VARIABLE TEMPERATURE EXPERIMENTS

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VARIABLE-TEMPERATURE NMR EXPERIMENTS

1. INTRODUCTION

1.1. About these Notes and Related Notes

These notes describe how to perform variable-temperature (VT) NMR experiments on the Inova 400 and Inova 600 spectrometers. Any experiment that can be performed at room temperature can also be performed above or below room temperature as well.

Table 1. Required and Recommended Notes for Performing VT NMR Experiments on the Inovas

If you would like to…	Then you…	
	…must consult these notes	**…should consult these notes**
Perform a 1D ^{1}H, ^{13}C ^{19}F, or ^{31}P VT experiment	• NMR Sample Preparation • NMR Spectrometer Capabilities and Specifications • Operation of the Inova 400 and 600 • Retrieving your NMR Data	• Processing 1D NMR Spectra using VNMRJ
Perform a 1D "odd-nucleus" VT experiment	• NMR Sample Preparation • NMR Spectrometer Capabilities and Specifications • Operation of the Inova 400 and 600 • Odd-Nuclei Experiments on the Inovas • Retrieving your NMR Data	• Processing 1D NMR Spectra using VNMRJ
Perform a 2D VT experiment	• NMR Sample Preparation • NMR Spectrometer Capabilities and Specifications • Operation of the Inova 400 and 600 • Retrieving your NMR Data	• Processing 2D NMR Spectra using VNMRJ
Perform a 2D "odd-nucleus" VT experiment	• NMR Sample Preparation • NMR Spectrometer Capabilities and Specifications • Operation of the Inova 400 and 600 • Odd-Nuclei Experiments on the Inovas • Retrieving your NMR Data	• Processing 2D NMR Spectra using VNMRJ

1.2. VT Capabilities of the NMR Spectrometers

VT experiments can only be performed on the Inova spectrometers and thus a brief summary of the capabilities of the Inova 400 and Inova 600 are presented here. For a full discussion, please see the "Spectrometer Capabilities and Specifications" sheet.

Table 2. Capabilities of the Inova 400 and Inova 600

Spectrometer	^{1}H	^{13}C	^{19}F	^{31}P	Odd nuclei	2D	VT Range	Cryogens?[a]
Inova 400	Yes	Yes★	Yes	Yes★	Yes	Yes	-80 °C to +130 °C	Yes
Inova 600	Yes★	Yes	No	Yes[b]	Yes[b]	Yes	-20 °C to +80 °C	No

★Preferred spectrometer

[a]The Inova 400 requires the use of liquid nitrogen for cooling, whereas the Inova 600 does not.

[b]Requires re-cabling of the probe.

1.3. Guidelines and Rules when Performing VT Experiments

CAUTION: Be patient! Variable-temperature experiments can cause severe damage to the probe if performed incorrectly or in a rush. The temperature must be reduced or increased slowly.

1) The temperature must be increased or decreased by no more than 25 °C at a time. After the requested temperature is reached, you must wait 5 minutes before moving to the next temperature.
2) For variable-temperature experiments performed during prime-time hours (9:00 am until 6:00 pm), a maximum of 3 hours may be booked upon approval by an NMR facility staff member.
3) The probe must be slowly warmed or cooled back to 25 °C before ejecting your sample.

1.4. Training

Before VT experiments are performed, users must receive spectrometer training from an NMR facility staff member. Training sessions are offered on demand and can be scheduled by contacting an NMR facility staff member. Users must have received Inova training and be comfortable using the Inovas before receiving VT training.

1.5. Deuterated Solvent Selection

When performing variable-temperature experiments, you must ensure that your solvent doesn't boil or solidify, both of which could cause the NMR tube to break and cause severe damage to the NMR probe. When performing high-temperature NMR experiments, the maximum temperature must be less than 10 °C below the solvent's boiling point. When performing low-temperature NMR experiments, the minimum temperature must be greater than 10 °C above the solvent's melting point.

Table 3. Melting and Boiling Points of Select Deuterated Solvents[a]

Solvent	VNMRJ Listing	Melting Point (°C)	Boiling Point (°C)
Acetone-d_6	Acetone	-94	56
Acetonitrile-d_3	Acetonitrile	-45	82
Benzene-d_6	Benzene	6	80
Chloroform-d	$CDCl_3$	-63	61
Cyclohexane-d_{12}	Cyclohexane	6	81
Deuterium Oxide	D2O	4	101
Dimethyl Formamide-d_7	DMF	-61	153
Dimethyl Sulfoxide-d_6	DMSO	18	189
Ethanol-d_6	Ethanol	-114	78
Methanol-d_4	Methanol	-98	65
Methylene Chloride-d_2	CD2Cl2	-95	40
Pyridine-d_5	Pyridine	-42	115
Tetrahydrofuran-d_8	THF	-108	66
Toluene-d_8	Toluene	-95	111

[a]These values were taken from the CIL solvent chart, which can be found at www.isotope.com/cil/products/images/nmrchart.pdf, and were rounded to the nearest integer.

1.6. Temperature Settings and Approximate Time Requirements

It is important to be patient and book a sufficient amount of time when performing variable-temperature experiments. The starting temperature is 25 °C and in general, the temperature changes at a rate of roughly 4 °C/minute change in temperature; 5 minutes for the temperature change and 5 minutes waiting for equilibrium. Use Table 4 below to estimate the time required for heating or cooling the sample only and returning back to 25 °C. Note the experiment time must be considered in addition to the heating/cooling cycle time as well when reserving your NMR time.

Table 4. Recommended Temperature Settings and Estimated Time Required for VT Experiments[ab]

Desired T	T Setting 1	T Setting 2	T Setting 3	T Setting 4	T Setting 5	Approximate Time Required for Heating/Cooling Cycle (minutes)
-80 (min T on I400)	0	-20	-40	-60	-80	105
-70	0	-25	-50	-70		90
-60	0	-20	-40	-60		85
-50	0	-25	-50			70
-40	0	-20	-40			65
-30	5	-15	-30			60
-20 (min T on I600)	0	-20				45
-10	5	-10				40
0	0					25

10	10					20
20	20					15
30	30					15
40	40					20
50	50					25
60	45	60				40
70	50	70				45
80 (max T on I600)	45	65	80			60
90	50	70	90			65
100	50	75	100			70
110	50	70	90	110		85
120 (max T on I400)	50	75	100	120		90

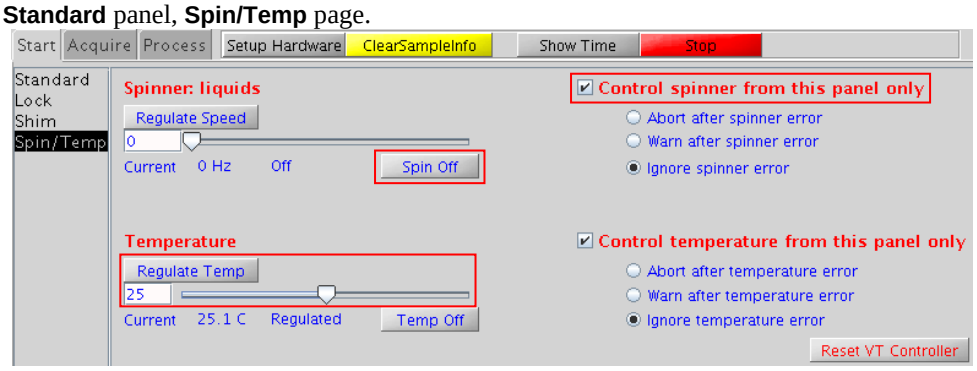
[a]All temperatures are in °C.

[b]To use the table above, find your desired experimental temperature from the left-most column. If your desired temperature is not listed, use the next coldest (eg. use -80 °C if you want to cool to -75 °C) or next warmest (eg. use 120 °C if you're heating to 115 °C) "Desired T" setting as a guide. First set the spectrometer to "T Setting 1". Once "T Setting 1" has been reached, wait 5 minutes for the temperature of the probe and sample to stabilize, gradient shim the sample, then proceed to "T Setting 2". Repeat waiting, gradient shimming and cooling/heating periods until the desired temperature is reached. After the experiment is preformed, return to room temperature using the same T settings and 5 minute waiting periods.

2. INITIAL STEPS FOR ALL VT EXPERIMENTS

These series of steps are performed for all VT experiments, regardless of the spectrometer or desired experiment temperature.

1) Log-in, insert your sample, select your solvent, lock, shim and perform a room-temperature experiment to verify the composition of your product. If necessary, see the "Operation of the Inova 400 and 600" sheet for guidance.
2) Go to the **Standard** panel, **Spin/Temp** page.



3) Ensure that box next to "Control spinner from this panel only" is checked.
4) VT experiments should be performed with the spinning turned off. If the sample is spinning, click Spin Off to stop the spinning.
5) The actual temperature will be displayed in three places:
   a) On the **Spin/Temp** page, next to "Current". A message saying "Regulated" will be displayed when the desired temperature has been reached or "NotReg" when the temperature is changing.
   b) On the bottom left-hand corner of the screen. The temperature will be blue when regulated, Temp 25.1 C, or purple when the temperature is changing, Temp 25.5 C. Clicking on Temp 25.5 C will bring up a chart of temperature vs. time.
   c) On the console panel, the temperature is displayed in large red numbers.
6) Depending on the spectrometer you are using and if you are performing low-temperature or high-temperature experiments, the VT procedure is slightly different. Therefore:
   a) if you are performing high-temperature experiments on the Inova 400 or Inova 600, go to section 3.
   b) if you are performing low-temperature experiments on the Inova 400, go to section 4.
   c) if you are performing low-temperature experiments on the Inova 600, go to section 5.

3. HIGH-TEMPERATURE EXPERIMENTS ON THE INOVA 400 OR 600

1) Consult Table 3 in Section 1.5 to ensure that your solvent will not boil before reaching the desired experimental temperature.

2) Consult Table 4 in Section 1.6. Use the table to determine the recommended temperature settings/cycle and to ensure you have booked enough spectrometer time.
3) In the **Spin/Temp** page, input the "T Setting 1" value in the box under Regulate Temp, followed by enter. The slider should change position. NOTE: You must hit the enter key after you input the temperature setting!
4) Click on Regulate Temp. This will initiate the VT gas flow and the temperature should start to change at a rate of about 4 °C/minute. The rate will decrease when approaching the temperature setting.
5) When the temperature is reached, wait 5 minutes, then gradient shim the sample before moving onto the next temperature.
6) Repeat steps 3-5, replacing the "T Setting 1" in step 3 with the appropriate temperature setting, until the desired experimental temperature is reached.
7) Wait a minimum of 5 minutes, gradient shim the sample, then perform your experiment.

TIP: Input the temperature of the spectrum in the Comments box found in the **Start** panel, **Standard** page. If you don't, there will be no indication of the temperature in which the spectrum was acquired.

8) When your experiment is complete, slowly cool the probe back to 25 °C using the same temperature values you used when heating the probe. Ensure you wait 5 minutes between temperature settings.
9) When the probe back to 25 °C, eject your sample and insert the standard $CDCl_3$ sample.
10) Uncheck the box next to "Control spinner from this panel only".
11) Go to the **Start** panel, **Standard** page and click on Logout.

4. LOW-TEMPERATURE EXPERIMENTS ON THE INOVA 400

The Inova 400 requires liquid nitrogen for cooling, which you must provide, and an overview of the Inova 400 VT equipment utilized when performing low-temperature NMR experiments is presented in Figure 1.

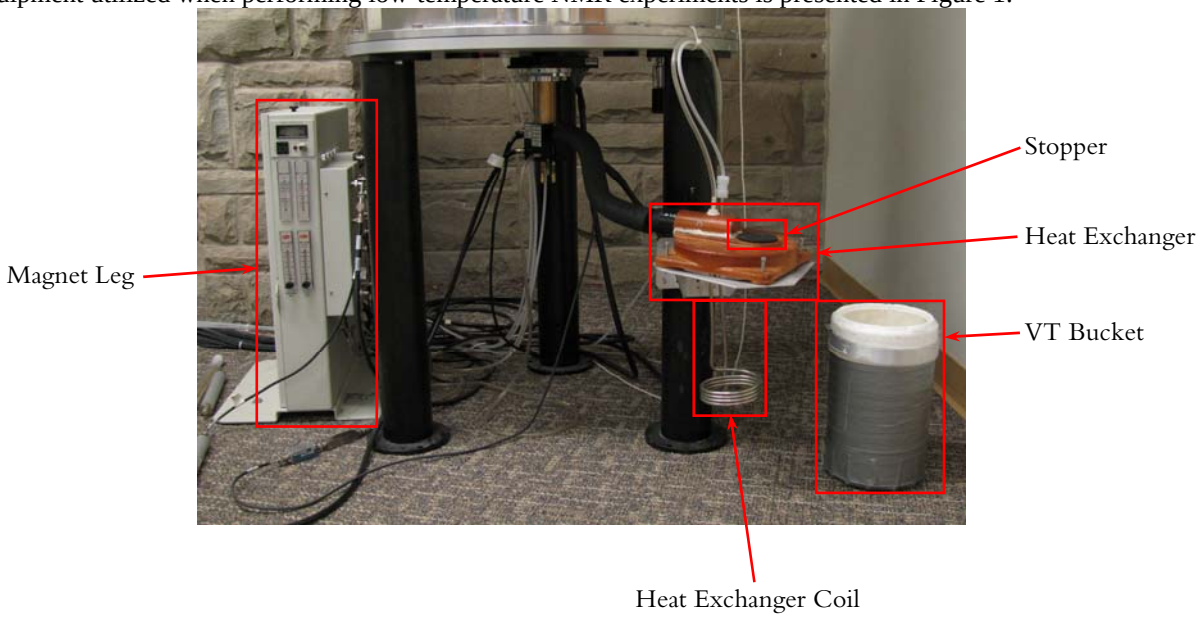


Figure 1. Overview of the VT equipment used when performing low-temperature NMR experiments on the Inova 400.

1) Consult Table 3 in Section 1.5 to ensure that your solvent will not freeze before reaching the desired experimental temperature.
2) Consult Table 4 in Section 1.6. Use the table to determine the recommended temperature settings/cycle and to ensure you have booked enough spectrometer time.
3) On the magnet's console leg, slowly turn the black knob above the "V.T. GAS" label counter-clockwise to increase the VT gas-flow to 20 LPM (see Figure 2). NOTE: Do not change the gas-flow on the "COOLING AIR".

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Figure 2. A close-up of the cooling air and VT gas controls located on the spectrometer's magnet leg. In order perform low-temperature NMR experiments, the VT gas should be increased to 20 LPM. The cooling air should not be increased.

4) Position and support the VT bucket about the heat-exchanger coil as follows:
   a) Tilt and gently slide the VT bucket under the heat-exchanger coil so the coil sits inside the VT bucket.
   b) Rotate the VT bucket as necessary to align the tabs on the bucket with the slots on the metal platform (see Figure 3).
   c) Lift the bucket so the tabs rise through the slots.
   d) Turn the bucket 45 degrees to secure the bucket in place.

Figure 3. (Left) The underside of the heat exchanger showing the slots in which the tabs of the VT bucket must be aligned. (Right) The top of the heat exchanger and VT bucket. The tabs of the VT bucket should be aligned as shown in order to lift the VT bucket into place.

5) On the **Spin/Temp** page, input the "T Setting 1" value in the box under Regulate Temp, followed by enter. The slider should change position. NOTE: You must hit the enter key after you input the temperature setting.
6) Click on Regulate Temp.
7) Remove the black rubber stopper and pour the liquid nitrogen through the hole into the VT bucket. Ensure that the heat-exchanger coil is completely surrounded by liquid nitrogen. For long runs, it may be necessary to top up the VT bucket with additional liquid nitrogen.
8) Place the black rubber stopper back on the VT bucket cap. The temperature should soon start to decrease.
9) When the temperature is reached, wait 5 minutes then gradient shim the sample.
10) Repeat steps 5-6, and 9, replacing the "T Setting 1" in step 5 with the appropriate temperature setting, until the desired experimental temperature is reached.
11) Wait a minimum of 5 minutes, then gradient shim and then perform your experiment.

TIP: Input the temperature of the spectrum in the Comments box found in the **Start** panel, **Standard** page. If you don't, there will be no indication of the temperature in which the spectrum was acquired.

12) When your experiment is complete, slowly warm the probe back to 25 °C using the same temperature values you used when heating the probe. Ensure you wait 5 minutes between temperature settings. NOTE: Do not remove the VT bucket when warming!
13) When the temperature has returned to 25 °C, wait a minimum of 5 minutes, then remove the VT bucket.

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CAUTION: Removing the VT bucket before the temperature has stabilized at 25 °C for at least 5 minutes will cause a dramatic spike in the temperature.

14) Wait at least 5 minutes to allow for the heat-exchanger coil to return to room temperature. The coil is at room temperature when there is no condensation on the heat-exchanger coil and the coil is warm to the touch.

CAUTION: Ejecting your sample before the heat-exchanger coil has returned to room-temperature will cause a dramatic spike in the temperature.

15) Eject your sample and insert the standard $CDCl_3$ sample.
16) On the magnet's console leg, slowly turn the black knob above the "V.T. GAS" label clockwise to decrease the VT gas-flow to 10 LPM. NOTE: Do not change the gas-flow on the "COOLING AIR".
17) Uncheck the box next to "Control spinner from this panel only".
18) Go to the **Start** panel, **Standard** page and click on Logout.

5. LOW-TEMPERATURE EXPERIMENTS ON THE INOVA 600

The Inova 600 has an FTS chiller which cools the air necessary for performed low-temperature experiments. The FTS chiller does not require liquid nitrogen and allows you to set the temperature of the VT gas. An overview of the Inova 600 VT equipment utilized when performing low-temperature NMR experiments is presented in Figure 4.

Figure 4. Overview of the VT equipment used when performing low-temperature NMR experiments on the Inova 600.

1) Consult Table 3 in Section 1.5 to ensure that your solvent will not boil before reaching the desired experimental temperature.
2) Consult Table 4 in Section 1.6. Use the table to determine the recommended temperature settings/cycle and to ensure you have booked enough spectrometer time.
3) On the magnet's console leg, slowly turn the black knob under the "VT GAS" counter-clockwise to increase the VT gas-flow to 20 (see Figure 5). NOTE: Do not change the gas-flow on the "COOLING AIR".

Figure 5. A close-up of the cooling air and VT gas controls located on the spectrometer's magnet leg. In order perform low-temperature NMR experiments, the VT gas should be increased to 20 LPM. The cooling air should not be increased.

4) Turn on the FTS chiller by flicking both power switches upwards (see Figure 6).

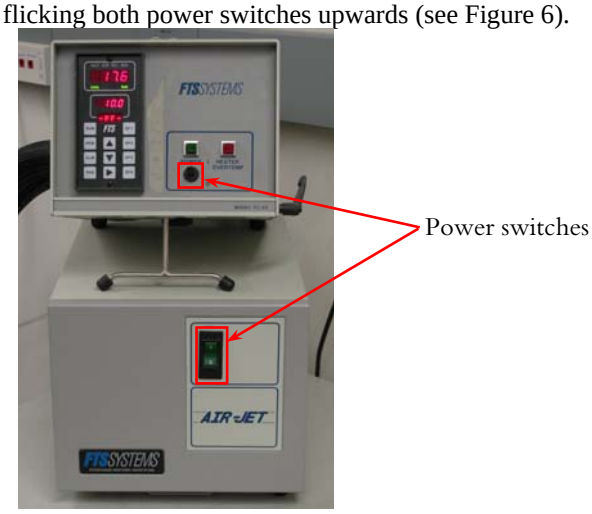

Figure 6. The FTS chiller and its two power switches.

5) On the FTS chiller control, use the pre-set temperature setting buttons or the down arrows to adjust the temperature to be at least 10 °C colder than your desired experiment temperature (see Figure 7). The temperature displayed on the FTS chiller is that of the VT gas (not the temperature of the probe); do not move onto the next step until the temperature displayed has stabilized.

Figure 7. A close-up of the FTS chiller control panel, the temperature readings, and the buttons used to control the temperature of the VT gas.

6) In the **Spin/Temp** page, input the "T Setting 1" value in the box under Regulate Temp, followed by enter. The slider should change position. NOTE: You must hit the enter key after you input the temperature setting.
7) Click on Regulate Temp. This will initiate the VT gas flow and the temperature should start to change at a rate of about 4 °C/minute. The rate will decrease when approaching the temperature setting.
8) When the temperature is reached, wait 5 minutes then gradient shim the sample.
9) Repeat steps 6-8, replacing the "T Setting 1" in step 5 with the appropriate temperature setting, until the desired experimental temperature is reached.
10) Wait a minimum of 5 minutes, gradient shim the sample and then perform your experiment.

TIP: Input the temperature of the spectrum in the Comments box found in the **Start** panel, **Standard** page. If you don't, there will be no indication of the temperature in which the spectrum was acquired.

11) When your experiment is complete and you've saved your data, slowly warm the probe back to 25 °C using the same temperature values you used when heating the probe. Ensure you wait 5 minutes between temperature settings.

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12) When the temperature has returned to 25 °C, set the FTS chiller temperature to 20 °C.
13) Once the temperature displayed on the chiller has stabilized, eject your sample and insert the $CDCl_3$ sample.
14) Turn off the FTS chiller by flicking both power switches down.
15) On the magnet's console leg, slowly turn the black knob above the "V.T. gas" label clockwise to decrease the VT gas-flow to 10 LPM. NOTE: Do not change the gas-flow on the "Cooling Air".
16) Uncheck the box next to "Control spinner from this panel only".
17) Go to the **Start** panel, **Standard** page and click on Logout.