



A non-classical view on the early stages of calcium phosphate formation and the role of citrate

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Improving our knowledge of calcium phosphate biomineralization is not only important to gain an understanding of how bones are formed *in vivo* but also because it may help in the design of new advanced functional materials. Very recently, citrate has been proposed to play a role at facilitating the infiltration of calcium phosphate into collagen fibrils during bone mineralization (Delgado-Lopez et al. 2017). However, the exact mechanism for this effect remains unresolved. Here we have analysed the formation of calcium phosphate in the presence of citrate by titration experiments in which pH, free calcium concentration and particle size distribution are continuously monitored. Additionally, reaction solution aliquots were drawn from solution at specific times and zeta potential measurements were performed. Samples were quenched in ethanol and analysed by means of TEM. Using these methods, we provide evidence that citrate contributes at stabilizing a dense calcium phosphate liquid phase that occurs at a critical concentration of bound calcium. We propose that this effect could facilitate the infiltration of calcium phosphate into the interstices of the collagen fibrils during the bone formation, a role that so far has been mainly attributed to macromolecules, i.e. non-collagenous proteins.

References

Delgado-López JM, Bertolotti F, Lyngsø J, Pedersen JS, Cervellino A, Masciocchi N, Guagliardi A. (2017) The synergic role of collagen and citrate in stabilizing amorphous calcium phosphate precursors with platy morphology. *Acta Biomater.* 49:555-562.