RSC Advances

Supplementary Information

Solid-state proton NMR study of bone using dipolar filter: Apatite hydroxyl content versus animal age

Agnieszka Kaflak[1]*, Stanisław Moskalewski[2], Waclaw Kolodziejski[1]

[1] Medical University of Warsaw, Department of Analytical Chemistry and Biomaterials, Banacha 1, Warsaw 02-097, Poland.

[2] Medical University of Warsaw, Department of Histology and Embryology, Chalubinskiego 5, Warsaw 02-004, Poland.

1. Determination of the hydroxyl content

Below, there is an outline of the procedure used in this work. We recommend to conform to the following rules in the DF MAS NMR analysis of the bone mineral hydroxylation:

(1) Properly excise and store bone specimens to avoid deterioration of the bone structure.[1] Do not grind the sample for MAS.[2] Instead, gently crush it or if possible, cut of it a cylinder fitting the NMR rotor. In our case, the latter manner was impossible, because the rat bone pieces were too small.

(2) Tune the dipolar filter on representative bone samples to expose hydroxyl region and attenuate as much as possible the neighbour lipid resonances (Fig. 1S).

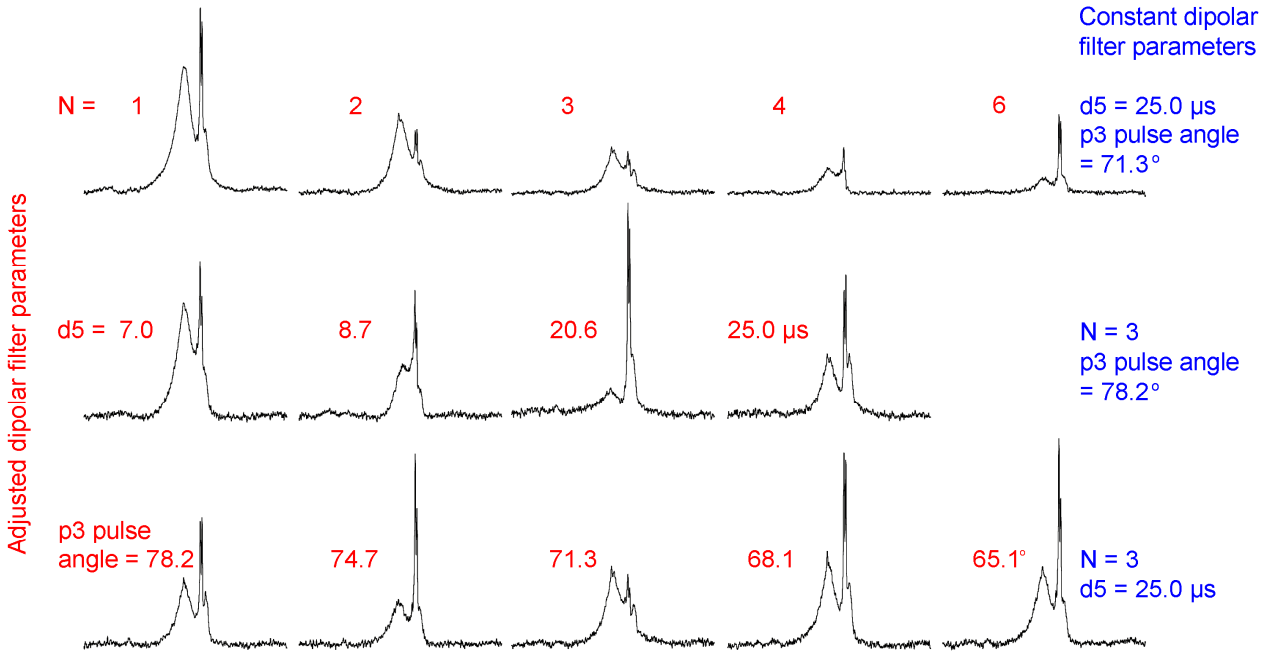

Figure 1S. Selected spectra showing the dipolar filter tuning in the applied pulse sequence presented in Figure 1a of the main text. In this comparison all the spectra were recorded for R30 with 8 scans, the recycle delay *d1* of 30 s and the *p1* pulse angle of 90° (3.2 µs long). The best filter parameters to expose the hydroxyl peak at 0 ppm, while minimizing the water and adipose peaks, were the following: *d5* = 25 µs, *N* = 3 and *p3* pulse angle of 71° (see the third spectrum in the upper and bottom rows).

(3) Record good quality (high S/N ratio) proton DF MAS NMR spectra of your bone samples and reference apatite materials using the same, formerly adjusted acquisition

parameters. The hydroxyl concentration of the reference materials should previously be accurately determined. As the reference materials can serve apatites synthesized in various conditions, for instance, having a different carbonate content and/or crystal size. For them, the hydroxyl concentration can be directly measured using BD MAS NMR, since there is no spectral interference from the organic peaks. We used the reference materials from earlier studies carried out in our group.[3-5]

(4) Process the FIDS as described in **Data processing and statistical analysis** in the main text. From the spectra deconvolutions (Fig. 2S) determine the hydroxyl peak areas per 1 mg of the sample for the bone and reference materials.

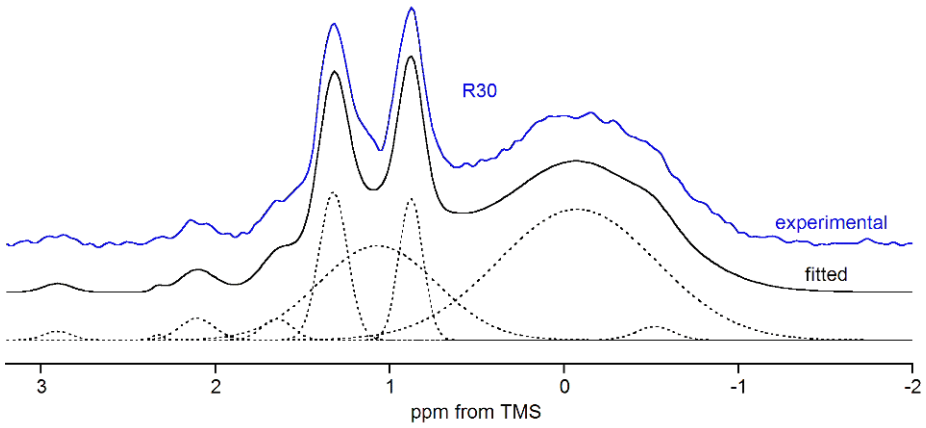

Figure 2S. Exemplary deconvolution (line fitting in GRAMS) of the ^{1}H DF MAS NMR spectrum of rat cranial bone.

(5) Perform the hydroxyl calibration on the reference materials, that is derive a function of the hydroxyl concentration (y) versus hydroxyl area (x). In our case, this was a linear function of the type $y = a\,x$ (Fig. 3S). The proportionality coefficient a is dependent on the spectrum acquisition and processing parameters, so it can be used only if they all are the same for all the studied samples. Inaccuracy of this procedural step may introduce a systematic error into the hydroxyl determination.

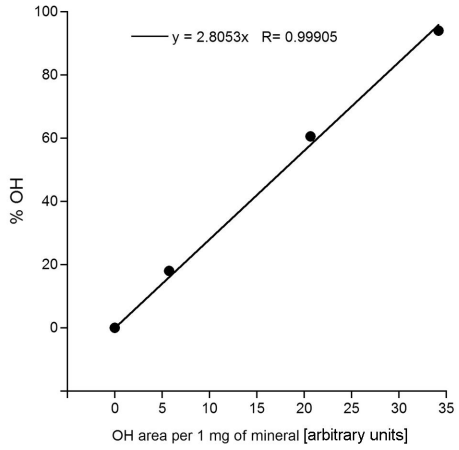

Figure 3S. The hydroxyl calibration line used in this work.

(6) Determine the mineral content in the studied bone samples. This can be traditionally done by heating in air, the technique usually called 'ashing'.[6] In our case, this was done using TGA in the air flow:[7,8]

(7) $[(m_{600} - m_{25})/m_{25}] \times 100\%$,

where m_t is the sample mass at temperature t ($^{\circ}$C).

(8) Using the mineral content calculate the hydroxyl peak areas per 1 mg of the mineral of the bone samples in the NMR rotor.

(9) Using the hydroxyl peak areas per 1 mg of the mineral of the sample in the NMR rotor and the hydroxyl calibration function determined in the step (5) calculate the hydroxyl content in the mineral of the studied bone samples.

References

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5. L. Pajchel and W. Kolodziejski, *J. Nanopart. Res.*, 2013, **15**, 1868–1882.

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2. Auxiliary figures

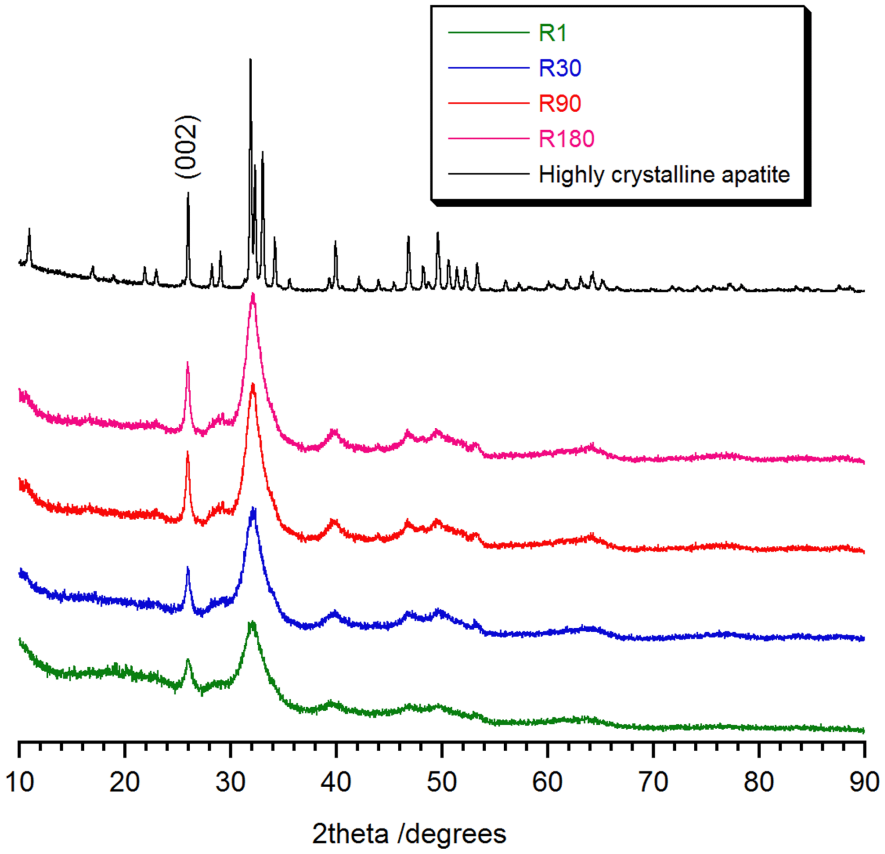

Figure 4S. PXRD diffractograms of the rat bone samples and crystalline apatite.

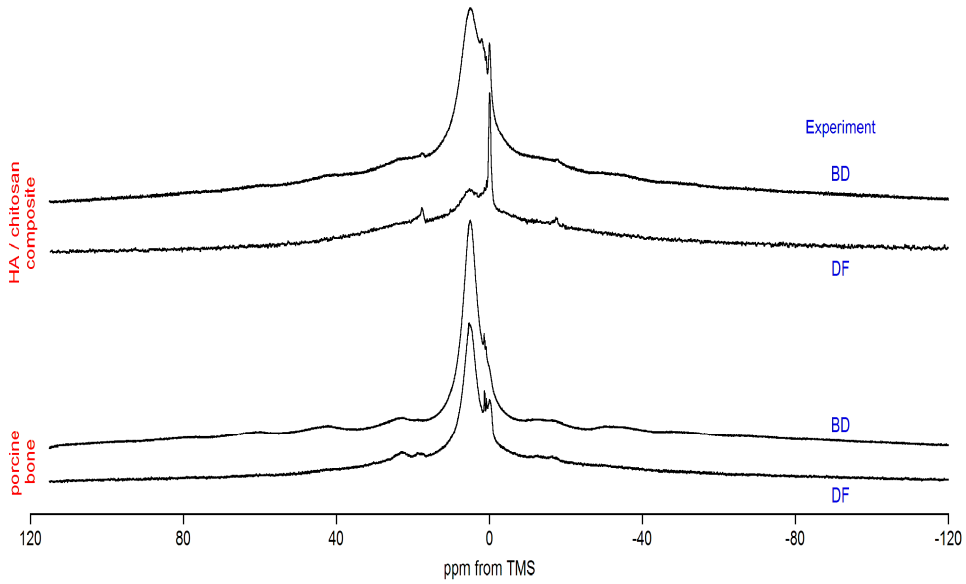

Figure 5S. Full-range proton NMR spectra of the HA/chitosan composite and porcine bone recorded with MAS at 7 kHz using the regular pulse-acquire experiment (Bloch decay; BD) and the dipolar filter pulse sequence (DF). The BD and DF spectra of each material were scaled to the same intensity at 0 ppm, that is at the position of the apatite hydroxyl peak.