Optimizing the location of the $^{13}$C inversion pulse in a modified INEPT sequence: Illustration with $^{13}$C$_3$ glutamate

Aliyah Yahya$^{1,2}$ and Peter S. Allen$^3$

$^1$Department of Medical Physics, Cross Cancer Institute, Edmonton, Alberta, Canada, $^2$Department of Oncology, University of Alberta, Edmonton, Alberta, Canada, $^3$Department of Biomedical Engineering, University of Alberta, Edmonton, Alberta, Canada

Target Audience: Researchers working in the field of $^{13}$C NMR (Nuclear Magnetic Resonance) spectroscopy.

**Purpose:** To optimize the signal to noise ratio (SNR) of $^{13}$C glutamate (Glu) signal obtained with the localized INEPT (Insensitive Nuclei Enhanced by Polarization Transfer) sequence, also referred to as LINEPT$^1$. The INEPT sequence shown in Fig. 1(a) allows for the direct detection of enhanced $^{13}$C signal from $^{1}$H-J-coupled $^{13}$C nuclei. Spatial localization was incorporated into the sequence by replacing the proton hard pulses by slice selective ones and separating the $^{13}$C inversion pulse from the proton refocusing pulse in time to allow the echo time (TE) to be longer than $1/(2J_{CH})$, which is only $\approx 3.7$ ms for the $C_3$ and $C_4$ carbons of Glu ($J_{CH}$ is the heteronuclear coupling constant). The repositioning of the $^{13}$C inversion pulse was based on heteronuclear product operator calculations which indicate that whether the $^{13}$C $180^\circ$ pulse is placed $1/(4J_{CH})$ after the first proton pulse or $1/(4J_{CH})$ before the third proton pulse, the required heteronuclear coupling evolution for polarization transfer will occur (Fig. 1(b)). The objective of this work is to demonstrate, using $^{13}$C$_3$-Glu at 3 T as an example, that the concurrent strong proton homonuclear coupling evolutions taking place causes the $^{13}$C signal yield of a refocused modified INEPT sequence (Fig. 1(c)) to be dependent on whether the $^{13}$C inversion pulse is placed at the beginning or at the end of the sequence. Our results show that the signal, which also varies with TE, is significantly higher if the pulse is placed $1/(4J_{CH})$ after the proton excitation pulse.

**Methods:** Numerical density matrix calculations were performed in MATLAB by representing $^{13}$C$_3$-Glu as an AMNPOQX spin system. The MN protons are strongly-coupled to each other and to the PQ protons and weakly-coupled to the $^{13}$C nuclei ($J_{CH} = 135$ Hz). The sequence of Figure 1(c) was implemented on a whole-body 3 T scanner; hard pulses were employed for simplicity and a refocused version of INEPT was employed to allow the formation of in-phase $^{13}$C signal (to enable proton decoupling). Two versions of the sequence were employed, one with the $^{13}$C inversion pulse applied $1/(4J_{CH})$ before third proton pulse and the other with the pulse placed $1.85$ ms prior to the third proton pulse. Experiments were conducted on a 2 cm diameter phantom containing $35$ mM of $99\%$ enriched $^{13}$C$_3$-Glu with a home-built 7 cm diameter $^1$H RF (radiofrequency) coil and a 3.5 cm diameter $^{13}$C RF coil. TE values were varied between 10 and 30 ms in steps of 5 ms and a TR (repetition time) of 3 seconds was employed. WALTZ-16 proton decoupling was applied during acquisition and a 16-step phase cycling scheme was used.

**Results:** Figure 2 shows the numerically calculated signal intensity of the decoupled $^{13}$C$_3$ − Glu peak as a function of TE in response to the two versions of the sequence displayed in Fig. 1(c). Intensities are normalized to the higher TE = 10 ms value. It can be seen that the signal decreases with increasing TE; furthermore, the signal is always higher when the $^{13}$C inversion pulse is placed $1/(4J_{CH})$ after the first proton pulse. For example, when TE = 30 ms the signal decreases by more than 50\% when the $^{13}$C $180^\circ$ pulse is applied towards the end of the sequence.

**Conclusion:** To obtain higher $^{13}$C$_3$ or $^{13}$C$_4$-glutamate signal from a modified localized INEPT (LINEPT) sequence, the $^{13}$C inversion pulse should be placed $1/(4J_{CH})$ after the first proton pulse rather than $1/(4J_{CH})$ before the third proton pulse.

**References:**


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**Figure 1:** The modified INEPT sequence$^1$ separates the $^{13}$C inversion pulse from the proton refocusing pulse in time placing it either $1/(4J_{CH})$ after the first proton pulse or $1/(4J_{CH})$ before the third proton pulse.

**Figure 2:** $^{13}$C$_3$-Glu signal as a function of TE in response to the two versions of the sequence shown in Fig. 1(c). The experimental data confirm the numerical calculations. The error bars are half the peak-to-peak noise.