

Artifacts in Sensitivity-Enhanced HSQC

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Proton-proton coupling can generate artifacts in sensitivity-enhanced HSQC spectra. These artifacts appear as cross-peaks involving remote protons. They are caused by relayed coherence transfer during the back-transfer portion of the pulse sequence. We present a product operator analysis of artifact formation and experimental results which demonstrate that the magnitude of these artifacts can exceed 10% of the main peak. © 1999 Academic Press

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Heteronuclear Single Quantum Correlation (HSQC) (1) has become a ubiquitous building block in multi-dimensional NMR. It is, therefore, essential to have a detailed understanding of such an important pulse sequence. Close inspection of Gradient-Enhanced HSQC (GEHSQC) (2) spectra reveals a large number of small artifacts. In situations of high dynamic range, these artifacts can interfere with spectral interpretation, since the artifacts associated with intense signals can obscure weak correlations of interest. Here we explain the origin of these artifacts and describe a simple solution to remove them.

HSQC uses an INEPT sequence (3) to transform proton magnetization into antiphase heteronuclear single-quantum coherence. Heteronuclear chemical shift evolution yields two orthogonal components. A subsequent reverse INEPT converts one of these components back into observable proton coherence. The other half of the initial proton magnetization is converted into unobservable heteronuclear multiple-quantum coherence. For a two-spin **IS** system, evolution of the product operators through the HSQC pulse sequence can be summarized as

$$\begin{aligned}
 I_z &\xrightarrow{\text{INEPT}} \\
 &\xrightarrow{t_1/2 - 180^\circ(I_x) - t_1/2} -2I_zS_y \\
 &\xrightarrow{2I_zS_y\cos(\Omega_s t_1) - 2I_zS_x\sin(\Omega_s t_1)} \quad [1]
 \end{aligned}$$

$$\begin{aligned}
 &\xrightarrow{\text{reverse INEPT}} -I_x\cos(\Omega_s t_1) - 2I_yS_x\sin(\Omega_s t_1). \quad [2]
 \end{aligned}$$

The basic HSQC experiment is often modified to include “sensitivity-enhancement” (4), also known as “Preservation of Equivalent Pathways” (PEP) (5), or “Coherence Order Selective INEPT” (COS-INEPT) (6–9). In practice, the most widely used method is GEHSQC. The advantages of GEHSQC are twofold. First, the process of “coherence-selection” offers a reduction in t_1 -noise. Second, the process of “sensitivity-enhancement” offers the prospect of a signal-to-noise ratio improvement of $\sqrt{2}$ compared to the basic HSQC experiment by transferring both orthogonal components present at the end of t_1 back into observable **I** spin coherence. This process is summarized below for a two-spin **IS** system,

$$\begin{aligned}
 I_z &\xrightarrow{\text{INEPT}} \\
 &\xrightarrow{t_1/2 - 180^\circ(I_x) - t_1/2} -2I_zS_y \\
 &\xrightarrow{2I_zS_y\cos(\Omega_s t_1) - 2I_zS_x\sin(\Omega_s t_1)} \quad [3]
 \end{aligned}$$

$$\begin{aligned}
 &\xrightarrow{\text{reverse INEPT}} -I_x\cos(\Omega_s t_1) - 2I_yS_x\sin(\Omega_s t_1) \quad [4]
 \end{aligned}$$

$$\begin{aligned}
 &\xrightarrow{90^\circ(I_y, S_y)} I_z\cos(\Omega_s t_1) + 2I_yS_z\sin(\Omega_s t_1) \quad [5]
 \end{aligned}$$

$$\begin{aligned}
 &\xrightarrow{\delta - 180^\circ(I_x, S_x) - \delta} -I_z\cos(\Omega_s t_1) - I_x\sin(\Omega_s t_1) \quad [6]
 \end{aligned}$$

$$\begin{aligned}
 &\xrightarrow{90^\circ(I_x)} I_y\cos(\Omega_s t_1) - I_x\sin(\Omega_s t_1), \quad [7]
 \end{aligned}$$

where δ symbolizes the delay $1/(4 J_{IS})$.

A comparison of expressions [2] and [7] demonstrates the sensitivity advantage of GEHSQC. This advantage is obtained by the addition of $\{90^\circ(I_y, S_y) - \delta - 180^\circ(I_x, S_x) - \delta - 90^\circ(I_x)\}$ after the reverse INEPT; the function of this extra pulse sequence element is to allow both components of the magnetization to be manipulated independently. The process involves

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three steps. First, the cosine-modulated **I** spin coherence is stored as longitudinal magnetization while the sine-modulated component is converted into antiphase coherence. Second, the antiphase coherence is refocused into **I** spin coherence. Finally, the cosine-modulated component which had been stored as longitudinal magnetization is brought back into the transverse plane. Unfortunately, this extra pulse sequence element is quite similar to that used for relayed coherence transfer (10, 11).

To gain a better understanding of the problems that may arise, it is easiest to consider a three-spin system **IKS**, consisting of two protons **I** and **K** and one heteronucleus **S** such that **I** and **S** are directly bonded, while **K** (a remote proton) exhibits homonuclear proton coupling to **I**. Long-range heteronuclear coupling J_{SK} is unnecessary. Ignoring product operator terms that do not contribute to the final spectrum, one can summarize the GEHSQC for a three-spin system **IKS** as

$$I_z \xrightarrow{\text{INEPT}} -2I_zS_y\cos(2\pi J_{IK}\delta) \quad [8]$$

$$\xrightarrow{t_1/2-180^\circ(I_x)-t_1/2} -2I_zS_y\cos(\Omega_s t_1)\cos(2\pi J_{IK}\delta) \\ -2I_zS_x\sin(\Omega_s t_1)\cos(2\pi J_{IK}\delta) \quad [9]$$

$$\xrightarrow{90^\circ(I_x, K_x, S_x)-\delta-180^\circ(I_x, K_x, S_x)-\delta-90^\circ(I_y, K_y, S_y)} \\ I_z\cos(\Omega_s t_1)\cos^2(2\pi J_{IK}\delta) + 2I_yS_z\sin(\Omega_s t_1)\cos^2(2\pi J_{IK}\delta) \quad [10]$$

$$\xrightarrow{\delta-180^\circ(I_x, K_x, S_x)-\delta} -I_z\cos(\Omega_s t_1)\cos^2(2\pi J_{IK}\delta) \\ -I_x\sin(\Omega_s t_1)\cos^3(2\pi J_{IK}\delta) \\ -2I_yK_z\sin(\Omega_s t_1)\cos^2(2\pi J_{IK}\delta)\sin(2\pi J_{IK}\delta) \quad [11]$$

$$\xrightarrow{90^\circ(I_x, K_x)} I_y\cos(\Omega_s t_1)\cos^2(2\pi J_{IK}\delta) \\ -I_x\sin(\Omega_s t_1)\cos^3(2\pi J_{IK}\delta) \\ +2I_zK_y\sin(\Omega_s t_1)\cos^2(2\pi J_{IK}\delta)\sin(2\pi J_{IK}\delta). \quad [12]$$

A comparison of expressions [6] and [11] shows that the term I_yK_z , which is ultimately responsible for the artifact, first appears while the sine component is being refocused from antiphase into **I** spin coherence. The efficiency of this process is affected by coupling among the protons. This I_yK_z term is subsequently transformed, in expression [12], into I_zK_y , resulting in an antiphase dispersive signal split by the coupling J_{IK} . This signal correlates the **K** proton with the **S** carbon, despite the fact that no coupling need exist between the two nuclei. Thus, the artifact is a consequence of PEP or COS-INEPT.

Strictly speaking, gradients are unnecessary; however, without them, the artifacts can become swamped by t_1 -noise.

To calculate the relative intensity of the artifact peaks, it is necessary to compare the numerical factors multiplying the I_x , I_y , and $2I_zK_y$ terms in expression [12]. The common factor of $\cos^2(2\pi J_{IK}\delta)$ can be ignored; it only has the effect of reducing the overall spectral intensity. Thus expression [12] can be reduced to

$$I_y\cos(\Omega_s t_1) \cdot 1 - I_x\sin(\Omega_s t_1)\cos(2\pi J_{IK}\delta) \\ + 2I_zK_y\sin(\Omega_s t_1)\sin(2\pi J_{IK}\delta). \quad [13]$$

The mixing of coherence transfer pathways generated by the gradient pulses (or by phase cycling for the non-gradient version) causes the factors of 1 and $\cos(2\pi J_{IK}\delta)$ multiplying the I_y and I_x terms in expression [13] to add coherently rather than in quadrature, and it also insures that both $\sin(\Omega_s t_1)$ and $\cos(\Omega_s t_1)$ phases of the artifact term $2I_zK_y$ are recorded. The fact that this term has the form $2I_zK_y$ means that the artifact peaks are dispersive and antiphase—but in fact, both the main peaks and the artifacts are of slightly mixed phase, neither purely absorptive nor purely dispersive, owing to evolution of the scalar coupling between spins **I** and **K** during the refocusing gradient period. Finally, the ratio of the artifact and main peak intensities is

$$\text{Artifact/main peak} = \sin(2\pi J_{IK}\delta)/(1 + \cos(2\pi J_{IK}\delta)) \\ = \tan(\pi J_{IK}\delta). \quad [14]$$

With $J_{IK} = 10$ Hz and $\delta = 1.7$ ms, this would lead to an artifact-to-main peak ratio of about 6%.

Figure 1 shows an expansion of part of a natural abundance ^{13}C GEHSQC spectrum of sucrose that demonstrates the ease with which these artifacts are generated. The spectrum exhibits many features characteristic of relayed spectroscopy. The artifacts (e.g., C4–H3 and C3–H4) and the main peaks stemming from direct transfer through one-bond coupling (e.g., C3–H3 and C4–H4) appear at the corners of a rectangle in 2D frequency space. This rectangular pattern is indicative of the fact that the C3–H3 and C4–H4 fragments are linked by a homonuclear coupling between H3 and H4. Integration of 1D slices in the proton dimension shows that the average artifact-to-main peak ratio is close to the predicted value of 6%.

While these artifacts will be easiest to see in small molecules, they can, given sufficient sensitivity, be observed in molecules lying in the size range for which it is possible to measure COSY spectra. These artifacts are not necessarily undesirable as they do contain useful information for spectral assignment. They can easily be suppressed by line broadening, but the relayed coherence transfer mechanism would remain in effect and cause a reduction in the efficiency of the coherence transfer process.

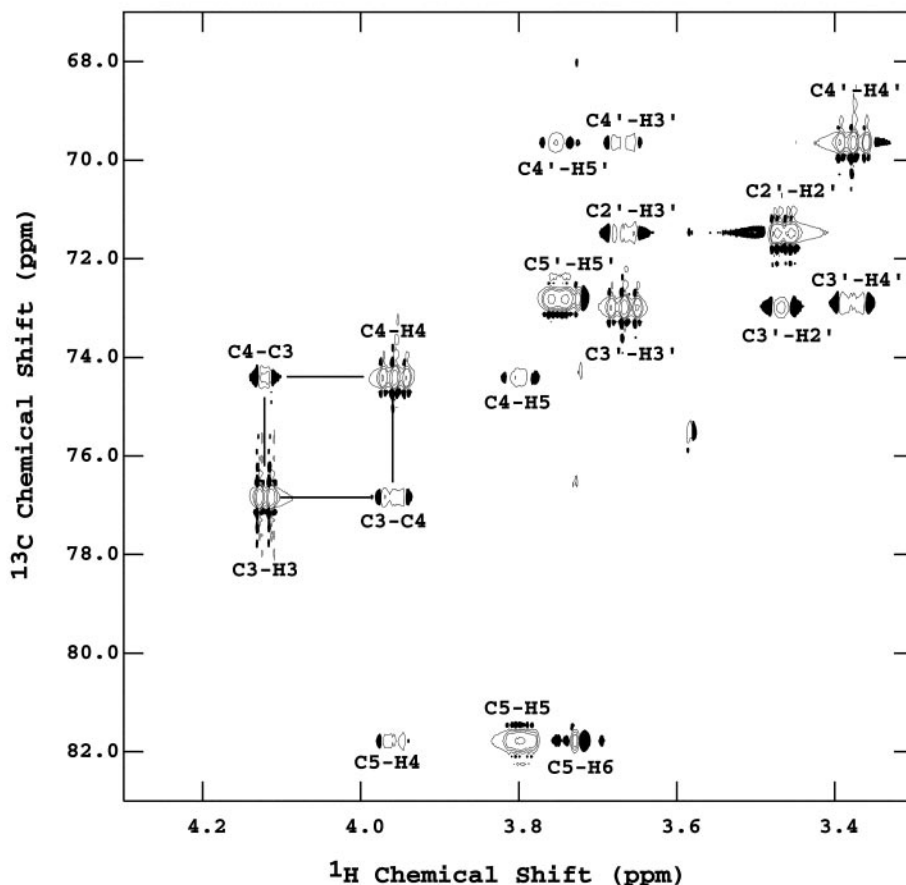


FIG. 1. Part of the natural abundance ^{13}C GEHSQC spectrum of sucrose, acquired at 500 MHz proton frequency. Positive signals are shown contoured; negative signals are shown as filled regions. Connecting lines have been drawn between the main C3-H3 and C4-H4 cross-peaks and the C4-H3 and C3-H4 artifact peaks; these artifacts arise from the vicinal H3-H4 coupling.

In sucrose, more than half the signals show two artifacts; thus the three-spin analysis used here is an oversimplification. It is generally assumed that the major practical problem in the application of COS-CT mixing sequences is related to the duration of the sequence, especially when dealing with proteins with high transverse relaxation rates (9). However, the side chains of amino acids such as Arg, Gln, Glu, Ile, Leu, Lys, Met, and Pro all form complicated spin systems. A logical extension of the three-spin analysis presented here would suggest that the greater the passive coupling among the protons, the greater the sensitivity loss. Thus, if a particular proton were coupled to three others with coupling constants similar in magnitude to those encountered here, then the losses might amount to about 20%. (Similar problems involving C-C couplings in uniformly ^{13}C -labeled molecules have been noted in the literature (8).) Presumably, attempts to increase the efficiency of coherence order selective coherence transfer processes must take into account the influence of proton-proton coupling.

In conclusion, we have shown that homonuclear proton coupling can generate artifacts in gradient-enhanced HSQC spectra by a relayed coherence transfer process. The artifact signals can easily be suppressed by line broadening. Despite this, proton-proton coupling still causes a reduction in the efficiency of the coherence transfer process.

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