Ex vivo characterization of calcium pyrophosphate-based osteoarticular calcifications by X-ray diffraction and Raman spectroscopy

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Although several techniques have been used for the characterization of *ex vivo* calcium pyrophosphate (CPP: $Ca_2P_2O_7 \cdot nH_2O$) deposits associated to osteoarthritis,little is known from a chemical point of view on these phases which are difficult to synthesize, could evolve during their formation *in vivo* and be altered after extraction during conservation, preparation and analysis. Recently, Gras *et al.*established a protocol allowing a one-step and fast synthesis of four phases of CPP of biological interest by monitoring the pH and temperature during the synthesis.The stability and evolution properties of these synthetic phases have been investigated and *ex vivo* specimen analyses were conducted on cryoground meniscii and synovial fluid specimens of arthritic patients to avoid any alteration of the samples. Synchrotron X-ray diffraction and Raman spectroscopy were used to analyse synthetic samples, including monoclinic and triclinic calcium pyrophosphate dihydrate (m-CPPD and t-CPPD: $Ca_2P_2O_7 \cdot 2H_2O$), the two phases detected in joints of arthritic patients, and *ex vivo*biological samples. This studyhighlights the good selectivity of these techniques to detect both of the CPPD phases in complex media such as meniscus and synovial fluid, and to determine their ratio. In accordance with their thermodynamic stability, the data suggests the evolution of CPP *in vivo* from m-CPPD to t-CPPD. Although precursor phases could be involved their identification seems yet difficult. Progresses in the fine characterization of the different synthetic CPP phases could improve their detection in patients suffering from calcium-salt crystal diseases and could contribute to clarifying the mechanism by which CPP crystals form and evolve *in vivo*.