# Heating experiments on bone apatite to observe structural alterations

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**Introduction:**

Thermal reactions on bones have been extensively studied in the fields of mineralogy, biology and archaeology. Besides the oxidation of organic materials, structural changes are observed in the inorganic component as well. The inorganic component of bone materials is a kind of calcium-phosphate which hydroxyl-apatite (HAp) ($Ca_5[PO_4]_3OH$), related structure characterized by ionic substitutions (Mg, Na, F, $CO_3$) and very small crystallite sizes. Earlier experiments suggest that hydroxyl-apatite s.str. and bone-apatite must be distinguished, mainly due to the differences in structural ordering.

**Material and methods:**

In this work, using a pieces of bovine cortical bone samples were heated temperature values ranging from 25 to 1100˚C, and studied their structural alterations. After each step of the thermal treatment, the sample was measured by X-ray powder diffraction (XRD, Bruker D8 Advance, Cu-$K_\alpha$ source with 40 kV and 40 mA, Göbel mirror, Vantec1 detector) applied to reveal the mineralogical composition and TEM (Philips CM20, 200 kV, $LaB_6$ cathode, and energy dispersive spectrometry, EDS), FEI Titan - THEMIS 80-200keV, Cs corrected objective lens (point resolution ~0.09nm in image mode and 0.16 nm in STEM mode) to determine crystallite size and nanostructural alterations. The structural change of bone-apatite on heat treatment is described as recrystallization. Upon heating to 100-150 °C, bone is progressively dehydrated and collagen is considered to be fully degraded nearly 400 °C. Most X-ray studies concluded an absence of mineral crystal structure modifications before 400 °C, while a rapid crystal growth has been reported at 700 °C. Weight loss of the bovine femur between 100 and 800 °C represents the breakdown of organics (e.g., collagen) and their volatilization. The decompositions of hydroxil and structurally incorporated carbonate release as $CO_2$ and water in different steps in this range.

Above 600 °C we observed stoichiometric crystallization to 800 °C. The heated bovine bone sample therefore presents two main phenomenon: 1, the possibility to fine-tune the nanocrystal size upon heating and 2, the existence of a structure transition at high temperatures.

Using a set of bovine bone we show structural alteration, phase transformation and crystal growth. These results provide new insight into the detailed effects of heating on bone as a heterogeneous material.

**Acknowledgements:**

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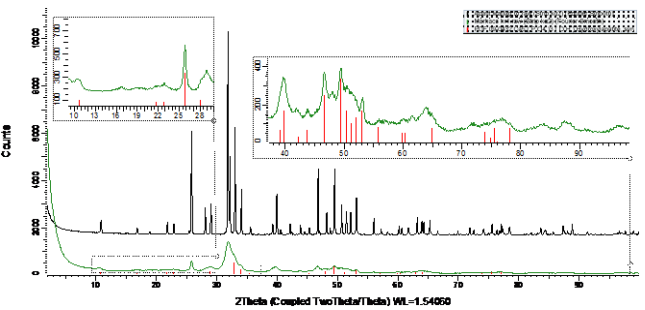


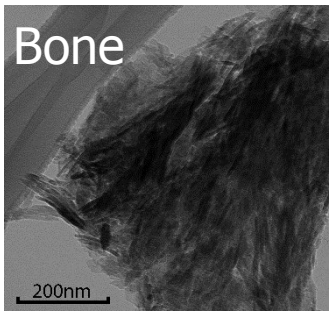


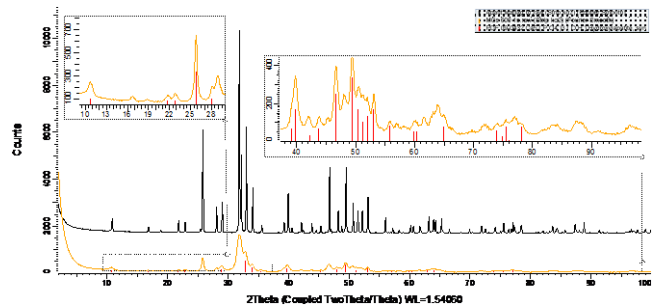


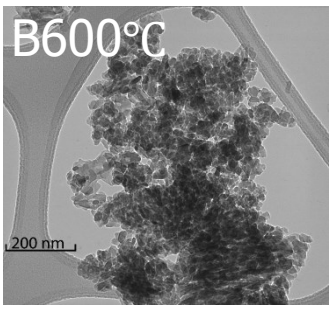


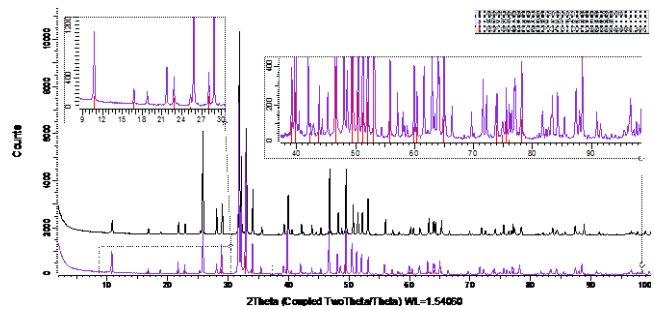


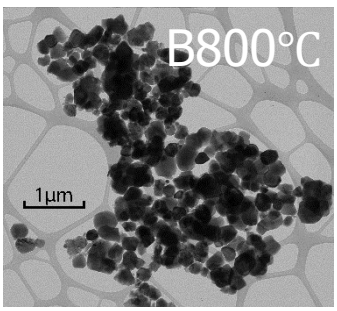


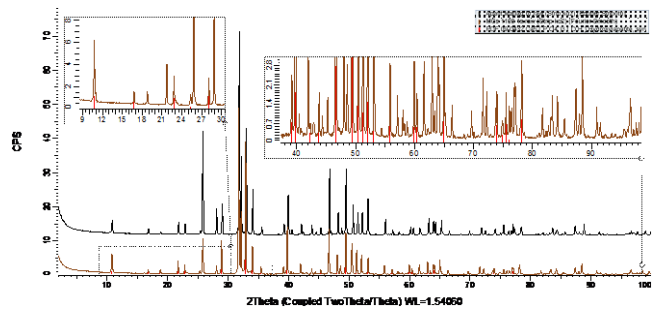


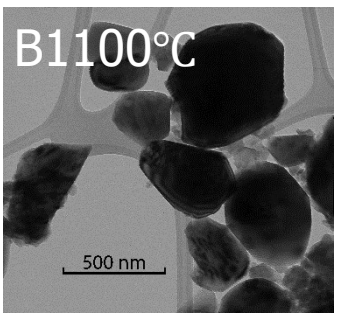


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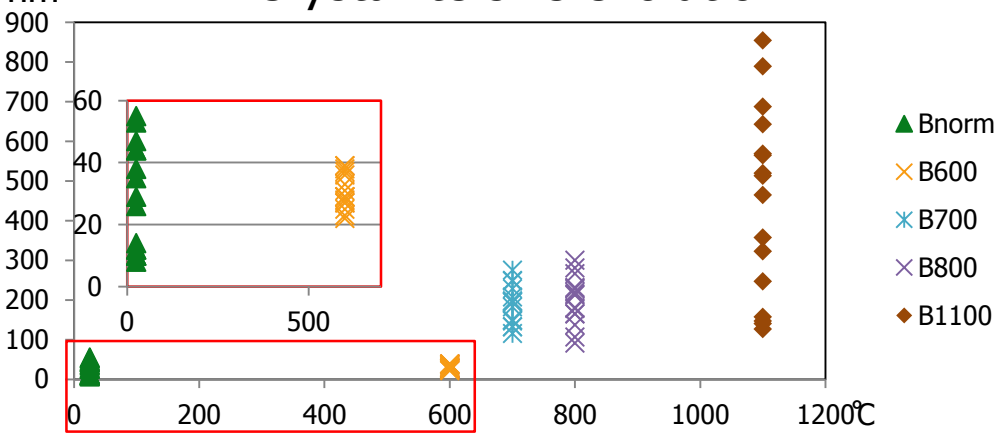
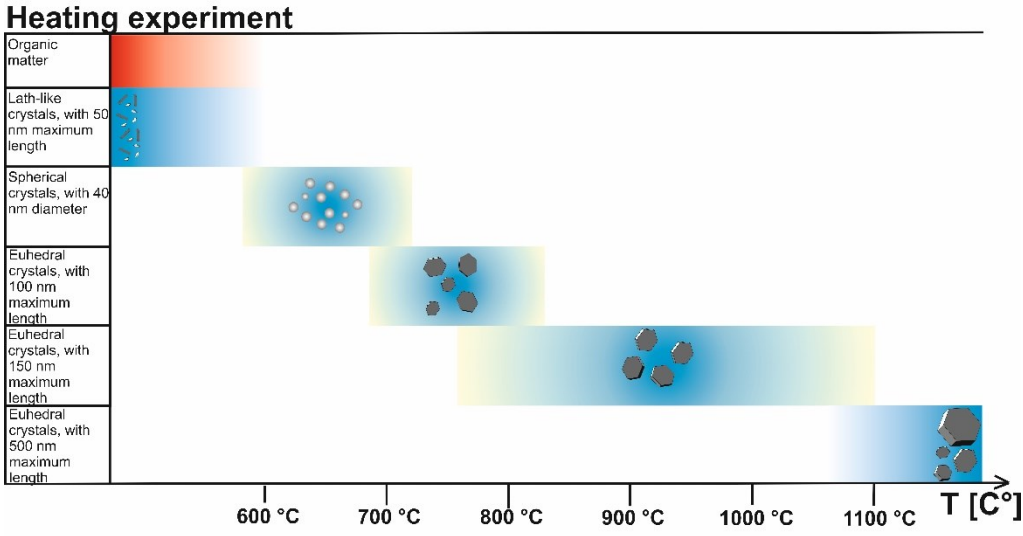
## Crystallite size evolution




## Heating experiment




Recrystallization begins over 400 °C, simultaneously with organic component breakdown. According to TEM observations, the crystallites are 3-7 nm wide and their lengths are between 10-50 nm. In the function of temperature elevation, we observed transformations from nanosized polycrystalline state to development of large single crystals. Crystallite size increased up to 700 nm at 1100 °C which is interpreted as recrystallization and sintering-like adherence of nanocrystals.

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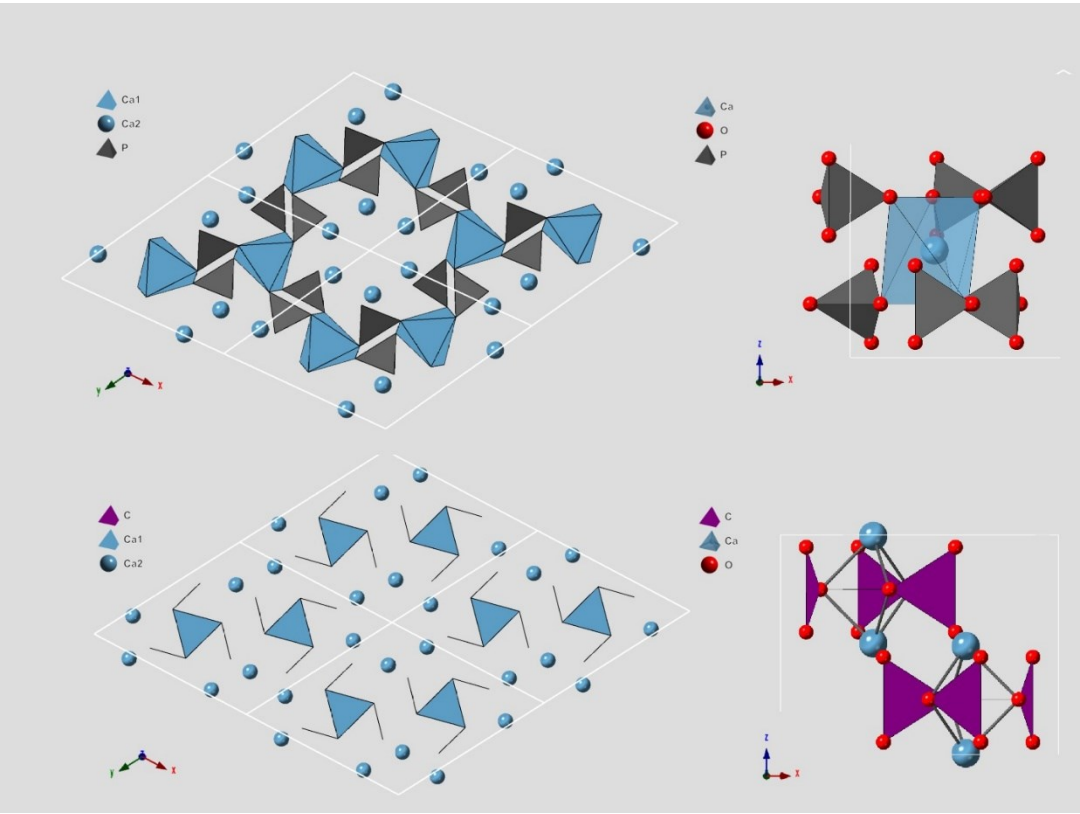


Two structural model of HAp-CAp end-members. CAp's model based on the finnemanite ($Pb_5(AsO_3)_3Cl$; Baikie et. al 2008) structure.

## Chemical composition:

Bone apatite is chemically more complex than end-member HAp, as well as being ionic replacement-rich and non-stoichiometric. The average Ca/P atomic ratio of the EDS analyses is 1.64±0.02, slightly lower than 1.67 of stoichiometric HAp.

| | Ca/P |
|---|---|
| Bone: | 1.59 |
| B600°C: | 1.64 |
| B700°C: | 1.64 |
| B800°C: | 1.66 |
| B1100°C: | 1.65 |

Baikie T., Ferraris C., Klosster W.T., Madhavi S., Pramana S. S., Pring A., Schmidt G., White T. J., (2008): Crystal chemistry of mimetite, $Pb_{10}(AsO_4)_6Cl_{1.48}O_{0.26}$, and finnemanite, $Pb_{10}(AsO_3)_6Cl_2$, *Acta Crystallographica B*, (64) 34-41.

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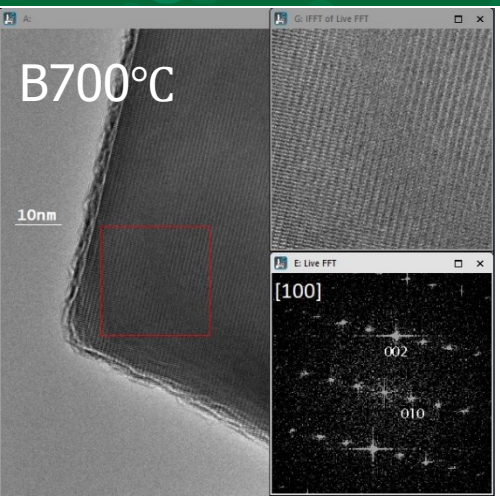


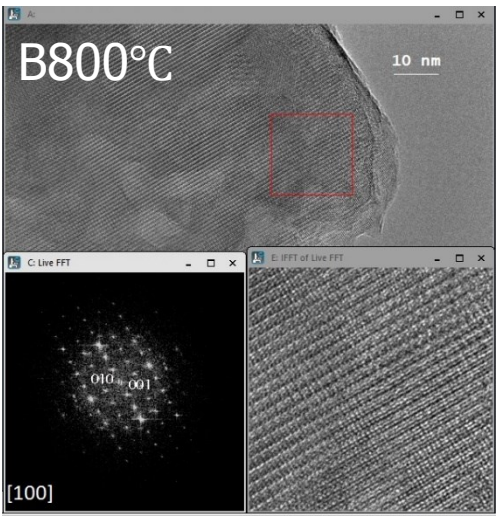


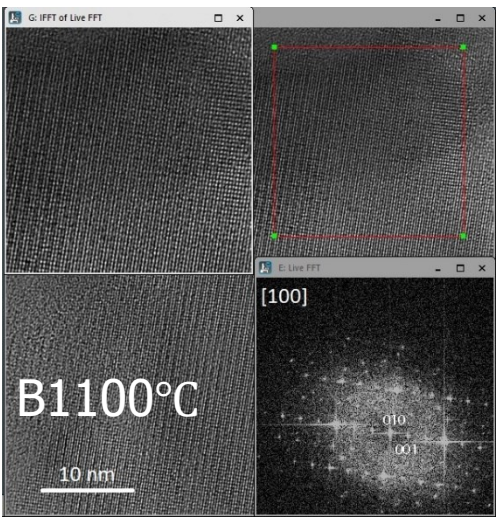


**Experimental:** FEI Titan - THEMIS 80-200keV, Cs corrected objective lens, FEG,
**Data processing:** Digital Micrograph (Gatan Inc.)

## Conclusion:

We observed alteration in *a:c* ratio. During heating this ratio became smaller:

**HAp**

$a$ = 9.422 Å; $c$ = 6.76 Å

**B1100°C**

$a$ = 9.422 Å; $c$ = 7.11 Å.

Although there are no changes in size and chemistry until 700 °C, the $P6_3/m$ space group symmetry decreased to $P6/m$ above 700 °C.

(the 00l = 2n+1 type reflections are forbidden in the case of $P6_3/m$ space group)

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