

XHCORR

Introduction

10.1

Heteronuclear (**X, H**) shift **COR**relation spectroscopy is a 2D technique that can be used to determine which ^1H 's of a molecule are bonded to which ^{13}C nuclei (or other X nuclei). Like DEPT, XHCORR makes use of the large one-bond heteronuclear J-coupling (J_{XH}) for polarization transfer, and so only ^{13}C 's bonded directly to ^1H 's are detected. For ^{13}C 's and directly attached ^1H 's, $J_{\text{XH}} = 100$ to 200 Hz, while for more distant ^1H 's $J_{\text{XH}} = 5$ to 20 Hz.

The XHCORR experiment yields a series of amplitude modulated ^{13}C spectra. The amplitude modulation contains information on ^1H chemical shifts and J_{HH} -couplings which are the source of ^1H evolution during t_1 . The ^1H chemical shifts and coupling constants that appear in t_1 are those that have controlled the ^{13}C population distribution during that time period.

The short delay between the final ^{13}C pulse and the start of acquisition is a refocusing time added so that the ^{13}C lines no longer have opposite phase and thus do not cancel one another when ^1H -decoupling is applied. The optimal refocusing time (Δ_2) depends on whether the ^{13}C belongs to a CH, CH_2 , or CH_3 group. Generally a compromise value of $\Delta_2 = 1/(3J_{\text{XH}})$ is chosen. ^{13}C couplings during t_1 are removed by adding a ^{13}C π pulse in the middle of t_1 , so that there is refocusing by the end of t_1 . To enable maximum polarization transfer, a fixed delay $\Delta_1 = 1/(2J_{\text{XH}})$ is added after t_1 . This delay allows anti-phase magnetization to be re-established.

Like DEPT, XHCORR requires phase cycling to eliminate signal from “natural magnetization,” i.e., ^{13}C z-magnetization during t_1 that is transformed into observable transverse magnetization by the final ^{13}C $\pi/2$ pulse. In a 2D experiment, such magnetization gives rise to an “axial” peak (large peak along $F1 = 0$). Extra phase cycling is required to obtain quadrature detection in F1.

The final 2D XHCORR spectrum has a projection onto the F2 axis which is the normal ^1H -decoupled ^{13}C spectrum with all quaternary carbons missing, and a projection onto the F1 axis which is the normal ^1H spectrum with reduced signal to noise since only ^1H 's directly attached to ^{13}C contribute to the signal. The XHCORR experiment is not phase-sensitive, and so the final 2D spectrum must be displayed in magnitude mode.

Reference: A. Bax and G. A. Morris, *J. Magn. Reson.*, **42**, 501 (1981).

Sample

The sample used to demonstrate XHCORR in this chapter is 1 g Cholesterylacetate in CDCl_3 . This is the same sample that was used to demonstrate DEPT.

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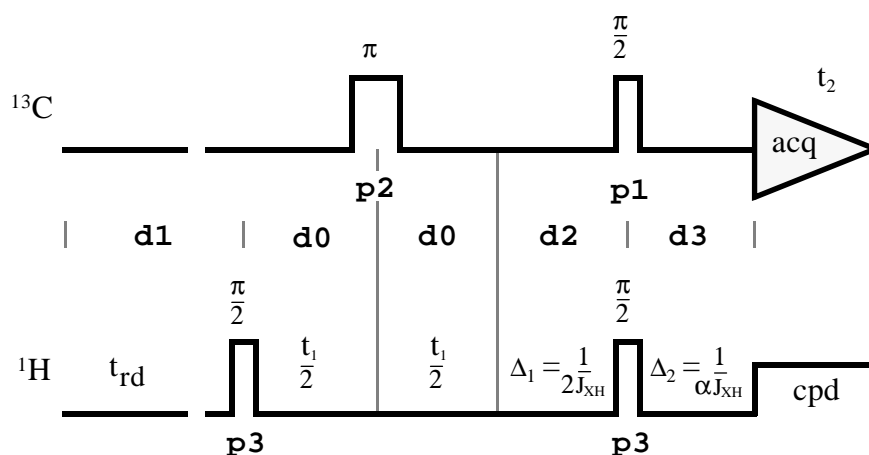
Pulse Sequence Diagram

10.2

The XHCORR pulse sequence is shown in Figure 31. Notice that the pulses **p1** and **p3** must be set to the appropriate 90° times found in Chapter 5 ‘Pulse Calibration’. Also, the cpd sequence used is WALTZ-16, which requires the calibrated 90° time **p31**. The 180° pulse length **p2** is determined by the pulse program itself.

In this pulse sequence, the delay time **d2** determines the length of the delay for the creation of anti-phase magnetization ($\Delta_1 = 1/(2J_{\text{XH}})$), and the time **d3** determines the length of the refocusing delay ($\Delta_2 = 1/(\alpha J_{\text{XH}})$, where α is usually chosen to be 3).

Figure 31: XHCORR Pulse Sequence



Acquisition and Processing

10.3

Make sure the following preliminary steps have been completed: Insert the sample in the magnet. Lock the spectrometer. Readjust the Z and Z^2 shims until the lock level is optimized. Tune and match the probehead for ^{13}C observation, ^1H decoupling.

It is generally recommended that XHCORR, like all 2D experiments, be run without sample spinning.

Note that while setting up to do an XHCORR experiment, the user may find it helpful to refer to Appendix A ‘Data Sets and Selected Parameters’, and Appendix B ‘Pulse Calibration Results’. Appendix A lists data sets generated throughout the course of this manual and also provides a table in which the user can record the **o1**, **o2**, and **sw** values appropriate for the various samples used. Appendix B provides a table in which the user can record the pulse lengths and power levels determined during the pulse calibration procedures described in Chapter 5 ‘Pulse Calibration’.

¹H reference spectrum

Since XHCORR is a ¹³C-observe, ¹H-decouple experiment, the first step is to obtain a reference ¹H spectrum of the sample. This reference spectrum will be used to determine the correct **o2** for ¹H decoupling, the correct **sw** for the F1 dimension, and can also be used as the F1 projection of the XHCORR spectrum.

Recall that in Chapter 3 ‘Basic 1H Acquisition and Processing’ we have already collected a few ¹H spectra of 100mg Cholesterylacetate, so one of these can be used as the starting point for this reference spectrum. Enter **re proton 2 1** to call up the data set proton/2/1. Enter **edc** and change the EXPNO to 4. Click **SAVE** to create the data set proton/4/1.

Enter **rga** to perform an automatic receiver gain adjustment. Acquire and process a standard ¹H spectrum. Calibrate the spectrum and optimize **sw** and **o1** so that the ¹H signals cover almost the entire spectral width.

Acquire an optimized spectrum to be used as the F1 projection of the XHCORR spectrum. (If desired, the number of scans may be increased for this spectrum.)

¹³C reference spectrum

The second step is to obtain a ¹H-decoupled ¹³C reference spectrum to determine the correct **o1** and **sw**, and to be the F2 projection of the XHCORR spectrum. Since XHCORR detects only ¹³C's bonded directly to ¹H's, a DEPT-45 spectrum is typically used as this reference spectrum.

Recall that in Chapter 6 ‘DEPT’ we already collected a DEPT-45 spectrum of this sample. Enter **re dept 1 1** to call up the data set dept/1/1. Enter **edc** and change EXPNO to 4. Click **SAVE** to create the data set dept/4/1.

Check **o2**. This should be set to the value of **o1** in the ¹H reference spectrum obtained above. Acquire and process a DEPT-45 spectrum. Optimize **sw** and **o1** so that the ¹³C signals cover almost the entire spectral width.

Create a new file directory for the 2D data set

From the data set dept/4/1, enter **edc** and change the following parameters:

NAME	xhcorr
EXPNO	1
PROCNO	1 .

Click **SAVE** to create the data set xhcorr/1/1. By creating the XHCORR data set from the data set of the DEPT reference spectrum, most of the F2 parameters for XHCORR are already set.

Change to 2D parameter mode

Enter **eda** and set PARMODE = 2D. Click on **SAVE** and ok the message “Delete ‘meta.ext’ files?”. The window now switches to a 2D display and the message “NEW 2D DATA SET” appears.

Set up the acquisition parameters

Enter **eda** and set the acquisition parameters as shown in Table 35. Use the values determined in Chapter 5 ‘Pulse Calibration’ for the parameters **p11** and **p1** (¹³C observe high power level and 90° pulse time), **p12** and **p3** (¹H decouple high power level and 90° pulse time), and **p112** and **pcpd2** (¹H decouple low power level and 90° pulse time). Note that the pulse program hxco calls an include file in which **cnst2** (defined to be J_{XH}) and **cnst11** (defined to be α, which is usually chosen

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to be 3) are used to calculate **d2** ($d2 = 1/(2 * cnst2)$) and **d3** ($d3 = 1/(cnst11 * cnst2)$). Thus, it is only necessary for the user to set the values of **cnst2** and **cnst11**. Similarly, the 180° pulse length **p2** is calculated from the corresponding 90° pulse length **p1**, so the user need only set the value of **p1**.

The F2 parameters **o1**, **o2**, and **sw** (not shown in the table) should be identical to the values used in the optimized DEPT-45 reference spectrum (dept/4/1). The F1 parameters **sfo1** and **sw** should be identical to the values used in the optimized ¹H reference spectrum (proton/4/1).

Finally, notice that **in0** and sw(F1) are not independent. A convenient way to set **in0** is to set the F1 parameters **NUC1** by clicking on **NUCLEI** for F1 parameters, **nd0**, and **sw** correctly. This automatically sets **in0** to the correct value.

Table 35. XHCORR Acquisition Parameters

F2 Parameters		
Parameter	Value	Comments
PULPROG	hxco	see Figure 31 for pulse sequence diagram.
TD	1k	
NS	8	the number of scans must be 4*n for the phase cycling to work properly.
DS	16	number of dummy scans.
PL1		high power level on f1 channel (see “An Important Note on Power Levels” on page 7).
PL2		high power level on f2 channel (see “An Important Note on Power Levels” on page 7).
PL12		power level for cpd on f2 channel.
P1		90° ¹³ C high power pulse on f1 channel.
P2		180° ¹³ C high power pulse on f1 channel; calculated internally.
P3		90° ¹ H high power pulse on f2 channel.
PCPD2		90° ¹ H pulse for cpd sequence.
D0	3μsec	incremented delay ($t_1/2$); predefined.
D1	2sec	relaxation delay; should be about $1.25 * T_1(^{13}C)$.
D2	3.45msec	delay for creation of anti-phase magnetization ($1/(2J_{XH})$); calculated internally.
D3	2.30msec	refocusing delay; choose $1/(3J_{XH})$ for all multiplicities; calculated internally.
D11	30msec	delay for disk I/O; predefined.
CNST2	145Hz	one-bond heteronuclear J-coupling (J_{XH}); used to calculate d2; 145Hz is a good intermediate value for ¹³ C.

CNST11	3	constant used with cnst2 to calculate d3; choose 3 for all multiplicities.
CPDPRG2	waltz16	composite pulse decoupling sequence.
F1 Parameters		
Parameter	Value	Comments
TD	256	number of experiments.
ND0	2	there are two d0 periods per cycle and MC2 = QF.
IN0	$1/(2*SW_H) = DW_H$	t ₁ increment.
SW		sw of the optimized ¹ H spectrum (proton/4/1).
NUC1		selects ¹ H frequency for F1.

Acquire the 2D data set

If this data set was created from the DEPT-45 reference spectrum dept/4/1, the receiver gain is already set correctly.

Enter **zg** to acquire the time domain data. The approximate experiment time for XHCORR with the acquisition parameters set as shown above is 2.5 hours.

Set up the processing parameters

Enter **edp** and set the processing parameters as shown in Table 36.

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Table 36. XHCORR Processing Parameters

F2 Parameters		
Parameter	Value	Comments
SI	1k	
SF		spectrum reference frequency (^{13}C).
WDW	EM	(for example).
LB	3Hz	a value of 2—5Hz is appropriate.
PH_mod	no	this is a magnitude spectrum.
PKNL	TRUE	necessary when using the digital filter.
BC_mod	quad	
F1 Parameters		
Parameter	Value	Comments
SI	512	
SF		spectrum reference frequency (^1H).
WDW	SINE	multiply data by phase-shifted sine function.
SSB	2	choose pure cosine wave.
PH_mod	mc	this is a magnitude spectrum.
BC_mod	no	
MC2	QF	determines type of FT in F1; QF results in a forward quadrature complex FT.

Process the 2D data set

Enter **xfb** to multiply the time domain data by the window functions and also perform the 2D Fourier transform. The 2D data set is displayed automatically.

Adjust the contour levels

The threshold level can be adjusted by placing the cursor on the  button, holding down the left mouse button, and moving the mouse up and down. The button **#colors** is used to set the number of levels. This means the intensity range of the displayed peaks.

The user can choose to display positive peaks only, negative peaks only, or both positive and negative peaks by clicking on **+/-** with the left mouse button. Since this is a magnitude spectrum, only positive peaks need to be displayed.

Phase correct the spectrum

Since this is a magnitude spectrum, no phase adjustment can be made.

Plot the spectrum

Read in the plot parameter file `standard2D` by entering **rpar**, selecting **standard2D** from the menu of parameter file names, and then selecting **plot** from the menu of parameter file types that appears. Equivalently, simply enter **rpar standard2D plot**. This sets most of the plotting parameters to values which are appropriate for this 2D spectrum, assuming that the paper size to be used here is the same as the default paper size defined when the spectrometer was configured.

More information about plotting parameters and the file `standard2D` can be found in Appendix C '1D and 2D Plotting Parameters'.

To set the region (full or expanded), threshold, and peak type (positive and/or negative) to be used in plotting the spectrum, first make sure the spectrum appears as desired on the screen, and then click **DefPlot** and answer the following questions.

```
Change levels?          y
Please enter number of positive levels?      6
Display contours?      n .
```

Enter **edg** to edit the plotting parameters.

Within the **edg** menu, it is a good idea to change the separation between tic marks on the F2 axis (i.e., the ^{13}C axis). Click the **ed** next to the parameter `EDAXIS` to enter the F1- and F2-axis parameters submenu. Change the value of the parameter `X2TICD` from 0.1 to 2.5. Click **SAVE** to save this change and return to the **edg** menu.

Next it is necessary to define the files that will be used as the F1- and F2-projections of the XHCORR spectrum. Click the **ed** next to the parameter `EDPROJ1` to enter the F1-projection parameters submenu. Edit the parameters from `PF1DU` to `PF1PROC` as follows:

```
PF1DU          u
PF1USER        (name of user for file proton/4/1)
PF1NAME        proton
PF1EXP         4
PF1PROC        1 .
```

Click **SAVE** to save these changes and return to the **edg** menu.

Click the **ed** next to the parameter `EDPROJ2` to enter the F2-projection parameters submenu. Edit the parameters from `PF2DU` to `PF2PROC` as follows:

```
PF2DU          u
PF2USER        (name of user for file dept/4/1)
PF2NAME        dept
PF2EXP         4
PF2PROC        1 .
```

Click **SAVE** to save these changes and return to the **edg** menu.

Click **SAVE** to save all the above changes and exit the **edg** menu.

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Next create a title for the spectrum. Enter **setti** to use the editor to open the title file. Write a title and save the file.

To plot the spectrum, simply enter **plot** (provided the correct plotter is selected in **edo**).

An XHCORR spectrum of 1g Cholesterylacetate in CDCl_3 is shown in Figure 32.

Figure 32: XHCORR Spectrum of 1 g Cholesterylacetate in $CDCl_3$

