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Determination of the Ca/P ratio in calcium-deficient hydroxyapatite using X-ray diffraction analysis

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The determination of the calcium to phosphate ratio (Ca/P) in Ca-deficient hydroxyapatite [d-HAP; $Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}$] using X-ray diffraction analysis (XRD) is reported. At temperatures above 700 °C HPO_4^{2-} groups are transformed to PO_4^{3-} groups, thereby producing β-tricalcium phosphate [β-TCP; $Ca_3(PO_4)_2$]. Thus, the deviation from stoichiometry, x, can be calculated from the mass fraction of β-TCP, which in turn can be determined from quantitative XRD analyses. In this study d-HAP powders with various Ca/P ratios were prepared following several procedures. It is shown that the Ca/P ratio determined by quantitative XRD correlates well with that measured by chemical analyses.

1. Introduction

A typical formula for carbonate-free HAP is $Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}$, where x ranges from 0 to almost 2, giving a Ca/P ratio of between 1.67 and 1.33 [1]. d-HAP is of greater biological interest than stoichiometric HAP (s-HAP) since the Ca/P ratio in bone is lower than 1.67. It has been suggested that d-HAP plays important roles in several processes such as bone remodelling and bone formation [1]. When d-HAP or s-HAP is used as a synthetic biomaterial, there are differences in their behaviour. It has been reported that d-HAP elicits an immediate precipitation of biologically equivalent apatite on its surface when immersed in a simulated physiological fluid, whereas precipitation on s-HAP requires some induction time [2]. The extent of deficiency of HAP is also important when this material is used as a catalyst in the dehydration and dehydrogenation of primary alcohols to aldehydes and ketones [3, 4]. It was found that the rate of catalytic action increased as the calcium deficiency of the HAP increased [3, 4]. It is apparent that in each of the fields mentioned, i.e. bone biochemistry, hard tissue biomaterials and catalysis of reactions involving alcohols, the actual chemical composition of the HAP is an important parameter. Therefore, the determination of the Ca/P ratio is an important goal.

In general, solution chemical analyses or electron-microprobe spectroscopy methods have been used to measure the Ca and P concentrations, and subsequently to determine the Ca/P ratio [5–7]. Solution chemical analytical methods, however, have drawbacks. Sample preparation and measurements are time-consuming, and Ca and P cannot be determined simultaneously. Furthermore, the phosphate concentration measurements using the molybdenum complex method [5–7] are subject to experimental error, since the stability of the molybdenum complex is highly sensitive to temperature and varies as a function of time. A second method, the electron-microprobe method, is effective for the determination of the Ca/P ratio on a microscopic scale. Its disadvantage, however, is that the value determined is not accurate. Thus, in this paper a simple, fast and reliable method of determining the Ca/P ratio in d-HAP is proposed, specifically XRD analysis. The accuracy of the determinations using this method is compared with results obtained using conventional chemical analyses.

2. Materials and methods

2.1. Materials

HAP powders were prepared using two methods. In method A $Ca(NO_3)\cdot 4H_2O$ (200 ml 2.0 mol l^{-1}) and $(NH_4)_2HPO_4$ (200 ml 1.2 mol l^{-1}) were added to 2000 ml deionized water in a three-necked flask using a roller pump (RP-NE1; Furue Science, Tokyo, Japan). The pH of the solution was maintained in the range 8.5–9.5 by adding 3.0 mol l^{-1} ammonium hydroxide. In method B H_3PO_4 (200 ml 2.0 mol l^{-1}) was added to a $Ca(OH)_2$ suspension (2000 ml 1.2 mol l^{-1}).

The mixing proceeded at several temperatures such that d-HAP precipitates with Ca/P ratios in the range 1.58–1.66 were obtained. The reactants were mixed after preheating to the selected temperature. Table I lists the various temperatures used. The temperature was kept constant overnight. Subsequently, the white precipitates were filtered and thoroughly washed with deionized water at the same temperature, removing any adsorbed surface. The products were dried under vacuum for 24 h and stored under vacuum. The products were identified as d-HAP by XRD and Fourier-transformed infrared spectroscopy (see Section 3). Commercially available HAPs (see Table I) were also

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TABLE I Molar ratios of calcium to phosphate and magnesium to phosphate determined by chemical analyses and preparation temperatures for several d-HAP, s-HAP and β-TCP

Calcium phosphate	Preparation method[a]	Preparation temperature (°C)	Molar ratio	
			Ca/P	Mg/P
HAP	A	40	1.58	0.0
HAP	A	70	1.60	0.0
HAP	A	95	1.66	0 0
HAP	B	40	1 61	0.0
HAP	B	70	1.63	0.0
HAP	B	95	1.66	0.0
HAP	Fisher[b]		1.63	n.d.
HAP	Asahi Glass[b]		1.67	n.d.
HAP	Central Glass[b]		1.65	n d.
HAP[c]	EM[b]		1.67	0.0
TCP	EM[b]		1.52	n.d.
TCP	Miter[b]		1.48	0 03
TCP[c]	C	900	1.50	0.00

[a] A, prepared from $Ca(NO_3)_2$ and $(NH_4)_2HPO_4$; B, prepared from $Ca(OH)_2$ and H_3PO_4; C, prepared from $CaHPO_4$ and $CaCO_3$.
[b] Obtained commercially and used as-received.
[c] Used as standard material. n.d., Not determined.

used as-received. β-TCP powders were prepared by heating the appropriate mixture of $CaHPO_4$ and $CaCO_3$ at 900 °C for 24 h [8]. Other chemicals for the chemical analyses and Fourier-transformed infrared spectroscopy measurements were used without further purification.

2.2. Methods

The XRD patterns were recorded on a Rigaku DMAX2 (Danvers, Massachusetts, USA) using Ni-filtered CuK_α radiation at 45 kV and 30 MA. The 2θ range from 25 to 35° was covered at a scan speed of 0.1 ° min^{-1}.

The mass fractions of β-TCP and s-HAP were calculated from the integrated peak using the program Diffrac 500 (Siemens, Madison, Wisconsin, USA) intensities of the peaks at $2\theta = 31.0$ and 31.8°, respectively. The integrated peak intensities were normalized by dividing by the integrated peak intensities of standard β-TCP and HAP.

Approximately 2 mg sample was carefully mixed with 800 mg ground spectroscopic-grade KBr. The mixtures were analysed in the diffuse reflectance mode over the wave number range 400–4000 cm^{-1} on a Fourier-transform infrared spectrometer (Nicolet 5DXC2; Madison, Wisconsin, USA).

For the chemical analyses, HAP and β-TCP powders were dissolved in distilled water containing 10^{-3} mol l^{-1} $HClO_4$, the filtrates were analysed for Ca on an atomic absorption spectrophotometer (Perkin–Elmer 5100 PC; Norwalk, Connecticut, USA). The phosphate concentration was determined by measuring the absorption of the molybdenum complex at 840 nm [7] on a spectrophotometer (Ultrospec 2; Pharmacia LKB Biotechnology, Piscataway, New Jersey, USA).

3. Results

Typical XRD patterns of powders prepared at various temperatures (40–95 °C) are shown in Fig. 1. They are apatitic patterns without supplementary peaks [9]. The patterns became sharper with increasing preparation temperature, indicating that the crystallinity of HAP powders increased with the synthesis temperature [10]. The XRD patterns of d-HAP are substantially equivalent to that of s-HAP, except for the peak width and absolute intensity. This substantiates that the structural deficiencies in d-HAP do not alter the basic crystalline arrangement. Infrared spectra, however, are useful to show the incorporation of HPO_4^{2-} rather than PO_4^{3-} groups in the lattice. Fourier-transformed infrared spectra of powder displayed the apatitic pattern (Fig. 2). The PO_4^{3-} bands occurred at 571 and 601 cm^{-1} (ν_4), 962 cm^{-1} (ν_1) and 1046 cm^{-1} (ν_3) [11]. The presence of HPO_4^{2-} in some of the HAP powders followed from the 875 cm^{-1} peak. Such peaks support the formula of d-HAP, $Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}$, which contains HPO_4^{2-} [12]. The Ca/P ratios of the various HAP and β-TCP powders, as determined by chemical analysis, are summarized in Table I. The Ca/P ratios of d-HAP powder increased with the preparation temperature for both preparation methods. The Ca/P ratios of the d-HAP prepared by method A were greater than or equal to those obtained with the d-HAP prepared by method B at identical preparation temperatures. All powders were then fired at 1000 °C for 3 h in air. As shown in Fig. 3, d-HAP partially transformed to β-TCP upon sintering. s-HAP remained unchanged.

4. Discussion

It is known that the high-temperature behaviour of d-HAP differs from that of s-HAP [12–14]. d-HAP

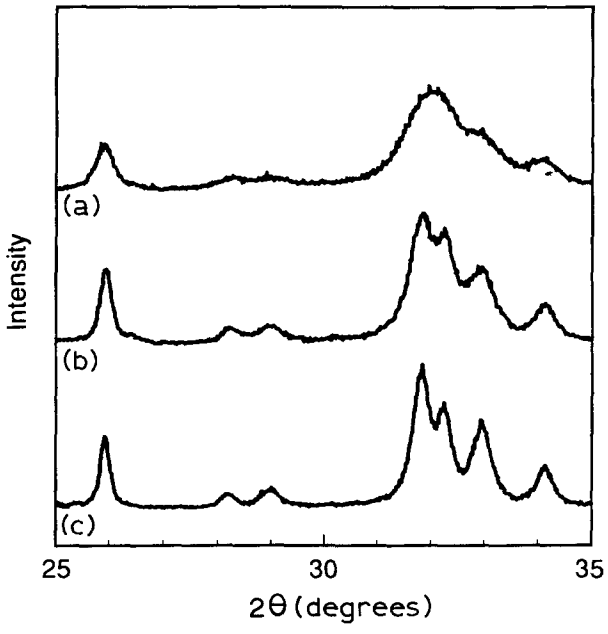

Figure 1 The XRD patterns of HAP powders before sintering, prepared from $Ca(NO_3)_2$ and $(NH_4)_2HPO_4$ (method A) in an aqueous solution at several temperatures: (a) 40 °C, (b) 70 °C and (c) 95 °C.

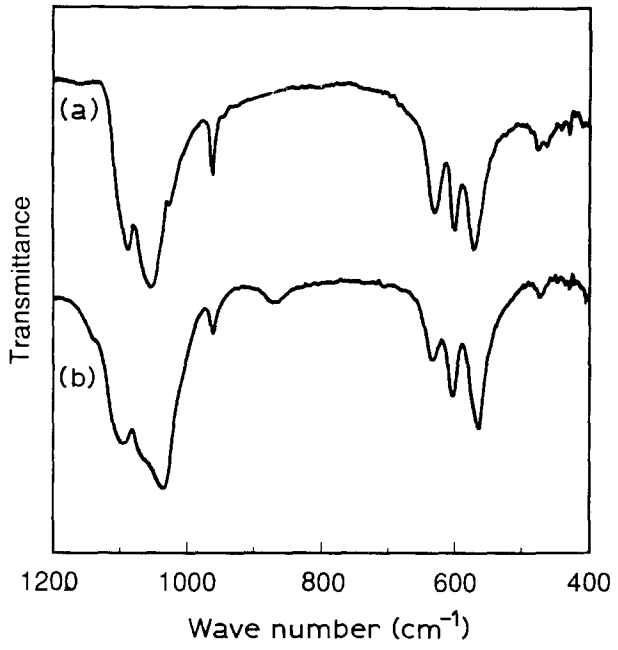

Figure 2 The Fourier-transformed infrared spectra of (a) s-HAP and (b) d-HAP.

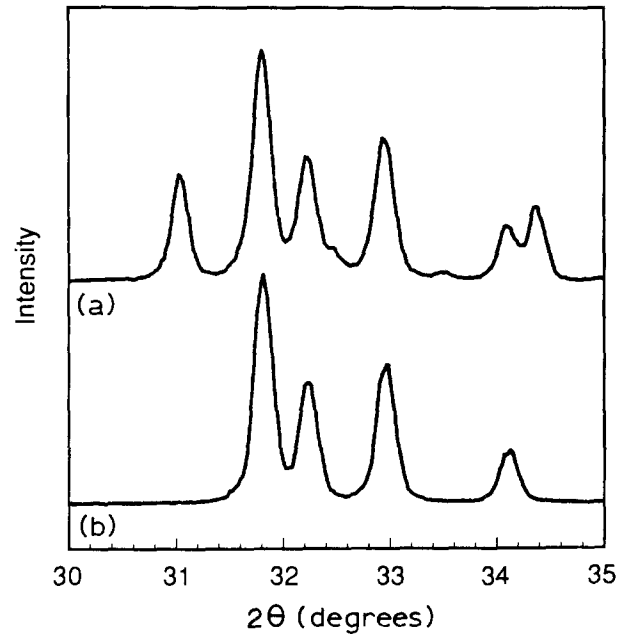

Figure 3 Typical XRD patterns of products obtained by heating (a) d-HAP and (b) s-HAP at 1000 °C for 3 h.

contains HPO_4^{2-} which transforms to $P_2O_7^{4-}$ as shown in Equation 1. Between 650 and 750 °C the reaction in Equation 2 takes place, together with a crystallographic change giving rise to β-TCP. Thus, the total reaction occurs as shown in Equation 3 [12, 14]

$$2HPO_4^{2-} \rightarrow P_2O_7^{4-} + H_2O \quad (1)$$

$$P_2O_7^{4-} + 2OH^- \rightarrow 2PO_4^{3-} + H_2O \quad (2)$$

$$Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x} \rightarrow (1-x)Ca_{10}(PO_4)_6(OH)_2 + 3xCa_3(PO_4)_2 + xH_2O \quad (3)$$

It follows from Equation 3 that the value of x can be calculated from the mass fraction of β-TCP formed in HAP. In the case of s-HAP $x = 0$, since s-HAP maintains its structure, regardless of the sintering duration at 1000 °C. In the case of d-HAP, which contains HPO_4^{2-} groups, HAP partially transforms to β-TCP upon heating. The mass fraction of this mixture of well-crystallized phases after firing can easily be determined by XRD. This is based on the occurrence of distinctively separate peaks for HAP and β-TCP. The strongest peaks are at $2\theta = 31.0$ and 31.8° for s-HAP and β-TCP, respectively (Fig. 3) [9, 15]. The mass fraction of each, i.e. HAP and β-TCP, are calculated using integrated peak intensities of the well-separated HAP and β-TCP peaks.

A comparison of the Ca/P ratio measured by using XRD with that measured by chemical analyses is shown in Fig 4. The full line represents the ideal result for singular, identical Ca/P values. Fig. 4 does not lend itself to the assessment of the error in the Ca/P ratio following from the XRD determination. This is because the value on the other axis, i.e. the Ca/P ratio following from chemical analysis, is not an independent variable, but is subject to experimental error as well. It would appear that the actual data points do not differ by more than 2% from the ideal (the full line). This differential can be ascribed as much, if not more, to the error in the chemical analysis than to the error in the XRD, since an error of 2% is inherent to chemical analysis [5–7].

The limitation of the XRD determination method arises from the principle of the technique. XRD patterns of different powders are the same provided the crystal structure is the same. This may create a problem when magnesium is incorporated into the structure. In such a case the Ca/P ratio of, for example, $Ca_{2.8}Mg_{0.2}(PO_4)_2$ determined by the XRD method would be 1.5. In reality the Ca/P ratio in our example was only 1.4. This phenomenon results from magnesium behaving in almost the same way as calcium in the

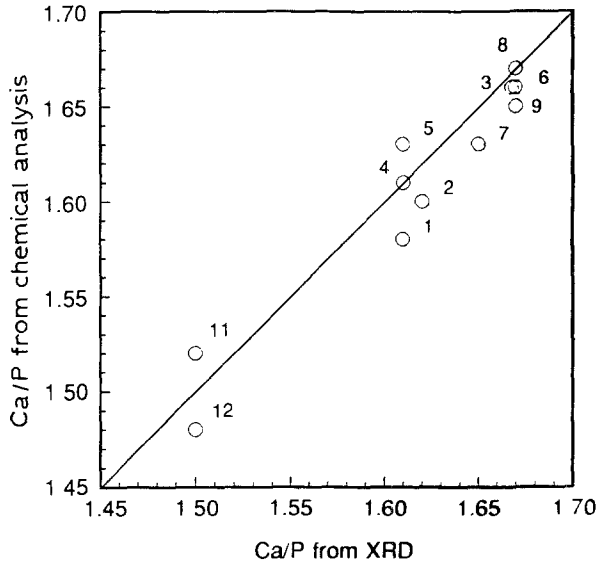

Figure 4 Relationship of the Ca/P ratio determined by chemical analyses and by XRD. The full line represents the ideal result for a singular, identical Ca/P value. The numbers refer to the calcium phosphate powders listed in Table I.

crystal structure. Mg and Ca have very similar ionic diameters, and therefore the XRD peak positions are barely shifted [13, 16, 17]. In the case of a mixture of d-HAP and other Ca-containing materials such as CaO and $Ca_4(PO_4)_2O$, it should also be noted that the Ca/P results yield the total Ca/P ratio of the mixture because, as a result of thermal reactions, the other Ca-containing materials are transformed to d-HAP or s-HAP. Also, a mixture of calcium salts and phosphate compounds can produce HAP and β-TCP when heated at 900 °C. Hence, this method requires previous identification that the specimen is d-HAP. Such a complication, however, is also inherent to the chemical analysis.

In conclusion, a new convenient method to determine the Ca/P ratio in d-HAP was presented. It is based on the measurement of the amount of HPO_4^{2-} that occurs in d-HAP, $Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}$. The amount of HPO_4^{2-} can be quantified after the transformation of HPO_4^{2-} to PO_4^{3-} as described in Equation 2.

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