Optimizing HMQC for ISₙ spin systems

Bosong Xiang,* Mark D. Winemiller, Timothy F. Briggs, David J. Fuller and David B. Collum

Department of Chemistry and Chemical Biology, Cornell University, Ithaca, New York 14853, USA

Received 25 September 2000; Revised 9 December 2000; Accepted 9 December 2000

A general analysis of the HMQC experiment for ISₙ nuclear spin systems is described. The optimal condition for setting up the HMQC experiment was derived. At this optimal condition, the expected HMQC signal was maximized and unwanted signals were satisfactorily reduced. The result was confirmed with the HMQC spectra of an organolithium compound. Copyright © 2001 John Wiley & Sons, Ltd.

KEYWORDS: magnetic resonance; NMR; HMQC; organometallic chemistry; organolithium compound

INTRODUCTION

HMQC (Fig. 1) is a powerful technique in detecting the J-coupling correlation between two heteronuclei.1 The most common use of the technique is to detect the one-bond proton (1H) to carbon (13C) or nitrogen (15N) connection in organic compounds and proteins. The proton is detected and the heteronucleus, 13C or 15N, is put on a decoupler channel of the NMR spectrometer. This 1H–13C or 1H–15N nuclear spin system can be described as IS, where I and S represent the proton and heteronucleus (13C or 15N), respectively. For the IS nuclear spin system, the time delay τ in the HMQC pulse sequence (Fig. 1) is usually set to 1/2J, where J is the one-bond (1H–13C or 1H–15N) J-coupling constant, for maximizing the HMQC signal.

HMQC can also be used in studying structures of aggregates of organometallic compounds.2–3 In such an aggregate the metal, such as 6Li, may be coordinated with more than one other nucleus, such as 13C or 15N. The HMQC experiment can be set up to detect the coordinated nucleus (13C or 15N) on a decoupler channel of the spectrometer. The nuclear spin system for this experiment can be described as ISₙ, where I and S represent the metal and the coordinated nucleus (13C or 15N), respectively, and n is the number of S-nuclei directly connected to the metal. If n is larger than 1, the time delay τ in the HMQC pulse sequence has to be set to an appropriate value, other than 1/2J, to optimize the HMQC signal.1,4 Otherwise, the expected HMQC signal is weak and unwanted peaks may appear in the spectrum such as that shown later. Here a general analysis is provided for setting up the HMQC experiment properly to maximize the expected HMQC signals and reduce the unwanted signals. The result is confirmed with the HMQC spectra of a sample containing organolithium compounds.

THEORY

For the ISₙ nuclear spin system, if S is a spin-1/2 nucleus and I is a spin-1/2 or spin-1 nucleus, the product operator formalism and coherence-transfer table method can be used to analyze the pulse sequence.5–9 Here the coherence-transfer pathways and NMR signals were derived by using the coherence-transfer table method and a new computer program called NMRPSA developed by the first author. The details of the derivation are omitted and only the results are discussed here. It is assumed here that the J-coupling constants for all the I–S connections of the ISₙ spin system are the same. From the detectable coherence-transfer pathways that survive the phase cycling, two different kinds of signals are obtained.

The first signal in time domain for any ISₙ spin system is

\[ 1S_n = [\sin(\pi/\tau) \cos^{n-1}(\pi/\tau)]^2 \cos(2\pi f_{I1} \tau) \sum_{i=1}^{n-1} \cos(2\pi f_{Si} \tau) \]  

(1)

where J is the J-coupling constant for the I–S connection, \( f_I \) is the resonance frequency of the I-nucleus, and \( f_S \) is the resonance frequency of the i-th S-nucleus. This signal generates an expected HMQC peak for each I–S connection at (\( f_S, f_I \)), where \( f_S \) and \( f_I \) are frequencies on first and second dimensions, respectively. The intensity of this signal is

\[ 1I_n = [\sin(\pi/\tau) \cos^{n-1}(\pi/\tau)]^2 \]  

(2)

It is maximized when

\[ \tau = \tau_m = (1/\pi f_I) \arccot(n - 1)^{1/2} \]  

(3)

which is the optimal value of \( \tau \). Table 1 shows \( \tau_m \) for different ISₙ systems.

For an ISₙ system where n is larger than two, in addition to the signal shown by Eqn (1), there are other signals that also survive the phase cycling. These additional signals are described by

\[ 2^{n+1} S_n = [\sin^{2^{n+1}}(\pi/\tau) \cos^{n-1}(\pi/\tau)]^2 \cos(2\pi f_{I1} \tau) \times \prod_{k=1,2} \cos(2\pi f_{Si} \tau) \]  

(4)

Copyright © 2001 John Wiley & Sons, Ltd.
The relative strength of the expected HMQC signal versus the unwanted signal is satisfactorily reduced. The unwanted signal [Eqn (6)] for an IS3 spin system generates peaks at (\[cos(fS_{k}f_{t})\cos(2\tilde{f}_{t}t_{2})\) \(x\) \([cos(2\pi f_{51}t_{1})\cos(2\pi f_{52}t_{1})\cos(2\pi f_{53}t_{1})\) for IS4 spin systems are

\[3S_{3} = \sin^{6}(\pi f_{t})\cos^{2}(\pi f_{t})\cos(2\tilde{f}_{t}t_{2})\]

\[\times [cos(2\pi f_{51}t_{1})+cos(2\pi f_{52}t_{1})+cos(2\pi f_{53}t_{1})\] for IS4 spin systems are

\[3S_{3} = \sin^{6}(\pi f_{t})\cos^{2}(\pi f_{t})\cos(2\tilde{f}_{t}t_{2})\]

and additional signals for the IS5 spin system are

\[3S_{5} = \sin^{6}(\pi f_{t})\cos^{2}(\pi f_{t})\cos(2\tilde{f}_{t}t_{2})\]

\[\times [cos(2\pi f_{51}t_{1})+cos(2\pi f_{52}t_{1})+cos(2\pi f_{53}t_{1})\]

\[\times [cos(2\pi f_{51}t_{1})+cos(2\pi f_{52}t_{1})+cos(2\pi f_{53}t_{1})\]

These additional signals generate peaks at \((f_{w}, f_{1})\), where the first dimension frequencies \(f_{w}\) are combinations of frequencies of the \(2i+1\) S-nuclei. For example, the additional signal [Eqn (6)] for an IS3 spin system generates peaks at \((f_{w}, f_{1})\) where

\[f_{w} = \pm f_{51} \pm f_{52} \pm f_{53}\]

which is a combination of frequencies of the three S-nuclei. Since these additional signals generate peaks at abnormal positions in addition to the expected HMQC peaks, they are called unwanted HMQC signals.

In general, the intensity of an unwanted HMQC signal \(2i+1S_{n}\), Eqn (4), is

\[2i+1I_{n} = 2^{-2i}[\tan^{2i+1}(\pi f_{t})\cos^{2}(\pi f_{t})]^{2}\]

where the factor \(2^{-2i}\) is obtained from converting the product \(\prod_{k=1}^{i}cos(2\pi f_{5j}t_{1})\) in Eqn (4) into a sum. It decreases with the increase of \(i\) when \(f_{t}\) is smaller than \(1/4\). Therefore, if \(f_{t}\) is set to be smaller than \(1/4\), the largest unwanted signal is \(3S_{3}\) for any ISn spin system with \(n\) larger than 2. The relative strength of the expected HMQC signal versus the unwanted signal can be described by the ratio

\[\frac{I_{n}}{I_{n}} = 4 \tan^{-4}(\pi f_{t})\]

where

\[n \geq 3\]

The \(I_{n}/I_{n}\) values at the optimal condition, \(\tau = \tau_{m}\), for different ISn spin systems are shown in Table 1. At the optimal condition, the expected HMQC signal is at least 16-fold stronger than the unwanted signal and, therefore, the unwanted signal is satisfactorily reduced. The unwanted

**Table 1.** \(1I_{n}\) and \(1I_{n}/1_{n}\) values at the optimal \(\tau_{m}\) for ISn spin systems

<table>
<thead>
<tr>
<th>(n)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\tau_{m})</td>
<td>1/2</td>
<td>1/4</td>
<td>1/5.1</td>
<td>1/6</td>
<td>1/6.8</td>
</tr>
<tr>
<td>(1I_{n})</td>
<td>1</td>
<td>0.25</td>
<td>0.148</td>
<td>0.106</td>
<td>0.082</td>
</tr>
<tr>
<td>(1I_{n}/1_{n})</td>
<td>16</td>
<td>36</td>
<td>64</td>
<td>81.6</td>
<td>100</td>
</tr>
</tbody>
</table>

where

\[1 \leq i \leq (n - 1)/2\]
signals may become dominant if \( \tau \) is increased above \( \tau_m \) according to Eqn (12).

**RESULTS AND DISCUSSION**

The above analysis was applied in setting up HMQC experiments for structure determinations of various \(^6\)Li compounds. The HMQC spectra (Fig. 2), of a sample recorded at different \( \tau \) values are presented as a demonstration in this paper. The major component of the sample is a molecule with the cage structure shown in Fig. 3. The lithium in the sample is the spin-1 isotope \(^6\)Li. The carbons connected to the \(^6\)Li were \( > 98\% \) \(^{13}\)C labeled. The \(^6\)Li–\(^{13}\)C \( J \)-coupling constants are around 6.0 (\( \pm 0.5 \)) Hz as determined by a \( J(13\text{C}, 6\text{Li}) \)-resolved experiment. \(^1\) The \( J \)-coupling between two \(^6\)Li nuclei is very small and not observable.

The three expected HMQC peaks (peaks 1–3) for the \(^6\)Li connected to three carbons (IS\(_3\) spin system) in Fig. 3 are maximized at \( D_1 = 5\) \( J \) as predicted by Eqn (2) while \( n = 3 \). Peaks 10–13 are the unwanted peaks for the same \(^6\)Li. As predicted by Eqn (10), the \(^{13}\)C frequencies of these unwanted peaks are combinations of the \(^{13}\)C frequencies of the three expected HMQC peaks (peaks 1–3):

\[
\begin{align*}
\nu_{10} &= -\nu_{1} - \nu_{2} - \nu_{3} \\
\nu_{11} &= \nu_{1} + \nu_{2} - \nu_{3} \\
\nu_{12} &= \nu_{1} + \nu_{2} + \nu_{3} \\
\nu_{13} &= -\nu_{1} + \nu_{2} + \nu_{3}
\end{align*}
\]

where \( \nu_{10}, \nu_{11}, \nu_{12} \) and \( \nu_{13} \) are \(^{13}\)C frequencies of peaks 10, 11, 12 and 13, respectively, and \( \nu_{1}, \nu_{2}, \nu_{3} \) are \(^{13}\)C frequencies of peaks 1, 2 and 3, respectively. These unwanted peaks are maximized at \( \tau = 1/5.1J \) as predicted by Eqn (11) while \( i \) and \( n \) equal 1 and 3, respectively, for the IS\(_3\) spin system. The unwanted peaks 10 and 11 are still noticeable at \( \tau = 1/4J \) [Fig. 2(b)]. At the optimal condition (\( \tau = 1/5.1J \)) for the IS\(_3\) spin system, the unwanted peaks disappeared, just as predicted by the above analysis.

Peaks 4–9 and 14–16 in Fig. 2 are expected HMQC peaks for the \(^6\)Li nuclei each of which is connected to two \(^{13}\)C nuclei (IS\(_2\) spin system). Peaks 4–9 are for three \(^6\)Li nuclei, each of which is connected to two \(^{13}\)C nuclei in Fig. 3. Peaks 14–16 are from minor components in the sample. These peaks are maximized at \( \tau = 1/4J \) as predicted by Eqn (2) while \( n = 2 \).

Peaks 17 and 18 are for two \(^6\)Li nuclei, each of them connected to only one \(^{13}\)C nucleus (IS spin system). They are from minor components in the sample. These peaks are maximized at \( \tau = 1/2J \) and decrease with decrease in \( \tau \) as predicted by Eqn (2) while \( n = 1 \).

The result from this example confirmed the theoretical analysis described in the above section. It shows that the HMQC experiment can be optimized for different IS\(_n\) spin systems. To maximize the HMQC signal for a particular IS\(_n\) system, \( \tau \) should be set at \( \tau_m \) calculated from Eqn (3). At this optimal condition, the unwanted signals for an IS\(_n\) spin system with \( n \) larger than 2 are satisfactorily reduced.

**Figure 2.** HMQC spectra recorded at \( \tau = (a) 1/2J, (b) 1/4J \) and \( (c) 1/5.1J \) and \( \tau \) is the time delay in the HMQC pulse sequence (Fig. 1). The value of \( J \) is 6.0 (\( \pm 0.5 \)) Hz. Peaks 1–3 are the expected HMQC peaks for the \(^6\)Li nucleus connected to three \(^{13}\)C nuclei in Fig. 3. Peaks 4–9 are the expected HMQC peaks for the three \(^6\)Li nuclei, each of which is connected to two \(^{13}\)C nuclei in Fig. 3. Peaks 10–13 are the unwanted peaks due to the incorrect setting of the time delay \( \tau \) in Fig. 1. Peaks 14–18 are HMQC peaks for the minor components in the sample.
For a sample containing several different IS \( n \) systems, the HMQC could be optimized for each particular IS \( n \) system. If it is desired to see all the HMQC peaks in one spectrum, \( \tau \) should be set at the \( \tau_m \) for the IS \( n \) system with the largest \( n \). For the sample used here, \( \tau \) is set at \( \tau_m = 1/5.1 \) for the IS \( 3 \) spin system, all the expected HMQC peaks (peaks 1–9) for IS \( 2 \) and IS \( 3 \) spin systems are observable and the unwanted peaks are satisfactorily reduced as shown in Fig. 2(c).

Since the HMQC peaks belonging to different IS \( n \) spin systems are maximized at different \( \tau_m \) values, they can be distinguished from HMQC spectra recorded at different optimal \( \tau_m \) values for the spin systems. This provides another method for assigning resonances in addition to the \( J \)-resolved experiment.

Since the nucleus I is defined as the nucleus to be observed in the HMQC, spin systems IS \( n \) and I(S are different from each other. The LS spin systems, such as CH\(_2\) and CH\(_3\) where H is observed, can be treated as an IS system if the \( J \)-coupling between the protons are ignored. It should be noticed that the results shown above only apply to the IS \( n \) spin systems where the nucleus I is a spin-1/2 or spin-1 nucleus and S is a spin-1/2 nucleus. Therefore, the results are applicable to \(^6\)Li compounds, but not \(^7\)Li compounds.

The result shown in this paper for HMQC is also applicable to the HSQC experiment for the IS \( n \) spin system. The HSQC experiment can be optimized by using the optimal value \( \tau_m \) of the time delay \( \tau \) in the HSQC pulse sequence (Fig. 1). The calculation of \( \tau_m \) is the same as discussed above for the HMQC. HMQC is often the method of choice owing to its simplicity with fewer pulses.

**EXPERIMENTAL**

The sample for spectroscopic analysis was prepared from the corresponding amino alcohol (0.015 mmol),\(^10\) \[^6\]Li][lithium \[^{13}\]C|cyclopropylacetylide (0.045 mmol)\(^11\) and \[^6\]Li][lithium hexamethyldisilazide\(^12\) (0.0175 mmol) in 1:1:3 THF–pentane (0.600 ml) using stock solutions and protocols described in detail previously.\(^13\) It was sealed in a 5 mm NMR tube for measurements at low temperature.

The HMQC spectra [Fig. 2(a)–(c)] were recorded at \(-115^\circ\)C on a Varian Inova-400 NMR spectrometer. The data were acquired consecutively with the same experimental set-up and processed with the same procedures so that the spectral intensities in Fig. 2(a)–(c) are comparable. A five-channel probe made by Nalorac was used to detect \(^6\)Li while \(^{13}\)C was put on another r.f. channel. The HMQC data acquisition time was 0.5 s and the recovery time before the first pulse in Fig. 1 was 5.5 s. The scan width in the direct dimension (\(^6\)Li) was set to 2000 Hz to enhance the sensitivity by over-sampling. The phase-sensitive States mode was used and 256 complex data points were taken for the indirect dimension (\(^{13}\)C). The number of transients per increment was 16. The NMRPipe program was used to process the two-dimensional spectra.\(^14\)

**Acknowledgments**

We thank the National Institutes of Health for direct support of this work. We also acknowledge the National Science Foundation Instrumentation Program (CHE 7904825 and PCM 8018643), the National Institutes of Health (RR02002, 08-S0RR13024A and 08-S0RR06248A) and IBM for support of the Cornell Nuclear Magnetic Resonance Facility.

**REFERENCES**