Origin and removal of mixed-phase artifacts in gradient sensitivity enhanced heteronuclear single quantum correlation spectra

Frans A. A. Mulder · Renee Otten · Ruud M. Scheek

Received: 31 May 2011 / Accepted: 8 July 2011 © The Author(s) 2011. This article is published with open access at Springerlink.com

Abstract Here we describe phasing anomalies observed in gradient sensitivity enhanced $^{15}$N-$^1$H HSQC spectra, and analyze their origin. It is shown that, as a result of $^{15}$N off-resonance effects, dispersive contributions to the $^1$H signal become detectable, and lead to $^{15}$N-offset dependent phase errors. Strategies that effectively suppress these artifacts are presented.

Keywords HSQC · Sensitivity enhancement · Artifact · Phase error · Line shape

The heteronuclear single quantum correlation (HSQC) experiment (Bodenhausen and Ruben 1980) has transfigured the analysis of chemical and three-dimensional molecular structure, including studies of conformational flexibility and interactions. Sensitivity enhanced (SE) versions of the HSQC experiment have been proposed (Palmer et al. 1991) that allow both anti-phase coherence terms ($2H_2N_X$ and $2H_2N_Y$) present during the indirect evolution domain to be detected as different linear combinations in separate scans. Subsequent addition/subtraction of the two data sets yields pure absorption 2D line shapes with an increase in signal-to-noise ratio of $\sqrt{2}$, neglecting pulse imperfections and relaxation. Without doubt, the most popular implementation of this principle is the one by Kay et al. (Kay et al. 1992), in which the collection of the orthogonal coherences is seamlessly integrated with coherence pathway selection using pulsed field gradients (PFGs). Most importantly, any imbalance during the contiguous reverse INEPT steps resulting from relaxation differences of the two coherence transfer pathways (CTPs) is erased, thereby avoiding quadrature artifacts. However, phase anomalies may still arise as a result of the limited bandwidth of the pulses applied to the heteronuclei ($X$-nuclei). Additional pairs of PFGs around the refocusing/inversion pulses in the reverse INEPT transfer periods have been suggested to reduce these artifacts (Bax and Pochapsky 1992). Surprisingly, the lengths and strengths of gradient pulses employed during the sensitivity enhancement scheme in various experimental implementations in the literature vary greatly, and appear to be chosen rather arbitrarily. Published versions are available that either use no gradients (Dayie and Wagner 1994; Kay et al. 1992; Mulder et al. 1996; Sattler et al. 1995; Schleucher et al. 1994; Stonehouse et al. 1995), matched gradient pairs (Muhandiram and Kay 1994; Weigelt 1998; Zhang et al. 1994), or unequal gradient pairs (Czisch and Boelens 1998; Muhandiram et al. 1993; Salzmann et al. 1999). To our knowledge, no detailed analysis of these choices has been presented so far, and, as a consequence, published experiments may not adequately suppress the spurious signals. Possibly, the effects have gone largely unnoticed, as the phase errors are relatively modest as long as the RF field strength applied to the $X$-nuclei significantly exceeds their...
under standard operating conditions—in our laboratory, on a 600 MHz spectrometer with a 53 μs nitrogen 90° pulse width—phase differences can be observed between proton traces taken at different nitrogen offsets in a typical 2D HSQC spectrum, unless the PFG strengths are judiciously chosen. As a result, quantitative analysis of peak intensity and position, which relies on spectra with pure-phase line shapes, is compromised. Understanding the origin of the artifacts, and finding effective methods for their removal, is expected to be of interest as (1) HSQC experiments are applied to cover larger chemical shift ranges, and (2) increasing static magnetic field strengths will lead to proportionally larger X-spin resonance frequency offsets, and concomitantly stronger artifacts.

In the present paper we present simulations of the nuclear spin coherence evolution during sensitivity enhanced HSQC experiments that explicitly include off-resonance effects and pulsed field gradients. The simulations identify the excitation and detection of unwanted coherences, allowing us to trace out their coherence transfer paths, and suggest effective strategies for their suppression. The efficacy of the approach is established via experimental PFG-SE 15N-1H HSQC experiments recorded on the small protein calbindin D9k.

Figure 1 shows the PFG-SE 15N-1H HSQC pulse sequence of Kay et al. (1992), in its water flip-back implementation (Zhang et al. 1994), as used in this study. The ‘encoding’ and ‘decoding’ gradients, G4 and G7, respectively, ensure proper ‘winding’ and successive ‘unwinding’ of the nuclear spin magnetizations to a degree proportional to coherence order and position in the sample. Following the recipe described in the original paper (Kay et al. 1992), two scans of opposite phase-modulated data are recorded, and manipulated prior to Fourier transformation, to generate a 2D spectrum with absorption line shapes in both frequency dimensions.

The encoding pulsed field gradient is applied as a pair of asymmetric bipolar gradients −1.2 × G4 and 0.8 × G4 to avoid the need for phase cycling on the 180° pulse, which is otherwise necessary to suppress quadrature artifacts due to pulse imperfection or limited band width. PFG pairs G5 and G6 around the 180° pulses in the reverse INEPT transfer periods suppress further artifacts that affect undesired coherence order changes (Bax and Pochapsky 1992).

To understand the origin of the phase errors we now present a product operator analysis of the time evolution of the main terms of the spin density operator for a two-spin HN system that lead to observable proton coherence at the time of detection. The first part of the HSQC experiment shown in Fig. 1 can be summarized as follows:

\[
\begin{align*}
1H & \quad \Delta \quad \Delta \\
\ ^{15}N & \quad \delta \quad \delta \\
\text{PFG} & \quad g_1 \quad g_2 \quad g_3 \\
& \quad \text{ gating} \\
& \quad \text{IN E P T} \\
& \quad \text{H} \quad \Delta \quad \Delta \quad \Delta \quad \Delta \\
\end{align*}
\]

\textbf{Fig. 1} Pulse sequence of the 2D PFG-SE $^1H$-$^{15}N$ HSQC experiment used in the simulation and in practice. Narrow (wide) filled bars indicate 90° (180°) RF pulses applied along the $x$ axis, unless otherwise indicated. The $^1H$ carrier is centered at the water resonance (4.76 ppm) and proton pulses are applied with a field strength of $\omega_0/2\pi = 37.3$ kHz. Proton decoupling is achieved using GARP-1 decoupling with $\omega_d/2\pi = 1.25$ kHz. The 90° water flip-back pulse after the first INEPT in the sequence (open dome) has a rectangular shape and a length of 2 ms. The $^{15}N$ carrier is centered at 119 ppm, and nitrogen pulses were applied with a field strength of $\omega_0/2\pi = 4.7$ kHz. Delays are: $\Delta = 2.3$ ms, $\delta = 1.5$ ms, and $\epsilon = 0.2$ ms. Phase cycling is: $\phi_1 = [x,-x]$, $\phi_2 = x$, and $\phi_{rot} = \{x,-x\}$. The gradient strengths (G/cm) and durations (ms) are: $g0 = 0.9$ ($t_2/2$), $g1 = 5.3$ (1.0), $g2 = 14.1$ (0.5), $g3 = 22.1$ (0.5), $g4 = 26.6$ (1.25), $g5 = \text{variable (0.15)}$, $g6 = \text{variable (0.15)}$, and $g7 = 52.3$ (0.125). Two data sets are recorded (in an interleaved manner) with G7 inverted for each data set together with inversion of $\phi_2$. The two data sets are manipulated in order to generate States type hypercomplex data (see text). Axial peaks are moved to the side of the spectrum by concomitant inversion of $\phi_1$ with the receiver phase for every other $t_1$ increment.

\[
\begin{align*}
\text{H}_2 \text{Y} \quad \text{INEPT} & \quad -2\text{H}_2\text{N}_Y \quad 1/2-180(\text{H}_X)-t_1/2-2-180(\text{N}_X)-2
\\
& \quad -2\text{H}_2\text{N}_Y \quad \text{cos(}\Omega\text{t}_1) \quad -2\text{H}_2\text{N}_X \quad \text{sin(}\Omega\text{t}_1)
\\
& \quad 90(\text{N}_X,\text{H}_X) \quad 2\text{H}_Y\text{N}_Z \quad \text{cos(}\Omega\text{t}_1) + 2\text{H}_Y\text{N}_X \quad \text{sin(}\Omega\text{t}_1)
\\
\end{align*}
\]

In the case of ideal pulses, and momentarily neglecting the gradient pulses and relaxation, the first of the two scans that make up the sensitivity enhancement scheme can be summarized by the following two simultaneous coherence transfer pathways:

\textbf{desired SQ pathway}:

\[
\begin{align*}
2\text{H}_Y\text{N}_Z \quad \text{cos(}\Omega\text{t}_1) & \quad \text{A-180(}\text{N}_X,\text{H}_X) \quad \text{A}
\\
& \quad -\text{H}_X \quad \text{cos(}\Omega\text{t}_1) \quad 90(\text{N}_X,\text{H}_Y) \quad \text{H}_Z \quad \text{cos(}\Omega\text{t}_1)
\\
& \quad \text{A-180(}\text{N}_X,\text{H}_X) \quad \text{A}
\\
& \quad -\text{H}_Y \quad \text{cos(}\Omega\text{t}_1)
\\
\end{align*}
\]

\textbf{desired MQ pathway}:

\[
\begin{align*}
2\text{H}_Y\text{N}_X \quad \text{sin(}\Omega\text{t}_1) & \quad \text{A-180(}\text{N}_X,\text{H}_X) \quad \text{A}
\\
& \quad -2\text{H}_Y\text{N}_X \quad \text{sin(}\Omega\text{t}_1)
\\
& \quad 90(\text{N}_Y,\text{H}_Y) \quad -2\text{H}_Y\text{N}_Z \quad \text{sin(}\Omega\text{t}_1)
\\
& \quad \text{A-180(}\text{N}_X,\text{H}_X) \quad \text{A}
\\
& \quad -\text{H}_X \quad \text{sin(}\Omega\text{t}_1)
\\
\end{align*}
\]
We refer to these two desired pathways as the “single quantum (SQ)” and “multiple quantum (MQ)” pathways, respectively, referring to the coherence state present during the first reverse INEPT transfer period. By simultaneous change of the sign of gradient $G_7$ and phase $\phi_2$, the signal after repetition of the experiment is described by:

$$H_Y \cos(\Omega N t_1) - H_X \sin(\Omega N t_1)$$

By subsequent addition/subtraction of the two signals, and a 90° phase shift to one of the outcomes, the resulting hypercomplex pair can be processed in the usual States (States et al. 1982) or States-TPPI (Marion et al. 1989) manner (Kay et al. 1992; Palmer et al. 1991).

In the case of non-negligible pulse widths additional observable signals will reach the receiver, and unwanted coherence transfer pathways may need to be suppressed by phase cycling or additional pulsed field gradients. Here we will focus in detail on the effect of finite pulse widths on the 15N channel, and will specifically consider the result of off-resonance rotations during the 15N pulses that follow $t_1$-evolution. An analogous situation in 13C-1H correlation spectroscopy can easily be envisaged, where a larger chemical shift range may lead to increased off-resonance effects and concomitant phasing artifacts.

Alternatively, to avoid off-resonance effects band selective, composite or adiabatic pulses may be employed. Because the sensitivity enhancement protocol carries forward orthogonal coherences, pulses are required that can simultaneously invert and refocus magnetization. Here we will consider REBUMP pulses for this purpose.

We will now trace out the coherence transfer pathways during sensitivity enhancement that lead to observable magnetization and mixed-phase artifacts. Table 1 presents the eight possible coherence pathways that will lead to proton SQ coherence at the moment of acquisition in case of imperfect 180° nitrogen pulses in the reverse INEPT periods. The influence of 90° pulse imperfections on the CTPs is minor, and can be neglected. For compactness we will express all coherence transfer amplitudes in terms of the dimensionless quantity $\alpha$, defined as the ratio of the resonance offset and the RF field strength, i.e. $\alpha = \Omega N/\omega_1$. Following the notation of Table 1, the desired coherences are referred to below as #1 (“SQ”) and #5 (“MQ”).

To gain insight in the type and magnitude of the relevant spurious signals that can arise during sensitivity enhancement, let us consider the specific case of a J-coupled amide spin pair $HN$, with a resonance offset $\Omega N/(2\pi)$. The 180° nitrogen pulses in the reverse INEPT periods are applied with RF field strength: $|N B_1| = \omega_1 = 2\pi/(4\ pw_N)$. All other pulses are considered ideal. We shall use notations like 90(H$_2$) for the effect of an ideal 90° 1H pulse with phase x, and 180(N$_2$,H$_x$) for the effect of simultaneous non-ideal 15N and ideal 1H 180° pulses, both with phase x. The main artifact then arises from the “SQ” and “MQ” pathways in the following manner:

| Table 1 Coherence transfer pathways$^a$ that lead to observable proton magnetization in the case of imperfect nitrogen pulses in the sensitivity enhancement scheme$^b$ |
|---|---|---|---|---|---|---|
| 1 | 2H$_2$N$_x$ | H$_x$ | $-z^2$ | H$_x$ | $-z^2$ | H$_x$ |
| 2 | 2H$_2$N$_x$ | H$_x$ | $\alpha \sqrt{2} \cos(\Omega N \Delta)$ | 2H$_2$N$_x$ | $-z^2$ | H$_x$ | $\alpha \sqrt{2} \cos(\Omega N \Delta)$ |
| 3 | 2H$_2$N$_x$ | H$_x$ | $\alpha \sqrt{2} \sin(\Omega N \Delta)$ | $-2H_2N_x$ | $z^2 \sin(\Omega N \Delta)$ | H$_x$ | $-1.11 z^2 \sin(\Omega N \Delta)$ |
| 4 | 2H$_2$N$_x$ | H$_x$ | $z^2$ | $-2H_2N_x$ | H$_x$ | $z^2 \sin(\Omega N \Delta)$ | H$_x$ |
| 5 | 2H$_2$N$_x$ | H$_x$ | $z^2$ | $-2H_2N_x$ | H$_x$ | $z^2 \sin(\Omega N \Delta)$ | H$_x$ |
| 6 | 2H$_2$N$_x$ | H$_x$ | $z^2 \cos(2\Omega N \Delta)$ | $2H_2N_x$ | H$_x$ | $z^2 \cos(2\Omega N \Delta)$ | H$_x$ |
| 7 | 2H$_2$N$_x$ | H$_x$ | $z^2 \sin(2\Omega N \Delta)$ | $-2H_2N_x$ | H$_x$ | $z^2 \sin(2\Omega N \Delta)$ | H$_x$ |
| 8 | 2H$_2$N$_x$ | H$_x$ | $z^2 \sin(\Omega N \Delta)$ | $-1.11 z^2 \sin(\Omega N \Delta)$ | H$_x$ | $z^2 \sin(\Omega N \Delta)$ | H$_x$ |

$^a$ The five columns a–e indicate the product operators generated in each of the eight CTPs, and correspond to the five time points a–e indicated in Fig. 1. The final column indicates the $t_1$-modulation that is carried forward by each of the CTPs

$^b$ $\alpha$ stands for $\Omega N/\omega_1$. Amplitudes are approximate, and hold for $|x|<1$ (for the practical situation with pw$_N = 50$ $\mu$s and a maximum offset of 17 ppm from the 15N carrier frequency on a 600 MHz spectrometer, $\Omega_N/(2\pi) < 1$ kHz $\alpha < 0.2$ and $x^2 < 0.008$; the entries with amplitude 0 in CTPs 4 and 6 start with terms in $x^2$ and are therefore neglected in this approximation). They pertain to the operators in columns b/c and d/e. Relaxation was not taken into account.
major undesired branch from SQ pathway (#2): 
\[ 2H_2YN_Z \cos(\Omega_{Nt1}) \xrightarrow{\Delta-180(N_X, H_X) - \Delta} 2H_2YN_X \cos(\Omega_{Nt1}) \]
\[ 90(N_Y, H_Y) \xrightarrow{\Delta} 2H_2YN_Z \cos(\Omega_{Nt1}) \xrightarrow{\Delta-180(N_X, H_X) - \Delta} \]
\[ - H_X \cos(\Omega_{Nt1}) \xrightarrow{90(H_X) - e^{-180(H_X)} - \varepsilon} - H_X \cos(\Omega_{Nt1}) \]

major undesired branch from MQ pathway (#8): 
\[ 2H_2YN_X \sin(\Omega_{Nt1}) \xrightarrow{\Delta-180(N_X, H_X) - \Delta} H_X \sin(\Omega_{Nt1}) \xrightarrow{90(N_Y, H_Y)} \]
\[ - H_Z \sin(\Omega_{Nt1}) \xrightarrow{\Delta-180(N_X, H_X) - \Delta} H_Z \sin(\Omega_{Nt1}) \]
\[ 90(H_X) - e^{-180(H_X)} - \varepsilon \xrightarrow{\Delta} H_Y \sin(\Omega_{Nt1}) \]

See Table 1 for the amplitudes of the terms that follow the different pathways.

This situation can be considered a case of “channel confusion”: the resulting \(^1\)H magnetizations that carry the orthogonal \(^{15}\)N chemical shift amplitude modulations are both \(90^\circ\) out of phase with respect to the desired signal.

Two important aspects of this dominant artifact need consideration. First, the undesired signals arise from the interconversion of \(^{15}\)N,\(^1\)H multiple-quantum coherences and \(^{15}\)N-orthogonal \(^1\)H chemical shift amplitude modulations, as the nitrogen evolution pathways stipulate that the artifacts will dem- 

In all CTPs that lead to artifacts the magnetization undergoes an unwanted change in coherence level (of +1 or -1) during at least one of the imperfect nitrogen \(\pi\) pulses. Hence, a symmetrical pair of PFGs around each of these \(\pi\) pulses will be effective in reducing the resulting phasing artifacts. However,
in pathways #3 and #7 this change in coherence level happens twice, so a fraction of the magnetization will not show any offset dependence, due to the refocusing effect of the second $\pi$ pulse. This same fraction will also survive gradient pairs ($G_5 = G_6$) when these gradient pairs are of equal strength ($G_5 = G_6$). For this reason the use of matched gradient pairs (Muhandiram and Kay 1994; Weigelt 1998; Zhang et al. 1994) must be advised against.

In pathway #4 and #6 the unwanted change in coherence level happens only during the second $\pi$ pulse. In order to suppress signals from these pathways it is necessary that gradients are applied around the second $\pi$ pulse. However, since the signals from CTP #4 and #6 are small relative to those from CTPs #2 and #8, the strength of the second gradient pair can be comparatively weaker.

Fig. 3 2D PFG-SE $^{15}$N-$^1$H HSQC spectra obtained for calbindin D$_{9k}$ together with traces at 114.1 ppm (upper) and 123.0 ppm (lower). a–b $G_5 = G_6 = 0$; c–d $G_5 = G_6 = 35.4$ G/cm; e–f $G_5 = 35.4$ G/cm, $G_6 = 7.1$ G/cm. The sample was contained in a Shigemi microcell.
Some of the CTPs that give rise to phase errors (see Table 1) lead to unbalanced contributions to the $\cos(\Omega_N t_1)$- and $\sin(\Omega_N t_1)$-modulated signals. This would cause quadrature artifacts in the final spectrum. However, the action of the encoding and decoding gradients (G4 and G7) ensures that any quadrature artifacts will be suppressed.

In what follows we will evaluate coherence transfer through a PFG-SE $^{15}$N-$^1$H HSQC experiment by computer simulation, using in-house routines programmed in Mathematica (Wolfram Research, Inc. 2010). The calculations emulate the situation on a 600 MHz spectrometer with ideal $^1$H pulses, a 50 $\mu$s nitrogen 90° pulse width, and single $z$ axis gradients. The sample is assumed to have a length of 10 mm. The length and strength of the applied gradients is specified with the calculations and figures.

Figure 2 shows the phase errors of the observable proton magnetization that are expected when the $^{15}$N resonance offset frequencies are increased from 0 to 3 kHz. The typical beat patterns observed in Fig. 2a are dominated by CTPs #2 and #8, and arise from imperfect $x$ rotations about the effective field, which is slightly tilted out of the transverse plane, and chemical shift evolution during only one of the $\Delta$ periods of the first reverse INEPT period. For resonance offsets $\Omega_N < \omega_1$ (i.e., $x < 1$) this results in modulations approximated by $\sqrt{2 \cos(\Omega_N \Delta) \times \Omega_N / \omega_1}$ (see Table 1). Panel (a) shows the consequence of incrementing only the first gradient pair, G5, and how this suppresses the major undesired CTPs #2 and #8. Panel (b) demonstrates that application of G6 alone hardly leads to any mitigation of artifacts at all, since only CTPs #4 and #6 are affected. Panel (c) shows that matched gradient pairs $G_5 = G_6$ lead to a reduction of the artifacts, but only in part, because CTPs #3 and #7 survive. In panel (d) the effect of mismatched gradient pairs ($G_5 \neq G_6$) is shown. Good suppression is obtained in the latter case, by choosing the second gradient pair to be weaker by about a factor four. Finally, panel (e) shows the expected offset profile when the 180° pulse in the first period is replaced by a 1.5 ms REBUP (Geen and Freeman 1991) shape with peak RF amplitude $\omega_1 / 2\pi = 4.2$ kHz. Although this pulse is capable of simultaneously refocusing and inverting the magnetizations of the “SQ” and “MQ” pathways to >98%
over a 2.5 kHz spectral width, it only marginally reduces the appearance of phasing artifacts, even for small chemical shift offsets.

Since we now understand the emergence of the mixed-phase artifacts from a theoretical point of view we can propose and test several solutions for recording sensitivity enhanced HSQC experiments in practice. A first solution would be to include phase cycling of the two 180° pulses. However, since this would increase the number of scans that need to be recorded per FID, we do not prefer this.

Fig. 5 2D PFG-SE $^{15}$N-$^1$H HSQC spectra obtained for calbindin D$_{9k}$ with the nitrogen carrier placed 2.0 kHz from the center of the spectrum (119 ppm). Traces are shown at 112.1 ppm (upper) and 121.0 ppm (lower). a-b G5 = G6 = 0 G/cm; c-d G5 = G6 = 35.4 G/cm; e-f G5 = 35.4 G/cm, G6 = 7.1 G/cm
option. Rather, we use PFGs G5 and G6 for this purpose. As borne out by the simulations, the optimal solution under these circumstances would require strong gradients G5, with G6 applied at about four-fold weaker strength.

Figure 3a shows a PFG-SE $^{15}\text{N}-^{1}\text{H}$ HSQC spectrum obtained for the small protein calbindin D$_{9k}$ (Oktaviani et al. 2011), when $G5 = G6 = 0$. In this case phase differences of up to 25° are observed, as exemplified by the traces taken along the $F_1$ frequency dimension at 114.1 ppm (upper) and 123.0 ppm (lower), respectively (Fig. 3b). The artifacts can be reduced significantly by placing bracketing purging PFGs around the 180° pulses during the reverse INEPT transfers to remove undesired coherence transfer pathways, viz. G5 and G6 in Fig. 1. For example, using strong gradient pairs $G5 = G6 = 35.4 \text{G/cm}$ the spectrum of Fig. 3c is obtained. Although much reduced, traces shown next to the 2D spectrum demonstrate that a phase difference of 7 degrees remains. Phase errors were no longer detectable when $G5 = 35.4 \text{G/cm}$ and $G6 = 7.1 \text{G/cm}$ (Fig. 3ef).

Figure 4 was obtained using experiments recorded with a 1.5 ms REBURP pulse (peak power 4.2 kHz) in the first reverse INEPT transfers. When $G5 = G6 = 0$ artifacts are still seen, as predicted by the simulations (Fig. 2e), indicating that wide band refocusing and/or inversion with this pulse does not eliminate artifacts sufficiently. However, with the same gradient setting as used for Fig. 3ef, phase errors were no longer detectable.

For purpose of illustration of the efficacy of artifact suppression using gradient pulses, Fig. 5 shows PFG-SE $^{15}\text{N}-^{1}\text{H}$ HSQC spectra obtained for calbindin D$_{9k}$ when the carrier is deliberately placed off-resonance by 2.0 kHz relative to the value used to obtain Fig. 3 (119 ppm). In panel (a) a phase difference of 72 degrees was observed for $G5 = G6 = 0$. Traces taken along the $F_1$ frequency dimension at 112.1 ppm (upper) and 121.0 ppm (lower), respectively show the severity of the artifacts in this situation (Fig. 5b). As before, the mixed-phase artifacts can be reduced significantly by setting $G5 = G6 = 35.4 \text{G/cm}$, which eliminates the dominant dispersive artifacts (Fig. 5c/d). In this case a phase difference of 10 degrees remains. Application of the mismatched gradient amplitudes $G5 = 35.4 \text{G/cm}$ and $G6 = 7.1 \text{G/cm}$ (Fig. 5ef) removed the remaining dispersive signal, but, as explained in detail above, also eliminated a fraction of absorptive signal. This can be clearly seen by comparison of panels (d) and (f). Unfortunately, REBURP pulses with sufficient band width to avoid these sensitivity losses would require a peak RF amplitude that is unattainable on our probe head (8.4 kHz), but alternative RF pulses could be considered. In many practical cases, however, such as those shown in Fig. 3, $\Omega_1 \ll \omega_1$ (i.e., $\Omega \ll 1$) only limited signal loss incurs. Nonetheless, undesired CTP amplitudes are not negligible, and phase errors are still observed. In such a situation mixed-phase artifacts can be satisfactorily reduced over a large offset range by pulsed field gradients alone.

To conclude, in any practical case the strategy to remove the mixed-phase artifacts described in this paper will have to be combined with effective control of the residual water magnetization at the point of acquisition. This is particular germane for cryogenically cooled probes with high quality factors, where efficient non-relaxation mechanisms can perturb the water polarization during the course of the pulse sequence and acquisition of the FID. Therefore, the recipe presented for the elimination of the mixed-phase artifacts may require optimization by the operator to achieve good water suppression in addition. It may well be that achieving good solvent suppression has prevailed in selecting alternative values for the gradients presented in the literature. In connection to this, PFGs G5 and G6 may also be expanded to fill the entire delay $\Delta$ in which they are placed. This will improve artifact suppression further, and may also help to prevent undesired effects from radiation damping.

In conclusion, we have presented here an explanation for phase errors that can be observed in gradient sensitivity enhanced correlation experiments and suggested a simple and effective procedure for their suppression. We anticipate that our results are of value for optimizing many contemporary triple-resonance NMR experiments that use the sensitivity enhanced HSQC and related building blocks as detection module.

Acknowledgments This paper is dedicated to Lewis E Kay on the occasion of his 50th birthday. Dr. Wolfgang Bermel (Bruker BioSpin) and one anonymous reviewer are acknowledged for valuable comments on the manuscript. This work was supported by a VIDI grant to F.A.A.M. from The Netherlands Organization for Scientific Research (NWO).

Open Access This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

References

Bax A, Pochapsky SS (1992) Optimized recording of heteronuclear multidimensional NMR spectra using pulsed field gradients. J Magn Reson 99(3):638–643

Bodenhausen G, Ruben DJ (1980) Natural abundance nitrogen-15 NMR by enhanced heteronuclear spectroscopy. Chem Phys Lett 69(1):185–189

Czisch M, Boelens R (1998) Sensitivity enhancement in the TROSY experiment. J Magn Reson 134(1):158–160

Dayie KT, Wagner G (1994) Relaxation-rate measurements for 15 N–1H groups with pulsed-field gradients and preservation of coherence pathways. J Magn Reson, Ser A 111(1):121–126
Geen H, Freeman R (1991) Band-selective radiofrequency pulses. J Magn Reson 93(1):93–141

Kay LE, Keifer P, Saarinen T (1992) Pure absorption gradient enhanced heteronuclear single quantum correlation spectroscopy with improved sensitivity. J Am Chem Soc 114(26):10663–10665

Marion D, Ikura M, Tschudin R, Bax A (1989) Rapid recording of 2D NMR spectra without phase cycling. Application to the study of hydrogen exchange in proteins. J Magn Reson 85(2):393–399

Muhandiram DR, Kay LE (1994) Gradient-enhanced triple-resonance three-dimensional NMR experiments with improved sensitivity. J Magn Reson Ser B 103(3):203–216

Muhandiram DR, Xu GY, Kay LE (1993) An enhanced-sensitivity pure absorption gradient 4D 15 N, 13C-edited NOESY experiment. J Biomol NMR 3(4):463–470

Mulder FAA, Spronk CAEM, Slijper M, Kapteijn R, Boelens R (1996) Improved HSQC experiments for the observation of exchange broadened signals. J Biomol NMR 8(2):223–228

Oktaviani NA, Otten R, Dijkstra K, Scheek RM, Thulin E, Akke M, Mulder FAA (2011) 100% complete assignment of non-labile H-1, C-13, and N-15 signals for calcium-loaded calbindin D-9 k P43G. Biomol NMR Assign 5(1):79–84

Palmer AG, Cavanagh J, Wright PE, Rance M (1991) Sensitivity improvement in proton-detected two-dimensional heteronuclear correlation NMR spectroscopy. J Magn Reson 93(1):151–170

Salzmann M, Wider G, Pervushin K, Wuthrich K (1999) Improved sensitivity and coherence selection for [15 N, 1H]-TROSY elements in triple resonance experiments. J Biomol NMR 15(2):181–184

Sattler M, Schmidt P, Schleucher J, Schedletzky O, Glaser SJ, Griesinger C (1995) Novel pulse sequences with sensitivity enhancement for in-phase coherence transfer employing pulsed field gradients. J Magn Reson Ser B 108(3):235–242

Schleucher J, Schwendinger M, Sattler M, Schmidt P, Schedletzky O, Glaser SJ, Sorensen OW, Griesinger C (1994) A general enhancement scheme in heteronuclear multidimensional NMR employing pulsed field gradients. J Biomol NMR 4(2):301–306

States DJ, Haberkorn RA, Ruben DJ (1982) A two-dimensional nuclear overhauser experiment with pure absorption phase in four quadrants. J Magn Reson 48(2):286–292

Stonehouse J, Clowes RT, Shaw GL, Keeler J, Laue ED (1995) Minimisation of sensitivity losses due to the use of gradient pulses in triple-resonance NMR of proteins. J Biomol NMR 5(3):226–232

Weigelt J (1998) Single scan, sensitivity- and gradient-enhanced TROSY for multidimensional NMR experiments. J Am Chem Soc 120(41):10778–10779

Wolfram Research, Inc. (2010) Mathematica, version 8. Champaign

Zhang O, Kay LE, Olivier JP, Forman-Kay JD (1994) Backbone 1H and 15 N resonance assignments of the N-terminal SH3 domain of drk in folded and unfolded states using enhanced-sensitivity pulsed field gradient NMR techniques. J Biomol NMR 4(6):845–858