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A general building block to introduce carbon multiplicity information into multi-dimensional HSQC-type experiments

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ABSTRACT: A general building block to achieve editing according to the multiplicity of the X nuclei in sensitivity-improved multidimensional ^{1}H–X HSQC experiments is proposed. Its simple implementation on standard experiments and the useful additional information obtained by such an approach are the two main reasons for its use even in routine NMR work. Illustrative examples on sensitivity-improved 2D ^{1}H–^{13}C HSQC, HSQC-TOCSY and 1,1-ADEQUATE experiments are given. © John Wiley & Sons Ltd.

KEYWORDS: carbon multiplicity editing; pulsed-field gradients; HSQC; HSQC-TOCSY; ADEQUATE

INTRODUCTION

The design of new methodologies plays an important role in the development of NMR spectroscopy. Thus, modifications improving the sensitivity, the resolution, the simplicity and/or ther performance of existing NMR experiments are always welcome. The basic HSQC pulse train has become one of the most useful building blocks for designing new multi-dimensional heteronuclear NMR experiments. In recent years, two major developments have favoured such an effect. First, the incorporation of pulsed-field gradients (PFG) for coherence selection affords a powerful way to obtain clean multi-dimensional spectra with excellent suppression of unwanted magnetization and with reduced phase cycles.[1] Second, the application of the preservation of equivalent pathways (PEP) methodology improves the sensitivity due to the selection of both orthogonal components of the magnetization existing during the evolution t_1 period.[2,3] Nowadays, the combination of the two techniques affords a universally accepted way for all spectroscopists to acquire and process such HSQC-type experiments with high quality and maximum improved sensitivity in reasonably short acquisition times.[4–6]

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DISCUSSION

Usually, the variable evolution t_1 period in gradient-based ^{1}H–X HSQC experiments consists in a period during which evolution of single-quantum coherences of the low-abundant X nucleus takes place. A hard proton 180° pulse is applied in the middle of this t_1 period in order to achieve broadband heteronuclear proton decoupling. On the other hand, a dephasing gradient G_1, of duration δ, is applied during this period, is

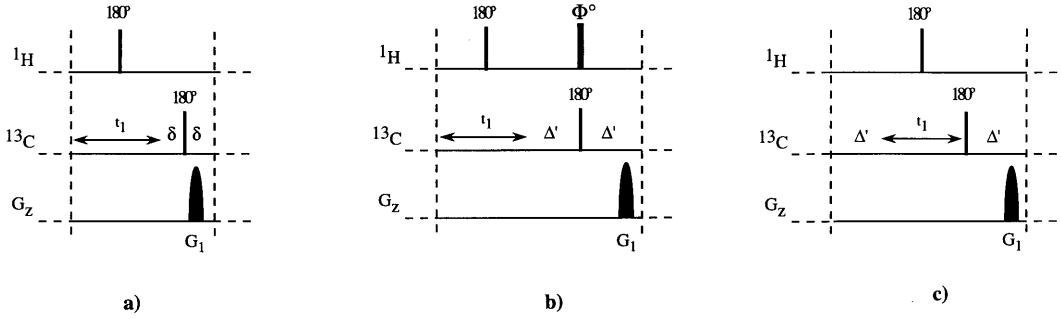

Figure 1. General building block to apply in HSQC-based experiments: (a) conventional, (b) carbon multiplicity editing and (c) concatenated carbon multiplicity editing derived from $\Phi = 180°$.

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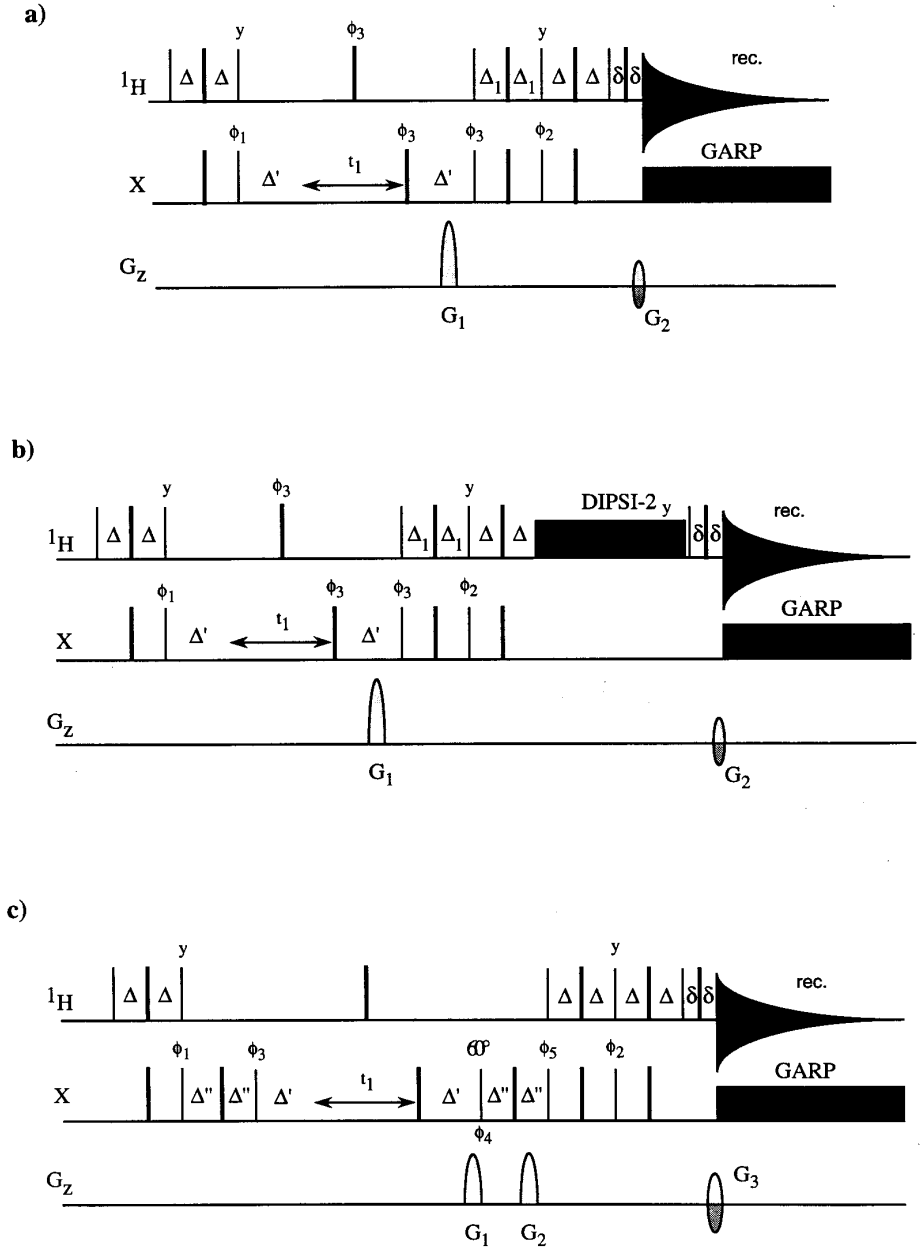

Figure 2. Pulse schemes of the gradient-enhanced sensitivity-improved multiplicity-edited (a) HSQC, (b) HSQC-TOCSY and (c) 1,1-ADEQUATE experiments. Hard 90° and 180° pulses are indicated by vertical narrow and wide black bars. All pulses are applied from the *x*-axis unless indicated otherwise. PFG of duration δ are also indicated by shaded shapes on the line G_z. All NMR experiments were recorded using a Bruker ARX400 spectrometer with an inverse broadband probe head incorporating a self-shielded *Z*-gradient coil. The length of all gradients (shaped as a 5% truncated Gaussian) was 1 ms, the PFG recovery time was 100 μs and the maximum strength was 20 G cm^{-1}.

inserted into a X spin-echo [δ–180°(X)–G_1] block to avoid offset and heteronuclear coupling evolutions during its application [Fig. 1(a)].

A very simple modification of this block enables one to introduce carbon multiplicity information into the HSQC experiment.[7] It is based in the application of a Φ proton pulse, simultaneous with the 180° X pulse, in order to allow heteronuclear coupling evolution during the carbon echo. On the other hand, the period δ is increased to a Δ' value in order to achieve proper refocusing at the end of the echo [Fig. 1(b)]. In this case, the amplitude and thus the sign of cross peaks depend on the flip angle of the Φ proton pulse. A $\cos^{n-1}\Phi$ dependence emerges for each I_nS system when $\Delta' = 2\Delta = 1/2^1 J_{CH}$, in which n is the number of protons directly bound to a specific X nucleus. In the most general case, Φ is set to 180°, so that peaks arising from XH_2 systems will appear as negative signals whereas those arising from XH (and XH_3) systems will all be positive. On the other hand, in cases where overlapping can become troublesome, XH_n-edited spectra can be recorded using other values of Φ and Δ'.

In this paper we propose a new and more general concatenated building block[8] that avoids the application of an extra 180° proton pulse [Fig. 1(c)]. In this case, X chemical shift evolution takes place during

$$\Delta' + t_1/2 + t_1/2 - \Delta' = t_1$$

whereas the heteronuclear coupling evolves during

$$\Delta' + t_1/2 - t_1/2 + \Delta' = 2\Delta'$$

In order to avoid artifacts due to imperfections of the 180° X pulse, in all schemes described in Fig. 1, the gradient pulse can be applied either in the delay directly following the t_1 period or even better in both δ (Δ') delays.

We have studied the scope and applications of this proposed building block by incorporating it in some widely used 2D experiments that include the basic HSQC pulse train. Figure 2 shows the sensitivity-improved multiplicity-edited versions of the 2D HSQC, 2D HSQC-TOCSY and 2D 1,1-ADEQUATE experiments (Fig. 2) that we have successfully tested in the case X = ^{13}C for typical organic samples.

An important point to be considered is the experimental implementation of this building block. In principle, the modification of the corresponding pulse programs can be made in a straightforward way and, in addition, both acquisition and processing steps for these new modified experiments do not change with respect to the conventional non-edited versions. On the other hand, although the incorporation of this new block into each basic sequence entails an increase in the overall length of the sequence in about 5 ms, in practice the possible sensitivity losses by T_2 relaxation effects or by mismatch of Δ' with respect to the $^1J_{XH}$ value can be neglected for small and medium-sized molecules.

Figure 3 shows the 2D ^{1}H–^{13}C edited-HSQC spectrum of strychnine using the pulse sequence of Fig. 2(a). It can be observed that with a single experiment, the multiplicity of each carbon resonance is additionally displayed in the otherwise conventional HSQC spectrum. With this approach, all laboratories equipped with gradient capabilities can avoid the recording of separate conventional DEPT and HSQC spectra to obtain this information. It can be even used for automated routine purposes. Another approach to record this experiment that uses a *z*-filter has been proposed,[9] but our method ensures much better sensitivity.

Multi-dimensional versions of the sensitivity-improved HSQC-TOCSY have been proposed recently.[10,11] In principle, the pure-adsorption 2D ^{1}H–^{13}C HSQC-TOCSY experiment should give a 2D map in which all cross peaks will have the same relative amplitude.[9] In contrast, if a relay-editing block were to be included at the end of the sequence,[12] the direct correlations would appear inverted in intensity with respect to the relayed ones or, alternatively, they could also be suppressed. The editing of direct and relay responses in a related gradient-based 2D HMQC-TOCSY experiment has been described.[13] When applying the HSQC-TOCSY sequence of Fig. 2(b), we obtain the carbon multiplicity information discussed above: all cross peaks arising from methine carbons are positive and those arising from methylene carbons are negative (Fig. 4). The editing of direct responses could also be included in the HSQC-TOCSY experiment of Fig. 2(a), as discussed above, giving cumulative results. Thus, in this doubly edited HSQC-TOCSY spectrum, negative peaks should be due to direct CH connectivities or to

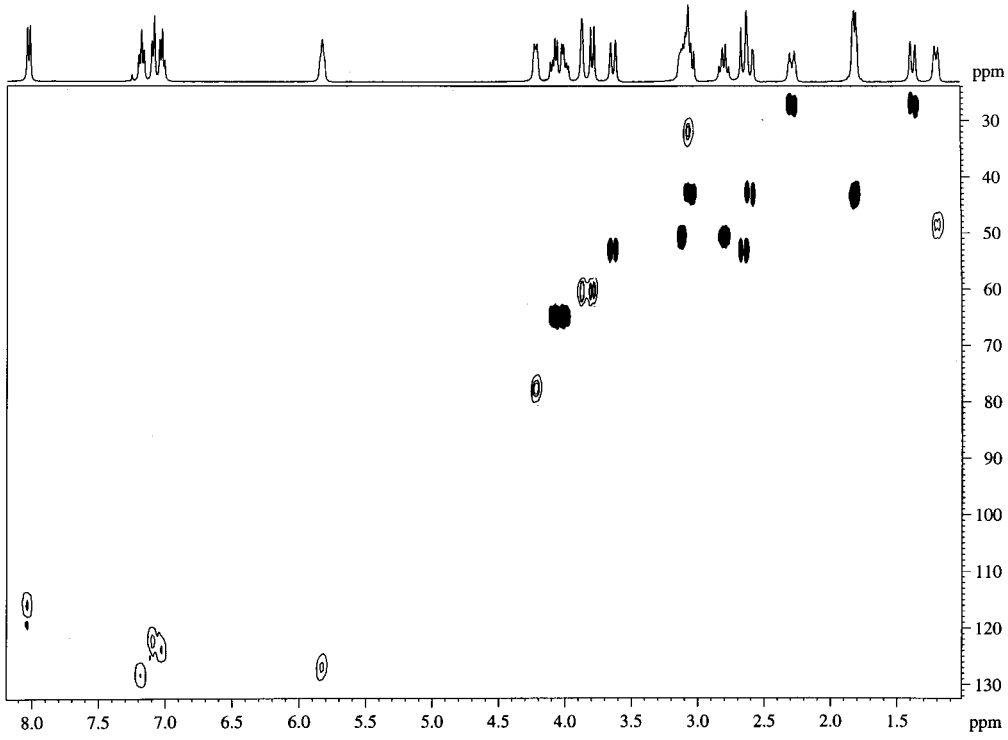

Figure 3. 2D ^{1}H–^{13}C multiplicity-edited HSQC spectrum of strychnine. Positive peaks appear as a white contour whereas negative peaks appear as a black filled contour. The pre-scan delay and the interpulse delays Δ ($= 1/4\,^1J_{CH}$), Δ_1 ($= 1/6\,^1J_{CH}$) and Δ' ($= 1/2\,^1J_{CH}$) were set to 1 s, 1.75 ms,1.15 ms and 3.5 ms, respectively. Data were acquired and processed according to the echo–antiecho procedure using a 4: −1 (ϕ_2) and 4:1 ($-\phi_2$) gradient ratio in alternate scans. Phase cycle: $\phi_1 = x, -x$; $\phi_2 = y_2, -y_2$; $\phi_3 = x_2, -x_2$; rec = $x, -x, -x, x$.

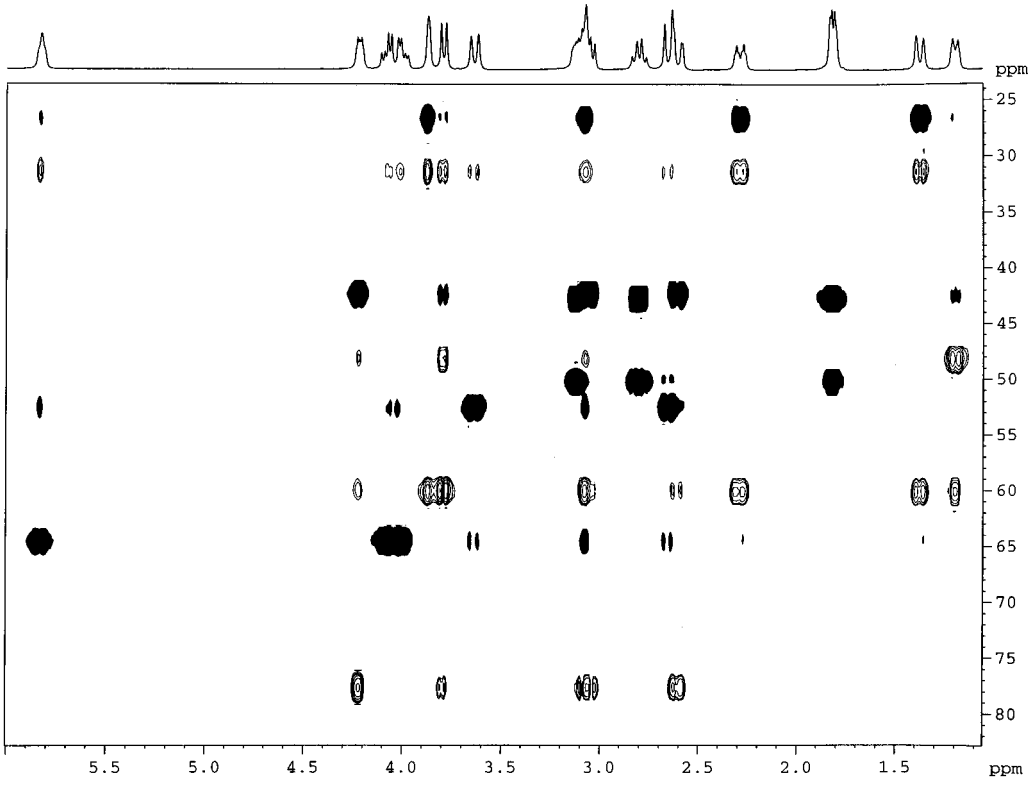

Figure 4. 2D ^{1}H–^{13}C multiplicity-edited HSQC-TOCSY spectrum of sucrose in D_2O. Positive peaks appear as a black contour and negative peaks as a white contour. A 7.4 kHz DIPSI-2 pulse train was applied during 130 ms as a mixing process. All other parameters as described in Fig. 3. Phase cycle: $\phi_1 = x, -x$; $\phi_2 = y_2, -y_2$; $\phi_3 = x_2, -x_2$; rec $= x, -x, -x, x$.

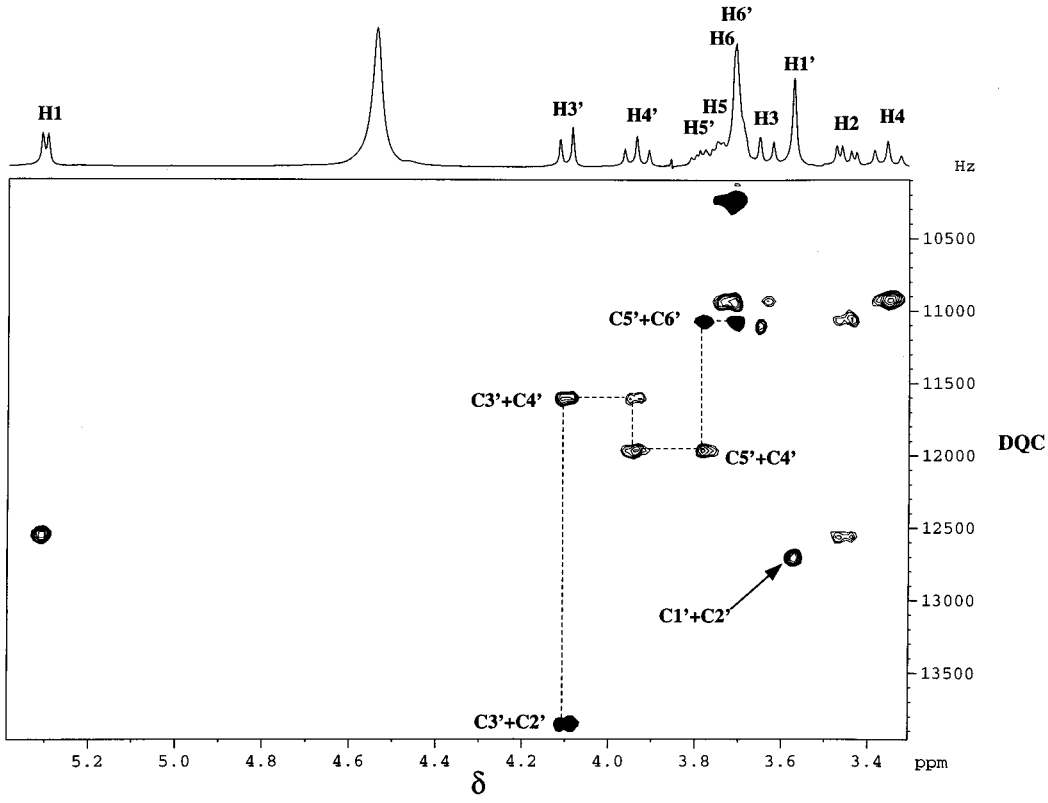

Figure 5. 2D ^{1}H–^{13}C multiplicity-edited ADEQUATE spectrum of sucrose. The delay Δ ($= 1/4\,{}^{1}J_{CC}$), was optimized to 5.5 ms. Data were acquired and processed according to the echo–antiecho procedure using a $-79:-78:59$ ($\phi_2 = y$) and $-79:-78:-59$ ($\phi_2 = y$) gradient ratio in alternate scans. All other parameters as described in Fig. 3. Phase cycle: $\phi_1 = x, -x$; $\phi_2 = y_2, -y_2$; $\phi_3 = x_4, -x_4$; $\phi_4 = x_8, -x_8$; $\phi_5 = x_2, -x_2$; rec $= x, -x, -x, x, -x, x, x, -x, -x, x, x, -x, x, -x, -x, x$.

relay peaks arising from a starting CH_2 connectivity. Conversely, positive peaks should be due to direct CH_2 connectivities or to relay peaks arising from a starting CH connectivity. As mentioned above, a CH_n-edited HSQC-TOCSY experiment could also be designed using other Φ and Δ' values (data not shown).

The analysis of such spectra can be performed in two different ways. From the F_1 columns the ^{13}C subspectrum of each subunit including the multiplicity of each carbon is obtained. On the other hand, from the F_2 rows a clean ^1H subspectrum of each residue is obtained in which the phase of each direct connectivity is related to its multiplicity and, in addition, it can appear inverted with respect to the other relay peaks. The exact topology of each subunit could be elucidated by varying the length of the mixing process in different experiments.

Finally, Fig. 5 shows the multiplicity-edited version of the 1,1-ADEQUATE experiment[14] obtained with the sequence of Fig. 2(c). From this spectrum, extra information can be obtained on the multiplicity of C–C systems, because double-quantum carbon–carbon coherence evolution takes place during the t_1 period. Thus, CH–CH and C–CH_2 correlations have positive intensity whereas C–CH and CH–CH_2 correlations have negative intensity. Note how the exact skeleton structure can be simply extracted for the fructose residue following the specified marks (the unconnected C-1 methylene is marked with an arrow). An analog INEPT–INADEQUATE spectrum of sucrose has been published but without the sensitivity-improved and multiplicity-editing approaches.[15] This same approach can be extended to the refocused version of this experiment[16] in which SQC evolves during t_1 and the resulting spectrum would have the same display as an HMBC spectrum in order to distinguish between two- and three-bond connectivities.

CONCLUSIONS

In summary, we have shown that the incorporation of a simple building block in some gradient-based HSQC-type experiments affords extra and useful carbon multiplicity information with minor penalties in sensitivity when compared with non-edited experiments. This approach can be implemented with great simplicity into any multi-dimensional experiment based on the sensitivity-improved HSQC pulse sequence and the resulting edited experiments can also be used in automation mode in the same way as the original sequences.

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