

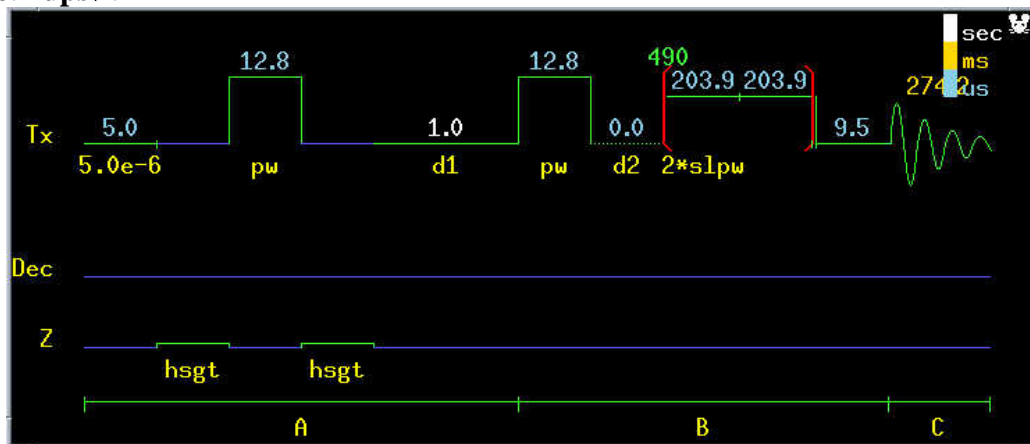
ROESY spectrum

ROESY (**R**otational nuclear **O**verhauser **E**ffect **S**pectroscop**Y**) is used to determine signals arisen from protons, which are close in space but not closely connected by chemical bonds. A ROESY spectrum yields through space correlations via the Rotaional nuclear Overhauser Effect (ROE). When one multiplet is irradiated, the intensities of multiplets arising from nearby nuclei are affected. ROESY is especially useful for cases where NOESY signals are weak because they are near the transition between negative and positive. **ROESY cross peaks are always negative.** The ROESY experiment also yields cross peaks arisen from chemical exchange. Exchange peaks and TOCSY type artifacts are always positive so they can be removed in ROESY. To maximise sensitivity, the mixing time should be half to one times the longitudinal relaxation time (T1) but not longer than 300 ms.

To avoid TOCSY artefacts, the power used to achieve spin-lock is reduced. Also to further reduce the chances of Hartmann-Hahn match, the Spin-Lock offset is shifted to one end of the spectra (instead of the center). The HOHAHA signals are very large compare to the ROE cross peaks. It is therefore essential to take as many precautions as necessary to cancel those signals. Also If HOHAHA peaks are present, relayed cross peaks can be observed (HOHAHA-ROESY or ROESY-HOHAHA).

Steps of doing ROESY experiment.

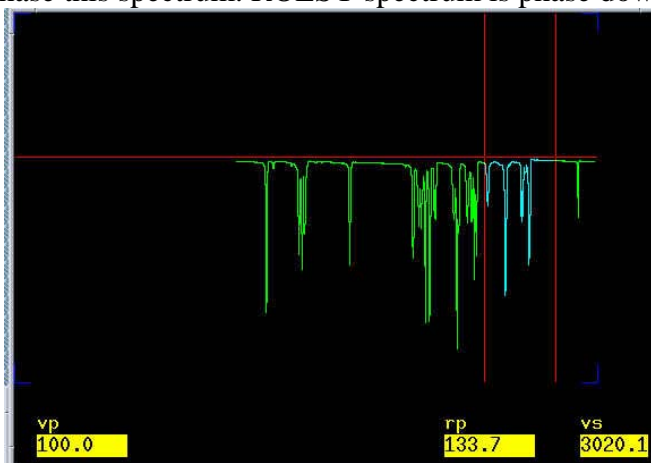
1. Acquire a normal 1D ¹H NMR spectrum first.
2. Calibrate pw90, Remember to set a lower power level; **tpwr=48** for organic solvent and **tpwr=52** for D2O. type **pw90=pw360/4 pw=pw90**, run the 1D spectrum again. Move sweep width and run the spectrum one more time.
3. **mf(1,3)**, **jexp3**.
4. **wft**.
5. **ROESY**.
6. **dps**.



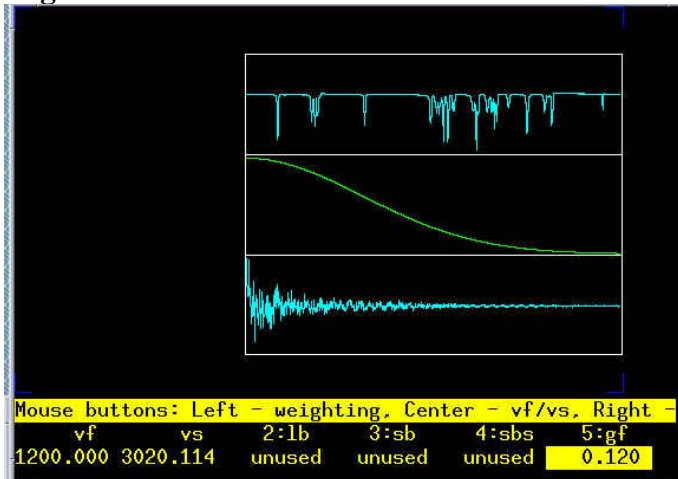
7. **dg**.

ACQUISITION		TRANSMITTER		FLAGS		PRESATURATION	
seqfil	ROESY	tn	H1	hs	n	satmode	nnnn
sw	3734.1	sfrq	399.729	sspul	y	satfrq	0
at	0.274	tof	-355.4	PFGflg	y	satdly	0
np	2048	tpwr	60	hsglvl	4854	satpwr	0
ss	64	pw	12.800	hsgt	0.002000	PROCESSING	
d1	1.000	DECOUPLER		SAMPLE		gf	0.063
nt	2	dn	H1	date	Sep 27 2000	gfs	not used
ct	0	dm	nnn	solvent	CDCl3	fn	2048
2D ACQUISITION		SPIN LOCK		sample	undefined	2D PROCESSING	
sw1	3734.1	slpwr	42	SPECIAL		gf1	0.049
ni	512	slpw	101.938	temp	not used	gfs1	not used
phase	arrayed	mix	0.200	spin	0	procl	lp
				gain	22	fn1	2048

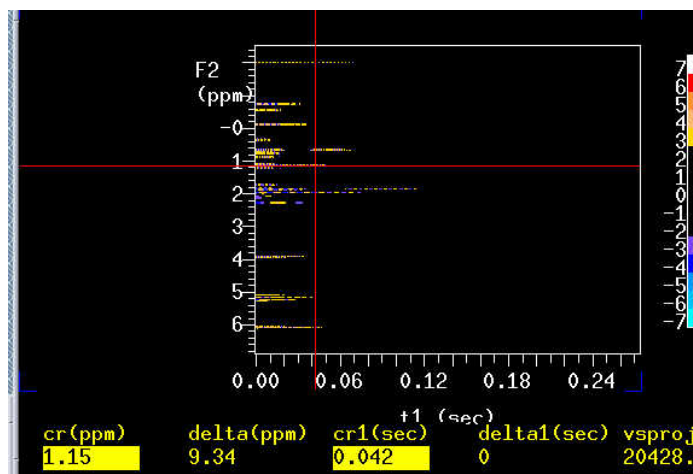
8. turn off spin.
9. **nt=8** ↓, or bigger to have a S/N ratio. So if you have time, nt=8.
10. **ni=512** ↓. This is the number of points in t1 dimension (second dimension) (ni), generally 200-400 are suggested, the greater the points, the better the resolution, and the less t1 noise observed
11. **np=2048** ↓. This is the number of points in t2 dimension.
12. **d1=1** ↓. This is relaxation delay; normally should be longer than in gCOSY to decrease t1 noise, and make ROEs between different types of protons more equivalent, so 2.0-2.5 seconds is normal, but aromatic (or vinyl or methyl) protons usually need 3.0-3.5 seconds for better spectra.
13. **mix=0.2** ↓. **The larger the mixing time the more ROEs will be observed, but the less difference in intensity by distance.** In addition, the mixing time is correlated to the size of the molecule, small molecule, long mixing time; generally 200-300 ms (so mix = 0.2 or mix = 0.3) for MW ~400-2000 for longer mixing time 100-150 ms for short mixing time; 300-500 ms for long mixing time for MW < ~400.
14. Spin lock pulse width(**slpw**), must be a 90° pulse with a pulse width of ~30-35μs.
15. Power of spin lock pulse(**slpwr**), cannot be more than 49 units, preferably ~43 units.
16. **go** ↓.
17. **svf('I_love_reosy')** ↓.
18. **wft(1)** ↓. Phase this spectrum. ROESY spectrum is phase down.



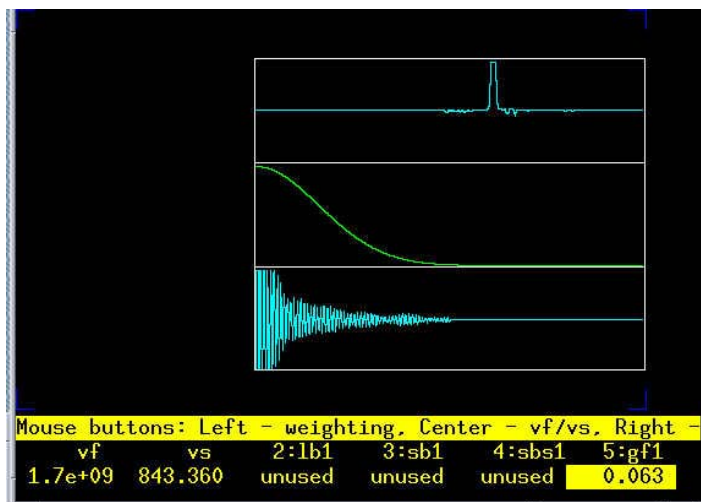
19. **wti**. Adjust **gf** to the end of the **fid**.



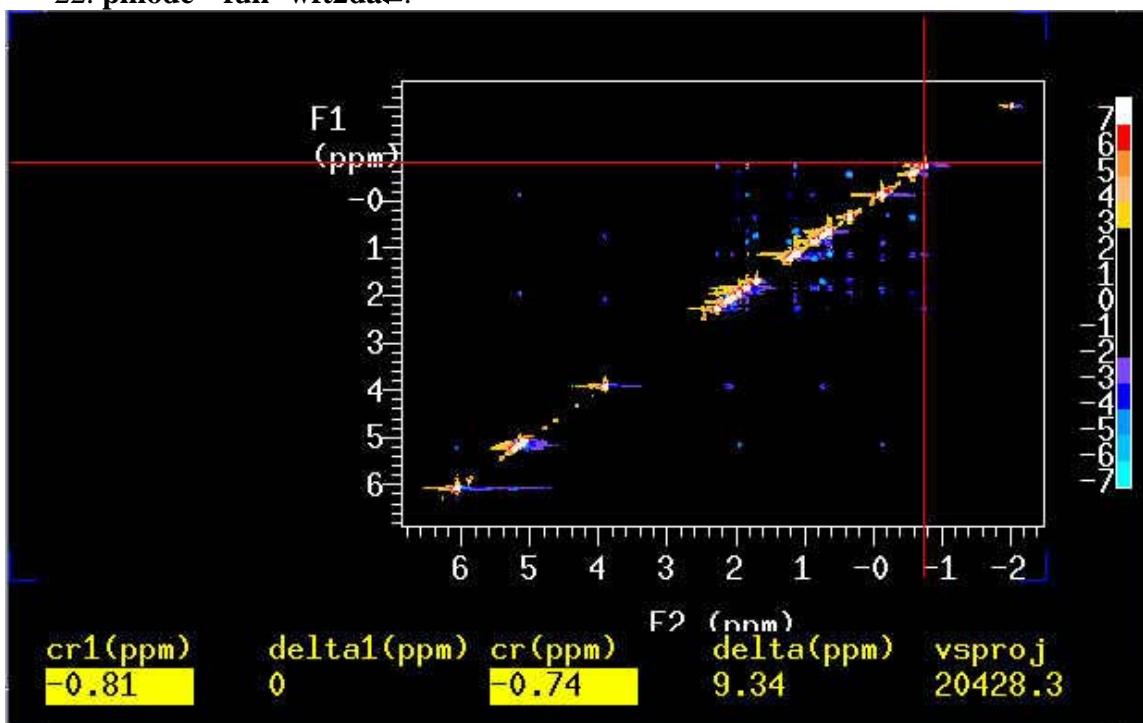
20. **wft1da**. Click on trace and choose a trace.



21. **wti**. adjust the **gf1** to the end of FID in t1 dimension.

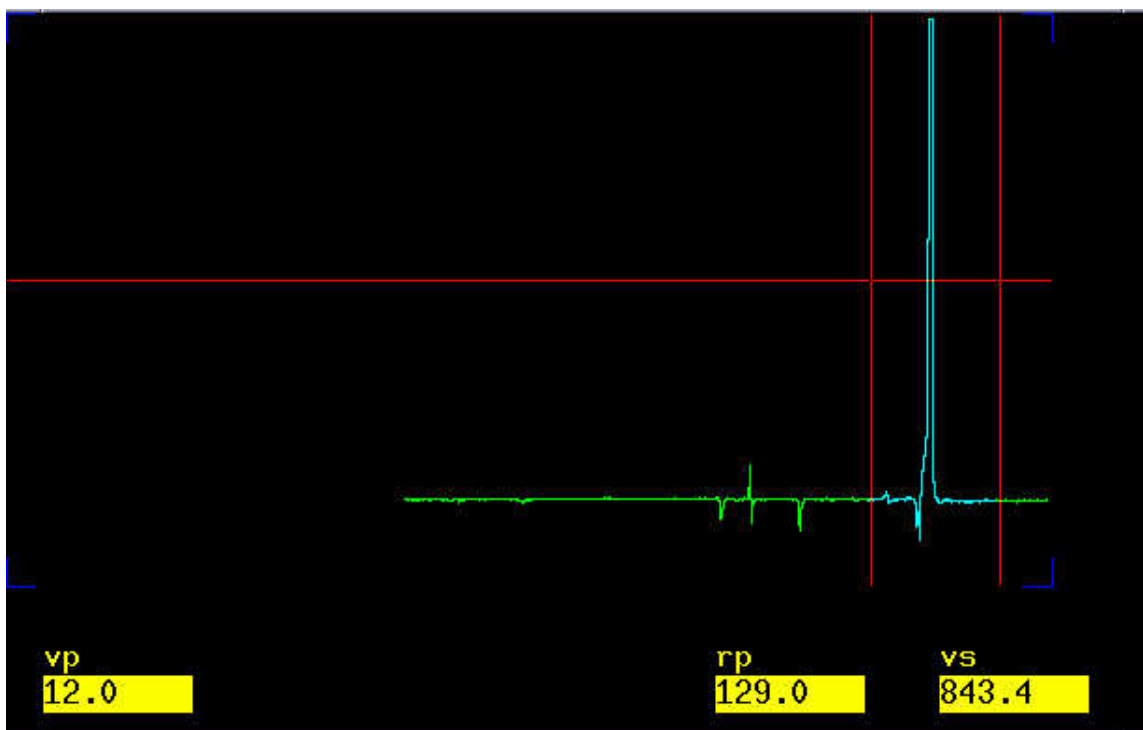


22. `pmode='full' wft2da`.



You might get a 2D spectrum like this, which needs to be phased on F2 dimension.

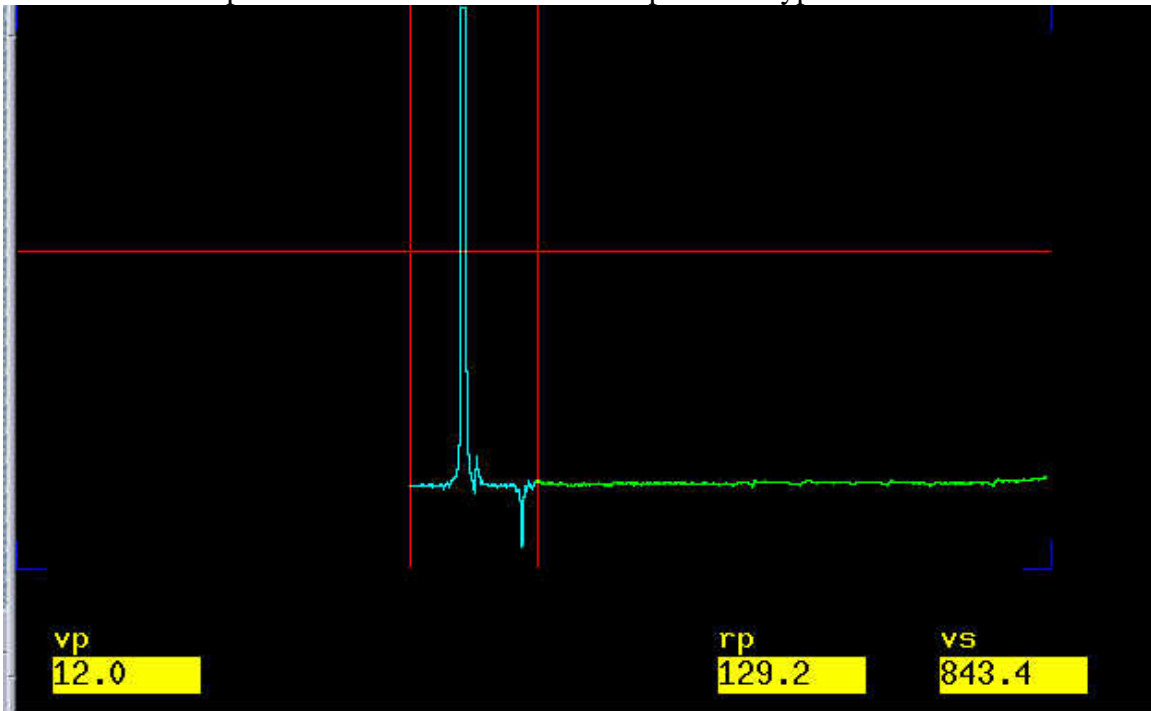
23. Point the mouse at the upper right peak (not solvent) and type `ds`.



24. Phase this spectrum.

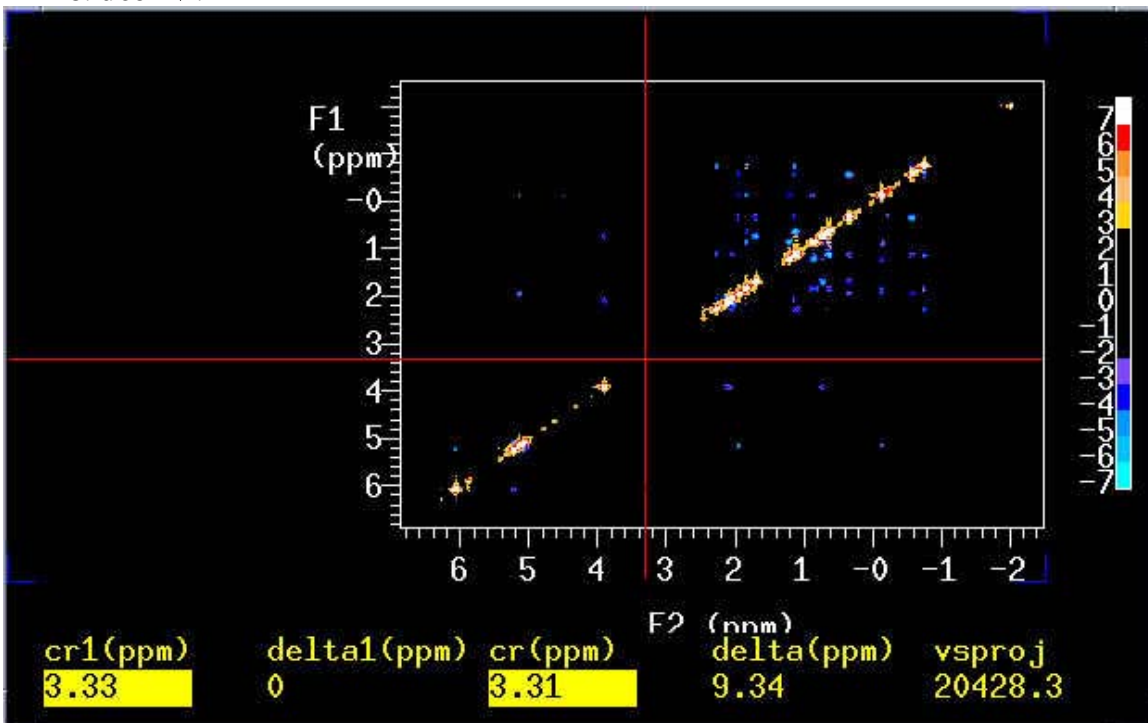
25. **dcon**. You a 2D spectrum again.

26. And then point the mouse at the lower left peak and type **ds**.



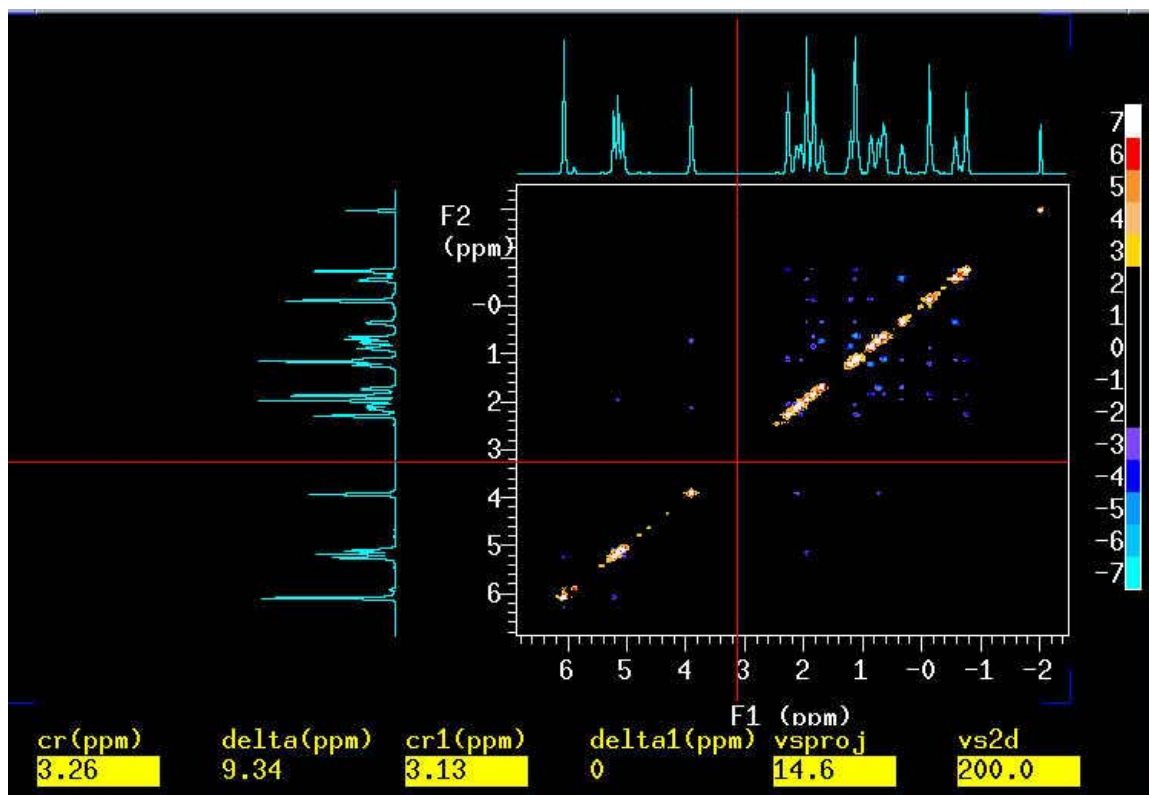
27. Phase this spectrum by adjusting the left peak. You need to click on the right side without adjustment.

28. **dcon**.



29. to change to F1 dimension, type **trace='f1'**.

30. If you like you can place 1D along both sides.



31. point the mouse at the solvent peak. **rl(7.27p) rl1(7.27p)** ↵.
32. *Adjust vertical scale with vs +20% and vs -20% menu buttons.*
33. **pcon(10,1.2) page** ↵.
34. You've got nice 2D ROESY spectrum.