Chapter 11

# COLOC

Goto

## Introduction 11.1

**CO**rrelation spectroscopy via **LO**ng-range **C**oupling, like XHCORR, is a 2D heteronuclear correlation technique that can be used to determine which $^1$H's of a molecule are bonded to which $^{13}$C nuclei (or other X nuclei). Unlike XHCORR, however, COLOC makes use of small long-range heteronuclear J-couplings ($^nJ_{XH}$, $n > 1$) for polarization transfer, and so all $^{13}$C's are detected, even those which are not bonded directly to $^1$H's.

In COLOC, the evolution time $t_1$ is incorporated in the polarization transfer period $\Delta_1 = 1/(2\,^nJ_{XH})$. Since the long-range heteronuclear coupling constants are small ($^nJ_{CH}$ = 5 to 20 Hz), the time period $\Delta_1$ is long. By eliminating the need for an additional $t_1$ period, COLOC reduces the magnetization loss due to relaxation that is inevitable during such a long pulse sequence. The evolution time is included in $\Delta_1$ by shifting a pair of $\pi$ pulses throughout $\Delta_1$. Homo- and heteronuclear couplings evolve during $\Delta_1$ but $^1$H chemical shift evolves only in $\Delta_1 - t_1$. Notice that $\Delta_1 - t_1$ is negative when the $\pi$ pulses happen after $\Delta_1/2$.

The delays $\Delta_1$ and $\Delta_2$ can be optimized using the refocused INEPT sequence. Such an optimization holds for the entire experiment whereas for XHCORR applied to long-range correlations, similarly optimized parameters do not hold entirely because of evolution of homonuclear coupling in $t_1$.

The 2D spectrum generated by a 2D Fourier transform with respect to $t_1$ and $t_2$ must be displayed in magnitude mode.

**Reference:** H. Kessler, C. Griesinger, J. Zarbock, and H. R. Loosli, *J. Magn. Reson.*, **57**, 331 (1984).

### Sample

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

## Pulse Sequence Diagram 11.2

The COLOC pulse sequence is shown in Figure 33. 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 lengths **p2** and **p4** are determined by the pulse program itself.

In this pulse sequence, the delay time **d6** determines the length of the delay for the creation of anti-phase magnetization ($\Delta_1 = 1/(2\,^nJ_{XH})$), and the time **d18** determines the length of the refocusing delay ($\Delta_2 = 1/(\alpha\,^nJ_{XH})$, where $\alpha$ is generally chosen to be 3).

To ensure that the $\pi$ pulses occur during $\Delta_1$, the user must make sure that **d6** Š **d0** + (**td**(F1)***in0**) + (**p2** or **p4**); in other words, that **d6** is at least as long as the maximum evolution time ($t_1$) plus the length of the longest $\pi$ pulse (**p2** or **p4**).

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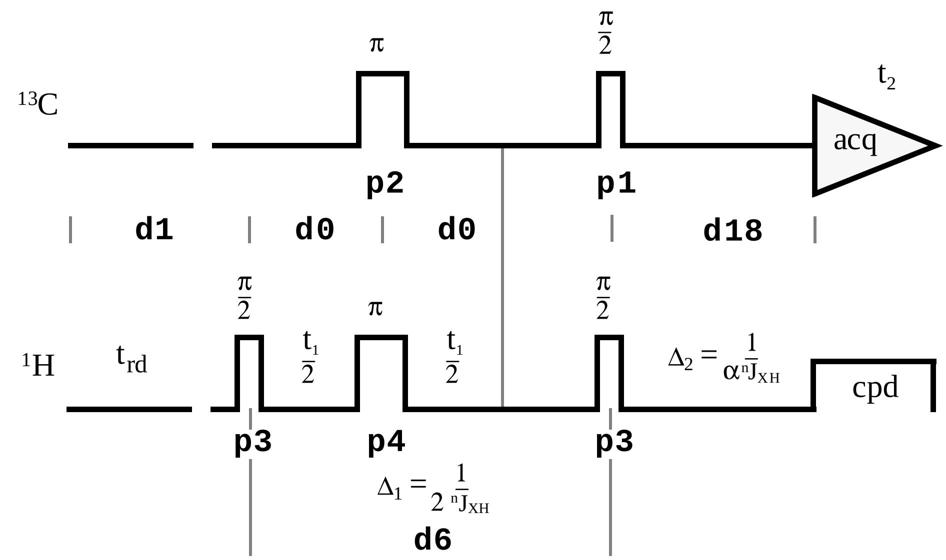
*Figure 33: COLOC Pulse Sequence*




## Acquisition and Processing 11.3

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, $^{1}H$ decoupling.

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

Note that while setting up to do a COLOC 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’.

### $^{1}H$ reference spectrum

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

A $^{1}H$ reference spectrum of this sample was obtained in Chapter 10 ‘XHCORR’, and can be found in the data set proton/4/1.

### $^{13}C$ reference spectrum

The second step is to obtain a $^{1}H$-decoupled $^{13}C$ reference spectrum to determine the correct **o1** and **sw**, and to be the F2 projection of the COLOC spectrum. Note that unlike XHCORR, COLOC detects all types of $^{13}$C's, not only those bonded directly to $^{1}$H's. Thus, a standard $^{1}$H-decoupled $^{13}$C spectrum, ***not*** a DEPT-45 spectrum, should be used as the reference spectrum.

Recall that in Section 4.3 we already collected a $^{1}$H-decoupled $^{13}$C spectrum of this sample. Enter **re carbon 3 1** to call up the data set carbon/3/1. Enter **edc** and change EXPNO to 4. Click **SAVE** to create the data set carbon/4/1.

Check **o2**. This should be set to the value of **o1** in the $^{1}$H reference spectrum above. Enter **eda** and set CPDPRG2 to waltz16. Also set **pl12** and **pcpd2** to the appropriate power level and pulse time for a $^{1}$H low power 90° decouple pulse, as determined in Section 5.4.3 on page 54. The acquisition parameters are now correctly set for a $^{13}$C spectrum with WALTZ-16 composite pulse decoupling.

Acquire and process a spectrum. Optimize **sw** and **o1** so that the $^{13}$C signals cover almost the entire spectral width.

### Create a new file directory for the 2D data set

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

| | |
|---|---|
| NAME | coloc |
| EXPNO | 1 |
| PROCNO | 1 . |

Click **SAVE** to create the data set coloc/1/1. By creating the COLOC data set from data set of the $^{13}$C reference spectrum, many of the F2 parameters for COLOC 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 37. Use the values determined in Chapter 5 'Pulse Calibration' for the parameters **pl1** and **p1** ($^{13}$C observe high power level and 90° pulse time), **pl2** and **p3** ($^{1}$H decouple high power level and 90° pulse time), and **pl12** and **pcpd2** ($^{1}$H decouple low power level and 90° pulse time). Note that the pulse program coloc calls an include file in which **p1** and **p3** are used to calculate **p2** and **p4**, respectively. Thus, it is only necessary for the user to set the value of **p1** and **p3**. On the other hand, the delays **d6** and **d18** are not defined in the include file, and so must be set explicitly in **eda**.

The F2 parameters **o1**, **o2**, and **sw** (not shown in the table) should be identical to the values used in the optimized $^{13}$C reference spectrum (carbon/4/1). The F1 parameters **sfo1** and **sw** should be identical to the values used in the optimized $^{1}$H 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.

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**Table 37. COLOC Acquisition Parameters**

| F2 Parameters | | |
|---|---|---|
| Parameter | Value | Comments |
| PULPROG | coloc | see Figure 33 for pulse sequence diagram. |
| TD | 1k | |
| NS | 64 | the number of scans should be 16*n in order 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. |
| P4 | | 180° $^{1}H$ high power pulse on f2 channel; calculated internally. |
| PCPD2 | | 90° $^{1}H$ pulse for cpd sequence. |
| D0 | 3µsec | incremented delay ($t_1/2$); predefined. |
| D1 | 2sec | relaxation delay; should be about $1.25*T_1(^{13}C)$. |
| D6 | ~50msec | delay for evolution of long-range couplings ($1/(2^nJ_{XH})$); make sure that d6 Š d0 + (td1*in0) + (p2 or p4). |
| D11 | 30msec | delay for disk I/O; predefined. |
| D12 | 20µsec | delay for power switching; predefined. |
| D18 | ~33.3msec | delay for evolution of long-range couplings ($1/(\alpha^nJ_{XH})$); generally a compromise value of $1/(3^nJ_{XH})$ is chosen. |
| CPDPRG2 | waltz16 | composite pulse decoupling sequence. |
| F1 Parameters | | |
| Parameter | Value | Comments |
| TD | 128 | 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 | | select $^1$H frequency for F1. |

### Acquire the 2D data set

If this data set was created from the $^{13}$C reference spectrum carbon/4/1, the receiver gain is already set correctly.

Enter **zg** to acquire the time domain data. The approximate experiment time for COLOC with the acquisition parameters set as shown above is 5 hours.

### Set up the processing parameters

Enter **edp** and set the processing parameters as shown in Table 38.

**Table 38. COLOC Processing Parameters**

| F2 Parameters | | |
|---|---|---|
| Parameter | Value | Comments |
| SI | 512 | |
| SF | | spectrum reference frequency ($^{13}$C). |
| WDW | EM | (for example). |
| LB | 2–5 Hz | |
| 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 ($^1$H). |
| 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. |

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### Process the 2D data set

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

### 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.

Since this is a magnitude spectrum, click on **+/-** with the left mouse button until only the positive peaks are displayed.

The optimum display (both the threshold and which peaks are displayed) may be saved by clicking on **DefPlot**.

### 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, e.g., 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.

Click the **ed** next to the parameter EDAXIS to enter the 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.

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 carbon/4/1) |
| PF2NAME | carbon |

| | |
|---|---|
| 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.

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**).

A COLOC spectrum of 1 g Cholesterylacetate in $CDCl_3$ is shown in Figure 34.

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*Figure 34: COLOC Spectrum of 1 g Cholesterylacetate in $CDCl_3$*