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Nanocrystalline apatites in biological systems: characterisation, structure and properties

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Nanocrystalline apatitic calcium phosphates play a crucial role in calcified tissues and biomaterials. One of the most interesting characteristics of biomimetic apatite nanocrystals is the existence of a surface hydrated layer essentially related to their formation process in solution. This hydrated layer shows specific spectroscopic characteristics. It seems to exist in its nascent state only in wet samples and is altered on drying. This surface-hydrated layer progressively disappears as the stable apatite domains develop. The surface ions can be rapidly and reversibly exchanged in solution, mainly with selected bivalent species. The exchange reactions clearly reveal the existence of two domains: the relatively inert apatite core and the very reactive surface-hydrated domains. The structure of the hydrated layer has been shown to be reversibly affected by the constituting ions. Such a surface layer in bone apatite nanocrystals could participate actively in homeostasis and probably other regulation processes. The specificity of biomimetic apatite nano-

crystals also opens interesting possibilities in materials science. The mobility of the mineral ions on the crystal surface, for example, allows strong bonding and interactions either with other crystals or with different substrates. Inter-crystalline interactions have been described as a "crystal fusion" process in vivo and they could be involved in the setting reaction of biomimetic calcium phosphate cements. Ceramic-like materials using the surface interaction capabilities of the nanocrystals can be produced at very low temperature (below 200 °C). The surface-hydrated layer could also be involved in interactions with macromolecules and polymeric materials or in the coating of implants. The ion exchange and adsorption capabilities of the nanocrystals could probably be used for drug release, offering a range of possible behaviours.

Keywords: Nanocrystalline apatites, biomaterials, calcium phosphate

1 Introduction

Nanocrystalline apatitic phosphates are the main constituents of mineralised tissues (bone, dentine, fish scales, horns of different animals) and they are involved in several pathological calcifications (e.g. dental calculi, tendinous mineralisation, calcification of blood vessels). In addition they play a crucial role in the biological activity of orthopaedic biomaterials and it has been shown that the formation of these apatites on the surface of orthopaedic implants can determine their integration in the living tissue. Indeed, one of the most widely used techniques for forecasting the behaviour of a material for orthopaedic applications consists in determining the formation rate of nanocrystalline apatite analogous to bone mineral when this material is immersed in SBF (Simulated Body Fluid) solution [1]. Recently, nanocrystalline apatites have been directly used as biomaterials especially as coatings for metallic prostheses [2], as the main constituent of calcium phosphate cements [3] and in mineral-organic composite materials [4]. Despite their biological role, nanocrystalline apatites are not very well known regarding their composition and structure and are difficult to characterise with accuracy due to their nanocrystalline nature, non-stoichiometry, heterogeneous composition and high reactivity. Currently the most common forms of analysis are electron microscopy observation and X-ray diffraction. The data obtained are poorly informative however. The global chemical composition is generally not given and specific structural details are almost always ignored. The aim of this work is to report on the different spec-

troscopic techniques which can be used to describe nanocrystalline apatites and to give an overview of their structural characteristics; to recall some of their physico-chemical and biological properties; and to present potential uses of such apatites in the field of biomaterials.

2 Materials and Methods

2.1 Synthesis of nanocrystalline apatites

Nanocrystalline biomimetic apatites were prepared by double decomposition in aqueous media, at physiologic pH, between a solution of calcium nitrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$: 17.4 g for 250 ml of deionised water) and an ammonium hydrogenphosphate solution which might or might not contain carbonate ions ($(\text{NH}_4)_2\text{HPO}_4$: 40 g and in some cases NaHCO_3 : 20 g for 500 ml of deionised water). The calcium solution was poured rapidly into the phosphate (and carbonate) solution. The large excess of phosphate (and carbonate) ions has a buffering effect and allows the pH to stabilise around 7.4 without the use of any foreign molecules or ions [5]. The suspensions were left to age at room temperature for variable periods of time (maturation). They were then filtered on a Büchner funnel and washed with de-ionised water until elimination of ammonium and nitrate counter ions. A few samples were analysed wet at this stage, but generally they were freeze-dried and stored at low temperature (-18 °C) to prevent any further alteration.

Several biological samples of mineralised tissues prepared in laboratory hospitals have also been studied. The soft tissues were removed and the samples were lyophilised. They were ground at liquid nitrogen temperature (Spex Freezer Mill) and washed with a methanol/chloroform mixture (2/1 ratio) to re-

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move fatty acids. After a second freeze-drying stage they were stored at -18°C .

2.2 Physico-chemical characterisations

The main constituents of the precipitated and biological apatites have been analysed. The calcium content was determined by complexometry with EDTA, the phosphate content by UV-vis spectrophotometry of the phosphovanadomolybdc complex and the carbonate content by coulometry of evolved CO_2 after acidic dissolution of the samples (Coulometrics CM 5130 UIC Inc.). In the case of non-carbonated apatites the HPO_4^{2-} content was determined using the method proposed by Gee and Dietz, involving the thermal condensation of hydrogrophosphate ions into pyrophosphate [6].

All samples were analysed by X-ray diffraction using a curved counter (INEL CP 120, Co $K\alpha$ radiation). They presented a pattern characteristic of poorly crystalline apatites, analogous to those observed for biological samples (not shown).

The Fourier transform infrared spectra (FTIR) were obtained on Perkin-Elmer 1760 and Nicolet 5700 instruments. The freeze-dried samples were dispersed in KBr pellets and analysed by transmission, while the spectra of wet samples were obtained using an Attenuated Total Reflectance (ATR) device (SpectraTech) equipped with a ZnSe crystal.

The solid state nuclear magnetic resonance (NMR) analyses were made at 125.77 MHz for ^{13}C and 202.47 MHz for ^{31}P , respectively with a Bruker ASX 500 spectrometer.

3 Results

Spectroscopic methods associated with chemical treatments of nanocrystalline apatites allow a precise description of their main surface characteristics, which can be linked to their reactivity.

3.1 Infrared spectroscopy

The infrared spectra of poorly crystalline apatites differ from those of well crystallised apatites by several features. In addition to the band-broadening effect and the loss in resolution generally observed for poorly crystalline samples, nanocrystalline apatites exhibit faint band shifts and additional bands in the different absorption domains of phosphate and carbonate ions, revealed by spectral treatments such as self-deconvolution and/or the use of the second derivative [7–9]. The $\nu_4 \text{PO}_4$ ($500\text{--}700 \text{ cm}^{-1}$) and $\nu_2 \text{CO}_3$ vibration modes are of special interest and allow a quantitative characterisation of the apatite nanocrystal.

The $\nu_4 \text{PO}_4$ mode observed for well crystallised stoichiometric apatites should exhibit three IR bands, according to group factor theory [10]. These can generally be distinguished and even non-stoichiometric apatites show three main bands in this domain, at $600 (\text{A}_g)$, $575 (\text{E}_{1u})$ and $560 (\text{E}_{1u}) \text{ cm}^{-1}$. In addition to these three main bands, nanocrystalline apatites, especially those obtained at very short maturation times, exhibit clear shoulders at 617 and 534 cm^{-1} [11]. Spectral decomposition (curve-fitting) allows the relative intensities of the bands constituting the spectra to be evaluated (Figure 1). These new bands do not correspond to other species

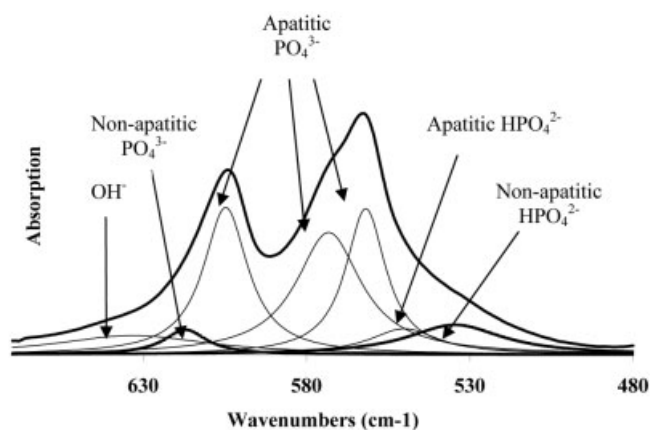


Figure 1. FTIR spectrum in the $\nu_4 \text{PO}_4$ domain of a synthetic nanocrystalline apatite (maturation time 3 days, no foreign ions present). Curve-fitting (Lorentzian band shape) shows the different absorption bands and their assignment.

like water molecules which could absorb in the same domain (no change on deuteration). The position of the band at 534 cm^{-1} suggests a possible assignment to HPO_4^{2-} ions as in other calcium salts containing HPO_4^{2-} groups [12]. Indeed, the chemical analyses of series of samples containing different proportions of HPO_4^{2-} show that the intensity of the 534 cm^{-1} band is well correlated with the HPO_4^{2-} content [11]. Other types of apatites, well crystallized and also containing HPO_4^{2-} species can be prepared by hydrolysis of relatively soluble calcium salts [7]. The comparison of their spectrum with that of nanocrystalline apatites reveals significant differences however. The HPO_4^{2-} band in well crystallised apatites is located at 550 cm^{-1} and appears narrower than in nanocrystalline samples. It therefore seems that two kinds of HPO_4^{2-} species can be observed in apatites. In some biological samples, dental enamel for example, both species can be clearly distinguished [7].

The intensities of the two additional bands observed in nanocrystalline apatites at 617 cm^{-1} and 534 cm^{-1} do not seem correlated in the various samples which have been analysed. They probably correspond to different phosphate groups, but the assignment of the rather weak 617 cm^{-1} band could not be determined.

The $\nu_2 \text{CO}_3$ mode corresponds to the out-of-plane vibration movement of the carbonate ion. This vibration mode is simply degenerated. Three bands can be observed in this domain in nanocrystalline apatites and bone (Figure 2): two relatively narrow bands at 880 and 873 cm^{-1} and a broader band around 866 cm^{-1} [13]. The first two bands can be assigned to carbonate groups in the apatite lattice. The band at 880 cm^{-1} corresponds to type A carbonate, located on the monovalent anionic site of the structure (OH sites of hydroxyapatite). The band at 873 cm^{-1} was assigned to type B carbonate substituting for phosphate groups in the structure. Although the presence of type A carbonate is rarely mentioned in bones [14] and sometimes questioned, it should be noted that another mineralised tissue, tooth enamel, indubitably contains such carbonate species [15]. In addition, the 880 cm^{-1} band is affected by substitutions in the monovalent anionic sites. Thus fluoridation of bone and synthetic nanocrystalline apatites for example, leads to a significant decrease of the relative intensity of this band due to the preferential location of fluoride ions on the monovalent anionic sites and the correlated displace-

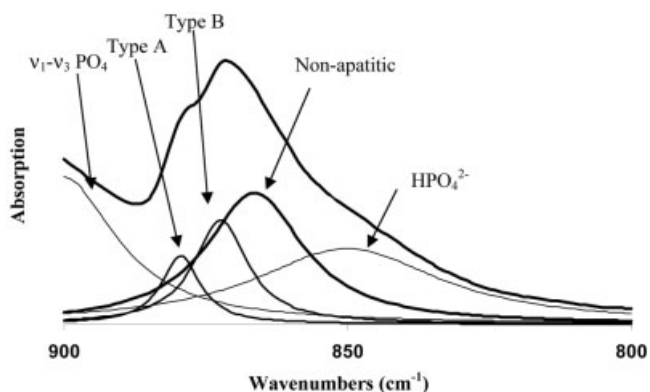


Figure 2. FTIR spectrum in the ν_2 CO_3 domain of a synthetic nanocrystalline apatite (maturation time 3 days, prepared in the presence of carbonate ions). Curve-fitting (Lorentzian band shape) shows the different absorption bands and their assignment.

ment of the other ions [16]. The third carbonate band, around 866 cm^{-1} , has never been observed in well crystallised apatites and occurs only in poorly crystalline apatites, including natural samples such as bone mineral. It is possible, using curve-fitting procedures, to estimate the relative proportions of these carbonate species (Figure 2), although the accuracy of such spectral treatments has not yet been corroborated by other types of analyses.

3.2 Solid State NMR

Solid State NMR studies on synthetic samples enriched with ^{13}C have confirmed the existence of three main chemical environments of carbonate ions in apatites. In addition to the two carbonate species located in apatite crystallographic sites (types A and B), a third type of carbonate site was clearly evidenced in fluoridated type B carbonated apatite containing a low amount of OH^- ions [17]. This site was found to correspond to hydrated domains distinct from the apatite domains. Indeed, the ^{13}C single pulse excitation (SPE) and $^1\text{H} \rightarrow ^{13}\text{C}$ cross-polarisation (CP) measurements (Figure 3) both combined with magic angle sample spinning (MAS) revealed two carbonate sites characterised by different chemical shifts (170 and 168.5 ppm) and cross polarisation times. The combined use of $^1\text{H} \rightarrow ^{13}\text{C}$ 2-D hetero-correlation chemical shift and $^1\text{H} \rightarrow ^{13}\text{C}$ differential cross polarisation (DCP) has allowed the identification of the two carbonate species. The car-

bonate groups corresponding to the line at 168.5 ppm (Figure 3) are mainly correlated to the water molecules (peak at ~ 5 ppm in the ^1H dimension) and those giving the line at 170 ppm (type B) to OH^- apatite groups (peak at ~ 0.2 ppm in the ^1H dimension).

Using ^{31}P solid state NMR, other research groups have also shown that HPO_4^{2-} ions in synthetic nanocrystalline apatites and bone mineral are mainly located in hydrated domains [18, 19]. Thus the specific environments of carbonate and HPO_4^{2-} ions, found in nanocrystalline apatites, appear to correspond to hydrated domains distinct from apatite domains.

Such a model of apatite compounds involving hydrated domains and apatite domains has already been proposed by Brown [20].

3.3 Brown's Model

Brown's model is based on the structure of triclinic octacalcium phosphate (OCP), $\text{Ca}_8(\text{PO}_4)_4(\text{HPO}_4)_2 \cdot 5\text{H}_2\text{O}$ formed by a succession of apatite-like and hydrated layers (Figure 4). The apatite layers contain Ca^{2+} and PO_4^{3-} ions, whereas the hydrated layers involve Ca^{2+} and HPO_4^{2-} ions. Several apatite layers could coalesce, during the hydrolysis of OCP, resulting in apatite domains separated by a hydrated layer (hydroxyapatite-OCP interlayering). The association of these domains with distinct chemical compositions would thus result in non-stoichiometric apatite formula. In this model, an OCP hydrated layer would always terminate an apatite crystal (Figure 4). In nanocrystalline apatites with a large specific surface area, the composition and non-stoichiometry would be determined by the size of the crystals. There are several justifications for this model, and a relationship between crystal size and non-stoichiometry has often been observed. Transmission electron microscopy experiments on hydrolysed OCP also support the existence of interlayering, at a greater scale, between apatite and OCP. However non-stoichiometry also exists in well crystallised apatites like those obtained by hydrolysis of relatively soluble calcium phosphate salts and in carbonate-containing apatites without the formation of interlayering. One major criticism of this model is that the carbonated equivalent of OCP has never been obtained despite several trials (see for example Hayek's work [21] and its discussion [22]) and this model lacks flexibility to account for the diversity of chemical composition and reactivity of nanocrystalline apatites. Additionally, the spectroscopic characteristics of non-apatitic environments detected in nanocrystalline apatites do not match those of OCP. Nevertheless, the concept of

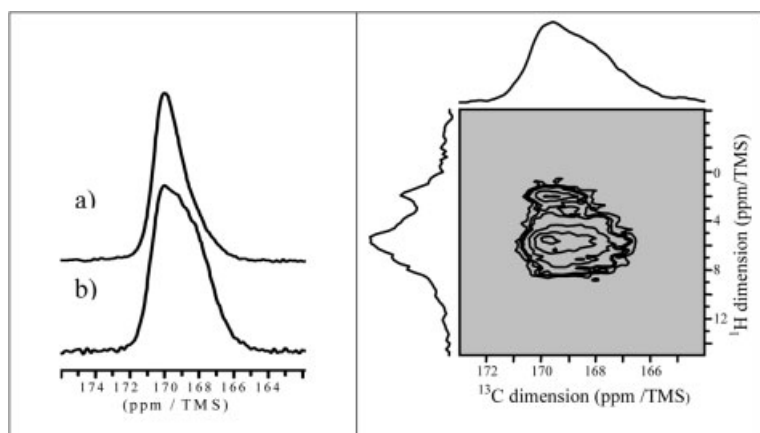


Figure 3. (Left) ^{13}C SPE-MAS (a) and $^1\text{H} \rightarrow ^{13}\text{C}$ CP-MAS (b) NMR spectra of carbonate fluorapatite weakly hydroxylated. (Right) Contours and projections of the $^1\text{H} \rightarrow ^{13}\text{C}$ 2-D HetCor-MAS NMR spectrum obtained on the same sample. The contact time was 5 ms and the spinning frequency 10 kHz.

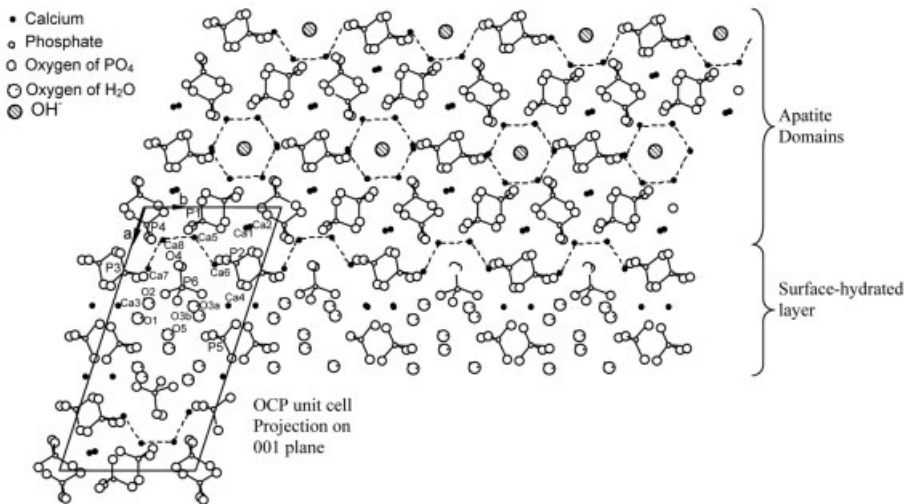


Figure 4. Brown's model of apatite surface [20], based on the structure of octacalcium phosphate (projection on the 001 plane of apatite structure),.

hydrated domains possibly existing at the surface of nanocrystals seems attractive and could explain several properties of these poorly crystalline apatites especially the ion-exchange properties.

3.4 Ion exchanges in nanocrystalline apatites

The ability of nanocrystalline apatites to exchange mineral ions with a solution was first studied by Neuman [23]. Both anionic and cationic species can be exchanged. These reactions are very rapid and an exchange equilibrium is reached within minutes. HPO_4^{2-} ions from nanocrystalline apatites can thus be replaced by carbonate ions from a solution and inversely. There is no alteration of the $\text{Ca}/(\text{P}+\text{C})$ ratio of the solid, indicating a simple ionic substitution, without other chemical alterations. The characteristics (size and morphology) of the crystalline apatite domains do not change, indicating that the entire crystals do not participate in the substitution reaction. Spectroscopic studies confirm that only non-apatitic species participate in the exchange reaction: thus the exchange of HPO_4^{2-} by CO_3^{2-} results in a decrease of the 534 cm^{-1} band accompanied by an increase of the broad featureless band corresponding to non-apatitic carbonates. These data indicate that HPO_4^{2-} and CO_3^{2-} in fact ions share the same domains distinct from the apatitic sites. Cationic exchanges are also possible and exhibit the same characteristics as the anionic exchanges.

The possibility of rapidly and reversibly exchanging mineral ions of nanocrystalline apatites without affecting the apatite domains suggests a location of these species on the surface of the nanocrystals. This model differs from the one proposed by Brown in the sense that the composition of the surface layer is not fixed and depends on preparation conditions. In addition, the apatite domain may also incorporate substituents and can be non-stoichiometric.

Such surface-hydrated domains could be very sensitive to drying conditions and additional spectroscopic investigations were carried out in a wet state.

3.5 Studies of wet samples

Most characterisation techniques applied to nanocrystalline apatites have been performed in a dry state. However these

compounds are generally formed and used in wet media and one may thus reasonably wonder if drying does not alter their surface characteristics (and therefore spectroscopic features). Interestingly, FTIR (Figure 5) and NMR [24] spectra obtained on wet suspensions of nanocrystalline apatites give spectra with very high resolution, better than those found for freeze-dried samples. On drying, the relatively thin spectroscopic bands become broader and many spectral details are lost [24].

The comparison of the wet spectrum of non-carbonated nanocrystalline apatites with that of OCP reveals strong analogies, although distinct differences (especially the absence of specific HPO_4^{2-} bands at 915 and 1200 cm^{-1} and small shifts of a few others) can be pointed out. This observation suggests that Brown's model could possibly be used as a basis for a structural representation of the hydrated layer in this case.

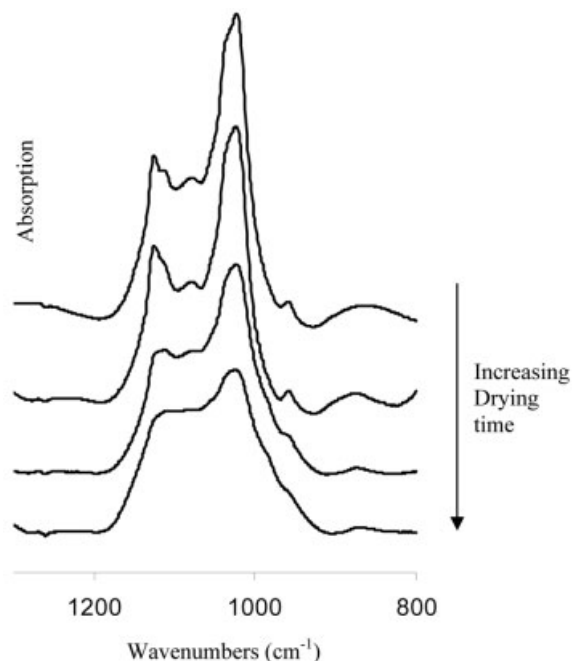


Figure 5. Evolution of FTIR spectra of nanocrystalline apatite on drying ($\nu_3\text{ PO}_4$ domain). In the wet state (top) narrow well resolved bands occur close to that of OCP. On drying bands broaden and their fine structure disappears.

However the NMR spectra of OCP and non-carbonated nanocrystalline apatites appear very different [24]. In addition, when the hydrated layer contains foreign ions such as carbonate and magnesium, which can be introduced by ion exchange, its spectroscopic characteristics appear very different from those of OCP [25]. However these modifications appear reversible and the initial spectroscopic features are restored by reverse exchange. It may then be inferred that the organisation of the hydrated layer depends on the constituent ions. In all cases the apatite domains remain essentially unchanged.

3.6 Spectroscopic studies of calcium environments.

The spectroscopic studies reported so far concern mainly carbonate and phosphate groups. Although the Mg-Ca exchange involves strong structural changes in the environment of phosphate, indicating the role of cations in the fine structure of the hydrated layer, there is no direct evidence of the observation, in biomimetic nanocrystals, of calcium environments different from those of apatite. In order to detect such environments, spectroscopic investigations have been carried out on calcium ions. X-ray Absorption Near Edge Structure (XANES) analyses of calcium ions at the K edge reveal faint differences which appear only in nanocrystalline apatites and have been assigned to non-apatitic environments of calcium ions, related to the hydrated layer [26]. Similarly, Energy Electron Loss Spectroscopy (EELS) analyses at the calcium L edge have shown specific characteristics related to nanocrystalline apatites, different from those of well crystallised apatite, thus confirming previous observation by XANES [27].

3.7 Model for a surface layer

This consistent set of data indicates the existence in nanocrystalline apatites of non-apatitic environments of the mineral ions. These ions can easily be exchanged in solution and belong possibly to a hydrated layer at the surface of the nanocrystals as schematically shown in *Figure 6*. Although well structured in aqueous media, this very fragile layer is altered on drying, leading to blurred spectroscopic features and broad

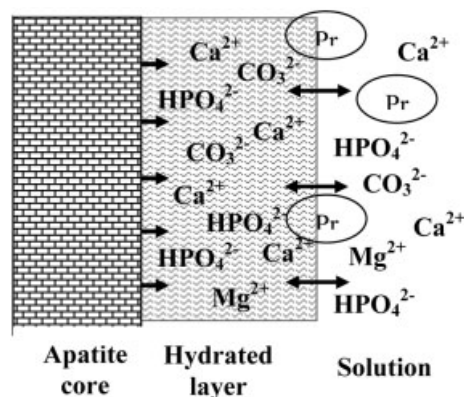


Figure 6. Schematisation of the surface-hydrated layer. The loosely bound ions can be incorporated in the growing apatite domains and can be exchanged with ions from the solutions and charged groups of proteins (Pr). Adapted from [37]

spectroscopic bands testifying to the lattice distortion resulting from the removal of water molecules. A precise structure of the hydrated surface layer cannot be reasonably proposed at this stage which, in fact, depends on the constituent ions. The surface properties of nanocrystalline apatites in wet media thus appear variable, being modified according to the surface ion composition. Similarly the thickness of the hydrated layer cannot be given with accuracy, especially in a wet state. An estimate has however been given after drying [19]. The amount of ions belonging to the hydrated layer has also been estimated from FTIR data [11]; it may reach about 30% of phosphate groups in fresh precipitate. In addition to ion exchange reactions, adsorption of different molecules can be observed most frequently involving the displacement of mineral ions from the surface [30–31] made easier due to the looser chemical bonds, resulting in high ionic mobility and more possibilities for structural re-arrangement.

4 Consequences of the structure of nanocrystalline apatites in the biological field

The structural characteristics of biomimetic nanocrystalline apatites determine not only their surface properties but also their evolution capabilities. The formation of a hydrated layer corresponds to a precipitation scheme presenting several advantages. It has been suggested that the hydrated layer decreases the energy at the crystal-solution interface, thus favouring the nucleation and the stabilisation of nanocrystals for which surface energy is a crucial factor [32]. Moreover apatite crystal growth has been shown to be a slow process, incompatible with the high/moderate supersaturation ratios which are frequently found in synthetic and biological systems. The hydrated layer thus provides, in addition to low interface energy, a reserve of mineral ions on the crystal surface nourishing the slow growth of apatite domains. The formation of the hydrated layer obviously does not correspond to a thermodynamic equilibrium. Thus the surface-hydrated layer tends to decrease progressively with time, in favour of the developing thermodynamically stable apatitic domain. The freshly-formed nanocrystals in synthetic as well as in biological systems exhibit a large amount of ions located in non-apatitic environments, corresponding to the hydrated layer. During ageing in wet media, the amount of non-apatitic environments decreases progressively as the apatite domain growth and organisation improves. However, in this irreversible process the crystals lose part of their surface reactivity and especially their exchange capabilities. This evolution is visible in bone tissue [5]: bones of young vertebrates, exhibit a high proportion of non-apatitic environments and with age, as the remodelling rate slows down, the global maturation time of the mineral increases and fewer non-apatitic environments are detected in the mineral fraction. This decrease is synonymous with a loss of surface reactivity.

Because of its ion exchange ability, the hydrated layer of bone crystals participates in homeostasis. However with age the capacity of this labile-ion reservoir decreases and its ability to regulate the concentration of mineral ions in blood diminishes accordingly. This phenomenon thus explains why a cyclic renewal of bone mineral (remodelling), especially in well-vascularised areas where active exchanges proceed easily, is a necessity for the organism. The hydrated layer

of bone mineral is also used to store vital elements such as magnesium for example which cannot easily replace calcium in the apatite lattice, but can easily penetrate the hydrated layer (magnesium in bone represents about 45% of the total body burden probably mainly bound to the surface of the crystals [33]). Another role, though still obscure, of the hydrated layer concerns mineral-organic interactions involved in the binding and regulation of bone proteins such as osteocalcin and osteopontin and in mineral-collagen interactions determining the mechanical properties of the tissue.

5 Possible uses of nanocrystalline apatites as biomaterials

The unique properties of biomimetic nanocrystalline apatites can be utilised in the field of materials. The ionic exchange capabilities allow the formation of an active-ion reservoir corresponding to the hydrated layer. It has been shown that strontium, magnesium and carbonate ions for example could be stored in the surface layer and released through a reverse exchange reaction with calcium or phosphate ions [28–29]. This property can probably be extended to other bioactive ions. It has also been shown that nanocrystalline apatites can adsorb much larger amounts of active molecules and proteins than well crystallised apatites for the same surface area [30–31]. The adsorption affinity and the maximum amount adsorbed vary considerably with the maturation state of the apatites and the extent of the hydrated layer. Thus freshly formed nanocrystalline apatites generally show a lower affinity but a higher maximum amount absorbed as compared to mature apatites, and they might similarly be able to take up more active molecules and to release them more easily. Adsorption and release properties can be modulated by acting on the maturation time and on the composition of the hydrated layer.

The existence of a high ionic mobility on the surface of such apatites may also allow new, low temperature processing methods to be set up (Figure 7). Nanocrystalline apatites can thus be consolidated by cold isostatic pressing or low temperature sintering (200 °C) [34, 35]. The mechanical properties of such ceramics are as good as those obtained at much higher temperature by natural sintering. In addition surface properties, non-stoichiometry and the nanocrystalline nature of the samples are preserved. The consolidation observed at low temperature could result from the junction of neighbouring nanocrystals via the interaction of their surface-hydrated layers. Such phenomena have been described in calcified tissue formation and are known as “crystal fusion” [36]. Analogous processes could be responsible for the setting and

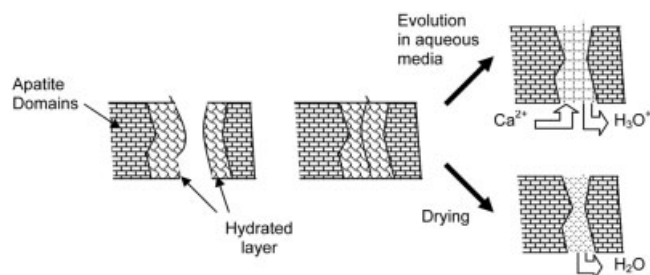


Figure 7. Interactions between nanocrystals and assembly processes (Adapted from [32])

hardening reactions of biomimetic calcium phosphate cements. Recently calcium phosphate coatings of metallic prostheses based on nanocrystalline apatites have been proposed. The existence of Mg ions at the interface suggests that interactions with the substrate, which determine the adhesion properties, could be established through the surface-hydrated layer [2]. The surface properties of nanocrystalline apatites could also be useful in the field of mineral-organic or hybrid materials involving an intimate association of organic molecules and mineral substances.

Conclusion

The surface reactivity of nanocrystalline apatites, related to the existence of a hydrated layer, opens new possibilities in the field of biomaterials and more specifically in material science. Nature has learned how to use and control surface properties of these nanocrystals to adapt calcified tissues to their different functions. These capabilities are however still not fully determined, and are in some ways rather difficult to explore considering the high reactivity of the nanocrystals and their alteration on drying and ageing in solution. It appears necessary however to achieve a better knowledge of these nanocrystals and their properties in order to understand all the subtle dynamic equilibria occurring in calcified tissues and to use them for elaborating more sophisticated and efficient biomaterials.

Acknowledgements

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