

# Double-quantum filtered heteronuclear correlation spectroscopy under magic angle spinning

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## Abstract

We present a simple experimental method to extract the van Vleck second moment of a multiple-spin system under high-resolution condition. The idea is to incorporate a double-quantum (DQ) filter into the pulse sequence of heteronuclear correlation spectroscopy so that a DQ excitation profile can be obtained by measuring a series of 2D spectra. The effects of spinning frequency and proton decoupling are demonstrated on the measurements of two model compounds, viz. hydroxyapatite and brushite. Based on the results obtained for the model compounds, the P-31 homonuclear second moment of the apatite component in rat dentin is characterized. The method is generally suited for the study of bone, enamel and dentin.

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## 1. Introduction

Studies of calcified tissues such as bone and teeth are of important interest to the development of biomaterials. The inorganic phase of tooth enamel, dentin and bone was identified as calcium phosphate with an apatite structure in the 1920s [1]. After decades of efforts, the composition and structure of these biological minerals remain poorly characterized at the molecular level. The major difficulty is that significant structural disorder is commonly found in biological hard tissues and therefore the utility of diffraction techniques is very limited. Because solid-state NMR spectroscopy is well suited to the study of amorphous systems, it is not surprising that <sup>31</sup>P NMR has been actively applied to the studies of bone minerals [2–12]. While the analysis of <sup>31</sup>P magic-angle spinning (MAS) spectra is severely plagued by the inherent poor resolution usually encountered in bone samples, it has been shown that the

<sup>31</sup>P{<sup>1</sup>H} heteronuclear correlation (HETCOR) spectroscopy can effectively alleviate the resolution problem [3,11]. Recently, it has been demonstrated that the distance distributions of phosphorus species in bone and dental enamel can be well characterized by the van Vleck second moments, which were obtained by measuring the <sup>31</sup>P spin-echo amplitudes as a function of echo time under static condition [13]. Second moments can provide useful insights concerning the structural arrangement of the spin nuclei even in amorphous systems. For the study of biominerals, it is therefore highly desirable to develop a method to determine the <sup>31</sup>P homonuclear second moments ( $M_2^{P-P}$ ) under MAS.

There are many different techniques developed for the recoupling of homonuclear dipolar interactions under MAS [14–17]. Interesting applications have been demonstrated for <sup>31</sup>P multiple-spin systems [18–22]. In particular, Schmedt auf der Günne [20] shows that for a multiple-spin system it is possible to describe the dephasing behavior of the DQ coherences by a fictitious two-spin system, for which the effective dipolar coupling can be calculated as a

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sum of squared dipolar coupling constants of the multiple-spin system. Empirically, Raghunathan et al. [21] also found that the dipolar dephasing curve of HAp can be reproduced both by a nine-spin simulation based on the X-ray structure and by a two-spin simulation corresponding to an effective  $^{31}\text{P}$ – $^{31}\text{P}$  distance of 3.1 Å. Alternatively, the  $M_2^{P-P}$  data can be estimated by monitoring the variation of a DQ-filtered signal as a function of the excitation time [23–25]. In this work, we show that a  $^{31}\text{P}$  double-quantum (DQ) filter can be incorporated to the HETCOR pulse sequence so that one can extract the corresponding  $M_2^{P-P}$  data of the apatite component in a dentin sample. To assist the data interpretation,  $M_2^{P-P}$  data were also obtained for two crystalline model compounds, viz. hydroxyapatite (HAp) and brushite.

## 2. Experimental methods

Hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ) and brushite ( $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ) were used as received (Acros Organics). The dentin sample was taken from the incisor of a Wistar rat (3 weeks old) after removing the enamel by a high-speed dental drill. The sample was washed with saline for three times and then ground into powder form by a Mortar Grinder KM 100 grinder.

All NMR experiments were measured at room temperature and carried out at  $^{31}\text{P}$  and  $^1\text{H}$  frequencies of 121.5 and 300.1 MHz, respectively, on a Bruker DSX300 NMR spectrometer equipped with commercial 2.5- and 4-mm probes. The sample was confined to the middle one-third of the rotor volume using Teflon spacers. The variation of MAS frequency was limited to  $\pm 3$  Hz using a commercial pneumatic control unit. Chemical shifts were externally referenced to 85% phosphoric acid and TMS for  $^{31}\text{P}$  and  $^1\text{H}$ , respectively.

The  $^{31}\text{P}\{^1\text{H}\}$  DQ filtered Lee–Goldburg cross-polarization (CP) heteronuclear correlation spectra were measured at a spin rate of 25 kHz. The flip angle of the pulse after the  $t_1$  evolution is adjusted so that the spin-temperature inversion can be realized by phase alternating the first  $^1\text{H}$   $\pi/2$  pulse. During the contact time, the  $^1\text{H}$  nutation frequency and the resonance offset were set equal to 50 and 35.35 kHz, respectively, to fulfill the Lee–Goldburg irradiation condition [26]. The amplitude of the  $^{31}\text{P}$  contact pulse was ramped adiabatically [27,28]. The  $^{31}\text{P}$  DQ filter was prepared based on the so-called HSMAS-DQ technique [29–31]. During the fp-RFDR recoupling periods, the  $^{31}\text{P}$   $\pi$  pulses were set to 12  $\mu\text{s}$  ( $\tau_p/\tau_r = 0.3$ ) [32]. The  $\pi$  pulse trains were phase cycled according to the XY-8 scheme [33]. The  $^{31}\text{P}$   $\pi/2$  pulses flanking the fp-RFDR pulse blocks were set to 5  $\mu\text{s}$  [29]. The DQ reconversion period was set equal to the excitation period. The tachometer signals for pulse sequence synchronization were filtered with a home-built phase-locked loop circuit. The standard four-step phase cycling of the excitation block (0,  $\pi/2$ ,  $\pi$ ,  $3\pi/2$ ) and receiver phase (0,  $\pi$ , 0,  $\pi$ ) was used to select the DQ coherence [34]. Together with spin-temperature inversion

and CYCLOPS [35] our phase cycling has a total of 32 steps. Quadrature detection in the  $F_1$  dimension was achieved by the hypercomplex approach [34]. Typically, for each  $t_1$  increment 128 transients were accumulated, and a total of 31 increments were done at steps of 80  $\mu\text{s}$ . Recycle delay was set to 3 s. The acquisition time for each DQF-HETCOR or HETCOR experiment was 7 h. The total experimental time required to generate a DQ excitation profile was 63 h.

## 3. Results and discussion

### 3.1. MAS and HETCOR

Fig. 1 shows the  $^{31}\text{P}$  and  $^1\text{H}$  MAS spectra measured for the dentin sample at a spin rate of 10 kHz. Additional experiment shows that the proton decoupling does not help improve the resolution of the  $^{31}\text{P}$  MAS spectrum. The full-width at half-maximum of the  $^{31}\text{P}$  MAS signal is about 600 Hz (5 ppm), which is comparable to the data measured for the apatite layer formed on the surface of bioactive glasses [24]. This observation is consistent with the notion that the apatite crystals in dentin have random orientation and their size is pretty small [1]. There are two major components in the  $^1\text{H}$  MAS spectrum. The sharp component at 1.1 ppm is difficult to assign but its shoulder at 0.2 ppm is readily assigned to the hydroxyl group of apatite [36]. The broad peak at 5.5 ppm is attributed to the structural water [36] and the organic matrix of dentin (20 wt%) [1]. Further analysis of the MAS spectra is plagued by the poor spectral resolution. As demonstrated previously, the CP-based HETCOR spectroscopy is particularly useful in the study of apatite materials [3,37]. To suppress the spin diffusion among the  $^1\text{H}$  spins during the CP contact time, LG irradiation was applied in the  $^1\text{H}$  channel. Fig. 2 shows the  $^{31}\text{P}\{^1\text{H}\}$  HETCOR spectrum measured at a spin rate of 25 kHz, in which there are two components at  $^1\text{H}$  chemical shifts of 0.2 and 5.5 ppm, which are henceforth referred to as the apatite and the amorphous components, respectively. The signals of these spectral components are assigned to the phosphorus species in the apatite domain and the disordered phase which contains substantial amount of structural water [3]. A careful inspection of the HETCOR spectrum reveals that the  $^{31}\text{P}$  chemical shifts of the two components are slightly different, showing that there are indeed two different phosphorus species which have different proton environments.

### 3.2. DQ excitation profile

It has been shown that the  $M_2^{P-P}$  data of  $^{31}\text{P}$  multiple-spin systems can be estimated by monitoring the variation of a DQ-filtered signal as a function of the excitation time [23–25]. By the pulse sequence shown in Fig. 3, the DQ excitation profiles were measured for HAp and brushite. There is one phosphorus site (orthophosphate,  $\text{PO}_4^{3-}$ ) in

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