

# Through-Bond Carbon–Carbon Connectivities in Disordered Solids by NMR

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**Abstract:** Using a refocused INADEQUATE NMR experiment we show that homonuclear carbon–carbon through-bond correlations can be obtained in disordered organic solids where the line widths greatly exceed the value of the scalar coupling. The feasibility of the experiment is demonstrated for inhomogeneous samples of <sup>13</sup>C-labeled wood and cellulose. The two-dimensional correlation maps are used to assign unambiguously the carbon resonances of this natural polymer. We show that the efficiency of the refocused INADEQUATE experiment depends on the ratio  $T_2'/T_2^*$ , where  $T_2^*$  is the apparent relaxation time, deduced from the line width, and  $T_2'$  is the transverse dephasing time measured in a spin–echo experiment. For cellulose, we find that  $T_2'$  is larger than  $T_2^*$  by more than 1 order of magnitude and that the experimental efficiency of the refocused INADEQUATE experiment is around 10%, in agreement with calculations. Even in ordinary crystalline organic solids, such as powdered amino acids, large differences are observed between  $T_2'$  and  $T_2^*$ .

## 1. Introduction

By combining cross-polarization (CP), magic angle spinning (MAS), and proton decoupling, high sensitivity solid-state NMR spectra of dilute spins such as carbon-13 can be recorded routinely for both crystalline and amorphous samples. For carbon-13 enriched samples, the assignment of the one-dimensional spectra is usually obtained by using carbon–carbon multidimensional correlation experiments which rely on dipolar-driven magnetization transfer between pairs of neighboring carbons. Thus, a number of methods are currently available to reintroduce the dipolar interaction (removed by MAS), yielding *through-space* nearest neighbor connectivities as well as longer range distance and angle information.<sup>1–13</sup>

In contrast to liquid-state NMR spectroscopy where experiments such as COSY<sup>14</sup> or TOCSY<sup>15,16</sup> usually constitute the first step toward assignment and structure determination, correlation techniques that probe *through-bond* connectivities are not yet commonly employed in solid-state NMR, mainly because the through-bond  $J$  coupling is usually much smaller than the (unaveraged) through-space dipolar interaction between adjacent nuclei. Nevertheless, a few correlation techniques that yield through-bond connectivities have been successfully used in solids as alternative techniques to dipolar recoupling methods. For example, Si–Si COSY-type experiments have been reported on glasses and zeolites.<sup>17–19</sup> Recently, a through-bond correlation technique, the TOBSY experiment (TOtal through-Bond correlation Spectroscopy), has been proposed that allows polarization transfer through the  $J$  couplings using a specially designed mixing scheme, and it has been applied to obtain <sup>13</sup>C–<sup>13</sup>C and <sup>31</sup>P–<sup>31</sup>P correlation spectra.<sup>20–22</sup>

We recently demonstrated that an INADEQUATE experiment, analogous to the liquid-state experiment, was feasible on ordinary crystalline organic solid samples.<sup>23</sup> The coherence transfer was demonstrated to be exclusively mediated by the  $J$

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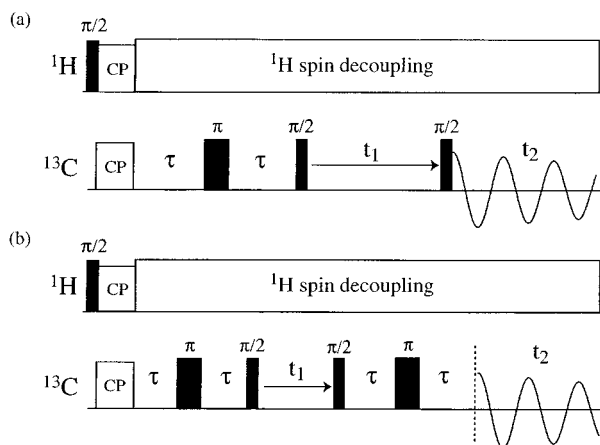
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**Figure 1.** Pulse sequences for solid-state INADEQUATE (a) and refocused INADEQUATE (b). Under ideal conditions, the degree of conversion into double quantum coherence is maximum for  $\tau = 1/(4J_{\text{CC}})$  and is zero for  $\tau = 1/(2J_{\text{CC}})$ . For all the experiments reported here, a 32-step phase cycle was used (pulse program and phase cycle available on our web site<sup>37</sup> or by request to the authors) and the  $\tau$  delay was synchronized to be an integral number of rotor periods.

interactions, leading to the unambiguous identification of through-bond connectivities. This simple and robust experiment was applied to crystalline samples such as powdered amino acids for which the line width in the one-dimensional carbon spectrum is comparable to the size of the scalar coupling (typically 50 Hz between two directly bonded carbons).

Through-bond connectivities in *disordered* solids represent a challenge to NMR since the interaction driving the coherence transfer may be over an order of magnitude smaller than the line width. In this paper, we report the feasibility of obtaining carbon-carbon through-bond correlations in amorphous solids exhibiting line widths of several hundreds of hertz using an inadequate type experiment. We show that a refocused INADEQUATE experiment can be performed on inhomogeneously broadened systems such as disordered polymers. This experiment has several intrinsic advantages over other schemes. The efficiency of the experiment, which is simple and robust, mainly depends on the *refocused line width*, i.e., on the line width corresponding to the dephasing time measured in a spin-echo experiment.

## 2. INADEQUATE Experiments in Solids

The pulse sequences for INADEQUATE and refocused INADEQUATE are shown in Figure 1, parts a and b. Apart from the cross-polarization period, the experiments are completely analogous to liquid-state versions.<sup>24</sup> After cross-polarization from  $^1\text{H}$ , the  $^{13}\text{C}$  magnetization evolves during the delay  $2\tau$  under the isotropic homonuclear  $J$  coupling Hamiltonian. The C-C homonuclear dipolar couplings are removed by fast magic angle spinning, and the chemical shift is refocused by the  $180^\circ$  pulse. The double quantum coherence created by the first carbon  $90^\circ$  pulse evolves during  $t_1$  at the sum frequency of the two spins,  $\omega_{\text{DQ}} = \omega_{\text{SQ}}^{\text{A}} + \omega_{\text{SQ}}^{\text{B}}$ , and is converted back into an anti-phase transverse coherence by the last  $90^\circ$  pulse. In the INADEQUATE experiment this anti-phase component is detected during  $t_2$ , whereas in the refocused INADEQUATE experiment, it is converted back during the second  $\tau$ - $\pi$ - $\tau$  delay, into an in-phase signal before detection. As in solution-state NMR, directly bonded carbon-13 resonances are identified by

the fact that they generate a common double-quantum frequency. As discussed previously,<sup>23</sup> the contribution of any residual dipolar coupling to the coherence transfer is negligible. Note that these experiments can be performed at high spinning rates.

If the  $\tau$  delay is synchronized with the rotation,  $\tau = n/\omega_r$ , the overall efficiency of an INADEQUATE sequence depends on one hand on the efficiency of excitation of the double quantum coherences, which depends periodically on  $\tau$ , and on the other hand on the decay of carbon magnetization during the  $\tau$  delays. Efficiency is also affected by cancellation or addition effects during the detection of anti-phase or in-phase line shapes respectively in  $t_2$ . Ordinary INADEQUATE yields anti-phase line shapes while refocused INADEQUATE yields in-phase line shapes. Thus, at first glance, for solid systems, refocused INADEQUATE may seem to be more sensitive because of the detection of in-phase line shapes. However, in this case loss of signal due to relaxation occurs during two  $\tau$ - $\pi$ - $\tau$  delays instead of only one for the ordinary INADEQUATE sequence (Figure 1). To compare the efficiency of the two experiments, two different time constants have to be introduced.

The apparent line width observed in the CPMAS spectra of dilute spin  $I = 1/2$  nuclei like carbon-13 originates from various sources<sup>25</sup> such as the following: (i) the degree of crystallinity of the sample, which yields a more or less broad chemical shift distribution; (ii) the local magnetic field homogeneity ( $B_0$  homogeneity, magnetic susceptibility effects, etc.); (iii) dipolar couplings; (iv) residual chemical shift anisotropy; or (v) the incoherent effects of stochastically fluctuating local fields that yield  $T_2$  relaxation. These contributions can be divided into two categories depending on whether they are inhomogeneous or homogeneous.<sup>26,27</sup> Inhomogeneously broadened line shapes consist of a continuum of independent lines that can be manipulated individually (and result, for example, from chemical shift distribution or field inhomogeneity) whereas homogeneously broadened lines react to pulses as an indivisible entity (and result, for example, from multispin dipolar couplings or  $T_2$  relaxation). This distinction is very important for INADEQUATE experiments.

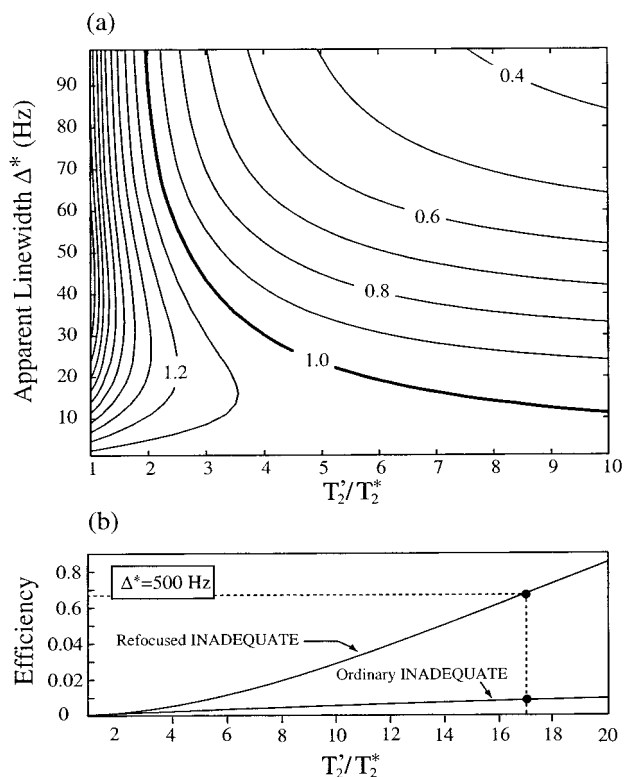
In this article we define the apparent full line width at half-height as  $\Delta^*$  (in Hz), and  $T_2^*$  (in s), the corresponding time constant, is given by  $T_2^* = 1/\pi\Delta^*$ . For spin  $I = 1/2$ , the inhomogeneous line broadening (or rather the contribution of all interactions which are described by a Hamiltonian linear in  $I_z$ ) can be refocused (i.e. removed) by a  $\pi$  pulse in a simple Carr-Purcell spin-echo experiment. We define  $T_2'$  as the time constant that can be measured in such a spin-echo experiment, and  $\Delta'$  the residual line width, or the "non-refocusable" line width,  $\Delta' = 1/\pi T_2'$ . In liquid samples the refocused line width is usually dominated by the motional behavior of the molecules through the spin-spin relaxation time  $T_2$  (i.e.  $T_2'$  is equal to  $T_2$ ). In solids, besides the genuine relaxation time  $T_2$ , there are additional nonrefocusable interactions which may be dominant, such as the high-order terms of the heteronuclear dipolar interaction which yield a coherent homogeneous line broadening and contribute to  $T_2'$  (i.e.  $T_2' < T_2$ ). To calculate the efficiency of INADEQUATE, we have to consider the time constant  $T_2'$  when considering the decay of the carbon magnetization *during the  $\tau$ - $\pi$ - $\tau$  delays*, and the time constant  $T_2^*$ , i.e., the apparent line width, when calculating the line shape detected *during acquisition*.

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**Figure 2.** (a) Ratio of the predicted efficiency of ordinary INADEQUATE to that of refocused INADEQUATE as a function of the apparent line width  $\Delta^* = 1/\pi T_2^*$  and the ratio  $T_2'/T_2^*$ . The efficiencies were calculated using the (numerical) optimum value of  $\tau$  for each ( $\Delta^*$ ,  $T_2'/T_2^*$ ) pair. (Note that, in fully  $^{13}\text{C}$ -enriched samples,  $\Delta^*$  is the line width for a single component of the  $J$  doublet.) The  $J$  coupling was fixed at 35 Hz. The calculations were carried out using an isolated two-spin system. (b) Absolute efficiencies of the refocused and ordinary INADEQUATE experiments as a function of  $T_2'/T_2^*$ . The theoretical efficiencies were calculated as the ratio of the maximum amplitude of the anti/in-phase doublet in 1D INADEQUATE to the amplitude of the in-phase doublet in a single-pulse experiment. These theoretical calculations were done for a  $J$  coupling of 35 Hz and an apparent line width at half-height of 500 Hz.

Figure 2a shows the ratio of the predicted efficiency of ordinary INADEQUATE to that of refocused INADEQUATE as a function of the apparent line width  $\Delta^*$  and the ratio  $T_2'/T_2^*$ . If we assume that the inhomogeneous line broadening is mainly due to a chemical shift distribution, large  $T_2'/T_2^*$  ratios correspond to highly disordered systems and small  $T_2'/T_2^*$  ratios to much more crystalline samples. For a given apparent line width, as the  $T_2'/T_2^*$  ratio increases the loss of magnetization due to relaxation during the  $\tau$ - $\pi$ - $\tau$  periods decreases and ordinary INADEQUATE becomes less efficient than refocused INADEQUATE (Figure 2a). The “degree” of disorder from which refocused INADEQUATE becomes the more sensitive experiment depends on the apparent line width. Indeed, the larger the apparent line width, the more the signal is attenuated by cancellation effects during the detection of anti-phase line shapes in ordinary INADEQUATE. Thus, for example, for an apparent line width of 30 Hz, refocused INADEQUATE offers a better signal-to-noise ratio when  $T_2'/T_2^*$  is larger than 4, whereas for an apparent line width of 100 Hz, this ratio only has to be larger than two for refocused INADEQUATE to be more efficient.

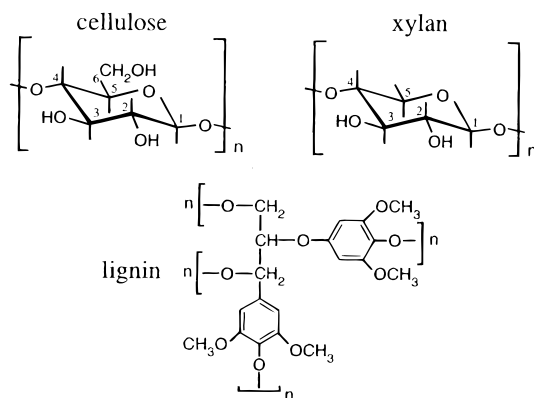
Figure 2b shows the absolute efficiency of the INADEQUATE sequences calculated for a line width of 500 Hz as a function of  $T_2'/T_2^*$ . This efficiency is calculated as the ratio of the maximum amplitude of the anti/in-phase doublet in a 1D

INADEQUATE spectrum to the amplitude of the in-phase doublet in a single-pulse experiment. We see that the more inhomogeneous the line broadening is, the more the efficiency of INADEQUATE increases. However, more importantly, whereas ordinary INADEQUATE remains very insensitive (<1%), the efficiency of refocused INADEQUATE rapidly reaches values that are suitable for running experiments. For example, for  $T_2'/T_2^* = 18$  (which is the order of magnitude that we found in cellulose, see below), the efficiency of refocused INADEQUATE is about 8% and is 8.5 times more sensitive than ordinary INADEQUATE. Note that this represents a factor of 72 in terms of experimental time. Thus from these calculations, we predict that for highly inhomogeneously broadened systems such as amorphous polymers and glasses, refocused INADEQUATE will be much more sensitive than ordinary INADEQUATE, and that this experiment should be sensitive enough to yield a reasonable signal in the 2D correlation map.

### 3. Application to Disordered Solids

We applied the refocused INADEQUATE sequence to the characterization of the carbon spectra of cellulose extracted from wood and to intact carbon-13 labeled wood chips. The one-dimensional CPMAS spectra are shown above the 2D maps in Figure 3a and b, respectively. In these 1D spectra the apparent line widths range from 450 to 650 Hz, and are known to correspond primarily to inhomogeneous broadening due to the amorphous character of these polymer samples.

The structural characterization of wood is a challenging problem, due to its complex chemical composition and to the conformational heterogeneity of the various components of this natural material, and the three-dimensional structure of wood is not yet fully understood. Wood (*Populus euramericana*) contains three major components: cellulose (45%); hemicellulose, mostly partially acetylated xylan (25%); and lignin (20%).



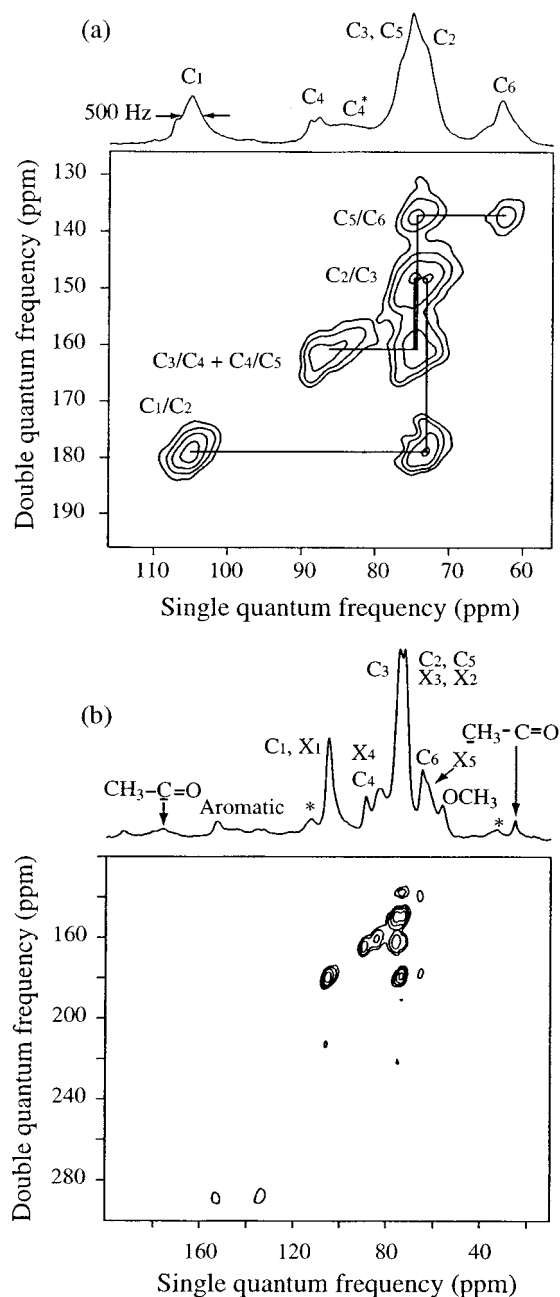
Cellulose, which is a linear polymer, forms fibrils which are held together by the hemicelluloses and the lignin. In the present study,  $^{13}\text{C}$ -enriched wood chips were obtained from aspen (*Populus euramericana*) grown in a 20%  $^{13}\text{CO}_2$ -enriched atmosphere.<sup>28</sup> An overall  $^{13}\text{C}$ -enrichment of 11% was obtained. Cellulose was isolated from extracted lignin fragments according to protocols described elsewhere.<sup>29,30</sup>

Figure 3a shows the refocused INADEQUATE spectrum of extracted cellulose recorded in a total experiment time of 16 h (250 mg of sample). As pointed out previously, in the 2D map

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**Figure 3.** (a) Two-dimensional refocused INADEQUATE spectrum of cellulose. The spectrum was obtained at 125 MHz on a Bruker DSX 500 spectrometer using a 7 mm double-tuned MAS probe. A total of 100  $t_1$  increments with 192 scans each were collected. The  $\tau$  delay was set to 3 ms, and the contact time for cross-polarization to 500  $\mu$ s. The spinning speed was 6 kHz and the proton decoupling field strength was 100 kHz using continuous wave decoupling. Quadrature detection was achieved using the TPPI method.<sup>38</sup> The repetition delay was 3 s. The various connectivities between the bonded carbons are indicated by solid lines. (b) Two-dimensional refocused INADEQUATE spectrum of wood chips. A total of 80  $t_1$  increments with 1280 scans each were collected. Other experimental parameters are the same as in Figure 3a except for the repetition delay which was 1.5 s. The 1D  $^{13}\text{C}$  spectra are shown above the 2D spectra together with the corresponding assignment as elucidated by this study and previous ones.<sup>31–33</sup>  $C_n$  and  $X_n$  refer to the carbon  $n$  of respectively the cellulose and the hemicellulose (xylan) unit. Note that, as the  $^{13}\text{C}$ -enrichment is 11%, there are only 1.2% of the molecules with two bonded carbon-13 which are observed in these two-dimensional spectra.

two directly bonded carbons share a common frequency in the double quantum dimension, and provided that one carbon

resonance can be identified a priori, assignment is quite straightforward. The cellulose spectrum contains resonances which have already been identified in previous studies, mainly by comparison with liquid spectra, and more recently by two-dimensional NMR.<sup>31–33</sup> Using the refocused INADEQUATE spectrum, and starting from the easily identifiable resonance of the anomeric carbon  $C_1$  at 105 ppm, it is possible to sequentially assign the carbon spectrum relatively straightforwardly. First, the  $C_2$  resonance can be identified at 73 ppm, since this is the only resonance correlated with  $C_1$ . Carbon  $C_2$  has a second clear correlation in the double quantum frequency dimension at 147.5 ppm which leads to the identification of  $C_3$  at 74.5 ppm. Two sets of correlation peaks are then possible for the  $C_3/C_4$  connectivity, one at 160 ppm and another at 137 ppm. On the basis of chemical shift considerations, the upfield carbon signal at 62 ppm is identified as the methylene  $C_6$  carbon, which allows the assignment of  $C_4$  to the resonances around 87 ppm. For the  $C_4$  resonance, the shoulder at lower frequency corresponds to less crystalline domains of cellulose, probably to units on the surface of the fibrils.<sup>34</sup> Carbon  $C_4$  is then connected to  $C_5$  (74.5 ppm) whose signal is in fact superimposed with  $C_3$ , and  $C_5$  is correlated with  $C_6$  (62 ppm).

Interestingly, our assignment differs in one point from that which has been recently proposed using two-dimensional spin diffusion exchange spectroscopy.<sup>33</sup> Indeed, in this previous assignment, carbon  $C_2$  was found to resonate at higher frequency than carbon  $C_3$ . From rows extracted parallel to the  $F_2$  dimension from the refocused INADEQUATE spectra (data not shown), it is quite clear that  $C_2$  resonates at lower frequency than  $C_3$ . Note that this observation is in agreement with chemical shifts measured for cellulose in the liquid state.<sup>32</sup> This discrepancy underlines the fact that data from dipolar correlation techniques must be interpreted with caution as they may yield incorrect assignments. *In this sense it is not surprising that through-bond correlation experiments provide more reliable methods to probe bonded connectivities.* Also note that in a dipolar-driven magnetization transfer experiment, inter-unit correlations can be potentially observed, i.e., correlations between spins belonging to nearby units on the same chain. Such correlations, which may hinder the assignment, would never be visible in an INADEQUATE-type spectrum. (Note that  $J$  couplings have other advantages, discussed in the conclusions below.)

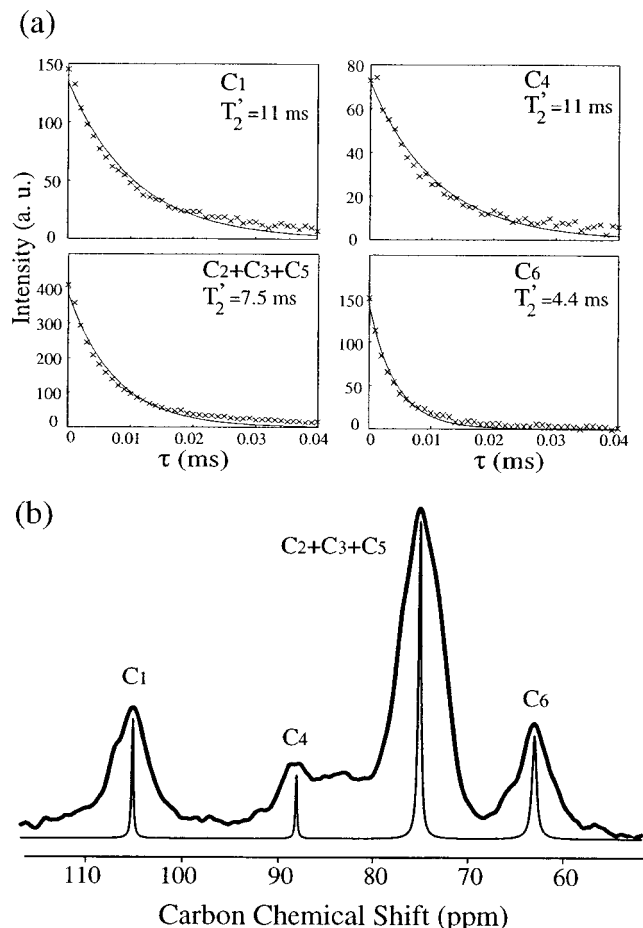
The refocused INADEQUATE spectrum of a sample of 80 mg of intact wood chips is illustrated in Figure 3b. Besides the connectivity pattern of cellulose and hemicellulose (except for  $X_5$ , the resonances of hemicellulose are superimposed on those of cellulose), the spectrum shows an additional set of correlation peaks at 287 ppm in the double quantum frequency dimension, corresponding to the connectivity between the aromatic carbons of lignin. This illustrates the fact that the experiment is sensitive enough to allow the observation of minor components (<20%) even in small samples (the spectrum was recorded in 42 h). Note that some connectivities corresponding to very weak signals are missing in the 2D map, such as those of the acetoxy group or the  $X_4-X_5$  correlation, but note also that the sample is only enriched in carbon-13 to a level of 11%, so that in the INADEQUATE spectra we only observe the 1.2% of the carbon

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**Figure 4.** (a) Measurement of carbon  $T_2$  dephasing times on enriched cellulose. The experimental points (8 scans each) were obtained using a  $\tau$ - $\pi$ - $\tau$  sequence on carbon after cross-polarization, with continuous wave proton decoupling (radio frequency field strength 100 kHz). The spinning speed was 6 kHz. The  $T_2$  dephasing constants were obtained by fitting the experimental points with a decaying monoexponential function. (b) Experimental (thick line) and schematic (thin line) cellulose  $^{13}\text{C}$  spectrum. In the schematic spectrum, each peak is a Lorentzian line whose line width was obtained from the fitted  $T_2$  values.

atoms in the sample that are carbon-13 and that have another carbon-13 as nearest neighbor.

#### 4. Carbon-13 Line Widths in Organic Solids

Under our experimental conditions, we found that the efficiency of these two refocused INADEQUATE experiments (measured for the  $\text{C}_1$  resonance) was about 7%. As pointed out previously, the feasibility of these experiments on samples having line widths of several hundreds of hertz implies that the line broadening is mainly inhomogeneous. To quantify the contributions of the inhomogeneous and homogeneous mechanisms on the line width, we have measured  $T_2$  on the cellulose sample, and compared the  $T_2/T_2^*$  values obtained with those measured in ordinary organic samples.

Figure 4a shows, for the four major peaks of the cellulose spectrum, the experimental data points recorded in a rotor synchronized spin-echo experiment as well as the curves obtained from a best fit to a monoexponential function. As mentioned above, for spin  $I = 1/2$ , the spin-echo experiment allows the measurement of the residual homogeneous line width  $\Delta'$  and thus the evaluation of the relative contribution of inhomogeneous interactions, such as a chemical shift distribu-

**Table 1.** Experimental  $T_2^*$  and  $T_2'$  (and equivalent line widths  $\Delta^*$  and  $\Delta'$ ) for 11% Carbon-13 Enriched Cellulose<sup>a</sup>

nuclei	$T_2^*$ ( $\Delta^*$ )	$T_2'$ ( $\Delta'$ )
C1	0.64 ms (500 Hz)	11 ms (29 Hz)
C2 + C3 + C5	0.49 ms (650 Hz)	7.5 ms (42 Hz)
C4	0.55 ms (580 Hz)	11 ms (29 Hz)
C6	0.71 ms (450 Hz)	4.4 ms (72 Hz)

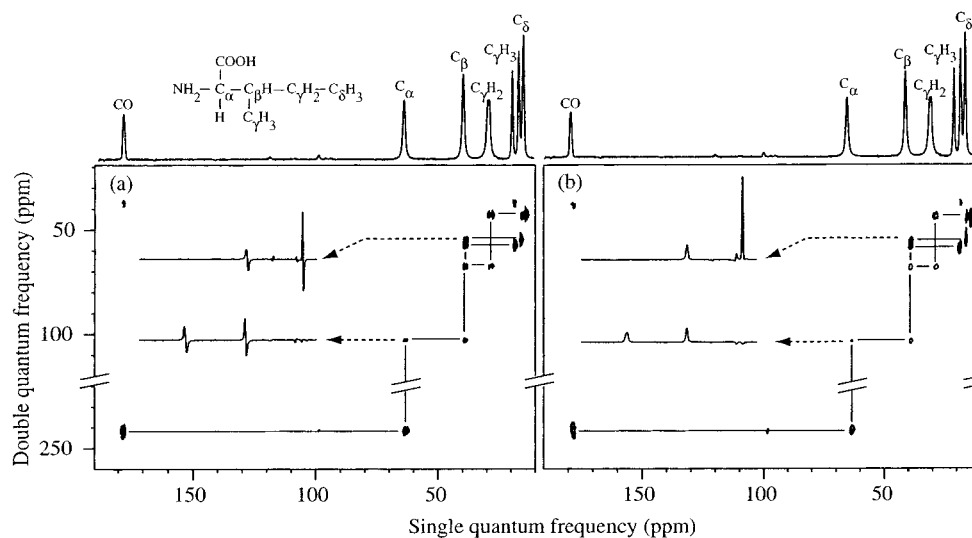
<sup>a</sup>  $T_2^*$  was calculated as  $T_2^* = 1/\pi\Delta^*$  where  $\Delta^*$  is the apparent line width, i.e., the full line width at half-height measured in the experimental 1D  $^{13}\text{C}$  spectrum.  $T_2'$  is the time constant obtained by a fitting procedure from experimental points recorded in a rotor synchronized spin-echo experiment as explained in the caption of Figure 4a.  $\Delta'$  is calculated as  $\Delta' = 1/\pi T_2'$ . Note that the values reported for  $T_2'$  are very likely to be dependent on the experimental conditions.

**Table 2.** Experimental  $T_2^*$  and  $T_2'$  (and equivalent line widths  $\Delta^*$  and  $\Delta'$ ) for Natural Abundance L-Isoleucine<sup>a</sup>

nuclei	$T_2^*$ ( $\Delta^*$ )	$T_2'$ ( $\Delta'$ )
CO	6.7 ms (47 Hz)	46 ms (7 Hz)
C $\alpha$	3.2 ms (100 Hz)	10 ms (32 Hz)
C $\beta$	3.1 ms (102 Hz)	13 ms (24 Hz)
C $\gamma\text{H}_2$	1.9 ms (166 Hz)	7.4 ms (43 Hz)
C $\gamma\text{H}_3$ (1)	11.8 ms (27 Hz)	120 ms (2.6 Hz)
C $\gamma\text{H}_3$ (2)	9.1 ms (35 Hz)	77 ms (4.1 Hz)
C $\delta\text{H}_3$ (3)	10.2 ms (31 Hz)	130 ms (2.5 Hz)
C $\delta\text{H}_3$ (4)	9.1 ms (35 Hz)	130 ms (2.5 Hz)

<sup>a</sup> The parameters were measured and calculated as for Table 1. The data were recorded with a spinning rate of 10 kHz and a proton decoupling field strength of 100 kHz using continuous wave decoupling (16 scans for each point). The delay  $\tau$  of the spin-echo sequence was varied from 0 to 30 ms. Note that some peaks have artificially large values of the apparent line width since there are two molecules per unit cell (yielding four distinct methyl group resonances) but since we only observe one peak for the CO, C $\alpha$ , C $\beta$ , and C $\gamma\text{H}_2$  resonances. The methyl groups are numbered in order of increasing frequency.

tion, to the apparent line width  $\Delta^*$ . Note that although each resonance in the carbon spectrum is a continuum of lines which probably have differing refocused line widths (this is especially true for the  $\text{C}_2 + \text{C}_3 + \text{C}_5$  peak that corresponds to three different types of carbons), the monoexponential decay model gives a relatively good fit of the data and yields a useful average value of  $T_2'$  for each peak. The results are summarized in Table 1, which compares the  $T_2^*$  and  $T_2'$  values (or the corresponding  $\Delta^*$  and  $\Delta'$  values) for each peak. For peaks C1, C4, C2 + C3 + C5, and C6, we find that the  $T_2'/T_2^*$  ratios were respectively 17, 15, 20, and 5. This means that (except for peak C6) about 95% of the line width is due to inhomogeneous (refocusable) effects, probably corresponding for this sample mainly to chemical shift dispersion. Note that such large differences between the two time constants have previously been observed for a sample of microcrystalline cellulose.<sup>34</sup> This difference in order of magnitude between the homogeneous and inhomogeneous line broadening is highlighted graphically in Figure 4b. Below each resonance of the experimental spectrum (thick line), we show a Lorentzian peak having a line width at half-height equal to the measured refocused line width  $\Delta'$ . (Note that such a spectrum could not be recorded experimentally since it implies the refocusing of all chemical shift information.) As mentioned above, the efficiency of the experiment measured for the C1 carbon was about 7%. This is in agreement with calculations (a theoretical efficiency for the refocused INADEQUATE experiment of about 6.5% is predicted in Figure 2b for an apparent line width of 500 Hz and  $T_2'/T_2^* = 17$ , corresponding to the measured values for carbon C1). Note that an ordinary INADEQUATE experiment would not be practicable on these cellulose samples (<1% predicted efficiency).



**Figure 5.** Two-dimensional INADEQUATE (a) and refocused INADEQUATE (b) spectra of 30 mg of fully  $^{13}\text{C}$  labeled L-isoleucine. The sample of L-isoleucine was purchased from Sigma and used without further recrystallization. The spectrum was obtained at 125 MHz on a Bruker DSX 500 spectrometer using a 4 mm triple-resonance MAS probe. A total of 384  $t_1$  increments with 32 scans each were collected. The  $\tau$  delay was set to 2 ms, and the contact time for cross-polarization to 1 ms. The spinning rate was 10 kHz and the proton decoupling field strength was 100 kHz using continuous wave decoupling. Quadrature detection was achieved using the TPPI method.<sup>38</sup> The connectivities are indicated by solid lines. Two rows extracted from the  $F_2$  dimension and corresponding to the  $\text{C}\alpha/\text{C}\beta$  and the  $\text{C}\beta/\text{C}\gamma\text{H}_3$  correlations are shown. Anti-phase line shapes are observed in the INADEQUATE spectrum (a) whereas in-phase peaks are obtained in the refocused INADEQUATE spectrum (b).

Using the same very simple approach, the contribution to the line broadening of the inhomogeneous interactions in crystalline samples was also investigated. If very large differences between the apparent and refocused line widths are expected for amorphous samples, it is less obviously the case in crystalline samples. We have made measurements on a powder of natural abundance L-isoleucine. The sample was purchased from Sigma and used without further recrystallization. The results are summarized in Table 2. Surprisingly, large differences between  $T_2^*$  and  $T_2'$  are also observed for L-isoleucine, which is however representative of what we usually designate as an ordered sample. Especially for the methyl groups  $T_2'/T_2^*$  ratios close to 10 are obtained, and when the contribution of the inhomogeneous interactions is subtracted, line widths as little as about  $3(\pm 2)$  Hz are observed. Similar studies by Cowans and Grutzner<sup>35</sup> on hexamethylbenzene showed similar effects, indicating that such observations are likely to correspond to a widespread effect. Note that these values for the refocused line width are of the same order of magnitude as those observed in liquid-state NMR. As pointed out above, the remaining (unrefocusable) homogeneous broadening can be due to coherent processes (like residual dipolar interactions) or to incoherent processes ( $T_2$  relaxation). We are currently investigating the respective contribution of these two types of processes, with the objective of determining the role of genuine  $T_2$  relaxation in line broadening of solid samples (i.e., to find out to what extent the refocused line width could possibly be reduced). As has been amply illustrated in pioneering studies of line widths in solids by VanderHart and co-workers,<sup>25</sup> the  $T_2'$  values measured in a spin-echo experiment largely depend (via the contribution of the residual dipolar interactions) on experimental parameters such as the spinning frequency or the proton decoupling field amplitude. Relatively modest conditions were used in this study for both cellulose and L-isoleucine ( $\omega_r = 6$  and 10 kHz, respectively,  $\omega_{1\text{H}} = 100$  kHz). Further studies of the refocused line width as a function of the spinning frequency and decoupling field strength using state of the art technology

to test current limits would be particularly informative. Note, for example, that in these studies we used continuous wave decoupling, but that more sophisticated decoupling schemes (such as TPPM<sup>36</sup>) should lead to increased values of  $T_2'$ .

For each carbon of natural abundance L-isoleucine, the  $T_2'/T_2^*$  ratio can be calculated and located on the diagram of Figure 2a. We find that the points are distributed to the right of the  $T_2'/T_2^* = 1$  contour level so that for this sample the efficiency of refocused INADEQUATE should already be slightly better than ordinary INADEQUATE (the efficiency ratio for the two experiments ranges from 0.5 to 0.9). Experimentally, for a 99% carbon-13 enriched sample of L-isoleucine, we found that the two types of experiments have about the same sensitivity, as illustrated in Figure 5. The connectivities between the carbon resonances are indicated and two rows extracted parallel to the  $F_2$  dimension are shown. The slight difference between our predictions and experiment may be due to multi-spin effects in this fully labeled sample, which were not included in the simulations of Figure 2. In addition, as pointed out in the caption of Table 2, the  $T_2'/T_2^*$  ratios are over estimated for the CO,  $\text{C}\alpha$ ,  $\text{C}\beta$ , and  $\text{C}\gamma\text{H}_2$  due to the overlap of the resonances from the two different molecules in the unit cell. Thus, it appears that even for ordinary organic molecules having line widths of a few tens of hertz, refocused INADEQUATE may be more sensitive than ordinary INADEQUATE.

## 5. Conclusions

In conclusion, we have shown that through-bond connectivities can be obtained in carbon-13 enriched disordered solids using a refocused INADEQUATE experiment. Despite large line widths, the experiment is practicable and provides the unambiguous assignment of the carbon resonances. Thus, this experiment, which is very easy to implement, should be useful for the characterization of carbon spectra of natural (as demonstrated here for wood) or synthetic polymers.

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One should note that, apart from its pure through-bond nature, there are several intrinsic advantages to using  $J$  couplings in solid-state NMR correlation experiments. These include the following: (i) the fact that the coupling is independent of orientation (no angle encoding); (ii) there is no constraint on the decoupling field amplitude; (iii) it is broadband (it does not depend on offset or on the value of the chemical shift anisotropy); (iv) it is insensitive to molecular motion; and (v) it can be used at any sample spinning rate. Indeed, the only real disadvantage of  $J$  couplings is their relatively small size, which results in sensitivity losses during the relatively long

excitation periods. However, we have shown that this problem can be overcome, yielding reasonably sensitive experiments, and further developments in decoupling performance and sample spinning (which will further increase  $T_2'$ ) will automatically lead to continuing improvements of the efficiency of these experiments.

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