

Supplementary Information for
Electron Paramagnetic Resonance Characterization of Sodium- and Carbonate-Containing
Hydroxyapatite Cement

Eaman T. Karim,^{1,}, Veronika Szalai², Lonnie Cumberland³, Alline F. Myers⁴, Shozo Takagi¹,
Stanislav A. Frukhtbeyn¹, Ileana Pazos³, and Laurence C. Chow¹*

- 1) American Dental Association Science and Research Institute, Gaithersburg, Maryland 20899, United States
- 2) Microsystems and Nanotechnology Division, National Institute of Standards and Technology, Gaithersburg, Maryland 20899, United States
- 3) Radiation Physics Division, National Institute of Standards and Technology, Gaithersburg, Maryland 20899, United States
- 4) Center for Nanoscale Science and Technology, National Institute of Standards and Technology, Gaithersburg, Maryland 20899, United States

* Corresponding author, E-mail: karime@ada.org

Table of Contents

Items	Description	Page No.
S1	The mechanism of setting reaction of calcium phosphate cement	S-2 to S-4
Table S2	Peak-to-peak intensities of EPR signals of the CO ₂ ^{•-} free radicals of the CHA cement samples irradiated with gamma radiation at a wide range of gamma doses from 1Gy to 150 kGy. The error bars are the standard deviations of at least three repeat measurements.	S-5 to S-6
Table S3	Intensities of the EPR signals determined by double-integration of the CHA cement samples	S-6 to S-7

	irradiated with gamma radiation at a wide range of gamma doses from 30 kGy to 150 kGy and exhibiting a signal from a second paramagnetic species were analyzed.	
Table S4	The intensities EPR signals of the $\text{CO}_3^{\bullet-}$ free radicals of the CHA cement samples irradiated with gamma radiation at a wide range of gamma doses from 30 kGy to 150 kGy. The errors are the standard deviations of at least three repeat measurements.	S-7 to S-8

S1. The mechanism of setting reaction of calcium phosphate cement:

As given in the main text, the setting reaction of a self-hardening calcium phosphate cement (CPC) results from the chemical reaction between calcium phosphate compounds in an aqueous environment that is driven by the solubility difference of the reactants and the product at a given pH value. Table S1 lists some of the calcium phosphates used as starting materials for preparing hydroxyapatite (HA) by the CPC method along with the Ca/P molar ratio. The thermodynamic solubility product constant, K_{sp} , is also listed because it is used to calculate the solubility isotherm of the compound and to establish the solubility phase diagram of reactants and product

Table S1. Calcium phosphate compounds and their calcium phosphate molar ratio, and solubility product constants, K_{sp} , at 25 °C.^{1,2}

Compound	Formula	Ca/P	K_{sp}
Dicalcium phosphate anhydrous (DCPA)	CaHPO_4	1.0	$10^{-6.90}$ [3]
Dicalcium phosphate dihydrate (DCPD)	$\text{CaHPO}_4 \bullet 2\text{H}_2\text{O}$	1.0	$10^{-6.59}$ [4]
Octacalcium phosphate (OCP)	$\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \bullet 5\text{H}_2\text{O}$	1.33	$10^{-96.6}$ [5]
α -Tricalcium phosphate (α -TCP)	$\alpha\text{-Ca}_3(\text{PO}_4)_2$	1.5	$10^{-25.5}$ [6]

β -Tricalcium phosphate (β -TCP)	β - $\text{Ca}_3(\text{PO}_4)_2$	1.5	$10^{-28.9}$ [7]
Tetracalcium phosphate (TTCP)	$\text{Ca}_4(\text{PO}_4)_2\text{O}$	2.0	10^{-38} [8]
Hydroxyapatite (HA)	$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$	1.67	$10^{-116.8}$ [9]

For example, a solubility phase diagram showing solubility isotherms of tetracalcium phosphate (TTCP), dicalcium phosphate anhydrous (DCPA), and hydroxyapatite (HA) at 25 °C is shown in Figure S1. Each curve represents the solubility of a salt described in the logarithm of the total calcium concentration of the saturated solution as a function of pH. At a given pH, a salt whose isotherm lies below another is less soluble and more stable. When two isotherms intersect, as shown in Figure S1 at approximately the physiological pH (≈ 8), the solution will be saturated with both salts and is highly supersaturated with respect to HA. Under this condition, the solubility difference between the reactants and the product is the driving force of the chemical reaction. Thus, the dissolution of the two more soluble salts, TTCP and DCPA, leads to re-precipitation of HA, and this process will continue until the TTCP and DCPA are exhausted. The selection of the TTCP and DCPA in this study is related to the fact that the TTCP has a high solubility in the neutral and acidic pH regions, and DCPA has a high solubility in the neutral and basic pH regions. In addition, the TTCP possesses a higher Ca/P molar ratio than other calcium phosphate salts, including HA (Table S1). Therefore, reaction of TTCP with other calcium phosphates with lower Ca/P ratios such as DCPA would produce HA without releasing acidic or basic by-products as illustrated in equation S1:



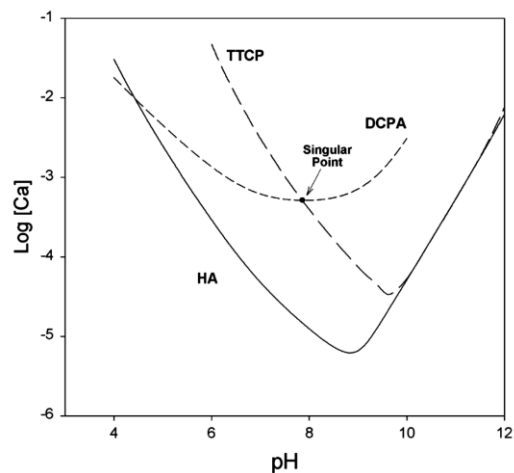


Figure S1. Solubility phase diagram for the ternary system showing solubility isotherms of TTCP, DCPA, and HA, at 25 °C. Reproduced with permission from ref 10.

Table S2. Peak-to-peak intensities of EPR signals of the $\text{CO}_2^{\bullet-}$ free radicals of the CHA cement samples irradiated with γ -radiation at a wide range of γ doses from 1Gy to 150 kGy. The error bars are the standard deviations of at least three repeat measurements.

Gamma dose, kGy	Mean of EPR intensity	Error bar (S.D.)
0.001	0.23	0
0.005	0.231	1E-4
0.01	0.46	0.001
0.025	1.24	0.29
0.05	2.5	0.17
0.1	5.54	0.67
0.25	11.7	3
0.5	15.42	0.56
1	28.76	0.96
5	89.57	16.85

10	221.33	18.6
20	377	94
30	185.32	29.71
40	272.7	7
50	377.7	104
60	249	16
70	324	64.5
80	390.56	23.21
100	354.621	30.32
120	290	17
150	337.983	51.11

Table S3. Intensities of the EPR signals determined by double-integration of the CHA cement samples irradiated with γ -radiation at a wide range of γ doses from 30 to 150 kGy and exhibiting a signal from a second paramagnetic species were analyzed.

Gamma dose, kGy	Double integral of EPR signals	Error bar (S.D.)
30	29	16.1
40	21	8.92
50	64	17.1
60	90	32.1
70	67.45	29
80	111.2	56.4
100	75	18.6

120	152	81.4
150	225	153

Table S4. The intensities EPR signals of the $\text{CO}_3^{\bullet-}$ free radicals of the CHA cement samples irradiated with γ radiation at a wide range of γ doses from 30 to 150 kGy. The errors are the standard deviations of at least three repeat measurements.

Gamma dose, kGy	Mean of EPR intensity	Error bar (S.D.)
30	1.67	0.34
40	4.87	1.5
50	7.80	1.6
60	10.22	0.65
70	15.82	3.8
80	12.25	1.6
100	17.27	1.34
120	30.95	2.13
150	32.74	2.63

REFERENCES

- (1) Chow, L. C. Next Generation Calcium Phosphate-Based Biomaterials. *Dent. Mater. J.* **2009**, 28(1), 1-10. DOI: [10.4012/dmj.28.1](https://doi.org/10.4012/dmj.28.1)
- (2) Ovenall, D. W.; Whiffen, D. H. Electron Spin Resonance and Structure of the CO_2^- Radical Ion. *Mol. Phys.* **1961**, 4 (2), 135-144. DOI: [10.1080/00268976100100181](https://doi.org/10.1080/00268976100100181)
- (3) Sutter, J. R.; McDowell, H.; Brown, W. E. Solubility Study of Calcium Hydrogen Phosphate. Ion-Pair Formation. *Inorganic Chem.* **1971**, 10 (8), 1638-1643. DOI: [10.1021/ic50102a020](https://doi.org/10.1021/ic50102a020)
- (4) Gregory, T. M.; Moreno, E. C.; Brown, W. E. Solubility of $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ in the System $\text{Ca}(\text{OH})_2\text{--H}_3\text{PO}_4\text{--H}_2\text{O}$ at 5, 15, 25 and 37.5°C. *J. Res. Nat. Bur. Stand. — A. Phys. Chem.* **1970**, 74A (4), 461-475.
- (5) Tung, M. S.; Eidelman, N.; Sieck, B.; Brown, W.E. Octacalcium Phosphate Solubility Product from 4 to 37°C. *J. Res. Nat. Bur. Stand.* **1988**, 93 (5), 613-624.
- (6) Fowler, B. O.; Kuroda, S. Changes in Heated and in Laser-Irradiated Human Tooth Enamel and their Probable Effects on Solubility. *Calcif. Tissue Int.* **1986**, 38 (4), 197-208. DOI: [10.1007/BF02556711](https://doi.org/10.1007/BF02556711)
- (7) Gregory, T. M.; Moreno, E.C.; Patel, J. M.; Brown, W. E. Solubility of $\beta\text{-Ca}_3(\text{PO}_4)_2$ in the System $\text{Ca}(\text{OH})_2\text{--H}_3\text{PO}_4\text{--H}_2\text{O}$ at 5, 15, 25, and 37°C. *J. Res. Nat. Bur. Stand. — A. Phys. Chem.* **1974**, 78A (6), 667-674. DOI: [10.6028/jres.078A.042](https://doi.org/10.6028/jres.078A.042)
- (8) Matsuya, S.; Takagi, S.; Chow, L. C. Hydrolysis of Tetracalcium Phosphate in H_3PO_4 and KH_2PO_4 . *J. Mater. Sci.* **1996**, 31 (7), 3263-3269. DOI: [10.1007/BF00354678](https://doi.org/10.1007/BF00354678)

- (9) Xu, H. H. K.; Carey, L. E.; Simon Jr., C. G.; Takagi, S.; Chow, L. C. Premixed calcium phosphate cements: Synthesis, physical properties, and cell cytotoxicity. *Dent. Mater.* **2007**, 23 (4), 433-441. DOI: [10.1016/j.dental.2006.02.014](https://doi.org/10.1016/j.dental.2006.02.014)
- (10) Chow, L. C.; Takagi, S. A Natural Bone Cement—A Laboratory Novelty Led to the Development of Revolutionary New Biomaterials. *J. Res. Natl. Inst. Stand. Technol.* **2001**, 106 (6), 1029-1033. DOI: [10.6028/jres.106.053](https://doi.org/10.6028/jres.106.053)