UVU422

MFL-15AUG91CD
VVM-17APR92UD

External Referencing of Samples on the U400

There are several ways to reference the chemical shifts of a given sample.

1. Reference to the solvent. This method is most commonly used for ^{1}H and ^{13}C NMR where an organic solvent such as $CDCl_3$ is used which has residual protons and carbon-13 at natural abundance.
2. Reference to an added standard. Tetramethylsilane (TMS) is the standard reference for ^{1}H, ^{13}C and ^{29}Si NMR.
3. Reference to a compound in a capillary tube which is placed in the research sample. In this case, the standard might be insoluble in the lock solvent chosen or it might be reactive with the solvent or research sample.
4. Reference to an "external" standard. This usually means that a standard sample is inserted in the spectrometer, a frequency measurement taken, the standard removed, the research sample inserted, a spectrum obtained and the frequency calibration applied to the spectrum.

This fourth method of referencing is the focus of this handout. Three possible cases exist and are given in order of preference for accuracy of the reference.

1. The standard sample and the research sample are both in the same lock solvent at similar concentration and ionic strenghth
2. The standard sample is in a different lock solvent than the research sample.
3. The standard sample is not in a lock solvent, or the research sample is not in a lock solvent.

Three examples are given below.

Example One: The standard sample has a lock solvent.
^{19}F research sample in $CDCl_3$ and $CF_3C_6H_5$ standard in C_6D_6. (Note that $CDCl_3$ at 7.26 ppm andC_6D_6 at 7.24 ppm are close enough in lock frequency that this will work.)

insert the research sample

use the standard setup for F19

jexp1 <>	join experiment 1
LC Main Menu	activate main menu display
LC Setup	activate setup menu
LC Nucleus,Solvent	select nucleus, solvent menu
LC F19	select nucleus
LC CDCl3	select solvent
rts('*current shim library*') <>	retrieve shims
su <>	perform experiment set up

When set up is complete:

load='n' <>	set load shim values to no
dg <>	display acquisition parameters

lock the sample and crudely shim Z1 and Z2 and disconnect the VNMR Acquistion window
set nt to some reasonable number that will allow the peak(s) to be visible

ga <>	start acquisition (will wft when complete)

when acquisition is complete:

f full <>	display full sweep width to screen
aph <>	autophase

make sure that the peak(s) are visible at this sweep width and number of transients

LC Connect (in the VNMR acquisition window)	IMPORTANT:
LC Lock	If you do not turn the lock off before
LC Lock off	ejecting the sample, your reference
eject the research sample	will not be correct.
insert the standard sample	
do not change any parameters	
lock on the standard by	
LC Lock off	turn lock off
LC Lock on	turn lock on

do not change Z0
it might be necessary to increase lockpower and/or lockgain to lock on the standard
once locked, crudely shim Z1 and Z2 and disconnect the VNMR Acquisition Window

nt=4 <>	set number of transients
ga <>	start acquisition (will wft when complete)

when acquisition is complete:

f full <>	display full sweep width to screen
aph <>	autophase
LC HOLD, then release	move first cursor to the left of reference line
RC HOLD, then release	move second cursor to the right of reference line
LC Expand	expand region inside cursors
LC HOLD, then release	move first cursor to center of line
nl <>	select nearest line
rl(-63.73p) <>	set reference (in ppm)
f full <>	display full sweep width to screen
LC HOLD, then release	move first cursor far right of spectrum, to the last data point on the screen
LC Mark	request frequency
cr = _____	write down the frequency (which is in hertz)

It is a good idea at this time to save the reference FID.

LC Connect (in the VNMR acquisition window)	IMPORTANT:
LC Lock	If you do not turn the lock off before
LC Lock off	ejecting the sample, your reference
eject the standard sample	will not be correct.
insert the research sample	
lock on the research sample without changing Z0	
the research sample should now be shimmed as normal and the VNMR Acquisition window disconnected	
do not change any other parameters	
set nt so that the peak(s) will be visible	
ga <>	start acquisition (will wft when complete)

When acquisition is complete:

f full <>	display full sweep width to screen
aph <>	autophase
LC HOLD, then release	move first cursor far right of spectrum, to the last data point on the screen
rl(cr value in hertz) <>	set reference (in hertz)
f full <>	display full sweep width to screen

At this point, it is a good idea to save the standard sweep width FID.

LC Next	access the next display menu
LC Thres	select threshold
LC HOLD, then release	move threshold line to include the peak(s)
axis='p' <>	set axis to ppm
pll page <>	print peak frequencies in Hz and ppm

The written record of the referenced chemical shifts is necessary if the sweep width and/or transmitter offset need to be changed. If the sweep width needs to be made larger or smaller, the spectrometer will not keep a record of the reference position and the peak references will be wrong in the next spectrum. However, using the prinout, the peaks can be easily re-referenced.

Example Two: The standard sample and the research sample have lock solvents with very different chemical shifts. The user will not be able to lock on the reference and should treat this like example three below.

Example Three: The standard sample has no lock solvent.
^{31}P research sample in $CDCl_3$ and 85% H_3PO_4 standard.

insert the research sample

use the standard setup for P31

jexp1 <>	join experiment 1
LC Main Menu	activate main menu display
LC Setup	activate setup menu
LC Nucleus,Solvent	select nucleus, solvent menu
LC P31	select nucleus
LC CDCl3	select solvent
rts('*current shim library*') <>	retrieve shims
su <>	perform experiment set up

when set up is complete:

load='n' <>	set load shim values to no
dg <>	display acquisition parameters

lock the sample and crudely shim Z1 and Z2 and disconnect the VNMR Acquistion window
set nt to some reasonable number that will allow the peak(s) to be visible

ga <>	start acquisition (will wft when complete)

When acquisition is complete:

f full <>	display full sweep width to screen
aph <>	autophase

make sure that the peak(s) are visible at this sweep width and number of transients

LC Connect (in the VNMR acquisition window)	IMPORTANT:
LC Lock	If you do not turn the lock off before
LC Lock off	ejecting the sample, your reference
eject the research sample	will not be correct.
insert the standard sample	
do not change any parameters	
nt=4 <>	set number of transients
ga <>	start acquisition (will wft when complete)

When acquisition is complete:

f full <>	display full sweep width to screen
aph <>	autophase
LC HOLD, then release	move first cursor to the left of reference line
RC HOLD, then release	move second cursor to the right of reference line
LC Expand	expand region inside cursors
LC HOLD, then release	move first cursor to center of line
nl <>	select nearest line
rl(0p) <>	set reference (in ppm)
f full <>	display full sweep width to screen
LC HOLD, then release	move first cursor far right of spectrum, to the last data point on the screen
LC Mark	request frequency
cr = _____	write down the frequency (which is in hertz)

It is a good idea at this time to save the reference FID.

LC Connect (in the VNMR acquisition window)	IMPORTANT:
LC Lock	If you do not turn the lock off before
LC Lock off	ejecting the sample, your reference
eject the standard sample	will not be correct.
insert the research sample	
lock on the research sample without changing Z0	
the research sample should now be shimmed as normal and the VNMR Acquisition window disconnected	
do not change any other parameters	
set nt so that the peak(s) will be visible	
ga <>	start acquisition (will wft when complete)

when acquisition is complete:

f full <>	display full sweep width to screen
aph <>	autophase
LC HOLD, then release	move first cursor far right of spectrum, to the last data point on the screen
rl(cr value in hertz) <>	set reference (in hertz)
f full <>	display full sweep width to screen

At this point, it is a good idea to save the standard sweep width FID.

LC Next	access the next display menu
LC Thres	select threshold
LC HOLD, then release	move threshold line to include the peak(s)
axis='p' <>	set axis to ppm
pll page <>	print peak frequencies in ppm

The written record of the referenced chemical shifts is necessary if the sweep width and/or transmitter offset need to be changed. If the sweep width needs to be made larger or smaller, the spectrometer will not keep a record of the reference position and the peak references will be wrong in the next spectrum. However, using the prinout, the peaks can be easily re-referenced.

NOTE: If vt is used for the research sample, the standard should be run at the same temperature, if possible. Under no circumstances should samples in D_20 or 85% H_3PO_4 be run below 0°C. If the reference sample can not be run at the same temperature as the research sample, note the conditions of the reference sample and the research sample and report these in appropriate experimental sections.