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# Hydroxyapatite/polyacrylic acid nanocrystals

**Enrico Bertoni, Adriana Bigi,* Giuseppe Falini, Silvia Panzavolta and Norberto Roveri**

*Department of Chemistry 'G.Ciamician', University of Bologna, Italy. E-mail: bigi@ciam.unibo.it*

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Hydroxyapatite nanocrystals have been synthesized in the presence of polyacrylic acid in solution. The method leads to apatite crystals with a polyelectrolyte relative content which increases as a function of its concentration in solution and reaches a limit value of about 15% wt. Polyacrylic acid affects the degree of crystallinity, the coherent length of the perfect crystalline domains, as well as the dimensions and the morphology of the crystals. TEM images indicate that on increasing polyelectrolyte content, the dimensions of the crystals are reduced, while their morphology changes from plate-like to acicular, and, at high polyacrylic acid content, the crystals tend to aggregate in clusters. In agreement, the broadening of the X-ray diffraction reflections indicate a reduction of the coherent length along the long dimension (002) and the cross section (310) of the apatite crystals. The reduction is greater along the direction orthogonal to the *c*-axis, which can be rationalized by a preferential interaction of the carboxylate groups of the polyelectrolyte with the phosphate sites on the crystalline faces parallel to the *c*-axis.

## Introduction

Biominerals offer exquisite examples of composite materials where function is controlled by shape.[1–4] Biomineralization processes take place under mild conditions and involve the conversion of ions in aqueous solution into solid minerals. Solution concentrations and the supersaturation of precipitating phases, as well as kinetics and direction of growth are generally controlled by soluble macromolecules. Furthermore, unusual crystal habitus, morphology and mechanical properties are obtained owing to the presence of an organic matrix inside the composite.[1,2,5] Thus, the solutions adopted by organisms to build biominerals represent a rich survey of ideas to develop composite materials, which can be successfully employed in different fields of applications, owing to their peculiar properties, usually unattainable by the individual constituents.[3–6] In the biomedical field, the synthesis of hydroxyapatite(HA)/polymer composite materials is of great interest for the development of biomaterials suitable to repair the skeletal system.[7–11] The inorganic phase of bone tissue, perhaps the most important biological composite material, is constituted of hydroxyapatite crystals embedded in a collagenous matrix.[1,6,12] Biological apatites are characterized by mean crystal sizes of the order of nanometers, poor crystallinity, non stoichiometry and a variety of ionic substitutions.[13,14] One of the main advantages of HA/polymer composites with respect to HA biomaterials is the possibility to modulate biodegradability, bioactivity and mechanical properties through variations in composition. Furthermore, the presence of the polymer could improve the interfacial bonding of the composite with bone tissue.[15] It is well known that the presence of some polymers in the reaction solution can affect the nucleation and growth of calcium salts. It has been verified that several polyelectrolytes may induce the nucleation of HA, but also quench crystal growth and control the apatite maturation rate.[16–18] In particular, it was verified that polyacrylic acid (PAA) reduces the crystallinity and the crystal dimensions of HA so as to even inhibit completely its precipitation.[16] PAA is a water soluble polymer, which is essentially non ionic in neutral solutions but undergoes ionization of its carboxyl side groups to carboxylate anions at basic pH. It is easily and irreversibly adsorbed on HA through a process which has been ascribed to electrostatic interactions and/or hydrogen bonding forces.[19] The adsorption of PAA on HA crystals induces significant modifications of the surface: it was verified that PAA coating leads to an improvement of the interfacial bonding between HA and a block copolymer, with a consequent improvement of the mechanical properties of the composite.[20] With respect to the simple coating of HA with PAA, the preparation of HA/PAA crystals through the synthesis of the inorganic phase in the presence of the polyelectrolyte offers the following advantages: it allows a wider range of PAA concentrations in the crystals and reduces their dimensions to values comparable to those characteristic of biological apatites.[21] These advantages have been explored here, which reports the results of a structural, chemical and morphological investigation carried out on hydroxyapatite synthesized in the presence of different amounts of PAA in solution in order to verify the effect of the polyelectrolyte on apatite crystallization.

## Materials and methods

Hydroxyapatite was synthesized in $N_2$ atmosphere using 100 ml of 1.08 M $Ca(NO_3)_2$ solution at pH adjusted to 10 with $NH_3$. The solution was heated at 90 °C and 100 ml of 0.65 M $(NH_4)_2HPO_4$ solution, pH = 10 adjusted with $NH_3$, was added dropwise under stirring. The precipitate was maintained in contact with the reaction solution for 5 h at 90 °C under stirring, then centrifuged at 10 000 rpm for 10 min and repeatedly washed with $CO_2$-free distilled water. The product was dried at 100 °C overnight.[21]

Hydroxyapatites at different polyelectrolyte contents (HAPAA) were obtained by adding low molecular weight (MW = 2000) PAA to the nitrate solution, before adjusting the pH to 10 with $NH_3$. The polyelectrolyte concentration ranged from 0.5 to 17 mM. Hereafter the samples are indicated as HAPAA0.5, HAPAA1, HAPAA5, HAPAA10, HAPAA17, where the last digits correspond to the concentration of the polyelectrolyte in solution.

X-Ray diffraction analysis was carried out using a Philips PW 1050/81 powder diffractometer equipped with a graphite monochromator in the diffracted beam. Cu-K$\alpha$ radiation was used. The $2\theta$ range was from 10 to 65° at a scanning speed of 0.5° $min^{-1}$. The lattice constants were determined by least square refinements from the well determined positions of the most intense reflections. Silicon was used as internal standard.

In order to evaluate the coherent length of the apatitic crystals, further X-ray powder data were obtained in two regions, peak (002) between $2\theta$ 24.4 and 26.4 and peak (310) between $2\theta$ 37.4 and 41.5 by means of step scans using a fixed counting time period of 30 s and a scan rate of 0.1° $step^{-1}$.

For IR absorption analysis, 1 mg of the powdered samples

was carefully mixed with 300 mg of KBr (IR grade) and pelletized under vacuum. The pellets were analyzed using a Nicolet FT 205 IR spectrophotometer to collect 32 scans in the range 4000–400 $cm^{-1}$ at a resolution of 4 $cm^{-1}$.

Thermogravimetric analysis was carried out using a Perkin Elmer TGA-7. Heating was performed in a platinum crucible in air flow (20 $cm^3$ $min^{-1}$) at a rate of 5 °C $min^{-1}$ up to 900 °C. The sample weights were in the range 5–10 mg.

Transmission electron microscopy (TEM) was carried out using a TEM Philips CM100. Calcium contents were determined using an atomic absorption spectrophotometer (Perkin Elmer 373); the samples were diluted to an appropriate volume with 10% lanthanum in 50% HCl.

Phosphorus content was determined spectrophotometrically as molybdovanadophosphoric acid.[22]

## Results

Fig. 1 shows the powder X-ray diffraction patterns of the samples synthesized in the presence of different amounts of PAA in solution. All the patterns indicate the presence of hydroxyapatite as the unique crystalline phase. However, the broadening of the diffraction reflections is in agreement with an overall reduction in crystallinity on increasing the polyelectrolyte concentration in solution. The values of the lattice constants of HA are $a=9.424(1)$ and $c=6.880(1)$ Å, and do not change appreciably on increasing PAA concentration, although the values of standard deviations increase. The reduction in the degree of crystallinity is confirmed by the results of the IR absorption analysis, as it can be deduced by the reduction of the absorption bands at 3572 and 630 $cm^{-1}$, due to $OH^-$ stretching and libration modes respectively, which are no longer appreciable in the IR spectra of the samples prepared at the highest PAA concentrations. Moreover, the spectra of HAPAA samples display, other than the bands characteristic of phosphate groups, several absorption bands at 2920, 1720, 1650, 1560, 1465 and 1410 $cm^{-1}$, due to polyacrylate.[7,23] The relative intensities of these bands increase on increasing PAA, as shown in Fig. 2. The absorption bands due to polyacrylate are no longer observable in the IR spectra of the samples treated at temperatures higher than 700 °C. Furthermore, heat treatment at high temperatures induces an overall increase of the degree of crystallinity of the apatitic phase. After heating at 1100 °C, all the samples display a very modest conversion into β-tricalcium phosphate, which averages around 5%, as evaluated from the relative areas of the most intense diffraction peaks of the two phases. Fig. 3 shows the powder X-ray diffraction patterns of the sample synthesized in the presence of 17 mM PAA and heat treated at different temperatures.

PAA content has been determined through thermogravimetric analysis. The TG–DTG plots of HAPAA samples display a thermal process centered at about 360 °C, due to PAA combustion,[7,21] as observed in Fig. 4. The weight losses associated to this process are given in Table 1 together with the Ca/P molar ratio of the different samples. The relative amount of polyelectrolyte in the crystals increases with PAA concentration in solution up to 5 mM, after which it remains almost constant. Both Ca and P contents decrease on increas-

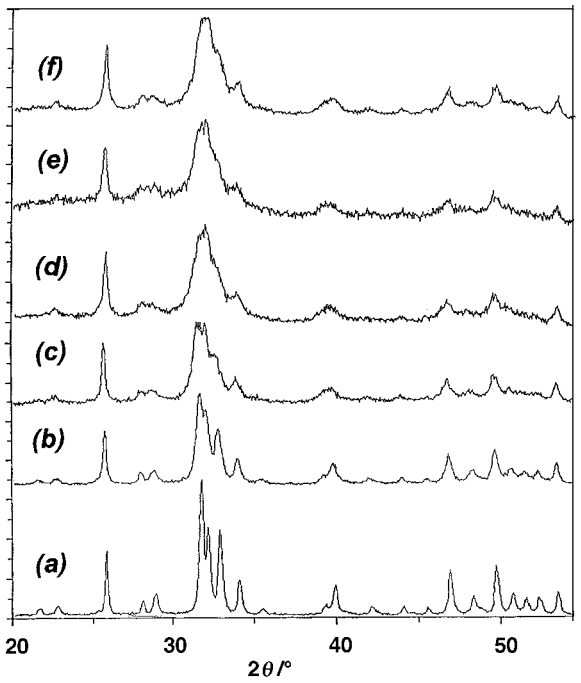


**Fig. 1** Powder X-ray diffraction patterns of the samples synthesized in the presence of different amounts of PAA in solution: (a) HA, (b) HAPAA0.5, (c) HAPAA1, (d) HAPAA5, (e) HAPAA10, (f) HAPAA17.

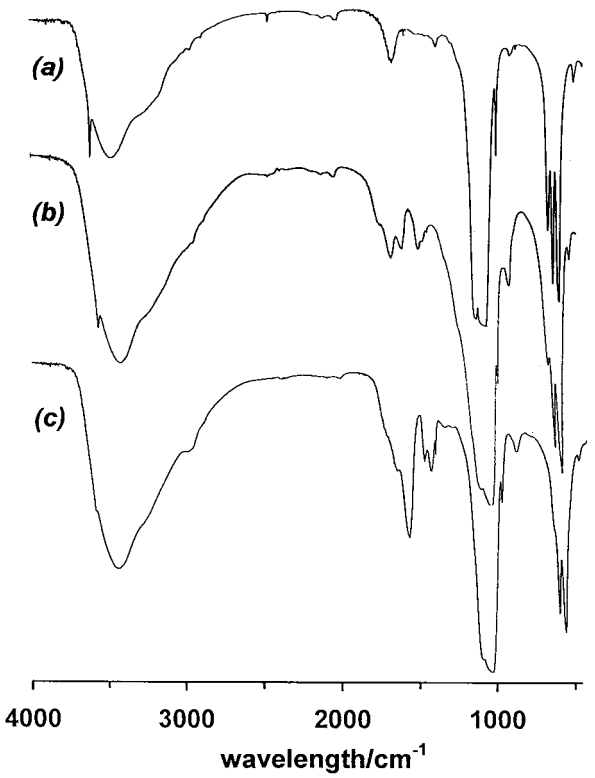


**Fig. 2** Infrared absorption spectra of (a) HA, (b) HAPAA1 and (c) HAPAA17.

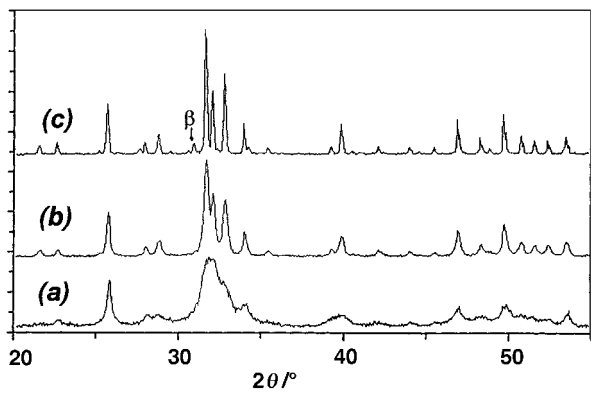


**Fig. 3** Powder X-ray diffraction patterns of HAPAA17 (a) air dried at 100 °C and after heat treatment at (b) 700 and (c) 1100 °C. The most intense diffraction peak of β-tricalcium phosphate is indicated.

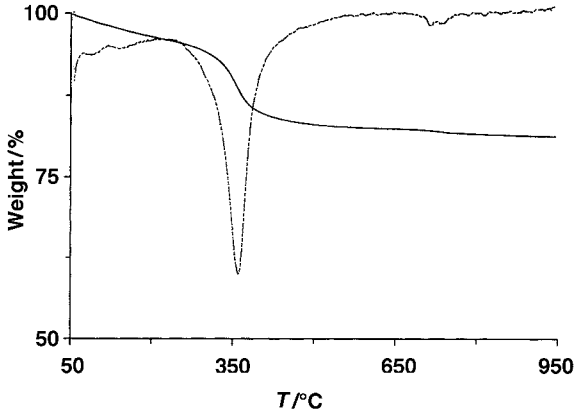


**Fig. 4** TG-DTG plots of HAPAA5.

**Table 1** Chemical composition of the apatitic samples

| Sample | $Ca^{2+}$/mmol $g^{-1}$ | P/mmol $g^{-1}$ | Ca/P | PAA (wt%) |
|---|---|---|---|---|
| HA | 9.11±0.03 | 5.41±0.03 | 1.68 | — |
| HAPAA0.5 | 8.92±0.02 | 5.35±0.05 | 1.67 | 4±1 |
| HAPAA1 | 8.44±0.02 | 5.05±0.05 | 1.67 | 7±1 |
| HAPAA5 | 7.60±0.08 | 4.62±0.03 | 1.65 | 14±1 |
| HAPAA10 | 7.27±0.07 | 4.60±0.05 | 1.58 | 12±1 |
| HAPAA17 | 7.27±0.08 | 4.65±0.05 | 1.56 | 15±1 |

ing PAA content with no significant variation in the Ca/P molar ratio up to HAPAA5. On the other hand, the Ca/P molar ratios of HAPAA10 and HAPAA17 appear slightly but appreciably reduced with respect to the stoichiometric value of 1.67. The line broadening of the (310) and (002) reflections was used to evaluate the coherence length of the perfect crystalline domains inside the crystals. $D_{hkl}$ values were calculated from the width at half maximum intensity ($\beta_{1/2}$) using the Scherrer equation[24]

$$D_{hkl}=k\lambda/\beta_{1/2}\cos\theta$$

where $\lambda$ is the X-ray wavelength and $\theta$ the diffraction angle. $K$ is a constant varying with crystal habit and chosen as 0.9. The results, which are summarized in Table 2, indicate that the $D_{hkl}$ values of HAPAA samples are significantly reduced with respect to those of HA. The reduction increases on increasing PAA content and is greater in the direction orthogonal to the $c$-axis.

TEM micrographs shown in Fig. 5 reveal that the dimensions, as well as the morphology of the apatitic crystals, change as a function of PAA concentration. HA is constituted of tiny crystals with plate shaped morphology. In the presence of polyacrylic acid the crystals exhibit reduced dimensions and become more acicular. At high PAA concentrations, most of the crystals are aggregated in clusters and the few isolated ones appear very small and exhibit ill defined faces.

**Table 2** Coherent lengths ($D_{hkl}$) evaluated from the width at half maximum intensity of the (002) and (310) reflections of HA and HAPAA as a function of PAA concentration in solution

| Sample | $D_{002}$/nm | $D_{310}$/nm |
|---|---|---|
| HA | 44.3±0.5 | 30.2±0.5 |
| HAPAA0.5 | 34.8±0.5 | 17.3±0.5 |
| HAPAA1 | 34.8±0.3 | 11.7±0.1 |
| HAPAA5 | 27.6±0.2 | 8.6±0.1 |
| HAPAA10 | 26.6±0.2 | 10.1±0.1 |
| HAPAA17 | 29.2±0.3 | 7.0±0.1 |

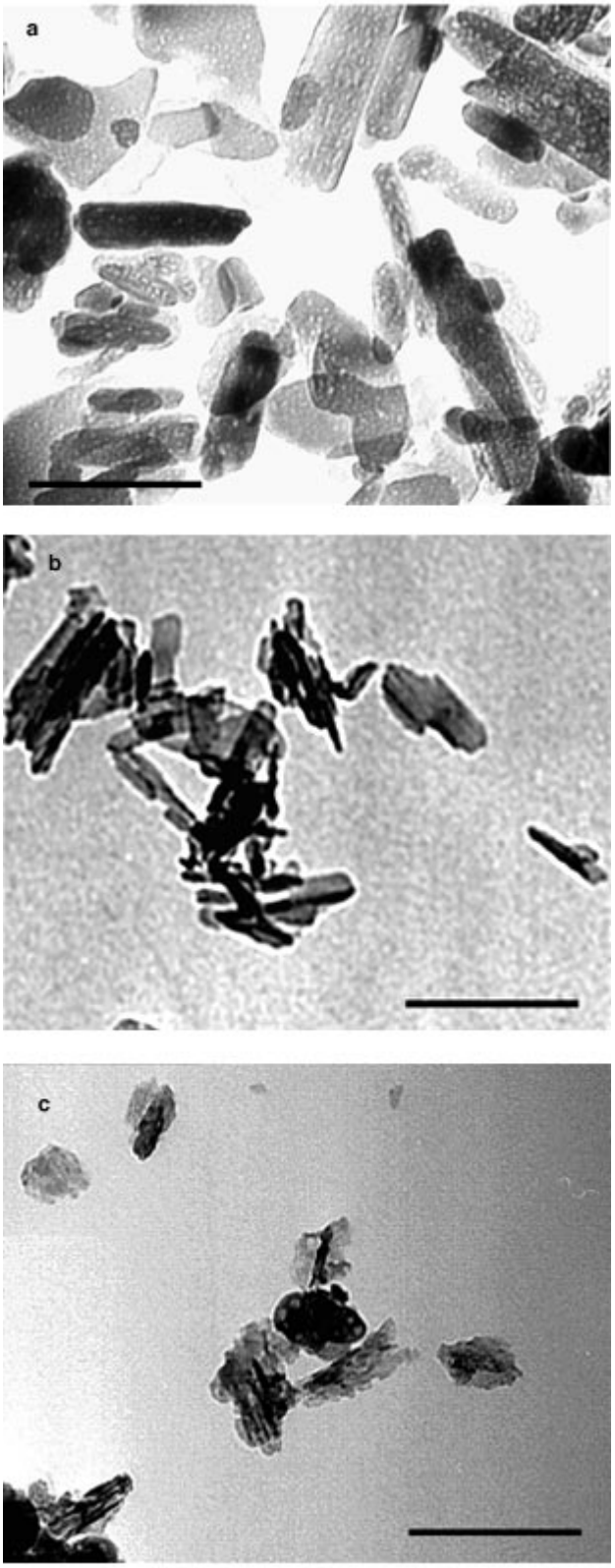


**Fig. 5** TEM images of (a) HA, (b) HAPAA1, (c) HAPAA10. Bar = 200 nm.

## Discussion

The results of this work indicate that both the size and shape of hydroxyapatite crystals can be controlled by varying PAA concentration in solution. The transmission electron microscopy images obtained from samples synthesized at increasing PAA concentration display not only a significant reduction of the crystal dimensions, but also variations in their

morphology. The characteristic HA prismatic faces are evident only in the images from samples prepared in the absence of the polyelectrolyte, and the sharpness of the crystal borders decreases as PAA content increases. At low PAA concentrations, crystal morphology becomes more acicular, while at concentrations higher than 5 mM most crystals are aggregated in clusters and they are so small that it is hard to describe their shape. Furthermore, the presence of the polyelectrolyte induces a reduction of the $D_{002}$ and $D_{310}$ coherent lengths, which could be ascribed to its incorporation inside the crystals. The reduction is greater along the direction orthogonal to the *c*-axis, as evident from the increase of the $D_{002}/D_{310}$ ratio, and in agreement with the morphology change seen in the TEM images. The coherent lengths decrease on increasing PAA and assume almost constant values for the HAPAA samples synthesized in the presence of polyelectrolyte concentrations greater than 5 mM. The PAA content of the solid phases, as determined by thermogravimetric analysis, increases up to a limit value of about 15 wt% from HA to HAPAA5, after which it does not change appreciably, suggesting that the values of the coherent lengths are related to the PAA content of the solid phase. The data do not allow us to distinguish between the amount of polymer inside the crystals and that just adsorbed on their surface, however the greater reduction observed on the direction orthogonal to the *c*-axis suggests a preferential adsorption of the polyelectrolyte on the crystal faces parallel to the *c*-axis. A preferential adsorption on the hydroxyapatite (100) faces was previously reported for a series of monosaccharides,[25] which were seen to change the morphology from anisotropic plates into elongated hexagonal prismatic crystals. Furthermore, it was found that the growth of carbonate apatite *in vitro* is affected by phosphophoryn, a protein which induces the formation of plates more elongated in *c*, in agreement with a preferential adsorption on (100) planes.[26] On the basis of HA structure and taking into account also the theories previously advanced to explain the release of calcium and phosphate ions during adsorption of PAA on apatite,[27] it could be suggested that polyacrylic acid interacts with the apatitic structure with the carboxylate groups substituting for phosphate groups, while the total calcium content is reduced in order to maintain electrical neutrality. Obviously this hypothesis needs further structural investigation to be substantiated. However, the interaction of PAA with the phosphate sites, which are more exposed on the crystal faces parallel to the *c*-axis direction, would explain the anisotropic reduction of the coherent length induced by the polyelectrolyte. Moreover, previous results obtained on HA and HAPAA synthesized in the presence of fluoride ions indicated that the polyelectrolyte content in the solid phase and the relative amount of fluoride substituted to hydroxyl ions are independent of each other, thus allowing us to exclude significant PAA interaction with the hydroxyl sites.[21] However, the presence of PAA does not affect significantly the values of the lattice constants, which exhibit just a slight increase in their standard deviations on increasing PAA content, in agreement with the simultaneous reduction of the degree of crystallinity. The degree of crystallinity of the apatitic phases increases on heat treatment: all the samples exhibit quite sharp IR absorption bands and well resolved X-ray diffraction reflections after treatment at temperatures higher then 700 °C, which leads to the decomposition of the polyelectrolyte. The presence of PAA does not alter the thermal stability of the apatitic phases, which display only a very modest conversion into β-tricalcium phosphate on heat treatment at 1100 °C, similar to that characteristic of bone apatite,[28] whatever their PAA content. Owing to the simultaneous decrease of Ca and P content, the value of Ca/P does not change appreciably on increasing PAA concentration and shows a significant reduction from the stoichiometric value of 1.67 only for the samples at high polyelectrolyte contents. However, the reduced Ca/P molar ratio does not affect the thermal stability of the apatitic phase, suggesting a stabilizing role of PAA on apatite structure. It can be concluded that the synthesis of hydroxyapatite in the presence of polyacrylic acid allows formation of HA/PAA crystals which could be of interest as biomaterials, due both to the presence of the polymer and to their dimensions, crystallinity and stability being very close to those characteristic of bone apatite.[28,29] Control of the morphology, crystallinity, as well as dimensions and coherent length, of the apatitic crystals is achieved through variations in the polyelectrolyte concentration.

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