

# Structural investigation of biogenic and synthetic calcium phosphate nanoparticles

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Calcium phosphate (CaP), usually in the form of hydroxyapatite (HAP), is the inorganic material of mammalian bone and teeth (dentin, enamel), where CaP occurs as platelet-like nanocrystals and/or arranged micrometer-sized rods, respectively. Synthetically produced calcium phosphate nanoparticles (CaP-NPs) with a high biocompatibility and good biodegradability possess a great potential in bone tissue engineering and are also used as efficient carriers for different kinds of biomolecules. As they are nanoparticulate, not much is known about their internal structure regarding crystallography.

This work focuses on the wet-chemical synthesis and characterization of CaP-NPs with an amorphous and/or HAP-like nanostructure functionalized with carboxymethyl cellulose (CMC), polyethyleneimine (PEI) or DNA, as an example for biomedical application. The internal structure of synthesized CaP-NPs was compared with biological apatite samples (human teeth, shark teeth) and reference materials, e.g. synthetic nano- (25 nm) and microcrystalline (500 nm) HAP powder and geological HAP with respect to crystallinity, crystal phase and chemical composition.

CaP-NPs were characterized by dynamic light scattering (DLS), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), atomic absorption spectroscopy (AAS), Fourier-transform infrared spectroscopy (FTIR), and thermogravimetric analysis (TGA). The crystal structure was analysed by X-ray powder diffraction (XRD) and atomic pair distribution function (PDF), as obtained with a laboratory-based instrument Bruker D8 Advance (Cu K $\alpha$ ) and the PDF beamline ID31 at the European Synchrotron Radiation Facility (ESRF, 75.00 keV) with the support of Momentum Transfer, respectively.

SEM showed spherical CaP-NPs with a solid core diameter of about 100 nm, whereas the hydrodynamic diameter of the dispersed CaP-NPs ranged from 100 to 200 nm (DLS). All NPs had a stoichiometry close to HAP (stoichiometric molar Ca:P ratio 1.67:1) as determined by EDX and AAS. They contained about 10-20 wt% organic material depending on functionalization as derived from TGA. Although the diffraction peaks were extremely broad and sometimes even absent due to the nanoscopic nature of the particles, a quantitative analysis by Rietveld refinement (TOPAS 7.0) indicated that the CaP-NPs had an apatite-like structure and consisted of much smaller domains of about 5 nm in size, which agreed well with the FTIR results. Based on PDF analysis and following refinement by TOPAS, the apatite-like nanostructure of CaP-NPs was confirmed and compared with the references (HAP powder and geological apatite) and biogenic tooth enamel from humans (consisting of HAP) and sharks (consisting of fluoroapatite, FAP).

The study showed that despite the nanoscopic/amorphous state of CaP-NPs, their smallest apatite domains with a dimension of about 5 nm can be successfully studied by a combination of microscopic, spectroscopic and diffraction methods.