Tips and Tricks
Part-II: Bits and pieces
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Overview

1H -13C correlation experiments

- Which ones to choose and a few pitfalls.

Non-uniform or sparse sampling:

- A good thing for small molecules too!

And an interesting case.......
1H -13C correlation experiments

- So many too choose from:

99 parameter sets, and 144 pulse sequences with “HSQC”
$^{1}H-^{13}C$ Heteronuclear 2D Experiments

**HSQC**

- "Bare Bones"
  - hsqcph
  - hsqcgpph

- hsqcetgp
  - Adiabatic Pulses
    - Shaped Pulses for Inversion (sp)
  - Sensitivity Improved
    - Shaped Pulses for Inversion and Refocusing (.2)
    - Shaped Pulses for Inversion (sp)
    - Gradients in Back INEPT (2)
  - Multiplicity Edited
    - COSY peak Suppression (2.3/4)
    - Shaped Pulses for Inversion (sp)
    - Matched Sweep Adiabatic Pulses (.3)
Adiabatic pulses have a wider band width

Adiabatic pulses are longer

At what field does it become important?

Are there any draw backs?
Adiabatic pulses have a wider band width

Adiabatic pulses are longer
Difference in duration is subtracted out of J/4J(C,H)
Adiabatic pulses have a wider band width

At what field does it become important to use adiabatic pulses?

Typical range of protonated carbons = 0 – 160 ppm + aldehydes

160 ppm
= 16,000 Hz @ 400 MHz
= 24,000 Hz @ 600 MHz
= 32,000 Hz @ 800 MHz
**1H-13C HSQC – Things to Consider**

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$1^H-1^{13}C$ HSQC – Things to Consider

Multiplicity Edited or Not?

$d_{21} = 1/2J_{xh} = 3.6 \text{ ms}$

$\delta = \text{gradient recovery delay} = .2\text{ms}$

$\sim 7 \text{ ms longer of a sequence}$

Depending on the $T_2$ relaxation rates of the molecule the non-edited version might be more sensitive:

But is it worth sacrificing the multiplicity information?
Are there any drawbacks?

What about the 13C 180 in the evolution period?

- Composite adiabatic pulse required (2 msec)
“Matched Sweep” Adiabatic Pulses
Removing the J Dependence

\[ d_{21} = \frac{1}{2}J_{xh} \]

- If \( J = 180 \text{ hz} \) \( \rightarrow \) 2.7 ms
- If \( J = 100 \text{ hz} \) \( \rightarrow \) 5 ms

The Matched Sweep Adiabatic Pulse

Sweeps through the \(^{13}\text{C}\) frequency range so that it inverts signals closer to when the time matches the \(1/2J\) condition.
$^1$H-$^{13}$C HSQC – Things to Consider

Benefit of Matched Sweep

hsqcedetgpsisp2.2  hsqcedetgpsisp2.3

Quinidine in DMSO
The Matched Sweep Adiabatic Pulse sweeps through the 13C frequency range so that it inverts signals closer to when the time matches the 1/2J condition.

- If J = 180 Hz → 2.7 ms
- If J = 100 Hz → 5 ms

\[ d_{21} = \frac{1}{2}J_{xh} \]

\[ \text{Chemical Shift (ppm)} \]

\[ \overset{1}{J}_{HC} \text{ (Hz)} \]
1H-13C HSQC – Things to Consider

Matched Sweep Adiabatic Pulse?

\[ \text{hsqcedetgpsisp2.3} \]
\text{Matched Sweep Pulse}

\[ \text{hsqcedetgpsisp2.2} \]
\text{Non Matched Sweep Pulse}

\[ J_{hc} = 158 \text{ Hz} \]

α-Thujone in DMSO
**1H-13C Heteronuclear 2D Experiments**

**HMBC – Low Pass Filter**

- **hmbcgplpndqf**
  \[ d_2 = \frac{1}{2J_{xh}} \]

- **hmbcetgpl3nd**
  \[ \Delta_1 = \frac{1}{2(J_{xh\text{-min}} + 0.07(J_{xh\text{-max}} - J_{xh\text{-min}}))} \]
  \[ \Delta_2 = \frac{1}{(J_{xh\text{-min}} + J_{xh\text{-max}})} \]
  \[ \Delta_3 = \frac{1}{2(J_{xh\text{-max}} - 0.07(J_{xh\text{-max}} - J_{xh\text{-min}}))} \]
$^{1}H-^{13}C$ Heteronuclear 2D Experiments

HMBC – Suppression of $^{1}J$ correlations

Gibberellic Acid in Acetone

Long Range $J_{xh}$

$8$ Hz

$^{1}J_{xh} (max) = 170$ Hz

$^{1}J_{xh} (min) = 120$ Hz

$^{1}J_{xh} = 145$ Hz
1H-13C Heteronuclear 2D Experiments
HMBC – Better resolution

Cyclosporine

HMBCETGPL3ND
MC in F2, PH in F1
xfb

HMBCGP
MC in F1

Long Range $J_{xh}$
8 Hz
**1H-13C Heteronuclear 2D Experiments**

**HMBC – Better resolution**

**Cyclosporine**

**HMBCETGPL3ND**
- MC in F2, PH in F1
- Xfb, xf2m

**HMBCCGP**
- MC in F1

Long Range $J_{\text{xy}}$
- 8 Hz
Non Uniform Sampling

- Non Uniform, Sparse or Random sampling (2D and nD)
  - Instead of systematically incrementing the evolution period the data is collected for only a smaller portion in a random order.

- The fraction of points collected is referred to as “sparseness” or with the TopSpin parameter NusAMOUNT and is expressed in percent of the total experiments.

- The data points or FID’s to be collected are determined in a file named “nuslist”. For a 2D this file has one column, for a 3D two etc.
Non Uniform Sampling

- Uniform Sampling (32 points)
Non Uniform Sampling

- Uniform Sampling (32 points) after FT
Non Uniform Sampling

- Non Uniform Sampling
  50% sparse (16 points)
Non Uniform Sampling

- Non Uniform Sampling
  50% sparse (16 points) after FT
Non Uniform Sampling

- **What parameters to choose**
  - NusJSP
    Give more weight to points where the signal is.
    - Not collecting data in nodes of coupling modulated data.
Non Uniform Sampling

- How to create the sampling schedule
  - **Outside of Topspin**
    - Generate schedule
    - Copy to a file in folder
      `<topspinhomedir>/exp/stan/nmr/lists/vc`
    - Set parameter NusLIST to name of file
    - Adjust NusAMOUNT
    - Start acquisition
Non Uniform Sampling

- How to create the sampling schedule
  - Outside of Topspin
    - hmsIST: Poisson Gap sampling
      [http://gwagner.med.harvard.edu/intranet/hmsIST/gensched_new.html](http://gwagner.med.harvard.edu/intranet/hmsIST/gensched_new.html)
Non Uniform Sampling

- How to create the sampling schedule
  - Outside of Topspin
    - UCHC NUS Schedule Tool
      http://sbtools.uchc.edu/nmr/sample_scheduler/
Non Uniform Sampling

- How to create the sampling schedule
  - Outside of Topspin
    - UNL: Powers Group, Deterministic Gap Sampling
      http://bionmr.unl.edu/dgs.php
Non Uniform Sampling

• **Back to this topic**
  • NusAMOUNT/NusPOINTS
    • Rules of thumb
      • for time savings: ~25 – 50 % per dimension
        25 – 50 % for 2D
        10 – 25 % for 3D
        5 – 10 % for 4D
      • for resolution enhancement
        keep total number of transients constant
        -> will result in equal or better S/N
Non Uniform Sampling

• **Back to this topic**
  • NusAMOUNT/NusPOINTS
    • Two most important reasons for using NUS
      1. Increasing the resolution of indirect dimensions
      2. Reduction of acquisition times
Non Uniform Sampling

- Most important reasons for using NUS in 2D experiments in organic chemistry
  - Increasing the resolution of indirect dimensions
  - Example: $^1H/^13C = HSQC$ on a typical small molecule

<table>
<thead>
<tr>
<th>Parameter @ 600 MHz</th>
<th>1D Carbon</th>
<th>2D HSQC</th>
</tr>
</thead>
<tbody>
<tr>
<td>SW</td>
<td>240 ppm (36 kHz)</td>
<td>160 ppm (24 kHz)</td>
</tr>
<tr>
<td>TD</td>
<td>64 k</td>
<td>256 - 512</td>
</tr>
<tr>
<td>AQ</td>
<td>1.1 sec</td>
<td>5 – 10 msec</td>
</tr>
<tr>
<td>Hz/pt</td>
<td>1.1</td>
<td>93</td>
</tr>
</tbody>
</table>
Non Uniform Sampling

- Most important reasons for using NUS
  - Increasing the resolution of indirect dimensions
    Example: $^1$H/$^{13}$C = HSQC on a typical small molecule
Non Uniform Sampling

- Most important reasons for using NUS
  - Increasing the resolution of indirect dimensions
    Example: $^1\text{H}/^\text{13C} = \text{HSQC}$ on a typical small molecule
Non Uniform Sampling

- Example 1: HSQC 25 mM Cyclosporine
  $\text{TD}\{F1\} = 512$, traditional acquisition
Non Uniform Sampling

- Example 1: HSQC 25 mM Cyclosporine
  TD\{F1\} = 512, traditional acquisition
Non Uniform Sampling

- Most important reasons for using NUS
  - Increasing the resolution of indirect dimensions
    Example: $^1\text{H}/^{13}\text{C} = \text{HSQC}$ on a typical small molecule
    2k/25% NUS sampling
Non Uniform Sampling

- Example 1: HSQC 25 mM Cyclosporine
  TD\{F1\} = 2048, Non Uniform Sampling 25%
Non Uniform Sampling

- Example 1: HSQC 25 mM Cyclosporine
  \[TD\{F1\} = 1024, \text{ Non Uniform Sampling 50\%}\]
Non Uniform Sampling

- Most important reasons for using NUS
  - Increasing the resolution of indirect dimensions
  Example: $^1\text{H}/^{13}\text{C} = \text{HSQC}$ on a typical small molecule
  16k/6.25% NUS sampling
Non Uniform Sampling

- Example 1: HSQC 25 mM Cyclosporine
  TD{F1} = 16k, Non Uniform Sampling 6.25 %
Non Uniform Sampling

- Example 1: HSQC 25 mM Cyclosporine
  TD\{F1\} = 16k, Non Uniform Sampling 6.25 %
Non Uniform Sampling

- Example 1: HSQC 25 mM Cyclosporine
  
  TD\{F1\} = 16k, Non Uniform Sampling 6.25%
  
  Comparison 16k/6.25% NUS sampling vs trad. 2D
Non Uniform Sampling

- Most important reasons for using NUS
  - Increasing the resolution of indirect dimensions

Example: $^1H/^\text{13}C = \text{HSQC}$ on a typical small molecule

Comparison 16k/6.25% NUS sampling vs 1D 13C
Non Uniform Sampling

- Example 2: HMBC 50 mM Cyclosporine
  $TD\{F1\} = 400$, traditional acquisition
Non Uniform Sampling

- Example 2: HMBC 50 mM Cyclosporine
  \( TD\{F1\} = 1600 \), non uniform sampling 25\%
Non Uniform Sampling

- Example 2: HMBC 50 mM Cyclosporine
  TD{F1} = 6400, non uniform sampling 6.25%
Non Uniform Sampling

- Example 2: HMBC 50 mM Cyclosporine
  Comparison: TD\{F1\} = 400 traditional sampling vs. TD\{F1\} = 6400, non uniform sampling 6.25%
And now something completely different

• Problem:
  Topshim did not give good results on a sample in 90\%H2O/10\%D2O in a Shigemi tube.

Shigemi option did not help,

Changing between 5\textsuperscript{th} order up to 8\textsuperscript{th} order did not help.

All the usual tricks did not resolve the issue.

Standard samples were ok.

Other shigemi tubes were ok.
And now something completely different

- Solution:
  To see what is going on a “topshim 3d map” was done.

This produces a lot of diagnostic data.

One dataset is the 3D acquisition data.

and it looked like this:
And now something completely different

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- **Solution:**
The Shigemi was a Advanced Microtube or thinwall tube

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**ADVANCED NMR MICROTUBES**

This advanced microtube is a combination of two of Shigemi's great products. The advanced microtube has significant improvements in the sensitivity of our existing NMR symmetrical microtube. This 5mm sample tube has a thinner glass wall in the sample area than the existing type (0.40mm -> 0.20mm), increasing the sample capacity held in the R, region by a factor of 1.2. Due to this improved space filling factor, the signal to noise ratio has been enhanced by 16%. Although the sample volume is increased, it is to be noted that the artifact caused by the RF inhomogeneity is very limited. Therefore, an increased sensitivity is expected.

They are magnetic susceptibility matched to each of the following solvents:

- D2O
- CDC13
- DMSO
- CD3OD

*The blue shaded area shown above is the magnetic susceptibility matched glass for each solvent.*
Optimal volume setting

- Shigemi Advanced NMR Microtube

- Optimal

- Too short

- Too long
And now something completely different

- **Solution:**

To prove that this was indeed the issue the plunger was then set too high
And now something completely different

- Solution:

And finally just right
And now something completely different

• One more word.

• Thin wall tubes are not a good idea with lossy samples.

• Chances are you are loosing the signal from the extra volume in the extra loss due to the fact that more of your sample is in the “bad” region of your probe.
Innovation with Integrity