Sample

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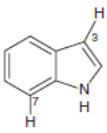
A correct sample preparation allows obtained the spectra with a good line shape



- The five-bond coupling (H3-H7) are very small – similar to the natural line width of a typical NMR signal.
- Only in the best conditions are possible to observe this couplings (differences about one part in 10^9)
- It is necessary that the magnetic field is very homogeneous, so that all molecules experience the same field regardless of position in the NMR tube

How Much Sample Do I need ?

- Nucleus to observe
- Probe
- Magnetic Field
- How pure is the sample ?
- Molecular weight
- What experiment you need to perform?

Started point

Field	1H	13C
400	10-5 mg	30-50 mg
500	5-2 mg	20-10 mg
600	2-1 mg	10-5 mg

If your spectra has a high noise level after 5 minutes, probably you need more than 1h 20 min (increase the signal by factor of four)

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The solvent effect

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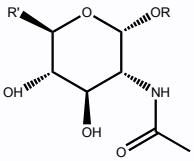
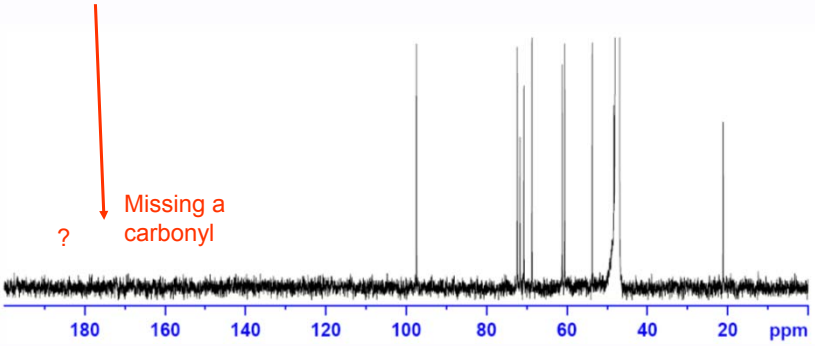


About 5 mg in CD_3OD. 2800 scans (~4 hrs)

Missing a carbonyl carbon
presumably due to insufficient relaxation



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Solution: Use H_2O

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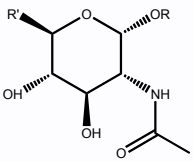
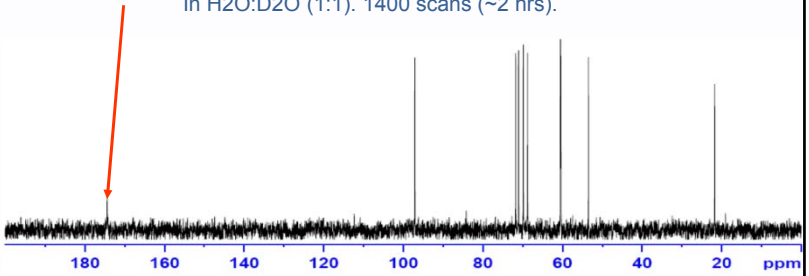


Why it works:

• Potential intra-molecular hydrogen bond is weakened or broken, and decoupled from ring movement

In H2O:D2O (1:1). 1400 scans (~2 hrs).



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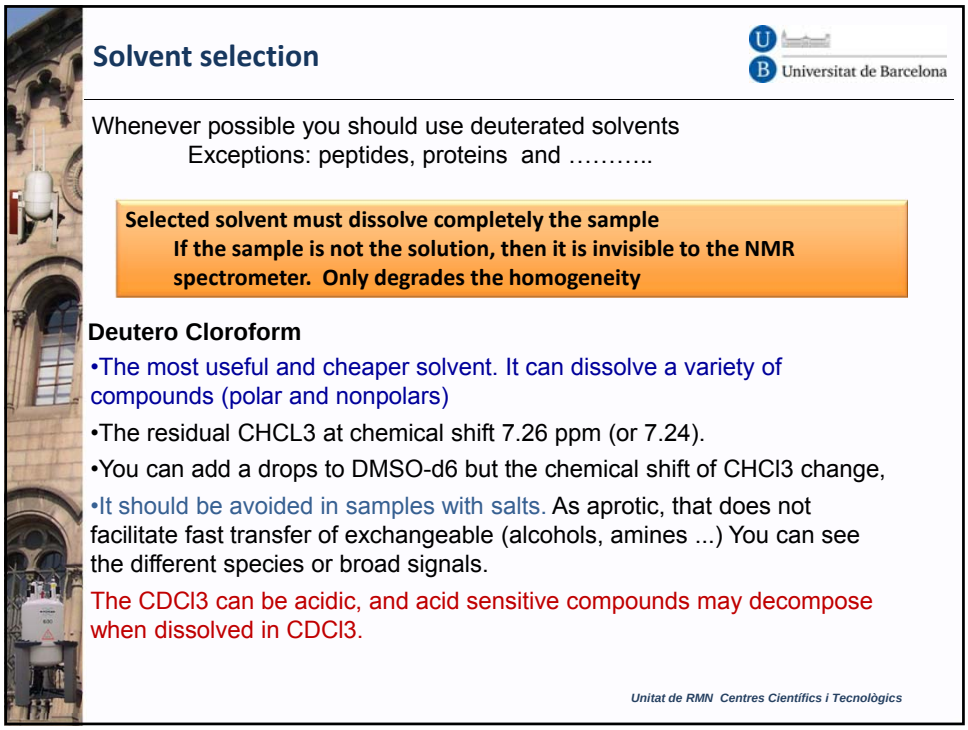

Solvent selection

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Whenever possible you should use deuterated solvents
Exceptions: peptides, proteins and ……..

Selected solvent must dissolve completely the sample
If the sample is not the solution, then it is invisible to the NMR spectrometer. Only degrades the homogeneity

Deutero Cloroform

- The most useful and cheaper solvent. It can dissolve a variety of compounds (polar and nonpolars)
- The residual CHCL3 at chemical shift 7.26 ppm (or 7.24).
- You can add a drops to DMSO-d6 but the chemical shift of CHCl3 change,
- It should be avoided in samples with salts. As aprotic, that does not facilitate fast transfer of exchangeable (alcohols, amines ...) You can see the different species or broad signals.

The CDCl3 can be acidic, and acid sensitive compounds may decompose when dissolved in CDCl3.

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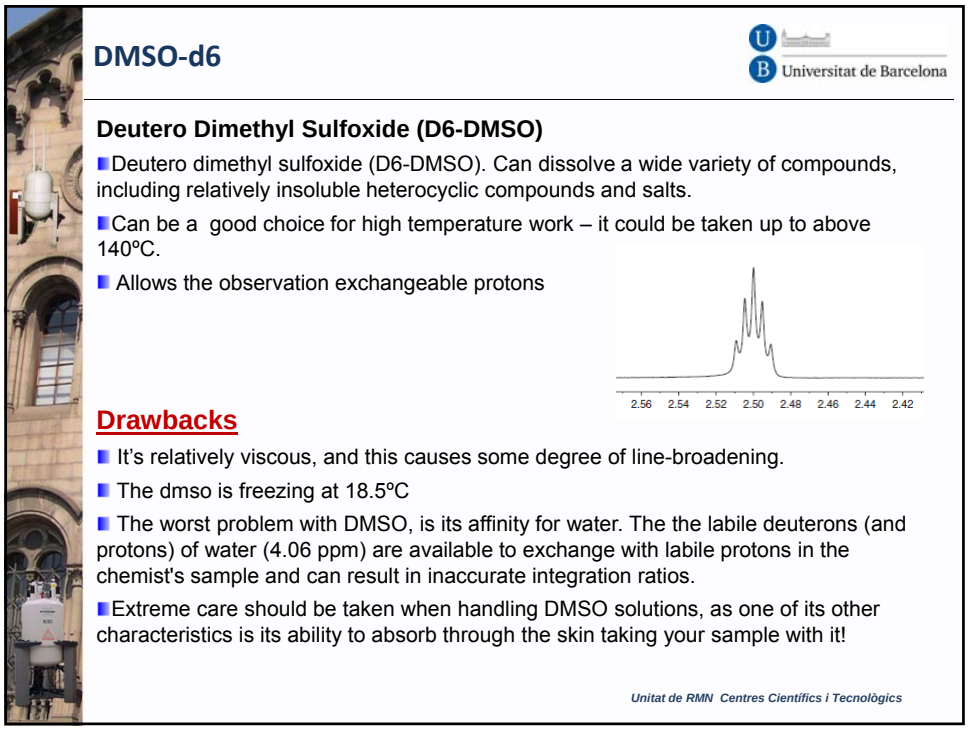

DMSO-d6

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Deutero Dimethyl Sulfoxide (D6-DMSO)

- Deutero dimethyl sulfoxide (D6-DMSO). Can dissolve a wide variety of compounds, including relatively insoluble heterocyclic compounds and salts.
- Can be a good choice for high temperature work – it could be taken up to above 140ºC.
- Allows the observation exchangeable protons

Drawbacks

- It's relatively viscous, and this causes some degree of line-broadening.
- The dmso is freezing at 18.5ºC
- The worst problem with DMSO, is its affinity for water. The the labile deuterons (and protons) of water (4.06 ppm) are available to exchange with labile protons in the chemist's sample and can result in inaccurate integration ratios.
- Extreme care should be taken when handling DMSO solutions, as one of its other characteristics is its ability to absorb through the skin taking your sample with it!

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Other Solvents

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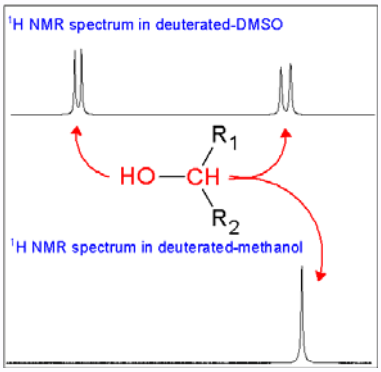
Deutero Methanol (CD3OD)

This is a very polar solvent, suitable for salts and extremely polar compounds. it has a very high affinity for water and is almost impossible to keep dry. Its water peak is sharper at around 4.8 ppm. The residual CD2HOD is observed at 3.3 ppm.

Its main disadvantage is that it will exchange ionizable protons in your sample for deuterons, and hence they will be lost from the spectrum, e.g., -OH, -NH and even -CONH2, though these can often be relatively slow to exchange. Also, protons α to carbonyl groups may exchange through the enol mechanism.



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More solvents

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Deutero Water (D2O)

- D2O is even more polar than D4-methanol and rather limited in its use for that reason usually for salts only.
- It exchanges all acidic protons readily and exhibits a strong HOD signal at about 4.9 ppm.
- Sometimes it can be used 1:9 mixtures D2O/H2O to observe amide protons

More solvents

Trifluoroacetic Acid (CF3COOH)

Pyridine- d5

C6D6 or CCl4

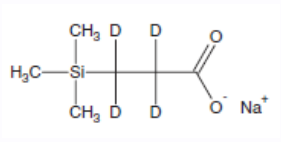
Not recomended (carcinogenic)

Reference

TMS (TetraMethyl Silane)

TSP (3-(Trimethylsilyl) propionic-2,2,3,3-D4 acid)

Residual solvent signals



J. Org. Chem. ***1997,*** *62, 7512-7515* . "NMR Chemical Shifts of Common Laboratory Solvents as Trace Impurities"

Organometallics **2010,** *29*, 2176-2179. "NMR Chemical Shifts of Trace Impurities: Common Laboratory Solvents, Organics, and Gases in Deuterated Solvents Relevant to the Organometallic Chemist"

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The most common solvents

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http://www.isotope.com/uploads/File/NMR_Solvent_Data_Chart.pdf

Solvent	Formula	H-NMR shift	C-NMR shift	multiplet	$J_{C\text{-}D}$(Hz)	mp (°C)	bp(°C)	comments
Chloroform-d	$CDCl_3$	7.24	77.0	triplet	32	-64	61	
Acetone-d_6	CD_3COCD_3	2.04	29.8	septet	20	-95	56	
			206.3	multiplet	<1			appears as singlet mostly
Benzene-d_6	C_6D_6	7.2	128.0	triplet	24	6	80	carcinogen
Acetonitrile-d_3	CD_3CN	1.93	1.3	septet	21	-45	82	
			117.7	multiplet	<1			appears as singlet mostly
Dichloromethane-d_2	CD_2Cl_2	5.32	53.5	quintet	21	-97	40	
Dimethylsulfoxide-d_6	CD_3SOCD_3	2.49	39.7	septet	21	19	189	
Methanol-d_4	CD_3OD	3.35, 4.78	49.3	septet	21	-98	64	
Tetrahydrofuran-d_8	C_4D_8O	1.73, 3.58	25.5	quintet	21	-108	66	
			67.7	quintet	22			
Toluene-d_8	C_7D_8	2.30, 7.19				-95	111	
Pyridine-d_5	C_5D_5N	7.19, 7.55, 8.71	123.5	triplet	25	-42	115	
			135.5	triplet	24			
			149.5	triplet	27			
Water-d_2	D_2O	4.65				0	100	
Acetic acid-d_4	CD_3COOD	2.03, 11.53	20.0	septet	20	17	118	
			178.4	multiplet	<1			appears as singlet mostly
Triluoroacetic acid-d	CF_3COOD	11.5	116.5	quartet	283	-15	72	C-F-coupling
			164.4	quartet	44			C-F-coupling
Dioxane-d_8	$C_4D_8O_2$	3.58	66.5	quintet	22	12	102	
Tetramethylsilane (TMS)	$Si(CH_3)_4$	0.00	0.00	singlet				internal reference

Effect of change of solvent

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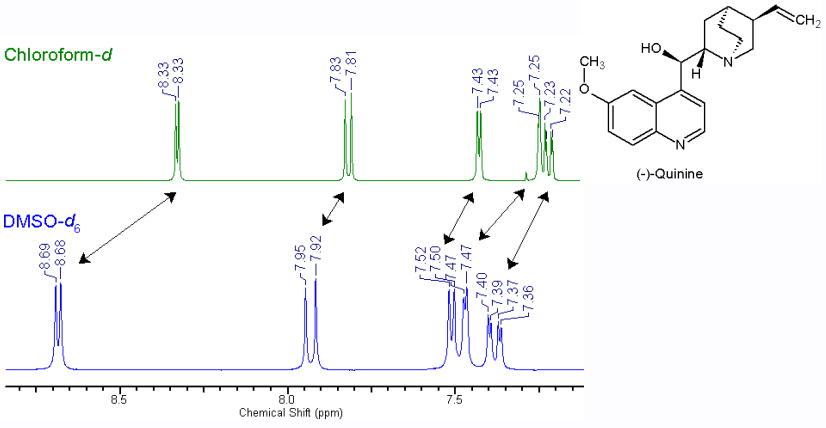
Certain experimental conditions, such as varying the temperature or using different solvents, may cause changes in chemical shifts
When it comes to referencing 2D NMR data, use the 1D NMR data collected at the same temperature and solvent.



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The Sample

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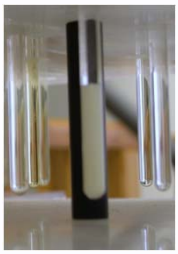
- Adjust the sample concentration to the solubility
  - Avoid product precipitation
- Use a single deuterated solvent
  - Reference for lock
- Avoid heterogeneous samples.
  - Avoid air bubbles, suspended particles, sample separation
- The low quality NMR tubes → distorts magnetic field homogeneity
  - Breaks easily → damage the NMR probe
- Adjust the Properly position NMR sample in the magnet with the NMR gauge



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NMR Sample Preparation

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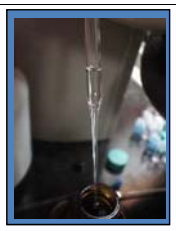
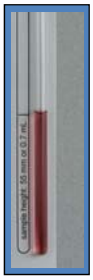
For 1H Use about 5-25 mg of product





1-0.75 ml deuterated solvent

The sample should be about 4.5-5.5 cm of liquid

The sample must be free of suspended material: You can filtering using a cotton wool

labeled your sample

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The quality of the spectra is not enough?

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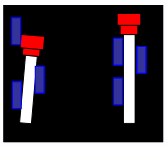
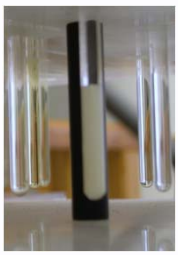
- **Failure due to sample preparation**
  - Dilute samples do not benefit from a short liquid length. The sample should be about 4.5-5.5 cm of liquid
  - The height of the NMR-tube in the spinner is not correct
  - Contaminations affecting spectral quality. You should: avoid:
    - Paramagnetic Substances
    - High salt concentration
    - Particles "fishes" and not dissolved compounds
    - Mixes of deuterated solvents





The time required for shimming increases significantly and may be impossible to obtain a quality spectra

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Effects due to incorrect sample preparation

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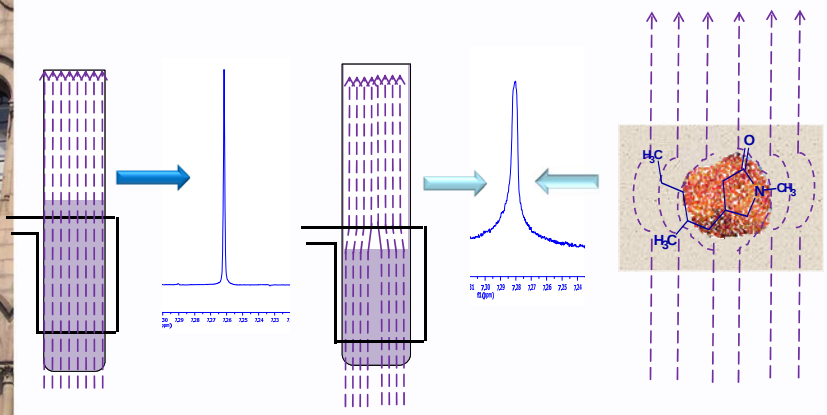
An insufficient volume or the presence of precipitate in the solution leads to a distortion of magnetic field lines. The result is a change in the line shape in the signals of the spectrum or a peak splitting



Overfilling the NMR tubes may cause problems due to temperature gradients in the sample

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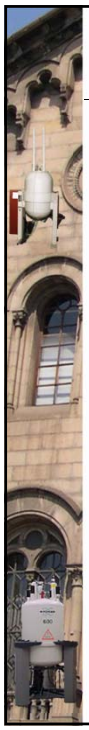

NMR Sample Tube Care of tube and cleaning

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- Do not heat the NMR tube in an oven, the tube will warp and may cause probe damage. Bent tubes may cause severe probe damage.
- Clean the NMR tube with a suitable solvent and dry with acetone and the nitrogen or air
- Tube caps are very cheap and disposable, dirty caps or old caps can contaminate your sample
- Use only EIGHT-INCH tubes on the SMS sample changer!
- The price of NMR tubes range from <2-3€ a piece to >50€. For high-quality NOE-based spectra at higher **fields use high-quality tubes**

Recomened for 400-500 MHz:
Wilmad ref PP 507 or PP 528 (7-14 $) Length: 8 inches

http://www.wilmad-labglass.com/services/technical_NMR_EPR.jsp

http://www.cortecnet.com

http://www.newera-spectro.com/

Wilmad
http://www.wilmad-labglass.com/services/NMR_001.jsp

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