2 Refocused INEPT, Delayed Decoupling and In-Phase Spectra

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The refocused INEPT sequence (Fig. 2.1) converts the anti-phase magnetization of the INEPT sequence into in-phase magnetization, while still benefiting from the signal enhancement of the INEPT sequence. This approach has the advantage that the spectrum can be decoupled to produce singlet peaks.

Burum D., Ernst R. Net polarization transfer via a J-ordered state for signal enhancement of low-sensitivity nuclei. J Magn Reson. 1980 Apr;39(1):163–168.

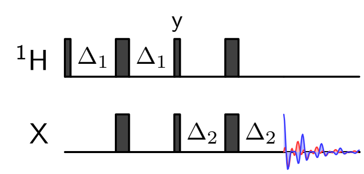

Fig. 2.1. The refocused INEPT experiment.

2.1. Theory

The INEPT sequence produces anti-phase magnetization (*e.g.* $-\boldsymbol{2H_zC_y}$) with peaks of opposite sign, and the refocused INEPT produces in-phase magnetization (*e.g.* $\boldsymbol{C_z}$) with peaks of the same sign. When ^{1}H decoupling is applied to the anti-phase magnetization of the INEPT sequence, the peaks cancel each other to produce a null spectrum. The objective of the refocused INEPT and DEPT experiments is to produce in-phase magnetization that can be decoupled to produce singlet peaks.

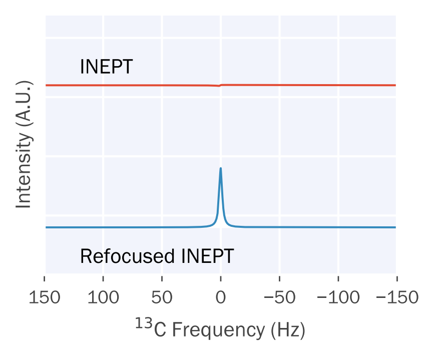

Fig. 2.2. Comparison of ^{13}C spectra for the INEPT sequence and refocused INEPT sequence with ^{1}H decoupling during ^{13}C acquisition.

2.1.1. Methine, Amide and the AX Spin System

The *refocused* INEPT sequence produces in-phase magnetization that can ^{1}H decoupled to produce high intensity singlet peaks.

In this example, we'll use a ^{1}H spin bonded to a ^{13}C spin. If we use a delay, $\Delta_1 = (4J_{CH})^{-1}$, the first step of the sequence is simply an INEPT sequence (Fig. 2.3).

$$\boldsymbol{H_z} \xrightarrow{\text{INEPT}} -\boldsymbol{2H_zC_y}$$

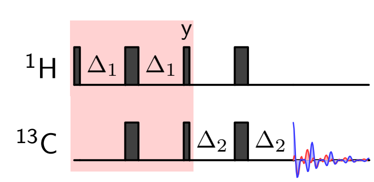

Fig. 2.3. The first step of the refocused INEPT sequence highlighted in red.

In the second step, we'll only propagate the J_{CH}-coupling since the '$\Delta_2 - 180^\circ_x(^{13}C) - \Delta_2$' pulse sequence block refocuses the ^{13}C chemical shifts (Fig. 2.4). Thereafter, we'll apply the two 180° pulses.

$$\xrightarrow{\Delta_2} -\boldsymbol{2H_zC_y}\cos(\pi J_{CH}\Delta_2) + \boldsymbol{C_x}\sin(\pi J_{CH}\Delta_2)$$

$$\xrightarrow{180^\circ_x(^1H),\ 180^\circ_x(^{13}C)} -\boldsymbol{2H_zC_y}\cos(\pi J_{CH}\Delta_2) + \boldsymbol{C_x}\sin(\pi J_{CH}\Delta_2)$$

The final and third step (Fig. 2.5) propagates the magnetization with another Δ_2 period.

$$\xrightarrow{\Delta_2} -\mathbf{2H_zC_y}\cos(\pi J_{CH}\Delta_2)\cos(\pi J_{CH}\Delta_2) + \mathbf{C_x}\cos(\pi J_{CH}\Delta_2)\sin(\pi J_{CH}\Delta_2) + \mathbf{C_x}\sin(\pi J_{CH}\Delta_2)\cos(\pi J_{CH}\Delta_2) + \mathbf{2H_zC_y}\sin(\pi J_{CH}\Delta_2)\sin(\pi J_{CH}\Delta_2)$$

$$= -\mathbf{2H_zC_y}\cos(\pi J_{CH}2\Delta_2) + \mathbf{C_x}\sin(2\pi J_{CH}\Delta_2)$$

The $\mathbf{C_x}$ term is maximum when $\Delta_2 = (4J_{CH})^{-1}$. As with the INEPT sequence, the $\mathbf{C_x}$ magnetization is enhanced by a factor $\frac{K_H}{K_C}$ over the unenhanced version.

For a ^{13}C-INEPT between for a ^{1}H spin bonded to a ^{13}C (J_{CH} = 145 Hz), the magnetization after the is $\mathbf{2H_zC_y}$. The refocused INEPT experiment produces in-phase magnetization, $\mathbf{C_x}$, suitable for ^{1}H decoupling (Fig. 2.6).

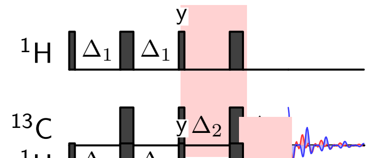

Fig. 2.4. The second step of the refocused INEPT sequence highlighted in red.

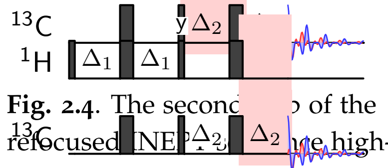

Fig. 2.5. The third step of the refocused INEPT sequence highlighted in red.

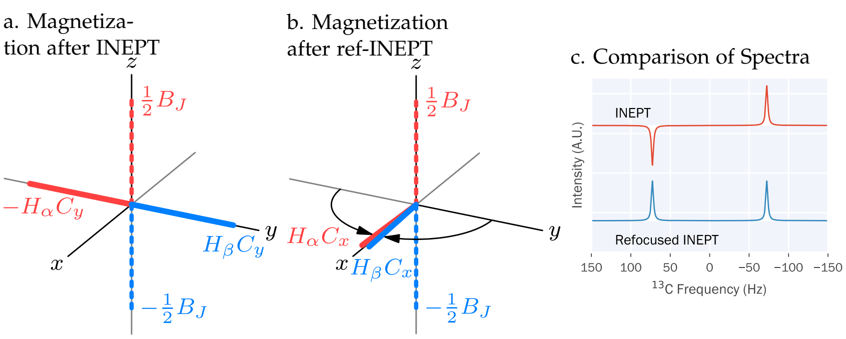

Fig. 2.6. Comparison of the INEPT sequence and the refocused INEPT sequence for a CH group.

2.1.2. *Cosine and Sine Modulation*

2.1.3. *Methylene, Methyl, AX2 and AX3 Spin Systems*

Different Δ_2 periods emphasize different types of spin systems. Varying Δ_2 periods is commonly used to differentiate between CH, CH_2 and CH_3 groups. This principal applies to other X spins, such as ^{15}N, but in the case of NH_2 and NH_3 groups, rapid hydrogen exchange with the solvent may impede the discrimination between these groups.

The initial INEPT period behaves the same for CH, CH_2 and CH_3 groups. This is because each ^{1}H spin is only bonded to one ^{13}C spin.

Once the magnetization is converted to transverse magnetization for the ^{13}C spin, the magnetization evolves with J-couplings to multiple ^{1}H spins during the rest of the refocused INEPT. This is because, from the ^{13}C spin's perspective, a CH group appears as a doublet, a CH_2 group appears as a triplet and a CH_3 group appears as a quartet.

The conversion to $\mathbf{C_x}$ magnetization follows different time dependencies for the CH (AX), CH_2 (AX2) and CH_3 (AX3) groups.