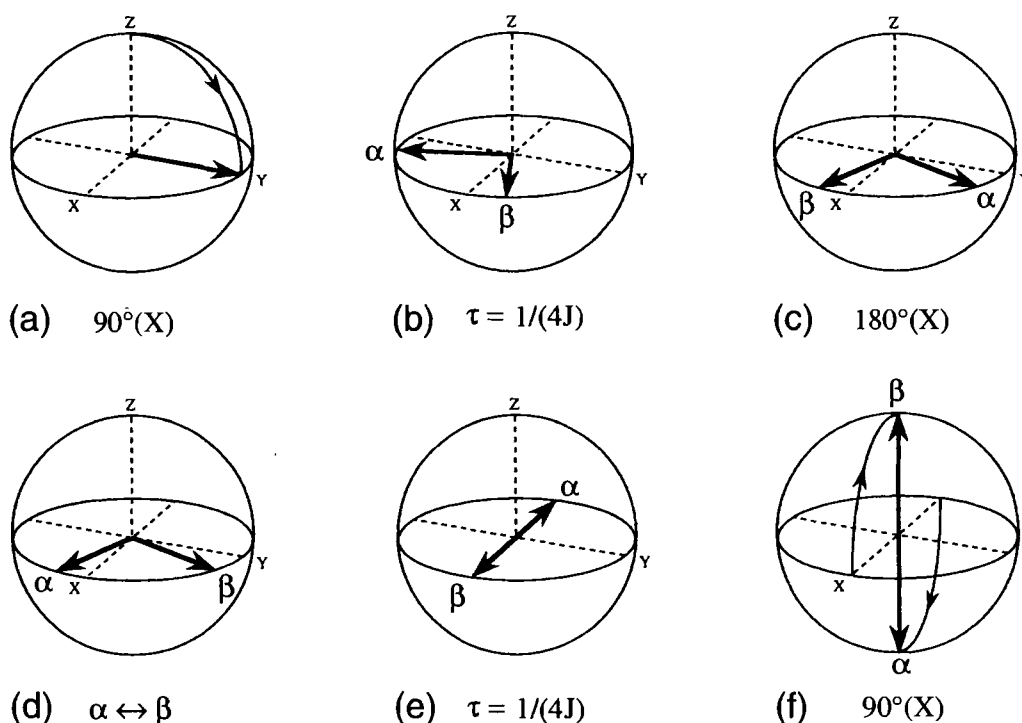


Fig. 2. The INEPT preparation of the I spins according to the vector model. (b) The vectors α and β are allowed to evolve for a time $\tau = 1/(4J_{IS})$ until they subtend an angle of 90° . (c) A $180^\circ(X)$ pulse applied to the I spins flips these vectors into mirror image positions with respect to the XZ plane. (d) A 180° pulse on the S spins interchanges the I-spin labels ($\alpha \leftrightarrow \beta$). (e) Free precession for a further period τ brings the I-spin vectors into opposition along the $\pm X$ axes. (f) A $90^\circ(Y)$ pulse realigns them along the $\pm Z$ axes, a condition equivalent to the selective population inversion of Fig. 1(c).

This leaves just the polarization transfer components, enhanced over their normal intensities by a factor $\pm(\gamma_I/\gamma_S)$.

In the product operator formalism these spin manipulations would be written as

$$\begin{aligned}
 I_Z \xrightarrow{I_x} & -I_Y \xrightarrow{(\pi/4) 2I_Z S_Z} -I_Y \cos(\pi/4) + 2I_X S_Z \sin(\pi/4) \xrightarrow{\pi I_x} \\
 & +I_Y \cos(\pi/4) + 2I_X S_Z \sin(\pi/4) \xrightarrow{\pi S_x} +I_Y \cos(\pi/4) - 2I_X S_Z \sin(\pi/4) \\
 & \xrightarrow{(\pi/4) 2I_Z S_Z} +2I_X S_Z \xrightarrow{I_y} -2I_Z S_Z \xrightarrow{S_x} +2I_Z S_Y. \quad [2]
 \end{aligned}$$

For simplicity we have neglected the chemical shift evolution of the I spins on the grounds that it is refocused at time $2\tau_1$. Note that an important simplification of the trigonometrical terms has resulted from the condition $\tau_1 = 1/(4J)$, ensuring that the evolution under the $2I_Z S_Z$ operator amounts to $\pi/4$ radians, where the sine and cosine are equal. The final term $2I_Z S_Y$ represents an S-spin absorption-mode doublet with the 'up-down' pattern of intensities. The signal enhancement factor (γ_I/γ_S) usually remains only implicit in the product operator treatment.

If we plan to record the enhanced S-spin spectrum in the coupled mode, the 'up-down' pattern causes little difficulty, but if we intend to decouple the I spins this would result in mutual signal cancellation. The remedy is to introduce a further interval $2\tau_2$ to allow the S-spin antiphase vectors to come back into alignment (5):

$$+2I_Z S_Y \xrightarrow{2I_Z S_Z} -S_X. \quad [3]$$

Then the decoupler can be safely switched on. To avoid any phase shifts that would build up from S-spin chemical shift precession during the $2\tau_2$ interval, it is usual to introduce refocusing and spin inversion 180° pulses at the mid-point of this interval (Fig. 3). Where there is only a single I spin attached to the S-spin site, then the optimum setting of the interval is $\tau_2 = 1/(4J)$, but if some of the S-spin sites have two or three directly attached I spins, then a compromise setting must be employed, usually $\tau_2 = 1/(6J)$.

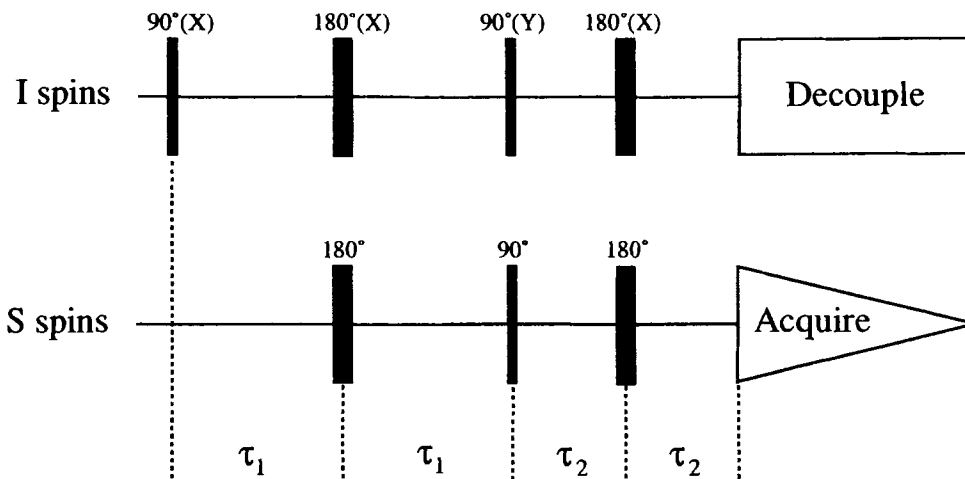


Fig. 3. The pulse sequence for the refocused INEPT experiment. The actual polarization transfer is effected by the simultaneous 90° pulses on the I and S spins. The ensuing evolution during $2\tau_2$ allows antiphase S-spin vectors to come to a focus along the $-X$ axis so that I-spin decoupling can be used.

RELAXATION EFFECTS

The actual sensitivity improvement by the INEPT scheme can be significantly higher than γ_I/γ_S in practice. This is because most experiments involve time averaging, and the rate at which the scans can be repeated is a critical factor. The nitrogen-15 nucleus can have quite long spin-lattice relaxation times compared with protons in the same material, so the direct observation of nitrogen-15 signals would involve long recycling intervals. In the INEPT experiment the natural nitrogen-15 signal is cancelled, all the intensity being obtained through the

polarization transfer. The experiment may therefore be cycled quite fast, limited only by proton spin–lattice relaxation. A clear demonstration of the importance of this relaxation factor is provided by an INEPT experiment performed by Rinaldi and Baldwin (6) using deuterium as the source (I spins). Even though deuterium has far less favourable Boltzmann populations than protons ($\gamma_H = 6.5\gamma_D$), the faster spin–lattice relaxation of this quadrupolar nucleus more than compensates for the population disadvantage.

'ROUND-TRIP' POLARIZATION TRANSFER

It was soon realized that even greater improvements in sensitivity could be achieved if we could return the S-spin polarization to protons before detection, making use of their high detection efficiency. We might describe this as 'round-trip' polarization transfer, $I \rightarrow S \rightarrow I$. The S-spin signal is only detected indirectly and the gyromagnetic ratio of the S spins does not enter into the expression for the sensitivity. Compared with the direct detection of the S-spin spectrum, this round-trip transfer scheme would be expected to enjoy a sensitivity advantage of approximately $(\gamma_I/\gamma_S)^{5/2}$ which is a factor of 32 if S is carbon-13, and 300 if S is nitrogen-15.

Bodenhausen and Ruben (7) were the first to exploit this idea in a technique now known as heteronuclear single-quantum correlation (HSQC). An initial INEPT sequence implements the polarization transfer $I \rightarrow S$, and the S spins are allowed to precess for an evolution period t_1 (decoupled from the I spins by a 180°

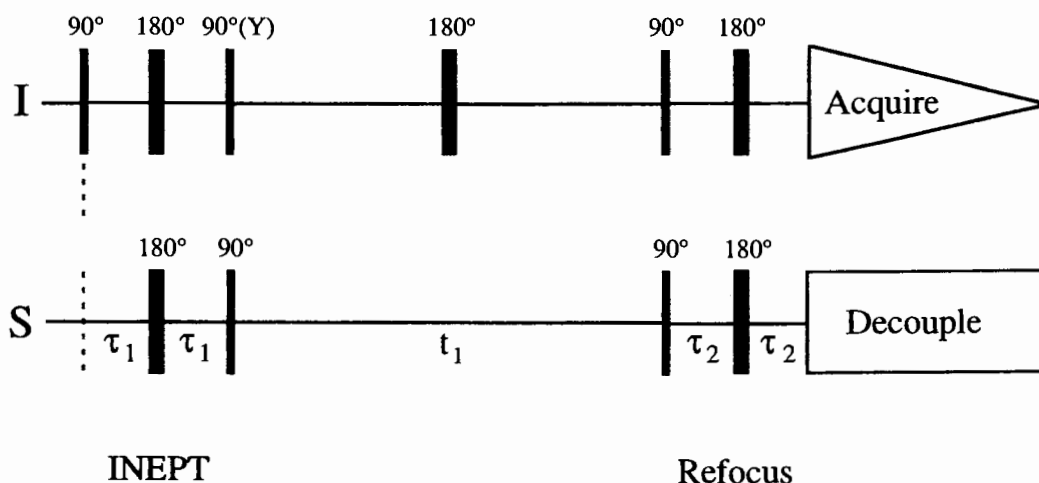


Fig. 4. Pulse sequence for heteronuclear single-quantum correlation (HSQC). All pulses are applied along the X axis except for the one marked $90^\circ(Y)$. The evolution period t_1 allows the S spins to evolve according to their chemical shifts, while the 180° pulse at the mid-point removes the J_{IS} splittings. The second delay τ_2 has a compromise setting since there may be one, two or three I spins at a given S-spin site.

refocusing pulse). At this point the S-spin signal is an antiphase multiplet, so the reverse transfer $S \rightarrow I$ is achieved by the application of simultaneous 90° pulses on both the I and S spins (Fig. 4). If the decoupled I-spin spectrum is required, the usual refocusing stage is tacked on to the end of the sequence.

This analysis has ignored an important complication – the intense signal from the I spins (protons) not coupled to carbon-13 or to nitrogen-15. It is not permissible to presaturate the I spins because their polarization is required to initiate the HSQC transfer. The usual strategy is to start the experiment with the ‘BIRD’ module (see Bilinear rotation decoupling*) which has the property that it leaves the coupled I spins unchanged, but inverts the spin populations of all the I spins not directly attached to the S spins (8). The inverted I-spin signal then recovers through spin–lattice relaxation, passing through the null condition after an interval $T_1 \ln 2$. At this point the HSQC experiment is started, and the only appreciable signals in the final spectrum are those that participate in the round-trip polarization transfer.

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Cross-references

Bilinear rotation decoupling
Multiple-quantum coherence
Nuclear Overhauser effect
Product operator formalism
Sensitivity enhancement
Vector model