

SURFACE STRUCTURE OF NANOCRYSTALLINE APATITES FOR BIOCERAMICS AND COATINGS

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Nanocrystalline apatites exhibit a very reactive surface characterised by the existence of a hydrated layer of mineral ions showing a relatively high mobility. The surface layer structure has been investigated by spectroscopic techniques (FTIR, MAS-NMR). The ionic environment is strongly dependant on the hydration ratio and evolves on drying. The ions mobility in the hydrated layer allows direct crystal-crystal bonding ("crystal fusion") often observed in bone and tooth enamel. This process permits the formation of "low temperature" ceramics through the association and bonding of nanocrystals and could be involved in self-setting Ca-P cements. The hydrated layer could also be responsible for the adhesion of nanocrystalline apatites on metal surfaces in "biomimetic" coatings.

1. INTRODUCTION

Biological Poorly Crystalline Apatites (PCA) are the main constituent of mineralised tissues (bone and dentine), they also occur in pathological calcifications such as arteriosclerosis, dental calculus or tendinous calcifications. PCA formed *in vivo* play a major role in the surface activity of most orthopaedic bioactive biomaterials¹. Recently synthetic PCA have been used directly in biomaterials and have been proposed as coatings for metallic prosthesis², as final constituent of orthopaedic calcium phosphate cements³ or in composite biomaterials⁴. Despite this widespread occurrence, the structure, properties and mode of formation of PCA are still the subject of much debate. The shape of PCA nanocrystals for example raises several questions: they form plate-like prismatic crystals rather inconsistent with a hexagonal unit-cell showing two equivalent directions⁵. In addition to this unusual shape, the numerous surface irregularities increase the surface energy and question the stability of the nanocrystals. It has been suggested that apatite crystals do not form directly but involve one or several metastable intermediate phases such as amorphous calcium phosphate (ACP), dicalcium phosphate

dihydrate (DCPD) or octacalcium phosphate (OCP)⁵. More recently it has been shown, using spectroscopic techniques, that in addition to the regular apatitic environments of the mineral ions found in any well crystallised apatites, nanocrystalline PCA exhibit specific, constant but extra features referred to as "non-apatitic environments"⁶⁻⁷. The non-apatitic environments occur mainly in freshly precipitated apatites in physiological conditions. They progressively disappear during ageing of the precipitate in the mother solution (maturation)⁸. It has been shown that the mineral ions in non-apatitic environments can be easily and reversibly exchanged. These ion exchange reactions however depend strongly on the sample preparation technique: wet samples especially exhibit a higher exchange rate than lyophilised samples. The present work was undertaken to determine the effect of wetness on PCA structure and properties.

2. MATERIALS AND METHODS

2.1. Sample preparation

The nanocrystalline apatites were prepared by double decomposition between a calcium nitrate solution ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 17,7 g in 250 ml deionized water) and an ammonium phosphate solution ($(\text{NH}_4)_2\text{HPO}_4$, 40g in 500 ml deionized water). The calcium solution was rapidly poured into the phosphate solution. The precipitate was filtered and washed immediately. Part was analysed wet and another part after lyophilisation.

2.2. Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of the wet gel-like samples were obtained by transmission through a polyethylene membrane coated with the gel on a Perkin-Elmer 1760 FTIR spectrometer. The lyophilised samples were also analysed by transmission using a KBr pellet.

2.3. Magic Angle Spinning - Nuclear Magnetic Resonance Spectroscopy (MAS-NMR)

The ³¹P MAS-NMR measurements were made at 202.47 MHz on Bruker ASX 500 spectrometer. The gel-like wet sample or the lyophilised samples were put in a zirconia rotor and spun at 10 kHz.

3. RESULTS:

The IR spectra, in the $\nu_3 \text{PO}_4$ domain, of a sample at different stages of drying is shown in figure 1.

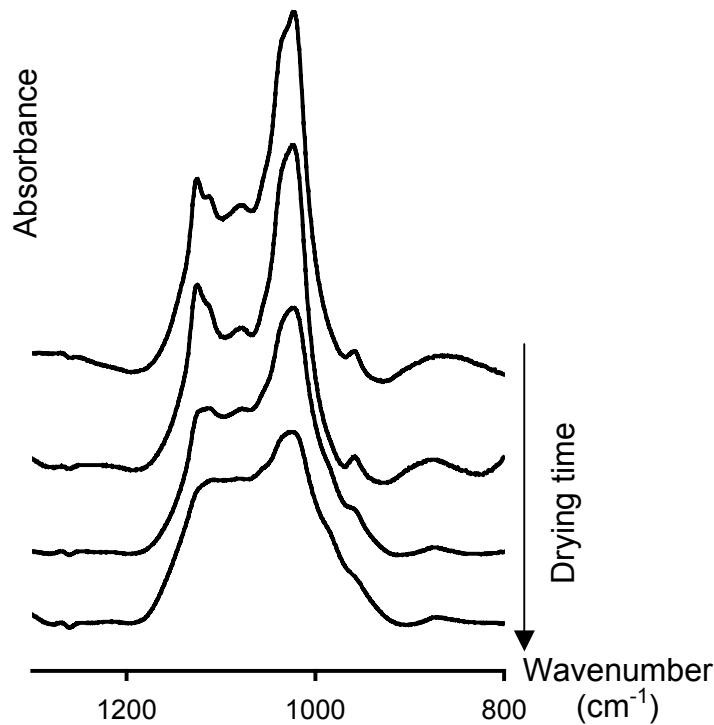


FIGURE 1: IR spectra in the ν_3 - ν_1 PO₄ domain of a gel-like PCA sample during drying (wet sample on the top, dry sample on the bottom).

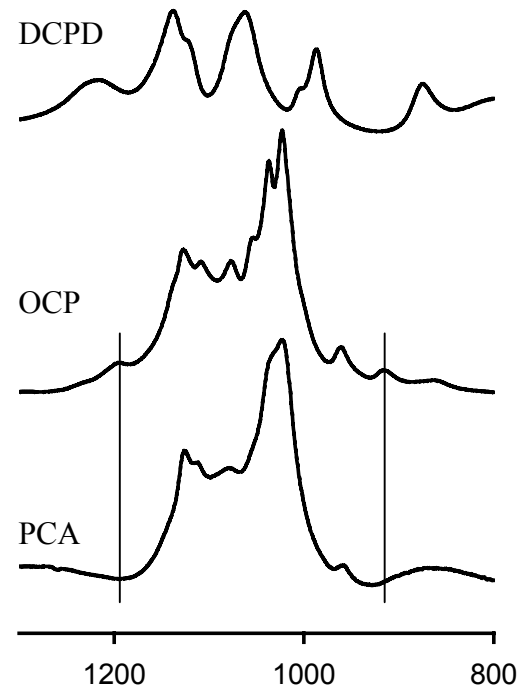


FIGURE 2: IR spectra in the ν_3 - ν_1 PO₄ domain of a wet PCA compared to supposed precursors OCP and DCPD (the lines show missing bands)

On the wet samples distinct bands are seen at 1125, 1110, 1021 and 960 cm⁻¹; clear shoulders at 1135, 1075 and 1035 cm⁻¹ and very weak shoulders at 1055, 1000 cm⁻¹. On drying however the spectral bands become broader and the fine structure observed in the wet state is lost. The dry sample spectrum is analogous to that of a lyophilised poorly crystalline apatite whereas the spectrum of the wet sample on the contrary looks similar to that of OCP (figure 2). More detailed analysis however shows that some bands are altered or missing. Thus the HPO₄²⁻ vibration at 915 and 1200 cm⁻¹ are not observed.

Large differences between wet and dry samples are also revealed by solid state ³¹P MAS-NMR (figure 3). The wet sample shows, here again, relatively narrow lines at 3.74 and 1.36 ppm. The former is highly asymmetric on the high field side, suggesting the existence of several phosphate groups with different chemical shifts in the region 2.3-6 ppm. ¹H→³¹P cross-polarisation experiments (CP-MAS, not shown) indicate that the phosphate groups corresponding to the line at 1.36 ppm are closer to protons than those corresponding to the line at 3.74 ppm. Therefore these phosphate groups could be assigned to HPO₄²⁻ groups. On the dry sample on the contrary the bands are

considerably broadened and the fine structure is lost: instead of a narrow HPO_4^{2-} band a very broad shoulder is observed.

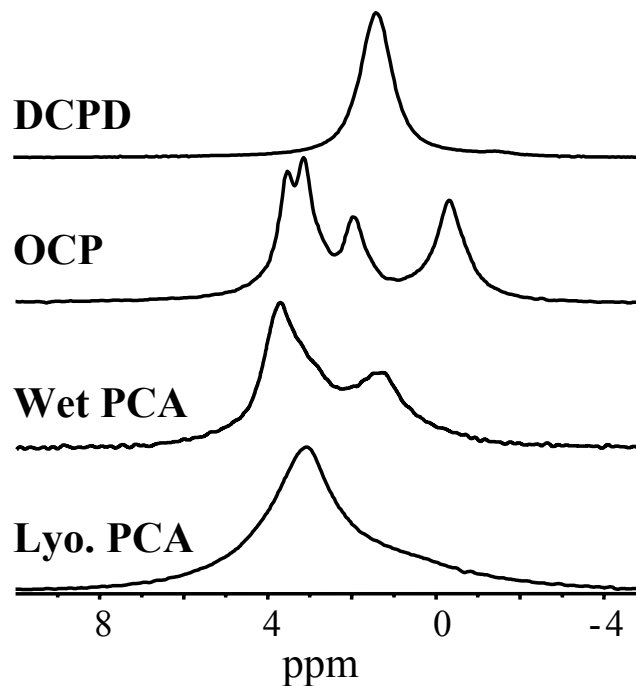


FIGURE 3 : Solid-state NMR spectra of wet and lyophilised PCA apatites compared to OCP and DCPD. (Chemical shifts reference is H_3PO_4)

Comparison with potential precursors of PCA do not confirm the analogies with OCP observed by FTIR. The most intense NMR band of the wet sample is close to that of apatitic PO_4^{3-} groups whereas the smaller band is close to that of HPO_4^{2-} ions in DCPC.

The chemical analysis of the precipitated PCA sample indicate a chemical composition similar to that of weakly hydrolysed OCP (Ca/P = 1.38 and 27.4% of P as HPO_4^{2-} ions)⁹.

The X-ray diffraction pattern of the lyophilised sample was characteristic of poorly crystalline apatite. The very strong (100) line of OCP was not detected. The wet sample did not reveal the presence of OCP structure either.

4. DISCUSSION

The data obtained clearly establish the structural evolution of poorly crystalline apatites during drying. One of the most intriguing question concerns the nature of the hydrated compound revealed by the data.

4.1 Nature of the hydrated compound

The spectroscopic data do not give a clear response to this essential question. The IR spectra suggest a strong analogy with OCP whereas the NMR data suggest

environments of the HPO_4^{2-} species closer to that of DCPD. A careful analysis of the IR spectra indicates however some differences with OCP concerning essentially HPO_4^{2-} ions. Recently the vibrational spectroscopic characteristics of OCP were re-considered by Fowler⁹. He suggested in fact the existence of two polymorphs in a reversible equilibrium linked to the water partial pressure. However none of these corresponds to our data and there is no complete analogy of the spectral characteristics of the wet PCA samples with well crystallised calcium phosphates. It does not seem surprising however that a nanocrystalline phase is not quite analogous to well crystallised three-dimensional crystals. The surface energy plays a dominant role in nanocrystals and may even affect the entire structure.

The data stress the importance of water molecules for the PCA nanocrystals. The amount of water associated with the gel-like sample is of course much higher than that possibly associated with the mineral phase and most of the water molecules are in environments analogous to that of liquid water. On drying the free water molecules are eliminated first and faint shoulders can be seen on the FTIR spectra, revealed by second derivation of the spectra (data not shown). These additional bands suggest that the remaining water molecules are associated with the mineral structure, the shifts observed indicate stronger hydrogen bonding than within liquid water. Drying leads to a progressive loss of resolution of both IR and NMR spectra. The ionic environments are less well structured and specified than in the hydrated compound, however the non-apatitic environments are present and can be detected spectroscopically. Even after drying some water molecules remain in the precipitate but the entire surface structure has collapsed. This process is partly reversible and a partial return to the wet structure is observed after re-hydration.

These data suggest the existence on the surface of the crystals of a hydrated structured layer which exists only in wet media. During dehydration a more disordered structure appears. The role of this hydrated layer is probably to reduce the surface energy of the nanocrystals. Although there is no data available on the surface energy of PCA, it has been shown recently¹⁰ that hydrated Ca-P showed a much lower surface energy in aqueous media than non-hydrated Ca-P, including well crystallised apatites, and one may expect that this is also the case for nanocrystals.

4.2 Properties of the hydrated layer

The surface hydrated layer should be conceived as a progressive transition between solid and liquid state. The ions belonging to this layer show a relatively high mobility.

Thus, as already reported, the cations as well as the anions can be readily and reversibly exchanged. These modifications of the composition may be associated with a modification of the surface structure.

The hydrated layer is not stable and it is progressively replaced by apatite during ageing in an aqueous media (maturation). The mechanism of this transformation is not yet well known but it involves a decrease of the amount of HPO_4^{2-} ions and an increase of the calcium content of the mineral phase. Simultaneously OH^- ions are included in the structure and water is excluded. The increase of ionic interactions leads to a more rigid, more stable structure with increased cohesive strength.

4.3 Consequences in materials science

The mobility of the surface ions related to the hydrated layer can be at the origin of inter-crystalline bonding as schematised in figure 4. When hydrated crystals surfaces come in close proximity, the ion mobility allows adaptation of the surfaces topologies. Then two patterns of evolution may occur:

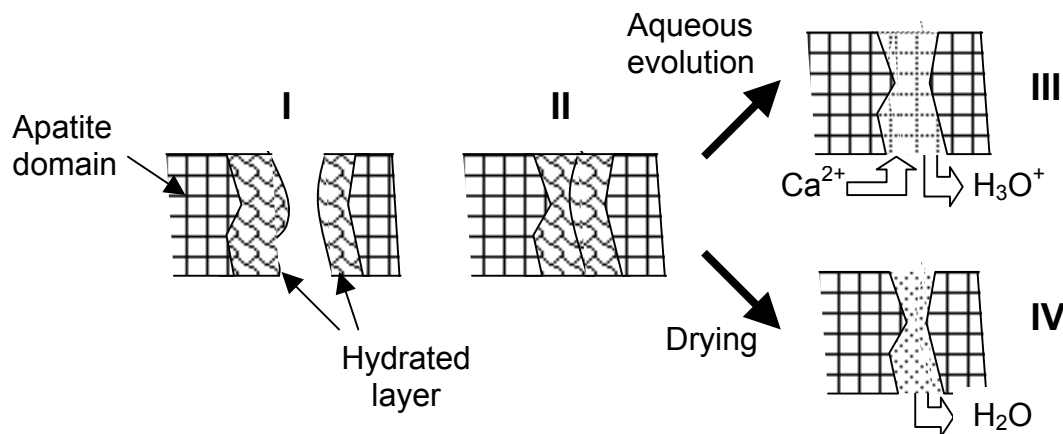


FIGURE 4: Role of the hydrated layer in the formation of materials from PCA nanocrystals. Junction of crystals and topological adaptation (I and II). "Crystal fusion" (III) corresponding to the evolution of the hydrated layer towards an apatite lattice. "Low temperature sintering" (IV) the elimination of water leads to direct ionic interactions.

-In wet media, the progressive elimination of the hydrated layer and its replacement by a regular apatite lattice leaves crystals in very close interaction. This phenomenon has been observed *in vivo* and has been described as "crystal fusion"¹¹. It contributes to the densification of tissues like dental enamel and may also probably be involved in the setting reactions of biomimetic cements formed by nanocrystalline apatites (SRS[®], Biobon[®], α -BSM[®])¹².

-On drying, another pattern may occur: the crystals tend to aggregate and to share their aqueous environment to minimise the surface energy, then the removal of water molecules puts the crystals surfaces in closer contact and eventually allows direct ionic interactions. At this stage the joined crystals cannot be separated because of the existence of strong inter-crystalline bonds. This phenomenon can be described as "low temperature sintering" and gives ceramic-like materials¹³.

These processes are rather complex and involve the stability of the hydrated layer and its transformation ability. Mature crystals partly loose these properties. However, several additives (magnesium, carbonate ions) can stabilise the hydrated layer and delay its evolution into apatite although they may also alter its structure. Besides antagonistic processes are involved: densification is always related to the elimination of water from the hydrated surface layer but simultaneously without water there is no surface mobility and weak inter-crystalline bonding results. Concerning "low temperature" ceramics a low rate of drying and a highly developed hydrated layer have been found crucial¹³.

This bonding phenomenon may also occur between heterogeneous surfaces. Synthetics nanocrystalline apatites have been proposed for coating various medical devices to improve their biological properties. During the initial stage of formation of apatites, the hydrated layer is well developed. On a hydrophilic surface the same process as that described for inter-crystalline bonding may occur: the ion mobility allows a topological adaptation to the metallic surface, and the progressive transformation into apatite tightens the adhesion of the nanocrystal to the surface. When drying is involved a very strong bonding may be formed by direct ionic interactions and very high adhesive strength have been reported for such "biomimetic" coatings¹⁴. The quality of the bonding depends on complex factors such as the crystal growth rate, the drying rate and/or the rate of evolution of the hydrated layer towards a regular apatite structure. A better understanding of these phenomena would certainly improve the technique and the quality of the coatings.

Another important property of the hydrated layer is its ability to adsorb proteins. However, the affinity varies considerably with the maturation stage. For Bovine Serum Albumin (BSA), for example, the affinity appears lower for freshly precipitated PCA than for mature samples¹⁵. This observation is consistent with a lower surface energy of highly hydrated Ca-P surface compared to that of well crystallised apatites. This variation of the surface affinity for proteins on ageing may be one way for the organism to control bone mineral performance and turn-over.

5. CONCLUSION

The existence of a labile hydrated layer might be one of the essential feature of nanocrystalline PCA which could explain most of their surface properties *in vivo*. This distinctive characteristic could be used to improve the performance and strength of biomimetic biomaterials. These very fragile temporary structure which exist only in aqueous media need more investigations and the development of characterisation techniques applicable to wet samples.

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