

Electronic Supplementary Information (ESI)

Growth mechanism of apatite nanocrystals assisted by citrate: relevance to bone biomineralization

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Crystallization method

Calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, BioXtra, $\geq 99,0\%$ pure), sodium citrate tribasic dihydrate ($\text{Na}_3(\text{Cit}) \cdot 2\text{H}_2\text{O}$ where Cit = citrate = $\text{C}_6\text{H}_5\text{O}_7$, ACS reagent, $\geq 99,0\%$ pure), sodium phosphate dibasic (Na_2HPO_4 , ACS reagent, $\geq 99,0\%$ pure) and sodium carbonate monohydrate ($\text{Na}_2\text{CO}_3 \cdot \text{H}_2\text{O}$, ACS reagent, 99.5% pure) were supplied by Sigma-Aldrich. All the solutions were prepared with ultrapure water ($0.22 \mu\text{S}$, 25°C , MilliQ, Millipore). The nanocrystals were obtained by a batch heating method described elsewhere.¹ Briefly, two solutions (1:1 v/v, 200 mL total) of (i) 0.1 M CaCl_2 + 0.4 M $\text{Na}_3(\text{Cit})$ and (ii) 0.12 M Na_2HPO_4 were mixed at 4°C . The mixture (pH=8.5 adjusted with HCl) was then introduced in a 250 mL round-bottom flask, sealed with a glass stopper and immersed in a water bath at 80°C . The precipitates were then matured in the mother solution for 30 s, 5 min, 1h, 4 h and 96 h at 80°C . After the precipitation, the particles were repeatedly washed with ultrapure water (MilliQ, Millipore) by centrifugation, freeze-dried (LyoQuest, Telstar, Spain) and stored at 4°C for further characterizations. The same syntheses were also performed but in the absence of citrate (blank experiments).

Characterization techniques

A hot-line pH probe (Sentron, Netherlands) immersed in the flask provided *in situ* measurements of the pH continuously during the precipitation process at 80 °C. Transmission electron microscopy (TEM) and high resolution TEM (HR-TEM) observations were performed with a STEM Philips CM 20 microscope operated at 80 kV (used also for selected area electron diffraction, SAED, analysis) and with a Jeol 3010 UHR microscope, operated at 300 kV, respectively. As apatite samples might evolve under the electron beam, potentially leading to further crystallization and/or loss of bulk water,²⁻⁴ observations were carried out under low illumination conditions (significantly lower than that indicated in the references) to avoid any modifications to the materials during the analysis. X-ray powder diffraction (XRPD) patterns were collected using a Cu K α radiation ($\lambda = 1.5418 \text{ \AA}$) on a PANalytical X'Pert PRO diffractometer equipped with a PIXcel detector operating at 45 kV and 40 mA. For the diffracted beam an automatic-variable anti-scatter slit with an irradiated length of 10 mm was used. The 2θ range was from $5^\circ 2\theta$ to $80^\circ 2\theta$ with a step size of $0.039^\circ 2\theta$. Atomic force microscopy (AFM) images were collected with a Nanoscope IIIA microscope (Digital Instruments, Veeco, Santa Barbara, USA) in tapping mode by scanning the surface in air with silicon probes (NCHV, spring constant of 42 N m^{-1} and resonant frequency of 320 kHz, Veeco Probes, Santa Barbara, USA).

Sample preparation for TEM, HR-TEM and AFM observations.

The freeze-dried powdered samples were dispersed in ultrapure water. A drop of this slurry was deposited either on Cu grids (HR-TEM and TEM) or on the atomically flat surface of a freshly cleaved mica substrate (AFM). They were subsequently dried by evaporation overnight at room temperature in a vacuum desiccator before the corresponding analysis.

Solution Chemistry Calculation

The supersaturations of the solutions were calculated by Visual MINTEQ (Version 3.0).⁵ The solubility product, K_{sp} , of the solid phases (HA: hydroxyapatite, OCP: Octacalcium phosphate, DCPD: Dicalcium phosphate, and ACP: Amorphous calcium phosphate) used in the calculation were extracted from the MINTEQ (type6) database (NIST 46.7).

Supplementary Figures

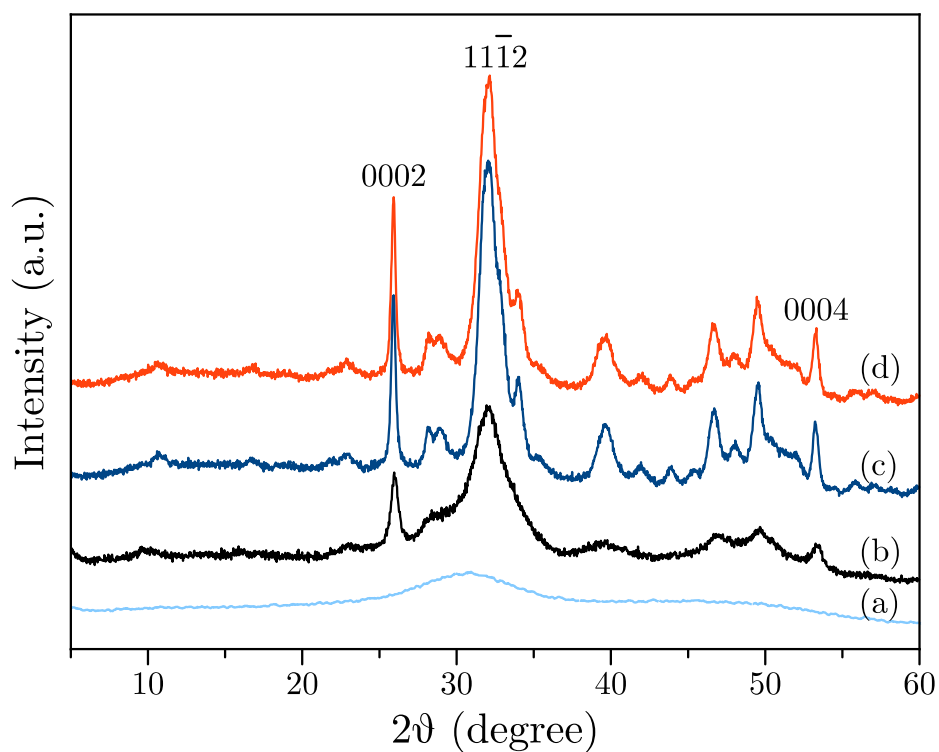


Figure S1. XRPD patterns of the calcium phosphates synthesized in presence of citrate at different maturation times: a) 30 s, b) 5 min, c) 4 h and d) 96 h.

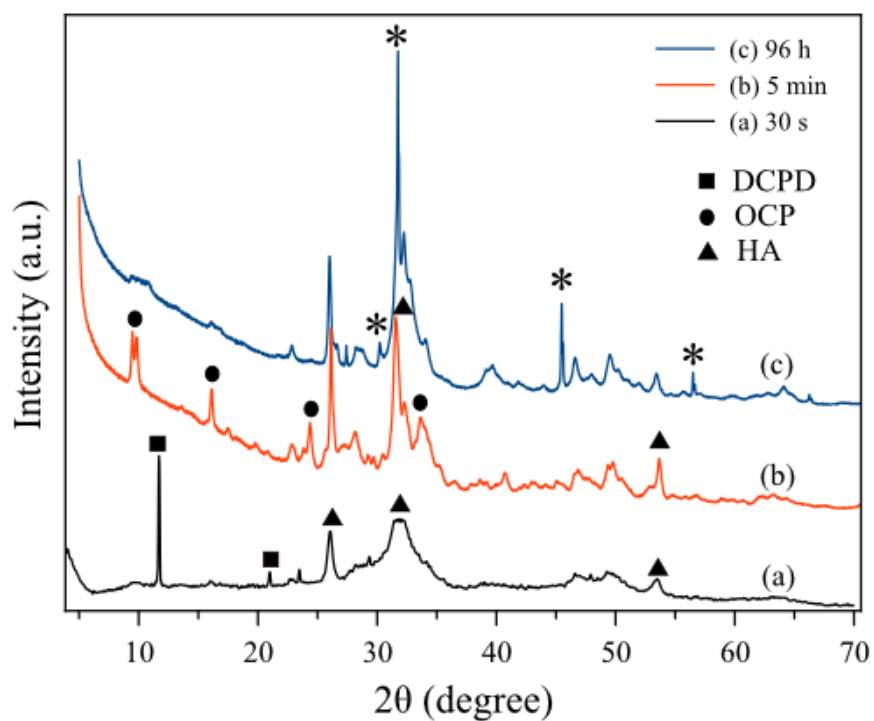


Figure S2. XRPD patterns of the calcium phosphates synthesized in the absence of citrate at different maturation times: (a) 30 s, (b) 5 min and (c) 96 hours. The asterisks indicate the presence of impurities of sodium chloride crystals.

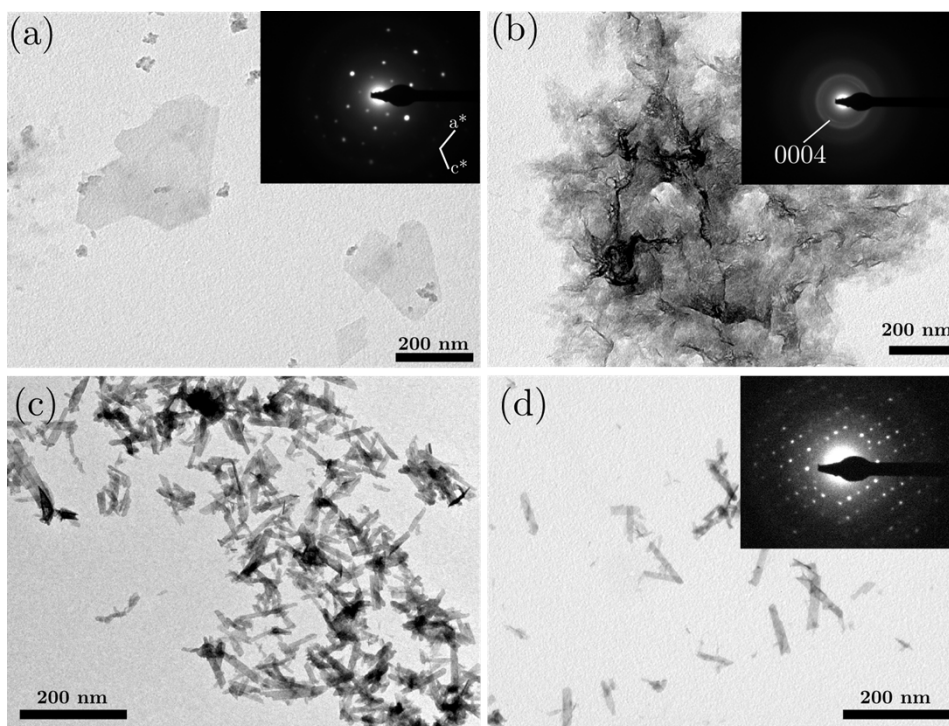


Figure S3. TEM micrographs of the calcium phosphates synthesized in the absence of citrate at different maturation times: (a,b) 30 s, (c) 5 min and (d) 96 hours.

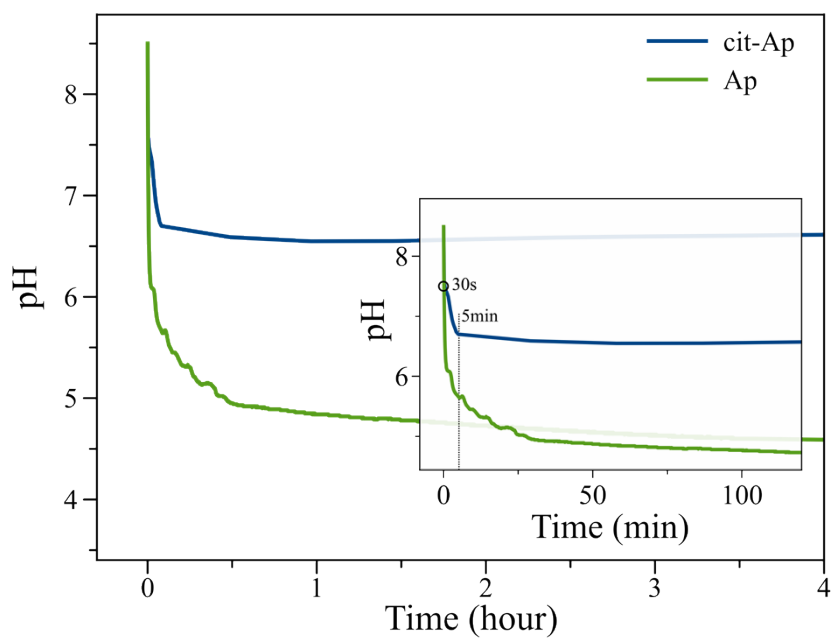


Figure S4. pH evolution during the crystallization experiments in the presence (blue curve) and in the absence (green curve) of citrate. The inset represents a enlarge view of the same curves.

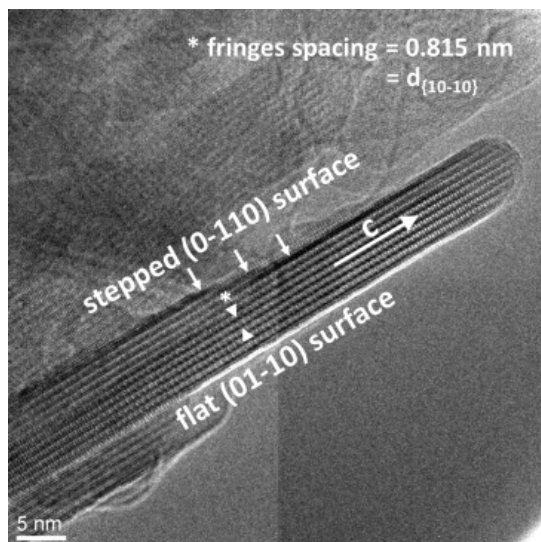


Figure S5. HR-TEM image of a cit-Ap-96h nanoparticles with the indexing of the lattice fringes. Original magnification: 400kx.

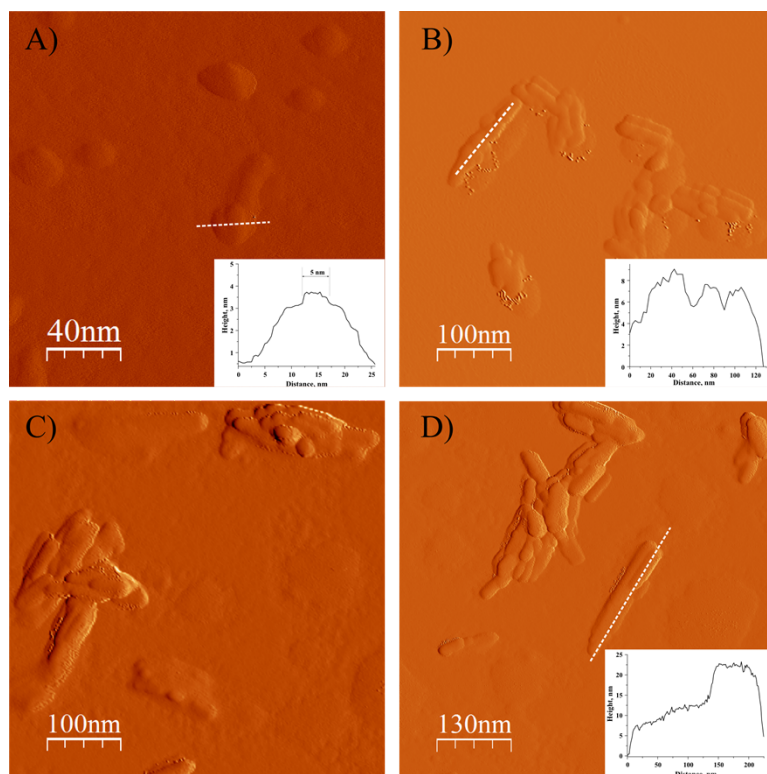


Figure S6. AFM images of calcium phosphate nanoparticles synthesized in presence of citrate at different maturation times: (a) 30 s, (b) 5 min, (c) 4 hours and (d) 96 hours. The insets show the vertical sections along the dotted lines of each figure.

Table S1. Ca concentration and Saturation Index (SI) calculated by Visual MINTEQ 3.0 [5]. [Ca]₀: initial Ca concentration; [Ca]_{eq}: Ca concentration in equilibrium with the solid phases; SI=log (IAP)-log K_{sp}, being IAP the ionic activity product and K_{sp} the solubility product. MINTEQ database (NIST 4.6.7) was used for these calculations [5].

	Citrate concentration	
	0 mM	200 mM
[Ca] ₀ /mM	50	50
[Ca] _{eq} / mM	14.4	7.2
SI (ACP)	8.4	6.2
SI (HA)	22.1	18.5
SI (OCP)	14.1	11.1
SI (DCPD)	1.4	0.6

Table S2. Structural water and citrate content of apatite nanoparticles synthesized in presence of citrate at different maturation times calculated by thermo-gravimetric analysis (TGA). Data extracted from reference [1]

Maturation Time	Structural water (%wt.)	Citrate (%wt.)
4 hours	3.9 ± 0.1	2.3 ± 0.1
24 hours	3.3 ± 0.1	1.9 ± 0.1
96 hours	2.6 ± 0.2	2.0 ± 0.1

References

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- [5] Gustafsson, J.P. Visual MINTEQ, Version 3.0. 2010. Stockholm. Available from: <http://www.lwr.kth.se/English/OurSoftware/vminteq>