



Analyses of Two Different Versions of HETCOR Techniques for Solids

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Abstract : Solid HETCOR (Hetero-Correlation) requires homo-dipolar decoupling between proton spins during the evolution and the mixing period in 2D-NMR. There are two different ways of achieving it with pulse sequences. One is based on the multiple pulse (MP) sequence where thousands of intense radio frequency (rf) pulses are used to remove the homo-dipolar interaction between protons. The other is utilizing the so-called Lee-Goldburg (LG) off-resonance scheme where a continuous rf-irradiation is used. In this report, the advantage of one technique to the other, is analyzed. LG version is evaluated better in S/N and easier in setup procedure with the same experimental time.

Keywords : HETCOR, solid-state NMR, 2D-NMR, Lee-Goldburg, multiple pulse sequence

INTRODUCTION

Compared to 1D-NMR techniques such as CP/MAS (Cross Polarization/Magic Angle Spinning) or CRAMPS (Combined Rotation And Multiple Pulses), the solid-HETCOR technique gives not only the chemical shifts of proton and X-nuclei but also their correlations which are vital in analyzing the molecular structure. Since the HETCOR is 2D-NMR technique, it has the evolution and the mixing period. In order to observe the proton chemical shift during the evolution period, the dipolar interaction between protons should be removed so that each proton spin is labeled with its chemical shift. Multiple pulse sequences such as WHH4, MREV8, BR24, can serve the purpose.¹⁻³ Since the sampling

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window is not required in HETCOR during the evolution period, BLEW24 is used, which is a variant of BR24 but does not require a strong rf pulse on the proton channel like BR24.⁴⁻⁵ The setup procedure of the multiple pulse sequence is not difficult but need some experience. It requires a precise tuning for rf-power and adjustment of matching capacitor to remove the so-called the phase glitch of the multiple pulses. Otherwise the error term accumulated by thousands of pulses, would ruin the resolution on the proton channel.

A continuous rf-irradiation technique is an alternative for the multiple pulse sequence in removing the dipolar interaction between protons. By the rf-irradiation, the strength of the dipolar interaction can be controlled depending on the resonance condition. With on-resonance condition, the dipolar interaction can be time-reversed as in the magic echo technique.⁶ By adjusting the off-resonance condition, the dipolar interaction can be disappeared, which is called the Lee-Goldburg condition.⁷ In rotating frame with LG condition, there are two components of magnetic field. One is the rf-field and the other is remnant external magnetic field due to the off-resonance condition. The effective field is a vector sum of two components. When the angle between the z-axis and the effective field becomes the magic angle, the dipolar interaction is to be diminished. So it can be viewed as MAS in the spin space like MP. It is fascinating to realize both LG and MP achieve the same objective in a similar fashion even though each pulse sequences look quite different. HETCOR pulse sequences utilizing these two different schemes, are presented and their experimental results are compared in the following sections.

EXPERIMENTALS

Like any other 2D-NMR techniques, the solid HETCOR is comprised of the preparation, the evolution, the mixing and the acquisition period, as presented in Fig. 1. Table 1 summarizes building blocks used in MP and LG version for each period. The details of pulse sequences are presented in references.^{8,9} In liquid state HETCOR, the mixing is mediated by J-coupling whereas it is mediated by the dipolar interaction in the solid HETCOR. Even though the origin of mixing is different, the spin Hamiltonian form is similar in both cases.

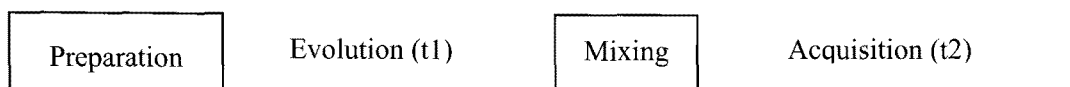


Fig. 1. General scheme of 2D-NMR experiment.

Table 1. Components of solid HETCOR in each period.

	Channel	Preparation	Evolution	Mixing	Acquisition
MP	¹ H	90	BLEW24	WIM24	Decoupling
	X	-	BB24	WIM24	Acquisition
LG	¹ H	90	LG	LG	Decoupling
	X	-	-	LG	Acquisition

During the mixing, a selective cross polarization between the specific carbon-proton pair occurs. In order to achieve this, homo-dipolar interaction between protons must be removed while the hetero-dipolar interaction between proton-carbon is maintained. In MP version, it is accomplished with WIM24 (Windowless Isotope Mixing).¹⁰⁻¹² In LG mode, off-resonance LG scheme is used. The correlation peak strength depends strongly on this mixing period where the spin diffusion or thermal contact between specific carbon-proton pair occurs. The actual spin diffusion depends on the interatomic distance and dynamics of proton-carbon pair. Therefore HETCOR can be used to estimate the interatomic distance qualitatively by varying the mixing time. This subject will be studied extensively in the future.

For 90-degree pulse, 5 μ s was used for LG version and 4 μ s for MP version since the shorter 90 degree pulse for MP is better. If 4 μ s was used for LG version, the power for the mixing on the carbon channel requires 76.5 kHz, which corresponds to 90 degree pulse of 3.26 μ s. Since our instrument cannot generate such strong pulse, a longer pulse length is chosen. The number of data point along t1 axis was 40. This can be adjusted by increasing t1 until no signal is observed during t2. Repetition delay was 3 sec and accumulated 32

times. The total experiment time was 1 hour 4 min for LG version and 4 times long for MP version to get comparable S/N ratio.

Monoethyl-fumerate is used for a test sample. It has one methyl, one methylen, two olefin and two carboxyl groups. Its molecular structure is given in Fig. 2.

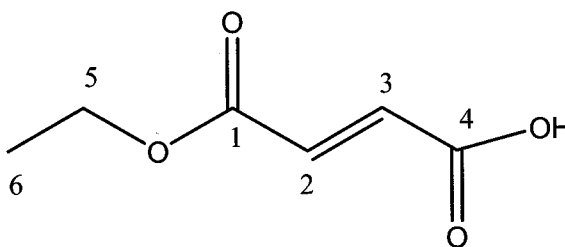


Fig. 2. The molecular structure of monoethyl-fumerate. The numbering is used for the referencing in the text.

RESULTS

The HETCOR results with MP and LG version, are presented in Fig. 4 and 5, respectively. Except the spinning sidebands observed in the HETCOR spectrum of MP version, they are comparable with each other. To achieve the comparable S/N ratio, however, the MP-HETCOR required four times more repetitions. With LG-HETCOR, the mixing time can be selected without restriction whereas only a certain time lapse is allowed in MP version. In the case of correlation detection for the proton-carbon pair with a longer distance, the MP version should have difficulty in observing it, which needs longer mixing time. Since the mixing should occur during the half rotation period, multiple mixing is needed to achieve the long mixing time for MP-HETCOR. This kind of complexity is due to the interference between the sample rotation and MP. Fig. 3 is the CP/MAS spectrum for the sample. Its peak assignment is C4, C1, C2, C3, C5, C6 from left to right.

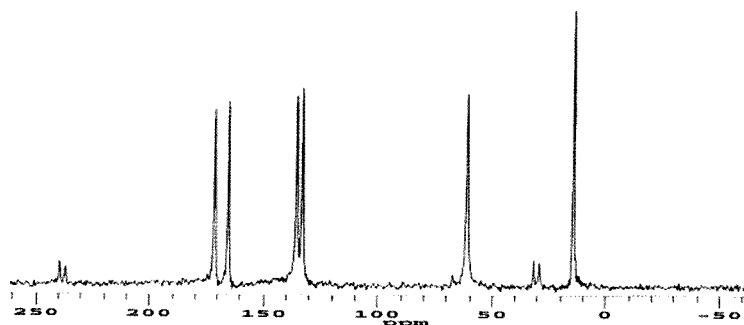


Fig. 3. The ^{13}C spectrum of monoethyl-fumerate obtained with CP/MAS. The small doublet peaks are spinning sidebands (SSB).

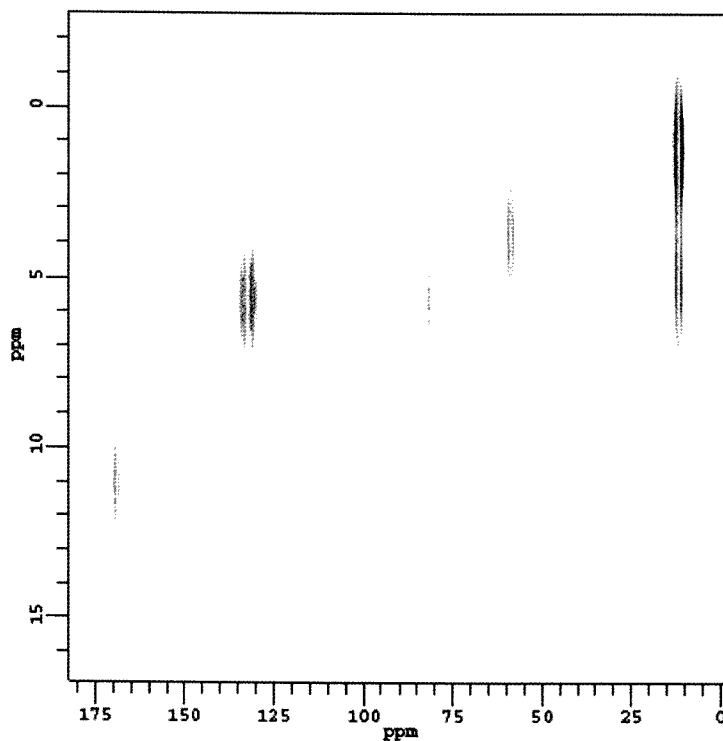


Fig. 4. HETCOR spectrum with MP version. Due to interference between MP and MAS, the spinning speed was set to 2603 Hz. The maximum mixing time is also limited to 192 μs . The SSB of olefin peaks are observed. The C4 peak from carboxyl acid is obtained with the maximum mixing time since its distance to neighboring proton is longer than directly bonded carbon-proton pair. The carboxylate correlation peak (C1) to H2 proton, is barely visible.

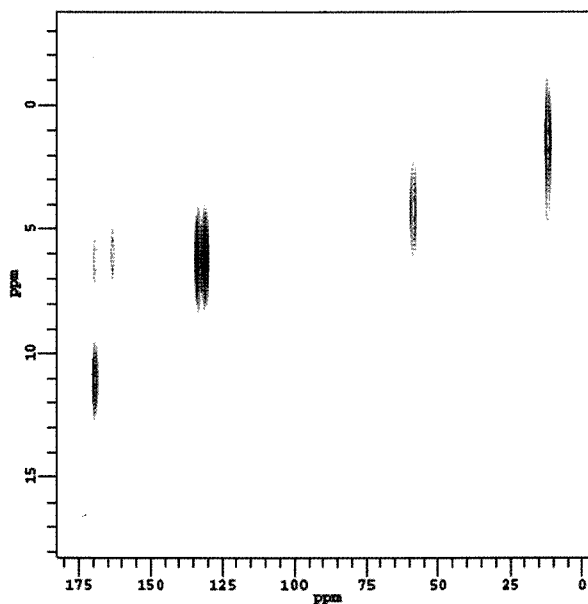


Fig. 5. HETCOR spectrum with LG version. Since there is no restriction on the spinning speed and the mixing time, it sets to 6 kHz and 200 μ s, respectively. There is no SSB. Overall, this HETCOR spectrum is clearer than that of Fig. 4

The projection onto F2 axis, coincides with the CP/MAS spectrum of ^{13}C . The projection of HETCOR spectrum onto F1 axis, should be ^1H spectrum, which can be obtained with CRAMPS or the ultrafast MAS.

Table 2. S/N ratio for each peaks with the mixing time of 192 μ s, which was the maximum mixing time for MP-HETCOR. The low value of the carboxylate peak indicates the interatomic distance between C1 and H2 is large. The low values of methyl and methylene peak are due to the effect of T1rho

	<i>Carboxyl acid</i>	<i>Carboxylate</i>	<i>Olefin</i>	<i>Methylene</i>	<i>Methyl</i>
MP	65.8	23.8	64	45	89.2
LG	63.6	28	70.2	59.7	48

Table 2 is S/N ratio for each peaks, obtained with MP and LG version of HETCOR. They are comparable with each other. The experimental time of MP version was four times longer than that of LG version.

CONCLUSION

Two different versions of HETCOR techniques are compared using the monoethyl-fumarate as a test sample. The spectrum resolution and the correlation peaks of the 2D-NMR spectrum, are comparable. The LG version is easier to setup for the experiment and gives a higher S/N with the same experiment time. Due to an interference of MP and sample spinning, there is a restriction in the mixing time for MP-HETCOR. With all these observations are considered, the LG version of HETCOR is superior to the MP version. Most of the recent HETCOR experiments seem to adopt the LG-HETCOR.¹³⁻¹⁵

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REFERENCES

1. H. G. Boman, *J. Intern. Med.* **254**, 197 (2003).
2. M. Zaslhoff, *Nature* **415**, 389 (2002).
3. D. Barra, and M. Simmaco, *Trends Biotechnol.* **13**, 205 (1995).
4. D. G. Lee, P. I. Kim, Y. Park, S. H. Jang, S. C. Park, E. R. Woo, and K. S. Hahm, *J. of Peptide Science* **8** **453** (2002).
5. K. H. Lee, D. G. Lee, Y. Park, D. I. Kang, S. Y. Shin, K. S. Hahm, and Y. Kim, *Biochem. J.* **394**, 1056 (2006).
6. A. Dercme and M. Williamson, *J. Magn. Reson.* **88**, 177 (1990).
7. A. Bax and D. G. Davis, *J. Magn. Reson.* **65**, 355 (1985).
8. S. Macura and R. R. Ernst, *Mol. Phys.* **41**, 95 (1980).

9. A. Bax and D. G. Davis, *J. Magn. Reson.* **63**, 207 (1985).
10. G. Bodenhausen and D. J. Ruben, *J. Chem. Phys. Lett.* **69**, 185 (1980).
11. A. T. Brünger, *X-PLOR Manual, Version 3.1*, Yale University, New Haven, CT (1993).
12. G. M. Clore and A. M. Gronenborn, *CRC. Rev. in Biochem. Biol.* **24**, 479 (1989).
13. G. M. Clore and A. M. Gronenborn, *Protein Science* **3**, 372 (1994).
14. K. Wüthrich, M. Billeter, and W. Braun, *J. Mol. Biol.* **169**, 949 (1983).
15. G. M. Clore, A. M. Gronenborn, M. Nilges, and C. A. Ryan, *Biochemistry* **26**, 8012-8023 (1987).
16. M. Nilges, G. M. Clore, and A.M. Gronenborn, *FEBS Lett.* **229**, 317 (1988).
17. A. Chattopadhyay, S. S. Rawat, D. V. Greathouse, D. A. Kelkar, and R. E. Koeppe, *Biophys. J.* **95**(1), 166 (2008).
18. R. Domanov, Y. Pietiäinen, V. P. Kontinen, and P. K. Kinnunen, *Biochim Biophys Acta* **1778**(4), 983 (2008).