Supporting Information for:
Multinuclear Solid State NMR Studies of
Ordered Mesoporous Bioactive Glasses

Ekaterina Leonova\textsuperscript{a}, Isabel Izquierdo-Barba\textsuperscript{b}, Daniel Arcos\textsuperscript{b}, Adolfo López-Noriega\textsuperscript{b}, Niklas Hedin\textsuperscript{c}, Maria Vallet-Regí\textsuperscript{b}, Mattias Edén\textsuperscript{a,*}

\textsuperscript{a}Physical Chemistry Division, Arrhenius Laboratory, Stockholm University, SE-106 91, Stockholm, Sweden
\textsuperscript{b}Departamento de Química Inorganica y Bioinorganica, F. Farmacia, Universidad Complutense de Madrid. 28040-Madrid, Spain
\textsuperscript{c}Inorganic Chemistry Division, Arrhenius Laboratory, Stockholm University, SE-106 91, Stockholm, Sweden

Contents

1. S.1: Assignment of the $Q^1$ $^{31}$P Resonance as P-O-Si Moieties.
2. S.2: $^1$H NMR Results on Heat-Treated Samples and Spin-Echo Experiments.
3. Table S1: Textural Properties.
4. Table S2: $^{31}$P 2QF Efficiencies from S85.
5. References.
6. Fig. S1: $^{31}$P Double-Quantum Filtration Results on S85.
7. Fig. S2: Spinning Sidebands Manifolds in $^{31}$P CPMAS Spectra of S85.
8. Fig. S3: $^1$H MAS Spectra of Heat-Treated Samples.
9. Fig. S4: $^1$H Spin-Echo Decays of S100 and S58.
10. Fig. S5: $^1$H Double-Quantum Filtration Results on S85.
11. Fig. S6: Transmission Electron Microscope Images.

Note that all references to the figures and tables are in this document numbered as S1, S2, etc.
S.1. Assignment of the $Q^1$ $^{31}$P Resonance to P-O-Si Moieties.

Here we motivate our assignment of the $^{31}$P resonance $\approx -7$ ppm as stemming from $Q^1$ [P-O-Si] constellations, i.e., to P atoms bonded to the silica surface. P-O-Si bonds are known to hydrolyze easily. This accords with the signal reduction from 6% (S85) to 3% (S85sbf) in the $^{31}$P NMR spectra prior to and on exposure to the ionic SBF solution (see Fig. 1), and favors the assignment of the signal $\approx -7$ ppm to stem from P-O-Si bonds. A direct answer could be provided by double-resonance $^{31}$P$\leftrightarrow^{29}$Si NMR experiments. However, the low natural abundance of the $^{29}$Si isotope coupled to a low phosphorus content of the MBG sample S85 (<2 at-%), renders such experiments very difficult at our moderate magnetic field of 9.4 T, unless large-volume rotors (≥5 mm outer diameter) and a HXY-probehead may be utilized. In the absence of this possibility, we have gathered information from a series of $^{31}$P experiments as follows:

(i) Calcium orthophosphates are typically associated with shortest $^{31}$P-$^{31}$P distances ranging between 4-5 Å. In HAp, for instance, the shortest distance is 4.1 Å, corresponding to a dipolar coupling constant of -285 Hz. However, due to the dipolar couplings to more distant $^{31}$P spins, 2QC excitation in inorganic phosphates is usually faster than that predicted solely from the shortest spin-pair internuclear distance. For instance, it has been demonstrated that the effective $^{31}$P-$^{31}$P contacts within crystalline hydroxyapatite may be numerically simulated assuming a spin-pair with a dipolar coupling constant $\approx -600$ Hz, i.e., an effective $^{31}$P-$^{31}$P distance of 2.9 Å.

The double-quantum filtered (2QF) $^{31}$P NMR spectra of Fig. S1, recorded from S85 and using the dipolar recoupling pulse sequence R20$^\circ_2$, show that for relatively short recoupling intervals ($\tau_{exc}$) up to 0.67 ms, the relative contributions from the two peaks appearing around 3 and -7 ppm are essentially equal to those in the CPMAS spectrum of Fig. S1(a). Table S2 lists the relative $Q^0$ and $Q^1$ signal contributions to each 2QF $^{31}$P spectrum, as well as their respective 2QF efficiencies. We also recorded a 2QC excitation profile (2QF efficiency vs. $\tau_{exc}$) for nanocrystalline HAp at 5.8 kHz spinning frequency (not shown). The maximum 2QF efficiency was found $\approx 1.4$ ms. Unfortunately, the low P content of the S85 sample prohibited the acquisition of a detailed 2QC excitation profile. However, it appears that the 2QF efficiency obtained at 1.3 ms (Fig. S2; Table S2) is likely to be around its optimum, while the “$Q^1$” signal around -7 ppm continue to increase. Altogether, this suggests that the $^{31}$P-$^{31}$P distances in the amorphous calcium orthophosphate phase are, on the average, similar to (or slightly longer than) those in crystalline HAp.

While further corroborating the assignment of the signal $\approx 3$ ppm to isolated $Q^0$ phosphate units, these results do not favor P-O-P species as the source of the “$Q^1$” signal; $^{31}$P in P-O-P linkages are expected to provide faster 2QC buildup due to their closer spatial proximity relative those of the isolated PO$_4^{3-}$ tetrahedra. Rather, the similar (or even slower) 2QC buildup suggests that the $^{31}$P-$^{31}$P internuclear distances within the “$Q^1$ phase” are comparable to (or longer than) those of the “$Q^0$ phase”. However, the assignment of the peak $\approx 7$ ppm to “$Q^1$” species would be consistent with a scenario of P binding to the mesoporous silica surface, provided that the P atoms are not evenly spread, but clustered so that the closest $^{31}$P-$^{31}$P distances are within 4-5 Å. For instance, the $^{31}$P-$^{31}$P distance between two neighboring surface silicon-centered tetrahedra, each involving one Si-O-P bond, is estimated as $\approx 3.6$ Å, whereas that in a SiO$_3$(OP)-SiO$_4$-SiO$_3$(OP) constellation is $\approx 7.2$ Å.
(ii) A direct proof of $^{31}$P-O-$^{31}$P segments could be provided by the scalar ($J$) coupling-based refocused 2Q-INADEQUATE experiment\(^6\) that requires P-O-P chemical bonds for 2QC excitation. Here, double-quantum coherences are excited from transverse magnetization using a $\left[\tau/2-\pi-\tau/2-\pi/2\right]$ block of pulses/delays, with the subsequent 2QC→1QC transfer implemented by reversing the sequence of pulses/delays.\(^6\) However, using this protocol, we failed to detect 2QF $^{31}$P-$^{31}$P signals from the S85 sample ($\tau=8$ ms) even after 13952 accumulated signal transients at 9.0 kHz spinning frequency. To consider $T_2$-relaxation losses, we note that the $\nu^{1}$ $^{31}$P resonance was clearly visible after 768 accumulated transients employing a spin-echo with the same total delay durations as used in the INADEQUATE experiment. We also verified the absence of signals from a 2Q-INADEQUATE acquisition using $\tau=12$ ms. Similar experiments (not shown) on a reference sample of Na$_4$P$_2$O$_7$·10H$_2$O (P in $Q^1$ environments) gave strong 2QC signals under the same experimental conditions. We note that whereas these INADEQUATE results on S85 cannot unambiguously preclude the presence of $Q^1$[P-O-P] units, they strongly suggest that their contributions to the signal $\approx-7$ ppm must at least be limited. Considering that typically $J^{\nu^{1}}P$-$^{31}$P$\approx20$ Hz,\(^7\) and that relaxation effects are accounted for by comparing with the signal of the $Q^1$ units available after a spin-echo experiment, we estimate that a net 2QF efficiency of 12-16% is expected by the refocused INADEQUATE experiment under our experimental conditions.\(^6,7\) Our results are sufficient to preclude that the $^{31}$P $Q^1$ signal would exclusively stem from $Q^1$[P-O-P] units. Nevertheless, a significant signal fraction (up to $\approx50\%$) may originate from such units, as it could pass undetected due to the limited S/N available.

In summary, our NMR data do not accord with either $^{31}$P–$^{31}$P spatial proximities or chemically bonded P-O-P segments that are characteristic of $Q^1$[P-O-P] units. While these observations cannot directly prove the presence of phosphorus atoms being connected to the silica surface through P-O-Si bonds, all our experimental data support this scenario.

Finally, we comment that the $Q^1$ $^{31}$P resonance is emphasized in $^1$H→$^{31}$P CPMAS NMR spectra compared to those recorded by single pulses. This is evident when comparing its contributions to the spectra of S85 in Fig. 1 (as well as by the data in Table S2). The relative integral of the $Q^1$ signal in the CPMAS spectrum amounts to $\approx11\%$, i.e., twice that of the spectrum recorded by direct excitation. While this suggests that the $Q^1$ $^{31}$P signal originates from $^{31}$P in closer spatial proximity to $^1$H than those of the orthophosphate phase, a series of CPMAS spectra recorded with increasing $^1$H→$^{31}$P CP contact intervals (verified at different spinning frequencies) did not reveal a faster signal buildup of the $Q^1$ resonance relative that of the $Q^0$ units (data not shown). We have currently no fully satisfactory explanation for these observations.

S.2. $^1$H NMR Results on Heat-Treated Samples and Spin-Echo Experiments.

Fig. S3(a, b) shows $^1$H NMR spectra recorded from the MBG samples S85 and S58 after an additional 3 hrs calcination at 700 °C. Both spectra display signal D (now centered around 3.8 ppm) which correspond to the remaining weakly hydrogen-bonded surface silanols. Besides this signal, peaks E and F ($\approx2.0$ ppm) are the dominating resonances, corresponding to isolated SiOH groups. This observation is expected from simultaneous dehydration and partial dehydroxylation processes following the heat treatment.\(^8\)
The absence of the set of narrow signals (B, C, G-I) in Fig. S3(a, b) confirms that they all originated from organic species. The relationship between the surface hydration level and the relative amounts of hydrogen-bonded silanols is further demonstrated in Fig. S3(c). This \(^1\)H NMR spectrum was recorded from S58 after first treating the sample at 700 °C for 2.5 hrs and next exposing the spread powder to air (~80% humidity) for 1 week. In this case, there are no signals (E, F) from isolated SiOH groups, and peak A is shifted up further to 4.95 ppm. This is consistent with a water-saturated S58 surface. However, the very small CH\(_3\) \(^1\)H resonances indicate minute remainings of organic groups.

We note that the features of the \(^1\)H MAS spectrum recorded from the “rehydrated” S58 specimen [Fig. S3(c)] is similar to that of the SBF-treated S85sbf sample of Fig. 4. Both spectra manifest an overall narrower A resonance relative that in all other spectra of Fig. 4, as well as a clear depletion of organic-moiety-deriving signals as compared to the pristine S85 or S58 samples. In the case of S85sbf, the removal of organic species and their associated \(^1\)H signals may readily be attributed to the exposure to the ionic SBF solution and the subsequent washing procedure using acetone.\(^9,10\) While this explains the reduction of signals from organic species, we have currently no obvious explanation for the apparent narrowing of peak A (particularly in the high-ppm region) upon treatments to SBF or high humidity. We speculate that for water-saturated MBG surfaces, the high water mobility leads to an apparent peak narrowing, stemming both from rapid chemical exchange of protons from water molecules and silanols, and the accompanying reduction in their mutual \(^1\)H-\(^1\)H dipolar interactions (which narrows the peak-width further).

The spectral assignments of the various hydrogen-bonded silica surface protons (A, D), isolated silanols (E, F) and organic moieties (B, C, G-I) are further corroborated by their \(^1\)H signal decays observed in spin-echo measurements made on the samples S100 and S58 in Fig. S4. Here signal A is associated with the fastest decay, presumably due to a high water mobility. The signals from the CH\(_3\) groups (G-I) diminish somewhat slower. The isolated SiOH groups (signals E and F) are least prone to relax, as expected from their overall restricted mobility and smaller \(^1\)H-\(^1\)H dipolar contacts available to promote spin diffusion with more rapidly relaxing protons. Interestingly, the fast decay of peak A reveals in the case of S58 the presence of a much broader signal component, extending towards high chemical shifts (inset spectrum of Fig. S4). This is attributed to SiOH groups experiencing the strongest H-bonding.\(^8,11-14\) Their presence in the Ca-rich MBG specimen is consistent with its overall highest water content.
Table S1: Textural Properties.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mesoporous structure</th>
<th>Unit cell dimension (nm)</th>
<th>Surface area (m² g⁻¹)</th>
<th>Pore volume (cm³ g⁻¹)</th>
<th>Pore diameter (nm)</th>
<th>Pore wall thickness (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S58</td>
<td>p6mm disordered</td>
<td>7.7</td>
<td>195</td>
<td>0.46</td>
<td>9.45</td>
<td>3.1</td>
</tr>
<tr>
<td>S75</td>
<td>p6mm p2mm</td>
<td>7.6</td>
<td>393</td>
<td>0.59</td>
<td>6.0</td>
<td>3.6</td>
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<tr>
<td>S85</td>
<td>1α3d</td>
<td>17.2</td>
<td>427</td>
<td>0.61</td>
<td>5.73</td>
<td>3.5</td>
</tr>
<tr>
<td>S100</td>
<td>1α3d</td>
<td>18.7</td>
<td>395</td>
<td>0.47</td>
<td>6.87</td>
<td>3.6</td>
</tr>
</tbody>
</table>

* Unit cell, surface area, pore volume and pore diameter data, reproduced from Ref.⁹
* The samples S58 and S75 comprise two separate phases, where the major component is listed at the top line. See Ref.⁹ for details.
* Calculated by combining XRD, N₂ adsorption porosimetry and potential mapping data.

Table S2: ³¹P 2QF Efficiencies from S85*  

<table>
<thead>
<tr>
<th>Spectrum</th>
<th>Relative population Q¹ (%) ⁶</th>
<th>2QF efficiency (%) ⁷</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Q⁰</td>
</tr>
<tr>
<td>CP</td>
<td>10.1</td>
<td>–</td>
</tr>
<tr>
<td>2QF: τexc=0.33 ms</td>
<td>10.9</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>9.9</td>
<td>10.8</td>
</tr>
<tr>
<td></td>
<td>16.0</td>
<td>13.2</td>
</tr>
</tbody>
</table>

* Data extracted from the set of ³¹P MAS spectra displayed in Fig. S1.
* Relative contribution of Q¹ units as obtained by spectral deconvolution.
* The 2QF efficiency was estimated as the ratio of the integrated signal intensity in the 2QF spectra [Fig. S1(b-d)] relative to the CPMAS spectrum [Fig. S1(a)], accounting for the different number of signal transients recorded in each case. Signals from the centerband and first-order spinning sidebands were included, and the integrals of the Q⁰ and Q¹ sites were obtained by deconvoluting each sideband. All percentages are uncertain within ±2 units.
REFERENCES

Fig. S1. $^{31}$P NMR spectra recorded from S85 at 9.4 T and 6.0 kHz MAS in the absence of $^1$H decoupling. (a) $^1$H→$^{31}$P CPMAS spectrum (3.0 ms contact interval), resulting from 640 accumulated signal transients and 4.5 s relaxation delays. (b-d) 2QF CP MAS spectra, obtained by the dipolar recoupling sequence R20 $^2$ (Ref.5) and using equal intervals for 2QC excitation ($\tau_{exc}$) and reconversion, as indicated in the figure. The acquisition conditions were otherwise identical to those in (a), except for the number of accumulated transients: (b) 12288, (c) 10688, (d) 6144. The spectra were recorded in the absence of $^1$H decoupling during $^{31}$P recoupling.5 Asterisks mark spinning sidebands from $^{31}$P in both $Q^0$ and $Q^1$ environments, whose centerband signals are assigned as indicated. Note that the spectra in (b-d) are displayed with a 10-fold vertical expansion relative that in (a), after accounting for the different numbers of signal transients.
Fig. S2. $^1\text{H} \rightarrow ^{31}\text{P}$ CP MAS spectra of S85 recorded at 9.4 T and (a) 4.7 kHz and (b) 8.5 kHz spinning frequencies, displayed together with vertical expansions of the spinning sidebands. A contact interval of 4.5 ms was used. The spectrum in (b) is the same as that shown in Fig. 1. Note the relatively larger contributions from the $Q^1$ signals to the spinning sideband intensities compared to that of the centerband, reflecting a larger chemical shift anisotropy (CSA) of $^{31}\text{P}$ in the $Q^1$ units relative that of $Q^0$. 
Fig. S3. \( ^1 \text{H} \) MAS spectra from S85 (a) and S58 (b) samples after additional 3 hr calcination at 700 °C; (c) S58 powder after 2.5 hrs calcination at 700 °C, followed by an exposure to air (80% humidity) for one week. (d) \( ^1 \text{H} \) MAS spectrum of S58 [Fig. 4(d)]. Background \( ^1 \text{H} \) signals are marked by asterisks.
Fig. S4. $^1$H MAS spectra (9.0 kHz spinning frequency) recorded by a Hahn-echo sequence ($\pi/2-\tau_{\text{echo}}/2-\pi-\tau_{\text{echo}}/2$-acquire) from S100 (left panel) and S58 (right panel), with echo delays as indicated. The peak labels are assigned in Table 3. Note that the spectra of the two bottom rows are expanded vertically by factors of 2 and 4, respectively. The inset spectrum at the top is a horizontal expansion of that from S58 using $\tau_{\text{echo}}=0.4$ ms. It reveals a broad spectral component extending out to high shifts as indicated by the grey box. This resonance is present in all spectra from S58 and originates from strongly hydrogen-bonded silanols.
Fig. S5. Double-quantum filtered (2QF) 1D $^1$H NMR spectra from S85, recorded by the POST-C7 pulse sequence$^{15}$ at 9.0 kHz MAS and using identical intervals ($\tau_{\text{exc}}$) for 2QC excitation and reconversion, as indicated at the right portion of each spectrum. Each acquisition resulted from 80 accumulated transients using 4.0 s relaxation delays and a $z$-filter delay of 222 $\mu$s between the POST-C7 reconversion block and the read pulse. Rf nutation frequencies were 60 kHz for both recoupling and $\pi/2$ pulses.
Fig. S6. High resolution transmission electron microscopy (HRTEM) images and their corresponding Fourier diffractograms of S100 (a, b); S58 (c); S75 (d) and S85 (e). The HRTEM images in (c-e) show that the pore wall is amorphous. The inset Fourier patterns also confirmed this, showing the characteristic halo of amorphous materials. In (a) and (b), the incident beam was parallel to the [110] and [111] directions, respectively, of the bicontinuous \( nlb \)3d structure. A JEOL 3000FEG electron microscope was used, fitted with a double tilting goniometer stage (±45°) and an Oxford LINK EDS analyser. All images were recorded using a CCD camera (MultiScan, model 794, Gatan), with 1024×1024 pixels, each of size 25×25 µm (a, b) and 24×24 µm (c-e) and using low-dose conditions at 3·10^5 times magnification. FT diffractograms were carried out using the images of thin parts of the crystal with Digital Micrograph (Gatan).