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Solution property of calcium phosphate cement hardening body

Changsheng Liu*, Wei Shen, Jianguo Chen

Institute of Technical Chemistry and Physics, East China University of Science and Technology, Shanghai 200237, People's Republic of China

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Abstract

In this paper, the solution property of calcium phosphate cement (CPC) hardening bodies with various ratios of calcium to phosphate were studied. The solution property included solubility and behavior in simulated blood phosphoric acid solutions with selected initial concentrations at 37°C. When the ratios of calcium to phosphate were 1.67, 1.60, 1.55, 1.50, the solubility products, $-\log K_{sp}$, were 99.2–102, 94.7–94.8, 89.6–90.5, 85.5–86.4, respectively. All these were significantly bigger than the values reported by the references due to the low crystallinity and calcium deficiency in the calcium phosphate cement hardening body. When CPC with Ca/P = 1.50 was immersed in fixed volumes of simulated blood plasma, both the calcium and phosphate concentrations in the solution increased, while for the samples with a ratio of 1.67, the concentrations decreased. These results showed that the simulated blood plasma was supersaturated when Ca/P = 1.67 but unsaturated Ca/P = 1.50. It was deduced that when CPC was implanted in the bodies, the specimen with the ratio of 1.5 was degraded faster than with the sample of 1.67. © 1999 Elsevier Science S.A. All rights reserved.

Keywords: Solubility; Calcium phosphate cement; Ratio of calcium to phosphate; Simulated blood plasma

1. Introduction

A kind of biomaterial for bone repair, calcium phosphate cement (CPC) which is a mixture of tetracalcium phosphate (TECP) and dicalcium phosphate anhydrous (DCPA) has been reported recently [1–3]. After mixing with water, CPC becomes a slurry which could be shaped according to the contour of the defect and could be capable of self-setting under ambient conditions. The final product of hydration was low crystallinity hydroxyapatite (OHAP).

CPC has received increasing interest because it has high biocompatibility and can be shaped easily. The difference between CPC and hydroxyapatite (HAP) ceramic is that the final product of hydration is formed under ambient conditions whereas HAP crystal is low in crystallinity and does not easily fuse to each other. When CPC was implanted in vivo, it would be adsorbed [4], but HAP ceramic could not be resolved due to the difference of solution property. The studies on the solution property of CPC hardening body will be beneficial to understand the behavior of CPC implanted in vivo.

A number of studies concerning the solubility of HAP have been carried out [5–9]. Mereno et al. [5,6] had explored the preparation and solubility of HAP which was prepared by aqueous precipitation and then was heated at 1000°C in

air. Solubility isotherms for HAP in the system $\text{Ca}(\text{OH})_2\text{-H}_3\text{PO}_4\text{-H}_2\text{O}$ were determined in the pH range 5–7 by equilibrating the solids with dilute H_3PO_4 solution, the solubility product, $[\text{Ca}]^{10}[\text{PO}]^6[\text{OH}]^2$, was found to be from $4.0 \pm 4.4 \times 10^{-117}$ to $1.4 \pm 0.3 \times 10^{-115}$. Mahapatra [7] studied the solubility of HAP prepared by aqueous precipitation at 37°C. And the solubility product obtained ranged from 5.4×10^{-114} to 1.08×10^{-114} in the pH range from 5.2 to 7.25. Driessens [8] pointed out that solubility product of HAP was 1.6×10^{-117} while the calcium-deficient hydroxyapatite (DAP) with Ca/P = 1.50, $\text{Ca}_9(\text{HPO}_4)_2(\text{PO}_4)_5(\text{OH})$, was 1.3×10^{-85} . Driessens also thought that the variation in the mole ratio of calcium to phosphate (Ca/P) greatly affected the solubility product due to the calcium deficiency in HAP crystal, but detailed data were not given. As all the data given above vary greatly, we cannot decide which data are suitable for the hardening body of CPC. Moreover, OHAP is different from HAP ceramics synthesized by aqueous precipitation and heat treatment at 1000°C. The solubility of HAP ceramic is not available. Meanwhile, OHAP formed by hydration is also different from the HAP powder synthesized by aqueous precipitation at 37°C, and the hardening body of OHAP contains traces of the remaining starting material, which would effect the solubility. Based on this, Berger et al. [9] investigated the dissolution of some calcium phosphate cement, but the main composition of the starting materials was tricalcium phosphate rather

*Corresponding author.

than TECP, so the hydration reaction routine and hydration product were different. Ishikawa et al. [10] has studied the behavior of CPC hardening bodies in simulated blood plasma in vitro, but a magnetic stirrer was introduced in his experiment which would strike the sample and make the results indistinct to define the behavior of the hardening bodies or the influence of operating mode. In this paper, the solubility products of OHAP with different Ca/P ratios formed by the hydration of CPC were investigated, and the behavior of CPC hardening bodies in simulated blood plasma by using glass stirrer to avoid the influence of operating mode was also carried out.

2. Material and methods

2.1. Preparation of the hardened body of calcium phosphate cement

The mixture of CPC used in this study consisted of different molar amounts of TECP and DCPA which were prepared in our laboratory and were phase-pure materials. When TECP and DCPA are equimolar amount, the final product of hydration is OHAP with Ca/P = 1.67. But if the amount of DCPA increased, the final product is the low crystallinity and calcium-deficient hydroxyapatite. So the OHAP specimen with different Ca/P ratio were prepared by changing the content of TECP and DCPA in CPC. The specific surface areas of TECP and DCPA were 0.732 and 6.9 m² g⁻¹, respectively. In the mixture, 3 wt.% HAP was also present as a seed to facilitate the cement setting reaction.

To prepare a cylinder, 0.6 g CPC was mixed with 0.15 ml distilled water with a spatula to form a paste in 1 min, then the paste was loaded into a stainless steel mold (6 mm diameter × 12 mm height) and periodically packed by means of a stainless steel rod (5.6 mm diameter) to about 2 kg. The specimen was taken out and put into a glass tube (8 mm diameter × 20 mm height), and then the glass tube was sealed with a film and incubated in a 100% humidity box at 37°C. After 48 h, the specimens were used for the study of CPC in simulated blood plasma. Alternatively, the specimens were also taken out and ground in a agate mortar, and then sieved through 20 and 40 mesh, respectively. The grains in the range of 20 and 40 mesh was used to determine the solubility product.

2.2. Equilibration and analysis

Leached experiments were carried out in rubber-stoppered bottles at 37°C in which 3.0 g CPC grains of the hardening body and 200 l dilute phosphoric acid were added and stirred by a magnetic stirrer for 8 h. Each sample was immersed in dilute phosphoric acid with different concentrations, 0.2, 0.5, 1.0 mM l⁻¹, respectively. Nitrogen was used to keep the solution free of CO₂. In order to remove the

influence of the ions adsorbed on the surface of lattice on the value of solubility product, pre-equilibration was adopted. After the equilibration was achieved, the leaching solution was siphoned up from the bottles with a long pipet. There were filtration-layers fixed around the top of the pipet with a sandwich-like structure to filter the leaching solution. The outer layers in the sandwich structure were gauze and the middle layer was filter paper. This method could avoid the decrease of calcium and phosphate concentration in the solution due to the temperature decrease by normal filtration.

The filtrates were analyzed for Ca²⁺ and total phosphate. Calcium was determined by atomic absorption spectrophotometer (PS-6, Baird Co.) and phosphorus was determined by the colorimetric method of Brabson et al. [11]. The estimated errors were ±3% of the amounts of Ca or P analyzed. The pH measurements were made with a null-point instrument of claimed accuracy ±0.01 pH units. The pH meter was standardized with certified buffers.

The solubility product constant is given by

$$K_{sp} = [\text{Ca}^{2+}]^{10} [\text{PO}_4^{3-}]^6 [\text{OH}^-]^2$$

where the parentheses denote ionic activities which were calculated by the procedure described by Moreno et al. [5] while the Davies equation was used to calculate activity coefficients [12].

Moreover, the solution of immersed CPC were taken out at various intervals, and the transmittances were measured immediately by spectrometer (722, Shanghai Third Factory of Analytic instrument) at 420 nm.

2.3. Behavior of calcium phosphate cement in simulated blood plasma

Simulated blood plasma having an electrolyte composition similar to that of human blood plasma [13] was prepared according to the following steps: (a) 1.0 mM l⁻¹ K₂HPO₄, 4.2 mM l⁻¹ NaHCO₃, 2.5 mM l⁻¹ CaCl₂, 1.5 mM l⁻¹ MgCl₂, 3.0 mM l⁻¹ KCl, 134.8 mM l⁻¹ NaCl, dissolved in about 800 ml H₂O and was buffered to pH 7.4 at 37°C with 30 mM l⁻¹ Tris and HCl, (b) the total volume of the solution was made up to 1000 ml and mixed uniformly.

Five calcium phosphate cement cylinders were leached in 200 ml simulated blood plasma which was contained in a water-circulated (37°C), double-walled crystal vessel. The solutions were taken out at various intervals by the method mentioned above, and then the concentrations of calcium and phosphate were analyzed.

3. Results and discussion

3.1. Determination of K_{sp}

After the samples were equilibrated in 0.2, 0.5, 1.0 mM l⁻¹ dilute phosphorus acid, the final pH of the

Table 1
Solubility data of OHAP with the ratio of calcium to phosphate being 1.5 and 1.67

Sample Ca/P	Initial concentration of H_3PO_4 ($mM l^{-1}$)	Final pH	Ca concentration ($\times 10^{-4}$ M)	Total phosphate ($\times 10^{-5}$ M)	Calculated results				log K_{sp}
					H_3PO_4	$H_2PO_4^-$	HPO_4^{2-}	PO_4^{3-}	
					($\times 10^{-9}$)	($\times 10^{-4}$)	($\times 10^{-4}$)	($\times 10^{-10}$)	
1.67	1.0	6.98	5.32	39.84	33.8	2.48	1.51	6.36	-101.95
	0.5	7.84	4.80	3.54	0.0126	0.0660	0.288	8.70	-100.15
	0.2	9.70	3.83	0.896	7.4×10^{-7}	2.81×10^{-4}	8.91×10^{-2}	195.0	-99.26
1.50	1.0	6.32	12.8	155.8	86.8	13.8	1.81	1.65	-86.36
	0.5	6.82	5.32	77.4	10.9	5.46	2.28	6.57	-86.20
	0.2	7.04	4.48	57.1	4.06	3.38	2.33	11.2	-85.49

solution, the concentration of calcium and phosphate were determined. These data and calculated results are listed in Table 1. It was shown that log K_{sp} of OHAP (Ca/P = 1.5) was in the range from -85.5 to -86.5 which was similar to the result reported (-85.1) by Driessens [8], while log K_{sp} of OHAP (Ca/P = 1.67) was in the range from -99.3 to -102 which was obviously different from the data reported (-117.2) by Driessens [8], but was similar to the upper limit claimed by Mahapatra [7] where the sample was prepared by aqueous precipitation. Because OHAP had low crystallinity (Fig. 1), the crystal development was not intact after the hydration of CPC. The deficiency presented in the inner crystal would result in deformation or disorder in lattice which would lead to the increase of solubility.

Table 1 also shows that the variation in Ca/P would lead to a great difference in solubility since the lower the Ca/P ratio in OHAP, the more calcium deficient will it be in the lattice.

In order to explore the relationship between the deficiency of calcium in OHAP lattice and its solubility, the

other samples with Ca/P equal to 1.55 and 1.6 were also employed. Table 2 summarizes the experimental data and the calculated results.

The log K_{sp} of OHAP with Ca/P = 1.55 ranged from -89.6 to -90.5 while that with Ca/P = 1.60 was from -94.7 to -94.8. Hence, these data combined with the data listed in Table 1 further confirmed the conclusion that the extent of calcium-deficiency in lattice also affected its solubility.

As OHAP has high ability to absorb ions, there would be significant amounts of calcium and phosphate ions adsorbed onto apatite surface during the crystallization process by hydration. When the crystals were inputted into the simulated blood plasma for the determination of solubility, the equilibration between the crystals and the solution would be interpreted by considering the surface state differences. In order to eliminate the influence of ions adsorbed on apatite surface on the value of K_{sp} , pre-equilibration was taken. The samples with Ca/P equal to 1.67 and 1.50 were leached in 0.5 mM dilute H_3PO_4 about 3 h for pre-equilibration, and then taken out and put into another vessel for equilibration. Table 3 gives the same information as Table 1 for the solubility except that the samples in Table 3 were pre-equilibration.

The data in Table 3 indicated that there were no marked differences in solubility product with Table 1, but the final pH increased and calcium and phosphate concentration decreased for the sample with Ca/P = 1.67 after pre-equilibration while the pH decreased but the concentration increased for the sample with Ca/P = 1.50.

All above data are of significance to the optimization and the application of calcium phosphate cement.

3.2. Behavior of CPC in fixed volumes of simulated blood plasma

The variation of calcium concentration in solution with immersing time was shown in Fig. 2. When CPC leached in the simulated blood plasma, the sample with Ca/P = 1.50 was dissolved, which made the calcium concentration in the solution to increase, but the concentration of calcium in the sample with Ca/P = 1.67 decreased. In other words, the

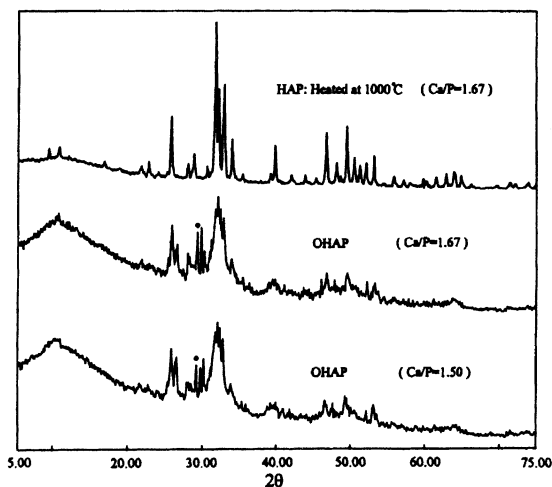


Fig. 1. XRD patterns of HAP and OHAP. Filled circles (*) denote the characteristic diffraction peaks of TECP.

Table 2
Solubility data of OHAP with the ratio of calcium to phosphate being 1.55 and 1.60

Sample Ca/P	Initial concentration (mM l ⁻¹)	Final pH	Ca concentration (× 10 ⁻⁴ M)	Total phosphate (× 10 ⁻⁵ M)	Calculated results				log K _{sp}
					H ₃ PO ₄ (× 10 ⁻⁹)	H ₂ PO ₄ ⁻ (× 10 ⁻⁴)	HPO ₄ ²⁻ (× 10 ⁻⁴)	PO ₄ ³⁻ (× 10 ⁻¹⁰)	
1.55	1.0	6.67	12.65	65.37	14.23	5.05	1.49	3.04	-89.60
	0.5	6.82	10.80	3.12	4.40	2.21	0.919	0.265	-90.51
1.60	1.0	6.65	11.73	45.99	7.96	3.40	1.20	29.56	-94.67
	0.5	7.14	8.59	13.68	0.698	0.731	0.637	3.84	-94.82

Table 3
Solubility data of OHAP with various Ca/P by pre-equilibration

Sample Ca/P	Initial concentration (mM l ⁻¹)	Final pH	Ca concentration (× 10 ⁻⁴ M)	Total phosphate (× 10 ⁻⁵ M)	Calculated results				log K _{sp}
					H ₃ PO ₄ (× 10 ⁻⁹)	H ₂ PO ₄ ⁻ (× 10 ⁻⁹)	HPO ₄ ²⁻ (× 10 ⁻⁴)	PO ₄ ³⁻ (× 10 ⁻¹⁰)	
1.67	0.5	9.02	1.72	0.461	9.05 × 10 ⁻⁶	7.02 × 10 ⁻⁴	4.54 × 10 ⁻²	20.27	-100.02
1.50	0.5	6.57	7.25	126.7	36.4	10.3	2.41	3.90	-86.35

simulated blood plasma was supersaturated with the sample when Ca/P = 1.67 but unsaturated when Ca/P = 1.5. The conclusion was different from that reported by Ishikawa [10] who found that both Ca and PO₄ concentration increased initially, then gradually decreased until leveling off after approximately 8 h. He deduced that the immersed discs initially underwent partial dissolution; TECP was most probably the major source. The author speculated that this conclusion was incorrect, because the striking of the magnetic stirrer to the disc would produce a lot of ultrafine particles which would increase the solubility of the sample. Meanwhile, the transmittance of the solution was down (Fig. 3). The process of dissolution of ultrafine particles and the ions reprecipitated onto the surface of apatite would undergo a long time. Finally, with the particle growing, the solubility of OHAP decrease, so the concentration of cal-

cium and phosphate would drop. With magnetic stirrer, we could not distinguish the solubility property of CPC from the influence of stirring on the specimen. In this study, as the glass stirrer at the upper part of the vessel was adopted to avoid the influence of the stirrer hitting the specimen, the results should be reasonable.

When CPC is implanted into the body of animals, the degradation of the material would be composed by two parts: biodegradation and chemical degradation. The former is the metabolic effect of osteoclast, the later is the equilibration between the ions in blood plasma and the surface of CPC which depends on the solubility product. To make clear the process in vitro it is beneficial to understand the behavior of CPC in vivo. When the specimen with Ca/P = 1.67 was immersed in the simulated blood plasma, the ions concentration in the solution would decrease due to the reprecipi-

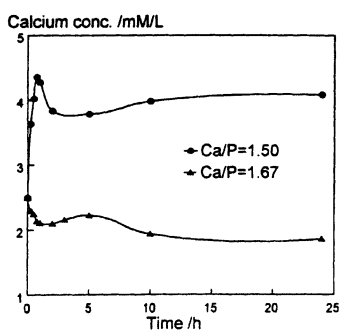


Fig. 2. Changes in calcium concentration with time in 200 ml of simulated blood plasma in the presence of five calcium phosphate cement specimens at 37°C (glass stirrer).

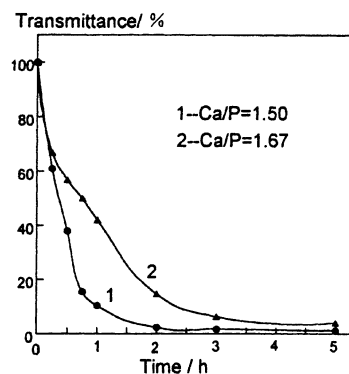


Fig. 3. Changes in the transmittance in 200 ml of simulated blood plasma in the presence of five CPC specimens at 37°C (magnetic stirrer).

tation, the layer of apatite would be precipitated onto the surface after a long time of leaching [9]. But when the specimen with $\text{Ca/P} = 1.5$ was immersed in the simulated blood plasma, the ion concentration increased. If the specimen with $\text{Ca/P} = 1.5$ was implanted in vivo, it would be degraded faster than that the specimen with 1.67. This deduction should be verified by experiments later.

4. Conclusions

The solution property of calcium phosphate cement hardening body was different from the hydroxyapatite ceramic. When the specimen with the ratio of calcium to phosphate of 1.67 were immersed in the dilute phosphoric acid solution, the solubility product, $-\log K_{\text{sp}}$, was -99.3 which was significantly higher than the values reported with reference to low crystallinity. Moreover, the calcium-deficiency existing in the lattice of hydroxyapatite would also result in the increase of solubility. When the ratios of calcium to phosphate were 1.60, 1.55, 1.50, the solubility product, $-\log K_{\text{sp}}$, were 94.7–94.8, 89.6–90.5, 85.5–86.4, respectively. The signal increases in the solubility were attributed to the deformation or disorder in the lattice resulted from the deficiency in the inner crystal. The ions adsorbed onto apatite surface did not interfere with the value of solubility product, but obviously affected the final pH and the concentration of ions in the leaching solution. The behavior of CPC hardening body in fixed volumes of simulated blood plasma confirmed the conclusion above. The glass stirrer in the upper part of the vessel was adopted instead of magnetic stirrer to avoid the influence of the operating mode on the behavior. The results showed that the specimen with $\text{Ca/P} = 1.50$ was unsaturated in the simulated blood plasma whereas with $\text{Ca/P} = 1.67$ it was supersaturated.

It could be deduced that CPC with $\text{Ca/P} = 1.50$ would degrade faster than the sample with $\text{Ca/P} = 1.67$ when CPC was implanted in vivo. The degradation rate in vivo could be controlled to a certain extent by changing the ratio of calcium to phosphate in the sample.

Acknowledgements

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