

Modification of hydroxyapatite during transmission electron microscopy

HUAXIA JI, P. M. MARQUIS

School of Metallurgy and Materials, University of Birmingham PO Box 363, Birmingham B15 2TT, UK

Hydroxyapatite ($\text{Ca}_5(\text{PO}_4)_3(\text{OH})$) is a challengeable biomaterial for clinical application and is found to be incorporated in bone tissue. Usually, either sintered hydroxyapatite compact implant or hydroxyapatite-coated implant is used as prostheses in surgery [1, 2]. The most interesting characteristics of hydroxyapatite crystallizing in the hexagonal system have been discussed [3]. This letter tries to show the structure of hydroxyapatite compacts examined using transmission electron microscopy (TEM), and the characterization of hydroxyapatite by X-ray diffraction. It is shown that the electron beam has an important effect on the formation of the artificial structure of hydroxyapatite on the TEM analysis.

Hydroxyapatite was prepared by the reaction of $\text{Ca}(\text{OH})_2$ and H_3PO_4 in aqueous solution. The hydroxyapatite powders were compacted into cylinders at approximately 4200 p.s.i. (29 MPa). The hydroxyapatite compacts were calcined at 800 °C for

3 h in vacuum. Calcined and non-calcined hydroxyapatite compacts were then sintered at 1200 °C for 12 min, 30 min and 12 h, respectively.

The hydroxyapatite powder and sintered compact were characterized by X-ray diffraction with $\text{Ca-K}\alpha$ ($\lambda = 0.15405 \text{ nm}$) radiation. The hydroxyapatite compact samples for TEM were prepared using both an ion-beam thinner and mechanical grinding to distinguish the effect of the techniques used to produce electron-transparent foils for TEM. The sample for TEM was prepared using an ion-beam thinner in the standard techniques. In the mechanical grinding, the hydroxyapatite compact sintered at 1200 °C for 12 min was ground into powder and then a TEM sample was prepared. The samples were coated with carbon film before being examined on a Philips EM400T and Jeol 4000FX at 100 and 400 kV.

Typical X-ray diffraction patterns of the hydroxyapatite materials are shown in Fig. 1. Compared with the standard diffraction patterns, the X-ray

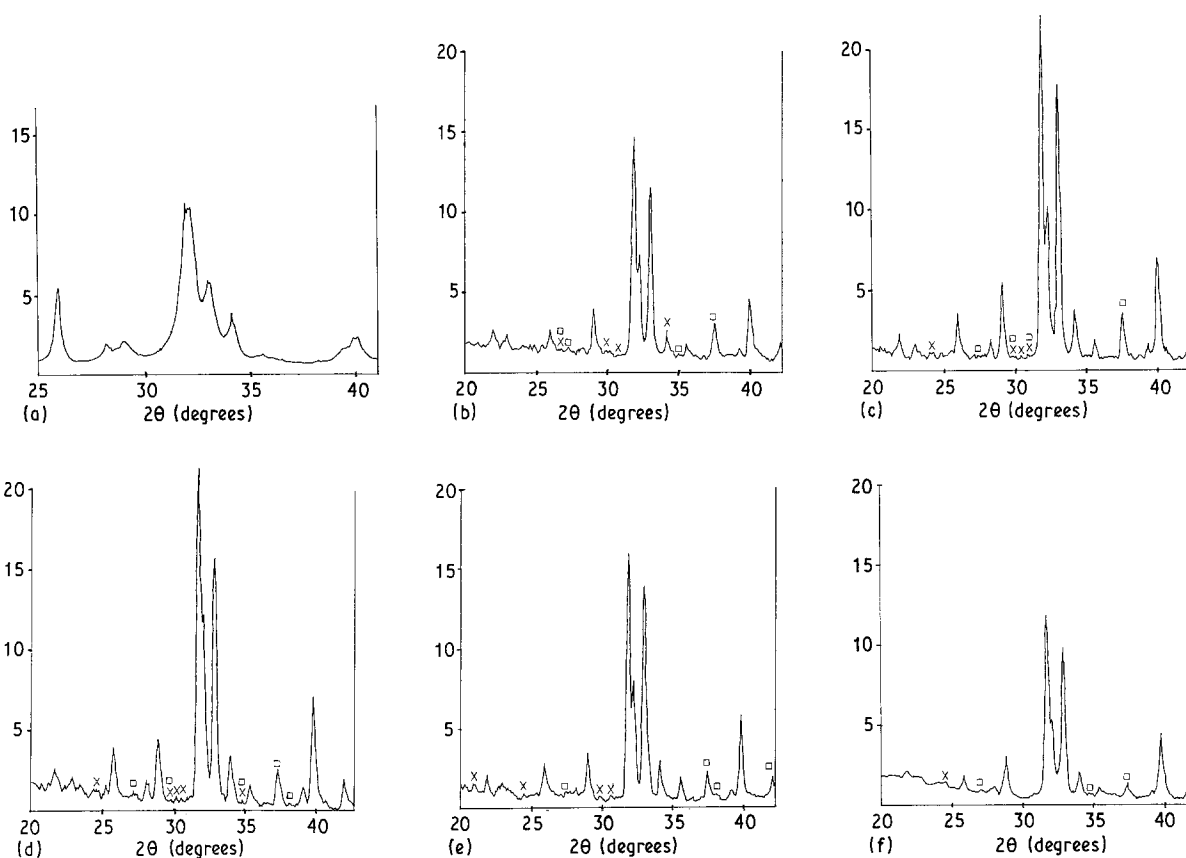


Figure 1 X-ray diffraction patterns of the hydroxyapatite: (x) $\alpha\text{-Ca}_3(\text{PO}_4)_2$ and (□) $\beta\text{-Ca}_3(\text{PO}_4)_2$. (a) Hydroxyapatite powder, (b) calcined hydroxyapatite sintered at 1200 °C for 12 min, (c) calcined hydroxyapatite sintered at 1200 °C for 30 min, (d) non-calcined hydroxyapatite sintered at 1200 °C for 30 min, (e) calcined hydroxyapatite sintered at 1200 °C for 12 h and (f) non-calcined hydroxyapatite sintered at 1200 °C for 12 h.

pattern of the powder is characteristic for hydroxyapatite. In contrast, the patterns for calcined and non-calcined hydroxyapatite compacts sintered at 1200 °C indicate that in addition to hydroxyapatite there are the extra peaks for α - and β -tricalcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$). Therefore, there would be a minor presence of α - and β -tricalcium phosphate in all of the sintered hydroxyapatite. Hydroxyapatite sintered at 1200 °C for 30 min appears highly crystalline. The calculated lattice constants of hydroxyapatite are given in Table I. Clearly, the lattice constants of hydroxyapatite sintered at 1200 °C are very close to the ASTM data (hexagonal structure with lattice parameters $a = 0.9442$ nm and $c = 0.6884$ nm).

Typical TEM micrographs of the hydroxyapatite compacts prepared using the ion-beam thinner are

TABLE I Calculated lattice parameters of hydroxyapatite sintered at 1200 °C from their X-ray diffraction patterns.

Sample	a (nm)	c (nm)
HAP* powder	0.677 25	0.937 04
HAP calcined and sintered for 12 min	0.686 48	0.938 06
HAP calcined and sintered for 30 min	0.685 49	0.9366
HAP sintered for 30 min	0.692 74	0.938 45
HAP calcined and sintered for 12 h	0.688 35	0.938 23
HAP sintered for 12 h	0.690 72	0.942 76

*HAP, hydroxyapatite.

shown in Fig. 2. It can be seen that hydroxyapatite is polycrystalline with calcium-rich phases as marked C in Fig. 2. The diffraction patterns taken from the hydroxyapatite compacts have been indexed in terms of the structure of $(\alpha\text{-Ca}_3(\text{PO}_4)_2)$ (orthorhombic structure with lattice parameters $a = 1.522$ nm, $b = 2.071$ nm and $c = 0.9109$ nm). It has been found from the indexing results that the measured values are in close agreement with the theoretical values for $\alpha\text{-Ca}_3(\text{PO}_4)_2$. Fig. 3 shows the typical diffraction patterns taken from the hydroxyapatite compact sintered at 1200 °C for 12 h. A TEM micrograph of hydroxyapatite prepared by grinding the compact sintered at 1200 °C for 12 min into powder is shown in Fig. 4. Basically, it exhibits the same feature as the sample prepared by ion-beam thinning. The diffraction patterns taken from this sample also show that hydroxyapatite has been converted to $\alpha\text{-Ca}_3(\text{PO}_4)_2$ under the electron beam.

Apparently, it is found in the TEM analysis that hydroxyapatite converts to $\alpha\text{-Ca}_3(\text{PO}_4)_2$. The effect of the ion-beam on the artificial structure of hydroxyapatite is not obvious because the sample prepared by mechanical grinding also shows the same transformation of hydroxyapatite into $\alpha\text{-Ca}_3(\text{PO}_4)_2$. This transformation is quite complicated in this case. Usually, it is known that hydroxyapatite can be decomposed into a mixture of $\text{Ca}_3(\text{PO}_4)_2$ plus tetracalcium phosphate ($\text{Ca}_4\text{P}_2\text{O}_9$) or calcium oxide

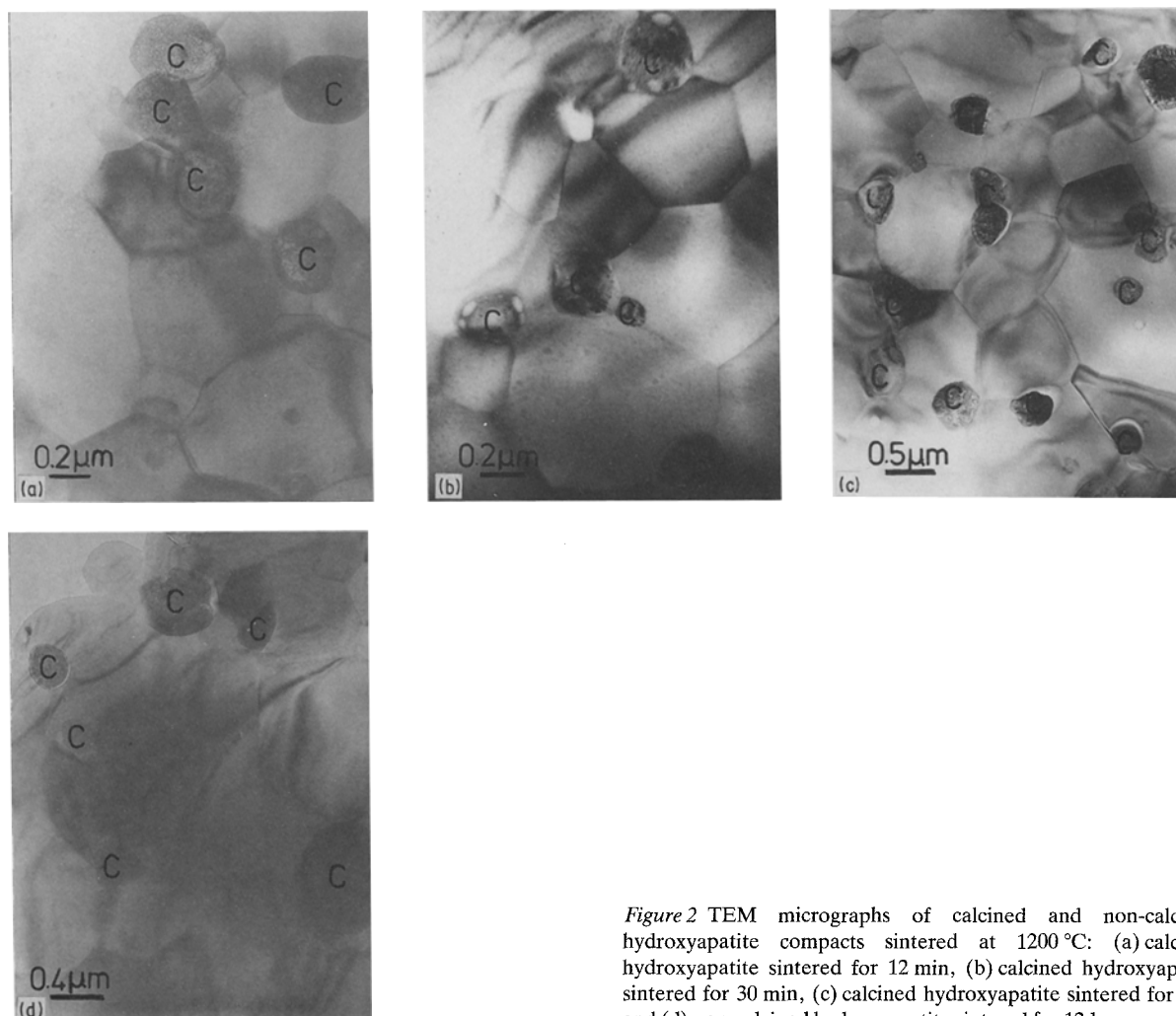


Figure 2 TEM micrographs of calcined and non-calcined hydroxyapatite compacts sintered at 1200 °C: (a) calcined hydroxyapatite sintered for 12 min, (b) calcined hydroxyapatite sintered for 30 min, (c) calcined hydroxyapatite sintered for 12 h and (d) non-calcined hydroxyapatite sintered for 12 h.

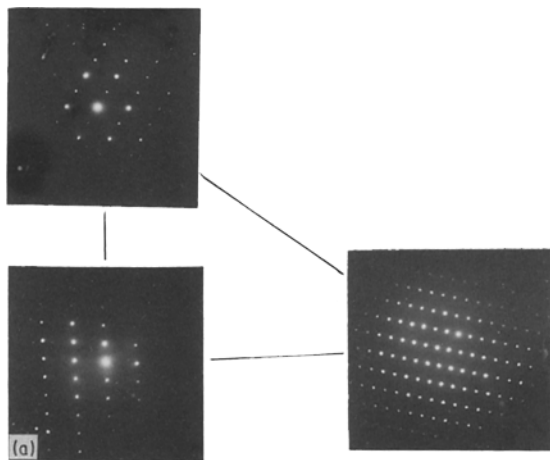
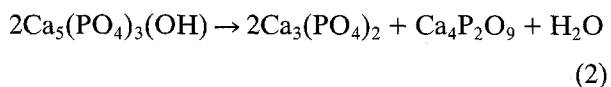
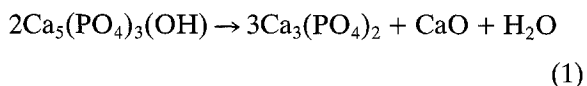


Figure 3 (a) Selected-area diffraction patterns taken from the hydroxyapatite compact sintered at 1200 °C for 12 h and (b) schematic diagram showing that the patterns are from $\alpha\text{-Ca}_3(\text{PO}_4)_2$.

(CaO) at high temperatures at any ambient pressure. The equations are



The $\text{Ca}_3(\text{PO}_4)_2$ exhibits an X-ray pattern distinctly different from that of hydroxyapatite. This $\text{Ca}_3(\text{PO}_4)_2$ exists in two crystalline forms [4]: a high-temperature form, $\alpha\text{-Ca}_3(\text{PO}_4)_2$, and a low-temperature form, $\beta\text{-Ca}_3(\text{PO}_4)_2$. This decomposition of hydroxyapatite may take place in the sample when it is subjected to the conditions of electron bombardment and high vacuum during observation

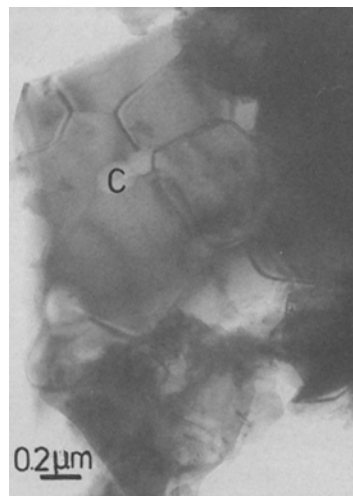


Figure 4 TEM micrograph of the hydroxyapatite sample prepared by grinding the compact into powder.

in a modern TEM. Otherwise, hydrogen ions are easily converted into hydrogen gas by electron bombardment in high vacuum because of weak bonding. The disappearance of the hydrogen due to electron bombardment may lead to such a transformation. It is significant that the calcium phosphate examined using the TEM is $\alpha\text{-Ca}_3(\text{PO}_4)_2$ rather than hydroxyapatite.

In conclusion, in these results the calcium phosphate material characterized by X-ray diffraction is hydroxyapatite, and may contain a small amount of $\alpha\text{-}$ and $\beta\text{-Ca}_3(\text{PO}_4)_2$. However, this hydroxyapatite will convert to $\alpha\text{-Ca}_3(\text{PO}_4)_2$ when it is examined in the TEM. The occurrence of this transformation would be due to the release of the hydrogen or decomposition of hydroxyapatite by the electron beam in high vacuum.

References

1. M. JARCHO, *Clin. Orthop.* **157** (1981) 259.
2. G. L. DE LANGE and K. DONATH, *Biomaterials* **10** (1989) 121.
3. M. I. KAY, R. A. YOUNG and A. S. POSNER, *Nature* **204** (1964) 1050.
4. A. L. MACKAY, *Catal. Cryst.* **6** (1953) 743.

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