New solid-state methods for investigating molecular structure: the CHCC approach

Carmen Tripon, Xenia Filip, Vasile V Morariu and Claudiu Filip

National Institute for Research and Development of Isotopic and Molecular Technologies, 65-103 Donath, 400293 Cluj-Napoca, Romania

E-mail: carmen.tripon@itim-cj.ro

Abstract. A new experimental technique based on cross polarization under magic angle spinning (CP/MAS) is introduced. This method, called CHCC, is used to quantify \(^1\text{H}–\(^{13}\text{C}\) polarization transfer efficiency in high resolution 2D \((^{13}\text{C}–^{13}\text{C})\) correlation spectra of uniformly \(^{13}\text{C}\)-labeled compounds. Comparing the intensities of the cross-peaks obtained by techniques that are usually employed to determine inter-nuclear distances in natural abundance compounds, and the CHCC experiment, it is shown that the latter has the advantage of measuring larger proton-carbon distances. This is experimentally demonstrated on \(^{13}\text{C}\) uniformly labelled L-Tyrosine hydrochloride.

1. Introduction

Structural refinement of supramolecular aggregates is one of the most important achievement of solid-state nuclear magnetic resonance (SSNMR). Recent work on this field reports on structure elucidation of different categories of materials, such as non-crystalline compounds [1-3], nanocomposites [4,5], paramagnetic systems [6], surface bond peptides [7], and proteins [8-10].

Several experimental approaches based on polarization transfer (CP) between \(^1\text{H}–^{13}\text{C}(^{15}\text{N})\) spin species, combined with fast magic angle sample spinning (MAS), are used to measure inter-atomic distances, and we mention here the CHHC[11,12], CPPI [13] and LG-CP [14-17] experiments. For instance, the LG-CP MAS experiment was successfully employed to measure the carbon signal enhancement, and implicitly the \(^{13}\text{C}–^{1}\text{H}\) heteronuclear interactions in naturally abundant compounds. The situation becomes more complex in uniformly \(^{13}\text{C}\)-labeled samples, where this procedure can be applied only for the case of strongly coupled or spatially isolated CH spin pairs. Moreover, in 2D LG-CP HETCOR correlation spectra individual \(^{13}\text{C}\) spin contributions are difficult to separate, due to the poor \(^{1}\text{H}\) spectral resolution.

Therefore, in the present paper we will introduce a new experimental approach, called CHCC, which is based on the polarization transfer between \(^1\text{H}\) and \(^{13}\text{C}\) under MAS conditions. This new technique has the following advantages: (a) it encodes \(^1\text{H}–^{13}\text{C}\) polarization transfer efficiency in 2D \((^{13}\text{C}–^{13}\text{C})\) correlation spectra with increased selectivity compared to 2D \((^{1}\text{H}–^{13}\text{C})\) spectra, due to the superior \(^{13}\text{C}\) resolution; (b) it gives more accurate structural details, being able to measure larger \(^{1}\text{H}–^{13}\text{C}\) distances than LG-CP MAS HETCOR. The above mentioned advantages will be verified experimentally on L-Tyrosine HCl.
2. Experimental
Solid state NMR spectra of uniformly $^{13}\text{C}$ labeled L-Tyrosine·HCl were recorded using the CHCC pulse sequence depicted in figure 1. All the experiments were carried out at room temperature, on a Bruker AVANCE-400 spectrometer (100 MHz $^{13}\text{C}$ Larmor frequency). Standard CP/MAS was calibrated at a spinning frequency $\nu_R = 10.5 \text{ kHz}$, using a $^1\text{H} 90^\circ$ pulse length of 3.75 $\mu$s. The 2D CHCC spectra were acquired under two-pulse phase-modulated (TPPM) $^1\text{H}$ decoupling at 60 kHz by averaging 16 scans. The CP transfer was optimized for the first Hartmann – Hahn matching condition ($\omega_{1\text{C}} = \omega_{1\text{H}} - \nu_R$), where the $^1\text{H}$ and $^{13}\text{C}$ rf fields have been calibrated to 50 and 40 kHz. A contact pulse of 1 ms was used for the first CP, and a shorter pulse-width has been employed for the second CP step (65 $\mu$s). Two values for the mixing time which corresponds to the third CP were considered, namely 500 $\mu$s and 1 ms, respectively. Suitable phase-cycling was applied during the experiment for selecting the polarization transfer path illustrated in figure 1 with dotted lines.

![Figure 1. The CHCC pulse sequence employed in the present work. Dotted line represents the selected $^1\text{H}$-$^{13}\text{C}$ inter-nuclear polarization transfer path.](image)

3. Results and discussion
CHCC cross-peaks are generated following a three step procedure: (i) standard $^1\text{H}$ – $^{13}\text{C}$ CP is followed by $t_1$-evolution under $^{13}\text{C}$ chemical shifts and simultaneous $^1\text{H}$ TPPM decoupling, which allows chemical site resolution on the carbon dimension; (ii) subsequently, a short CP pulse is applied to transfer the polarization from the $^{13}\text{C}$ nuclei mostly to their directly bonded $^1\text{H}$; (iii) finally, by incrementing the last CP time ($t_{\text{mix}}$) this $^1\text{H}$ polarization is transferred back not only to the strongly bonded carbon with the polarized proton, but also, to other neighboring $^{13}\text{C}$ nuclei. The individual CP transfer efficiencies are reflected in the corresponding cross-peaks intensities. Also, the evolution with the mixing time, $t_{\text{mix}}$, of all cross-peak buildup curves are expected to depend on the distances between the polarized $^1\text{H}$ nucleus and individual $^{13}\text{C}$ sites.

The CHCC technique is used to selectively measure the efficiency of proton-carbon polarization exchange in uniformly $^{13}\text{C}$-labeled compounds, where the initial polarization comes primarily from the protonated carbons. To test its accuracy in providing structural information reflected by ($^{13}\text{C}$, $^{13}\text{C}$) correlations, numerical simulations using the SPINEVOLUTION [18] program were performed on representative spin systems. For this purpose, the simulated cross polarization buildup curves are obtained using the LG-CP HETCOR pulse sequence, and CHCC sequence, respectively. Comparing the intensities of the CP signal for different C-H distances, in the LG-CP HETCOR case the value of the buildup curve intensity becomes almost zero for a $^{13}\text{C}$–$^1\text{H}$ distance of ~3 Å, while in the CHCC case the buildup curve, which corresponds to a 4.5 Å distance, is still observable. These preliminary results clearly show the increased sensitivity of the CHCC experiment to structural details in molecular systems. To analyze the precision of this newly introduced procedure, the CHCC 2D ($^{13}\text{C}$-$^{13}\text{C}$) spectra of 100% U[$^{13}\text{C}$]L-Tyrosine·HCl are discussed next.

The experimental 2D ($^{13}\text{C}$-$^{13}\text{C}$) CHCC correlation spectra of uniformly $^{13}\text{C}$-labeled L-Tyrosine·HCl, for two values of the mixing time, $t_{\text{mix}}$, i.e. 0.5 ms and 1 ms, respectively are presented in figures 2a, 2b. The intra-molecular $^1\text{H}$–$^{13}\text{C}$ distances span a relatively broad range, from 2.13 Å (H7–C8), to 5.65 Å (H3–C8). The discussion of the experimental results is confined to inter-molecular $^1\text{H}$–$^{13}\text{C}$ distances less than 6 Å.
Figure 2. 2D CHCC spectra recorded at 0.5 (a) and 1 ms (b) mixing times on uniformly-$^{13}$C labeled L-Tyrosine·HCl. 2D ($^{13}$C–$^{13}$C) correlation CHCC spectra were acquired under TPPM $^1$H decoupling at 60 kHz by averaging 16 scans. Horizontal slices for the C2 and C8 diagonal peaks are shown in the lower side of the figure, which enables one to illustrate the C2–C4, and C8–C1 cross-peaks discussed in the text.

At a close examination of the CHCC spectra, one can notice that the most intense cross-peaks (C7–C8, C3–C5, C2–C4) are those which correspond to shortest $^1$H–$^{13}$C distances, of about 2.15 Å, indicating that the selected mixing time encodes information about short intra-molecular contacts. This is in agreement with the theoretical findings that such cross-peaks will dominate the spectrum at all mixing time values (see figure 2b). However, cross-peaks which are due to the correlations between more distant spins are also well shaped, such as C5 (C4)–C7 (2.7 Å), C3–C4 (3.9 Å), and C2–C7 (4.68 Å).

The comparison between theoretical and experimental results will be confined in the remainder of this work to two relevant CHCC cross-peaks, namely C2–C4 and C8–C1, which imply short- or medium-size distances (C2–C4), as well as large distances (C8–C1). The intensities of these cross-peaks are due to both contributions, the intra-molecular $^1$H–$^{13}$C contacts (2.1 Å for C2–C4; 5.1 Å for C8–C1), and inter-molecular contacts (4 Å for C2–C4; 5.6 Å for C8–C1). The most significant outcome achieved using this approach is the occurrence of C8–C1 cross-peak, proving the ability of the CHCC experiment to generate the correlation between two carbon sites which imply large intra- and inter-molecular distances, up to 6 Å, which are commonly found in complex solid-state compounds. In contrast with the 2D LG-CP HETCOR experiment, where the information is limited by the $^1$H poor spectral resolution, this approach being sensitive to proton-carbon distances up to 3 Å, in the CHCC case the cross-peaks corresponding to long range carbon-carbon correlations are well defined. The present approach hence, allows for the study of structural environment in supra-molecular aggregates with relevance in biophysics and chemistry.
4. Conclusions
In summary, we have introduced here the so called CHCC mixing sequence, designed to probe the $^1$H–$^{13}$C polarization transfer efficiency in high resolution SS-NMR 2D ($^{13}$C, $^{13}$C) correlation spectra of uniformly-labeled compounds. This method was implemented using numerical simulations on representative spin systems, and verified experimentally on U[$^{13}$C] L-Tyrosine HCl. For the investigated values of the mixing time, the experimental results indicate that the CHCC technique can be used to qualitatively describe large inter-molecular distances in solid-state systems within a broad range, up to 6 Å. Therefore, the increased accuracy of the CHCC experiment in investigating intermolecular conformation in complex systems makes this approach suitable for structural refinement in a large variety of supra-molecular compounds.

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