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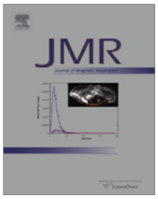

# Triple resonance, triple-quantum NMR studies of dipolar coupled spin systems

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ABSTRACT

Multiple quantum-single quantum correlation experiments are employed for spectral simplification and determination of the relative signs of the couplings. In this study, we have demonstrated the excitation of three nuclei, triple quantum coherences and discussed the information obtainable from such experiments. The experiments have been carried out on doubly labeled acetonitrile and fluoroacetonitrile aligned in liquid crystalline media. The experiment is advantageous in providing many spectral parameters from a single experiment. The coherence pathways involved in the pulse sequence are described using product operators.

## 1. Introduction

The concept of multiple quantum (MQ) experiments has been utilized in isotropic systems, molecules oriented in liquid crystal media and in solid state NMR either to study the spin dynamics or for the determination of structure of small molecules, bio-molecules and solids [1–7]. NMR is an excellent test bed for quantum mechanical demonstration of spin dynamics. The knowledge of spin dynamics of the coupled spin systems aids in the designing of desired experiments for the extraction of appropriate spectral information [8–20]. Multiple quantum excitations are feasible when two or more coupled nuclei are present in a system. The spins that are involved in the creation of MQ coherences are known as active spins and the remaining as passive spins. The collection of active spins can be considered as a single spin during the evolution of multiple quantum coherences and which evolve at the sum of chemical shifts of active spins and under the additive value of the couplings between active spins and a passive spin [21,22]. The direct detection of MQ coherences is forbidden and the two dimensional experiments are utilized where higher quantum coherences evolve in the indirect dimension and are subsequently converted to single quantum coherences [7]. Numerous homo- and hetero- nuclear MQ studies involving two nuclei have been reported [1–20]. In this work we have extended our earlier study of two heteronuclear multiple quantum experiment to three nuclei multiple quantum experiment by simultaneous flipping of three different nuclei. This aids in the extraction of additional information about the couplings, than the heteronuclear double resonance MQ experiments. Consequent to the involvement of more active spins, more information about couplings can be determined in the indirect dimension in a single experiment. Thus it circumvents the need for the utilization of several double resonance MQ experiments for obtaining identical information. The evolution and detection of these coherences is discussed through product operator formalism [20].

## 2. Spin dynamics in the designed pulse sequence

The designed pulse sequence is reported in Fig. 1. For the conceptual understanding of excitation and evolution of triple quantum (TQ) coherences, a coupled heteronuclear three spin (I, P and S) system is considered.

The first $\pi/2$ pulse applied on the I-spins brings the magnetization of I-spins to the transverse plane. Thus the magnetization at stage 1 of the sequence is;

$$I_x = P_z + S_z \tag{1}$$

These spins evolve under the coupling with P spins to create I-spin anti-phase terms at the end of $\tau_1$ period, where the duration $\tau_1$ corresponds to $1/2J_{IP}$, which is essential to excite MQ coherences. The magnetization just before the application of the $\pi/2$ pulse on P spins (stage 2) is:

$$I_x + I_xP_z + P_z + S_z \tag{2}$$

The $\pi/2$ pulse on P-spins creates MQ coherences from the anti-phase terms and the magnetization at stage 3 is:

$$I_x + I_xP_y + P_y + S_z \tag{3}$$

The created $I_xP_y$ multiple quantum coherences are then allowed to evolve during $\tau_2$ under the coupling to S spin. The effective coupling experienced by these coherences is the sum of the couplings

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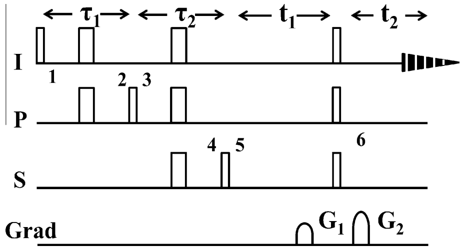

**Fig. 1.** Pulse sequence for triple resonance, triple quantum experiment. The thin and thick bars represent $\pi/2$ and $\pi$ pulses respectively. All the pulses are applied along *x*. If the third nucleus is at low natural abundance, the first $\pi/2$ pulse on the third nuclei and receiver need a two-step phase cycling *x*, −*x*. The gradients G1 and G2 were appropriately chosen, depending on the heteronuclei and the coherence order to be retained during $t_1$.

$T_{IS}$ and $T_{PS}$, wher T refers to the sum of scalar and dipolar couplings and the superscript to the number of bonds between the coupled nuclei [21]. The simultaneous application of $\pi$ pulses on all the three channels results in anti-phase double quantum coherence with respect to the third spin S when the delay $\tau_2$ is half of the inverse of the sum of these two couplings. The magnetization at stage 4 of the sequence is:

$$I_x + I_xS_z + I_xP_yS_z + P_z + P_xS_z + S_z \tag{4}$$

Therefore one of the requirements of this experiment is the use of two optimum delays $\tau_1$ and $\tau_2$. The last pulse before the $t_1$ period, on S-spins, will excite desired three spin multiple quantum coherences (stage 5).

$$I_xP_yS_y \tag{5}$$

These TQ coherences evolve during $t_1$ period and are converted into single quantum coherences by the simultaneous application of $\pi/2$ pulses on all the three channels. In the present experiments, we have retained only three nuclei TQ coherences during $t_1$ by selecting the appropriate pathway using gradients. These TQ coherences evolve at the sum of the chemical shifts of the three spins involved and under the sum of the couplings between the active spins and a passive spin.

## 3. Experimental

To demonstrate the application of the pulse sequence, we have chosen two molecules, viz., doubly labeled acetonitrile and fluoroacetonitrile, aligned in the liquid crystal ZLI-1132. In the acetonitrile sample, $^1H$, $^{13}C$ and $^{15}N$ and in the fluoroacetonitrile sample, $^1H$, $^{19}F$ and $^{13}C$, with $^{13}C$ at natural abundance, are the participating spins. All the experiments were carried out on Bruker AV-500 NMR spectrometer at 298 K. The acquisition and processing parameters are reported in the corresponding figure captions.

## 4. Results and discussion

The pulse sequence was initially demonstrated on doubly labeled acetonitrile, an $I_3P_1P_2S$ spin system, where $I_3$ are methyl protons, $P_1$ and $P_2$ are respectively methyl and nitrile carbons and S is nitrogen. The corresponding two dimensional TQ–SQ correlated spectrum is shown in Fig. 2. Consequent to the labeling of both the carbons, there is a possibility of simultaneous excitation of two $^1H^{13}C^{15}N$ triple quantum coherences, with the participation of methyl carbon ($P_1$) in one and the nitrile carbon ($P_2$) in the other. The two TQ coherences along $F_1$ dimension are represented by the doublet headed arrows **k** and **l** respectively and are split into triplets and then doublets. This is due to the fact that each of the TQ coherence is coupled to two passive protons and a passive carbon respectively. In the TQ spectrum marked as **k**, the triplet splitting

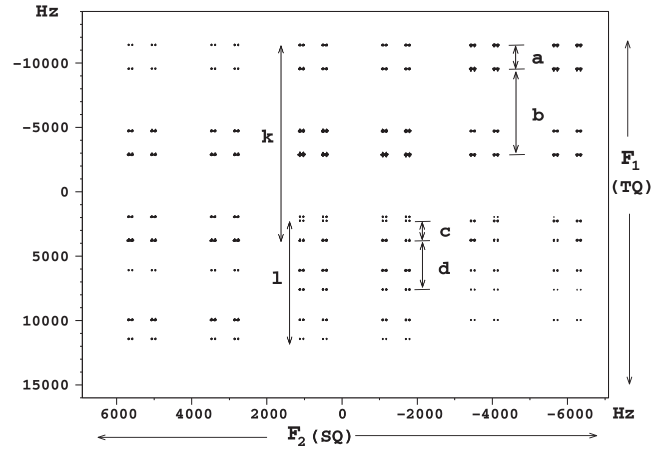

**Fig. 2.** Two dimensional triple resonance TQ–SQ spectrum of doubly labeled acetonitrile. The delay $\tau_1$ was set to 1.5 ms and $\tau_2$ to 1 ms. Four FIDs were accumulated with 512 and 8 k data points in $F_1$ and $F_2$ dimensions respectively. The total duration of the experiment was 2 h. The data was zero filled to double the data points and exponential window functions were used in both the dimensions before processing. The markings **k** and l represent two possible TQ coherences pertaining to methyl and nitrile carbons, as the active spins, respectively. The marked separations yield the coupling information as discussed in the text. The gradient ratio G1:G2 was 40:54.

**b**, due to the presence of two passive protons, is the sum of the couplings $^2T_{HH}$, $^1T_{CH}$ and $^3T_{NH}$. This splitting is 6660 Hz and corresponds to the situation when $^2T_{HH}$ (4556 Hz) and $^1T_{CH}$ (2206 Hz) are of same sign and $^3T_{NH}$ (95 Hz) is of opposite sign. Based on the earlier report [22], $^2T_{HH}$ is taken as positive, it is possible to assign positive sign to $^1T_{CH}$ and negative to $^3T_{NH}$. The smaller doublet **a** is due to the presence of nitrile carbon with the splitting of 1836 Hz. This is true only when $^2T_{CH}$ (617 Hz), $^1T_{CC}$ (665 Hz) and $^1T_{CN}$ (560 Hz) all have the same sign. In the TQ labeled as **l** the triplet splitting **d** is 3844 Hz and this pertains to the sum of the couplings $^2T_{HH}$ (4556 Hz) of one sign, and $^2T_{CH}$ (617 Hz) and $^3T_{NH}$ (95 Hz) different from $^2T_{HH}$. This establishes that $^2T_{CH}$ is negative. From these arguments we can assign $^1T_{CC}$ and $^1T_{CN}$ to be negative. The smaller doublet **c** is due to the presence of methyl carbon with the splitting of 1490 Hz. This is equal to the sum of the couplings $^1T_{CH}$ (2206 Hz), $^1T_{CC}$ (665 Hz) and $^2T_{CN}$ (55 Hz) only when the first term has a different sign with respect to the remaining two. This concludes that $^2T_{CN}$ is negative. All the determined couplings are reported in Table 1.

After successful implementation of the pulse sequence on the labeled sample, to ascertain its generality and applicability, the experiment was carried out on the sample fluoroacetonitrile, with $^{13}C$ at its natural abundance. In this situation the three participating spins are $^1H$, $^{19}F$ and $^{13}C$. Because of natural abundant $^{13}C$, to retain the magnetization of $^{13}C$ attached $^1H$ and $^{19}F$ nuclei the first $\pi/2$ pulse on S spin requires a two-step phase cycling *x*, −*x* and the receiver should follow identical phase cycling. The 2D TQ–SQ spectrum of this molecule is reported in Fig. 3. As in the previous case, in this system the two possible TQ coherences are marked as **k** and **l**, respectively with $^1H$, $^{19}F$ and $^{13}C$ (methyl) and $^1H$, $^{19}F$ and $^{13}C$ (nitrile) as the active spins. The separation **k** pertains to the sum of $^2T_{HH}$, $^1T_{HC}$ and $^2T_{HF}$ and the separation **l** to the sum of $^2T_{HF}$, $^2T_{HH}$ and $^2T_{CH}$. In Fig. 3, the observed doublet separation **k** is 7010 Hz and is equal to the sum only when $^2T_{HH}$ (4321 Hz), $^1T_{HC}$ (2337 Hz) and $^2T_{HF}$ (359 Hz) are of same sign (marked as **a**, **c** and **b** respectively). The separation **l**, which is 4505 Hz, is equal to the sum of $^2T_{HH}$ (4321 Hz), $^2T_{HF}$ (359 Hz) and $^2T_{HC}$ (175 Hz) with the last being opposite (marked as **a**, **c** and **d** respectively). Since $^2T_{HH}$ is assumed to be positive and $^1T_{HC}$ and $^2T_{HF}$ are known to be positive, $^2T_{CH}$ has to be negative to satisfy this value.

**Table 1**
The couplings measured from the experiment and determined based on the relative signs for labeled acetonitrle and fluoroacetonitrile oriented in liquid crystal ZLI-1132. The signs of the magnetogyric ratios of the nuclei are not included during the calculations [22].

| Couplings | Value with sign (Hz) | $F_2$ dimension splitting (Hz) | | |
|---|---|---|---|---|
| | | | Calculated | Measured |
| *Acetonitrile* | | | | |
| $^2T_{HH}$ | 4556 | **a** | 1842 | 1836 |
| $^1T_{HC}$ | 2206 | **b** | 6667 | 6660 |
| $^2T_{HC}$ | −617 | **c** | 1486 | 1490 |
| $^3T_{NH}$ | −95 | **d** | 3834 | 3844 |
| $^1T_{CC}$ | −665 | | | |
| $^2T_{CN}$ | −55 | | | |
| $^1T_{CN}$ | −560 | | | |

| Couplings | Value with sign (Hz) | Splitting in the $F_2$ dimension (Hz) | | |
|---|---|---|---|---|
| | | | Calculated | Measured |
| *Fluoroacetonitrile* | | | | |
| $^2T_{HH}$ | 4321 | **k** | 7018 | 7017 Hz |
| $^2T_{HF}$ | 359 | **l** | 4510 | 4505 |
| $^1T_{CH}$ | 2337 | | | |
| $^1T_{CF}$ | 788[a] | | | |
| $^2T_{CH}$ | −175 | | | |
| $^2T_{CF}$ | [b] | | | |

[a] The value was obtained from $^{13}C\{^1H\}$ spectrum consequently sign is unknown.
[b] This value could not be determined.

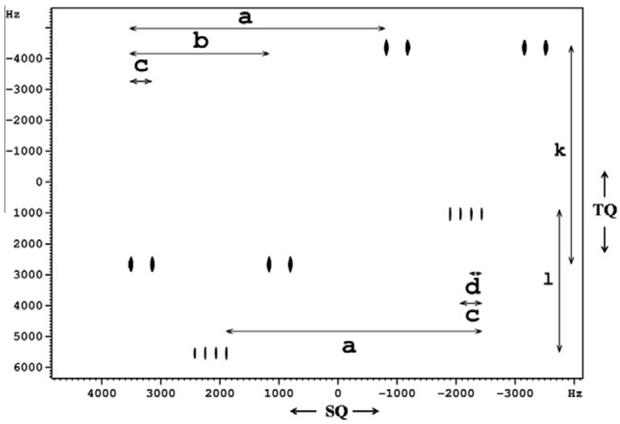

**Fig. 3.** The 2D triple resonance 3Q–SQ spectrum of fluoroacetonitrile with $^{13}C$ at its natural abundance. The delay $\tau_1$ was set to be 1.4 ms and $\tau_2$ to 0.2 ms. For each $t_1$ increment eight scans were accumulated with 512 and 2 k data points in $F_1$ and $F_2$ dimensions respectively. The data was zero filled to double the data points and Fourier transformed with exponential window function in both the dimensions. The marked separations are discussed in the text. The gradient ratio G1:G2 was 40:87.6.

This concept can be extended to any molecule with MQ excitation for the determination of relative signs of the couplings. In a double resonance MQ experiments, one can compare signs of only two couplings from one separation in the indirect dimension, where as in this experiment three of them can be compared to get the relative signs of any two. All the coupling information could be derived in a single experiment unlike in our previously reported two nuclei DQ experiments. The precise values of the dipolar couplings can be obtained by knowing the magnitudes and signs of scalar couplings [22].

## 5. Conclusions

The tri-nuclear triple resonance pulse sequence has been developed for the creation and detection of higher quantum coherences. The spin dynamics at different stages of the pulse sequence has been understood using product operators. The application of tri-nuclear TQ–SQ technique to dipolar coupled spin systems reveals that the single experiment is able to provide all the magnitudes and relative signs of the couplings. The technique can be extended, with the similar logic, to larger number of heteronuclear spin systems provided the spectrometer has multiple frequencies channels.

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## References

[1] G. Bodenhausen, Multiple-quantum NMR, Progress in Nuclear Magnetic Resonance Spectroscopy 14 (1980) 137.
[2] T.J. Norwood, Multiple-quantum NMR methods, Progress in Nuclear Magnetic Resonance Spectroscopy 24 (1992) 295.
[3] J.W. Emsley, J.C. Lindon, NMR Spectroscopy Using Liquid Crystal Solvents, Pergamon Press, Oxford, 1975.
[4] R.R. Ernst, G. Bodenhausen, A. Wokaun, Principles of Nuclear Magnetic Resonance in One and Two Dimensions, Clarendon Press, Oxford, 1990.
[5] J. Cavanagh, W.J. Fairbrother, A.G. Palmer, N.J. Skelton, M. Rance, Protein NMR Spectroscopy, Principles and Practice, second ed., Elsevier Publishers, 2007.
[6] M. Mehring, Principles of High Resolution NMR in Solids, Springer-Verlag, New York, 1982.
[7] G. Drobny, A. Pines, S. Sinton, D. Weitekamp, D. Wemmer, Fourier transform multiple quantum nuclear magnetic resonance, Faraday Division of the Chemical Society Symposium 13 (1979) 49.
[8] L. Braunschweiler, G. Bodenhausen, R.R. Ernst, Analysis of networks of coupled spins by multiple quantum NMR, Molecular Physics 48 (1983) 535.
[9] W.S. Warren, A. Pines, Analogy of multiple-quantum NMR to isotopic spin labeling, Journal of the American Chemical Society 103 (1981) 1613.
[10] W.S. Warren, D.P. Weitekamp, A. Pines, Theory of selective excitation of multiple-quantum transitions, Journal of Chemical Physics 73 (1980) 2084.
[11] J. Keeler, Understanding NMR Spectroscopy, John Wiley and Sons, England, 2005.
[12] J. Jarvet, P. Allard, Phase-sensitive two-dimensional heteronuclear zero- and double-quantum-coherence spectroscopy, Journal of Magnetic Resonance. Series B 112 (1996) 240.
[13] P. Permi, S. Heikkinen, I. Kilpeläinen, A. Annila, Measurement of homonuclear 2J-couplings from spin-sate selective double-/zero-quantum two-dimensional NMR spectra, Journal of Magnetic Resonance 139 (1999) 273.
[14] G. Otting, A DQ/ZQ NMR experiment for the determination of the signs of small $J(^1H, ^{13}C)$ coupling constants in linear spin systems, Journal of Magnetic Resonance 124 (1997) 503.
[15] P. Anderson, J. Weigelt, G. Otting, Spin-state selection filters for the measurement of heteronuclear one-bond coupling constants, Journal of Biomolecular NMR 12 (1988) 435.
[16] B. Brutscher, Accurate measurement of small spin-spin couplings in partially aligned molecules using a novel J-mismatch compensated spin-state-selection filter, Journal of Magnetic Resonance 151 (2001) 332.
[17] P. Permi, A spin-state-selective experiment for measuring heteronuclear one-bond and homonuclear two-bond couplings from an HSQC-type spectrum, Journal of Biomolecular NMR 22 (2002) 27.
[18] S. Sinton, A. Pines, Study of liquid crystal conformation by multiple quantum NMR: n-pentylcyanobiphenyl, Chemical Physics Letters 76 (1980) 263.
[19] A. Bax, R. Freeman, Investigation of $^{13}C$–$^{13}C$ couplings in natural abundance samples: the strong coupling case, Journal of Magnetic Resonance 41 (1980) 507.
[20] O.W. Sørensen, G.W. Eich, M.H. Levitt, G. Bodenhausen, R.R. Ernst, Product operator formalism for the description of NMR pulse experiments, Progress in Nuclear Magnetic Resonance Spectroscopy 16 (1984) 163.
[21] B. Bikash, N. Suryaprakash, Spin selective multiple quantum NMR for spectral simplification, determination of relative signs, and magnitudes of scalar couplings by spin state selection, Journal of Chemical Physics 127 (2007) 214510.
[22] S. Hebbar, N. Suryaprakash, Spin-selective multiple quantum excitation: Relative signs of the couplings and ambiguous situations, Journal of Magnetic Resonance 194 (1998) 192.