

## The Heteronuclear Single-Quantum Correlation (HSQC) Experiment: Vectors versus Product Operators

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**Supporting Information** 

**ABSTRACT:** A vectorial representation of the full sequence of events occurring during the 2D-NMR heteronuclear single-quantum correlation (HSQC) experiment is presented. The proposed vectorial representation conveys an understanding of the magnetization evolution during the HSQC pulse sequence for those who have little or no quantum mechanical background. This vectorial depiction is compared to results from the product operator formalism, and thus uncovers several parallelisms and subtle differences between both models. This approach permits a nice introduction to product operators at a level comprehensible to advanced upper-division undergraduate and graduate students without the use of the spin-density matrix.



**KEYWORDS:** Upper-Division Undergraduate, Graduate Education/Research, Physical Chemistry, Analogies/Transfer, NMR Spectroscopy

## INTRODUCTION

Nuclear magnetic resonance (NMR) is a core educational issue in chemistry. Many papers have been published in the educational literature dealing with NMR for structure elucidation,<sup>1-12</sup> reviews of books and media,<sup>13,14</sup> and other practical applications.<sup>15-20</sup> However, very few deal with the theory behind spin evolution,<sup>21,22</sup> which is ultimately responsible for the appearance of NMR spectra.

Indeed, why two-dimensional nuclear magnetic resonance (2D-NMR) spectra look as they do has always been hard to explain. As the queen of the microworld, quantum mechanics has been successfully employed to treat complex multiple-pulse NMR techniques. One of the quantum mechanical procedures to predict the appearance of 2D-NMR spectra is the product operator formalism.<sup>23-28</sup> However, as was pointed out in this *Journal*,<sup>21</sup> teaching product operators (PO) has not been part of undergraduate curricula mostly because it needs to be developed from the spin-density matrix. We adhere to the opinion that PO might be satisfactorily introduced by taking the intuitive vector model as the starting point.<sup>21</sup> Even though the simple vector model might sacrifice the most interesting and subtle features of 2D-NMR, it is undoubtedly useful because it provides visual and comfortable representations, and it is very intuitive for those who have little background in quantum mechanics. Consequently, the vector model could serve as bridge toward more rigorous explanations.

Among multiple-pulse NMR experiments, the heteronuclear single-quantum correlation (HSQC) experiment has mostly been approached using quantum mechanics.<sup>29-34</sup> However, HSQC is a good candidate for a vectorial illustration for two

reasons: (i) its heteronuclear nature permits treating the evolution of carbon and proton magnetizations independently; and (ii) even though multiple quantum coherence appears during the experiment, it may be ignored at a basic level.<sup>33</sup>

Herein we present a vectorial depiction of the full sequence of events occurring during the HSQC experiment. This representation allows a full understanding of the appearance of the resulting spectrum for those who are novices in 2D-NMR. The further comparison with PO results allows an interesting pedagogical link between both models, and also gives a nice introduction to the meaning of PO. This approach could be beneficial in courses dealing with advanced organic chemistry, biophysical chemistry, and other graduate or advanced undergraduate courses concerning NMR.

## GENERAL STRUCTURE OF 2D-NMR EXPERIMENTS

All 2D-NMR experiments contain four well-defined stages: (i) preparation; (ii) evolution; (iii) mixing; and (iv) detection. Preparation and mixing stages comprise pulse sequences or delay times that vary depending on the nature of the experiment. The detection period is entirely analogous to that in one-dimensional techniques, during which the spectrometer detects a free induction decay (FID) with all information saved in a frequency dimension known as  $F_2$ . The other dimension is generated during the evolution period. Any observable, offset, heteronuclear or homonuclear couplings that are not refocused during this period would be observed in the indirect frequency known as  $F_1$ .



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For the HSQC, carbon offset is the only observable that changes during the evolution period, thus carbon chemical shifts are presented along  $F_1$ . To clarify differences between offset and chemical shift, see the glossary in the Supporting Information. Along  $F_2$ , proton chemical shifts and homonuclear coupling are presented, indicating that these observables evolve during the detection stage. The HSQC allows for the correlation of protons with the carbon atoms to which they are directly attached. Observe in Figure 1 how the most shielded <sup>13</sup>C signal presents a cross peak with the <sup>1</sup>H triplet (methyl group). A similar analysis may be performed with the other cross peak.



**Figure 1.** Drawing of the HSQC spectrum of chloroethane. This 2D-NMR technique allows correlation of the protons and carbons directly attached.

## BUILDING THE HSQC: SPIN ECHO AND POLARIZATION TRANSFER

Before dealing with the vector description of HSQC, there are two main concepts that should be familiar to the reader: these are spin echo and polarization transfer.

In general, a spin echo consists of two equal time delays with a 180° pulse in the middle. Such a sequence allows a particular experiment to be constructed so that offsets or couplings could be intentionally suppressed, thus allowing one to handle spins according to their couplings with neighbors.

Conversely, in a system of coupled nuclei, polarization transfer takes place when the excitation of one transition of a nucleus changes the overall spin population distribution. Polarization transfer was initially devised to enhance the signals of a nucleus with low magnetogyric ratio (such as <sup>13</sup>C) by population (or polarization) transfer from its spin-coupled nucleus with a larger magnetogyric ratio (such as <sup>1</sup>H).

Further explanations concerning spin echo and polarization transfer may be found in the Supporting Information and in specialized books.<sup>30–33</sup>

## GENERAL DESCRIPTION OF THE HSQC PULSE SEQUENCE

The pulse sequence of the HSQC experiment is depicted at the top of Figure 2. The preparation stage is an INEPT sequence, which incorporates a spin echo as well. Then comes another spin echo as the evolution period, followed by an inverse INEPT that is a spin echo too, as the mixing period, before the detection at the end.

From this point on, the terms proton and carbon shall refer to  ${}^{1}$ H and  ${}^{13}$ C respectively, unless otherwise stated. In the vector schemes of Figure 2, red color corresponds to protons, and blue corresponds to carbon atoms. The arrows represent magnetization, and the labels indicate the respective coupling partner. Hence, if arrows are blue (carbon magnetization), labels are red (corresponding proton coupling partner), and vice versa.

All subsequent analyses dealing with vectors will be described as they would be observed in the rotating coordinate system. Axes in the rotating frame are referred to as x' and y'. Pulses to proton and carbon are assumed to be on resonance to avoid scheme complications due to offset evolution. Pulses are designated by the rotation angle, in degrees, with a subscript indicating the axis, while the angle between split vectors is presented in radians.

#### VECTOR DESCRIPTION

## **Preparation: INEPT**

The first  $90^{\circ}_{x}$  pulse to proton creates proton transverse magnetization along the -y' axis (Figure 2, scheme 1). The vector splits according to the proton-coupling partner, in this case carbon (Figure 2, scheme 2). The system is allowed to evolve a time equal to  $(4^{1}J_{CH})^{-1}$ , where  ${}^{1}J_{CH}$  stands for the single bond proton to carbon coupling constant. After this time, the angle between the two vector components is  $\pi/2$  radians (see the Supporting Information). The  $180^{\circ}_{x}$  pulse to proton rotates the transverse magnetization in the x'y' plane around 180° about the x' axis (Figure 2, scheme 3), and the  $180^{\circ}_{x}$ pulse to carbon changes the net carbon magnetization from +zto -z (Figure 2, scheme 4). The effect of applying this pulse on carbon spins is to invert the relative labels. Note that in real time these  $180^{\circ}$ , pulses occur simultaneously, even though they are analyzed sequentially for the sake of simplicity. At this point, proton vectors are still  $\pi/2$  radians to one another. After an additional  $(4^{1}J_{CH})^{-1}$  delay time, the proton components are in opposite directions, forming an angle of  $\pi$  radians; in this state the vectors are said to be antiphase (Figure 2, scheme 5). The 90° pulse to proton brings half of the proton vectors to +z(those protons attached to carbons with spin  $\beta$ ) and the other half to -z (those protons attached to carbons with spin  $\alpha$ ) (Figure 2, scheme 6). This state corresponds to inversion of the population of all protons, as occurs in selective population inversion (SPI, see the Supporting Information), but for all spin pairs simultaneously. At this point in the pulse sequence, a subsequent  $90^{\circ}_{x}$  pulse to carbon permits polarization transfer from proton to carbon (Figure 2, scheme 7). In fact, the vector model is unable to provide a convincing explanation concerning polarization transfer. At the end of the preparation period, proton offset is refocused while homonuclear coupling is not.

## **Evolution: Internal Spin Echo**

The evolution period is simply a spin echo (see the Supporting Information). From Figure 2, scheme 7, to Figure 2, scheme 10, there is no evolution due to proton offset and homonuclear coupling because all proton magnetization lies on the  $\pm z$  axis. Instead, carbon evolves according to its offset (not represented) and to the coupling with protons. In Figure 2, scheme 8, carbon vectors have moved toward each other during a time equal to  $t_1/2$ . The  $180^{\circ}_{x}$  pulse to proton inverts proton magnetization

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180°, 90°, 90°, 180°<sub>x</sub> 90°<sub>x</sub> 180°<sub>x</sub>  $(4^{1}J_{CH})^{\cdot 1}$  $(4^{1}J_{CH})^{-1}$  $t_{1}/2$  $(4^{1}J_{CH})$ (4<sup>1</sup>*J*<sub>CH</sub>)  $t_1/2$ ±x Mm 5 6 2 3 12 13 14 16 180°, 90°±x 90°<sub>x</sub> **180°**<sub>x</sub> <sup>13</sup>C Decouple 7 8 9 10 11 15 Preparation **Evolution** Mixing Detection  $(4^{1}J_{CH})^{-1}$ 90°<sub>x</sub> 180°<sub>x</sub> 180°, 2 3 1 (4<sup>1</sup>*J*<sub>CH</sub>) **90°**<sub>y</sub> 90°±x  $t_{1}/2$ 5 6 7 8 180°,  $t_{1}/2$ 90°x **90°**<sub>x</sub> 9 10 11 12  $(4^{1}J_{CH})^{-1}$  $(4^{1}J_{CH})^{-1}$ **180°**<sub>x</sub> **180°**<sub>x</sub>

Figure 2. Vectorial representation of magnetization evolution during the HSQC experiment. The pulse sequence of the experiment is presented on top. Pulses are designated by the rotation angle, in degrees, with a subscript indicating the axis. Magnetization evolution is presented in the schemes for each stage in the pulse sequence. Red represents proton; blue represents carbon. The arrows indicate magnetization, and the labels indicate the respective coupling partner.

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along the  $\pm z$  axis, which is seen in the x'y' plane as an inversion of proton labels (Figure 2, scheme 9). Consequently, carbon vectors reverse their evolution direction during another  $t_1/2$ period, until they are antiphase (Figure 2, scheme 10). Whatever the value of  $t_1$ , the net result of the evolution period shall always be antiphase magnetization of carbon. In Figure 2, scheme 10, magnetization of carbon lies along the y' axis because carbon offsets have not been represented. The

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experiment is repeated many times with the  $t_1$  period incremented at each stage. This procedure allows the sampling of carbon offsets and, therefore, permits the construction of the indirect dimension of the spectrum. As a result of this spin echo, heteronuclear coupling has been refocused, so only carbon offset evolves and shall be the sole observable in  $F_1$ .

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#### **Mixing: Inverse INEPT**

The 90 $^{\circ}_{r}$  pulse to carbon brings carbon magnetization to the  $\pm z$  axis (Figure 2, scheme 11). Note that this spin state is similar to that represented in Figure 2, scheme 6, but in this case there are proton magnetization on  $\pm z$  and inversion of carbon population. A subsequent  $90^{\circ}_{x}$  pulse applied to proton allows polarization transfer back from carbon to proton (Figure 2, scheme 12). Indeed, this is the same as in the INEPT, but in reverse. Now transverse magnetization is due to protons and shall evolve according to heteronuclear coupling. Proton vectors precess a period equal to  $(4^{1}J_{CH})^{-1}$  until the angle between both components is  $\pi/2$  radians (Figure 2, scheme 13). The  $180^{\circ}_{x}$  pulse to proton interchanges the vectors (Figure 2, scheme 14), and the simultaneous  $180^{\circ}_{x}$  pulse to carbon changes carbon labels (Figure 2, scheme 15). Then, proton vectors continue to close another time equal to  $(4^{1}J_{CH})^{-1}$  until both components are in-phase (Figure 2, scheme 16).

Finally, proton offset and homonuclear coupling are observed in  $F_2$  during the detection period. The procedure to suppress the resonances of those protons attached to <sup>12</sup>C has been described in the literature.<sup>31,33</sup>

## COMPARISON WITH PRODUCT OPERATORS

## **Representation of Spin States with Product Operators**

The vector model represents spin states through Cartesian components of each magnetization vector. However, PO allows for such a representation in a rather simple manner. Table 1 presents PO for one-spin and two-spin systems, along with a brief description of their meaning. Proton and carbon operators are represented by I and S, respectively.

# Table 1. Product Operators for One-Spin and Two-Spin Systems<sup>29</sup>

Product Operator	Coherence Name
$I_z, S_z$	Longitudinal I-spin (S-spin) magnetization
$I_{x}$ , $I_{y}$ , $S_{x}$ , $S_{y}$	Transverse I-spin (S-spin) magnetization
$2I_zS_z$	Nonequilibrium longitudinal two-spin magnetization
$2I_xS_z$ , $2I_yS_z$	Transverse <i>I</i> -spin magnetization antiphase with respect to the coupling to <i>S</i> -spin
$2I_z S_{x}$ , $2I_z S_y$	Transverse S-spin magnetization antiphase with respect to the coupling to <i>I</i> -spin
$2I_xS_y,\ 2I_yS_x,\ 2I_xS_x,\ 2I_yS_y$	Multiple quantum coherence

The operator  $I_x$  (or  $S_x$ ) means that the nucleus whose operator is denoted by I (or S) presents transverse magnetization along the x' axis. The operator  $2I_xS_z$  represents transverse magnetization of the nucleus I along x', which is antiphase with respect to the coupling to S. In the same way, the operator  $2I_zS_y$  stands for antiphase magnetization of the nucleus S along y' with respect to the coupling to I. The factor of 2 in these operators is needed for normalization purposes. Multiple quantum operators, such as  $2I_xS_y$ , represent transverse magnetization for both nuclei at the same time. The reason why these are unobservable escapes the possibilities of the vector model even though efforts have been made to provide a graphical explanation.<sup>23,25,29,35</sup> A physical description for the operator  $2I_zS_z$  is not straightforward; it has been defined as a particular type of nonequilibrium population distribution.<sup>33</sup>

PO formalism presents pulses and magnetization evolution in a particular manner. For example, eq 1 represents a pulse to the nucleus *I* about *x'*, which causes a rotation by an angle  $\beta$ . If  $\beta = 90^{\circ}$ , then  $\cos \beta = 0$  and  $\sin \beta = 1$ . The net result is precession of +z magnetization toward -y' with a sinusoidal dependence on the flip angle  $\beta$ . Besides, if a pulse is applied to an operator (say  $I_x$ ) about its own axis (say about *x'*), the operator remains unaltered.

$$I_z \xrightarrow{\beta I_x} I_z \cos\beta - I_y \sin\beta \tag{1}$$

#### **Preparation: INEPT**

From here on, a comparison of PO<sup>29,33</sup> with vector schemes provided in Figure 2 is presented, with *I* representing the proton spins, and *S* representing the carbon spins. The first 90°<sub>x</sub> pulse converts  $I_z$  into  $-I_y$  in agreement with Figure 2, scheme 1, and eq 1. Evolution under heteronuclear coupling ( $J_{IS}$ ) makes  $-I_y$  split according to eq 2.

$$-I_{y} \xrightarrow{(2\pi J_{IS})I_{z}S_{z}} -I_{y} \cos(\pi J_{IS})t + 2I_{x}S_{z} \sin(\pi J_{IS})t$$
(2)

Since  $t = (2J_{IS})^{-1}$ , substitution into eq 2, with  $\cos(\pi/2) = 0$ and  $\sin(\pi/2) = 1$ , shows that there is complete conversion of in-phase into antiphase proton magnetization represented by the PO  $2I_xS_z$  (Figure 2, scheme 5). The other  $90^\circ_x$  pulse to spin *I* turns this to  $2I_zS_z$ , very similar to Figure 2, scheme 6, in which all vectors are along *z*. The last  $90^\circ_x$  pulse brings the system to the state  $-2I_zS_y$  in accordance with Figure 2, scheme 7.

## **Evolution: Internal Spin Echo**

During this period, proton offset and homonuclear coupling do not evolve because all proton magnetization lies on  $\pm z$ . The  $180^{\circ}_{x}$  pulse to spin *I* in the middle of the evolution period refocuses heteronuclear coupling. Therefore, only the offset of carbon affects the evolution, and shall be the sole observable in  $F_1$ . Equation 3 represents free evolution of the corresponding PO  $-2I_zS_y$  under the offset of  $S(\Omega_s)$  after an evolution time equal to  $t_1$ .

$$-2I_z S_y \xrightarrow{\Omega_S t_1} 2I_z S_y \cos \Omega_S t_1 - 2I_z S_x \sin \Omega_S t_1 \tag{3}$$

The spin state after the evolution period is represented by the right side of eq 3. After a time equal to  $t_1$  there is antiphase magnetization of carbon, represented as a combination of  $2I_zS_x$ and  $2I_zS_y$ . In the corresponding Figure 2, scheme 10, pure antiphase magnetization in the x'y' plane may be observed, while the same state is represented with PO as a combination of two antiphase operators.

#### Mixing: Inverse INEPT

The next two 90°<sub>x</sub> pulses transfer the magnetization from *S* to *I* as expressed by the first terms in the left and right sides of eq 4. Observe how  $2I_zS_y$  is transformed to  $2I_yS_z$ . On the other hand, the second term in the left of eq 4 is turned to unobservable multiple quantum coherence ( $2I_yS_{xy}$  second term in the right of eq 4). Details of the four-step phase cycling to eliminate multiple quantum terms may be found in the literature.<sup>29</sup>

$$2I_z S_y \cos \Omega_S t_1 - 2I_z S_x \sin \Omega_S t_1$$

$$\xrightarrow{(90^0 I_x + 90^0 S_x)} 2I_y S_z \cos \Omega_S t_1 - 2I_y S_x \sin \Omega_S t_1$$
(4)

This state is approximated to Figure 2, scheme 12. Antiphase magnetization of proton is depicted there, corresponding to the PO  $2I_yS_z$ .

The term  $2I_yS_z \cos \Omega_S t_1$  then evolves to in-phase magnetization and finally results in  $I_x \cos \Omega_S t_1$ , which is the final



Figure 3. Vectorial representation of multiple quantum coherence in the HSQC experiment. The scheme numbers in this figure correspond to the numbering in Figure 2.

observable in the FID in  $F_2$ . Nicely, the final result is proton transverse magnetization along  $x'(I_x)$  evolving at the offset of carbon  $(\Omega_s)$ , giving rise to the characteristic cross peak between carbons and the directly attached protons. There are two differences with the corresponding Figure 2, scheme 16: (i) proton magnetization lies along -x'; and (ii) there is no way of representing proton magnetization evolving at the offset of carbon. The spectrum contains an in-phase doublet in  $F_2$  centered at the offset of proton, and in  $F_1$  at the offset of carbon.

## MULTIPLE QUANTUM COHERENCE: WHAT DO THE VECTORS SAY?

The last section shows an unexpected result for the HSQC experiment, namely, the apparition of multiple quantum terms in PO. Interestingly, the origin of multiple quantum coherence may be explained by the vector model as follows.

Figure 3 presents vector schemes similar to those presented in Figure 2, but now carbon offset has been represented. In Figure 3, the numeration of schemes was set to agree with Figure 2. Evolution of carbon offset during the evolution period gives rise to two components along the x' and y' axes, respectively (Figure 3, scheme 10), in agreement with  $2I_zS_y \cos$  $\Omega_s t_1 - 2I_z S_x \sin \Omega_s t_1$  (eq 4, left side). Since the 90° value applied to carbon is about x', only the component onto y' shall be affected, while the x' component remains in the x'y' plane (Figure 3, scheme 11). The next pulse applied brings proton magnetization to the x'y' plane. The coexistence of both carbon and proton magnetization in the transverse plane is a way to envision multiple quantum coherence, useful for a first approach. Therefore, Figure 3, scheme 12, would be equivalent to  $2I_vS_z \cos \Omega_s t_1 - 2I_vS_x \sin \Omega_s t_1$ .

## CONCLUSION

A complete description of the HSQC experiment using vectors has been provided. This intuitive model permits a basic comprehension of the experiment for those who do not have a background in quantum mechanics. Comparing the vector model and the product operator results shows many common points. Nevertheless, subtle features of the experiments are revealed only by means of the more rigorous PO formalism. Additionally, a careful examination of the vector model uncovered an explanation regarding the apparition of multiple quantum coherence in a simple and understandable fashion. We recommend this comparative approach to introduce 2D-NMR whenever possible.

#### ASSOCIATED CONTENT

#### Supporting Information

Short glossary containing important NMR terminology and further explanations concerning spin echo and polarization transfer. This material is available via the Internet at http:// pubs.acs.org.

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## Notes

The authors declare no competing financial interest.

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