9 Coherence Selection: Phase Cycling and Gradient Pulses[†]

9.1 Introduction

The pulse sequence used in an NMR experiment is carefully designed to produce a particular outcome. For example, we may wish to pass the spins through a state of multiple quantum coherence at a particular point, or plan for the magnetization to be aligned along the z-axis during a mixing period. However, it is usually the case that the particular series of events we designed the pulse sequence to cause is only one out of many possibilities. For example, a pulse whose role is to generate double-quantum coherence from anti-phase magnetization may also generate zero-quantum coherence or transfer the magnetization to another spin. Each time a radiofrequency pulse is applied there is this possibility of branching into many different pathways. If no steps are taken to suppress these unwanted pathways the resulting spectrum will be hopelessly confused and uninterpretable.

There are two general ways in which one pathway can be isolated from the many possible. The first is *phase cycling*. In this method the phases of the pulses and the receiver are varied in a systematic way so that the signal from the desired pathways adds and signal from all other pathways cancels. Phase cycling requires that the experiment is repeated several times, something which is probably required in any case in order to achieve the required signal-to-noise ratio.

The second method of selection is to use *field gradient pulses*. These are short periods during which the applied magnetic field is made inhomogeneous. As a result, any coherences present dephase and are apparently lost. However, this dephasing can be undone, and the coherence restored, by application of a subsequent gradient. We shall see that this dephasing and rephasing approach can be used to select particular coherences. Unlike phase cycling, the use of field gradient pulses does not require repetition of the experiment.

Both of these selection methods can be described in a unified framework which classifies the coherences present at any particular point according to a *coherence order* and then uses *coherence transfer pathways* to specify the desired outcome of the experiment.

9.2 Phase in NMR

In NMR we have control over both the phase of the pulses and the *receiver phase*. The effect of changing the phase of a pulse is easy to visualise in the usual rotating frame. So, for example, a 90° pulse about the x-axis rotates magnetization from z onto $-y$, whereas the same pulse applied about the y-axis rotates the magnetization onto the x-axis. The idea of the receiver phase is slightly more complex and will be explored in this section.

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The NMR signal – that is the free induction decay – which emerges from the probe is a radiofrequency signal oscillating at close to the Larmor frequency (usually hundreds of MHz). Within the spectrometer this signal is shifted down to a much lower frequency in order that it can be digitized and then stored in computer memory. The details of this down-shifting process can be quite complex, but the overall result is simply that a fixed frequency, called the *receiver reference* or *carrier*, is subtracted from the frequency of the incoming NMR signal. Frequently, this receiver reference frequency is the same as the transmitter frequency used to generate the pulses applied to the observed nucleus. We shall assume that this is the case from now on.

The rotating frame which we use to visualise the effect of pulses is set at the transmitter frequency, ω_{rf}, so that the field due to the radiofrequency pulse is static. In this frame, a spin whose Larmor frequency is ω_0 precesses at $(\omega_0 - \omega_{\text{rf}})$, called the offset Ω. In the spectrometer the incoming signal at ω_0 is down-shifted by subtracting the receiver reference which, as we have already decided, will be equal to the frequency of the radiofrequency pulses. So, in effect, the frequencies of the signals which are digitized in the spectrometer are the offset frequencies at which the spins evolve in the rotating frame. Often this whole process is summarised by saying that the "signal is detected in the rotating frame".

9.1.1 Detector phase

The quantity which is actually detected in an NMR experiment is the transverse magnetization. Ultimately, this appears at the probe as an oscillating voltage, which we will write as

$$S_{\text{FID}} = \cos \omega_0 t$$

where ω_0 is the Larmor frequency. The down-shifting process in the spectrometer is achieved by an electronic device called a *mixer*; this effectively multiplies the incoming signal by a locally generated reference signal, S_{ref}, which we will assume is oscillating at ω_{rf}

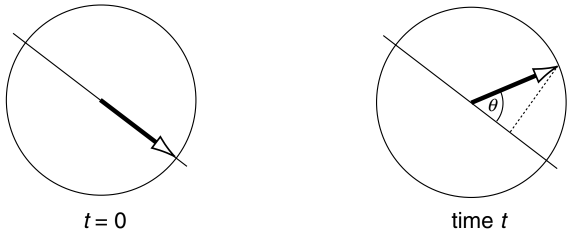
$$S_{\text{ref}} = \cos \omega_{\text{rf}} t$$

The output of the mixer is the product $S_{\text{FID}} S_{\text{ref}}$

$$\begin{aligned} S_{\text{FID}} S_{\text{ref}} &= A \cos \omega_{\text{rf}} t \cos \omega_0 t \\ &= \tfrac{1}{2} A \left[\cos(\omega_{\text{rf}} + \omega_0) t + \cos(\omega_{\text{rf}} - \omega_0) t \right] \end{aligned}$$

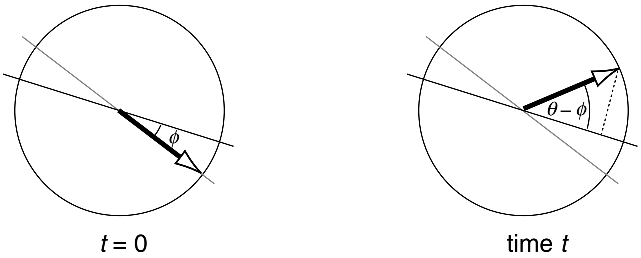
The first term is an oscillation at a high frequency (of the order of twice the Larmor frequency as $\omega_0 \approx \omega_{\text{rf}}$) and is easily removed by a low-pass filter. The second term is an oscillation at the offset frequency, Ω. This is in line with the previous comment that this down-shifting process is equivalent to detecting the precession in the rotating frame.

We can go further with this interpretation and say that the second term represents the component of the magnetization along a particular axis (called the *reference axis*) in the rotating frame. Such a component varies as $\cos \Omega t$, assuming that at time zero the magnetization is aligned along the chosen axis; this is illustrated below



At time zero the magnetization is assumed to be aligned along the reference axis. After time t the magnetization has precessed through an angle $\theta = \Omega t$. The projection of the magnetization onto the reference axis is proportional to $\cos \Omega t$.

Suppose now that the phase of the reference signal is shifted by ϕ, something which is easily achieved in the spectrometer. Effectively, this shifts the reference axis by ϕ, as shown below



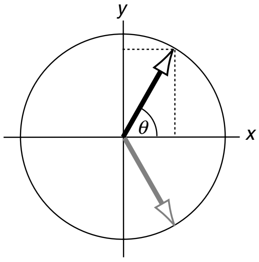
Shifting the phase of the receiver reference by ϕ is equivalent to detecting the component along an axis rotated by ϕ from its original position (the previous axis is shown in grey). Now the apparent angle of precession is $\theta = \Omega t - \phi$. and the projection of the magnetization onto the reference axis is proportional to $\cos(\Omega t - \phi)$.

The component along the new reference axis is proportional to $\cos(\Omega t - \phi)$. How this is put to good effect is described in the next section.

9.1.2 Quadrature detection

We normally want to place the transmitter frequency in the centre of the resonances of interest so as to minimise off-resonance effects. If the receiver reference frequency is the same as the transmitter frequency, it immediately follows that the offset frequencies, Ω, may be both positive and negative. However, as we have seen in the previous section the effect of the down-shifting scheme used is to generate a signal of the form $\cos \Omega t$. Since $\cos(\theta) = \cos(-\theta)$ such a signal does not discriminate between positive and negative offset frequencies. The resulting spectrum, obtained by Fourier transformation, would be confusing as each peak would appear at both $+\Omega$ and $-\Omega$.

The way out of this problem is to detect the signal along two *perpendicular* axes. As is illustrated opposite, the projection along one axis is proportional to $\cos(\Omega t)$ and to $\sin(\Omega t)$ along the other. Knowledge of both of these projections enables us to work out the sense of rotation of the vector *i.e.* the sign of the offset.



The x and y projections of the black vector are both positive. If the vector had precessed in the opposite direction (shown shaded), and at the same frequency, the projection along x would be the same, but along y it would be minus that of the black vector.

The sin modulated component is detected by having a second mixer fed with a reference whose phase is shifted by $\pi/2$. Following the above discussion the output of the mixer is

$$\cos(\Omega t - \pi/2) = \cos\Omega t \cos\pi/2 + \sin\Omega t \sin\pi/2$$
$$= \sin\Omega t$$

The output of these two mixers can be regarded as being the components of the magnetization along perpendicular axes in the rotating frame.

The final step in this whole process is regard the outputs of the two mixers as being the real and imaginary parts of a complex number:

$$\cos\Omega t + \mathrm{i}\sin\Omega t = \exp(\mathrm{i}\Omega t)$$

The overall result is the generation of a complex signal whose phase varies according to the offset frequency Ω.

9.1.3 Control of phase

In the previous section we supposed that the signal coming from the probe was of the form $\cos\omega_0 t$ but it is more realistic to write the signal as $\cos(\omega_0 t + \phi_{\mathrm{sig}})$ in recognition of the fact that in addition to a frequency the signal has a phase, ϕ_{sig}. This phase is a combination of factors that are not under our control (such as phase shifts produced in the amplifiers and filters through which the signal passes) and a phase due to the pulse sequence, which certainly is under our control.

The overall result of this phase is simply to multiply the final complex signal by a phase factor, $\exp(\mathrm{i}\phi_{\mathrm{sig}})$:

$$\exp(\mathrm{i}\Omega t)\exp(\mathrm{i}\phi_{\mathrm{sig}})$$

As we saw in the previous section, we can also introduce another phase shift by altering the phase of the reference signal fed to the mixer, and indeed we saw that the cosine and sine modulated signals are generated by using two mixers fed with reference signals which differ in phase by $\pi/2$. If the phase of each of these reference signals is advanced by ϕ_{rx}, usually called the receiver phase, the output of the two mixers becomes $\cos(\Omega t - \phi_{\mathrm{rx}})$ and $\sin(\Omega t - \phi_{\mathrm{rx}})$. In the complex notation, the overall signal thus acquires another phase factor

$$\exp(\mathrm{i}\Omega t)\exp(\mathrm{i}\phi_{\mathrm{sig}})\exp(-\mathrm{i}\phi_{\mathrm{rx}})$$

Overall, then, the phase of the final signal depends on the *difference* between the phase introduced by the pulse sequence and the phase introduced by the receiver reference.

9.1.4 Lineshapes

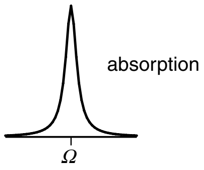
Let us suppose that the signal can be written

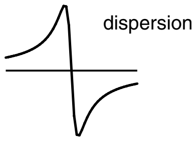
$$S(t) = B\exp(\mathrm{i}\Omega t)\exp(\mathrm{i}\Phi)\exp(-t/T_2)$$

where Φ is the overall phase ($= \phi_{\mathrm{sig}} - \phi_{\mathrm{rx}}$) and B is the amplitude. The term, $\exp(-t/T_2)$ has been added to impose a decay on the signal. Fourier transformation of $S(t)$ gives the spectrum $S(\omega)$:

$$S(\omega) = B[A(\omega) + \mathrm{i}D(\omega)]\exp(\mathrm{i}\Phi) \qquad [1]$$

where $A(\omega)$ is an absorption mode lorentzian lineshape centred at $\omega = \Omega$ and $D(\omega)$ is the corresponding dispersion mode lorentzian:





$$A(\omega) = \frac{T_2}{1 + (\omega - \Omega)^2 T_2^2} \qquad D(\omega) = \frac{(\omega - \Omega)T_2^2}{1 + (\omega - \Omega)^2 T_2^2}$$

Normally we display just the real part of $S(\omega)$ which is, in this case,

$$\mathrm{Re}[S(\omega)] = B[\cos\Phi\ A(\omega) - \sin\Phi\ D(\omega)]$$

In general this is a mixture of the absorption and dispersion lineshape. If we want just the absorption lineshape we need to somehow set Φ to zero, which is easily done by multiplying $S(\omega)$ by a phase factor $\exp(\mathrm{i}\Theta)$.

$$\begin{aligned} S(\omega)\exp(\mathrm{i}\Theta) &= B[A(\omega) + \mathrm{i}D(\omega)]\exp(\mathrm{i}\Phi)\exp(\mathrm{i}\Theta) \\ &= B[A(\omega) + \mathrm{i}D(\omega)]\exp(\mathrm{i}[\Phi + \Theta]) \end{aligned}$$

As this is a numerical operation which can be carried out in the computer we are free to choose Θ to be the required value (here $-\Phi$) in order to remove the phase factor entirely and hence give an absorption mode spectrum in the real part. This is what we do when we "phase the spectrum".

9.1.5 Relative phase and lineshape

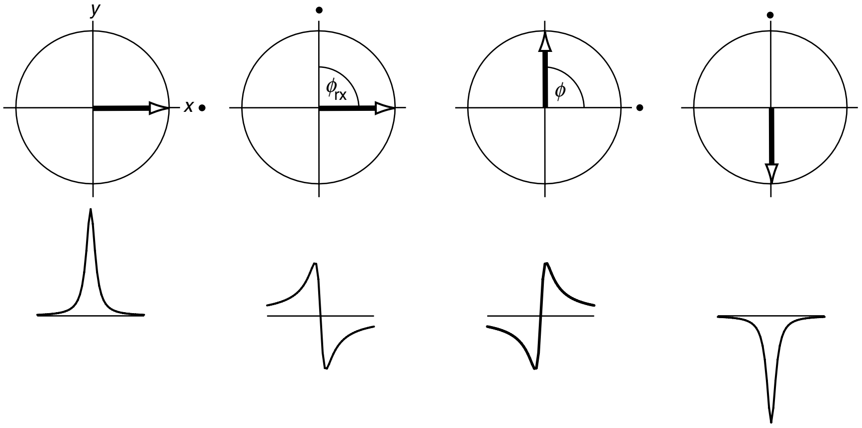
We have seen that we can alter the phase of the spectrum by altering the phase of the pulse or of the receiver, but that what really counts is the difference in these two phases.

We will illustrate this with the simple vector diagrams shown below. Here, the vector shows the position of the magnetization at time zero and its phase, ϕ_{sig}, is measured anti-clockwise from the x-axis. The dot shows the axis along which the receiver is aligned; this phase, ϕ_{rx}, is also measured anti-clockwise from the x-axis.

If the vector and receiver are aligned along the same axis, $\Phi = 0$, and the real part of the spectrum shows the absorption mode lineshape. If the receiver phase is advanced by $\pi/2$, $\Phi = 0 - \pi/2$ and, from Eq. [1]

$$\begin{aligned} S(\omega) &= B[A(\omega) + \mathrm{i}D(\omega)]\exp(-\mathrm{i}\pi/2) \\ &= B[-\mathrm{i}A(\omega) + D(\omega)] \end{aligned}$$

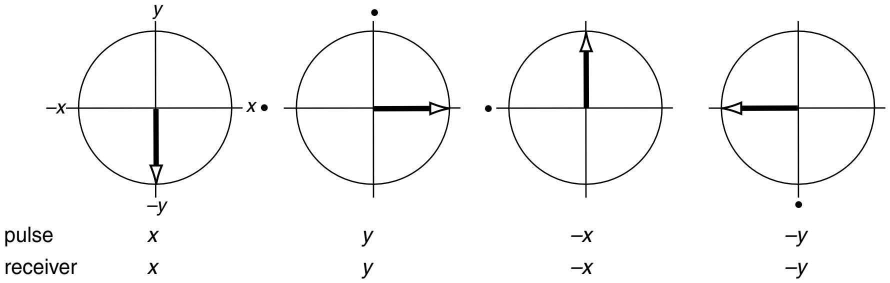
This means that the real part of the spectrum shows a dispersion lineshape. On the other hand, if the magnetization is advanced by $\pi/2$, $\Phi = \phi_{\mathrm{sig}} - \phi_{\mathrm{rx}} = \pi/2 - 0 = \pi/2$ and it can be shown from Eq. [1] that the real part of the spectrum shows a negative dispersion lineshape. Finally, if either phase is advanced by π, the result is a negative absorption lineshape.



9.1.6 CYCLOPS

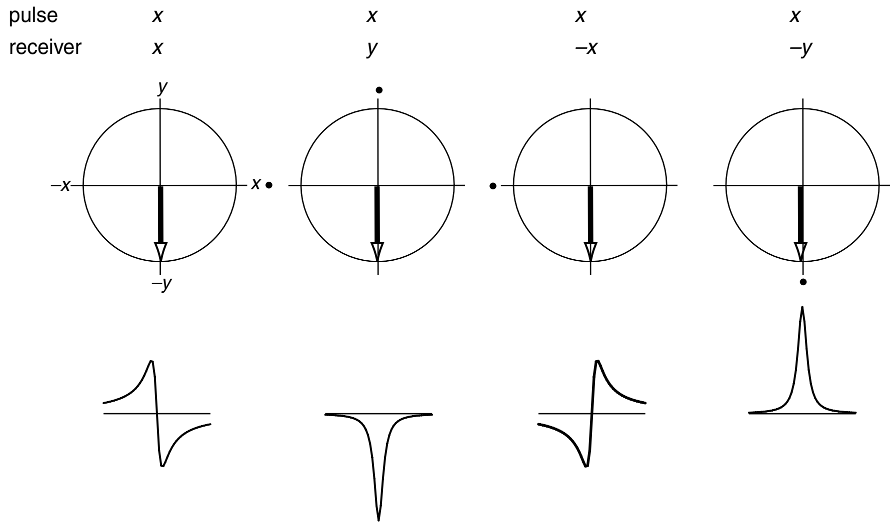
The CYCLOPS phase cycling scheme is commonly used in even the simplest pulse-acquire experiments. The sequence is designed to cancel some imperfections associated with errors in the two phase detectors mentioned above; a description of how this is achieved is beyond the scope of this discussion. However, the cycle itself illustrates very well the points made in the previous section.

There are four steps in the cycle, the pulse phase goes *x*, *y*, –*x*, –*y* *i.e.* it advances by 90° on each step; likewise the receiver advances by 90° on each step. The figure below shows how the magnetization and receiver phases are related for the four steps of this cycle



Although both the receiver and the magnetization shift phase on each step, the phase difference between them remains constant. Each step in the cycle thus gives the same lineshape and so the signal adds on all four steps, which is just what is required.

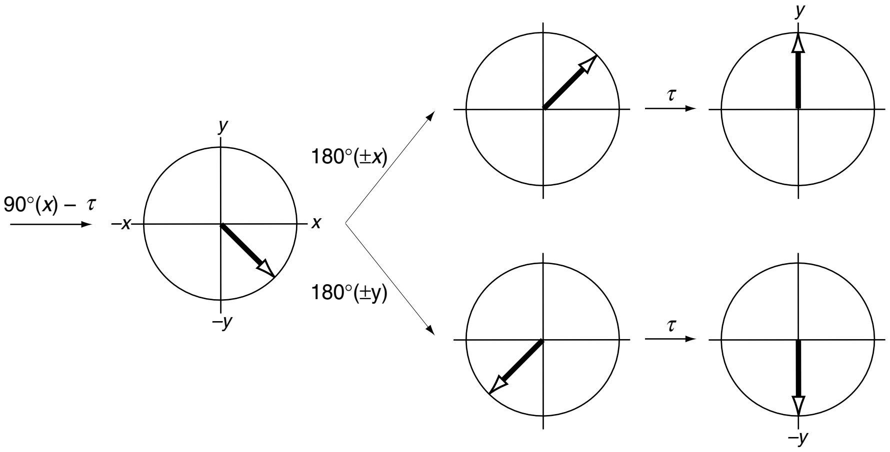
Suppose that we forget to advance the pulse phase; the outcome is quite different



Now the phase difference between the receiver and the magnetization is no longer constant. A different lineshape thus results from each step and it is clear that adding all four together will lead to complete cancellation (steps 2 and 4 cancel, as do steps 1 and 3). For the signal to add up it is clearly essential for the receiver to follow the magnetization.

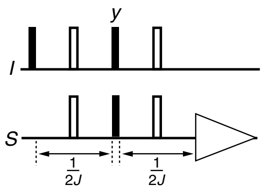
9.1.7 EXORCYLE

EXORCYLE is perhaps the original phase cycle. It is a cycle used for 180° pulses when they form part of a spin echo sequence. The 180° pulse cycles through the phases *x*, *y*, –*x*, –*y* and the receiver phase goes *x*, –*x*, *x*, –*x*. The diagram below illustrates the outcome of this sequence



If the phase of the 180° pulse is +*x* or –*x* the echo forms along the *y*-axis, whereas if the phase is ±*y* the echo forms on the –*y* axis. Therefore, as the 180° pulse is advanced by 90° (*e.g.* from *x* to *y*) the receiver must be advanced by 180° (*e.g.* from *x* to –*x*). Of course, we could just as well cycle the receiver phases *y*, –*y*, *y*, –*y*; all that matters is that they advance in steps of 180°. We will see later on how it is that this phase cycle cancels out the results of imperfections in the 180° pulse.

9.1.8 Difference spectroscopy



Pulse sequence for INEPT. Filled rectangles represent 90° pulses and open rectangles represent 180° pulses. Unless otherwise indicated, all pulses are of phase *x*.

Often a simple two step sequence suffices to cancel unwanted magnetization; essentially this is a form of difference spectroscopy. The idea is well illustrated by the INEPT sequence, shown opposite. The aim of the sequence is to transfer magnetization from spin *I* to a coupled spin *S*.

With the phases and delays shown equilibrium magnetization of spin *I*, I_z, is transferred to spin *S*, appearing as the operator S_x. Equilibrium magnetization of *S*, S_z, appears as S_y. We wish to preserve only the signal that has been transferred from *I*.

The procedure to achieve this is very simple. If we change the phase of the second *I* spin 90° pulse from *y* to –*y* the magnetization arising from transfer of the *I* spin magnetization to *S* becomes $-S_x$ *i.e.* it changes sign. In contrast, the signal arising from equilibrium *S* spin magnetization is unaffected simply because the S_z operator is unaffected by the *I* spin pulses. By repeating the experiment twice, once with the phase of the second *I* spin 90° pulse set to *y* and once with it set to –*y*, and then subtracting the two resulting signals, the undesired signal is cancelled and the desired signal adds. It is easily confirmed that shifting the phase of the *S* spin 90° pulse does not achieve the desired separation of the two signals as both are affected in the same way.

In practice the subtraction would be carried out by shifting the receiver by 180°, so the I spin pulse would go *y*, –*y* and the receiver phase go *x*, –*x*. This is a two step phase cycle which is probably best viewed as difference spectroscopy.

This simple two step cycle is the basic element used in constructing the phase cycling of many two- and three-dimensional heteronuclear experiments.

9.2 Coherence transfer pathways

Although we can make some progress in writing simple phase cycles by considering the vector picture, a more general framework is needed in order to cope with experiments which involve multiple-quantum coherence and related phenomena. We also need a theory which enables us to predict the degree to which a phase cycle can discriminate against different classes of unwanted signals. A convenient and powerful way of doing both these things is to use the coherence transfer pathway approach.

9.2.1 Coherence order

Coherences, of which transverse magnetization is one example, can be classified according to a coherence order, *p*, which is an integer taking values 0, ± 1, ± 2 ... Single quantum coherence has $p = \pm 1$, double has $p = \pm 2$ and so on; *z*-magnetization, "*zz*" terms and zero-quantum coherence have $p = 0$. This classification comes about by considering the phase which different coherences acquire is response to a rotation about the *z*-axis.

A coherence of order *p*, represented by the density operator $\sigma^{(p)}$, evolves under a *z*-rotation of angle ϕ according to

$$\exp(-\mathrm{i}\phi F_z)\sigma^{(p)}\exp(\mathrm{i}\phi F_z) = \exp(-\mathrm{i}\,p\phi)\sigma^{(p)} \qquad [2]$$

where F_z is the operator for the total z-component of the spin angular momentum. In words, a coherence of order p experiences a phase shift of $-p\phi$. Equation [2] is the definition of coherence order.

To see how this definition can be applied, consider the effect of a z-rotation on transverse magnetization aligned along the x-axis. Such a rotation is identical in nature to that due to evolution under an offset, and using product operators it can be written

$$\exp(-\mathrm{i}\phi I_z)I_x\exp(\mathrm{i}\phi I_z) = \cos\phi\, I_x + \sin\phi\, I_y \qquad [3]$$

The right hand sides of Eqs. [2] and [3] are not immediately comparable, but by writing the sine and cosine terms as complex exponentials the comparison becomes clearer. Using

$$\cos\phi = \tfrac{1}{2}\left[\exp(\mathrm{i}\phi) + \exp(-\mathrm{i}\phi)\right] \quad \sin\phi = \tfrac{1}{2\mathrm{i}}\left[\exp(\mathrm{i}\phi) - \exp(-\mathrm{i}\phi)\right]$$

Eq. [3] becomes

$$\begin{aligned}&\exp(-\mathrm{i}\phi I_z)I_x\exp(\mathrm{i}\phi I_z)\\&= \tfrac{1}{2}\left[\exp(\mathrm{i}\phi) + \exp(-\mathrm{i}\phi)\right] I_x + \tfrac{1}{2\mathrm{i}}\left[\exp(\mathrm{i}\phi) - \exp(-\mathrm{i}\phi)\right] I_y\\&= \tfrac{1}{2}\left[I_x + \tfrac{1}{\mathrm{i}}I_y\right]\exp(\mathrm{i}\phi) + \tfrac{1}{2}\left[I_x - \tfrac{1}{\mathrm{i}}I_y\right]\exp(-\mathrm{i}\phi)\end{aligned}$$

It is now clear that the first term corresponds to coherence order –1 and the second to +1; in other words, I_x is an equal mixture of coherence orders ±1.

The cartesian product operators do not correspond to a single coherence order so it is more convenient to rewrite them in terms of the raising and lowering operators, I_+ and I_-, defined as

$$I_+ = I_x + \mathrm{i}I_y \quad I_- = I_x - \mathrm{i}I_y$$

from which it follows that

$$I_x = \tfrac{1}{2}\left[I_+ + I_-\right] \quad I_\mathrm{y} = \tfrac{1}{2\mathrm{i}}\left[I_+ - I_-\right] \qquad [4]$$

Under z-rotations the raising and lowering operators transform simply

$$\exp(-\mathrm{i}\phi I_z)I_\pm\exp(\mathrm{i}\phi I_z) = \exp(\mp\mathrm{i}\phi)I_\pm$$

which, by comparison with Eq. [2] shows that I_+ corresponds to coherence order +1 and I_- to –1. So, from Eq. [4] we can see that I_x and I_y correspond to mixtures of coherence orders +1 and –1.

As a second example consider the pure double quantum operator for two coupled spins,

$$2I_{1x}I_{2y} + 2I_{1y}I_{2x}$$

Rewriting this in terms of the raising and lowering operators gives

$$\tfrac{1}{\mathrm{i}}\left(I_1^+I_2^+ - I_1^-I_2^-\right)$$

The effect of a z-rotation on the term $I_1^+I_2^+$ is found as follows:

$$\begin{aligned}&\exp(-\mathrm{i}\phi I_{1z})\exp(-\mathrm{i}\phi I_{2z})I_{1+}I_{2+}\exp(\mathrm{i}\phi I_{2z})\exp(\mathrm{i}\phi I_{1z})\\&= \exp(-\mathrm{i}\phi I_{1z})\exp(-\mathrm{i}\phi)I_{1+}I_{2+}\exp(\mathrm{i}\phi I_{1z})\\&= \exp(-\mathrm{i}\phi)\exp(-\mathrm{i}\phi)I_{1+}I_{2+} = \exp(-2\mathrm{i}\phi)I_{1+}I_{2+}\end{aligned}$$

Thus, as the coherence experiences a phase shift of -2ϕ the coherence is classified according to Eq. [2] as having $p = 2$. It is easy to confirm that the term $I_{1-}I_{2-}$ has $p = -2$. Thus the pure double quantum term, $2I_{1x}I_{2y} + 2I_{1y}I_{2x}$, is an equal mixture of coherence orders +2 and –2.

As this example indicates, it is possible to determine the order or orders of any state by writing it in terms of raising and lowering operators and then simply inspecting the number of such operators in each term. A raising operator contributes +1 to the coherence order whereas a lowering operator contributes –1. A *z*-operator, I_{iz}, has coherence order 0 as it is invariant to *z*-rotations.

Coherences involving heteronuclei can be assigned both an overall order and an order with respect to each nuclear species. For example the term $I_{1+}S_{1-}$ has an overall order of 0, is order +1 for the *I* spins and –1 for the *S* spins. The term $I_{1+}I_{2+}S_{1z}$ is overall of order 2, is order 2 for the *I* spins and is order 0 for the *S* spins.

9.2.2 Evolution under offsets

The evolution under an offset, Ω, is simply a *z*-rotation, so the raising and lowering operators simply acquire a phase Ωt

$$\exp(-\mathrm{i}\Omega t I_z) I_{\pm} \exp(\mathrm{i}\Omega t I_z) = \exp(\mp \mathrm{i}\Omega t) I_{\pm}$$

For products of these operators, the overall phase is the sum of the phases acquired by each term

$$\begin{aligned}&\exp\left(-\mathrm{i}\Omega_j t I_{jz}\right)\exp\left(-\mathrm{i}\Omega_i t I_{iz}\right) I_{i-} I_{j+} \exp\left(\mathrm{i}\Omega_i t I_{iz}\right)\exp\left(\mathrm{i}\Omega_j t I_{jz}\right) \\ &= \exp\left(\mathrm{i}\left(\Omega_i - \Omega_j\right)t\right) I_{i-} I_{j+}\end{aligned}$$

It also follows that coherences of opposite sign acquire phases of opposite signs under free evolution. So the operator $I_{1+}I_{2+}$ (with $p = 2$) acquires a phase $-(\Omega_1 + \Omega_2)t$ *i.e.* it evolves at a frequency $-(\Omega_1 + \Omega_2)$ whereas the operator $I_{1-}I_{2-}$ (with $p = -2$) acquires a phase $(\Omega_1 + \Omega_2)t$ *i.e.* it evolves at a frequency $(\Omega_1 + \Omega_2)$. We will see later on that this observation has important consequences for the lineshapes in two-dimensional NMR.

The observation that coherences of different orders respond differently to evolution under a *z*-rotation (*e.g.* an offset) lies at the heart of the way in which gradient pulses can be used to separate different coherence orders.

9.2.3 Phase shifted pulses

In general, a radiofrequency pulse causes coherences to be transferred from one order to one or more different orders; it is this spreading out of the coherence which makes it necessary to select one transfer among many possibilities. An example of this spreading between coherence orders is the effect of a non-selective pulse on antiphase magnetization, such as $2I_{1x}I_{2z}$, which corresponds to coherence orders ±1. Some of the coherence may be transferred into double- and zero-quantum coherence, some may be transferred into two-spin order and some will remain unaffected. The precise outcome depends on the phase and flip angle of the pulse, but in general we can see that there are many possibilities.

If we consider just one coherence, of order p, being transferred to a coherence of order p' by a radiofrequency pulse we can derive a very general result for the way in which the phase of the pulse affects the phase of the coherence. It is on this relationship that the phase cycling method is based.

We will write the initial state of order p as $\sigma^{(p)}$, and the final state of order p' as $\sigma^{(p')}$. The effect of the radiofrequency pulse causing the transfer is represented by the (unitary) transformation U_ϕ where ϕ is the phase of the pulse. The initial and final states are related by the usual transformation

$$U_0\sigma^{(p)}U_0^{-1} = \sigma^{(p')} + \text{terms of other orders} \qquad [5]$$

which has been written for phase 0; the other terms will be dropped as we are only interested in the transfer from p to p'. The transformation brought about by a radiofrequency pulse phase shifted by ϕ, U_ϕ, is related to that with the phase set to zero, U_0, in the following way

$$U_\phi = \exp(-\mathrm{i}\phi F_z)U_0\exp(\mathrm{i}\phi F_z) \qquad [6]$$

Using this, the effect of the phase shifted pulse on the initial state $\sigma^{(p)}$ can be written

$$\begin{aligned}&U_\phi\sigma^{(p)}U_\phi^{-1}\\&= \exp(-\mathrm{i}\phi F_z)U_0\exp(\mathrm{i}\phi F_z)\sigma^{(p)}\exp(-\mathrm{i}\phi F_z)U_0^{-1}\exp(\mathrm{i}\phi F_z)\end{aligned} \qquad [7]$$

The central three terms can be simplified by application of Eq. [2]

$$\exp(\mathrm{i}\phi F_z)\sigma^{(p)}\exp(-\mathrm{i}\phi F_z)U_0^{-1} = \exp(\mathrm{i}p\phi)\sigma^{(p)}$$

giving

$$U_\phi\sigma^{(p)}U_\phi^{-1} = \exp(\mathrm{i}p\phi)\exp(-\mathrm{i}\phi F_z)U_0\sigma^{(p)}U_0^{-1}\exp(\mathrm{i}\phi F_z)$$

The central three terms can, from Eq. [5], be replaced by $\sigma^{(p')}$ to give

$$U_\phi\sigma^{(p)}U_\phi^{-1} = \exp(\mathrm{i}p\phi)\exp(-\mathrm{i}\phi F_z)\sigma^{(p')}\exp(\mathrm{i}\phi F_z)$$

Finally, Eq. [5] is applied again to give

$$U_\phi\sigma^{(p)}U_\phi^{-1} = \exp(\mathrm{i}p\phi)\exp(-\mathrm{i}p'\phi)\sigma^{(p')}$$

Defining $\Delta p = (p' - p)$ as the change is coherence order, this simplifies to

$$U_\phi\sigma^{(p)}U_\phi^{-1} = \exp(-\mathrm{i}\Delta p\phi)\sigma^{(p')} \qquad [8]$$

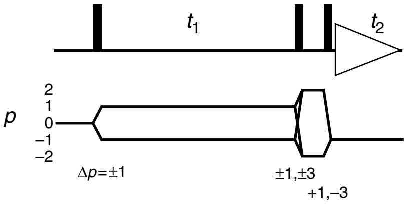
Equation [8] says that if the phase of a pulse which is causing a change in coherence order of Δp is shifted by ϕ the coherence will acquire a phase label $(-\Delta p\ \phi)$. It is this property which enables us to separate different changes in coherence order from one another by altering the phase of the pulse.

In the discussion so far it has been assumed that U_ϕ represents a single pulse. However, any sequence of pulses and delays can be represented by a single unitary transformation, so Eq. [8] applies equally well to the effect of phase shifting all of the pulses in such a sequence. We will see that this property is often of use in writing phase cycles.

If a series of phase shifted pulses (or pulse sandwiches) are applied a phase ($-\Delta p\ \phi$) is acquired from each. The total phase is found by adding up these individual contributions. In an NMR experiment this total phase affects the signal which is recorded at the end of the sequence, even though the phase shift may have been acquired earlier in the pulse sequence. These phase shifts are, so to speak, carried forward.

9.2.4 Coherence transfer pathways diagrams

In designing a multiple-pulse NMR experiment the intention is to have specific orders of coherence present at various points in the sequence. One way of indicating this is to use a *coherence transfer pathway* (CTP) diagram along with the timing diagram for the pulse sequence. An example of shown below, which gives the pulse sequence and CTP for the DQF COSY experiment.



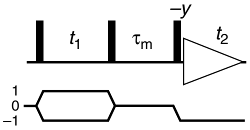
The solid lines under the sequence represent the coherence orders required during each part of the sequence; note that it is only the pulses which cause a change in the coherence order. In addition, the values of Δp are shown for each pulse. In this example, as is commonly the case, more than one order of coherence is present at a particular time. Each pulse is required to cause different changes to the coherence order – for example the second pulse is required to bring about no less than four values of Δp. Again, this is a common feature of pulse sequences.

It is important to realise that the CTP specified with the pulse sequence is just the *desired* pathway. We would need to establish separately (for example using a product operator calculation) that the pulse sequence is indeed capable of generating the coherences specified in the CTP. Also, the spin system which we apply the sequence to has to be capable of supporting the coherences. For example, if there are no couplings, then no double quantum will be generated and thus selection of the above pathway will result in a null spectrum.

The coherence transfer pathway must start with $p = 0$ as this is the order to which equilibrium magnetization (z-magnetization) belongs. In addition, the pathway has to end with $|p| = 1$ as it is only single quantum coherence that is observable. If we use quadrature detection (section 9.1.2) it turns out that only one of $p = \pm1$ is observable; we will follow the usual convention of assuming that $p = -1$ is the detectable signal.

9.3 Lineshapes and frequency discrimination

9.3.1 Phase and amplitude modulation



The selection of a particular CTP has important consequences for lineshapes and frequency discrimination in two-dimensional NMR. These topics are illustrated using the NOESY experiment as an example; the pulse sequence and CTP is illustrated opposite.

If we imagine starting with I_z, then at the end of t_1 the operators present are

$$-\cos \Omega t_1 I_y + \sin \Omega t_1 I_x$$

The term in I_y is rotated onto the z-axis and we will assume that only this term survives. Finally, the z-magnetization is made observable by the last pulse (for convenience set to phase $-y$) giving the observable term present at $t_2 = 0$ as

$$\cos \Omega t_1 I_x$$

As was noted in section 9.2.1, I_x is in fact a mixture of coherence orders $p = \pm 1$, something which is made evident by writing the operator in terms of I_+ and I_-

$$\tfrac{1}{2} \cos \Omega t_1 \left(I_+ + I_- \right)$$

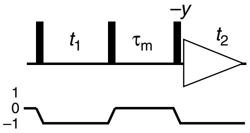
Of these operators, only I_- leads to an observable signal, as this corresponds to $p = -1$. Allowing I_- to evolve in t_2 gives

$$\tfrac{1}{2} \cos \Omega t_1 \exp\left(\mathrm{i} \Omega t_2 \right) I_-$$

The final detected signal can be written as

$$S_{\mathrm{C}}\left(t_1, t_2 \right) = \tfrac{1}{2} \cos \Omega t_1 \exp\left(\mathrm{i} \Omega t_2 \right)$$

This signal is said to be *amplitude modulated* in t_1; it is so called because the evolution during t_1 gives rise, via the cosine term, to a modulation of the amplitude of the observed signal.



The situation changes if we select a different pathway, as shown opposite. Here, only coherence order –1 is preserved during t_1. At the start of t_1 the operator present is $-I_y$ which can be written

$$-\tfrac{1}{2\mathrm{i}} \left(I_+ - I_- \right)$$

Now, in accordance with the CTP, we select only the I_- term. During t_1 this evolves to give

$$\tfrac{1}{2\mathrm{i}} \exp\left(\mathrm{i} \Omega t_1 \right) I_-$$

Following through the rest of the pulse sequence as before gives the following observable signal

$$S_{\mathrm{P}}\left(t_1, t_2 \right) = \tfrac{1}{4} \exp\left(\mathrm{i} \Omega t_1 \right) \exp\left(\mathrm{i} \Omega t_2 \right)$$

This signal is said to be *phase modulated* in t_1; it is so called because the evolution during t_1 gives rise, via exponential term, to a modulation of the phase of the observed signal. If we had chosen to select $p = +1$ during t_1 the signal would have been

$$S_N(t_1,t_2) = \tfrac{1}{4}\exp(-i\Omega t_1)\exp(i\Omega t_2)$$

which is also phase modulated, except in the opposite sense. Note that in either case the phase modulated signal is one half of the size of the amplitude modulated signal, because only one of the two pathways has been selected.

Although these results have been derived for the NOESY sequence, they are in fact general for any two-dimensional experiment. Summarising, we find

- If a single coherence order is present during t_1 the result is phase modulation in t_1. The phase modulation can be of the form $\exp(i\Omega t_1)$ or $\exp(-i\Omega t_1)$ depending on the sign of the coherence order present.
- If both coherence orders $\pm p$ are selected during t_1, the result is amplitude modulation in t_1; selecting both orders in this way is called *preserving symmetrical pathways.*

9.3.2 Frequency discrimination

The amplitude modulated signal contains no information about the sign of Ω, simply because $\cos(\Omega t_1) = \cos(-\Omega t_1)$. As a consequence, Fourier transformation of the time domain signal will result in each peak appearing twice in the two-dimensional spectrum, once at $F_1 = +\Omega$ and once at $F_1 = -\Omega$. As was commented on above, we usually place the transmitter in the middle of the spectrum so that there are peaks with both positive and negative offsets. If, as a result of recording an amplitude modulated signal, all of these appear twice, the spectrum will hopelessly confused. A spectrum arising from an amplitude modulated signal is said to *lack frequency discrimination* in F_1.

On the other hand, the phase modulated signal is sensitive to the sign of the offset and so information about the sign of Ω in the F_1 dimension is contained in the signal. Fourier transformation of the signal $S_P(t_1,t_2)$ gives a peak at $F_1 = +\Omega$, $F_2 = \Omega$, whereas Fourier transformation of the signal $S_N(t_1,t_2)$ gives a peak at $F_1 = -\Omega$, $F_2 = \Omega$. Both spectra are said to be frequency discriminated as the sign of the modulation frequency in t_1 is determined; in contrast to amplitude modulated spectra, each peak will only appear once.

The spectrum from $S_P(t_1,t_2)$ is called the P-type (P for positive) or echo spectrum; a diagonal peak appears with the same sign of offset in each dimension. The spectrum from $S_N(t_1,t_2)$ is called the N-type (N for negative) or anti-echo spectrum; a diagonal peak appears with opposite signs in the two dimensions.

It might appear that in order to achieve frequency discrimination we should deliberately select a CTP which leads to a P– or an N-type spectrum. However, such spectra show a very unfavourable lineshape, as discussed in the next section.

9.3.3 Lineshapes

In section 9.1.4 we saw that Fourier transformation of the signal

$$S(t) = \exp(\mathrm{i}\Omega t)\exp(-t/T_2)$$

gave a spectrum whose real part is an absorption lorentzian and whose imaginary part is a dispersion lorentzian:

$$S(\omega) = A(\omega) + \mathrm{i}D(\omega)$$

We will use the shorthand that A_2 represents an absorption mode lineshape at $F_2 = \Omega$ and D_2 represents a dispersion mode lineshape at the same frequency. Likewise, A_{1+} represents an absorption mode lineshape at $F_1 = +\Omega$ and D_{1+} represents the corresponding dispersion lineshape. A_{1-} and D_{1-} represent the corresponding lines at $F_1 = -\Omega$.

The time domain signal for the P-type spectrum can be written as

$$S_\mathrm{P}(t_1,t_2) = \tfrac{1}{4}\exp(\mathrm{i}\Omega t_1)\exp(\mathrm{i}\Omega t_2)\exp(-t_1/T_2)\exp(-t_2/T_2)$$

where the damping factors have been included as before. Fourier transformation with respect to t_2 gives

$$S_\mathrm{P}(t_1,F_2) = \tfrac{1}{4}\exp(\mathrm{i}\Omega t_1)\exp(-t_1/T_2)[A_2 + \mathrm{i}D_2]$$

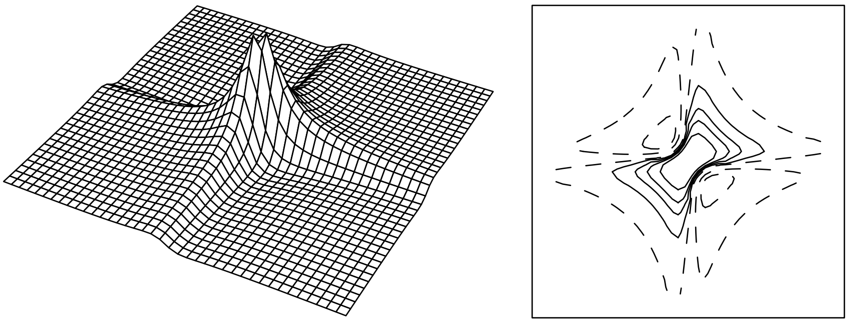
and then further transformation with respect to t_1 gives

$$S_\mathrm{P}(F_1,F_2) = \tfrac{1}{4}[A_{1+} + \mathrm{i}D_{1+}][A_2 + \mathrm{i}D_2]$$

The real part of this spectrum is

$$\mathrm{Re}\{S_\mathrm{P}(F_1,F_2)\} = \tfrac{1}{4}[A_{1+}A_2 - D_{1+}D_2]$$

The quantity in the square brackets on the right represents a phase-twist lineshape at $F_1 = +\Omega$, $F_2 = \Omega$



Perspective view and contour plot of the phase-twist lineshape. Negative contours are shown dashed.

This lineshape is an inextricable mixture of absorption and dispersion, and it is very undesirable for high-resolution NMR. So, although a phase modulated signal gives us frequency discrimination, which is desirable, it also results in a phase-twist lineshape, which is not.

The time domain signal for the amplitude modulated data set can be written as

$$S_\mathrm{C}(t_1,t_2) = \tfrac{1}{2}\cos(\Omega t_1)\exp(\mathrm{i}\Omega t_2)\exp(-t_1/T_2)\exp(-t_2/T_2)$$

Fourier transformation with respect to t_2 gives

$$S_\mathrm{C}(t_1,F_2) = \tfrac{1}{2}\cos(\Omega t_1)\exp(-t_1/T_2)[A_2 + \mathrm{i}D_2]$$

which can be rewritten as

$$S_C(t_1,F_2) = \tfrac{1}{4}\left[\exp(\mathrm{i}\Omega t_1) + \exp(-\mathrm{i}\Omega t_1)\right]\exp(-t_1/T_2)\left[A_2 + \mathrm{i}D_2\right]$$

Fourier transformation with respect to t_1 gives, in the real part of the spectrum

$$\mathrm{Re}\left\{S_C(F_1,F_2)\right\} = \tfrac{1}{4}\left[A_{1+}A_2 - D_{1+}D_2\right] + \tfrac{1}{4}\left[A_{1-}A_2 - D_{1-}D_2\right]$$

This corresponds to two phase-twist lineshapes, one at $F_1 = +\Omega$, $F_2 = \Omega$ and the other at $F_1 = -\Omega$, $F_2 = \Omega$; the lack of frequency discrimination is evident. Further, the undesirable phase-twist lineshape is again present.

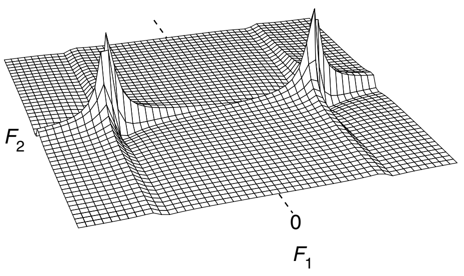
The lineshape can be restored to the absorption mode by discarding the imaginary part of the time domain signal after the transformation with respect to t_2, *i.e.* by taking the real part

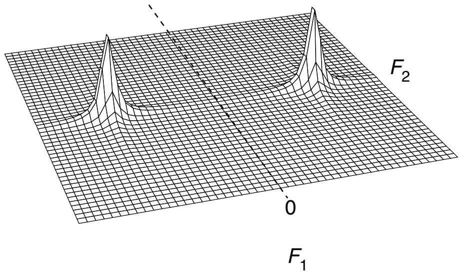
$$\mathrm{Re}\left\{S_C(t_1,F_2)\right\} = \tfrac{1}{2}\cos(\Omega t_1)\exp(-t_1/T_2)A_2$$

Subsequent transformation with respect to t_1 gives, in the real part

$$\tfrac{1}{4}A_{1+}A_2 + \tfrac{1}{4}A_{1-}A_2$$

which is two double absorption mode lineshapes. Frequency discrimination is lacking, but the lineshape is now much more desirable. The spectra with the two phase-twist and two absorption mode lines are shown below on the left and right, respectively.





9.3.4 Frequency discrimination with retention of absorption mode lineshapes

For practical purposes it is essential to be able to achieve frequency discrimination and at the same time retain the absorption mode lineshape. There are a number of ways of doing this.

9.3.4.1 States-Haberkorn-Ruben (SHR) method

The key to this method is the ability to record a cosine modulated data set and a sine modulated data set. The latter can be achieved simply by changing the phase of appropriate pulses. For example, in the case of the NOESY experiment, all that is required to generate the sine data set is to shift the phase of the first 90° pulse by 90° (in fact in the NOESY sequence the pulse needs to shift from *x* to *–y*). The two data sets have to kept separate.

The cosine data set is transformed with respect to t_1 and the imaginary part discarded to give

$$\mathrm{Re}\left\{S_C(t_1,F_2)\right\} = \tfrac{1}{2}\cos(\Omega t_1)\exp(-t_1/T_2)A_2 \qquad [9]$$

The same operation is performed on the sine modulated data set

$$S_S(t_1,t_2) = \tfrac{1}{2}\sin(\Omega t_1)\exp(\mathrm{i}\Omega t_2)\exp(-t_1/T_2)\exp(-t_2/T_2)$$

$$\mathrm{Re}\{S_S(t_1,F_2)\} = \tfrac{1}{2}\sin(\Omega t_1)\exp(-t_1/T_2)A_2 \qquad [10]$$

A new complex data set is now formed by using the signal from Eq. [9] as the real part and that from Eq. [10] as the imaginary part

$$\begin{aligned} S_{\mathrm{SHR}}(t_1,F_2) &= \mathrm{Re}\{S_C(t_1,F_2)\} + \mathrm{i}\,\mathrm{Re}\{S_S(t_1,F_2)\} \\ &= \tfrac{1}{2}\exp(\mathrm{i}\Omega t_1)\exp(-t_1/T_2)A_2 \end{aligned}$$

Fourier transformation with respect to t_1 gives, in the real part of the spectrum

$$\mathrm{Re}\{S_{\mathrm{SHR}}(F_1,F_2)\} = \tfrac{1}{2}A_{1+}A_2$$

This is the desired frequency discriminated spectrum with a pure absorption lineshape.

As commented on above, in NOESY all that is required to change from cosine to sine modulation is to shift the phase of the first pulse by 90°. The general recipe is to shift the phase of *all* the pulses that precede t_1 by $90°/|p_1|$, where p_1 is the coherence order present during t_1. So, for a double quantum spectrum, the phase shift needs to be 45°. The origin of this rule is that, taken together, the pulses which precede t_1 give rise to a pathway with $\Delta p = p_1$.

In heteronuclear experiments it is not usually necessary to shift the phase of all the pulses which precede t_1; an analysis of the sequence usually shows that shifting the phase of the pulse which generates the transverse magnetization which evolves during t_1 is sufficient.

9.3.4.2 Echo anti-echo method

We will see in later sections that when we use gradient pulses for coherence selection the natural outcome is P- or N-type data sets. Individually, each of these gives a frequency discriminated spectrum, but with the phase-twist lineshape. We will show in this section how an absorption mode lineshape can be obtained provided *both* the P- and the N-type data sets are available.

As before, we write the two data sets as

$$\begin{aligned} S_P(t_1,t_2) &= \tfrac{1}{4}\exp(\mathrm{i}\Omega t_1)\exp(\mathrm{i}\Omega t_2)\exp(-t_1/T_2)\exp(-t_2/T_2) \\ S_N(t_1,t_2) &= \tfrac{1}{4}\exp(-\mathrm{i}\Omega t_1)\exp(\mathrm{i}\Omega t_2)\exp(-t_1/T_2)\exp(-t_2/T_2) \end{aligned}$$

We then form the two combinations

$$\begin{aligned} S_C(t_1,t_2) &= S_P(t_1,t_2) + S_N(t_1,t_2) \\ &= \tfrac{1}{2}\cos(\Omega t_1)\exp(\mathrm{i}\Omega t_2)\exp(-t_1/T_2)\exp(-t_2/T_2) \\ S_S(t_1,t_2) &= \tfrac{1}{\mathrm{i}}\left[S_P(t_1,t_2) + S_N(t_1,t_2)\right] \\ &= \tfrac{1}{2}\sin(\Omega t_1)\exp(\mathrm{i}\Omega t_2)\exp(-t_1/T_2)\exp(-t_2/T_2) \end{aligned}$$

These cosine and sine modulated data sets can be used as inputs to the SHR method described in the previous section.

An alternative is to Fourier transform the two data sets with respect to t_2 to give

$$S_{\mathrm{P}}(t_1,F_2) = \tfrac{1}{4}\exp(\mathrm{i}\Omega t_1)\exp(-t_1/T_2)[A_2 + \mathrm{i}D_2]$$

$$S_{\mathrm{N}}(t_1,F_2) = \tfrac{1}{4}\exp(-\mathrm{i}\Omega t_1)\exp(-t_1/T_2)[A_2 + \mathrm{i}D_2]$$

We then take the complex conjugate of $S_{\mathrm{N}}(t_1,F_2)$ and add it to $S_{\mathrm{P}}(t_1,F_2)$

$$S_{\mathrm{N}}(t_1,F_2)^* = \tfrac{1}{4}\exp(\mathrm{i}\Omega t_1)\exp(-t_1/T_2)[A_2 - \mathrm{i}D_2]$$

$$\begin{aligned} S_+(t_1,F_2) &= S_{\mathrm{N}}(t_1,F_2)^* + S_{\mathrm{P}}(t_1,F_2) \\ &= \tfrac{1}{2}\exp(\mathrm{i}\Omega t_1)\exp(-t_1/T_2)A_2 \end{aligned}$$

Transformation of this signal gives

$$S_+(F_1,F_2) = \tfrac{1}{2}[A_{1+} + \mathrm{i}D_{1+}]A_2$$

which is frequency discriminated and has, in the real part, the required double absorption lineshape.

9.3.4.3 Marion-Wüthrich or TPPI method

The idea behind the TPPI (time proportional phase incrementation) or Marion–Wüthrich (MW) method is to arrange things so that all of the peaks have positive offsets. Then, frequency discrimination is not required as there is no ambiguity.

One simple way to make all offsets positive is to set the receiver carrier frequency deliberately at the edge of the spectrum. Simple though this is, it is not really a very practical method as the resulting spectrum would be very inefficient in its use of data space and in addition off-resonance effects associated with the pulses in the sequence will be accentuated.

In the TPPI method the carrier can still be set in the middle of the spectrum, but it is made to *appear* that all the frequencies are positive by phase shifting some of the pulses in the sequence *in concert* with the incrementation of t_1.

It was noted above that shifting the phase of the first pulse in the NOESY sequence from x to $-y$ caused the modulation to change from $\cos(\Omega t_1)$ to $\sin(\Omega t_1)$. One way of expressing this is to say that shifting the pulse causes a phase shift ϕ in the signal modulation, which can be written $\cos(\Omega t_1 + \phi)$. Using the usual trigonometric expansions this can be written

$$\cos(\Omega t_1 + \phi) = \cos\Omega t_1 \cos\phi - \sin\Omega t_1 \sin\phi$$

If the phase shift, ϕ, is $-\pi/2$ radians the result is

$$\begin{aligned} \cos(\Omega t_1 + \pi/2) &= \cos\Omega t_1 \cos(-\pi/2) - \sin\Omega t_1 \sin(-\pi/2) \\ &= \sin\Omega t_1 \end{aligned}$$

This is exactly the result we found before.

In the TPPI procedure, the phase ϕ is made proportional to t_1 *i.e.* each time t_1 is incremented, so is the phase. We will suppose that

$$\phi(t_1) = \omega_{\mathrm{add}} t_1$$

The constant of proportion between the time dependent phase, $\phi(t_1)$, and t_1 has been written ω_{add}; ω_{add} has the dimensions of rad s^{-1} *i.e.* it is a frequency. Following the same approach as before, the time-domain function with the inclusion of this incrementing phase is thus

$$\begin{aligned}\cos\left(\Omega t_1 + \phi(t_1)\right) &= \cos\left(\Omega t_1 + \omega_{\text{add}} t_1\right)\\ &= \cos\left(\Omega + \omega_{\text{add}}\right) t_1\end{aligned}$$

In words, the effect of incrementing the phase in concert with t_1 is to add a frequency ω_{add} to *all* of the offsets in the spectrum. The TPPI method utilizes this in the following way.

In one-dimensional pulse-Fourier transform NMR the free induction signal is sampled at regular intervals Δ. After transformation the resulting spectrum displays correctly peaks with offsets in the range $-(SW/2)$ to $+(SW/2)$ where SW is the spectral width which is given by $1/\Delta$ (this comes about from the Nyquist theorem of data sampling). Frequencies outside this range are not represented correctly.

Suppose that the required frequency range in the F_1 dimension is from $-(SW_1/2)$ to $+(SW_1/2)$. To make it appear that all the peaks have a positive offset, it will be necessary to add $(SW_1/2)$ to all the frequencies. Then the peaks will be in the range 0 to (SW_1).

As the maximum frequency is now (SW_1) rather than $(SW_1/2)$ the sampling interval, Δ_1, will have to be halved *i.e.* $\Delta_1 = 1/(2SW_1)$ in order that the range of frequencies present are represented properly.

The phase increment is $\omega_{\text{add}} t_1$, but t_1 can be written as $n\Delta_1$ for the nth increment of t_1. The required value for ω_{add} is $2\pi(SW_1/2)$, where the 2π is to convert from frequency (the units of SW_1) to rad s^{-1}, the units of ω_{add}. Putting all of this together $\omega_{\text{add}} t_1$ can be expressed, for the nth increment as

$$\begin{aligned}\omega_{\text{additional}} t_1 &= 2\pi\left(\frac{SW_1}{2}\right)\left(n\Delta_1\right)\\ &= 2\pi\left(\frac{SW_1}{2}\right)\left(n\frac{1}{2SW_1}\right)\\ &= n\frac{\pi}{2}\end{aligned}$$

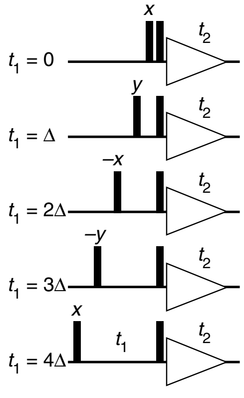


Illustration of the TPPI method. Each time that t_1 is incremented, so is the phase of the pulse preceding t_1.

In words this means that each time t_1 is incremented, the phase of the signal should also be incremented by 90°, for example by incrementing the phase of one of the pulses.

A data set from an experiment to which TPPI has been applied is simply amplitude modulated in t_1 and so can be processed according to the method described above for cosine modulated data so as to obtain absorption mode lineshapes. As the spectrum is symmetrical about $F_1 = 0$, it is usual to use a modified Fourier transform routine which saves effort and space by only calculating the positive frequency part of the spectrum.

9.3.4.4 States-TPPI

When the SHR method is used, axial peaks (arising from magnetization which has not evolved during t_1) appear at $F_1 = 0$; such peaks can be a nuisance as they may obscure other wanted peaks. We will see below (section 9.4.6) that axial peaks can be suppressed with the aid of phase cycling, all be it at the cost of doubling the length of the phase cycle.

The States-TPPI method does not suppress these axial peaks, but moves them to the edge of the spectrum so that they are less likely to obscure wanted peaks. All that is involved is that, each time t_1 is incremented, both the phase of the pulse which precedes t_1 and the receiver phase are advanced by 180° *i.e.* the pulse goes x, $-x$ and the receiver goes x, $-x$.

For non-axial peaks, the two phase shifts cancel one another out, and so have no effect. However, magnetization which gives rise to axial peaks does not experience the first phase shift, but does experience the receiver phase shift. The sign alternation in concert with t_1 incrementation adds a frequency of $SW_1/2$ to each peak, thus shifting it to the edge of the spectrum. Note that in States-TPPI the spectral range in the F_1 dimension is $-(SW_1/2)$ to $+(SW_1/2)$ and the sampling interval is $1/2SW_1$, just as in the SHR method.

The nice feature of States-TPPI is that is moves the axial peaks out of the way without lengthening the phase cycle. It is therefore convenient to use in complex three- and four-dimensional spectra were phase cycling is at a premium.

9.4 Phase cycling

In this section we will start out by considering in detail how to write a phase cycle to select a particular value of Δp and then use this discussion to lead on to the formulation of general principles for constructing phase cycles. These will then be used to construct appropriate cycles for a number of common experiments.

9.4.1 Selection of a single pathway

To focus on the issue at hand let us consider the case of transferring from coherence order +2 to order –1. Such a transfer has $\Delta p = (-1 - (2)\ ) = -3$. Let us imagine that the pulse causing this transformation is cycled around the four cardinal phases (*x, y, –x, –y, i.e.* 0°, 90°, 180°, 270°) and draw up a table of the phase shift that will be experienced by the transferred coherence. This is simply computed as $-\Delta p\ \phi$, in this case $= -(-3)\phi = 3\phi$.

step	pulse phase	phase shift experienced by transfer with $\Delta p = -3$	equivalent phase
1	0	0	0
2	90	270	270
3	180	540	180
4	270	810	90

The fourth column, labelled "equivalent phase", is just the phase shift experienced by the coherence, column three, reduced to be in the range 0 to 360° by subtracting multiples of 360° (*e.g.* for step 3 we subtracted 360° and for step 4 we subtracted 720°).

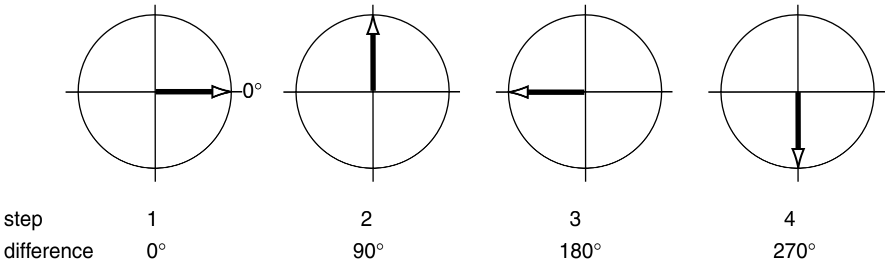
If we wished to select $\Delta p = -3$ we would simply shift the phase of the receiver in order to match the phase that the coherence has acquired; these are the phases shown in the last column. If we did this, then each step of the cycle would give an observed signal of the same phase and so they four contributions would all add up. This is precisely the same thing as we did when considering the CYCLOPS sequence in section 9.1.6; in both cases the receiver phase follows the phase of the desired magnetization or coherence.

We now need to see if this four step phase cycle eliminates the signals from other pathways. As an example, let us consider a pathway with $\Delta p = 2$, which might arise from the transfer from coherence order –1 to +1. Again we draw up a table to show the phase experienced by a pathway with $\Delta p = 2$, that is computed as $-(2)\phi$

step	pulse phase	phase shift experienced by transfer with $\Delta p = 2$	equivalent phase	rx. phase to select $\Delta p = -3$	difference
1	0	0	0	0	0
2	90	–180	180	270	270 – 180 = 90
3	180	–360	0	180	180 – 0 = 180
4	270	–540	180	90	90 – 180 = –90

As before, the equivalent phase is simply the phase in column 3 reduced to the range 0 to 360°. The fifth column shows the receiver (abbreviated to rx.) phases determined above for selection of the transfer with $\Delta p = -3$. The question we have to ask is whether or not these phase shifts will lead to cancellation of the transfer with $\Delta p = 2$. To do this we compute the difference between the receiver phase, column 5, and the phase shift experienced by the transfer with $\Delta p = 2$, column 4. The results are shown in column 6, labelled "difference". Looking at this difference column we can see that step 1 will cancel with step 3 as the 180° phase shift between them means that the two signals have opposite sign. Likewise step 2 will cancel with step 4 as there is a 180° phase shift between them. We conclude, therefore, that this four step cycle cancels the signal arising from a pathway with $\Delta p = 2$.

An alternative way of viewing the cancellation is to represent the results of the "difference" column by vectors pointing at the indicated angles. This is shown below; it is clear that the opposed vectors cancel one another.



Next we consider the coherence transfer with $\Delta p = +1$. Again, we draw up the table and calculate the phase shifts experience by this transfer, which are given by $-(+1)\phi = -\phi$.

step	pulse phase	phase shift experienced by transfer with $\Delta p = +1$	equivalent phase	rx. phase to select $\Delta p = -3$	difference
1	0	0	0	0	0
2	90	–90	270	270	270 – 270 = 0
3	180	–180	180	180	180 – 180 = 0
4	270	–270	90	90	90 – 90 = 0

Here we see quite different behaviour. The equivalent phases, that is the phase shifts experienced by the transfer with $\Delta p = 1$, match exactly the receiver phases determined for $\Delta p = -3$, thus the phases in the "difference" column are all zero. We conclude that the four step cycle selects transfers both with $\Delta p = -3$ *and* +1.

Some more work with tables such as these will reveal that this four step cycle suppresses contributions from changes in coherence order of –2, –1 and 0. It selects $\Delta p = -3$ and 1. It also selects changes in coherence order of 5, 9, 13 and so on. This latter sequence is easy to understand. A pathway with $\Delta p = 1$ experiences a phase shift of –90° when the pulse is shifted in phase by 90°; the equivalent phase is thus 270°. A pathway with $\Delta p = 5$ would experience a phase shift of $-5 \times 90° = -450°$ which corresponds to an equivalent phase of 270°. Thus the phase shifts experienced for $\Delta p = 1$ and 5 are identical and it is clear that a cycle which selects one will select the other. The same goes for the series $\Delta p = 9, 13$ ...

The extension to negative values of Δp is also easy to see. A pathway with $\Delta p = -3$ experiences a phase shift of 270° when the pulse is shifted in phase by 90°. A transfer with $\Delta p = +1$ experiences a phase of –90° which corresponds to an equivalent phase of 270°. Thus both pathways experience the same phase shifts and a cycle which selects one will select the other. The pattern is clear, this four step cycle will select a pathway with $\Delta p = -3$, as it was designed to, and also it will select any pathway with $\Delta p = -3 + 4n$ where $n = \pm 1, \pm 2, \pm 3$ ...

9.4.2 General Rules

The discussion in the previous section can be generalised in the following way. Consider a phase cycle in which the phase of a pulse takes N evenly spaced steps covering the range 0 to 2π radians. The phases, ϕ_k, are

$$\phi_k = 2\pi k/N \text{ where } k = 0, 1, 2 \ldots (N-1).$$

To select a change in coherence order, Δp, the receiver phase is set to $-\Delta p \times \phi_k$ for each step and the resulting signals are summed. This cycle will, in addition to selecting the specified change in coherence order, also select pathways with changes in coherence order $(\Delta p \pm nN)$ where $n = \pm 1, \pm 2$ ..

The way in which phase cycling selects a series of values of Δp which are related by a harmonic condition is closely related to the phenomenon of aliasing in Fourier transformation. Indeed, the whole process of phase cycling can be seen as the computation of a discrete Fourier transformation with respect to the pulse phase; in this case the Fourier co-domains are phase and coherence order.

The fact that a phase cycle inevitably selects more than one change in coherence order is not necessarily a problem. We may actually wish to select more than one pathway, and examples of this will be given below in relation to specific two-dimensional experiments. Even if we only require one value of Δp we may be able to discount the values selected at the same time as being improbable or insignificant. In a system of m coupled spins one-half, the maximum order of coherence that can be generated is m, thus in a two spin system we need not worry about whether or not a phase cycle will discriminate between double quantum and six quantum coherences as the latter simply cannot be present. Even in more extended spin systems the likelihood of generating high-order coherences is rather small and so we may be able to discount them for all practical purposes. If a high level of discrimination between orders is needed, then the solution is simply to use a phase cycle which has more steps *i.e.* in which the phases move in smaller increments. For example a six step cycle will discriminate between $\Delta p = +2$ and +6, whereas a four step cycle will not.

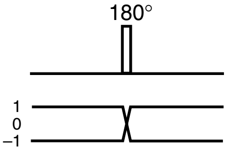
9.4.3 Refocusing Pulses

A 180° pulse simply changes the sign of the coherence order. This is easily demonstrated by considering the effect of such a pulse on the operators I_+ and I_-. For example:

$$I_+ \equiv \left(I_x + \mathrm{i}I_y\right) \xrightarrow{\pi I_x} \left(I_x - \mathrm{i}I_y\right) \equiv I_-$$

corresponds to $p = +1 \rightarrow p = -1$. In a more complex product of operators, each raising and lowering operator is affected in this way so overall the coherence order changes sign.

We can now derive the EXORCYLE phase cycle using this property. Consider a 180° pulse acting on single quantum coherence, for which the CTP is shown opposite. For the pathway starting with $p = 1$ the effect of the 180° pulse is to cause a change with $\Delta p = -2$. The table shows a four-step cycle to select this change



A 180° pulse simply changes the sign of the coherence order. The EXORCYLE phase cycling selects both of the pathways shown.

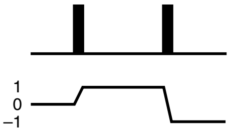
Step	phase of 180° pulse	phase shift experienced by transfer with $\Delta p = -2$	Equivalent phase = rx. phase
1	0	0	0
2	90	180	180
3	180	360	0
4	270	540	180

The phase cycle is thus 0, 90°, 180°, 270° for the 180° pulse and 0° 180° 0° 180° for the receiver; this is precisely the set of phases deduced before for EXORCYLE in section 9.1.7.

As the cycle has four steps, a pathway with $\Delta p = +2$ is also selected; this is the pathway which starts with $p = -1$ and is transferred to $p = +1$. Therefore, the four steps of EXORCYLE select both of the pathways shown in the diagram above.

A two step cycle, consisting of 0°, 180° for the 180° pulse and 0°, 0° for the receiver, can easily be shown to select all *even* values of Δp. This reduced form of EXORCYCLE is sometimes used when it is necessary to minimise the number of steps in a phase cycle. An eight step cycle, in which the 180° pulse is advanced in steps of 45°, can be used to select the refocusing of double-quantum coherence in which the transfer is from $p = +2$ to -2 (*i.e.* $\Delta p = -4$) or *vice versa.*

9.4.4 Combining phase cycles



Suppose that we wish to select the pathway shown opposite; for the first pulse Δp is 1 and for the second it is -2. We can construct a four-step cycle for each pulse, but to select the overall pathway shown these two cycles have to be completed *independently* of one another. This means that there will be a total of sixteen steps. The table shows how the appropriate receiver cycling can be determined

Step	phase of 1st pulse	phase for $\Delta p = 1$	phase of 2nd pulse	phase for $\Delta p = -2$	total phase	equivalent phase = rx. phase
1	0	0	0	0	0	0
2	90	–90	0	0	–90	270
3	180	–180	0	0	–180	180
4	270	–270	0	0	–270	90
5	0	0	90	180	180	180
6	90	–90	90	180	90	90
7	180	–180	90	180	0	0
8	270	–270	90	180	–90	270
9	0	0	180	360	360	0
10	90	–90	180	360	270	270
11	180	–180	180	360	180	180
12	270	–270	180	360	90	90
13	0	0	270	540	540	180
14	90	–90	270	540	450	90
15	180	–180	270	540	360	0
16	270	–270	270	540	270	270

In the first four steps the phase of the second pulse is held constant and the phase of the first pulse simply goes through the four steps 0° 90° 180° 270°. As we are selecting $\Delta p = 1$ for this pulse, the receiver phases are simply 0°, 270°, 180°, 90°.

Steps 5 to 8 are a repeat of steps 1–4 except that the phase of the second pulse has been moved by 90°. As Δp for the second pulse is -2, the required pathway experiences a phase shift of 180° and so the receiver phase must be advanced by this much. So, the receiver phases for steps 5–8 are just 180° ahead of those for steps 1–4.

In the same way for steps 9–12 the first pulse again goes through the same four steps, and the phase of the second pulse is advanced to 180°. Therefore, compared to steps 1–4 the receiver phases in steps 9–12 need to be advanced by $-(-2) \times 180° = 360° = 0°$. Likewise, the receiver phases for steps 13–16 are advanced by $-(-2) \times 270° = 540° = 180°$.

Another way of looking at this is to consider each step individually. For example, compared to step 1, in step 14 the first pulse has been advanced by 90° so the phase from the first pulse is – (1) × 90° = –90°. The second pulse has been advanced by 270° so the phase from this is – (–2) × 270° = 540°. The total phase shift of the required pathway is thus –90 + 540 = 450° which is an equivalent phase of 90°. This is the receiver phase shown in the final column.

The key to devising these sequences is to simply work out the two four-step cycles independently and then merge them together rather than trying to work on the whole cycle. One writes down the first four steps, and then duplicates this four times as the second pulse is shifted. We would find the same steps, in a different sequence, if the phase of the *second* pulse is shifted in the *first* four steps.

We can see that the total size of a phase cycle grows at an alarming rate. With four phases for each pulse the number of steps grows as 4^l where l is the number of pulses in the sequence. A three-pulse sequence such as NOESY or DQF COSY would therefore involve a 64 step cycle. Such long cycles put a lower limit on the total time of an experiment and we may end up having to run an experiment for a long time not to achieve the desired signal-to-noise ratio but simply to complete the phase cycle.

Fortunately, there are several "tricks" which we can use in order to shorten the length of a phase cycle. To appreciate whether or not one of these tricks can be used in a particular sequence we need to understand in some detail what the sequence is actually doing and what the likely problems are going to be.

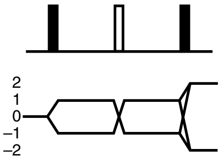
9.4.5 Tricks

9.4.5.1 The first pulse

All pulse sequences start with equilibrium magnetization, which has coherence order 0. It can easily be shown that when a pulse is applied to equilibrium magnetization the only coherence orders that can be generated are ±1. If retaining both of these orders is acceptable (which it often is), it is therefore not necessary to phase cycle the first pulse in a sequence.

There are two additional points to make here. If the spins have not relaxed completely by the start of the sequence the initial magnetization will not be at equilibrium. Then, the above simplification does not apply. Secondly, the first pulse of a sequence is often cycled in order to suppress axial peaks in two-dimensional spectra. This is considered in more detail in section 9.4.6.

9.4.5.2 Grouping pulses together



Pulse sequence for generating double-quantum coherence. Note that the 180° pulse simply causes a change in the sign of the coherence order.

The sequence shown opposite can be used to generate multiple quantum coherence from equilibrium magnetization; during the spin echo anti-phase magnetization develops and the final pulse transfers this into multiple quantum coherence. Let us suppose that we wish to generate double quantum, with $p = \pm 2$, as show by the CTP opposite.

As has already been noted, the first pulse can only generate $p = \pm 1$ and the 180° pulse only causes a change in the sign of the coherence order. The only pulse we need to be concerned with is the final one which we want to generate only double quantum. We could try to devise a phase cycle for the last pulse alone or we could simply group all three pulses together and imagine that, as a group, they achieve the transformation $p = 0$ to $p = \pm 2$ *i.e.* $\Delta p = \pm 2$. The phase cycle would simply be for the three pulses together to go 0°, 90°, 180°, 270°, with the receiver going 0°, 180°, 0°, 180°.

It has to be recognised that by cycling a group of pulses together we are only selecting an *overall* transformation; the coherence orders present within the group of pulses are not being selected. It is up to the designer of the experiment to decide whether or not this degree of selection is sufficient.

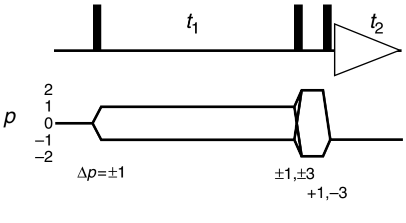
The four step cycle mentioned above also selects $\Delta p = \pm 6$; again, we would have to decide whether or not such high orders of coherence were likely to be present in the spin system. Finally, we note that the Δp values for the final pulse are ±1, ±3; it would not be possible to devise a four step cycle which selects *all* of these pathways.

9.4.5.3 The last pulse

We noted above that only coherence order –1 is observable. So, although the final pulse of a sequence may cause transfer to many different orders of coherence, only transfers to $p = -1$ will result in observable signals. Thus, if we have already selected, in an unambiguous way, a particular set of coherence orders present just before the last pulse, no further cycling of this pulse is needed.

9.4.5.4 Example – DQF COSY

A good example of the applications of these ideas is in devising a phase cycle for DQF COSY, whose pulse sequence and CTP is shown below.



Note that we have retained symmetrical pathways in t_1 so that absorption mode lineshapes can be obtained. Also, both in generating the double quantum coherence, and in reconverting it to observable magnetization, all possible pathways have been retained. If we do not do this, signal intensity is lost.

One way of viewing this sequence is to group the first two pulses together and view them as achieving the transformation 0 → ±2 *i.e.* $\Delta p = \pm 2$. This is exactly the problem considered in section 9.4.5.2, where we saw that a suitable four step cycle is for the first two pulses to go 0°, 90°, 180°, 270° and the receiver to go 0°, 180°, 0°, 180°. This unambiguously selects $p = \pm 2$ just before the last pulse, so phase cycling of the last pulse is not required (see section 9.4.5.3).

An alternative view is to say that as only $p = -1$ is observable, selecting the transformation $\Delta p = +1$ and –3 on the last pulse will be equivalent to selecting $p = \pm 2$ during the period just before the last pulse. Since the first pulse can only generate $p = \pm 1$ (present during t_1), the selection of $\Delta p = +1$ and –3 on the last pulse is sufficient to define the CTP completely.

A four step cycle to select $\Delta p = +1$ involves the pulse going 0°, 90°, 180°, 270° and the receiver going 0°, 270°, 180°, 90°. As this cycle has four steps is automatically also selects $\Delta p = -3$, just as required.

The first of these cycles also selects $\Delta p = \pm 6$ for the first two pulses *i.e.* filtration through six-quantum coherence; normally, we can safely ignore the possibility of such high-order coherences. The second of the cycles also selects $\Delta p = +5$ and $\Delta p = -7$ on the last pulse; again, these transfers involve such high orders of multiple quantum that they can be ignored.

9.4.6 Axial peak suppression

Peaks are sometimes seen in two-dimensional spectra at co-ordinates $F_1 = 0$ and F_2 = frequencies corresponding to the usual peaks in the spectrum. The interpretation of the appearance of these peaks is that they arise from magnetization which has not evolved during t_1 and so has not acquired a frequency label.

A common source of axial peaks is magnetization which recovers due to longitudinal relaxation during t_1. Subsequent pulses make this magnetization observable, but it has no frequency label and so appears at $F_1 = 0$. Another source of axial peaks is when, due to pulse imperfections, not all of the original equilibrium magnetization is moved into the transverse plane by the first pulse. The residual longitudinal magnetization can be made observable by subsequent pulses and hence give rise to axial peaks.

A simple way of suppressing axial peaks is to select the pathway $\Delta p = \pm 1$ on the first pulse; this ensures that all signals arise from the first pulse. A two-step cycle in which the first pulse goes 0°, 180° and the receiver goes 0°, 180° selects $\Delta p = \pm 1$. It may be that the other phase cycling used in the sequence will also reject axial peaks so that it is not necessary to add an explicit axial peak suppression steps. Adding a two-step cycle for axial peak suppression doubles the length of the phase cycle.

9.4.7 Shifting the whole sequence – CYCLOPS

If we group *all* of the pulses in the sequence together and regard them as a unit they simply achieve the transformation from equilibrium magnetization, $p = 0$, to observable magnetization, $p = -1$. They could be cycled as a group to select this pathway with $\Delta p = -1$, that is the pulses going 0°, 90°, 180°, 270° and the receiver going 0°, 90°, 180°, 270°. This is simple the CYCLOPS phase cycle described in section 9.1.6.

If time permits we sometimes add CYCLOPS-style cycling to all of the pulses in the sequence so as to suppress some artefacts associated with imperfections in the receiver. Adding such cycling does, of course, extend the phase cycle by a factor of four.

This view of the whole sequence as causing the transformation $\Delta p = -1$ also enables us to interchange receiver and pulse phase shifts. For example, suppose that a particular step in a phase cycle requires a receiver phase shift θ. The same effect can be achieved by shifting all of the pulses by $-\theta$ and leaving the receiver phase unaltered. The reason this works is that all of the pulses taken together achieve the transformation $\Delta p = -1$, so shifting their phases by $-\theta$ shift the signal by $-(-\theta) = \theta$, which is exactly the effect of shifting the receiver by θ. This kind of approach is sometimes helpful if hardware limitations mean that small angle phase-shifts are only available for the pulses.

9.4.8 Equivalent cycles

For even a relatively simple sequence such as DQF COSY there are a number of different ways of writing the phase cycle. Superficially these can look very different, but it may be possible to show that they really are the same.

For example, consider the DQF COSY phase cycle proposed in section 9.4.5.4 where we cycle just the last pulse

step	1st pulse	2nd pulse	3rd pulse	receiver
1	0	0	0	0
2	0	0	90	270
3	0	0	180	180
4	0	0	270	90

Suppose we decide that we do not want to shift the receiver phase, but want to keep it fixed at phase zero. As described above, this means that we need to subtract the receiver phase from all of the pulses. So, for example, in step 2 we subtract 270° from the pulse phases to give –270°, –270° and –180° for the phases of the first three pulses, respectively; reducing these to the usual range gives phases 90°, 90° and 180°. Doing the same for the other steps gives a rather strange looking phase cycle, but one which works in just the same way.

step	1st pulse	2nd pulse	3rd pulse	receiver
1	0	0	0	0
2	90	90	180	0
3	180	180	0	0
4	270	270	180	0

We can play one more trick with this phase cycle. As the third pulse is required to achieve the transformation $\Delta p = -3$ or $+1$ we can alter its phase by 180° and compensate for this by shifting the receiver by 180° also.

Doing this for steps 2 and 4 only gives

step	1st pulse	2nd pulse	3rd pulse	receiver
1	0	0	0	0
2	90	90	0	180
3	180	180	0	0
4	270	270	0	180

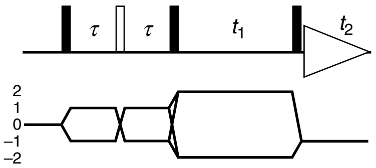
This is exactly the cycle proposed in section 9.4.5.4.

9.4.9 Further examples

In this section we will use a shorthand to indicate the phases of the pulses and the receiver. Rather than specifying the phase in degrees, the phases are expressed as multiples of 90°. So, EXORCYCLE becomes `0 1 2 3` for the 180° pulse and `0 2 0 2` for the receiver.

9.4.9.1 Double quantum spectroscopy

A simple sequence for double quantum spectroscopy is shown below

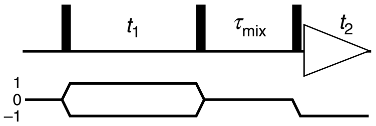


Note that both pathways with $p = \pm1$ during the spin echo and with $p = \pm2$ during t_1 are retained. There are a number of possible phase cycles for this experiment and, not surprisingly, they are essentially the same as those for DQF COSY. If we regard the first three pulses as a unit, then they are required to achieve the overall transformation $\Delta p = \pm2$, which is the same as that for the first two pulses in the DQF COSY sequence. Thus the same cycle can be used with these three pulses going `0 1 2 3` and the receiver going `0 2 0 2`. Alternatively the final pulse can be cycled `0 1 2 3` with the receiver going `0 3 2 1`, as in section 9.4.5.4.

Both of these phase cycles can be extended by EXORCYCLE phase cycling of the 180° pulse, resulting in a total of 16 steps.

9.4.9.2 NOESY

The pulse sequence for NOESY (with retention of absorption mode lineshapes) is shown below



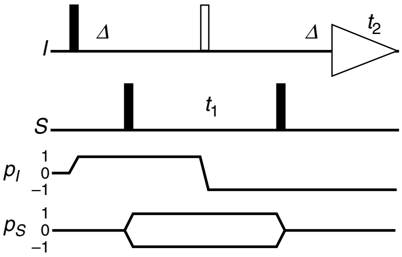
If we group the first two pulses together they are required to achieve the transformation $\Delta p = 0$ and this leads to a four step cycle in which the pulses go `0 1 2 3` and the receiver remains fixed as `0 0 0 0`. In this experiment axial peaks arise due to *z*-magnetization recovering during the mixing time, and this cycle will *not* suppress these contributions. Thus we need to add axial peak suppression, which is conveniently done by adding the simple cycle `0 2` on the first pulse and the receiver. The final 8 step cycle is 1st pulse: `0 1 2 3  2 3 0 1`, 2nd pulse: `0 1 2 3  0 1 2 3`, 3rd pulse fixed, receiver: `0 0 0 0  2 2 2 2`.

An alternative is to cycle the last pulse to select the pathway $\Delta p = -1$, giving the cycle `0 1 2 3` for the pulse and `0 1 2 3` for the receiver. Once again, this does not discriminate against z-magnetization which recovers during the mixing time, so a two step phase cycle to select axial peaks needs to be added.

9.4.9.3 Heteronuclear Experiments

The phase cycling for most heteronuclear experiments tends to be rather trivial in that the usual requirement is simply to select that component which has been transferred from one nucleus to another. We have already seen in section 9.1.8 that this is achieved by a `0 2` phase cycle on one of the pulses causing the transfer accompanied by the same on the receiver *i.e.* a difference experiment. The choice of which pulse to cycle depends more on practical considerations than with any fundamental theoretical considerations.

The pulse sequence for HMQC, along with the CTP, is shown below



Note that separate coherence orders are assigned to the I and S spins. Observable signals on the I spin must have $p_I = -1$ and $p_S = 0$ (any other value of p_S would correspond to a heteronuclear multiple quantum coherence). Given this constraint, and the fact that the I spin 180° pulse simply inverts the sign of p_I, the only possible pathway on the I spins is that shown.

The S spin coherence order only changes when pulses are applied to those spins. The first 90° S spin pulse generates $p_S = \pm 1$, just as before. As by this point $p_I = +1$, the resulting coherences have $p_S = +1$, $p_I = -1$ (heteronuclear zero-quantum) and $p_S = +1$, $p_I = +1$ (heteronuclear double-quantum). The I spin 180° pulse interconverts these midway during t_1, and finally the last S spin pulse returns both pathways to $p_S = 0$. A detailed analysis of the sequence shows that retention of both of these pathways results in amplitude modulation in t_1 (provided that homonuclear couplings between I spins are not resolved in the F_1 dimension).

Usually, the I spins are protons and the S spins some low-abundance heteronucleus, such as ^{13}C. The key thing that we need to achieve is to suppress the signals arising from vast majority of I spins which are not coupled to S spins. This is achieved by cycling a pulse which affects the phase of the required coherence but which does not affect that of the unwanted coherence. The obvious targets are the two S spin 90° pulses, each of which is required to give the transformation $\Delta p_S = \pm 1$. A two step cycle with either of these pulses going 0 2 and the receiver doing the same will select this pathway and, by difference, suppress any I spin magnetization which has not been passed into multiple quantum coherence.

It is also common to add EXORCYCLE phase cycling to the I spin 180° pulse, giving a cycle with eight steps overall.

9.4.10 General points about phase cycling

Phase cycling as a method suffers from two major practical problems. The first is that the need to complete the cycle imposes a minimum time on the experiment. In two- and higher-dimensional experiments this minimum time can become excessively long, far longer than would be needed to achieve the desired signal-to-noise ratio. In such cases the only way of reducing the experiment time is to record fewer increments which has the undesirable consequence of reducing the limiting resolution in the indirect dimensions.

The second problem is that phase cycling always relies on recording all possible contributions and then cancelling out the unwanted ones by combining subsequent signals. If the spectrum has high dynamic range, or if spectrometer stability is a problem, this cancellation is less than perfect. The result is unwanted peaks and t_1-noise appearing in the spectrum. These problems become acute when dealing with proton detected heteronuclear experiments on natural abundance samples, or in trying to record spectra with intense solvent resonances.

Both of these problems are alleviated to a large extent by moving to an alternative method of selection, the use of field gradient pulses, which is the subject of the next section. However, as we shall see, this alternative method is not without its own difficulties.

9.5 Selection with field gradient pulses

9.5.1 Introduction

Like phase cycling, field gradient pulses can be used to select particular coherence transfer pathways. During a pulsed field gradient the applied magnetic field is made spatially inhomogeneous for a short time. As a result, transverse magnetization and other coherences dephase across the sample and are apparently lost. However, this loss can be reversed by the application of a subsequent gradient which undoes the dephasing process and thus restores the magnetization or coherence. The crucial property of the dephasing process is that it proceeds at a different rate for different coherences. For example, double-quantum coherence dephases twice as fast as single-quantum coherence. Thus, by applying gradient pulses of different strengths or durations it is possible to refocus coherences which have, for example, been changed from single- to double-quantum by a radiofrequency pulse.

Gradient pulses are introduced into the pulse sequence in such a way that only the wanted signals are observed in each experiment. Thus, in contrast to phase cycling, there is no reliance on subtraction of unwanted signals, and it can thus be expected that the level of t_1-noise will be much reduced. Again in contrast to phase cycling, no repetitions of the experiment are needed, enabling the overall duration of the experiment to be set strictly in accord with the required resolution and signal-to-noise ratio.

The properties of gradient pulses and the way in which they can be used to select coherence transfer pathways have been known since the earliest days of multiple-pulse NMR. However, in the past their wide application has been limited by technical problems which made it difficult to use such pulses in high-resolution NMR. The problem is that switching on the gradient pulse induces currents in any nearby conductors, such as the probe housing and magnet bore tube. These induced currents, called *eddy currents*, themselves generate magnetic fields which perturb the NMR spectrum. Typically, the eddy currents are large enough to disrupt severely the spectrum and can last many hundreds of milliseconds. It is thus impossible to observe a high-resolution spectrum immediately after the application of a gradient pulse. Similar problems have beset NMR imaging experiments and have led to the development of *shielded gradient coils* which do not produce significant magnetic fields outside the sample volume and thus minimise the generation of eddy currents. The use of this technology in high-resolution NMR probes has made it possible to observe spectra within tens of microseconds of applying a gradient pulse. With such apparatus, the use of field gradient pulses in high resolution NMR is quite straightforward, a fact first realised and demonstrated by Hurd whose work has pioneered this whole area.

9.5.2 Dephasing caused by gradients

A field gradient pulse is a period during which the B_0 field is made spatially inhomogeneous; for example an extra coil can be introduced into the sample probe and a current passed through the coil in order to produce a field which varies linearly in the z-direction. We can imagine the sample being divided into thin discs which, as a consequence of the gradient, all experience different magnetic fields and thus have different Larmor frequencies. At the beginning of the gradient pulse the vectors representing transverse magnetization in all these discs are aligned, but after some time each vector has precessed through a different angle because of the variation in Larmor frequency. After sufficient time the vectors are disposed in such a way that the net magnetization of the sample (obtained by adding together all the vectors) is zero. The gradient pulse is said to have dephased the magnetization.

It is most convenient to view this dephasing process as being due to the generation by the gradient pulse of a *spatially dependent phase*. Suppose that the magnetic field produced by the gradient pulse, B_g, varies linearly along the z-axis according to

$$B_g = Gz$$

where G is the gradient strength expressed in, for example, T m^{-1} or G cm^{-1};

the origin of the z-axis is taken to be in the centre of the sample. At any particular position in the sample the Larmor frequency, $\omega_L(z)$, depends on the applied magnetic field, B_0, and B_g

$$\omega_L = \gamma\left(B_0 + B_g\right) = \gamma\left(B_0 + Gz\right) \ ,$$

where γ is the gyromagnetic ratio. After the gradient has been applied for time t, the phase at any position in the sample, $\Phi(z)$, is given by $\Phi(z) = \gamma(B_0 + Gz)t$. The first part of this phase is just that due to the usual Larmor precession in the absence of a field gradient. Since this is constant across the sample it will be ignored from now on (which is formally the same result as viewing the magnetization in a frame of reference rotating at γB_0). The remaining term γGzt is the *spatially dependent phase* induced by the gradient pulse.

If a gradient pulse is applied to pure x-magnetization, the following evolution takes place at a particular position in the sample

$$I_x \xrightarrow{\gamma GztI_z} \cos\left(\gamma Gzt\right)I_x + \sin\left(\gamma Gzt\right)I_y \ .$$

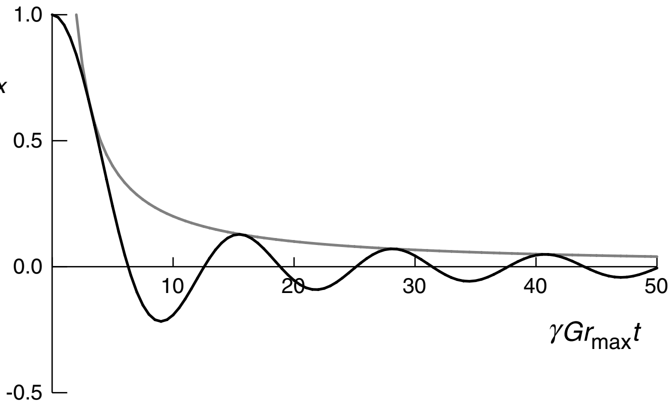
The total x-magnetization in the sample, M_x, is found by adding up the magnetization from each of the thin discs, which is equivalent to the integral

$$M_x(t) = \frac{1}{r_{\text{max}}} \int_{-\frac{1}{2}r_{\text{max}}}^{\frac{1}{2}r_{\text{max}}} \cos\left(\gamma Gzt\right) \mathrm{d}z$$

where it has been assumed that the sample extends over a region $\pm\frac{1}{2}r_{\text{max}}$. Evaluating the integral gives an expression for the decay of x-magnetization during a gradient pulse

$$M_x(t) = \frac{\sin\left(\frac{1}{2}\gamma G r_{\text{max}} t\right)}{\frac{1}{2}\gamma G r_{\text{max}} t} \qquad [11]$$

The plot below shows $M_x(t)$ as a function of time



The black line shows the decay of magnetization due to the action of a gradient pulse. The grey line is an approximation, valid at long times, for the envelope of the decay.

Note that the oscillations in the decaying magnetization are imposed on an overall decay which, for long times, is given by $2/(\gamma G t r_{\text{max}})$. Equation [11] embodies the obvious points that the stronger the gradient (the larger G) the faster the magnetization decays and that magnetization from nuclei with higher gyromagnetic ratios decays faster. It also allows a quantitative assessment of the gradient strengths required: the magnetization will have decayed to a fraction α of its initial value after a time of the order of $2/(\gamma G\alpha r_{\text{max}})$ (the relation is strictly valid for $\alpha << 1$). For example, if it is assumed that r_{max} is 1 cm, then a 2 ms gradient pulse of strength 0.37 T m^{-1} (37 G cm^{-1}) will reduce proton magnetization by a factor of 1000. Gradients of such strength are readily obtainable using modern shielded gradient coils that can be built into high resolution NMR probes

This discussion now needs to be generalised for the case of a field gradient pulse whose amplitude is not constant in time, and for the case of dephasing a general coherence of order p. The former modification is of importance as for instrumental reasons the amplitude envelope of the gradient is often shaped to a smooth function. In general after applying a gradient pulse of duration τ the spatially dependent phase, $\Phi(r,\tau)$ is given by

$$\Phi(r,\tau) = sp\gamma B_{\text{g}}(r)\tau \qquad [12]$$

The proportionality to the coherence order comes about due to the fact that the phase acquired as a result of a z-rotation of a coherence of order p through an angle ϕ is $p\phi$, (see Eqn. [2] in section 9.2.1). In Eqn. [12] s is a shape factor: if the envelope of the gradient pulse is defined by the function $A(t)$, where $|A(t)| \leq 1$, s is defined as the area under $A(t)$

$$s = \frac{1}{\tau}\int_0^{\tau} A(t)\,\text{d}t$$

The shape factor takes a particular value for a certain shape of gradient, regardless of its duration. A gradient applied in the opposite sense, that is with the magnetic field decreasing as the z-coordinate increases rather than *vice versa*, is described by reversing the sign of s. The overall amplitude of the gradient is encoded within B_{g}.

In the case that the coherence involves more than one nuclear species, Eqn. [12] is modified to take account of the different gyromagnetic ratio for each spin, γ_i, and the (possibly) different order of coherence with respect to each nuclear species, p_i:

$$\Phi(r,\tau) = sB_{\text{g}}(r)\tau\sum_i p_i\gamma_i$$

From now on we take the dependence of Φ on r and τ, and of B_{g} on r as being implicit.

9.5.3 Selection by refocusing

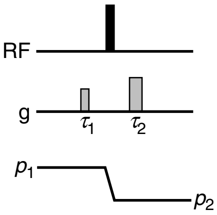


Illustration of the use of a pair of gradients to select a single pathway. The radiofrequency pulses are on the line marked "RF" and the field gradient pulses are denoted by shaded rectangles on the line marked "g".

The method by which a particular coherence transfer pathway is selected using field gradients is illustrated opposite. The first gradient pulse encodes a spatially dependent phase, Φ_1 and the second a phase Φ_2 where

$$\Phi_1 = s_1 p_1 \gamma B_{g,1} \tau_1 \quad \text{and} \quad \Phi_2 = s_2 p_2 \gamma B_{g,2} \tau_2 \quad .$$

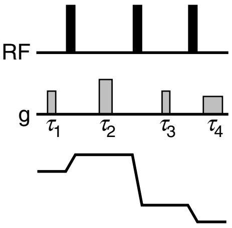
After the second gradient the net phase is ($\Phi_1 + \Phi_2$). To select the pathway involving transfer from coherence order p_1 to coherence order p_2, this net phase should be zero; in other words the dephasing induced by the first gradient pulse is undone by the second. The condition ($\Phi_1 + \Phi_2$) = 0 can be rearranged to

$$\frac{s_1 B_{g,1} \tau_1}{s_2 B_{g,2} \tau_2} = \frac{-p_2}{p_1} \quad . \qquad [13]$$

For example, if $p_1 = +2$ and $p_2 = -1$, refocusing can be achieved by making the second gradient either twice as long ($\tau_2 = 2\tau_1$), or twice as strong ($B_{g,2} = 2B_{g,1}$) as the first; this assumes that the two gradients have identical shape factors. Other pathways remain dephased; for example, assuming that we have chosen to make the second gradient twice as strong and the same duration as the first, a pathway with $p_1 = +3$ to $p_2 = -1$ experiences a net phase

$$\Phi_1 + \Phi_2 = 3sB_{g,1}\tau_1 - sB_{g,2}\tau_1 = sB_{g,1}\tau_1 \quad .$$

Provided that this spatially dependent phase is sufficiently large, according the criteria set out in the previous section, the coherence arising from this pathway remains dephased and is not observed. To refocus a pathway in which there is no sign change in the coherence orders, for example, $p_1 = -2$ to $p_2 = -1$, the second gradient needs to be applied in the opposite sense to the first; in terms of Eqn. [13] this is expressed by having $s_2 = -s_1$.



The procedure can easily be extended to select a more complex coherence transfer pathway by applying further gradient pulses as the coherence is transferred by further pulses, as illustrated opposite. The condition for refocusing is again that the net phase acquired by the required pathway be zero, which can be written formally as

$$\sum_i s_i p_i \gamma_i B_{g,i} \tau_i = 0 \quad .$$

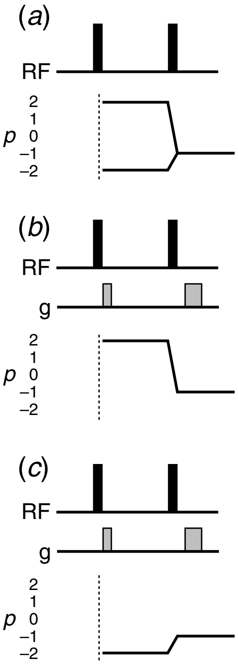
With more than two gradients in the sequence, there are many ways in which a given pathway can be selected. For example, the second gradient may be used to refocus the first part of the required pathway, leaving the third and fourth to refocus another part. Alternatively, the pathway may be consistently dephased and the magnetization only refocused by the final gradient, just before acquisition.

At this point it is useful to contrast the selection achieved using gradient pulses with that achieved using phase cycling. From Eqn. [13] it is clear that a particular pair of gradient pulses selects a particular *ratio* of coherence orders; in the above example any two coherence orders in the ratio –2 : 1 or 2 : –1 will be refocused. This selection according to ratio of coherence orders is in contrast to the case of phase cycling in which a phase cycle consisting of N steps of $2\pi/N$ radians selects a particular *change* in coherence order $\Delta p = p_2 - p_1$, and further pathways which have $\Delta p = (p_2 - p_1) \pm mN$, where $m = 0, 1, 2 ...$

It is straightforward to devise a series of gradient pulses which will *select* a single coherence transfer pathway. It cannot be assumed, however, that such a sequence of gradient pulses will *reject* all other pathways *i.e.* leave coherence from all other pathways dephased at the end of the sequence. Such assurance can only be given be analysing the fate of *all* other possible coherence transfer pathways under the particular gradient sequence proposed. In complex pulse sequences there may also be several different ways in which gradient pulses can be included in order to achieve selection of the desired pathway. Assessing which of these alternatives is the best, in the light of the requirement of suppression of unwanted pathways and the effects of pulse imperfections may be a complex task.

9.5.3.1 Selection of multiple pathways

As we have seen earlier, it is not unusual to want to select two or more pathways *simultaneously*, for example either to maximise the signal intensity or to retain absorption-mode lineshapes. A good example of this is the double-quantum filter pulse sequence element, shown opposite.



The ideal pathway, shown in (a), preserves coherence orders $p = \pm 2$ during the inter-pulse delay. Gradients can be used to select the pathway –2 to –1 or +2 to –1, shown in (b) and (c) respectively. However, no combination of gradients can be found which will select simultaneously both of these pathways. In contrast, it is easy to devise a phase cycle which selects both of these pathways (section 9.4.5.4). Thus, selection with gradients will in this case result in a loss of *half* of the available signal when compared to an experiment of equal length which uses selection by phase cycling. Such a loss in signal is, unfortunately, a very common feature when gradients are used for pathway selection.

9.5.3.2 Selection versus suppression

Coherence order zero, comprising z-magnetization, zz-terms and homonuclear zero-quantum coherence, does not accrue any phase during a gradient pulse. Thus, it can be separated from all other orders simply by applying a single gradient. In a sense, however, this is not a gradient selection process; rather it is a *suppression* of all other coherences. A gradient used in this way is often called a *purge gradient*. In contrast to experiments where selection is achieved, there is no inherent sensitivity loss when gradients are used for suppression. We will see examples below of cases where this suppression approach is very useful.

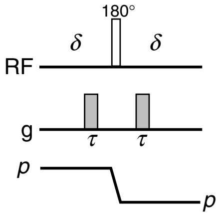
9.5.3.3 Gradients on other axes

The simplest experimental arrangement generates a gradient in which the magnetic field varies in the z direction, however it is also possible to generate gradients in which the field varies along x or y. Clearly, the spatially dependent phase generated by a gradient applied in one direction *cannot* be refocused by a gradient applied in a different direction. In sequences where more than one pair of gradients are used, it may be convenient to apply further gradients in different directions to the first pair, so as to avoid the possibility of accidentally refocusing unwanted coherence transfer pathways. Likewise, a gradient which is used to destroy all coherences can be applied in a different direction to gradients used for pathway selection.

9.5.4 Refocusing and inversion pulses

Refocusing and inversion pulses play an important role in multiple-pulse NMR experiments and so the interaction between such pulses and field gradient pulses will be explored in some detail. As has been noted above in section 9.4.3, a perfect refocusing pulse simply changes the sign of the order of any coherences present, $p \rightarrow -p$. If the pulse is imperfect, there will be transfer to coherence orders other than $-p$.

A perfect inversion pulse simply inverts z-magnetization or, more generally, all z-operators: $I_z \rightarrow -I_z$. If the pulse is imperfect, it will generate transverse magnetization or other coherences. Inversion pulses are used extensively in heteronuclear experiments to control the evolution of heteronuclear couplings.



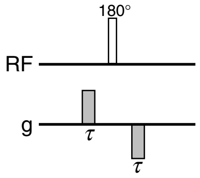
Gradient sequence used to "clean up" a refocusing pulse. Note that the two gradients are of equal area. The refocused pathway has $p' = -p$.

We start out the discussion by considering the refocusing of coherences, illustrated opposite. The net phase, Φ, at the end of such a sequence is

$$\Phi = \Omega^{(p)}\delta + sp\gamma B_g\tau + \Omega^{(p')}\delta + sp'\gamma B_g\tau$$

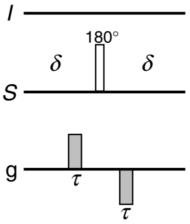
where $\Omega^{(p)}$ is the frequency with which coherence of order p evolves in the absence of a gradient; note that $\Omega^{(-p)} = -\Omega^{(p)}$. The net phase is zero if, and only if, $p' = -p$. With sufficiently strong gradients all other pathways remain dephased and the gradient sequence thus selects the refocused component. As is expected for a spin echo, the underlying evolution of the coherence (as would occur in the absence of a gradient) is also refocused by the selection of the pathway shown. Any transverse magnetization which an imperfect refocusing pulse might create is also dephased.

Placing equal gradients either side of a refocusing pulse therefore selects the coherence transfer pathway associated with a perfect refocusing pulse. This selection works for all coherence orders so, in contrast to the discussion in section 9.5.3.1, there is no loss of signal. Such a pair of gradients are often described as being used to "clean up" a refocusing pulse, referring to their role in eliminating unwanted pathways.



Gradient sequence used to "clean up" an inversion pulse.

We cannot use gradients to select the pathway associated with an inversion pulse as $p = 0$ both before and after the pulse. However, we can apply a gradient *after* the pulse to dephase any magnetization which might be created by an imperfect pulse. Taking the process a step further, we can apply a gradient both *before* and *after* the pulse, with the two gradients in *opposite* directions. The argument here is that this results in the maximum dephasing of unwanted coherences – both those present before the pulse and those that might be generated by the pulse. Again, this sequence is often described as being used to "clean up" an inversion pulse.

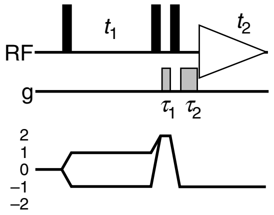


In a heteronuclear experiment (separate pulses shown on the *I* and *S* spins) a 180° pulse on the *S* spin refocuses the *IS* coupling over the period 2*δ*. The gradient pulses shown are used to "clean up" the inversion pulse.

In heteronuclear experiments an inversion pulse applied to one nucleus is used to refocus the evolution of a coupling to another nucleus. For example, in the sequence shown opposite the centrally placed *S* spin 180° pulse refocuses the *IS* coupling over the period 2δ. The pair of gradients shown have no net effect on *I* spin coherences as the dephasing due to the first gradient is exactly reversed by the second. The gradient sequence can be thought of as "cleaning up" the *S* spin inversion pulse.

9.5.4.1 Phase errors due to gradient pulses

For the desired pathway, the spatially dependent phase created by a gradient pulse is refocused by a subsequent gradient pulse. However, the underlying evolution of offsets (chemical shifts) and couplings is not refocused, and phase errors will accumulate due to the evolution of these terms. Since gradient pulses are typically of a few milliseconds duration, these phase errors are substantial.



Simple pulse sequence for DQF COSY with gradient selection and retention of symmetrical pathways in t_1. The second gradient is twice the area of the first.

In multi-dimensional NMR the uncompensated evolution of offsets during gradient pulses has disastrous effects on the spectra. This is illustrated here for the DQF COSY experiment, for which a pulse sequence using the gradient pulses is shown opposite. The problem with this sequence is that the double quantum coherence will evolve during delay τ_1. Normally, the delay between the last two pulses would be a few microseconds at most, during which the evolution is negligible. However, once we need to apply a gradient the delay will need to be of the order of milliseconds and significant evolution takes place. The same considerations apply to the second gradient.

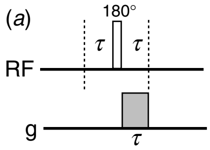
We can investigate the effect of this evolution by analysing the result for a two-spin system. In the calculation, it will be assumed that only the indicated pathway survives and that the spatially dependent part of the evolution due to the gradients can be ignored as ultimately it is refocused. The coherence with order of + 2 present during τ_1 evolves according to

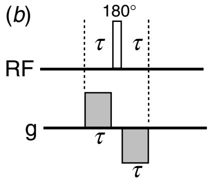
$$I_{1+}I_{2+} \xrightarrow{\Omega_1\tau_1 I_{1z}+\Omega_2\tau_1 I_{2z}} I_{1+}I_{2+}\exp\left(-i\left(\Omega_1+\Omega_2\right)\tau_1\right) \quad ,$$

where Ω_1 and Ω_2 are the offsets of spins 1 and 2, respectively. After the final 90° pulse and the second gradient the observable terms on spin 1 are

$$\tfrac{i}{2}\exp\left(-i\left(\Omega_1+\Omega_2\right)\tau_1\right)\left[\cos\Omega_1\tau_2\; 2I_{1x}I_{2z}+\sin\Omega_1\tau_2\; 2I_{1y}I_{2z}\right] \qquad [14]$$

where it has been assumed that τ_2 is sufficiently short that evolution of the coupling can be ignored. It is clearly seen from Eqn. [14] that, due to the evolution during τ_2, the multiplet observed in the F_2 dimension will be a mixture of dispersion and absorption anti-phase contributions. In addition, there is an overall phase shift due to the evolution during τ_1. The phase correction needed to restore this multiplet to absorption depends on both the frequency in F_2 *and* the double-quantum frequency during the first gradient. Thus, no single linear frequency dependent phase correction could phase correct a spectrum containing many multiplets. The need to control these phase errors is plain.

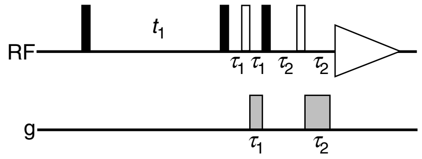




The general way to minimise these problems is to associate each gradient with a refocusing pulse, as shown opposite. In sequence (*a*) the gradient is placed in one of the delays of a spin echo; the evolution of the offset during the first delay τ is refocused during the second delay τ. So, overall there are no phase errors due to the evolution of the offset.

An alternative is the sequence (*b*). Here, as in (a), the offset is refocused over the whole sequence. The first gradient results in the usually spatially dependent phase and then the 180° pulse changes the sign of the coherence order. As the second gradient is opposite to the first, it causes *further* dephasing; effectively, it is as if a gradient of length 2τ is applied. Sequence (*b*) will give the same dephasing effect as (*a*) if each gradient in (*b*) is of duration $\tau/2$; the overall sequence will then be of duration τ. If losses due to relaxation are a problem, then clearly sequence (*b*) is to be preferred as it takes less time than (*a*).

The sequence below shows the gradient-selected DQF COSY pulse sequence modified by the inclusion of extra 180° pulses to remove phase errors. Note that although the extra 180° pulses are effective at refocusing offsets, they do not refocus the evolution of homonuclear couplings. It is essential, therefore, to keep the gradient pulses as short as is feasible.



In many pulse sequences there are already periods during which the evolution of offsets is refocused. The evolution of offsets during a gradient pulse placed within such a period will therefore also be refocused, making it unnecessary to include extra refocusing pulses. Likewise, a gradient may be placed during a "constant time" evolution period of a multi-dimensional pulse sequence without introducing phase errors in the corresponding dimension; the gradient simply becomes part of the constant time period. This approach is especially useful in three- and four-dimensional experiments used to record spectra of ^{15}N, ^{13}C labelled proteins.

9.5.5 Sensitivity

The use of gradients for coherence selection has consequences for the signal-to-noise ratio of the spectrum when it is compared to a similar spectrum recorded using phase cycling. If a gradient is used to *suppress* all coherences other than $p = 0$, *i.e.* it is used simply to remove all coherences, leaving just z-magnetization or zz terms, there is no inherent loss of sensitivity when compared to a corresponding phase cycled experiment. If, however, the gradient is used to *select* a particular order of coherence the signal which is subsequently refocused will almost always be half the intensity of that which can be observed in a phase cycled experiment. This factor comes about simply because it is likely that the phase cycled experiment will be able to retain two symmetrical pathways, whereas the gradient selection method will only be able to refocus one of these.

The foregoing discussion applies to the case of a selection gradient placed in a *fixed* delay of a pulse sequence. The matter is different if the gradient is placed within the incrementable time of a multi-dimensional experiment, *e.g.* in t_1 of a two-dimensional experiment. To understand the effect that such a gradient has on the sensitivity of the experiment it is necessary to be rather careful in making the comparison between the gradient selected and phase cycled experiments. In the case of the latter experiments we need to include the SHR or TPPI method in order to achieve frequency discrimination with absorption mode lineshapes. If a gradient is used in t_1 we will need to record separate P- and N-type spectra so that they can be recombined to give an absorption mode spectrum. We must also ensure that the two spectra we are comparing have the same limiting resolution in the t_1 dimension, that is they achieve the same maximum value of t_1 and, of course, the total experiment time must be the same.

The detailed argument which is needed to analyse this problem is beyond the scope of this lecture; it is given in detail in *J. Magn. Reson Ser. A*, **111**, 70-76 (1994)[†]. The conclusion is that the signal-to-noise ratio of an absorption mode spectrum generated by recombining P- and N-type gradient selected spectra is lower, by a factor $1/\sqrt{2}$, than the corresponding phase cycled spectrum with SHR or TPPI data processing.

The potential reduction in sensitivity which results from selection with gradients may be more than compensated for by an improvement in the *quality* of the spectra obtained in this way. Often, the factor which limits whether or not a cross peak can be seen is not the *thermal* noise level by the presence of other kinds of "noise" associated with imperfect cancellation *etc*.

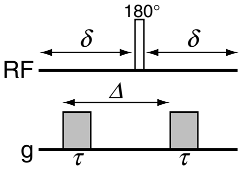
9.5.6 Diffusion

The process of refocusing a coherence which has been dephased by a gradient pulse is inhibited if the spins move either during or between the defocusing and refocusing gradients. Such movement alters the magnetic field experienced by the spins so that the phase acquired during the refocusing gradient is not exactly opposite to that acquired during the defocusing gradient.

In liquids there is a translational diffusion of both solute and solvent which causes such movement at a rate which is fast enough to cause significant effects on NMR experiments using gradient pulses. As diffusion is a random process we expect to see a smooth attenuation of the intensity of the refocused signal as the diffusion contribution increases. These effects have been known and exploited to measure diffusion constants since the very earliest days of NMR.

[†] There is an error in this paper: in Fig. 1(b) the penultimate S spin 90° pulse should be phase y and the final S spin 90° pulse is not required.

The effect of diffusion on the signal intensity from the simple echo sequence shown opposite is relatively simple to analyse and captures all of the essential points. Note that the two gradient pulses can be placed anywhere in the intervals δ either side of the 180° pulse. For a single uncoupled resonance, the intensity of the observed signal, S, expressed as a fraction of the signal intensity in the absence of a gradient, S_0 is given by



$$\frac{S}{S_0} = \exp\left(-\gamma^2 G^2 \tau^2 \left(\Delta - \frac{\tau}{3}\right) D\right) \qquad [15]$$

where D is the diffusion constant, Δ is the time between the start of the two gradient pulses and τ is the duration of the gradient pulses; relaxation has been ignored. For a given pair of gradient pulses it is diffusion during the interval between the two pulses, Δ, which determines the attenuation of the echo. The stronger the gradient the more rapidly the phase varies across the sample and thus the more rapidly the echo will be attenuated. This is the physical interpretation of the term $\gamma^2 G^2 \tau^2$ in Eqn. [15].

Diffusion constants generally decrease as the molecular mass increases. A small molecule, such as water, will diffuse up to twenty times faster than a protein with molecular weight 20,000. The table shows the loss in intensity due to diffusion for typical gradient pulse pair of 2 ms duration and of strength 10 G cm^{-1} for a small, medium and large sized molecule; data is given for $\Delta = 2$ ms and $\Delta = 100$ ms. It is seen that even for the most rapidly diffusing molecules the loss of intensity is rather small for $\Delta = 2$ ms, but becomes significant for longer delays. For large molecules, the effect is small in all cases.

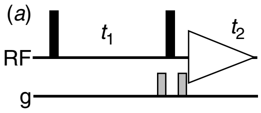
Fraction of transverse magnetization refocused after a spin echo with gradient refocusing[a]

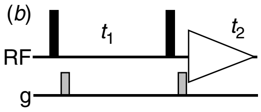
Δ/ms	small molecule[b]	medium sized molecule[c]	macro molecule[d]
2	0.99	1.00	1.00
100	0.55	0.88	0.97

[a] Calculated for the pulse sequence shown above for two gradients of strength 10 G cm^{-1} and duration, τ, 2 ms; relaxation is ignored. [b] Diffusion constant, D, taken as that for water, which is 2.1×10^{-9} m^2 s^{-1} at ambient temperatures. [c] Diffusion constant taken as 0.46×10^{-9} m^2 s^{-1}. [d] Diffusion constant taken as 0.12×10^{-9} m^2 s^{-1}.

9.5.6.1 Minimisation of Diffusion Losses

The foregoing discussion makes it clear that in order to minimise intensity losses due to diffusion the product of the strength and durations of the gradient pulses, $G^2\tau^2$, should be kept as small as is consistent with achieving the required level of suppression. In addition, a gradient pulse pair should be separated by the shortest time, Δ, within the limits imposed by the pulse sequence. This condition applies to gradient pairs the first of which is responsible for dephasing, and the second for rephasing. Once the coherence is rephased the time that elapses before further gradient pairs is irrelevant from the point of view of diffusion losses.





In two-dimensional NMR, diffusion can lead to line broadening in the F_1 dimension if t_1 intervenes between a gradient pair. Consider the two alternative pulse sequences for recording a simple COSY spectrum shown opposite. In (*a*) the gradient pair are separated by the very short time of the final pulse, thus keeping the diffusion induced losses to an absolute minimum. In (*b*) the two gradients are separated by the incrementable time t_1; as this increases the losses due to diffusion will also increase, resulting in an extra decay of the signal in t_1. The extra line broadening due to this decay can be estimated from Eqn. [15], with $\Delta = t_1$, as $\gamma^2 G^2 \tau^2 D/\pi$ Hz. For a pair of 2 ms gradients of strength 10 G cm^{-1} this amounts ≈ 2 Hz in the case of a small molecule.

This effect by which diffusion causes an extra line broadening in the F_1 dimension is usually described as *diffusion weighting*. Generally it is possible to avoid it by careful placing of the gradients. For example, the sequences (*a*) and (*b*) are in every other respect equivalent, thus there is no reason *not* to chose (*a*). It should be emphasised that diffusion weighting occurs only when t_1 intervenes between the dephasing and refocusing gradients.

9.5.7 Some examples of gradient selection

9.5.7.1 Introduction

Reference has already been made to the two general advantages of using gradient pulses for coherence selection, namely the possibility of a general improvement in the quality of spectra and the removal of the requirement of completing a phase cycle for each increment of a multi-dimensional experiment. This latter point is particularly significant when dealing with three- and four-dimensional experiments.

The use of gradients results in very significant improvement in the quality of proton-detected heteronuclear experiments, especially when unlabelled samples are used. In such experiments, gradient selection results in much lower dynamic range in the free induction decay as compared to phase cycled experiments.

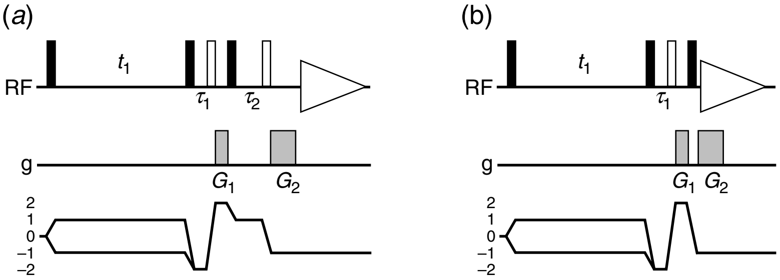
As has been discussed above, special care needs to be taken in experiments which use gradient selection in order to retain absorption mode lineshapes.

In the following sections the use of gradient selection in several different experiments will be described. The gradient pulses used in these sequences will be denoted G_1, G_2 *etc.* where G_i implies a gradient of duration τ_i, strength $B_{g,i}$ and shape factor s_i. There is always the choice of altering the duration, strength or, conceivably, shape factor in order to establish refocusing. Thus, for brevity we shall from now on write the spatially dependent phase produced by gradient G_i acting on coherence of order p as $\gamma p G_i$ in the homonuclear case or

$$\sum_j \gamma_j p_j G_i$$

in the heteronuclear case; the sum is over all types of nucleus.

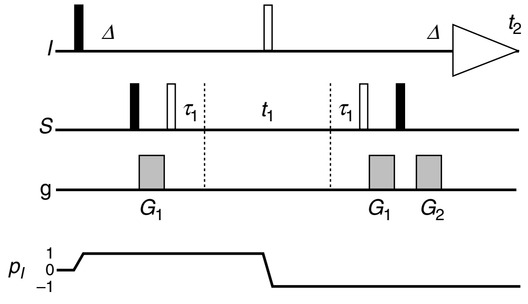
9.5.7.2 Double-quantum Filtered COSY



This experiment has already been discussed in detail in previous sections; sequence (*a*) is essentially that described already and is suitable for recording absorption mode spectra. The refocusing condition is $G_2 = 2G_1$; frequency discrimination in the F_1 dimension is achieved by the SHR or TPPI procedures. Multiple quantum filters through higher orders can be implemented in the same manner.

In sequence (*b*) the final spin echo is not required as data acquisition is started *immediately* after the final radiofrequency pulse; phase errors which would accumulate during the second gradient pulse are thus avoided. Of course, the signal only rephases towards the end of the final gradient, so there is little signal to be observed. However, the crucial point is that, as the magnetization is all in antiphase at the start of t_2, the signal grows from zero at a rate determined by the size the couplings on the spectrum. Provided that the gradient pulse is much shorter that $1/J$, where J is a typical proton-proton coupling constant, the part of the signal missed during the gradient pulse is not significant and the spectrum is not perturbed greatly. An alternative procedure is to start to acquire the data *after* the final gradient, and then to right shift the free induction decay, bringing in zeroes from the left, by a time equal to the duration of the gradient.

9.5.7.3 HMQC



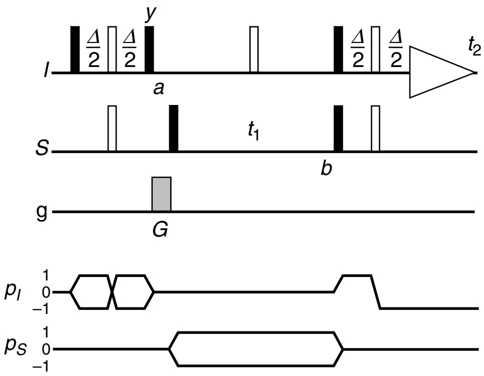
There are several ways of implementing gradient selection into the HMQC experiment, one of which, which leads to absorption mode spectra, is shown above. The centrally placed I spin 180° pulse results in no net dephasing of the I spin part of the heteronuclear multiple quantum coherence by the two gradients G_1 *i.e.* the dephasing of the I spin coherence caused by the first is undone by the second. However, the S spin coherence experiences a net dephasing due to these two gradients and this coherence is subsequently refocused by G_2. Two 180° S spin pulses together with the delays τ_1 refocus shift evolution during the two gradients G_1. The centrally placed 180° I spin pulse refocuses chemical shift evolution of the I spins during the delays Δ and all of the gradient pulses (the last gradient is contained within the final delay, Δ). The refocusing condition is

$$\mp 2\gamma_s G_1 - \gamma_I G_2 = 0$$

where the + and – signs refer to the P- and N-type spectra respectively. The switch between recording these two types of spectra is made simply by reversing the sense of G_2. The P- and N-type spectra are recorded separately and then combined in the manner described in section 9.3.4.2 to give a frequency discriminated absorption mode spectrum.

In the case that I and S are proton and carbon-13 respectively, the gradients G_1 and G_2 are in the ratio 2:±1. Proton magnetization not involved in heteronuclear multiple quantum coherence, *i.e.* magnetization from protons not coupled to carbon-13, is refocused after the second gradient G_1 but is then dephased by the final gradient G_2. Provided that the gradient is strong enough these unwanted signals, and the t_1-noise associated with them, will be suppressed.

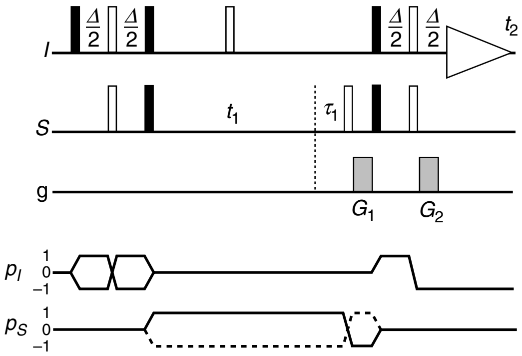
9.5.7.4 HSQC



The sequence above shows the simplest way of implementing gradients into the HSQC experiment. An analysis using product operators shows that at point *a* the required signal is present as the operator $2I_zS_z$ whereas the undesired signal (from I spins not coupled to S spins) is present as I_y. Thus, a field gradient applied at point *a* will dephase the unwanted magnetization and leave the wanted term unaffected. This is an example of using gradients not for selection, but for suppression of unwanted magnetization (see section 9.5.3.2).

The main practical difficulty with this approach is that the unwanted magnetization is only along *y* at point *a* provided all of the pulses are perfect; if the pulses are imperfect there will be some *z*-magnetization present which will not be eliminated by the gradient. In the case of observing proton-^{13}C or proton-^{15}N HSQC spectra from natural abundance samples, the magnetization from uncoupled protons is very much larger than the wanted magnetization, so even very small imperfections in the pulses can give rise to unacceptably large residual signals. However, for globally labelled samples the degree of suppression is often sufficient and such an approach is used successfully in many three- and four-dimensional experiments applied to globally ^{13}C and ^{15}N labelled proteins.

The key to obtaining the best suppression of the uncoupled magnetization is to apply a gradient when transverse magnetization is present on the *S* spin. An example of the HSQC experiment utilising such a principle is shown below



HSQC pulse sequence with gradient selection. The CTP for an N-type spectrum is shown by the full line and for the P-type spectrum by the dashed line.

Here, G_1 dephases the *S* spin magnetization present at the end of t_1 and, after transfer to the *I* spins, G_2 refocuses the signal. An extra 180° pulse to *S* in conjunction with the extra delay τ_1 ensures that phase errors which accumulate during G_1 are refocused; G_2 is contained within the final spin echo which is part of the usual HSQC sequence. The refocusing condition is

$$\mp\gamma_S G_1 - \gamma_I G_2 = 0$$

where the – and + signs refer to the N- and P-type spectra respectively. As before, an absorption mode spectrum is obtained by combining the N- and P-type spectra, which can be selected simply by reversing the sense of G_2.

9.6 Zero-quantum dephasing and purge pulses

Both *z*-magnetization and homonuclear zero-quantum coherence have coherence order 0, and thus neither are dephased by the application of a gradient pulse. Selection of coherence order zero is achieved simply by applying a gradient pulse which is long enough to dephase all other coherences; no refocusing is used. In the vast majority of experiments it is the *z*-magnetization which is required and the zero-quantum coherence that is selected at the same time is something of a nuisance.

A number of methods have been developed to suppress contributions to the spectrum from zero-quantum coherence. Most of these utilise the property that zero-quantum coherence evolves in time, whereas *z*-magnetization does not. Thus, if several experiments in which the zero-quantum has been allowed to evolve for different times are co-added, cancellation of zero-quantum contributions to the spectrum will occur. Like phase cycling, such a method is time consuming and relies on a difference procedure. However, it has been shown that if a field gradient is combined with a period of spin-locking the coherences which give rise to these zero-quantum coherences can be dephased. Such a process is conveniently considered as a modified purging pulse.

9.6.1 Purging pulses

A purging pulse consists of a relatively long period of spin-locking, taken here to be applied along the *x*-axis. Magnetization not aligned along *x* will precess about the spin-locking field and, because this field is inevitably inhomogeneous, such magnetization will dephase. The effect is thus to purge all magnetization except that aligned along *x*. However, in a coupled spin system certain anti-phase states aligned *perpendicular* to the spin-lock axis are also preserved. For a two spin system (with spins *k* and *l*), the operators preserved under spin-locking are I_{kx}, I_{lx} and the anti-phase state $2I_{ky}I_{lz} - 2I_{kz}I_{ly}$. Thus, in a coupled spin system, the purging effect of the spin-locking pulse is less than perfect.

The reason why these anti-phase terms are preserved can best be seen by transforming to a tilted co-ordinate system whose *z*-axis is aligned with the *effective* field seen by each spin. For the case of a strong B_1 field placed close to resonance the effective field seen by each spin is along *x*, and so the operators are transformed to the tilted frame simply by rotating them by –90° about *y*

$$I_{kx} \xrightarrow{-\pi/2 I_{ky}} I_{kz}^{\mathrm{T}} \qquad I_{lx} \xrightarrow{-\pi/2 I_{ly}} I_{lz}^{\mathrm{T}}$$

$$2I_{ky}I_{lz} - 2I_{kz}I_{ly} \xrightarrow{-\pi/2\left(I_{ky}+I_{ly}\right)} 2I_{ky}^{\mathrm{T}}I_{lx}^{\mathrm{T}} - 2I_{kx}^{\mathrm{T}}I_{ly}^{\mathrm{T}}$$

Operators in the tilted frame are denoted with a superscript T. In this frame the *x*-magnetization has become *z*, and as this is parallel with the effective field, it clearly does not dephase. The anti-phase magnetization along *y* has become

$$2I_{ky}^{\mathrm{T}}I_{lx}^{\mathrm{T}} - 2I_{kx}^{\mathrm{T}}I_{ly}^{\mathrm{T}}$$

which is recognised as *zero-quantum coherence in the tilted frame*. Like zero-quantum coherence in the normal frame, this coherence does not dephase in a strong spin-locking field. There is thus a connection between the inability of a field gradient to dephase zero-quantum coherence and the preservation of certain anti-phase terms during a purging pulse.

Zero-quantum coherence in the tilted frame evolves with time at a frequency, $\Omega_{\mathrm{ZQ}}^{\mathrm{T}}$, given by

$$\Omega_{\mathrm{ZQ}}^{\mathrm{T}} = \left| \sqrt{\left(\Omega_k^2 + \omega_1^2\right)} - \sqrt{\left(\Omega_l^2 + \omega_1^2\right)} \right|$$

where Ω_i is the offset from the transmitter of spin *i* and ω_1 is the B_1 field strength. If a field gradient is applied during the spin-locking period the zero quantum frequency is modified to

$$\Omega_{\mathrm{ZQ}}^{\mathrm{T}}(r) = \left| \sqrt{\left(\left\{\Omega_k + \gamma B_{\mathrm{g}}(r)\right\}^2 + \omega_1^2\right)} - \sqrt{\left(\left\{\Omega_l + \gamma B_{\mathrm{g}}(r)\right\}^2 + \omega_1^2\right)} \right|$$

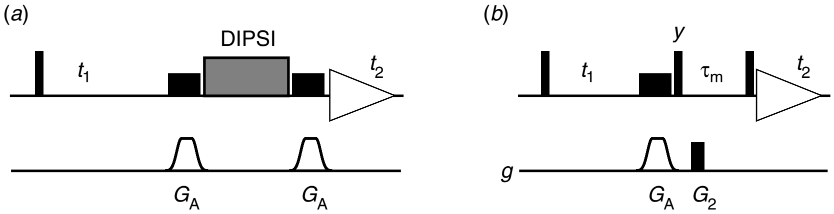
This frequency can, under certain circumstances, become spatially dependent and thus the zero-quantum coherence in the tilted frame will dephase. This is in contrast to the case of zero-quantum coherence in the laboratory frame which is not dephased by a gradient pulse.

The principles of this dephasing procedure are discussed in detail elsewhere (*J. Magn. Reson. Ser. A* **105**, 167-183 (1993)). Here, we note the following features. (a) The optimum dephasing is obtained when the extra offset induced by the gradient at the edges of the sample, $\gamma B_g(r_{\max})$, is of the order of ω_1. (b) The rate of dephasing is proportional to the zero-quantum frequency in the absence of a gradient, $(\Omega_k - \Omega_l)$. (c) The gradient must be switched on and off adiabatically. (d) The zero-quantum coherences may also be dephased using the inherent inhomogeneity of the radio-frequency field produced by typical NMR probes, but in such a case the optimum dephasing rate is obtained by spin locking off-resonance so that

$$\tan^{-1}\left(\omega_1/\Omega_{k,l}\right) \approx 54°.$$

(e) Dephasing in an inhomogeneous B_1 field can be accelerated by the use of special composite pulse sequences.

The combination of spin-locking with a gradient pulse allows the implementation of essentially perfect purging pulses. Such a pulse could be used in a two-dimensional TOCSY experiment whose pulse sequence is shown below as (a).



Pulse sequences using purging pulses which comprise a period of spin locking with a magnetic field gradient. The field gradient must be switched on and off in an adiabatic manner.

In this experiment, the period of isotropic mixing transfers in-phase magnetization (say along *x*) between coupled spins, giving rise to cross-peaks which are absorptive and in-phase in both dimensions. However, the mixing sequence also both transfers and generates anti-phase magnetization along *y*, which gives rise to undesirable dispersive anti-phase contributions in the spectrum. In sequence (*a*) these anti-phase contributions are eliminated by the use of a purging pulse as described here. Of course, at the same time all magnetization other than *x* is also eliminated, giving a near perfect TOCSY spectrum without the need for phase cycling or other difference measures.

These purging pulses can be used to generate pure *z*-magnetization without contamination from zero-quantum coherence by following them with a 90°(*y*) pulse, as is shown in the NOESY sequence (*b*). Zero-quantum coherences present during the mixing time of a NOESY experiment give rise to troublesome dispersive contributions in the spectra, which can be eliminated by the use of this sequence.