

# Carbonate-doped hydroxyapatite

E. G. NORDSTRÖM, K. H. KARLSSON

Department of Chemical Engineering, Åbo Akademi, 20500 Åbo, Finland

A hydroxyapatite slurry was prepared by stirring into water a stoichiometric mixture of tricalcium phosphate and calcium hydroxide until the solid content was 50 wt%. Samples were then prepared by slip casting, drying and firing in vacuum at 1000 to 1300°C for 1 h. The fired samples were crushed and soaked in ordinary mineral water for up to 2 months and examined by differential thermal analysis and thermogravimetry, infrared spectroscopy, X-ray diffraction, scanning electron microscopy and energy-dispersive X-ray analysis, and direct-current plasma emission spectroscopy. One phosphate ion was found to be replaced by carbonate and practically all hydroxide by water, regardless of heat treatment. The infrared spectra showed a close resemblance to that obtained from natural bone.

## 1. Introduction

Calcium phosphates have been found to be both resorbable and non-resorbable in a biological environment. Tricalcium phosphate (TCP) is resorbable. Hydroxyapatite (HA) is reported to be fully non-resorbable when tested *in vivo* [1]. However, there are some studies on long-term tests with HA showing that also HA resorption starts gradually after 4 to 5 years [2].

The unit cell of HA and natural bone is the same, and this is the main reason why HA has been proposed as an implant material. On the other hand, natural bone contains carbonate, as can be shown by infrared (IR) spectroscopy [3], and some reports show that carbonated hydroxyapatites are biologically more reactive than pure HA [4-6]. The aim of this study was to simulate natural bone by incorporating carbonate in HA by soaking in a saturated solution of carbon dioxide.

## 2. Materials and methods

The carbonate ions were incorporated by soaking carbonate-free samples in a saturated solution of carbon dioxide. The intention was to study whether a pure HA ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ), soaked in mineral water, results in a mixed A- and B-type or only B-type carbonate-apatite (phosphate replacement). It has been shown that the pure A-type,  $\text{Ca}_{10}(\text{PO}_4)_6\text{CO}_3$ , does not occur in natural apatite [7]. There is always some  $\text{OH}^-$  or  $\text{F}^-$  present.

The HA was prepared as follows. The starting products were commercial TCP and  $\text{Ca}(\text{OH})_2$  of analytical grade. The powders were mixed in the molar ratio 3:1, to correspond to  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ . The mixed powder was stirred into distilled water until the solid content of the slurry was 50 wt%. The slurry was then slip cast into rods in plaster moulds. The rods were sintered in vacuum. As a reference material for the IR and X-ray diffraction (XRD) measurements, however, a standard sample was prepared by sintering a rod in

air at 950°C for 30 h. This gives a pure HA without TCP.

Four different rods sintered for 1 h at 1000, 1100, 1200 and 1300°C in vacuum were crushed and pulverised. The fine material was then soaked in ordinary mineral water for 1 to 2 months. After soaking, the apatite was dried and studied by differential thermal analysis and thermogravimetric analysis (DTA-TGA), IR Fourier-transformed spectroscopy (IR), XRD, scanning electron microscopy and microanalysis (SEM-EDXA) and direct-current plasma emission spectroscopy (DCP). The sample codes are given in Table I. By TGA it is possible to determine quantitatively the sum of  $\text{CO}_2$  and  $\text{H}_2\text{O}$  in a sample. From this sum an approximate value for the carbonate content can be determined, when TGA is used together with DTA, assuming that most of the water has disappeared around 600°C. Water is partly found as humidity, evaporating around 100°C, and partly as lattice water and hydroxyl ions. For DTA and TGA 50 mg samples were heated in a platinum crucible. The heating interval was 50 to 1400°C and the heating rate was  $10^\circ\text{C min}^{-1}$ . Carbonate contents reported in this paper were determined by TGA and IR. The IR range studied was 400 to  $4000\text{ cm}^{-1}$ . This interval contains

TABLE I Carbonate and hydroxide content after soaking for 2 months. Values are in wt%

Sample	Sintering temperature (°C)	$\text{CO}_3^{2-}$ (IR)	$\text{CO}_3^{2-}$ (TGA)	$\text{OH}^-$ (TGA)	$\text{H}_2\text{O}$ (TGA)
VU10	1000	3.9	3.9	0.6	0
VU11	1100	1.9	1.3	0.5	0
VU12	1200	0.8	0.9	0.4	0
VU13	1300	n.d.	n.d.	n.d.	n.d.
RB01		4.0	4.6	n.d.	n.d.
VS10	1000	5.5	5.5	0.4	0.6
VS11	1100	4.8	4.5	0.5	7.6
VS12	1200	4.8	5.1	0.6	8.3
VS13	1300	4.4	3.8	0.5	2.4

V, Vacuum sintered; U, unsoaked, S, soaked; RB, rabbit bone.

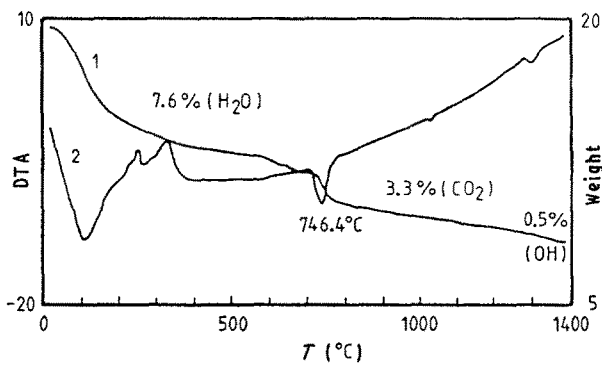


Figure 1 TGA and DTA curves for sample VS11.

the important information and is commonly used for studying apatites [3, 8, 9]. IR spectra were recorded on all samples as well as on natural rabbit bone. The possible presence of carbonate apatite was confirmed by XRD. The calcium-to-phosphorus (Ca/P) ratio was determined by using EDXA and DCP analysis.

### 3. Results and discussion

The investigation showed that it is possible to exchange ions in HA by soaking. A soaking time of 2 months gives a carbonate content of 4 to 5.5 wt % as determined by TGA. The presence of carbonate is shown by an endothermic reaction in DTA at 746°C. At 1100°C for VS11 a 3.3 wt % CO<sub>2</sub> loss was registered. When determined by IR the result was roughly the same (Table I). Soaking times of < 1 month gave very small carbonate contents. Natural bone contains 4 to 8 wt % carbonate [10]. In the soaked samples VS11 and VS12 the water content was about 8 wt %. Some two-thirds of this was humidity and one-third lattice water (Fig. 1).

The composition of the carbonated apatites is closer to natural bone than to HA. This is shown by IR. In the soaked sample VS11 the carbonate absorption bands occur at 1410 to 1460 cm<sup>-1</sup> and at 872 cm<sup>-1</sup>. In the unsoaked sample OH absorption occurs at 3630 cm<sup>-1</sup> (Fig. 2); this peak is missing from the spectrum of the soaked sample (Fig. 3). This sample of soaked synthetic carbonated apatite, VS11, was compared with a sample of rabbit bone and a high similarity could be seen. Even the absorption peak at

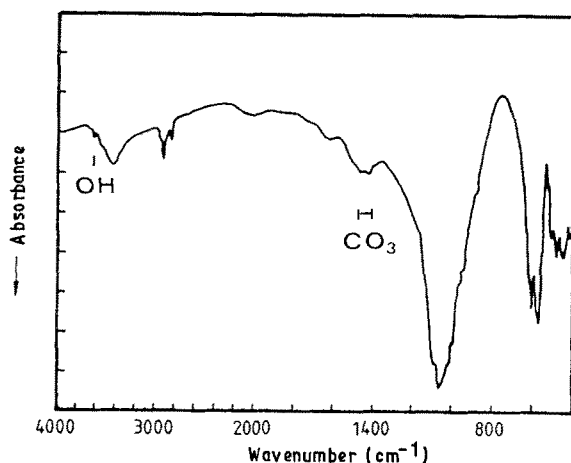


Figure 2 IR spectrum of vacuum-sintered HA at 1100°C and unsoaked.

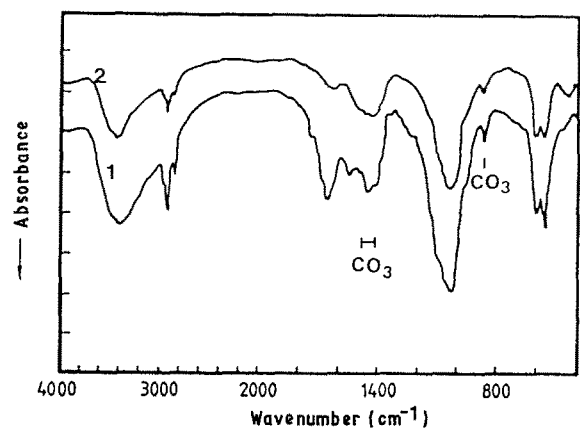


Figure 3 IR spectra of (curve 1) rabbit bone and of (curve 2) apatite sintered at 1100°C in vacuum and soaked in mineral water (VS11).

2930 cm<sup>-1</sup> referred to in earlier texts as the decomposition of organic components [3] was found in both samples. Actually the only difference is the lower intensity of the H<sub>2</sub>O band at 1640 to 1650 cm<sup>-1</sup> in the synthetic products. Although treated in exactly the same way, VS10 contained even less water than VS11 and VS12. The reason for this is not known. Finally, VS13 had already partially decomposed and was no longer a pure apatite.

In Fig. 4 the IR spectra of the unsoaked samples VU12 and VU13 are shown. For VU11 (Fig. 2) and VU12 a minute carbonate peak can be seen, which is missing for VU13. When the samples were treated in mineral water the absorption peaks for carbonate appeared in each sample.

From the determined carbonate (C) and phosphate (P) contents, the molar ratio  $P/(P + C)$  can be calculated (Table II). On average the value is 0.85 in the soaked samples, with very little deviation. Stoichiometrically this corresponds to one carbonate ion occupying one phosphate site out of six. This can be taken as an indication of a B-type apatite, although a mixed A- and B-type is also possible. An A-type carbonate apatite should contain 35.3 mol % phosphate when all OH-sites are replaced with carbonate. In a B-type, Ca<sub>10</sub>[(PO<sub>4</sub>)<sub>6-x</sub>(CO<sub>3</sub>)<sub>x</sub>](OH)<sub>2</sub> carbonate occupies phosphate sites and the apatite contains 27.8 mol % phosphate when  $x = 1$ . However, the  $P/(P + C)$  ratio gives a better description of the type,

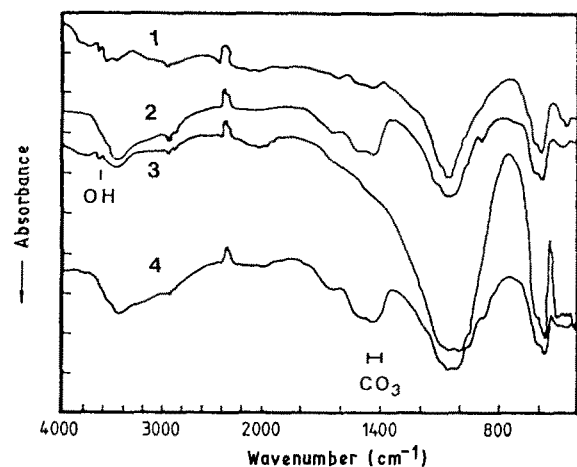


Figure 4 IR spectra of (1) VU12, (2) VS12, (3) VU13 and (4) VS13.

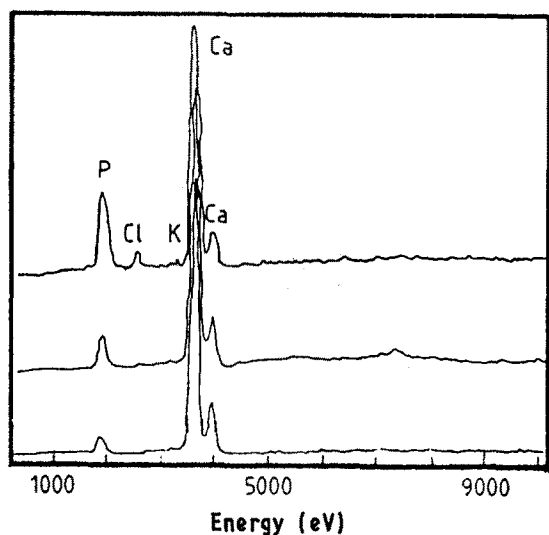


Figure 5 Three different EDXA spot analyses from sample VS11.

because other ions may also be incorporated during soaking and change the phosphate content/phosphate site ratio. When  $x = 1$  the  $P/(P + C)$  ratio is 0.83. As can be seen from Table II,  $x$  is close to unity in the unsoaked samples and between 0.84 and unity (corresponding to the ratios 1 to 0.83) in the soaked samples. Thus, by soaking only one phosphate can be replaced.

XRD showed that the HA structure is preserved in the soaked samples. The only sample that did not have a clear apatite structure was the one sintered at 1300°C (VS13). This showed a number of unidentified peaks, some of which might be due to calcium carbonate. Although other identifications are possible, it can be concluded that the structure of HA is unstable when sintered in vacuum at temperatures as high as 1300°C. Up to 1200°C the crystalline phase is mainly HA and the full width at half-maximum (FWHM) of the diffraction peaks increased with increasing carbonate content, which is in accordance with earlier reports [11].

EDXA showed for the carbonate HA Ca/P ratios which varied between 1.44 and 1.98. The Ca/P ratio of HA is 1.67. However, one phosphate replaced by carbonate gives Ca/P = 2, if no calcium deficiency occurs. Values below 2.0 can be explained by some  $\text{Ca}^{2+}$  being replaced by other cations from mineral water ( $\text{K}^+$ ,  $\text{Na}^+$  and  $\text{Mg}^{2+}$ ). In EDXA there is no sign of sulphur in the carbonated HA (Fig. 5), although sulphate from mineral water could be expected to occupy phosphate sites in the apatite structure.

TABLE II Analytically determined phosphate (P) and carbonate (C) contents

Sample	P (mol %)	C (mol %)	P + C (mol %)	$\frac{P}{P + C}$	$\frac{\text{Ca}}{\text{P}}$
VU10	34.8	3.6	38.4	0.91	
VU11	32.9	1.2	34.1	0.96	
VU12	24.4	0.8	25.2	0.97	
VU13	29.7	0.0	29.7	1.00	
RB01	28.5	4.3	32.8	0.87	
VS10	31.0	5.1	36.1	0.86	1.44
VS11	23.8	4.2	28.0	0.85	1.91
VS12	21.0	4.7	25.7	0.82	1.98
VS13	21.9	3.5	25.4	0.86	1.70

Instead, chloride can be found by EDXA. This is not unexpected, because chloride is the main ion at OH sites in chlorapatite. The DCP analysis showed that potassium, magnesium and sodium were present in amounts corresponding to 1  $\text{K}^+$ , 0.5  $\text{Mg}^{2+}$  and 0.1  $\text{Na}^+$  ion per formula unit.

As seen in the IR spectrum in Fig 3, no OH-bands are found in the carbonate apatite VS11, or in the rabbit bone RB01. On the other hand, the soaked samples have very broad  $\text{H}_2\text{O}$  absorption bands at 3320 to 3600  $\text{cm}^{-1}$ . This is also true for the IR spectrum of natural bone. This indicates that OH-sites are occupied by  $\text{H}_2\text{O}$ . As they were shown to be in a synthetic carbonated apatite called CDCA [3]. In this a deficiency in calcium occurs, which is compensated by some  $\text{HPO}_4^{2-}$  groups. Also in the present investigation, a calcium deficiency was found, but this may have been caused by ion exchange with sodium, magnesium, potassium and possibly even hydronium. Mineral water contains all of these ingredients and the metals were also found in the present samples as well as in natural bone.

#### 4. Conclusions

By soaking synthetic HA in carbonated water, one phosphate ion is replaced by carbonate and OH is replaced by water. This was found to be true also for natural bone. Long-term exposure of HA to body fluid may therefore be expected to result in a structure that is very similar to natural bone. The extra reaction step involved could account for the reported slower biological reactivity of HA than carbonate apatite [4–6]. However, this was unambiguously seen in an *in vivo* study where carbonated hydroxyapatite was compared with pure HA. Results from this study will be reported in the near future.

#### References

- C. P. A. T. KLEIN, A. A. DRIESSEN, K. DE GROOT and A. VAN DEN HOOFF, *J. Biomed. Mater. Res.* **17** (1983) 769.
- K. DE GROOT, R. G. T. GEESINK, J. G. C. WOLKE and C. P. A. T. KLEIN, in Transactions of the 3rd World Biomaterials Congress, Kyoto, Japan, April 1988, Vol. XI, (Free University, Amsterdam) p. 306.
- R. M. BILTZ and E. D. PELLEGRINO, *J. Dent. Res.* **62** (1983) 1190.
- D. G. A. NELSON, *ibid.* **60C** (1981) 1622.
- D. G. A. NELSON, J. D. B. FEATHERSTONE, J. F. DUNCAN and T. W. CUTRESS, *Caries Res.* **17** (1983) 200.
- L. G. ELLIES, J. M. CARTER, J. R. NATIELLA, J. D. B. FEATHERSTONE and D. G. A. NELSON, *J. Biomed. Mater. Res.* **22** (1988) 137.
- D. McCONNELL, "Apatite: Its Crystal Chemistry, Mineralogy, Utilization, and Geologic and Biologic Occurrences", 1st Edn (Springer-Verlag, New York, 1973) p. 39.
- L. G. ELLIES, D. G. A. NELSON and J. D. B. FEATHERSTONE, *J. Biomed. Mater. Res.* **22** (1988) 541.
- K. C. BLAKESLEE and R. A. CONDRATE, Sr., *J. Amer. Ceram. Soc.* **54** (1971) 559.
- F. C. M. DRIESSENS, *Bull. Soc. Chim. Belg.* **89** (1980) 663.
- J. E. HARRIES, S. S. HASNAIN and J. S. SHAH, *Calcif. Tissue Int.* **41** (1987) 346.

Received 25 October 1989

and accepted 26 February 1990