

Understanding NMR Multiplet Structure with WinDNMR

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Spectrometer technology has developed at a surprising rate over the past ten years, allowing us to perform experiments that were previously inconceivable. In addition, the speed and efficiency of computers and the sophistication of the software developed for many NMR applications have contributed to the arsenal of techniques available to the research scientist wishing to extract maximum information from an NMR experiment. Unfortunately, many researchers who use NMR as a technique for characterization opt for the quickest experiment that will give the minimum amount of information needed to characterize a compound but which may largely ignore crucial structural information. It is too often the case that a quick two-dimensional COSY experiment will tell us which protons are coupled to each other (1, 2). Unfortunately, we seldom analyze the multiplets in a one- or two-dimensional spectrum, which are encoded with a wealth of structural information. The conformational dependence of a coupling constant described by the Karplus equations (3) can be used in tandem with molecular modeling programs to develop a detailed picture of a molecular structure (2a). This might be important in the way a molecule interacts with, say, living systems—for instance, in the design of efficient drug receptors.

In this paper, we wish to encourage the analysis of multiplet structure, particularly to an undergraduate audience, with the aid of a simulation program (WinDNMR) (4). While two- and three-spin systems are relatively easy to interpret, there are instances where the more complicated four-spin system defies the expected appearance as a result of second-order effects and accidental line overlap. For this reason we will concentrate on the potentially more complicated four-spin system as a convenient “teaching aid” and use the WinDNMR simulation program as a teaching tool capable of illustrating these complicating effects. A basic understanding of this type of analysis will arm the research student with the confidence to eventually simulate a complete spectrum, and in so doing test the interpretation of a structural assignment based on a one-dimensional spectral analysis.

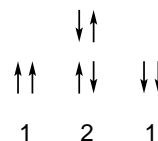
It has been our experience that students are willing to accept the description and composition of a conventional multiplet presented in a lecture, but struggle to visualize changes in the appearance of a multiplet when selected coupling constants are changed. A simulation program (in this case WinDNMR) provides visual evidence for any changes, and allows the student the freedom to change any combination of coupling constants or the chemical shift difference between any combination of coupled partners. We suggest that students, in small groups, might be offered exercises similar to the ones described in this paper and then given the freedom to set their own parameters in order to test their predictions. We do not, however, recommend this *practical exercise* without prior exposure to a *theory-based* NMR course, which would introduce some basic concepts that will not be treated in detail in this paper.

Spin–Spin (*J*) Coupling

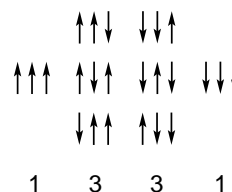
The theory underlying spin–spin coupling has been described at great length (1) and will not be treated here. We need only consider the simplest case of a doublet and triplet in order to illustrate why we observe the number of lines we do in the simplest possible way, avoiding complicated energy diagrams that often overwhelm the student.

A proton will appear as a singlet in the absence of neighboring protons with which to interact at a chemical shift value (δ in ppm) that depends on the electron density around it and on the effect of neighboring groups and atoms in the molecule (induced magnetic moments, electric fields, ring currents, etc.). By introduction of a second proton, the single line is transformed into two lines of half the original intensity. The distance between these lines is known as the coupling constant (J in Hz) and reflects the degree of electronic communication between the two spins (strictly a through bond effect, technically referred to as the electron-coupled spin–spin interaction), the value of which generally decreases as a function of the increasing number of bonds that separate the spins.

A doublet results from the two possible spin states that the neighboring proton may adopt, either along the direction of the magnetic field (\uparrow) or against it (\downarrow), and has little to do with the proton under consideration. The appearance of a triplet is a simple extension of this idea, whereby a proton has two identical neighbors (spins) and four possible combinations for interaction of these spins. Of the possible combinations, two are degenerate (of the same energy) and result in the central line's exhibiting twice the intensity of the outer lines. *Tree diagrams* (also known as *splitting diagrams*) are often used to represent the nature of the splitting pattern and should have been encountered in course work prior to the use of this program.



A quartet therefore results from the interaction of the neighboring three spins in a four-spin system (shown below). Two degenerate combinations contribute to the threefold intensity of the two central lines relative to the two outer lines.



In the simplest manifestation (Fig. 1a), the splitting pattern for each multiplet of a four-spin system will appear as a doublet of doublets of doublets (ddd). The resulting eight lines, from which the coupling constants will be trivial to extract, will be easily recognized and of equal intensity.

Unfortunately, eight lines are usually not observed, as accidental line overlap and second-order effects turn the ideal appearance of the multiplet (eight equal intensity lines) into a complex multiplet depending on the severity of the effects acting on the system. Multiplet pattern recognition depending on the various coupling constants for assigning the relative configuration among two or more stereocenters in a molecule has been treated (5) and is used extensively in protein work (2, 6). While certain combinations of coupling constants result in line overlap, which acts to simplify the appearance of a multiplet, second-order effects can complicate the assignment of a multiplet. Both features will be discussed below.

As far as line overlap is concerned, there are *particular* relationships among the J values that make some lines in the

multiplet overlap. As a consequence, the number of lines can be reduced from eight, and their relative intensities can also vary.

Selected combinations of J values for a proton coupled to three other spins are presented in Figure 1. In each case, the parameters used to generate the particular splitting pattern are shown next to it, so that the multiplet can be easily reproduced by the reader. It is important to emphasize that the line-shape, which can be affected by a number of factors such as viscosity, size of molecule, or temperature, might also complicate the interpretation of multiplet structure by merging lines. In all the cases presented here, the line-width (a variable that might be changed in the program) has been set to 0.1 Hz, allowing us to identify all the lines in a multiplet.

The simplest multiplet in appearance arises when all three coupling constants are of equal magnitude, in which case a “quartet” is observed with relative line intensities 1:3:3:1 (Fig. 1b)—a simplification in the appearance of the spectrum by a factor of two! Perturbing the degeneracy of the coupling constants by setting the three coupling constant almost, but not exactly, equal results in an eight-line system (Fig. 1c) resembling the appearance of Figure 1b and makes it possible to see the cause of the two central lines in Figure 1b. In all the simulations, we activate the “1st order” option in the menu of WinDNMR, ensuring that the appearance of the multiplet is affected only by overlapping lines. Second-order effects, which complicate the appearance of a multiplet in an unpredictable manner, will be treated in more detail below.

If we now consider the case when two coupling constants are the same and the third one is twice or half the value of the other two, then five or six equally spaced lines arise, with intensities in the ratio 1:2:2:2:1 or 1:1:2:2:1:1, respectively (Fig. 1d,e). Two superimposed but not overlapping triplets (six lines overall) are observed in all the other cases in which two J s are the same. The appearance can change dramatically depending on whether the two identical J values are the largest or the smallest values (Fig. 1f,g). Finally, it is worth pointing out the special relationship in which the sum of two of the coupling constants is equal to the third, in which case seven lines are observed with intensities in the ratio 1:1:1:2:1:1:1 (Fig. 1h).

Second-Order Effects

As a rule of thumb, if the chemical shift difference ($\Delta\nu$ in Hz) between coupled spins is greater than their coupling constant (i.e., $\Delta\nu/J > 10$), the multiplets will be first order in appearance, allowing trivial analysis of the multiplet structure (i.e., determining the coupling pattern and measuring reliable coupling constants directly from the spectrum). In cases where $\Delta\nu/J$ is less than 10, second-order effects arise and the assignment of the multiplet becomes more and more complicated as the magnitude of $\Delta\nu/J$ diminishes. If $\Delta\nu/J$ is comparable to a value of 10, the intensity distribution of the multiplet is affected in such a way that the resulting “roofing” or “leaning” directs us to the coupled partner in the spectrum. In a congested one-dimensional spectrum, this effect can act more as an aid than a hindrance, sometimes making it possible to identify coupled partners in a congested region.

Increasing the field strength of the magnet (which has the effect of increasing the number of Hz per unit of ppm) results in a larger separation ($\Delta\nu$ in Hz) between two coupled

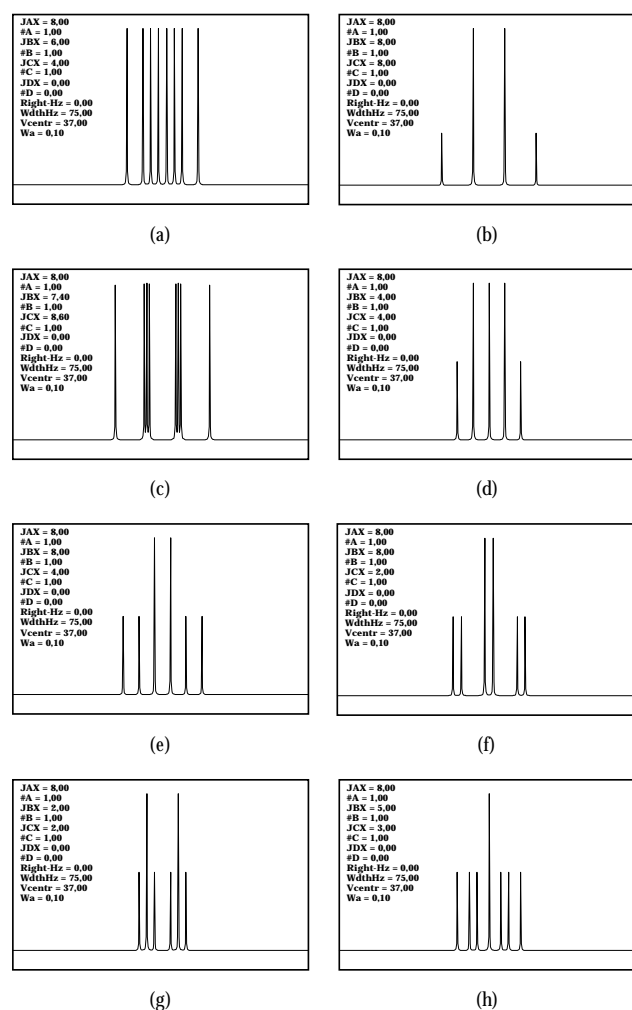


Figure 1. The appearance of a multiplet (ddd) based on the selected relationship of the J -values; (a) $J_1 > J_2 > J_3$; (b) $J_1 = J_2 = J_3$; (c) $J_1 \equiv J_2 \equiv J_3$; (d) $J_1 = 2J_2$, $J_2 = J_3$; (e) $J_1 = J_2$, $J_2 = 2J_3$; (f) $J_1 < J_2$, $J_2 = J_3$; (g) $J_1 > J_2$, $J_2 = J_3$; (h) $J_1 = J_2 + J_3$.

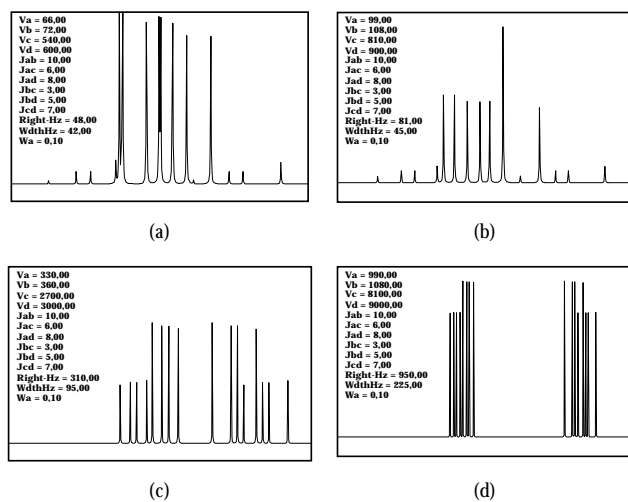


Figure 2. Transformation of an ABXY into an AMXY spin system upon increasing the magnetic field strength: (a) 60 MHz; (b) 90 MHz; (c) 300 MHz; (d) 900 MHz.

multiplets, while the J value (in Hz), which is characteristic of a coupled pair, remains unaffected. In this way, a second-order multiplet is progressively conferred first-order character by increasing the field strength of the magnet (as $\Delta\nu/J$ increases with it). The transformation of a heavily roofed or leaning second-order multiplet into a first-order system is illustrated in Figure 2. The AB component of a 4-coupled spin system is shown at four magnetic field strengths (60, 90, 300, and 900 MHz). The chemical shifts of A and B have been set to 1.10 and 1.20 ppm and their coupling constant to 10 Hz. Most students will find it difficult to extract information from the spectra at 60 or 90 MHz (where $\Delta\nu/J < 1$), as neither the number of signals nor the intensities of the component lines resemble the expected structure of the multiplet at first glance. An additional feature of a second-order spectrum is dramatically illustrated here, whereby, apart from a “roofing” distortion of the intensities, there also appear many low-intensity lines that defy any symmetry. In an experimental spectrum, these small lines contribute to a significant complication in the appearance of a multiplet, especially when recorded near to or overlapping with other peaks.

One would need to “acquire” the spectrum at 300 MHz before the A and B components of the multiplet are separated enough to suppress the second-order derived complications (note that roofing is still evident). Acquiring a spectrum on a 900-MHz instrument (not yet commercially available) would render the spectrum suitable for first-order analysis ($\Delta\nu/J = 9$) with the expected line intensities in each multiplet.

From the examples described here, we can begin to understand how the appearance of an ABCD spin system can differ depending on the relationships among the J values or the chemical shift difference between coupled protons. In addition to these effects, *magnetic nonequivalence* can also complicate the shape of a multiplet. For example, two protons at identical chemical shift (*isochronous nuclei*) coupled to a common third proton with different coupling constants become magnetically nonequivalent. Under such a condition, the magnetically nonequivalent nuclei (also referred to as

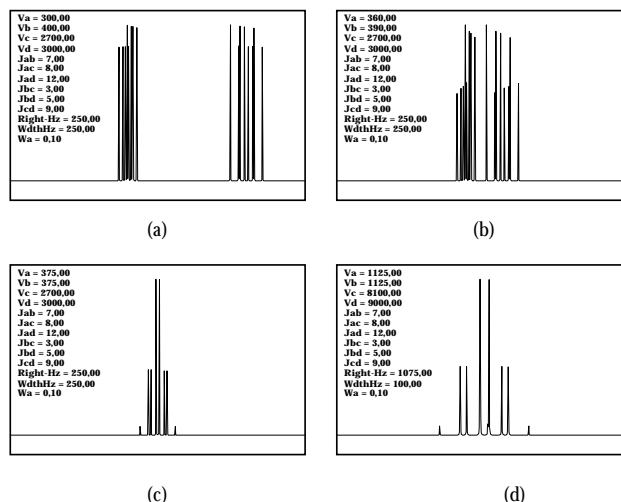


Figure 3. Transformation of an ABXY into an AA'XY spin system: (a) 300 MHz spectrum where $\delta_A \neq \delta_B$; (b) 300 MHz spectrum where $\delta_A \approx \delta_B$; (c) 300 MHz spectrum where $\delta_A = \delta_B$; (d) 900 MHz spectrum where $\delta_A = \delta_B$.

anisogamous) exhibit a nontrivial multiplet pattern, the effects of which cannot be removed by increasing the magnetic field strength. This effect and how it affects the appearance of the spectrum are shown in Figure 3, which represents the signals of two protons in a 4-spin system. The chemical shifts of these two protons are made different in the first case and eight lines are observed for each multiplet, as expected for a proton coupled to 3 neighbors with different coupling constants. In the second case, the chemical shift difference between these two protons is reduced while the coupling constants remain unchanged. Apart from some roofing, the appearance of the multiplets does not change strikingly. The condition of anisogamy is introduced in Figure 3c, where the chemical shifts of the two protons coincide (1.25 ppm) and their coupling constants to a third proton are different. The shape of the multiplet has transformed to a symmetrical envelope of lines, for which the analysis is served by no simple rules. Quantum mechanical calculations would be required to characterize this spin system; a prospect avoided by all synthetic chemists that might use this technique. No change in the shape of the multiplet is observed in the spectrum upon increasing the strength of the chemical field (Fig. 3d).

The examples above, generated with the aid of a simulation program, show how to best explain the potentially deceptive appearance of a multiplet (due to various combinations of J values) to an undergraduate audience. In order to do this, it is important to discriminate between first- and second-order effects and to be able to reproduce these effects in an attempt to generate a spectrum that will resemble an experimental spectrum. An understanding of these simple concepts will allow a student to extract the maximum information from the most complicated of spectra.

Acknowledgments

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Literature Cited

1. While many excellent texts deal with the concepts that are introduced here, the ones listed below represent a selection that might be found in most academic libraries. For a general treatment of NMR spectroscopy and experiments related to coupling constant analysis, refer to Günther, H. *NMR Spectroscopy*, 2nd ed.; Wiley: Chichester, 1995. Sanders, J. K. M.; Hunter, B. K. *Modern NMR Spectroscopy, A Guide for Chemists*, 2nd ed.; Oxford University Press: New York, 1993. Frebolin, H. *Basic One- and Two-Dimensional NMR Spectroscopy*; VCH: Weinheim, 1991. Hore, P. J. *Nuclear Magnetic Resonance*; Oxford Chemistry Primers; Oxford University Press: New York, 1995. Lash, T. D.; Lash, S. S. *J. Chem. Educ.* **1987**, *64*, 315. Mann, B. E. *J. Chem. Educ.* **1995**, *72*, 614. Thoben, D. A.; Lowry, T. H. *J. Chem. Educ.* **1997**, *74*, 68.
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3. Karplus, M. *J. Am. Chem. Soc.* **1963**, *85*, 2870.
4. Spectra were simulated using Reich, H. J. WinDNMR: Dynamic NMR Spectra for Windows; *JCE Software* **1995**, *3D(2)*. This program is intuitive and easy to use, requiring no prior knowledge of quantum mechanics to construct the multiplets. A set of clearly labeled dialogue boxes allows the simulation of a variety of NMR multiplets (AB, AB₂, ABX, ABC, ABCD, ABCDE, AA'BB', and AA'XX') invoking a first- or second-order treatment. Varying the spectral parameters to generate single multiplets or entire spectra can be achieved in a number of ways, after which the output can be presented as a plot (as in this paper) or as a file for incorporation into other software packages. The effects of exchange dynamics can also be investigated using this program, but this feature has not been dealt with in this paper.
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