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# Reaction of calcium phosphate cements with different amounts of tetracalcium phosphate and dicalcium phosphate anhydrous

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**Abstract:** Calcium phosphate cements (CPCs) with different amounts of tetracalcium phosphate (TTCP) and dicalcium phosphate anhydrous (DCPA) (TTCP/DCPA molar ratio from 0.25 to 2.00) were prepared to further understand the setting reaction and the factors that could influence the properties of CPCs. Quantitative X-ray diffraction patterns, Fourier transform IR spectra, and diametral tensile strength of the set mass were measured along with pH measurements of the CPC suspension. Calcium-deficient hydroxyapatite (d-HAP) with a calcium to phosphate molar ratio of approximately 1.5 was formed initially in the CPC setting consisting of an equimolar mixture of TTCP and DCPA. This gradually transformed into stoichiometric HA (s-HA) with increasing incubation time. The s-HA was formed in the initial stage

when the CPC contained an excess amount of TTCP. In contrast, maturation to s-HAP was slow when the CPC contained excess amounts of DCPA. The highest mechanical strength of set CPC was associated with an equimolar mixture of TTCP and DCPA, and the mechanical strength decreased as the TTCP/DCPA molar ratio deviated from 1.00. We concluded, therefore, that the setting reaction and the nature of the resulting set mass are dependent on the molar ratios of TTCP and DCPA. © 1999 John Wiley & Sons, Inc.\* *J Biomed Mater Res*, 46, 504–510, 1999.

**Key words:** calcium phosphate cement; hydroxyapatite; hydroxyapatite cement; self-setting cement; biomaterial; tetracalcium phosphate; dicalcium phosphate anhydrous

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## INTRODUCTION

A calcium phosphate cement (CPC) composed of tetracalcium phosphate [TTCP;  $\text{Ca}_4(\text{PO}_4)_2\text{O}$ ] and di-

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calcium phosphate anhydrous (DCPA;  $\text{CaHPO}_4$ ) or dicalcium phosphate dihydrate (DCPD;  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ )<sup>1–4</sup> has attracted considerable attention because it sets like a dental cement and forms hydroxyapatite (HA, the major mineral component of teeth and bone) as the end product. A number of studies using animal models showed that CPC produced excellent tissue response.<sup>4–12</sup> In addition to its good osteoconductivity, CPC was also reported to be replaced slowly by bone without a loss of volume.<sup>4,6–9,12</sup> Therefore, CPC is a highly promising biomaterial for the reconstruction of bony defects. Several recent studies showed that the handling properties of CPC can be improved to make the material useful in a wider range of clinical applications. For example, conventional CPC (c-CPC) that employs water as a liquid phase hardens in about 30 min at 37°C, but fast-setting CPC (FSCPC) that employs a sodium phosphate solution as the liquid phase hardens in approximately 5 min.<sup>13–16</sup> Addition of a gelling agent such as sodium alginate or chitosan to FSCPC significantly improves the CPC's resistance to wash-out before the cement hardens.<sup>17–20</sup> The addition of carboxymethylcellulose, hydroxypropyl methylcellulose, or chitosan to a c-CPC also improves the wash-out resistance of cement pastes.<sup>21</sup> The use of

gelling agents widened the possible application of CPC because the cement can be used even when complete homeostasis is difficult. Another important factor that may influence the properties of CPC is the nature of the apatitic phase formed in the cement. While an equimolar mixture of TTCP and DCPA is believed to form stoichiometric HA having the composition of  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ , a mixture with the TTCP/DCPA molar ratio of 0.5 was reported to form a calcium deficient HA having a composition of  $\text{Ca}_9(\text{HPO}_4)(\text{PO}_4)_5(\text{OH})$ .<sup>22</sup> A previous study showed<sup>23</sup> that mixtures of TTCP and DCPA with molar ratios in the range of 0.51–2.0 formed cements with apatite and residual starting compounds in the products. In the present investigation, cements with varying TTCP/DCPA molar ratios were further studied to understand the effect of the TTCP/DCPA ratio on the setting reaction and physical properties of CPC.

## MATERIALS AND METHODS

### Preparation of CPC

TTCP, which was prepared from  $\text{CaHPO}_4$  and  $\text{CaCO}_3$  as described previously,<sup>13,14</sup> was first crushed with a mortar and pestle and then ground for 1 h in an agate jar in dried cyclohexane using a planetary ball mill (Retsch PM4, Brinkman, NY) to obtain a median particle size of 10.1  $\mu\text{m}$ . Commercial ultrapure DCPA (J. T. Baker Chemical Co.) was also ground for 24 h in the agate jar in a volume fraction of 95% ethanol to obtain a median particle size of 1.2  $\mu\text{m}$ . The particle sizes of the TTCP and DCPA were measured in isopropanol by a sedimentation method based on Stokes law using a centrifugal particle size analyzer (SA-CP3, Shimadzu, Kyoto, Japan). Powder X-ray diffraction (XRD) analysis confirmed that no other solid phases were present in the samples after grinding.

### Preparation of set CPC specimens

CPC powder and distilled water with a powder to liquid ( $P/L$ ) ratio of 4.0 g/mL were mixed on a glass slab with a spatula.  $P/L$  ratios of 2 and 3 were also used for mechanical strength evaluation. The resultant paste was placed in a split mold (6 × 3 mm, diameter × height). A force of approximately 1 MPa was applied in packing the CPC paste into the mold. Both sides of the mold were covered by glass plates and clamped, and the mold was placed in an incubator for prescribed periods at 37°C and 100% relative humidity. For quantitative XRD measurements, the specimens were removed from the incubator at the prescribed time, quenched

in liquid nitrogen, and dried using a freeze dryer (FD-1, Tokyo Rikakiki Co., Tokyo).

### Mechanical strength measurements

The diametral tensile strength (DTS) was used as a measure of the mechanical strength of the set CPC. CPC specimens kept in an incubator for 24 h were used for the DTS measurement, which was performed within 30 min after the specimens were removed from the incubator. The DTS values were measured using an Instron Universal Testing Machine (United Calibration Corp., Canton, MA). The diameter and length of each specimen were measured with a micrometer. The samples were then placed between steel platens, which were each covered with one thickness of wet filter paper, and tested at a loading rate of 10.0 mm/min. The DTS values given are the average of 10 specimens. For statistical analysis, one-way factorial ANOVA and Fisher's protected least significant difference method as a *post hoc* test were performed using Stat View 4.02 (Abacus Concepts, Berkeley, CA).

### Powder XRD

The XRD patterns of the vacuum dried samples were recorded with a vertically mounted diffractometer system (ADG-301, Toshiba Co. Ltd., Tokyo) using Ni filtered  $\text{CuK}\alpha$  radiation ( $\lambda = 0.1540$  nm) generated at 30 kV and 10 mA. The specimens were first scanned from 3 to 60° 2 $\theta$  (where  $\theta$  is the Bragg angle) in a continuous mode (1.0° 2 $\theta$ /min, 2-s time constant) to obtain XRD patterns. Slower scans (0.25° 2 $\theta$ /min, 4-s time constant) were then performed from 20 to 40° 2 $\theta$  for the quantitative measurements of HA, TTCP, and DCPA. The baselines of the XRD patterns were estimated manually; the areas under the 014 (29.2°, 2 $\theta$ ) and 040 peaks (29.8°, 2 $\theta$ ) of TTCP, the 110 peak (26.6°, 2 $\theta$ ) of DCPA, and the 002 peak (25.9°, 2 $\theta$ ) of HA were measured from XRD patterns obtained from four independently prepared specimens. The amount of TTCP or DCPA present in the sample was calculated from the ratio of peak intensities of the phase at time  $t$  and time 0; the amount of HA was calculated based on the peak intensities of HA, TTCP, and DCPA as described previously.<sup>13,14</sup> The estimated standard uncertainty of the 2 $\theta$  measurement is 0.01°, and the minimum amount of a phase to be detected by XRD in the present system is about 0.03 mass fraction.

### Fourier transform IR spectroscopy (FTIR)

The set CPC was removed from the incubator, ground into a powder, and freeze-dried. Approximately 2 mg of each sample was thoroughly mixed with 800 mg of ground spectroscopic grade KBr and pressed into transparent pellets. The IR spectra were measured between 4000 and 400

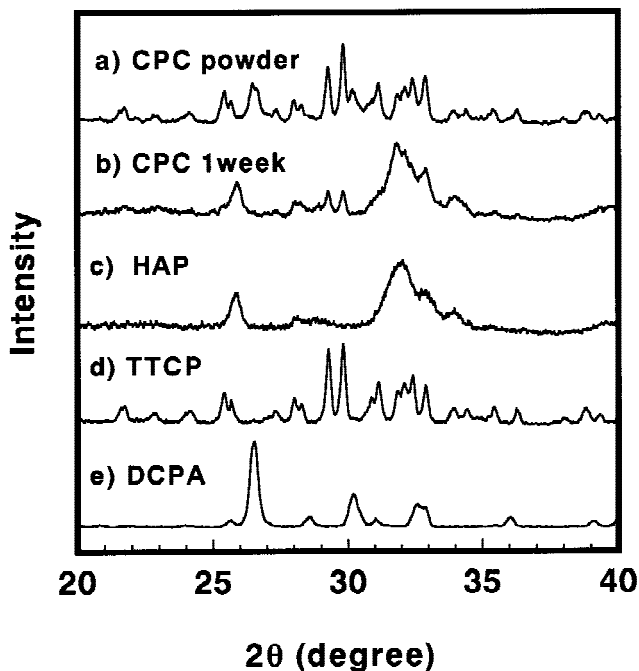
$\text{cm}^{-1}$  at a resolution of  $2 \text{ cm}^{-1}$  using a FTIR spectrometer (FTS-40, Bio-rad, Cambridge, MA).

### pH Measurement

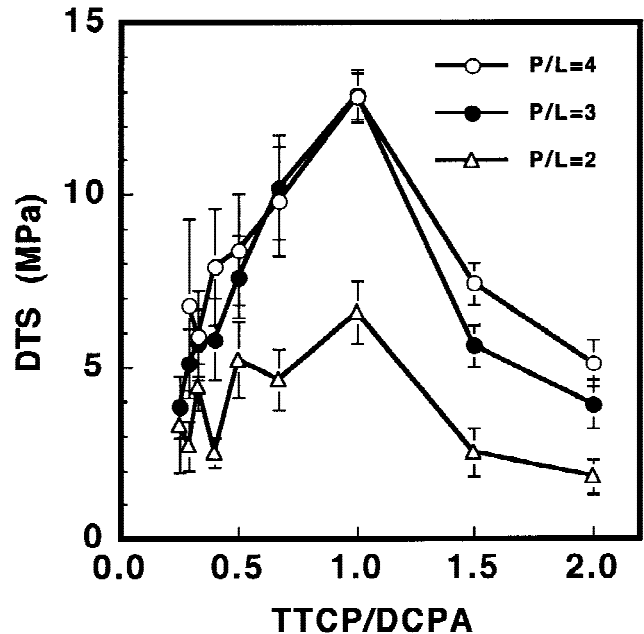
A suspension of CPC prepared by the addition of 2 g of CPC powder to 8 mL of distilled water was stirred continuously under a  $\text{CO}_2$ -free  $\text{N}_2$  atmosphere in a thermostated ( $37^\circ\text{C}$ ) glass vessel. The pH of the suspension was continuously recorded using a pH meter (TP-100, Toko Chemical Laboratories Co., Ltd., Tokyo) connected to a strip chart recorder. Results from a previous study<sup>24</sup> showed that the standard uncertainty in similar slurry pH measurements was about 0.15 pH units ( $n = 3$ ).

## RESULTS

Figure 1 shows the XRD patterns of unreacted CPC(1:1) and of the set cement. The XRD patterns of a poorly crystalline HA, TTCP, and DCPA are also shown for comparison. The DTS values for the 24-h CPC specimens are plotted against the TTCP/DCPA ratios in Figure 2. Three  $P/L$  ratios (4.0, 3.0, and 2.0) were employed in the preparation of these CPC specimens. For a given  $P/L$  used, the CPC mixture having



**Figure 1.** Powder X-ray diffraction patterns of the (a) unreacted powder phase of CPC(1:1), (b) set CPC(1:1) kept in an incubator at  $37^\circ\text{C}$  and 100% relative humidity for 1 week, (c) poorly crystalline HAP, (d) TTCP, and (e) DCPA.

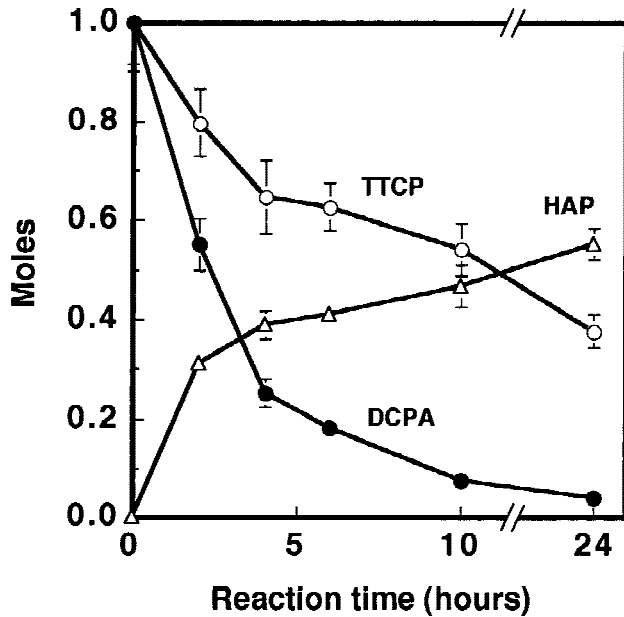


**Figure 2.** Diametral tensile strength of set CPCs with different TTCP/DCPA mixing ratios after storage in an incubator at  $37^\circ\text{C}$  and 100% humidity for 24 h. The powder to liquid ratios employed in this experiment were ( $\circ$ ) 4.0, ( $\bullet$ ) 3.0, and ( $\triangle$ ) 2.0. Vertical lines indicate the standard uncertainty ( $n = 10$ ).

a TTCP/DCPA molar ratio of 1.0 always showed the highest DTS value. The DTS value decreased as the TTCP/DCPA ratio diverged from 1.0. Within the range of the  $P/L$  studied, the DTS value increased with the  $P/L$  value. However, a  $P/L$  of 4 represents the maximum  $P/L$  that will produce a smooth cement paste. A ratio greater than 4 was found to produce a dry and flaky paste that did not have satisfactory handling properties for most applications.

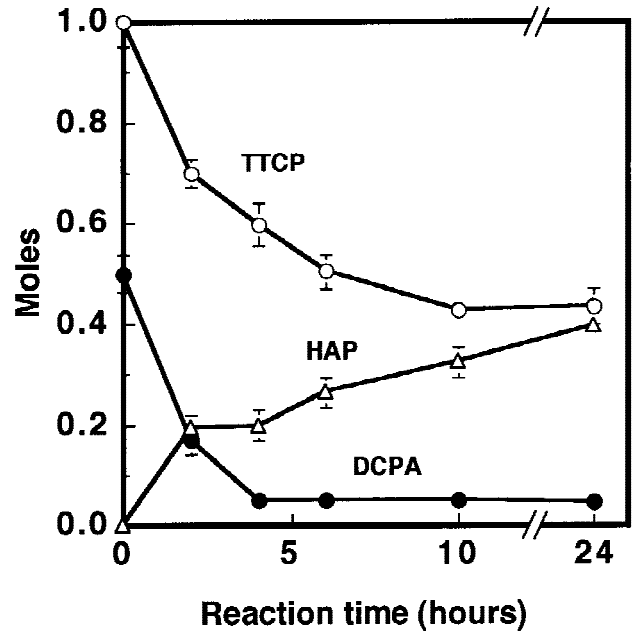
Figures 3, 4, and 5 show the amounts of the reactants (TTCP and DCPA) and product (HA) of the cement setting reaction present in the specimens as a function of time for CPC mixtures CPC(1:1), CPC(1:2), CPC(2:1), respectively. The amounts of the various phases shown in each figure are normalized to 1 mol of TTCP initially present in the CPC mixture. Decreases in the amount of TTCP and DCPA and a concomitant increase in the amount of HA were observed over time, indicating that HA was formed from the TTCP and DCPA in all cases. Figure 6 shows the FTIR spectra of various set CPC samples. The intensity of the absorption peak at  $895 \text{ cm}^{-1}$ , which is attributable to  $\text{HPO}_4^{2-}$ , increased with a decreasing TTCP/DCPA ratio of the cement powder.

The pH changes of the CPC suspensions are shown in Figure 7 in which representative pH curves for cements with various TTCP/DCPA ratios are plotted against time. The pH of the CPC suspension increased initially in all cases before decreasing with time (data



**Figure 3.** Calculated phase composition of CPC(1:1) prepared with a powder to liquid ratio of 4.0 and kept in an incubator at 37°C and 100% humidity. The amounts of the phases are normalized to 1 mol of TTCP initially present in the cement mixture. The vertical lines indicate the standard uncertainty ( $n = 4$ ).

not shown). After approximately 2–3 h, the pH increased again for samples with TTCP/DCPA ratios greater than 0.67 [i.e., CPC(2:1) and CPC(1:1)] while the pH leveled off in the case where the TTCP/DCPA



**Figure 5.** Calculated phase composition of CPC(1:2) prepared with a powder to liquid ratio of 4.0 and kept in an incubator at 37°C and 100% humidity. The amounts of the phases are normalized to 1 mol of TTCP initially present in the cement mixture. The vertical lines indicate the standard uncertainty ( $n = 4$ ).

ratio was 0.67 [i.e., CPC(2:3)] and continued to decrease when the TTCP/DCPA ratios were less than 0.67 [i.e., CPC(1:2), CPC(1:3), and CPC(1:4)].

## DISCUSSION

For CPC(1:1) the ideal cement setting reaction, expressed by Equation (1), forms stoichiometric HA (s-HA).

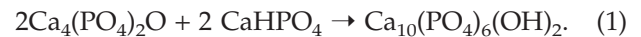
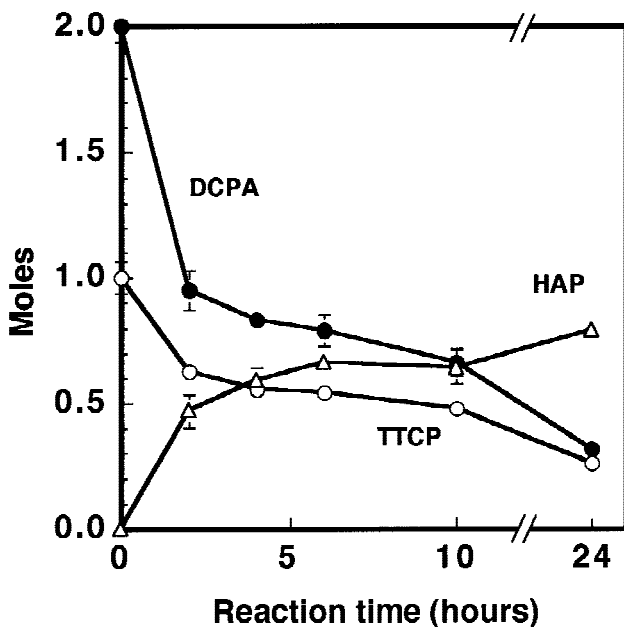
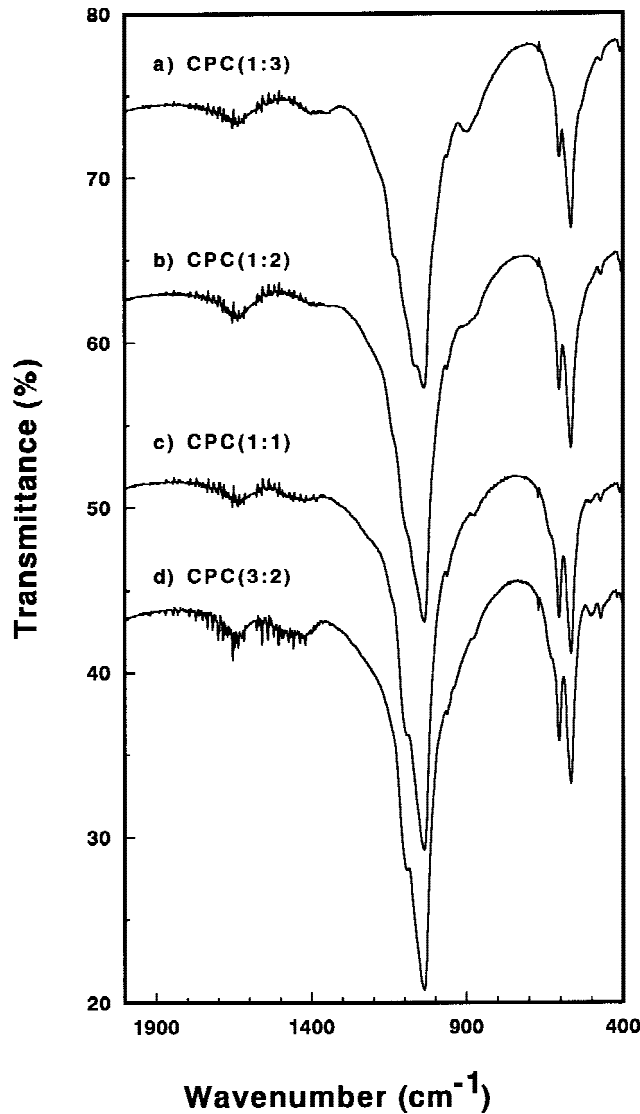


Figure 1 shows that the 24-h CPC(1:1) sample contained a significant amount of TTCP but not DCPA. Because TTCP is the calcium-rich component of the cement, this suggests that the cement setting did not follow Equation (1) and instead formed a nonstoichiometric, calcium deficient HA (d-HA),  $\text{Ca}_{10-x}(\text{HPO}_4)_x(\text{PO}_4)_{6-x}(\text{OH})_{2-x}$ . Although this nonstoichiometric mineral retains the fundamental HA structure and thus produces a similar XRD pattern, there are some differences in the biological and chemical behavior of s-HA and d-HA. When d-HA or s-HA are used as synthetic biomaterials, d-HA elicits an immediate precipitation of biologically equivalent HA on its surface whereas precipitation of HA on s-HA requires an induction time.<sup>25</sup> The extent of the calcium deficiency in HA is also an important factor when the material is used as a catalyst (i.e., in the dehydra-

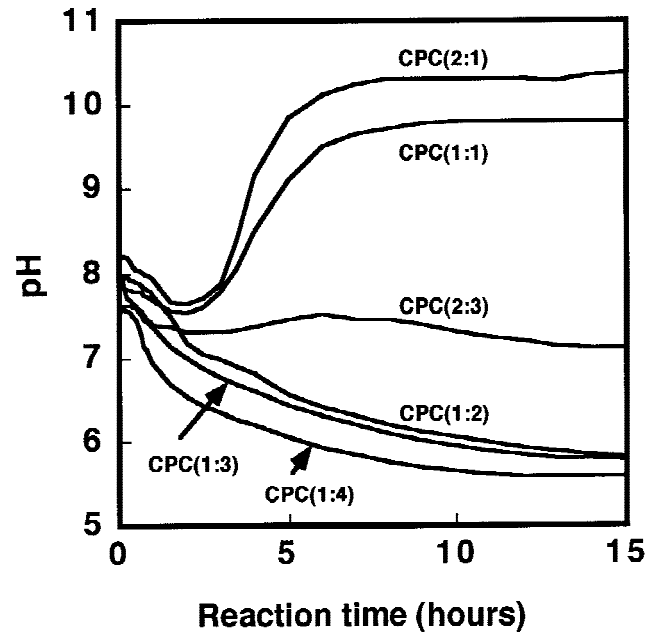
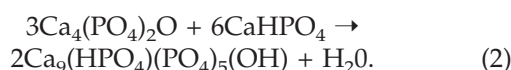


**Figure 4.** Calculated phase composition of CPC(2:1) prepared with a powder to liquid ratio of 4.0 and kept in an incubator at 37°C and 100% humidity. The amounts of the phases are normalized to 1 mol of TTCP initially present in the cement mixture. The vertical lines indicate the standard uncertainty ( $n = 4$ ).



**Figure 6.** IR spectra of CPCs prepared with a powder to liquid ratio of 4.0 and kept in an incubator at 37°C and 100% humidity: (a) CPC(1:3), (b) CPC(1:2), (c) CPC(1:1), and (d) CPC(3:2).

tion and dehydrogenation of primary alcohols to aldehydes and ketones).<sup>26,27</sup> Two research groups found that the catalytic activity increased as the calcium deficiency of the HA increased.<sup>26,27</sup> In general, d-HA transforms to s-HA, a thermodynamically more stable HA, with time if calcium ions are present in the aqueous milieu around d-HA. d-HA can have a wide range of molar Ca/P ratios, reaching approximately as low as 1.33 when  $x = 2.25$ . Brown et al. proposed that a cement that consists of 1 mol TTCP and 2 mol DCPA would produce a d-HA with a Ca/P ratio of 1.5 as predicted from the reaction stoichiometry [Equation (2)]<sup>22</sup>:



**Figure 7.** The pHs of several CPCs as a function of time at 37°C. Slurries were prepared by mixing 2 g of CPC powder with 8 mL of twice distilled water.

The formation of d-HA was also confirmed by the FTIR spectra where the  $\text{HPO}_4$  peak typical for d-HA was seen at around  $890 \text{ cm}^{-1}$ .

To further analyze the stoichiometry of the cement setting reaction, the data presented in Figures 3–5 (i.e., the amounts of TTCP and DCPA remaining in the cement as a function of time) were used to calculate the number of moles of calcium (Ca) and phosphate (P) consumed by the reaction at the various times. The overall ratio of Ca and P consumed from the start of the reaction and the differential ratio (i.e., the ratio of Ca and P consumed during each time interval) are plotted against time in Figures 8 and 9, respectively. Although for CPC(1:1) and CPC(1:2) during the first 6 h the Ca/P consumed was approximately 1.5 (Fig. 9), indicating that 2 mol of DCPA reacted with 1 mol of TTCP, the reaction subsequently took different pathways (Table I). For CPC(1:2) the Ca/P consumed remained at 1.5, reflecting the stoichiometry of the mixture. In contrast, for CPC(1:1) the Ca/P consumed increased to 1.6 and 1.9 at 10 and 24 h, respectively. A ratio of 1.5 indicates that the setting reaction followed Equation (2) and formed d-HA, and a ratio of 1.67 indicates the reaction followed Equation (1) and formed s-HA. Because the maximum Ca/P ratio of HA is 1.67 if it is carbonate free, a value of 1.9 may be the result of two different reactions occurring simultaneously. One is the formation of d-HA from TTCP and DCPA via Equation (2). The other is maturation of d-HA by transformation to s-HA [Equation (3)] using the  $\text{Ca}^{2+}$  formed by the hydrolysis of TTCP to s-HA [Equation (4)] or d-HA [Equation (5)].



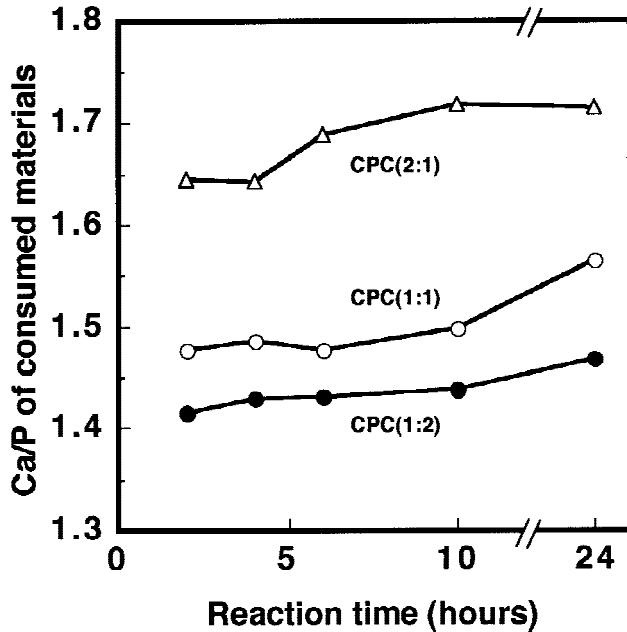


Figure 8. Overall Ca/P molar ratio of the materials consumed by the cement setting reaction from the beginning of the reaction.

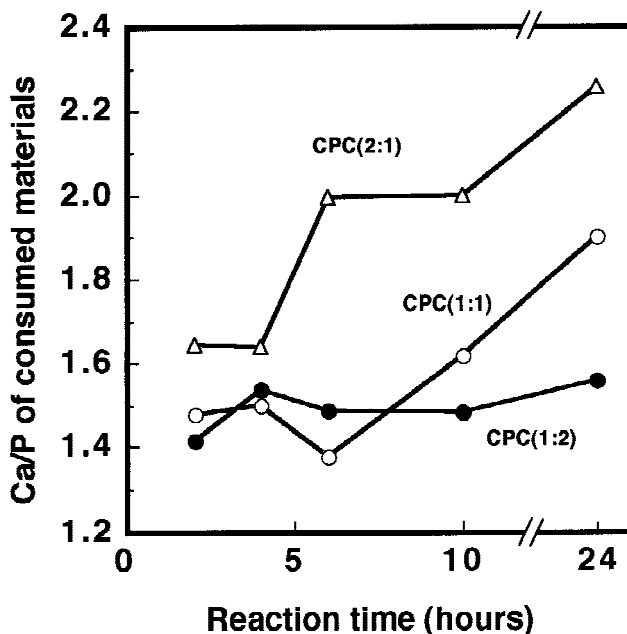
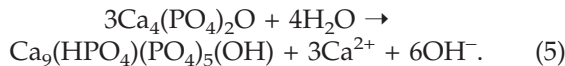
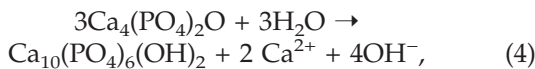
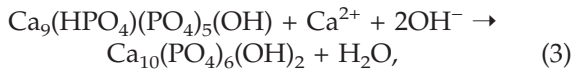


Figure 9. Differential Ca/P molar ratio of the materials consumed by the cement setting reaction during the indicated time intervals.

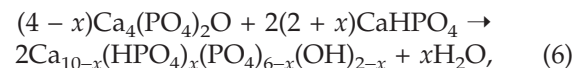
TABLE I  
Molar TTCP/DCPA Ratios and Molar Ca/P Ratios of Various Calcium Phosphate Cements Used in Study

Sample	Molar Ratio		Molar Ca/P Ratio
	TTCP:DCPA	TTCP/DCPA	
CPC(2:1)	2:1	2.00	1.80
CPC(3:2)	3:2	1.50	1.75
CPC(1:1)	1:1	1.00	1.67
CPC(2:3)	2:3	0.67	1.57
CPC(1:2)	1:2	0.50	1.50
CPC(2:5)	2:5	0.40	1.44
CPC(1:3)	1:3	0.33	1.40
CPC(2:7)	2:7	0.29	1.36
CPC(1:4)	1:4	0.25	1.33

The high pHs observed for CPC(1:1) and CPC(2:1) at >5 h indicate accumulation of  $\text{OH}^-$ , probably by hydrolysis of TTCP through Equation (4) or (5). This would suggest that TTCP hydrolysis had occurred early in the reaction and more rapidly than the transformation of d-HA to s-HA [Equation (3)].

Although the composition of the CPC(1:1) mixture is in accordance with the reaction for the formation of s-HA [Equation (1)], the data on Ca/P consumed (Figs. 8, 9) suggest that the actual reaction essentially followed Equation (2) and formed d-HA. Some of the d-HA was later converted to s-HA such that the apatitic phase in the 24-h product had a Ca/P ratio of about 1.55 (Fig. 8). In the case of CPC(2:1) that contains twice the required amount of TTCP for forming s-HA, the Ca/P ratio during the first 4 h was about 1.62 (Fig. 9), indicating that the product formed was primarily s-HA. Figure 8 shows that the overall ratio of the consumed Ca/P was close to 1.67, suggesting that the final product was s-HA.

The above results clearly demonstrate that the HA formed by the setting reaction of CPC might exhibit various Ca/P ratios, depending on the TTCP/DCPA ratio in the mixture. A general reaction may be written [Equation (6)] to describe the range of stoichiometry of the reaction



where  $x = 0$  for s-HA with a Ca/P ratio of 1.67 and  $x = 1$  for d-HA with a Ca/P of 1.50. The particle sizes and therefore the specific surface areas of TTCP and DCPA were kept constant in this study. However, the particle size distribution is likely to be another important factor that may affect the kinetics and stoichiometry of the reaction. Thus, even for a given TTCP/DCPA ratio, the properties of the HA formed from mixtures with different particle distributions may be different.

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