

# POLYMER MATRIX EFFECTS ON THE PROPERTIES OF AMORPHOUS CALCIUM PHOSPHATE-FILLED COMPOSITES

D. Skrtic,<sup>1</sup> J.M. Antonucci,<sup>2</sup> and E.D. Eanes<sup>2</sup>

<sup>1</sup>American Dental Association Health Foundation-Paffenbarger Research Center, <sup>2</sup>Polymers Division, NIST, Gaithersburg, MD 20899-8545

## Introduction

Calcium phosphates are finding increasing use as biomaterials, e.g. in the treatment of defective mineralized tissues. Crystalline hydroxyapatite (HAP) is considered to be the final stable product in the precipitation of calcium and phosphate ions from neutral or basic solutions and it is also the structural prototype of the major mineral component of bones and teeth. Over the broad range of solution conditions when precipitation occurs spontaneously, amorphous calcium phosphate (ACP) precedes the formation of HAP. ACP, a plausible key precursor in the biological formation of HAP, is characterized by a high degree of aqueous solubility and facile conversion to HAP, particularly at low pHs (1). These properties, undesirable for applications where structural, mechanical and chemical stabilities are desired, make ACP suitable as a remineralizing agent.

We have recently shown (2) that a new class of polymeric composite materials can be formulated that utilize ACP as a filler with dental monomers such as 2,2-bis[p-(2'-hydroxy-3'-methacryloxypropoxy)phenyl]propane (Bis-GMA), triethylene glycol dimethacrylate (TEGDMA), and 2-hydroxyethyl methacrylate (HEMA). Previous studies have shown that certain variations in methacrylate resin chemical structure and composition may affect the rate and extent of the ion release from ACP-filled composites, the internal conversion of ACP into HAP and the mechanical properties of the composites (4, 5).

The objective of this study was to further explore the effects of the chemical structure and compositional variations of the resins on the release of mineral ions (Ca<sup>2+</sup> and phosphate). The resins included those previously examined and other types of resin matrices that include urethane dimethacrylate (UDMA), and a carboxylic acid-containing monomer, pyromellitic glycerol dimethacrylate (PMGDMA). The effect of adding a potential coupling agent such as zirconyl methacrylate (ZrMA) was also investigated.

## Experimental\*

Six photoactivated resins, designated BT, BTHZ, TP, U0H, U66H and U132H were formulated as shown in Table 1. The photoinitiator system comprised camphorquinone (CQ) and ethyl-4-N,N-dimethylaminobenzoate (EDMAB) for BT, BTHZ, U0H, U66H and U132H composites. However, for the TP composites a mixture of 2-benzyl-2-(dimethylamino)-1-(4-(4-morpholinyl)phenyl)-1-butanone (IRGACURE<sup>®</sup> 369), and 2-hydroxy-2-methyl-1-phenyl-1-propanone plus diphenyl(2,4,6-trimethylbenzoyl) phosphine oxide (DAROCUR<sup>®</sup> 4265) was combined with CQ to enhance photopolymerization and storage stability. Three types of ACPs were prepared and evaluated as fillers: unhybridized, P<sub>2</sub>O<sub>7</sub>-stabilized ACP (Pyro-ACP), silica- (TEOS-ACP) or zirconia-hybridized ACP (Zr-ACP) (6).

Table 1. Composition of Resins (mass fraction, %)

Component	BT	BTHZ	TP	U0H	U66H	U132H
BisGMA	49.5	35.1	-	-	-	-
TEGDMA	49.5	35.1	48.65	-	-	-
HEMA	-	28.0	-	-	6.6	13.2
PMGDMA	-	-	48.65	-	-	-
UDMA	-	-	-	99.0	92.4	85.8
ZrMA	-	0.8	-	-	-	-
CQ	0.2	0.2	0.40	0.2	0.2	0.2
EDMAB	0.8	0.8	-	0.8	0.8	0.8
IRGACURE	-	-	0.8	-	-	-
DAROCUR	-	-	1.5	-	-	-

Composite pastes were made up of mass fraction of 40 % filler and a mass fraction of 60 % resin. Uncured pastes were examined by x-ray diffractometry (XRD) and Fourier-transform infrared spectroscopy (FTIR) to discern that no conversion of the ACP filler to HAP occurred at this stage. Composite disks were prepared in teflon molds (15.8 mm to 19.6 mm in

diameter and 1.55 mm to 1.81 mm thick) by visible light photocuring for 2 min/side (TRIAD 2000, Dentsply).

The remineralizing ability of the composites was tested in triplicate by immersing individual disk specimens in buffered saline solutions with conditions: pH = 7.40, ionic strength = 0.13 mol/L, 37 °C, continuous magnetic stirring and time up to 300 h. Aliquots were taken at predetermined time intervals, filtered, and the filtrates analyzed for their [Ca]<sub>tot</sub> and [PO<sub>4</sub>]<sub>tot</sub> contents using atomic absorption spectroscopy and UV/VIS spectrophotometry, respectively.

The thermodynamic stability of immersion solutions was calculated as their supersaturation relative to stoichiometric HAP and expressed as the Gibbs free energy, ΔG°:

$$\Delta G^\circ = -2.303 (RT/n) \ln(IAP/K_{sp}) \quad (1)$$

where IAP is the ion activity product for HAP, K<sub>sp</sub> is the corresponding thermodynamic solubility product, R is the ideal gas constant, T is the absolute temperature (K) and n is the number of ions in the IAP (n=18). IAP and ΔG° were calculated by the solution equilibrium calculation program EQUIL (MicroMath Scientific Software, Salt Lake City, UT).

Upon completion of the immersion tests, the disks were removed, dried at 37 °C and evaluated for the extent of ACP to HAP conversion that occurred during soaking by XRD and FTIR analysis. Experimental data were analyzed by multifactorial ANOVA (α=0.05). To determine significant differences between specific groups, appropriate multiple comparison tests were performed.

## Results and Discussion

All the composites were capable of releasing mineral ions at levels above the minimum necessary for remineralization as derived from data in Figure 1.

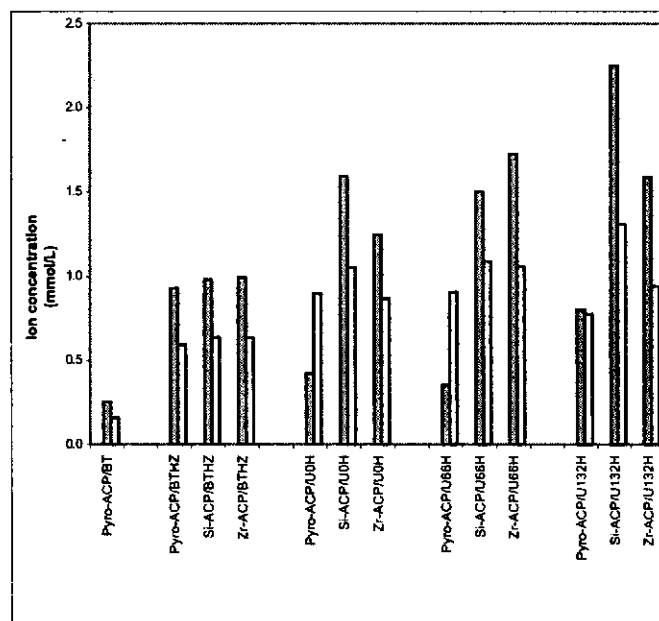


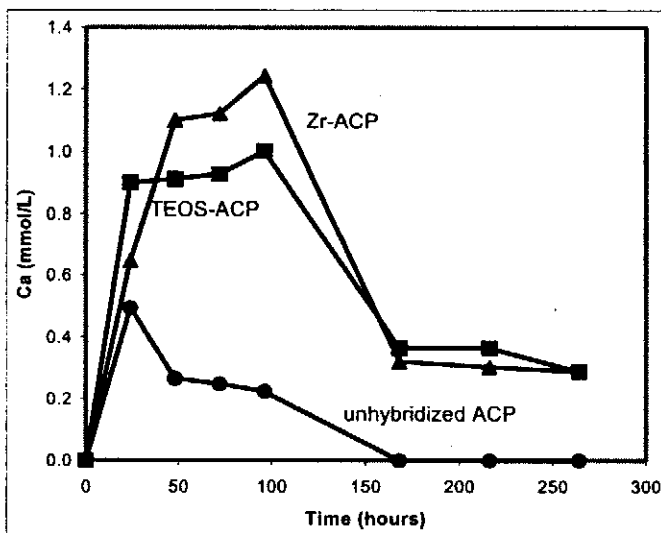
Figure 1. Maximum concentrations Ca (filled bars) and PO<sub>4</sub> (unfilled bars) released from different resins. Number of runs ≥ 3 in each experimental group. Standard deviations of values were < 0.127 mmol/L for [Ca]<sub>tot</sub> and < 0.057 mmol/L for [PO<sub>4</sub>]<sub>tot</sub>.

Mean ΔG° values ± standard deviation of the mean (SD) of the immersion solutions containing maximum concentrations of Ca and PO<sub>4</sub> released from different ACP composites are given in Table 2. A more negative ΔG° value represents a solution more supersaturated with respect to stoichiometric HAP.

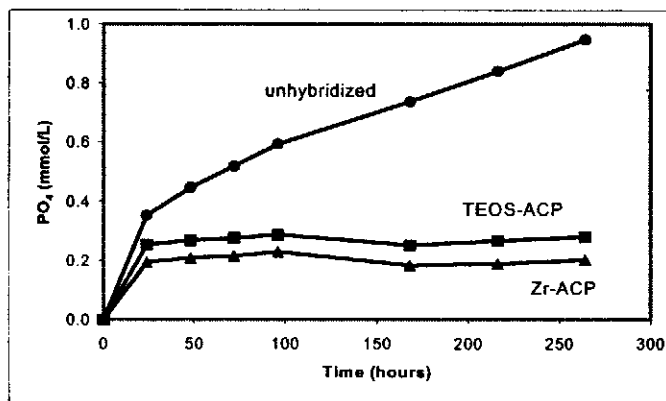
**Table 2. Remineralization Potential of ACP Composites Expressed as  $\Delta G^\circ$  (kJ/mol).**

	Pyro-ACP	TEOS-ACP	Zr-ACP
BT	-3.33±0.22	-	-
BTHZ	-4.88±0.22	-5.00±0.20	-5.02±0.18
TP	-4.42±0.11	-4.28±0.10	-4.33±0.13
U0H	-4.17±0.11	-6.01±0.18	-5.53±0.19
U66H	-3.95±0.11	-5.96±0.18	-6.12±0.10
U132H	-4.90±0.17	-6.62±0.19	-5.92±0.14

Elevated Ca and PO<sub>4</sub> concentrations were sustained by BTHZ and UH resins (data not shown). However, with increased time TP composites failed to maintain a favorable remineralization potential due to matrix uptake of released Ca via ion binding by the carboxylic acid groups of PMGDMA (Figures 2 a,b).



**Figure 2a.** Ca release from TP composites. Values are averages of triplicate runs. Standard deviations were < 0.028 mmol/L (unhybridized ACP), < 0.030 mmol/L (TEOS-ACP) and < 0.044 mmol/L (Zr-ACP).



**Figure 2b.** PO<sub>4</sub> release from TP composites. Values are averages of triplicate runs. Standard deviations were < 0.015 mmol/L (unhybridized ACP), < 0.014 mmol/L (TEOS-ACP) and < 0.010 mmol/L (Zr-ACP).

The ranking of the maximal remineralizing ability of Pyro-ACP composites was: (BTHZ, U132H) > TP > (U0H, U66H) >> BT. Composites based on the TEOS- or Zr-ACP, and U0H, U66H or U132H had significantly higher ( $p \leq 0.0013$ ; Student-Newman-Keuls test) remineralization potential compared to the similarly prepared Pyro-ACP UH composites. Hybridization of the fillers had no effect on Ca or PO<sub>4</sub> ion releases from BTHZ composites. However, internal conversion of ACP to HAP was significantly reduced or almost completely inhibited in the case of BTHZ and UH/ hybridized ACP

composites. On the other hand, Ca and PO<sub>4</sub> ion releases from TEOS-ACP and Zr-ACP based UDMA composites was significantly higher than that from Pyro-ACP based UDMA composites.

The most probable mechanism by which the hydrophilic HEMA-enriched matrix composites increased internal mineral saturation was by allowing the uptake of more water and/or better accessibility of the ACP to the water already entrained. In addition to the hydrophilicity of the matrix, cross-link density may effect ion release. The elevated Ca and PO<sub>4</sub> ion releases observed with hybridized ACP-U0H, U66H, or U132H composites was probably due to the enhanced area of contact between the hybrid ACP particulates and UDMA-based resins.

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**\*Disclaimer.** Certain commercial materials and equipment are identified in this article to specify the experimental procedure. In no instance does such identification imply recommendation or endorsement by the National Institute of Standards and Technology or the ADA Health Foundation or that the material or equipment identified is necessarily the best available for the purpose.

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