NEW METHODS OF BROADBAND DECOUPLING IN HIGH RESOLUTION NMR

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INTRODUCTION

Since the advent of Fourier transform methods in high resolution NMR, the organic chemists and, to a lesser extent, the biochemists, have exploited the immense promise of carbon-13 spectroscopy. But to do this effectively they needed a reliable method of broadband proton decoupling, for this makes the carbon-13 spectra very much simpler to interpret and it increases the sensitivity by gathering multiplet intensity into a single line and by introducing a significant nuclear Overhauser enhancement.

For many years the technique of pseudo-random phase modulation, or noise decoupling, was the method of choice (1) and worked remarkably well considering that the background theory was somewhat doubtful, being based on an analogy with decoupling through random chemical exchange events. But as spectrometers were built at higher applied magnetic fields B_0, the range of proton chemical shifts to be covered was extended, and extended again, and the only option seemed to be to turn up the radiofrequency decoupler level B_2 in proportion to the bandwidth to be covered. The crisis in our own laboratory came when an extremely precious sample consisting of the world supply of a particular snake venom neurotoxin was inadvertently cooked by our noise decoupler during an overnight run.

Alternative modulation schemes began to emerge, for example square-wave phase modulation (2), chirp frequency modulation (3) and some mysterious proprietory concoctions that were never described in the literature at all although they seemed to offer some small advantage in bandwidth for a given decoupler level, B_2. It was becoming quite clear that a general problem existed and that it would only get worse as higher-field spectrometers came into use.

PULSED DECOUPLING

It began to look as if a completely new approach was needed. Consider the following experiment. Two carbon-13 resonances, represented by two vectors C(+) and C(-), corresponding to two possible proton spin states, are allowed to evolve during a short time interval τ. If they start in phase at time 0 aligned along the Y axis of the rotating frame, then at time τ they have precession angles

$$2\pi(\delta_C \pm 0.5\ J_{CH})\tau$$

If at this time a proton spin inversion pulse is applied then the identities of

the two vectors are interchanged and precession occurs at frequencies

$$2\pi(\delta_C \mp 0.5\ J_{CH}).$$

Consequently at time 2τ the two precessions come back into phase at a total angle $(4\pi\delta_C\tau)$ radians, which contains no term representing the CH coupling. The proton inversion pulses may be repeated, giving a phase evolution diagram in which there is a periodic realignment of the vectors. If sampling of the carbon-13 free induction decay is restricted to these points in time, then the result is just the same as if the carbon-13 magnetization had precessed at the chemical shift frequency δ_C without any effects of coupling to protons. This suggests that average Hamiltonian theory (coherent averaging theory) could be usefully applied to the analysis of the problem.

Of course this is not a very practicable scheme for carbon-13 spectroscopy because the carbon sampling rate must be equal to the rate of application of proton pulses, and the latter is strictly limited by considerations of power dissipation and the radiofrequency voltage limits of the probe and decoupler circuitry. As the proton pulse repetition rate is increased, it is no longer permissible to neglect the width of the radiofrequency pulse in comparison with the interval between pulses, so the spin evolution picture becomes rather complicated, involving rotation of proton vectors about tilted effective fields in the rotating frame.

THE MLEV SEQUENCES

But this impractical experiment did constitute a new approach and it was very fortunate that at this time Levitt (3) invented the concept of a composite inversion pulse — a sandwich of three pulses [90^0(+X) 180^0(+Y) 90^0(+X)] which compensates for the influence of resonance offset effects over an appreciable bandwidth. This allows us to operate with appreciable radiofrequency pulsewidths and yet still have good spin inversion for off-resonance protons. Indeed we may even put the inversion pulses in contact, with no periods of free precession between them, so that the protons are continually inverted and reinverted almost like the "stirring" motion in the imagery introduced by Abragam (4). Naturally we must expect that cumulative errors will creep into the problem since there is an appreciable spatial inhomogeneity of the decoupler field B_2. The simple way to prevent this is to reverse the phase of the B_2 field periodically, but this must not be done after every inversion pulse but only after two pulses of the same phase. If R is a composite spin inversion pulse and $\bar{R}$ is its phase-inverted counterpart, then this gives the first "magic cycle" $RR\bar{R}\bar{R}$, the so-called MLEV-4 sequence (5). This method of broadband decoupling shows significant improvements in proton bandwidth coverage compared with noise decoupling performed with the same radiofrequency level B_2.

If we thought that the matter ended there we were sadly mistaken; indeed the exploration of broadband decoupling was taking on many of the attributes of a saga. Guided by ideas being used in

multipulse experiments in solid-state NMR, Frenkiel (6) experimentally and Levitt (6) theoretically showed that the residual imperfections of the MLEV-4 cycle could be largely compensated in a supercycle called MLEV-16. This was constructed by joining together MLEV-4 with a cyclically permuted form of MLEV-4, then repeating them both in a phase-inverted form. The manner in which the imperfections cancelled was predictable by average Hamiltonian theory (7) although in a rather cumbersome way.

The expansion to higher-order supercycles such as MLEV-64 and beyond was straightforward although the bandwidth improvements showed diminishing returns. These sequences worked extremely well for broadband decoupling provided that the carbon-13 resonances were not scrutinized under the very highest resolution conditions, for then small residual splittings of the order of 0.1 Hz could be detected under certain conditions. It turned out that the phase shifts required to implement the $90^0(X)$ $180^0(Y)$ $90^0(X)$ composite pulse sequence had to be extremely finely adjusted, and the optimum setting did not quite correspond to exact orthogonality of the phases. Although the effects were too small to inconvenience the organic chemist doing routine carbon-13 spectroscopy, they could nevertheless be troublesome for particular applications where a narrow window in the carbon-13 spectrum was examined with high digital and spectroscopic resolution.

THE WAUGH THEORY

At this point a seminal contribution to the understanding of these experiments was made by Waugh (8) who introduced an exact theory to replace the power series approximations essential to average Hamiltonian theory. Waugh pointed out that the treatment meted out to the protons in these experiments was simply a sequence of pure rotations, and that the resultant at the end of a cycle or supercycle must necessarily be another pure rotation. In fact two proton resonances must be considered, H(+) and H(-), corresponding to the two possible carbon-13 spin states. These are the carbon-13 satellites observed in the proton spectrum at 1% abundance. Waugh was able to show quite simply that there is a residual splitting of the carbon-13 resonance related to the difference between the residual rotation angles β(+) and β(-) of the two proton satellites H(+) and H(-). If, at the end of a cycle, H(+) and H(-) had evolved in such a way that it appeared that they were influenced identically by the decoupler, then the residual splitting of the carbon-13 resonance was zero; indeed it behaved as if it were not coupled to the protons, just as they behaved as if they were not coupled to carbon-13.

In practice good decoupling is more easily assured by making both β(+) and β(-) small, the condition that the decoupling sequence is a good cycle. The beauty of this exact treatment is that it allows the decoupling performance to be simulated on a computer for all the possible instrumental imperfections. It was soon confirmed in this way that the MLEV sequences are indeed extremely sensitive to the exact radiofrequency phase shifts, as are all sequences that employ four nominally orthogonal phases.

Waugh [8,9] also highlighted the importance of calculating the axes n(+) and n(-) about which the residual rotations occurred, since these determine the procedure required to compensate residual imperfections. He suggested that if a starting sequence had n(+) and n(-) aligned near to the Z axis of the rotating frame, then cyclic permutation of a 90^0 pulse element from one end of the sequence to the other would have the effect of rotating n(+) and n(-) until they were both close to the XY plane. If the new cyclically permuted sequence is C, then the combination $C\bar{C}$ is a better supercycle because radiofrequency phase inversion reverses the XY coordinates of n(+) and n(-). This turns out to be a very good expansion procedure because the 90^0 pulse is self-compensating with respect to resonance offset effects over an appreciable range.

WALTZ-16 DECOUPLING

For these reasons, Shaka [10] set out to design a composite inversion pulse that needs only 0^0 and 180^0 radiofrequency phases. He exploited the concept of a "reversed precession pulse" [11], a hypothetical pulse that rotates the spins in the sense opposite to that defined by the sign of the magnetogyric ratio. At exact resonance this effect may be achieved simply by reversing the radiofrequency phase, but in the general case of off-resonance spins, reversed precession must be approximated by a composite pulse. Suppose we start with a broadband cycle

$$270^0(-X) \; 270^0(+X) \; \simeq \; 1$$

which may be factorized into

$$90^0(-X) \; 180^0(-X) \; 270^0(+X) \; \simeq \; 1$$

This is an exact cycle at resonance and also at offsets where $\Delta B = 0.88 \; B_2$, and is a good approximation in the intermediate region. Now a conventional pulse followed by its reversed precession counterpart must also constitute a cycle, hence

$$[90^0(-X)]^{-1} \; \simeq \; 180^0(-X) \; 270^0(+X)$$

where $[\quad]^{-1}$ represents reversed precession.

Since a simple $90^0(+X)$ pulse takes magnetization from the north pole and leaves it very close to the equator, the sequence $90^0(+X) \; [90^0(-X)]^{-1}$ must carry magnetization from the north to the south pole over a considerable range of resonance offsets. Shaka's composite inversion pulse was therefore

$$R \; = 90^0(+X) \; 180^0(-X) \; 270^0(+X)$$

which may be written $1\bar{2}3$ in a convenient shorthand notation. A broadband decoupling cycle would be

$$RRRR = \bar{1}\bar{2}3 \quad 1\bar{2}\bar{3} \quad \bar{1}2\bar{3} \quad \bar{1}\bar{2}3$$

which naturally enough came to be called WALTZ-4. Expansion involves the cyclic permutation of a $90^0(+X)$ pulse from the front to the back of this sequence, followed by the same sequence inverted in phase

$$KKKK = \bar{2}\bar{4}23\bar{1} \quad 24\bar{2}\bar{3}1 \quad \bar{2}\bar{4}23\bar{1} \quad 24\bar{2}\bar{3}1$$

This is known as WALTZ-8 and a similar expansion gives WALTZ-16:

$$QQQQ \quad \text{where } Q = \; 3\bar{4}2\bar{3}1\bar{2}4\bar{2}3$$

The efficiency of WALTZ-16 is quite insensitive to missetting the phase shift; an error of as much as 10^0 can

be tolerated without serious problems (12). It is easy to implement with present-day digital electronics and most high-resolution spectrometers may be readily converted to WALTZ-16 operation. Further expansion appears to be unnecessary and experimentally the higher supercycles do not seem to offer the expected improvement, probably because other small imperfections (for example channel imbalance) become important.

WALTZ-16 covers a proton bandwidth about double the strength of the decoupler field ($\gamma B_2/2\pi$). This is adequate for the majority of applications in carbon-13 spectroscopy at present-day magnetic field strengths, but for decoupling fluorine or phosphorus, an extended bandwidth would be desirable. This may be achieved by relaxing the artificial requirement that the pulses be integral multiples of 90^0. One of the most successful broadband sequences of this kind is GARP, which was designed by numerical optimization with the constraint that the sequence must simultaneously compensate for small variations in radiofrequency intensity as well as resonance offset effects (13).

CYCLING SIDEBANDS

During the decoupler cycle the carbon-13 free precession signal deviates slightly from the "ideal" value expected from the chemical shift alone. This is because the proton motions are not ideal and only return to the ideal condition at the end of each decoupler supercycle. If we use "stroboscopic" sampling, where carbon-13 is only observed at the end of each decoupler supercycle these imperfections are not detected.

However we are compelled to sample at a faster rate than this, and the spurious modulation of the carbon-13 free induction decay shows up as "cycling sidebands" in the decoupled spectra. They can be minimized in time-averaging applications by desynchronizing the decoupler pulsing from the carbon-13 sampling operation, but a small proportion of these sidebands have constant phase, and persist even after time-averaging (12). For WALTZ-16, these would normally be below 0.3% of the intensity of the main decoupled peak. All decoupling schemes produce these cycling sidebands, although procedures such as pseudo-random phase modulation may spread them into a large number of different frequency components contributing to the baseline "noise". A decoupling sequence can usually be written in several equivalent forms, depending on the details of the expansion procedure. Concatenation of these different forms usually delivers equivalent performance as a decoupler but can split the cycling sidebands into many weak components. In situations where it is useful to reduce the general level of cycling sidebands another order of magnitude, one procedure is cyclic permutation of one of the pulse elements each time the sequence is repeated (14). Normally this generates a sequence which has a period of the same order as the length of the carbon-13 free induction decay.

THREE-SPIN EFFECTS

For reasons of simplicity, the various theoretical formulations of broadband decoupling took as a model a two-spin system – one proton coupled to one carbon-13 nucleus. If two or three equivalent protons are attached to the

same carbon atom this causes no particular problem, but when there are two coupled protons with different chemical shifts then the decoupler performance is slightly impaired. This is because both protons experience the influence of the decoupler pulses, introducing bilinear terms into the propagator describing the evolution of the spin system. This "three-spin effect" introduces some new, very small splittings into the decoupled carbon-13 resonance, usually not resolved (15). The magnitude of these splittings depends on the proton-proton coupling J(HH), the proton-carbon coupling ^{1}J(CH), and the inverse of the strength of the decoupler field $\gamma B_2/2\pi$. The long-range coupling J(CCH) has no significant effect under these conditions. Since the decoupler field B_2 is normally inhomogeneous in space this is often the dominant broadening effect, so the spurious splittings are seldom resolved. The three-spin effect depends in a complex way on the two proton offset parameters. It disappears if both protons have the same resonance offset (equivalence) or if one of the protons lies outside the effective range of the decoupler. Under normal operating conditions, [$\gamma B_2/2\pi$ = 2 kHz, ^{1}J(CH) $\approx$ 200 Hz, J(HH) $\approx$ 5 Hz] the result is only a slight broadening of the decoupled carbon-13 resonance (a few tenths of 1 Hz) only apparent if very high resolution conditions are employed.

REFERENCES

[1] R.R. Ernst, J. Chem. Phys., 45, 3845 (1986).

[2] J.B. Grutzner and R.E. Santini, J. Magn. Reson., 19, 173 (1975).

[3] V.J. Basus, P.D. Ellis, H.D.W. Hill and J.S. Waugh, J. Chem. Phys., 35, 19 (1979).

[4] A. Abragam, "The Principles of Nuclear Magnetism" (Oxford University Press, 1961).

[5] M.H. Levitt and R. Freeman, J. Magn. Reson., 43, 502 (1981).

[6] M.H. Levitt, R. Freeman and T. Frenkiel, J. Magn. Reson., 47, 328 (1982)

[7] M.H. Levitt, R. Freeman and T. Frenkiel, Advances in Magnetic Resonance, 11, 47 (1983).

[8] J.S. Waugh, J. Magn. Reson., 50, 30 (1982).

[9] J.S. Waugh, J. Magn. Reson., 49, 517 (1982).

[10] A.J. Shaka, J. Keeler, T. Frenkiel and R. Freeman, J. Magn. Reson., 52, 335 (1983).

[11] M.H. Levitt, private communication.

[12] A.J. Shaka, J. Keeler and R. Freeman, J. Magn. Reson., 53, 313 (1983).

[13] A.J. Shaka, P.B. Barker and R. Freeman, J. Magn. Reson., 64, 547 (1985).

[14] A.J. Shaka, P.B. Barker, C.J. Bauer and R. Freeman, J. Magn. Reson., 67, 396 (1986).

[15] P.B. Barker, A.J. Shaka and R. Freeman, J. Magn. Reson., 65, 535 (1985).