THREE-DIMENSIONAL CORRELATION SPECTROSCOPY THE MOST COMPLICATED SPECTRUM OF AN AMX SPIN SYSTEM?

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Three-dimensional NMR has recently been demonstrated to be a viable technique for improving the dispersion of data in highly congested spectra. It is thus finding application in the assignment of spectra of proteins, oligosaccharides and other large biomolecules. With our sights set on performing such assignments we have been developing our own methodology for acquiring and processing three-dimensional experiments.

In the initial stages of our work we performed the simplest three-dimensional experiment possible - the so-called COSY-COSY experiment. This consists of three pulses and two independently incremented delays:

$$90_x - t_1 - 90_x - t_2 - 90_x - \text{acquire } (t_3)$$

We chose to study the simple AMX spin system of 2,3-dibromopropionic acid. The use of hard pulses gives a complicated spectrum. Naively, one might expect to observe 3x3x3=27 crosspeaks in a hard COSY-COSY experiment, with each crosspeak having 4x4x4=64 elements of fine structure. However, when the experiment is actually performed, in addition to the 27 crosspeaks expected, a further 27 crosspeaks are observed in the spectrum.

The appearance of these extra crosspeaks can be traced to the presence of three-spin coherence during the second evolution period. Product operator analysis shows that the coherences corresponding to operators of the type $4I_{Ay}I_{My}I_{Xx}$ are generated during t_2. These terms can be demonstrated to represent a combination of triple quantum coherence and single quantum coherence. It is the latter coherences that are of interest because they cannot be suppressed by phase cycling. These terms evolve during t_2 under effective chemical shifts given by combinations of the shifts of A, M and X: this results in crosspeaks which are found at three new frequencies in f_2.

The multiplet structure of these three-spin crosspeaks is of some interest. Magnetisation which is described by the operator $4I_{Ay}I_{My}I_{Xx}$ during t_2 must pass through a doubly antiphase state in t_1. This operator does not evolve under couplings during t_2, so no multiplet structure is found in f_2. $4I_{Ay}I_{My}I_{Xx}$ is converted into $4I_{Az}I_{Mz}I_{Xx}$ by the final pulse, and thus double antiphase structure is found in f_3.

Product operator analysis can also be used to predict the phase properties of the different types of crosspeak. No one set of phase corrections can be applied to a spectrum to obtain all the crosspeaks in pure absorption mode. If corrections are applied so that the true three-dimensional crosspeaks ($f_3 \neq f_2 \neq f_1$) are in pure absorption, then the back transfer peaks ($f_1 = f_3 \neq f_2$) will also be in pure absorption. This is not surprising, since both types of crosspeak arise from the same coherence transfer process. Note that the true three-dimensional crosspeaks are doubly antiphase in f_2. A further result of the analysis of phase is that the diagonal peaks ($f_1 = f_2 = f_3$) have the same phase properties as the three-spin crosspeaks.

In conclusion, we have shown that although the hard three-dimensional COSY-COSY experiment produces a complex spectrum, it can be interpreted. It also demonstrates that three-dimensional spectroscopy is not always a straightforward extension of the corresponding two-dimensional experiments.