Journal of Magnetic Resonance **140,** 300–303 (1999)
Article ID jmre.1999.1880, available online at http://www.idealibrary.com on IDEAL®

INADEQUATE-CR Experiments in the Solid State

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Received June 16, 1999; revised July 14, 1999

Through-bond connectivity can be probed by *J* couplings. For effective two-spin systems, the INADEQUATE experiment is highly valuable in liquid-state spectroscopy. It is the purpose of this Communication to show that *in-phase* INADEQUATE-CR spectra, where the intensity is concentrated in only one line of the *J* splitted doublet, can be obtained from solid-state samples. The problem of the cancellation of nonresolved multiplet lines, as experienced typically in INADEQUATE spectra in the solid, is resolved and the ^{13}C spectra become simpler because the number of resonance lines is reduced. Furthermore, a gain in signal intensity by $\sqrt{2}$ can, theoretically, be achieved. We limit the discussion to two-spin systems. In the present context, a two-spin system is defined considering the *J* coupling only. When the dipolar coupling is also taken into account, the two-spin system will usually become a many-spin system, but in the present context this is not relevant.

Because of the small size of the *J* couplings, compared to the dipolar coupling and the chemical shielding anisotropy, its use in rigid solids has only recently been demonstrated for carbon nuclei. Two approaches have been taken for homonuclear systems: (a) Total Through Bond Spectroscopy (TOBSY) (*1*, *2*) leads to a net transfer of polarization between the coupled spins and is the equivalent of the liquid-state TOCSY (*3*) experiment. No spectral resolution of the *J* splitting is required. During the mixing period (polarization-transfer period), a rotor-synchronous pulsed spin-lock is applied to the ^{13}C spins. The rotor synchronization is particularly important at slower spinning speed; it may become unnecessary at faster spinning rates and for small isotropic chemical-shift differences (*1*): (b) Lesage *et al.* (*4*) used the liquid-state INADEQUATE experiment (*5, 6*) and combined it with cross-polarization and MAS. The INADEQUATE experiment prepares double-quantum coherence by an excitation period of length $1/(2J)$. The double-quantum coherence is then converted to observable single-quantum coherence by a single RF pulse. If the antiphase multiplet is not well resolved, the transfer efficiency drops because of destructive interference between the multiplet components. For a linewidth exceeding the *J* coupling by more than a factor of two, the efficiency of the experiments is greatly deteriorated (*4*). The INADEQUATE experiment does not require RF-irradiation during the preparation of the multiple-quantum coherence and is expected to work particularly well for fast MAS spinning.

It should be noted, that the TOBSY and the INADEQUATE experiment have some different properties and have different fields of application. In the following, we will concentrate on INADEQUATE experiments on effective two-spin systems. For the size of the spin system, only *J*-coupling interactions have to be accounted for. When the dipolar coupling is also considered, the two-spin system will usually become a many-spin system (for an example see (*7*)).

In liquid-state INADEQUATE experiments, it has been demonstrated that the single pulse used for reconversion of double-quantum to single-quantum coherence can be replaced by a sequence similar to the one applied for excitation. Then, the multiplet structure of the resulting spectrum is in-phase and no cancellation takes place. However, another time period of length $1/(2J)$ must be introduced into the experiment. (For an overview see Buddrus *et al.* (*8*) and the monograph by Ernst *et al.* (*9*), Chap. 8.)

The INADEQUATE-CR (Composite Refocusing) sequence goes even a step further. At the cost of introducing yet another period of length $1/(2J)$, the magnetization is channeled into one line of the *J* doublet (α or β) only (*10–12*). This reduces the number of lines in the spectrum and improves, in the absence of transverse relaxation processes, the signal-to-noise ratio.

We aim to demonstrate in this Communication that it is not the linewidth *per se* which deteriorates the INADEQUATE experiment but rather the homogeneous part of it. It will turn out that the INADEQUATE-CR experiment is superior to the INADEQUATE experiment if the heterogeneous line broadening (e.g., caused by chemical inhomogeneities, or packing inhomogeneities of the sample, or by field inhomogeneities) is larger than the homogeneous broadening. The latter is mainly caused by insufficient proton decoupling or by dipolar ^{13}C–^{13}C couplings which are not fully averaged by MAS. Because only homogeneous line broadening mechanisms deteriorate the INADEQUATE-CR experiment, it can deliver results superior to the INADEQUATE experiment in many practical cases where the linewidth in MAS NMR is dominated by inhomogeneous effects which, for instance, can be caused by hetero-

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1090-7807/99 $30.00

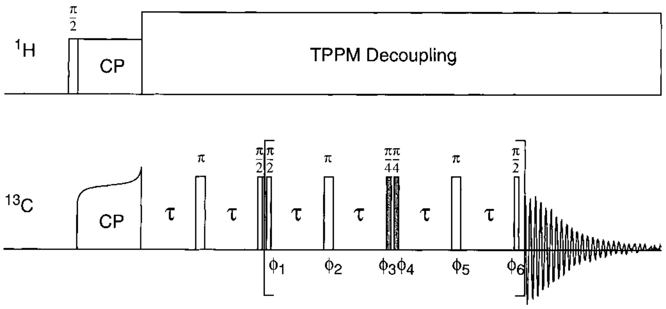

FIG. 1. Pulse sequence of the INADEQUATE-CR experiment. The part in brackets represents the Composite Rotation part. The delay τ was adjusted to be $1/(4J_{CC})$. The phases ϕ_1 to ϕ_6 were chosen in accordance with Nielsen *et al.* (*11, 12*) and were such that the α components of the doublets were selected. All experiments were performed on a Varian/Chemagnetics Infinity spectrometer operating at a ^{1}H larmor frequency of 300 MHz. A magic-angle-spinning double-resonance probe with 2.5 mm outer diameter rotors was used. The spinning speed for all experiments was set at ν_r = 24.0 kHz. The pulse sequences used an initial APHH cross polarization (*17*) of 1.5 ms duration with an amplitude of 100 kHz for the ^{1}H spin lock pulse and a tangential variation of the ^{13}C pulse around 76 kHz (-1 matching condition) with an initial offset of 12 kHz. The field strength of the TPPM decoupling (*18*) was set at 147 kHz with an optimum pulse length of 3.2 μs and a phase excursion of $\pm$9 degrees. The RF field strength for the ^{13}C pulses was set to 100 kHz. The delays between the two consecutive $\pi/2$ pulses and between the two $\pi/4$ pulses was set to 3.0 μs and to 1.0 μs, respectively.

geneity of the structure. Of course, the homogeneous linewidth must be optimized (e.g., on a test sample) prior to application of the INADEQUATE-CR experiment.

In Fig. 2 one-dimensional INADEQUATE-CR experiments (for the experimental scheme see Fig. 1) are compared with INADEQUATE spectra and CP-MAS spectra for a sample of spider dragline silk from *Nephila madagascariensis.* The spiders were fed with doubly ^{13}C labeled glycine. The preparation of the sample is described in Ref. (*13*). Spider silk is a heterogeneous material and the resonances are generally broad. The two most intense peaks in the CP-MAS spectrum (Fig. 2a) are assigned (see (*13*)) to the carbonyl (172 ppm) and C_α (43 ppm) resonance of glycine. They have a linewidth (FWHH) of 450 and 270 Hz, respectively, which exceeds the J coupling of approximately 52 Hz by a factor of approximately 8.5 and 5. Also detected are the serine C_β (61 ppm), serine C_α (54 ppm), alanine C_α (49 ppm), and alanine C_β (17 ppm and 20 ppm) resonances. The presence of the relatively strong signals of other amino acids can be explained by scrambling of the ^{13}C labels by the spider's metabolism. In particular, the signals of serine and alanine, which are thought to make up a sizeable part of the spider silk, are found.

INADEQUATE and INADEQUATE-CR spectra of dragline silk are shown in Figs. 2b and 2c, respectively. The superior properties of the INADEQUATE-CR spectrum in Fig. 2c are clearly seen. The slightly narrower linewidths in Fig. 2c, compared to the CP spectrum in Fig. 2a, has to do with the fact that only one part of the J doublet is selected.

The signal-to-noise ratio of the INADEQUATE-CR spectrum is better than that of the normal INADEQUATE but it is reduced with respect to the CP-MAS spectrum by a factor of almost 10. Nevertheless, the experiment is practical for labeled samples. The INADEQUATE-CR shows indications for a splitting of the carbonyl resonance. This splitting is too large to be a J coupling. It could be related to two different types of glycine environments for glycine in the sample and a similar splitting has been observed before in glycine-labeled samples (*13*).

To understand the limitations of the experiment, a simple test-system of [2,3-$^{13}C_2$]sodium propionate is now described in some detail. The results are shown in Fig. 3. In particular, we will separately study the influence of homogeneous and heterogeneous line broadening. Optimum TPPM decoupling (Fig. 3a) yields a linewidth of ~20 Hz for each component of the doublet (mainly of homogeneous character). Under these conditions the INADEQUATE-CR and INADEQUATE experiments both perform well as can be seen from Figs. 3d and 3g. The signal intensity of the selected component of the multiplet in the INADEQUATE-CR experiment (Fig. 3d) should be enhanced, in this 1D experiment, by $\sqrt{2}$ (*14*). Due to relaxation, the signal gain with respect to the CP spectrum cannot be realized in the INADEQUATE-CR spectra and a line intensity slightly less than for the CP experiment is detected. The small residual intensities at the position of the suppressed multiplet lines in the INADEQUATE-CR spectra are explained by ex-

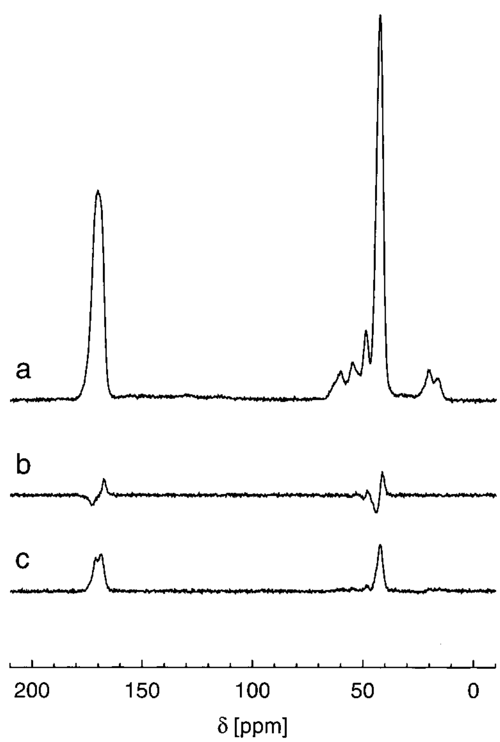

FIG. 2. Experiments on [1,2-$^{13}C_2$]glycine enriched *N. madagascariensis* drag line silk. Cross polarization experiment (a), normal INADEQUATE experiment (b), and INADEQUATE-CR experiment (c). A total of 512 transients was acquired for each spectrum and a delay τ = 4.8 ms was used with an estimated J-coupling constant of 52 Hz.

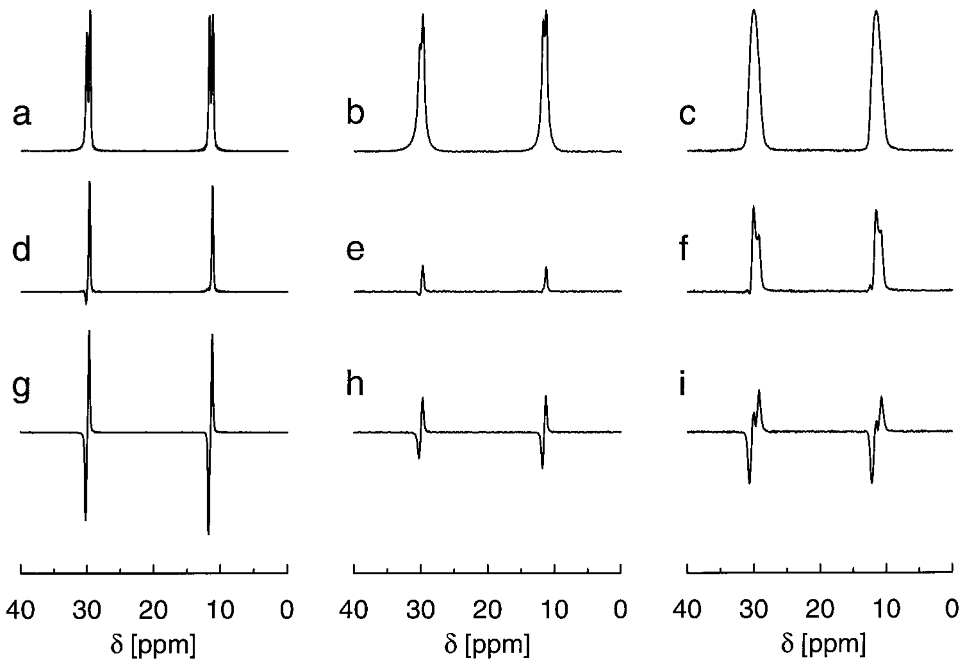

FIG. 3. Experiments on [2,3-$^{13}C_2$]sodium propionate with optimum decoupling (a, d, and g), with detuned decoupling (b, e, and h), and with a linear Z gradient over the sample volume (c, f, and i). From top to bottom the experiments are cross polarization (a, b, and c), INADEQUATE-CR (d, e, and f), and INADEQUATE (g, h, and i). The spectra in each column are normalized to the maximum intensity of the corresponding CP experiment on the top row. Small artifacts from the β component due to pulse imperfections or delay missettings can be observed in the INADEQUATE-CR experiments. These imperfections were not investigated further. In Fig. 3f the deviation from a pure linear Z gradient can clearly be seen in the lineshape of the resonances. A total of 16 scans were acquired for each experiment and a delay of $\tau = 7.25$ ms was found to be optimum. This is close to the expected delay for the J-coupling constant of 37 Hz extracted from the spectrum in 3a.

perimental imperfections, in particular by RF-inhomogeneity which turns out to be about 25% in our MAS probe.

To study the effect of the homogeneous linewidth, the parameters of the decoupling sequence were detuned to obtain an increase in the homogeneous linewidth. This effect can clearly be seen in Fig. 3b. A deconvolution of the lines indicated a linewidth (FWHH) of ~38 Hz. The reduction of the experimental amplitudes of the lines in the INADEQUATE-CR (Fig. 3e) and INADEQUATE (Fig. 3h) spectra can then be observed as a function of the homogeneous linewidth. In this case the INADEQUATE-CR experiment is less efficient than the ordinary INADEQUATE experiment, a fact which can be rationalized because the time needed for preparation and mixing is three times as long for the INADEQUATE-CR experiment. Nevertheless, the pure absorption spectrum can be obtained, however, at the cost of signal-to-noise. Detuning of the decoupling parameters even further, to an extent where no resolved splitting can be observed in the CP-MAS experiment, resulted in a reduction of the signal intensity for both INADEQUATE experiments to approximately twice the noise level (results not shown).

In contrast to the homogeneous linewidth, an increase in the inhomogeneous linewidth was experimentally achieved by applying a linear Z shim gradient over the sample volume while keeping the decoupling parameters at optimum. The experimental data are shown in Figs. 3c, 3f, and 3i. Figure 3c, which is a normal CP experiment with TPPM decoupling, illustrates the increase of linewidth caused by the gradient: the J-coupled doublet is not resolved anymore. The full linewidth at half height (FWHH) is estimated from Fig. 3f to be ~95 Hz. Since the spectrum in Fig. 3f shows no overlap between the two components of the doublets (as is the case in Fig. 3c) the increase in linewidth is purely inhomogeneous in nature and is caused by the gradient. Indeed the linewidth estimated from Fig. 3c is 125 Hz and the difference between the two values is approximately the value of the J-coupling constant.

In this regime, where the linewidth is dominated by inhomogeneous effects, the normal INADEQUATE spectrum (Fig. 3i) still shows considerable intensity but this intensity is mainly at the edges of the line and the antiphase doublet does not represent the J coupling arising from a single spectral component. The INADEQUATE-CR experiment (Fig. 3f), in contrast, recovers a single component of the doublet with its "fine-structure," the inhomogeneous lineshape which is hidden by the superposition of the two J-split lines in the spectra in 3c and 3i. It does so with considerable efficiency. This detail of the "fine-structure" however should be interpreted with care since the experiment is very sensitive to flip-angle errors, which are unavoidable when dealing with an inhomogeneous B_1 field. In the experiment of Figs. 3c, 3f, and 3i the RF inhomogeneity is highly correlated to the linear Z gradient and this leads to some distortions of the “fine-structure.” Nevertheless, the integrated intensity of Fig. 3f represents about 35% of the intensity of Fig. 3c, approximately equal to the ratio in the same sample without broadening (Figs. 3a and 3d).

Pulse sequences with improved flip-angle error dependence are currently under investigation.

In conclusion, it has been demonstrated experimentally that the applicability of the INADEQUATE-CR (as well as of the refocused INADEQUATE, not investigated here) experiment is only limited by the homogeneous linewidth of the sample and that the CR experiment has an added advantage over the original sequence in that it can recover to some extent inhomogeneous lineshapes without the overlap caused by the J doublet. This removal of one of the components of the doublet gives rise to a seemingly homonuclear decoupled spectrum, although with the line position shifted by $J/2$, with the associated reduction of the number of resonance lines.

An extension of the 1D experiments presented here to a 2D INADEQUATE experiment should pose no problems. In the case of heterogeneous broadening, the INADEQUATE-CR can be combined with whole-echo acquisition (*15*) to obtain pure phase two-dimensional spectra (*16*).

ACKNOWLEDGEMENTS

This research has been supported by the Swiss National Science Foundation and by the European Science Foundation through the Network: Silk, Properties and Production.

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