THE ION RELEASE BEHAVIORS AND WATER SORPTION OF NOVEL RESIN-BASED CALCIUM PHOSPHATE CEMENT

by

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DEDICATION

This thesis is dedicated to my family, a great source of motivation, inspiration, and support since the beginning of my studies.

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TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

INTRODUCTION

Dental caries is a site-specific, multifactorial disease. The development of caries is the result of each individual's biofilm composition and metabolism, which is influenced by biological determinants, including saliva and possibly genetic factors. Dental caries is the outcome of the metabolism of micro-organisms in the biofilm that leads to demineralization and ultimately loss of the tooth structure; this occurs when there is a drop in the pH after CHO fermentation and acid production from micro-organisms.[1] Dental caries is a dynamic process with cycles of loss and gain of minerals. Remineralization occurs when the pH is restored by mineral gain from saliva and plaque. When the demineralization and remineralization processes are in balance, no net mineral loss occurs at the tooth surface, but when one of the processes exceeds the other, this will lead to net demineralization, or alternatively, remineralization of a previously formed white spot lesion.[2]

Fluoride (F), Calcium (Ca) and phosphate (PO_4) are some of the ions that have been shown to play a role in remineralization of the tooth structure, a process which aids in preventing and managing dental caries.[3]

It is believed nowadays that the main action of fluoride in preventing enamel demineralization is the promotion of fluorapatite formation in the presence of Ca and PO_4 ions that are lost from the tooth during enamel demineralization by plaque bacterial organic acids.[4]

Calcium and phosphate are naturally available in the body and released through stimulation of the salivary glands. However, the salivary glands may not produce adequate amounts of Ca and PO_4 to maintain balance between demineralization and remineralization cycles, a situation may that result in caries lesion formation.[5] Fluoride ions also promote the remineralization of previously demineralized enamel if there is enough plaque or salivary Ca and PO_4 ions available when F is applied. However, to form one unit cell of fluorapatite ($Ca_{10}(PO_4)_6F_2$); two F ions, 10 Ca ions and six PO_4 ions are required. Therefore, when F is applied topically, the availability of Ca and PO_4 ions can be the limiting factor for the net remineralization of enamel to occur.[6] Based on this current understanding of the caries process, $CaPO_4$-filled restorative materials were developed.

$CaPO_4$-filled restorative materials are biocompatible.[3] These materials provide Ca and PO_4 ions that aid in the conversion to hydroxyapatite crystals.[7] Sustained Ca and PO_4 release is necessary to achieve the supersaturation needed to arrest mineral dissolution, and promote subsequent hard tissue remineralization by increasing the mineral content of incipient carious lesions.[8-11] In the past, the clinical use of Ca and PO_4 ions for remineralization has not been successful due to the low solubility of $CaPO_4$ especially in the presence of F ions.[6] Therefore, the amount of $CaPO_4$ released and its solubility is important. If the solubility of $CaPO_4$ is low, only a few mineral ions will interact with the demineralized tooth. That is because the insoluble calcium phosphates are not easy to apply and do not effectively localize at the tooth surface; further, acid is required for its solubility in order to produce ions that are capable of diffusing into the enamel surface lesions.[6] On the other hand, if the $CaPO_4$ solubility is relatively high, mineral deposition on the enamel surface will occur too rapidly negatively affecting subsurface mineral deposition.[12-14] Additionally, soluble Ca and PO_4 ions can be used only at very low concentrations due to the intrinsic solubility of calcium phosphates, particularly the calcium fluoride phosphates. Soluble Ca and PO_4 ions do not significantly incorporate into dental plaque and do not localize on the tooth surface to produce effective concentration to drive diffusion into the subsurface enamel.[6]

Some $CaPO_4$ technologies that have been developed are: calcium sodium phosphosilicate bioactive glass, milk-derived protein complexes containing calcium also known as casein phosphopeptide amorphous calcium phosphate nanocomplexes (CPP-ACP), amorphous calcium phosphate (ACP),[6, 8, 15] and cements containing tetracalcium phosphate (TTCP) and dicalcium phosphate (DCPA) fillers.[9] Recently, tricalcium phosphate (TCP)-filled restorative materials were introduced.

In the present study, TCP treated with fumaric acid (FA) was used as the filler and mixed with monomers to formulate a novel resin composite.

AIM OF THE STUDY

The aim of this study was to measure the concentrations of Ca and PO_4 ions that are released from a TCP-filled resin composite at various weight percent levels and to measure water sorption at eight time intervals for up to 21 days.

HYPOTHESES

1. Null Hypothesis – The tricalcium phosphate (TCP) filler levels will not have a significant effect on the ion release of Ca and PO_4 ions. The different TCP filler levels will have no significant effect on water sorption.

2. Alternative Hypothesis – The TCP filler levels will have a significant effect on the ion release of Ca and PO_4 ions. The different TCP filler levels will have a significant effect on water sorption.

REVIEW OF LITERATURE

$CaPO_4$ systems have been developed and continue to be improved. The purpose of these systems is to modify the oral environment to favor remineralization by supplying Ca and PO_4 ions.

SELECTED CALCIUM PHOSPHATE-FILLED RESTORATIVE MATERIALS

Casein Phosphopeptide Amorphous Calcium Phosphate Nanocomplexes (CPP-ACP)

This technology is based on inorganic amorphous calcium phosphate (ACP) made by combining soluble salts of Ca and PO_4 stabilized by casein phosphopeptide (CPP). The purpose of this combination is to keep Ca and PO_4 in an amorphous soluble state[16] together with F ions at the tooth surface by binding to pellicle and plaque.[6] Therefore, that binding prevents the Ca and PO_4 from spontaneous precipitation by forming a carrier complex around ACP.[16] Even though Ca, PO_4 and F ions are stabilized by CPP to prevent the formation of dental calculus, these ions are freely available to diffuse down the concentration gradients into the enamel subsurface lesions, and thus effectively promoting remineralization *in vivo*.[6]

The CPP-ACP technology has been shown to have anticariogenic activity *in vitro*, animal, human *in situ*, also in randomized, controlled caries clinical trials.[6] However, researchers have found insufficient clinical trial evidence in quantity and quality to make a recommendation regarding the long-term effectiveness of casein derivatives in preventing dental caries *in vivo*.[17]

Calcium Sodium Phosphosilicate Bioactive Glass Technology

This technology is based on calcium sodium phosphosilicate bioactive glass.[6] This technology is a result of medical bone research. It is claimed that this technology releases Ca and PO_4 ions intra-orally to help and promote remineralization of teeth.[6] Calcium and PO_4 are protected by the silicate, and when particles come in contact with water and saliva, they react and release Ca and PO_4.

However, there are no published studies supporting the remineralization of enamel surface lesions *in vitro* or *in situ*. Also, no published studies have been found showing anticariogenic efficacy of NovaMin in animal models or other caries model systems, or randomized, controlled caries clinical trials. This technology appears to be still in a very early stage of development.[6]

Amorphous Calcium Phosphate (ACP) Technology

Amorphous Calcium Phosphate technology is based on unstabilized ACP, where Ca salts and PO_4 salts are delivered separately (e.g., a dual chamber device) intraorally. When the salts mix with saliva, they dissolve releasing Ca and PO_4 ions producing an ion activity product for ACP which exceeds its solubility product. This results in the immediate precipitation of ACP, or in the presence of F ions, amorphous calcium fluoride phosphate (ACFP). The ACP and ACFP phases are very unstable in the oral environment and rapidly transform to a more thermodynamically stable, insoluble crystalline phase (e.g., hydroxyapatite and fluoroapatite). Nevertheless, before the phases are transformed to hydroxyapatite and fluoroapatite, Ca and PO_4 ions should be transiently bioavailable to inhibit demineralization of enamel and promote enamel surface remineralization.[6]

There are concerns by some authors about the unstable nature of ACP. The ACP rapidly transforms to crystalline phases in the mouth and may promote dental calculus formation. In the presence of F ions, the unstabilized ACP may produce fluoroapatite and therefore sequester the available F ions and consequently reduce their subsurface enamel remineralization ability during acid challenge.[6]

A review was done by Reynolds[6] in 2008 concerning the role of these remineralization systems in the non-invasive treatment of early carious lesions. The review revealed that there is evidence for an anticariogenic efficacy of ACP for root caries and for CPP-ACP technology in slowing the progression of coronal caries significantly and in promoting the regression of lesions in randomized, controlled clinical trials. Furthermore, the Ca-PO_4 -based remineralization technologies show promise as an adjunct to fluoride therapy in management of early carious lesions.[6]

ACP-filled restorative materials are able to provide a continuous supply of Ca and PO_4 ions.[3, 8] Facilitating the repair of demineralized tooth structures by preventing demineralization and enhancing remineralization.[3] The Ca and PO_4 release rate is increased in a changing environment, such as the oral cavity.[8] When ACP is incorporated in photo-polymerizable methacrylate matrices[18-19] and exposed to an aqueous environment, it promotes re-deposition of a thermodynamically stable, apatitic tooth mineral[3, 8] and formation of hydroxyapatite crystals (HAP).[18-19] ACP is relatively highly soluble in aqueous solutions and is rapidly transformed to HAP. This property makes ACP a suitable remineralizing agent in dental materials.[18-19] However, ACP-filled resin composites are not mechanically as strong as glass-filled resin composites. Therefore, zirconia and silica are sometimes added to give strength to ACP-filled resin composites[19-

[20] while allowing them to retain their remineralization potential.[19] The addition of these fillers can result in the release of higher concentrations of Ca and PO_4 ions.[20]

The amount of Ca and PO_4 ions released from ACP-filled resin was shown to be affected by resin matrix type, and to a lesser extent, by the type of the ACP filler (i.e. hybridized silica or zirconia vs. unhybridized ACP).[10, 19]

A study was conducted by Regnault et al.[11] in 2008, to see the amount of Ca and PO_4 released from different matrix types. Poly (ethelene glycol) extended urethane dimethacrylate (PEG-U) ACP-filled resin composites disk samples of 15 mm to 20 mm in diameter and 1 mm to 2 mm in thickness were soaked in saline for about 24 h to 72 h. Results showed a high initial release of Ca and PO_4 from (0.29 to 0.49 Ca mmol/L and 0.18 to 0.35 mmol/L PO_4) followed by a significant decrease in concentrations (0.24 mmol/L Ca and 0.25 mmol/L PO_4). The samples had a maximum water sorption (WS) after 35 days of 2.7 percent for PEG composite. Urethane dimethacrylate (UDMA) resin showed a sustained release for about 15 days. After 15 days, 0.43 mmol/L Ca and 0.4 mmol/L PO_4 was released with a maximum WS of 1.2 percent. Also, UDMA with a blend of PEG-U resin showed sustained release for about 15 days of 0.58 mmol/L Ca and 0.43 mmol/L PO_4, with a maximum WS of 1.5 percent.[11]

In 2000, a study by Skrtic et al.[19] also observed the amount of Ca and PO_4 released from different matrix types. Disk samples of 15 mm diameter and 1 mm thick were soaked in 100 ml buffered saline solution for 500 h (20 days). It was shown that Ca and PO_4 released from unhybridized ACP composites was ≤ 0.0118 mmol/L and ≤ 0.075 mmol/L, respectively, whereas the release from hybrid ACP fillers ranged between 0.087-0.137 mmol/L Ca and 0.084-0.073 mmol/L PO_4.[19]

Another study to compare Ca and PO_4 release from different matrix types was conducted by Skrtic and Antonucci[3] in 2007. Disk samples of 15-mm diameter and 1-mm thick were soaked in buffered saline solution stored at 37˚C for 216 hours (9 days). It was found that after that time period, significantly more Ca and PO_4 ions were released from an ethoxylated bisphenol A dimethacrylate (EBPADMA) matrix (3.4 ± 0.5 mmol/L of Ca and 0.8 ± 0.2 mmol/L of PO_4) compared to Bis-GMA and UDMA resin matrices (1.1 ± 0.2 mmol/L of Ca and 0.6 ± 0.1 mmol/L of PO_4). EBPADMA matrix has less cross-linked density and, therefore, a more open network structure which enhanced the diffusion of ions into the immersion environment.[3] Moreover, it was observed that hexamethylene dimethacrylate (HmDMA)- containing composites absorbed 29 percent less water compared to composites without HmDMA. Maximum WS values after 30 days for Bis-GMA/ TEGDEMA matrix were 2.9 percent, for EBPADMA/HmDMA/HEMA matrix was 3.5 percent and for UDMA/TEGDMA matrix was 3 percent .[3] In addition, it is believed that hydrophilic hydroxyethyl methacrylate (HEMA) has the potential for promoting water uptake and mineral saturation.[3, 11] Consequently, higher Ca and PO_4 levels were achieved in HEMA/EBPADMA-containing polymers.[3, 10] However, there were no significant differences in biaxial flexural strength between the different resin formulations and matrices.[3]

O'Donnell et al.[21] did a study in 2008 to explore how resin types affect the physicochemical properties of ACP-filled and glass-filled composites. They compared Bis-GMA/TEGDMA/HEMA/methacryloyloxyethyl phthalate (MEP), which is a less hydrophilic carboxylic acid monomer than HEMA with EBPADMA/TEGDMA/HEMA/MEP filled with 30-percent, 35-percent and 40-percent

ACP filler concentrations. Previous studies suggested that when comparing Bis-GMA-based composites with similar EBPADMA-based composite formulations that the later had lower WS while maintaining adequate mechanical strength and ion release concentrations. They soaked disk samples of 15 mm in diameter and 1.3 mm to 1.5 mm in thickness in saline solution for 1 month, 3 months, and 6 months. Generally, the ion release increased with the increase in filler concentration and time of immersion. After 6 months, the release of Ca ions was approximately 0.48 mmol/L, 0.9 mmol/L, 1.1 mmol/L, and PO_4 ion release was 0.18 mmol/L, 0.3 mmol/L, 0.38 mmol/L for 30-percent, 35-percent and 40-percent ACP filler concentration respectively. It was concluded that a minimum of 35-percent ACP was required to achieve the desired solution supersaturation with respect to hydroxyapatite for both Bis-GMA-based composites with similar EBPADMA-based composite formulations. They also found that Bis-GMA-based ACP composites absorbed more water (13.2 percent to 14.4 percent) than EBPADMA-based ACP composites (8.6 percent to 9.8 percent), where the 35-percent ACP concentration absorbed more water than the 30-percent and 40-percent ACP concentrations.[21]

In 2000, Skrtic et al.[19] also evaluated different monomer types with ACP used as fillers. They wanted to see whether hybridization strengthened the composites by improving interfacial interactions with polymer phase without compromising the mineral ion release. Two resin formulations were used; the first was Bis-GMA, triethylene glycol dimethacrylate (TEGDMA), HEMA and zirconyl methacrylate (ZrM). The other formulation was TEGDMA and pyromellitic glycerol dimethacrylate (PMGDM). The Ca and PO_4 ion release after soaking in 100-ml buffered saline solution at 37˚C for 500 hours for TEGDMA/PMGDM formulation were ≤0.137 mmol/L and ≤0.084 mmol/L respectively; for Zr hybridized composite formulation, ≤0.087 mmol/L Ca and ≤0.073 mmol/L PO_4; for unhybridized ACP composite, ≤0.118 mmol/L Ca and ≤0.075 mmol/L PO_4. It was concluded that the biflexural strength and remineralization ability of TEGDMA/PMGDM formulation deteriorated upon their exposure to an aqueous environment. However, ACP-filled Bis-GMA/TEGDMA/Zr (or silica) hybridized composite formulation has the potential for utilization in a more demanding restorative, sealant and adhesive application, while retaining its high remineralization potential.[19]

In a study conducted by Skrtic et al.[20] in 1996, it was reported that the use of ACP Zr and silica modified fillers can enhance the strength of methacrylate composites containing APC. After disk samples of 15 mm to 20 mm in diameter and 1 mm to 2 mm thickness were soaked for 260 hours (about 11 days) in saline solution and stored in 37˚C, their results showed ≤0.108 mmol/L Ca release and ≤0.062 mmol/L PO_4 ion release. There was a 27-percent increase in strength compared with unmodified ACP fillers while retaining, if not enhancing, the high mineralization potential for these composites.[20]

In 2006 O'Donnell et al.[10] conducted research to assess the effect of the particle size of the filler on the mechanical properties of hybridized Zr-ACP-filled methacrylate composites. The unmilled Zr-ACP was 27.48 µm and milled Zr-ACP was 9.98 µm. It was found that the mechanical milling of the filler increased the volume of fine particles in the composite specimens, which resulted in a more homogenous distribution of ACP and reduction in voids. Consequently, less WS was observed in the milled composites. The maximum WS observed for copolymers (unfilled resin) was 1.4 percent, 2.3 percent for unmilled ACP composites, and 1.6 percent for milled ACP composites. In addition, there was a significant improvement in biaxial flexural strength and a moderate improvement in flexural strength compared to composites with unmilled ACP. It was suggested that the improvement in the mechanical properties of milled Zr-ACP composites may help in extending their dental applicability.[10]

Although these $CaPO_4$-filled restorative materials may show potential, the generation of unstable $CaPO_4$ phases[6, 22] or insufficient enamel remineralization has been noted.[17, 23] Therefore, the rapid conversion to HAP is not desirable, because HAP is not soluble to be effective as a remineralizing agent.[19-20] Also, materials that contain and release $CaPO_4$ have lower mechanical strength than materials with traditional fillers and cannot be used in stress-bearing areas.[24] Therefore, additional research regarding these $CaPO_4$-filled restorative materials is desired to develop a promising $CaPO_4$ remineralizing material.

Cements Containing Tetracalcium Phosphate (TTCP) and Dicalcium Phosphate (DCPA) Fillers

Tetracalcium phosphate (TTCP:$Ca_4(PO_4)_2O$) and dicalcium phosphate (DCPA: $CaHPO_4$) can be considered as possible Ca-PO_4 candidates for remineralization, because when mixed**,** they potentially supply minerals for the conversion to HAP.[22] Novel-resin based Ca-PO_4 cements consisting of TTCP, DCPA, carboxylated and other monomers were recently reported being potential pulp-capping materials or lining cements.[25] These Ca-PO_4 fillers in these cements are similar to those used in water-based self-setting bone cement.[26] When Ca-PO_4 is combined with carboxylated, acidic monomers, polymerization initiators and water, the resulting set materials are Ca-PO_4 crystallites surrounded by a polymer matrix. The mineral phase of the set cement consists of Ca-PO_4

and HAP.[9] Resin-based cements have higher strength compared with water-based bone cements,[25-26] suggesting that the newer material may be suitable as a base when placed under restorative materials. Typically, Ca-PO_4 conversion to HAP is observed during the setting of water-based cements. This conversion is delayed in the resin-based cements. Therefore, Ca-PO_4 ions may be released freely from the cement and in theory can form a mineral deposit in demineralized tooth structure.[9]

In a study conducted by Dickens et al.[9] in 2003, they evaluated the Ca and PO_4 release after soaking for four weeks in 100-ml buffered saline solution and saliva-like solution of two Ca-PO_4 cements. One was a powder/liquid formulation, and the other was a two-paste formulation of a fluoride-releasing resin-based Ca-PO_4 cement. Both formulations were soaked in buffered saline solution and saliva like solution up to 90 days. After 90 days of soaking in buffered saline solution, Ca and PO_4 released were 0.48 mmol/L and 0.04 mmol/L from the first formulation, and 0.25 mmol/L Ca and 0.09 PO_4 mmol/L from the second formulation. After 90 days of soaking in saliva-like solution, Ca and PO_4 released were 0.8 mmol/L and 0.45 mmol/L from the first formulation, and 0.85 mmol/L Ca and 0.47 PO_4 mmol/L from the second formulation. It was concluded that the second two-paste formulation released more Ca and PO_4 and was mechanically stronger than the first powder/liquid formulation and could serve as lining material in areas where complete removal of carious tissue is contraindicated.[9]

In 2008, Dickens and Flaim[27] tested the remineralization potential of a resin-based TTCP and DCPA Ca-PO_4 ion releasing cement with or without fluoride, and with or without an acidic bonding agent. They soaked disk samples of 15 mm in diameter and 1 mm thickness in a 100-ml, saliva-like solution stored at 37˚C for 45 days. The Ca and

PO_4 released from the cements formulated without fluoride were 1.6 mmol/L and 0.4 mmol/L respectively, without fluoride but with a bonding agent 1.4 mmol/L and 0.6 mmol/L respectively. Moreover, Ca and PO_4 released from cements formulated with fluoride was 1.1 mmol/L and 0.3 mmol/L, with fluoride and bonding agent 1.2 mmol/L and 0.6 mmol/L respectively. It was concluded that there was no correlation between the Ca levels in solution or the saturation levels and the actually measured remineralization. Also, combining the cement with a bonding agent to enhance the adhesion may show a lower level of remineralization.[27]

Tricalcium Phosphate (TCP) Technology

Recently, tricalcium phosphate (TCP)-filled restorative materials were introduced. Like TTCP and DCPA; TCP was also used as bone cement.[28] TCP ($Ca_3(PO_4)_2$) has a more crystalline structure than ACP, and is therefore potentially stronger than ACP. There are different types of TCP compounds; some examples include α-TCP, β-TCP, and milled TCP (mTCP). α-TCP releases almost 10 times more calcium than β-TCP.[29] When α-TCP was added to the restorative material, larger apatite crystals formed, and the mechanical strength was increased by approximately 30 percent.[28] β-TCP is biocompatible, bioactive,[30-31] may appear as a transient phase in HAP conversion,[29] and may show lattice defects that permit crystal modification.[32] A modified β-TCP with carboxylic acids was used to create a promising surface and subsurface enamel remineralization system. Furamic acid (FA) was chosen among the carboxylic acids because it has a relatively high melting point and thermal stability, relatively slow dissolution, and strong calcium chelation tendency.[33] This β-TCP and FA combination can formulate a material that has a greater potential to remineralize demineralized tooth structure in comparison to other types of TCP compounds.[7]

RATIONALE FOR THE STUDY

In the present study, concentrations of Ca and PO_4 ions released from composite formulated with this FA-modified TCP were characterized and compared at various weight percent levels at different time intervals. We expected to see higher levels of Ca and PO_4 ions released and lower WS from resin composites with higher TCP filler concentration.

Moreover, we expected to observe an initial burst of Ca and PO_4 ions from the TCP filler in the first 24 hours, followed by a decrease in the release.[9] We also expected to see more PO_4 released than Ca.

MATERIALS AND METHODS

SAMPLE PREPARATION

Resin Formulation

The resin was formulated from commercially available monomers and photo-initiator systems. The monomers used were:

1. A relatively flexible low viscosity base monomer: ethoxylated bisphenol A dimethacrylate (EBPADMA) (Figure1).
2. A hydrophilic diluent monomer: hexamethylene dimethacrylate (HmDMA) (Figure 2).
3. A very hydrophilic monomer: 2-hydroxyethyl methacrylate (HEMA) (Figure 3).

Photo-initiator and co-initiator were added to the resins. They are:

1. Photo-initiator: camphorquinone (CQ) (Figure 4) 0.2 percent by weight.
2. Co-initiator: ethyl-4-N,N-dimethylaminobenzoate (4EDMAB) (Figure 5) 0.8 percent by weight.

The resin components and weight percents are summarized in Table I.

TCP Filler Preparation

TCP fillers (Figure 6) were provided by Indiana Nanotech. Filler size is 1 μm to 4 μm.

Preparation of the Composites and Specimens

Resin composites were prepared at four different filler levels by weight percent. The TCP fillers were prepared with 30-percent, 40-percent, 50-percent, and 60-percent by weight were mixed with resins of 70-percent, 60-percent, 50-percent, and 40-percent by weight respectively, using hand spatulation. The four filler-level groups were labeled A, B, C, and D, respectively. Monomers were mixed with a magnetic stir bar on a magnetic stir plate. After several minutes of rapid mixing, the filler was added in small amounts to ensure incorporation into the monomer mixture. After all filler was added the monomer and filler mixtures were left on the magnetic stir plate overnight to ensure homogenization. After that, initiator and co-initiator were added in the same manner. Afterwards, the homogenized pastes were kept under vacuum overnight for elimination of the air entrapped during mixing.

The samples were prepared according to ISO 4049 guidelines for water sorption. The pastes were molded into disks (15 mm in diameter and 1 mm in thickness) by filling the circular openings of flat Teflon molds, covering each side of the mold with a Mylar film plus a glass slide.[3] The Sample disks were cured by three overlapping irradiation cycles of 40 seconds with visible light (DEMI LED light curing system, Kerr) and power density output of 1463 mW/cm^2 on each side of the mold assembly to insure curing. The power density of the DEMI was monitored throughout sample preparation with the aid of a Cure Rite Visible Curing Light Meter (Caulk, Milford, DE, USA).

After samples were removed from the Teflon molds, they were inspected in the light for any voids. Then the peripheries were finished to remove flash and irregularities with 1000-grit abrasive paper on a non-rotating grinding table with water so that the periphery was abraded. Peripheries of each sample were visually inspected to insure they were smooth. Each sample was washed with distilled water, wiped with tissues and then blown with a dust off blower to insure all debris was removed. Then each sample dimensions was measured with a caliper. Two measurements of the diameter at right angles were measured and were maximum 15 mm ± 0.1. The thicknesses of the samples were measured at the center of the sample and at four equally spaced points on the circumference where they were maximum 1± 0.1. The area was calculated in square millimeters using the mean diameter, and the volume was calculated in cubic millimeters using the mean diameter and mean thickness values.

MINERAL IONS RELEASE TESTS

A total of 160 samples were prepared by the student investigator using 40 samples from each group of filler concentration: 30-percent (group A), 40-percent (group B), 50-percent (group C), and 60-percent (group D) by weight. The study included soaking samples at eight time points; four hours (4 h), eight hours (8 h), 12 hours (12 h), 24 hours (24 h), three days (3 d), seven days (7 d), 14 days (14 d), and 21 days (21 d). The time points were chosen based on the study by Dickens.[9] We expected to see an initial fast release between time zero and 24 hours. Since the concentration change would be fast, we decided to have four measurement time points in the first 24 hours. We then expected the ion concentration change to slow down until seven days and maintain at a constant level after 14 days. We therefore chose to have one time point every seven days and chose to use 21 days as the end point of measurement.

Each immersion time point included five samples of each group of different filler concentrations (30-percent, 40-percent, 50-percent, and 60-percent by weight) making a total of 20 samples. Samples were weighed, then immersed in 100-ml deionized water and then placed in a 37˚C incubator for the specified time points.

The ion release from the composite disk specimens was examined by the student investigator at 37°C.

Table II includes the details of the sample grouping at different filler concentrations and immersion times.

Calcium analysis of samples was conducted using atomic absorption spectroscopy:

1. Fifty-µl (50-ul) aliquot of each sample was transferred to a 7-ml vial.
2. One ml (1 ml) of lanthanum chloride ($LaCl_3$) was added to the vial.
3. Three-point-nine-five ml (3.95 ml) of DI water was added to the vial.

Each sample was analyzed with a Perkin Elmer Analyst 200 (Figure 7).

Calcium concentration was measured (as above) by comparison with a standard curve. The volume of sample and water (50 µl and 3.95 ml) was adjusted so that the final calcium concentration was above the detection limit of the instrument and below the highest standard. Total volume was 5 ml and the volume of $LaCl_3$ was constant.

The volumes of sample and water are listed below.

1. For groups A, B and C samples that were immersed for 4 h, 8 h, 12 h, 24 h and 3 d:
    a) Two-ml (2-ml) aliquot of each sample was transferred to a 7-ml vial.
    b) One ml (1 ml) of lanthanum chloride ($LaCl_3$) was added to the vial.
    c) Two ml (2 ml) of DI water was added to the vial.

2. For group D samples immersed for 3 d, and groups A, B and C immersed for 7 d, 14 d, and 21 d:
    a) One-ml (1-ml) aliquot of each sample was transferred to a 7-ml vial.
    b) One ml (1 ml) of lanthanum chloride ($LaCl_3$) was added to the vial.
    c) Three ml (3 ml) of DI water was added to the vial.
3. For group D samples immersed for 7 d, 14 d, and 21 d:
    a) Zero-point-five ml (0.5-ml) aliquot of each sample was transferred to a 7-ml vial.
    b) One ml (1 ml) of lanthanum chloride ($LaCl_3$) was added to the vial.
    c) Three-point-five ml (3.5 ml) of DI water was added to the vial.

Phosphate analysis of samples was conducted using light spectroscopy by the student investigator.

1. Two-point-five ml (2.5 ml) aliquot of each sample was transferred to a 3-oz plastic cup with a stir bar.
2. Two-point-five ml (2.5 ml) of ammonium molybdate (MOLY) was added to the cup.
3. One ml (1 ml) of para-amino naphtholsulfonic acid (ANSA) was added to the cup.
4. Nineteen ml (19 ml) of DI water was added to the cup.

The mixture was covered, stirred for 30 seconds, and allowed to sit for 20 minutes. Each sample was then stirred again for 30 seconds and analyzed with a Milton Roy Spectronic 601 (Figure 8).

Phosphate concentration was measured (as above) by comparison with a standard curve. The volume of sample and water (2.5 ml and 19 ml) was adjusted so that the final phosphate concentration was above the detection limit of the instrument and below the highest standard. Total volume was 25 ml, and the volume of MOLY and ANSA was constant.

The volumes used for group A and B were transferred to a 3-oz plastic cup with a stir bar.

1. Ten-ml (10-ml) aliquot sample was added to the cup.
2. Two-point-five ml (2.5 ml) of MOLY was added to the cup.
3. One ml (1 ml) of ANSA was added to the cup.
4. Eleven-point-five ml (11.5 ml) of DI water was added to the cup.

The volumes used for group C were transferred to a 3-oz plastic cup with a stir bar.

1. Five-ml (5-ml) aliquot sample was added to the cup.
2. One-point-five ml (1.5 ml) of MOLY was added to the cup.
3. One ml (1 ml) of ANSA was added to the cup.
4. Sixteen-point-five ml (16.5 ml) of DI water was added to the cup.

The volumes used for group D were transferred to a 3-oz plastic cup with a stir bar.

1. Two-point-five ml (2.5 ml) aliquot sample was added to the cup.
2. Two-point-five ml (2.5 ml) of MOLY was added to the cup.
3. Twenty-one ml (21 ml) of ANSA was added to the cup.
4. Nineteen ml (19 ml) of DI water was added to the cup.

WATER SORPTION TEST

According to ISO 4049 specifications regarding water sorption, samples were removed at each time point from the solutions, the surface water was blotted away using absorbent tissues until free from visible moisture, waved in the air for 15 seconds, and weighed 1 minute after removal from the solution. This measurement recording was m_2.

After that, the samples were placed in small metal containers and transferred to desiccators containing silica gel freshly dried for 5 h at 130˚C maintained at 37±1˚C. After 22 h, the samples were removed and stored in a second desiccator maintained at 23±1˚C for 2 h and then weighed to an accuracy of ± 0.1 mg. The cycle was repeated every day until a constant mass m_3 was obtained, i.e. until the mass loss of each sample was not more than 0.1 mg in any 24 h period.

Afterwards, the values of W_s were calculated according to the equation:

$$W_s = \frac{m_2 - m_3}{V}$$

Where:

m_2 : The mass of the specimen in micrograms (µg).

m_3 : The mass of the reconditioned samples in micrograms (µg).

V : The volume of the sample in cubic millimeters (mm^3).

DIFFUSION COEFFICENT

Diffusion coefficient was utilized previously for estimating the water uptake behavior of dental composites[34-35] and calculated from Fick's Law that predicts the initial stages of water sorption:[36]

$$\frac{M_t}{M_e} = 2\left(\frac{Dt}{\pi l^2}\right)^{\frac{1}{2}}$$

Where:

M_t : The mass uptake in (g) at time (t).

M_e : The mass uptake in (g) at equilibrium.

l : The specimen thickness in (m).

D: The diffusion coefficient (m^2/s) and was calculated from the gradient of the plot M_t/M_e against $t^{1/2}$.

STATISTICAL ANALYSIS

This sample size was determined by a power analysis using data published by Dickens[9]. From that study, we expected our calcium release data to fall in the range of 0.3-0.5 mmol/L with a group standard deviation of 0.1. With an alpha level of 0.05 and a group number of 4, a sample size of 5 in each group gave us a power of 80 percent.

Mean, standard deviation (SD), and regression for Ca and PO_4 ion release was calculated. Moreover, water sorption was calculated, WS mean, SD and diffusion coefficient were calculated.

The ion concentration and WS at each time point for each group was compared using two-way ANOVA with main variables of time and filler concentration. The significant level was set at $p = <0.001$.

Also, ion concentration and WS at the last time point (21 days) for each group was compared using one-way ANOVA with the filler concentration as the variable. The significant level was set at $p = <0.001$.

RESULTS

CALCIUM ION RELEASE

Mean and standard deviation (SD) were calculated for Ca release and presented in Table III, Figures 9 and 10. Figure 9 shows the Ca release short and long-term data from 4 hours-21 days. Figure 10 shows only the short term Ca release data from 4-24 hours. The short-term data demonstrate an initial burst of Ca ions in the first 24 hours, followed by a constant linear release in the long-term data between 1-21 days.

Two-way ANOVA test revealed that there is a statistically significant interaction between time and filler level percent ($p = <0.001$) on the Ca released.

One-way ANOVA test for only 21 day data revealed that there is a statistically significant difference in filler level percent at ($p = <0.001$).

REGRESSION ANALYSIS FOR CALCIUM

Regression analysis of Ca release data is presented in Figure 11 and Figure 12. Figure 11 shows the linear regression for 1-day through 21-day data, and Figure 12 shows the logarithmic regression for the initial release (first 24-hour) data. Table IV shows the release rate and R^2 for linear regression analysis, and Table V shows the R^2 for logarithmic regression analysis. From the regression analysis presented in Table IV, we found that the release rate from the TCP-filled composites is 0.0075 mmol/L/day for group A; 0.0109 mmol/L/day for group B; 0.0229 mmol/L/day for group C, and 0.033 mmol/L/day for group D.

The regression analysis of the short-term data showed that the initial burst reaction follows a logarithmic pattern, which continued for a longer period for group D. However, groups A, B and C follow a logarithmic pattern that continued to a linear pattern.

In other words, a transition from logarithmic pattern to a linear pattern happened earlier in group A, followed by group B, then group C and lastly group D.

PHOSPHATE ION RELEASE

Mean and SD were calculated for PO_4 release and presented in Table VI, Figure 13 and Figure 14. Figure 13 shows the PO_4 release of short-term and long-term data from 4 h to 21 d. Figure 14 shows only the short-term PO_4 release data from 4 h to 24 h. The short-term data also demonstrate an initial burst of PO_4 ions in the first 24 h, followed by a constant linear release in the long-term data from 1 d to 21 d.

A two-way ANOVA test revealed a statistically significant interaction between time and filler level percent ($p = <0.001$) on the PO_4 released.

One-way ANOVA testing for only 21-day data revealed a statistically significant difference in filler level percent ($p = <0.001$).

REGRESSION ANALYSIS FOR PHOSPHATE

Regression analysis of PO_4 release data are presented in Figure 15 and Figure 16. Figure 15 shows the linear regression for 1-d to 21-d data, and Figure 16 shows the logarithmic regression for the initial release (first 24-h) data. Table VII shows the release rate and R^2 for linear regression analysis, and Table VIII shows the R^2 for logarithmic regression analysis. From the regression analysis presented in Table VII, we found that the release rate from the TCP-filled composites is 0.1386 mmol/L/day for group A, 0.199 mmol/L/day for group B, 0.4675 mmol/L/day for group C, and 0.3819 mmol/L/day for group D.

The regression analysis of the short-term data showed that the initial burst reaction follows a logarithmic pattern, which continued for a longer period for group D. However, groups A, B and C followed a logarithmic pattern that continued to a linear pattern.

In other words, a transition from a logarithmic pattern to a linear pattern happened earlier in group A, followed by group B, then group C and lastly group D. This transition was observed to happen earlier in Ca than PO_4.

WATER SORPTION

Water sorption was calculated according to the ISO 4049 specification standards by calculating the weight and volume of the disk samples. Diameter of the samples ranged from 14.91 mm to 15.14 mm. Thickness of the samples ranged from 0.94 mm to 1.088 mm.

The means of the weights and volumes of the disk samples are presented in Table IX. Water sorption mean and SD were calculated and presented in Table X, Figures 17 and 18. Figure 17 shows the WS of all the data from 4 h to 21 d, and Figure 18 shows only the short-term WS data from 4 h to 24 h. It is evident from Figure 17 that there was an initial high WS in the first three days that reached a plateau after that time.

A two-way ANOVA test revealed a statistically significant interaction between the time and the filler level percent ($p = <0.001$) on WS.

A one-way ANOVA test for only 21-d data revealed a statistically significant difference in filler level percent (p = <0.001.)

DIFFUSION COEFFICIENT

Diffusion coefficient of water for the TCP-filled composite was calculated from plotting M_t/M_e against $t^{1/2}$ for the initial stages of WS and presented in Figure 19. The diffusion coefficient of each group calculated is presented in Table XI where group A had the most and group D had the least diffusion coefficient. It is evident from the figure that all groups behaved similarly.

TABLES AND FIGURES

TABLE I

Resin components and weight percent

Component	Chemical Compound	Abbreviation	Percent
Resin	Ethoxylated bisphenol A dimethacrylate	EBPADMA	34.3 percent
	Hexamethylene dimethacrylate	HmDMA	34.2 percent
	2-hydroxyethyl methacrylate	HEMA	30.5 percent
Photo initiator	Camphorquinone	CQ	0.2 percent
Co-initiator	Ethyl-4-N,N-dimethylaminobenzoate	4EDMAB	0.8 percent

TABLE II

Sample grouping at different filler concentrations and immersion times

Immersion time (20 samples/time point)	Filler concentration (5 samples/group)
4 hours	30% (group A)
	40% (group B)
	50% (group C)
	60% (group D)
8 hours	30% (group A)
	40% (group B)
	50% (group C)
	60% (group D)
12 hours	30% (group A)
	40% (group B)
	50% (group C)
	60% (group D)
24 hours	30% (group A)
	40% (group B)
	50% (group C)
	60% (group D)
3 days	30% (group A)
	40% (group B)
	50% (group C)
	60% (group D)
7 days	30% (group A)
	40% (group B)
	50% (group C)
	60% (group D)
14 days	30% (group A)
	40% (group B)
	50% (group C)
	60% (group D)
21 days	30% (group A)
	40% (group B)
	50% (group C)
	60% (group D)

TABLE III

Mean and standard deviation (SD) of Ca ion release in mmol/L

Groups	A		B		C		D	
Times	Mean	SD	Mean	SD	Mean	SD	Mean	SD
4 h	0.0078	0.0045	0.0278	0.0062	0.0605	0.0081	0.1224	0.0267
8 h	0.0142	0.0046	0.0400	0.0030	0.0841	0.0126	0.1580	0.0300
12 h	0.0167	0.0026	0.0482	0.0054	0.1048	0.0089	0.2371	0.0644
24 h	0.0187	0.0012	0.0749	0.0113	0.1076	0.0271	0.2868	0.0268
3 d	0.0342	0.0047	0.1081	0.0142	0.2004	0.0121	0.4716	0.0626
7 d	0.0767	0.0454	0.1699	0.0328	0.3293	0.0601	0.8379	0.1418
14 d	0.1260	0.0283	0.2118	0.0517	0.4488	0.0384	1.0131	0.1244
21 d	0.1684	0.0152	0.3063	0.0602	0.6298	0.0397	1.2993	0.0199

TABLE IV

Linear regression of Ca release

Group	Equation	R^2
A	$y = 0.0075 x + 0.0154$	0.9899
B	$y = 0.0109 x + 0.0739$	0.9784
C	$y = 0.0229 x + 0.1445$	0.9895
D	$y = 0.033 x + 0.5887$	0.9811

TABLE V

Logarithmic regression of Ca release

Group	Equation	R^2
A	$y = 0.0061\ln(x) + 0.0005$	0.9298
B	$y = 0.026\ln(x) - 0.0116$	0.9536
C	$y = 0.0275\ln(x) + 0.0265$	0.8912
D	$y = 0.0968\ln(x) - 0.0198$	0.9469

TABLE VI

Mean and standard deviation (SD) of PO_4 ion release in mmol/L

Groups	A		B		C		D	
Times	Mean	SD	Mean	SD	Mean	SD	Mean	SD
4 h	0.21	0.1158	0.46	0.0877	1.13	0.1119	1.88	0.6966
8 h	0.46	0.4401	0.88	0.0865	1.92	0.1127	3.92	0.6651
12 h	0.34	0.0813	1.21	0.1080	2.09	0.2415	4.47	1.2793
24 h	0.38	0.0295	1.09	0.2783	2.63	0.4976	6.18	0.7321
3 d	0.84	0.0802	2.46	0.3017	3.84	0.2461	10.85	1.1704
7 d	2.97	0.7801	3.30	0.4632	5.55	1.0900	15.36	2.3660
14 d	2.55	0.4640	3.09	0.8493	9.02	0.5870	18.87	2.0888
21 d	3.42	0.2773	6.01	1.0126	12.02	0.8414	20.70	0.5458

TABLE VII

Linear regression of PO_4 release

Group	Equation	R^2
A	y = 0.1386 x + 0.7555	0.7244
B	y = 0.199 x + 1.3592	0.8364
C	y = 0.4675 x + 2.3118	0.9987
D	y = 0.3819 x + 12.964	0.9683

TABLE VIII

Logarithmic regression of PO_4 release

Group	Equation	R^2
A	y = 0.3611ln(x) - 0.2888	1
B	y = 0.6747ln(x) - 0.4869	0.9926
C	y = 0.8174ln(x) + 0.0776	0.9742
D	y = 2.3481ln(x) - 1.2458	0.9877

TABLE IX

Mean dimensions and weights of water sorption (WS) samples

Group	Time	Mean Diameter (D)	Mean Thickness (T)	Mean Volume (V)	Mean m_2	Mean m_3
A	4 h	15.00	1.01	178.39	0.26	0.25
B		14.98	0.99	175.09	0.29	0.29
C		15.01	1.03	182.85	0.32	0.32
D		15.02	1.03	182.60	0.36	0.35
A	8 h	15.08	1.06	188.91	0.27	0.26
B		15.08	1.03	183.59	0.29	0.28
C		15.08	1.03	183.32	0.33	0.32
D		15.05	1.08	191.90	0.38	0.37
A	12 h	15.04	1.00	177.16	0.27	0.26
B		15.04	1.00	178.35	0.30	0.29
C		15.05	1.01	179.92	0.33	0.32
D		15.02	1.09	192.18	0.38	0.36
A	24 h	15.02	1.03	182.16	0.28	0.27
B		15.03	1.04	185.11	0.31	0.30
C		15.04	1.05	187.13	0.34	0.33
D		14.99	1.06	187.85	0.40	0.37
A	3 d	15.08	1.03	183.32	0.29	0.27
B		15.06	1.00	178.01	0.30	0.29
C		15.07	1.06	188.02	0.35	0.33
D		15.03	1.06	188.61	0.39	0.36
A	7 d	15.01	1.02	180.39	0.28	0.26
B		15.01	1.03	182.63	0.30	0.29
C		15.05	1.05	186.64	0.34	0.31
D		15.04	1.06	187.88	0.38	0.34
A	14 d	15.06	1.01	178.85	0.28	0.27
B		15.00	1.02	180.54	0.29	0.28
C		15.01	1.05	185.70	0.33	0.31
D		15.02	1.07	189.54	0.38	0.34
A	21 d	15.03	0.97	172.40	0.28	0.27
B		15.03	1.00	176.82	0.30	0.28
C		15.04	0.98	173.70	0.32	0.30
D		15.06	1.00	177.38	0.36	0.33

TABLE X

Mean and standard deviation (SD) of WS in µg/mm^3

Groups	A		B		C		D	
Times	Mean	SD	Mean	SD	Mean	SD	Mean	SD
4 h	30.6	0.891	34.7	2.135	35.4	1.922	41.6	5.576
8 h	41.06	2.372	43.9	1.295	51.2	2.676	58.4	4.429
12 h	50.0	0.876	55.1	1.768	60.7	2.975	75.9	2.577
24 h	53.1	24.759	72.4	1.076	86.6	3.292	134.3	11.632
3 d	79.5	3.534	95.6	5.121	117.4	3.584	160.3	2.807
7 d	79.4	2.230	95.2	2.519	119.3	4.374	164.6	6.192
14 d	82.6	4.449	95.1	7.767	124.1	11.327	166.9	8.143
21 d	86.1	2.874	100.8	3.702	130.2	3.065	180.2	11.548

TABLE XI

Diffusion coefficient of WS data

Group	Equation	Diffusion Coefficient (m^2/s)
A	$y = 0.029x$	6.74×10^{-12}
B	$y = 0.028x$	6.03×10^{-12}
C	$y = 0.024x$	4.80×10^{-12}
D	$y = 0.021x$	3.67×10^{-12}

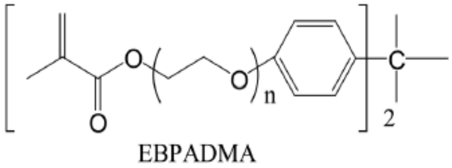


FIGURE 1. Chemical structure of ethoxylated bisphenol A dimethacrylate monomer (EBPDMA).

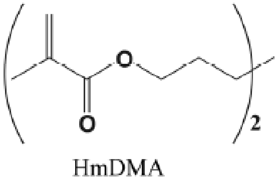


FIGURE 2. Chemical structure of hexamethylene dimethacrylate monomer (HmDMA).

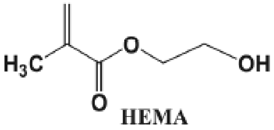
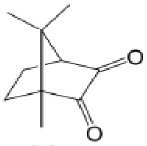
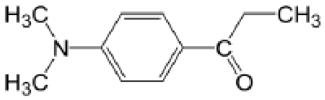


FIGURE 3. Chemical structure of 2-hydroxyethyl methacrylate monomer (HEMA).



CQ

FIGURE 4. Chemical structure of camphorquinone initiator (CQ).



4EDMAB

FIGURE 5. Chemical structure of ethyl-4-N,N-dimethylaminobenzoate co-initiator (4EDMAB).

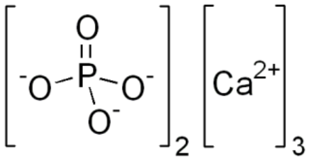


FIGURE 6. Chemical structure of tricalcium phosphate filler (TCP).

FIGURE 7. Calcium analysis by atomic absorption spectroscopy
(Perkin Elmer Analyst 200).

FIGURE 8. Phosphate analysis using light spectroscopy (Milton Roy Spectronic 601).

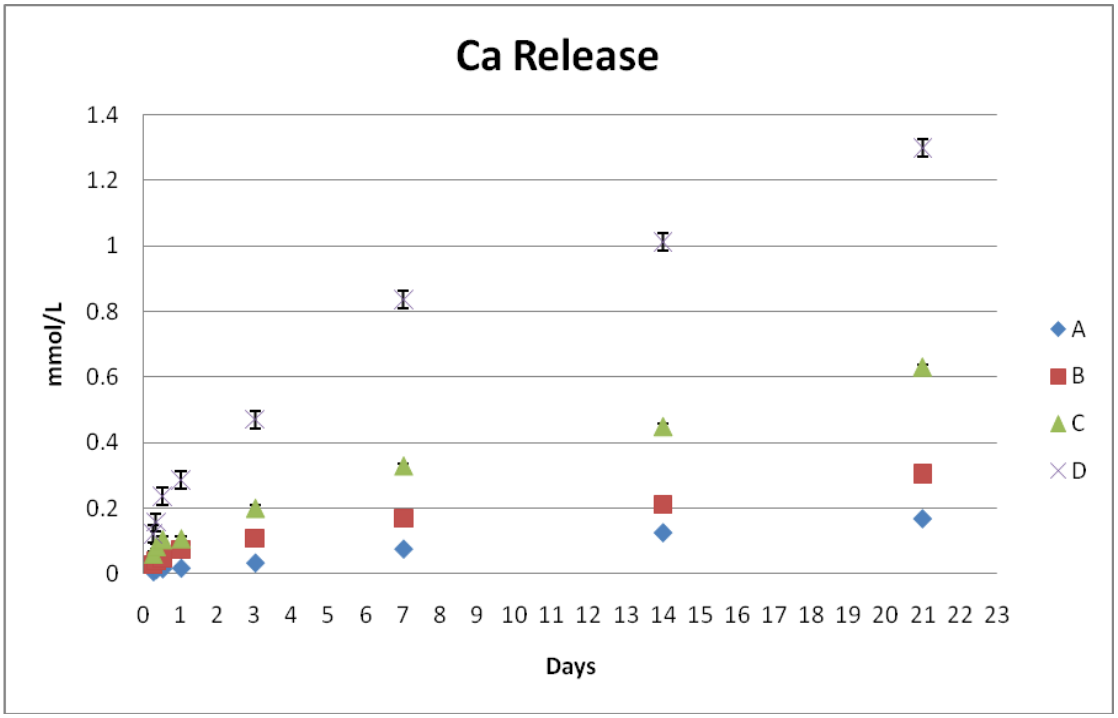


FIGURE 9. Mean and standard deviation (SD) for Ca ion release.

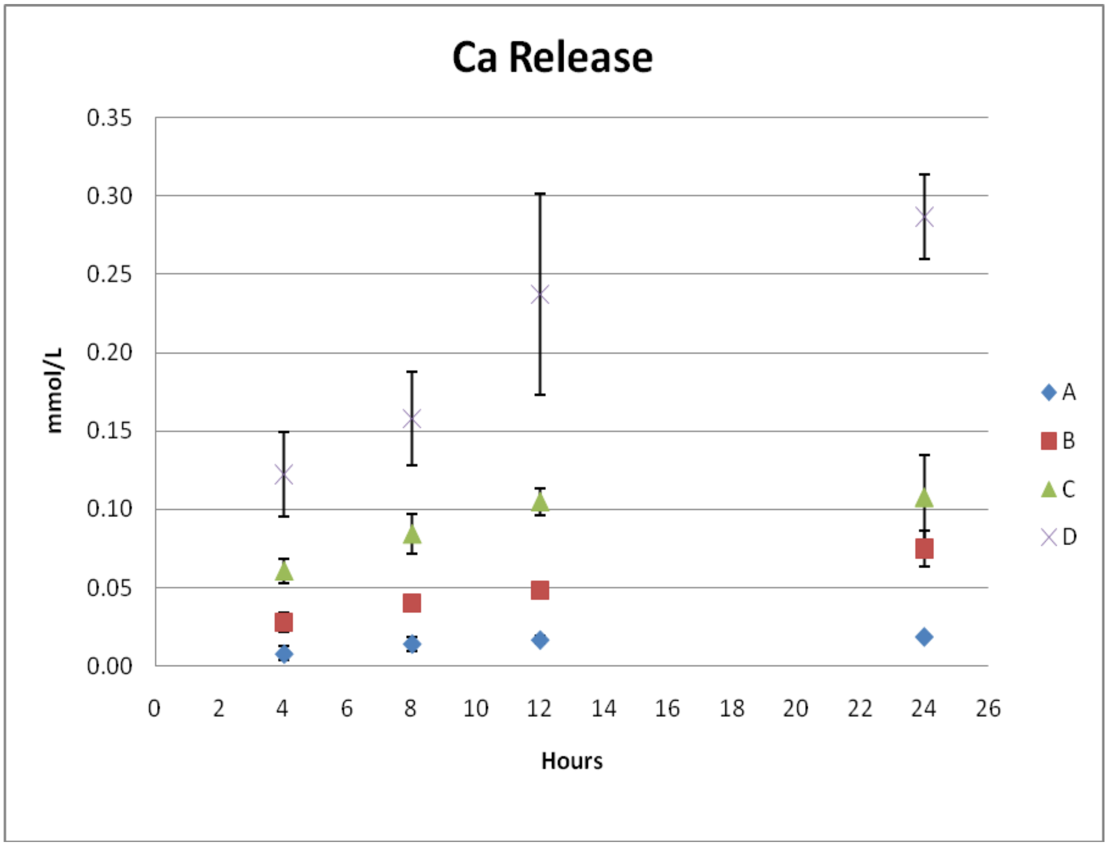


FIGURE 10. Mean and SD for short-term Ca ion release
(4-h to 24-h data) from Figure 9.

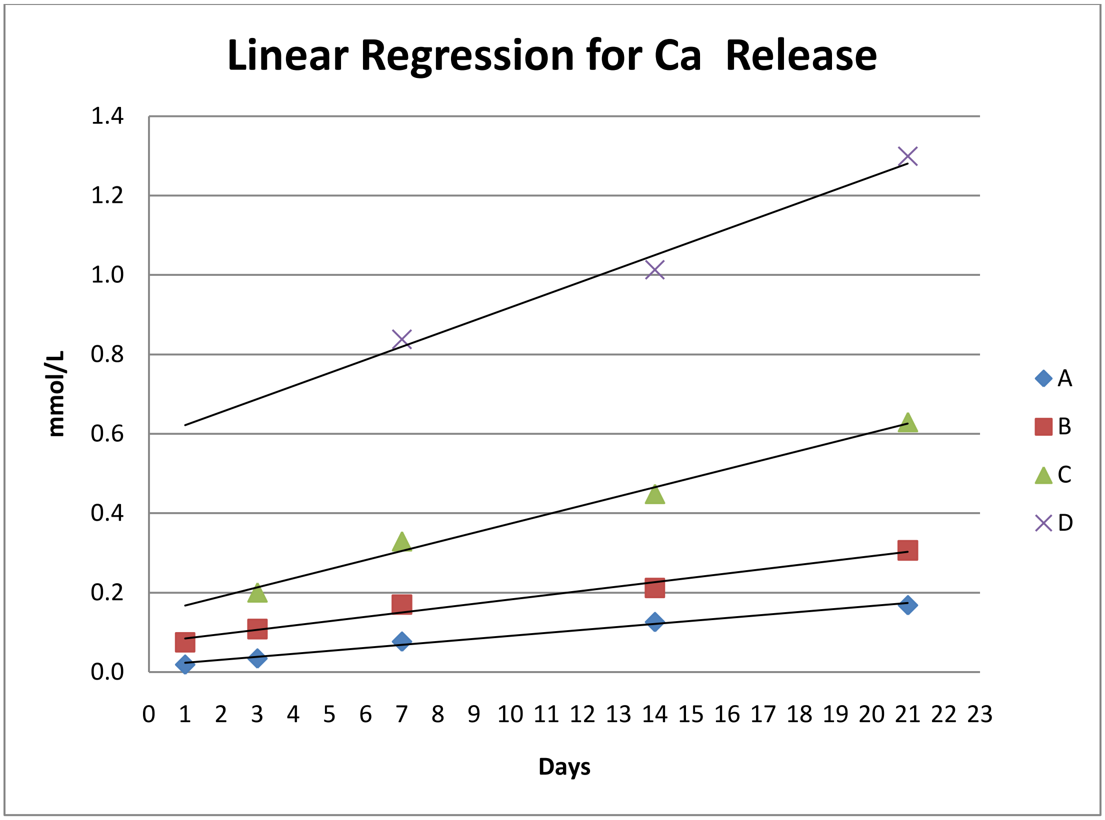


FIGURE 11. Linear regression of Ca release.

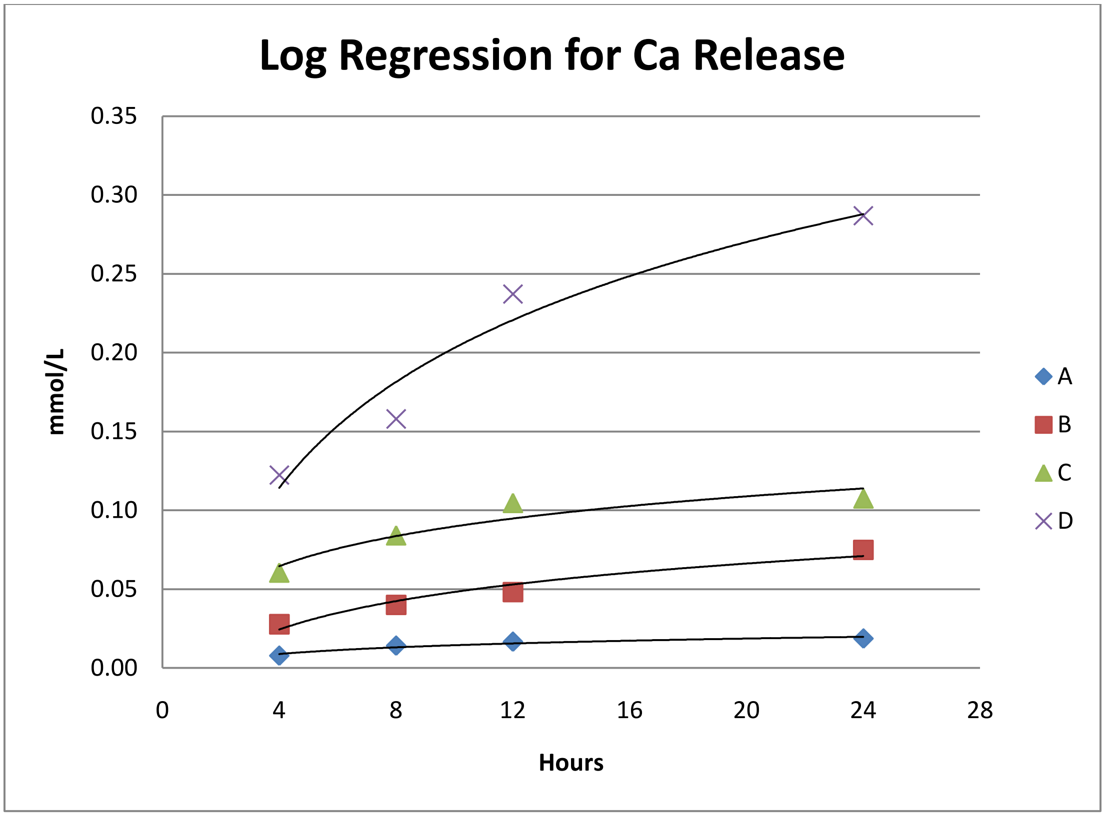


FIGURE 12. Logarithmic regression of Ca release.

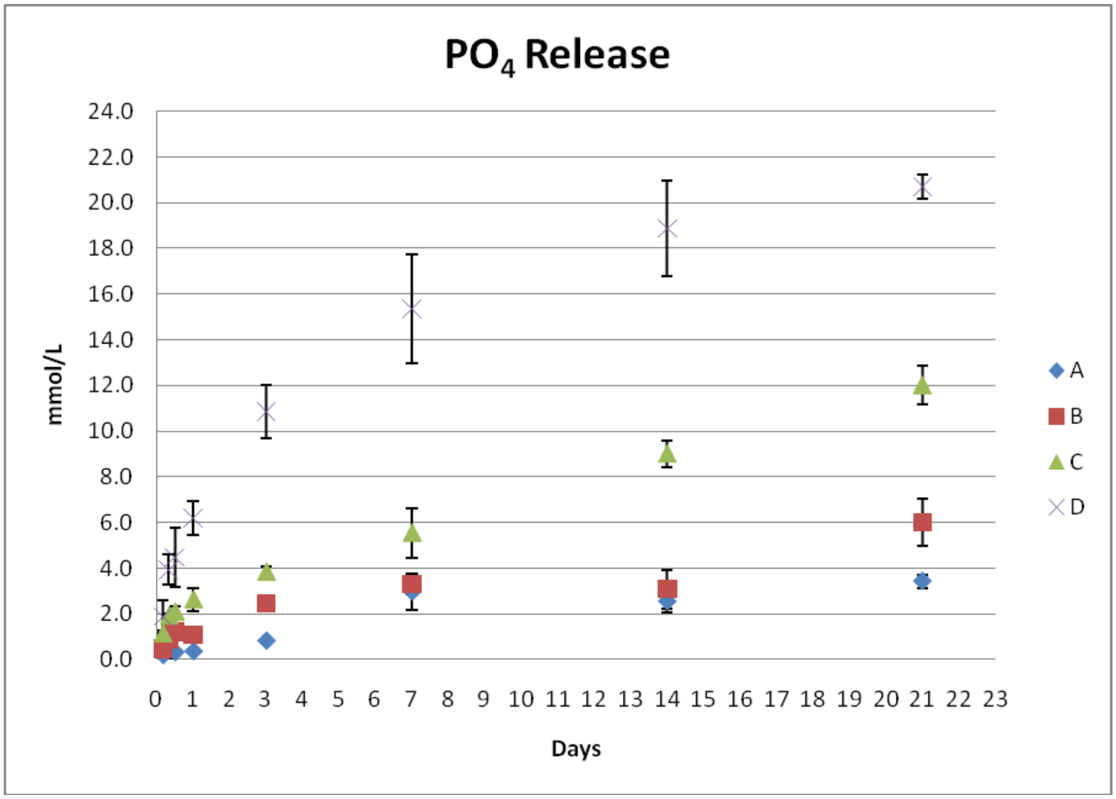


FIGURE 13. Mean and SD of PO_4 ion release.

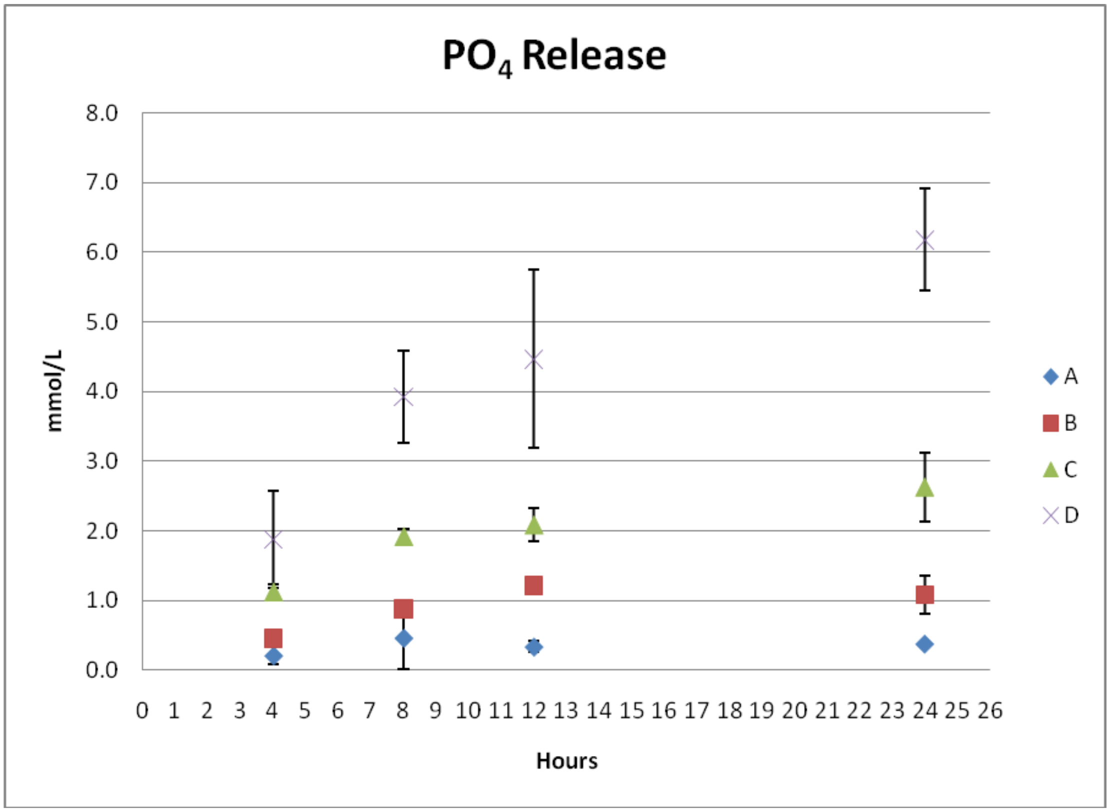


FIGURE 14. Mean and SD of short-term PO_4 ion release (4-hr to 24-h data) from Figure 13.

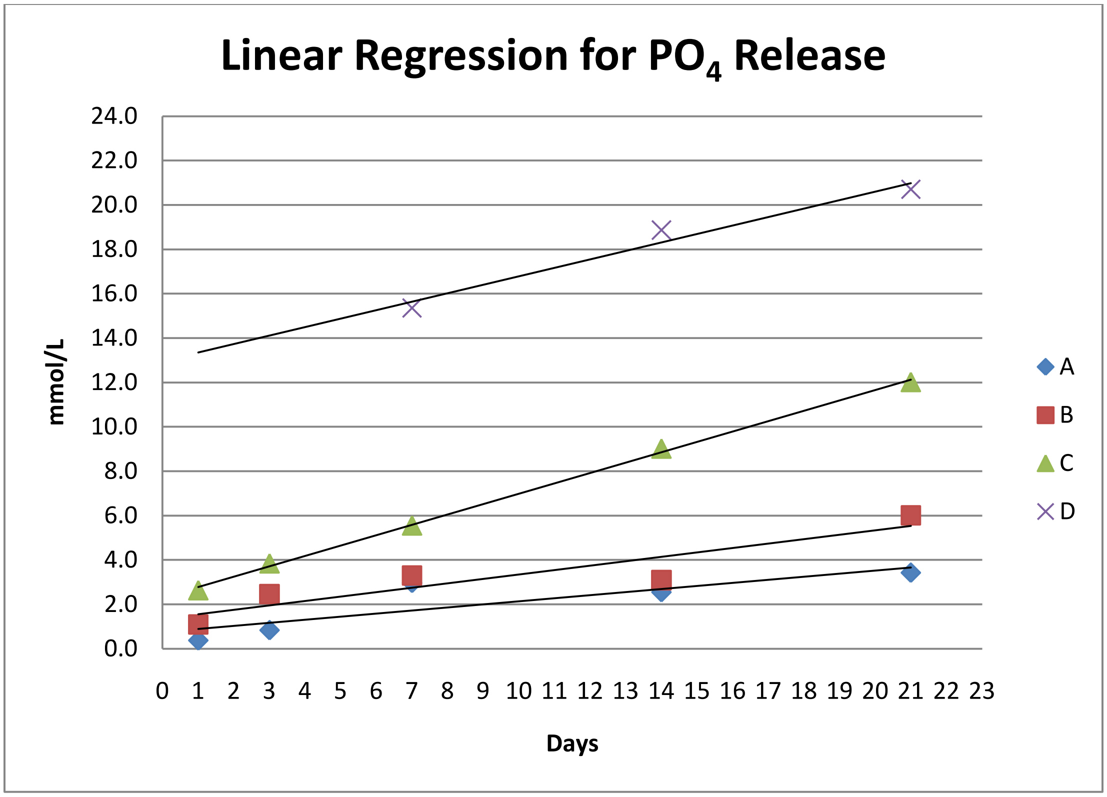


FIGURE 15. Linear regression of PO_4 ion release.

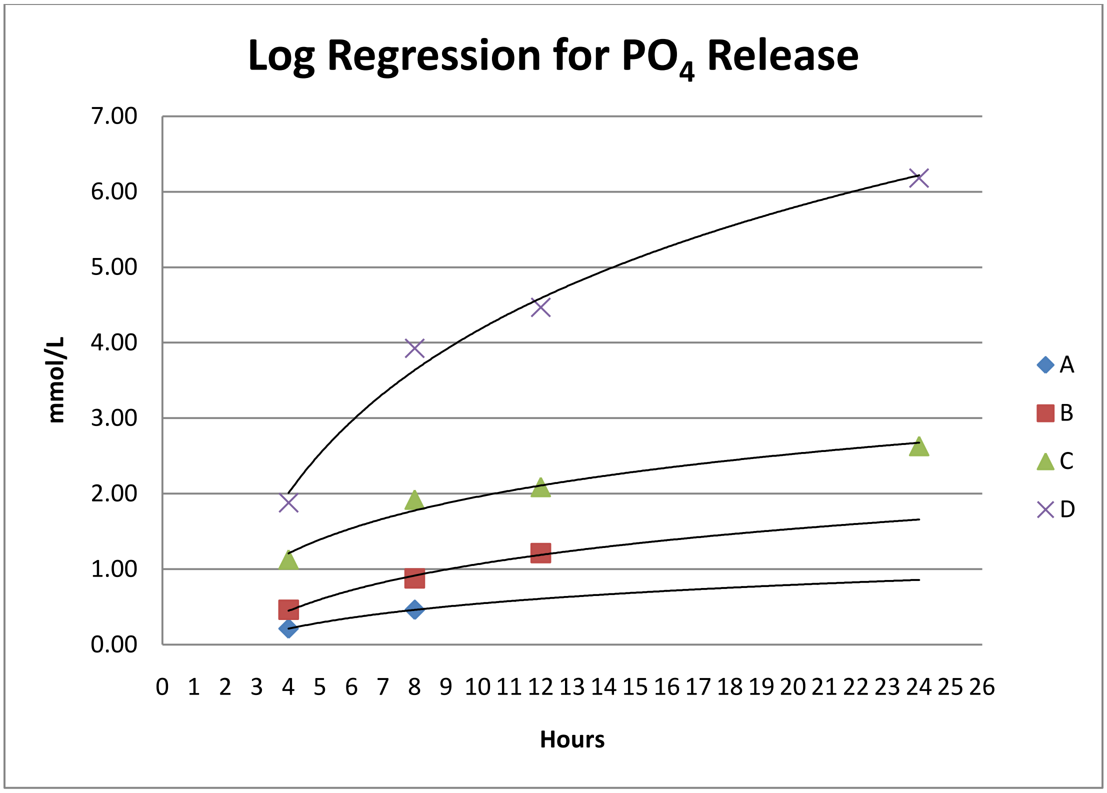


FIGURE 16. Logarithmic regression of PO_4 ion release.

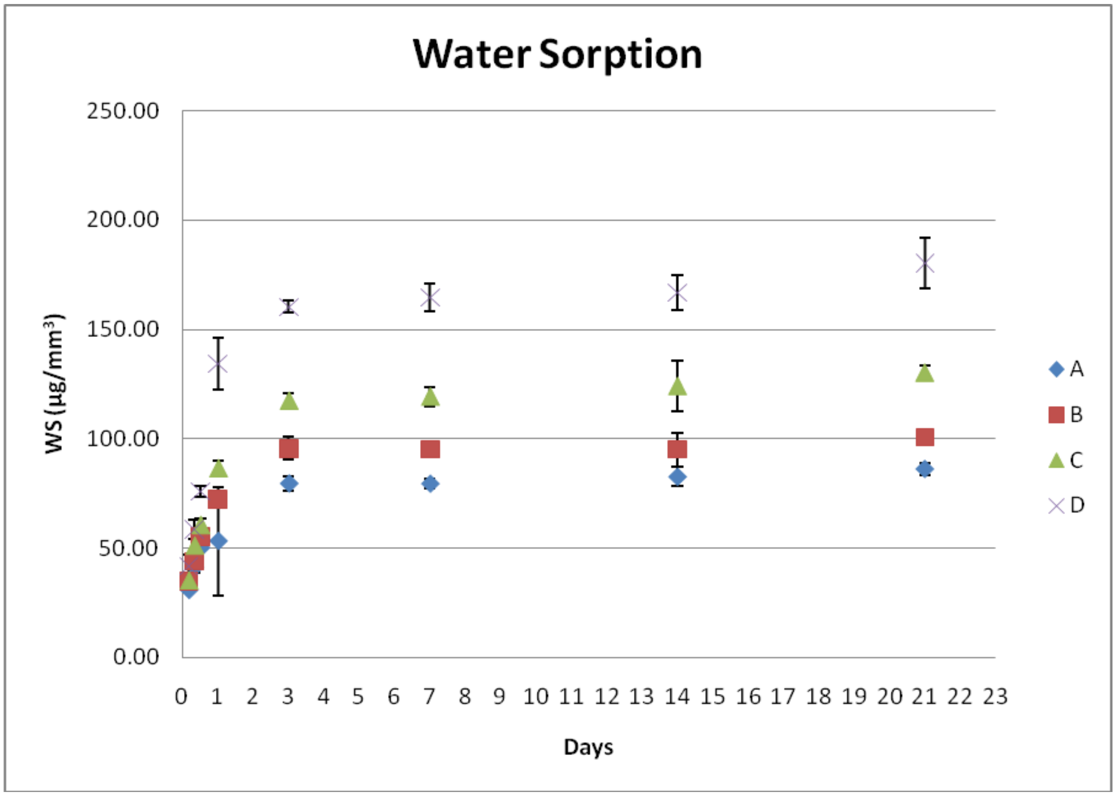


FIGURE 17. Mean and SD for WS data.

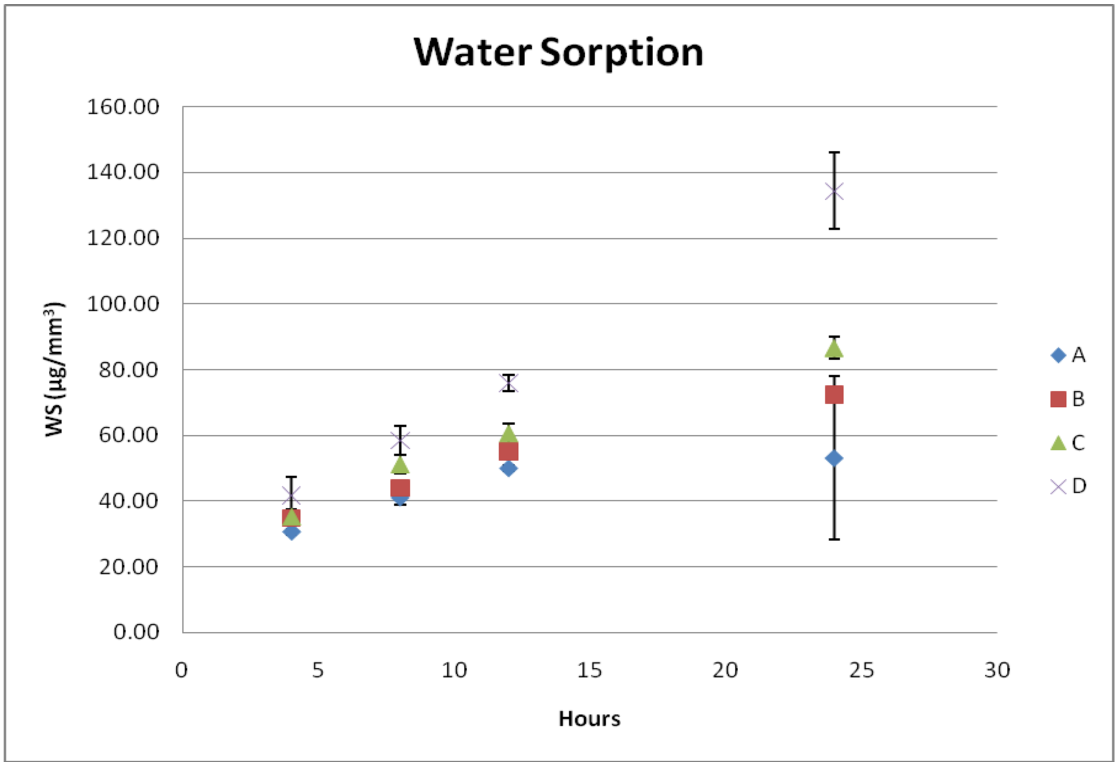


FIGURE 18. Mean and SD for short-term WS (4-h to 24-h data) from Figure 17.

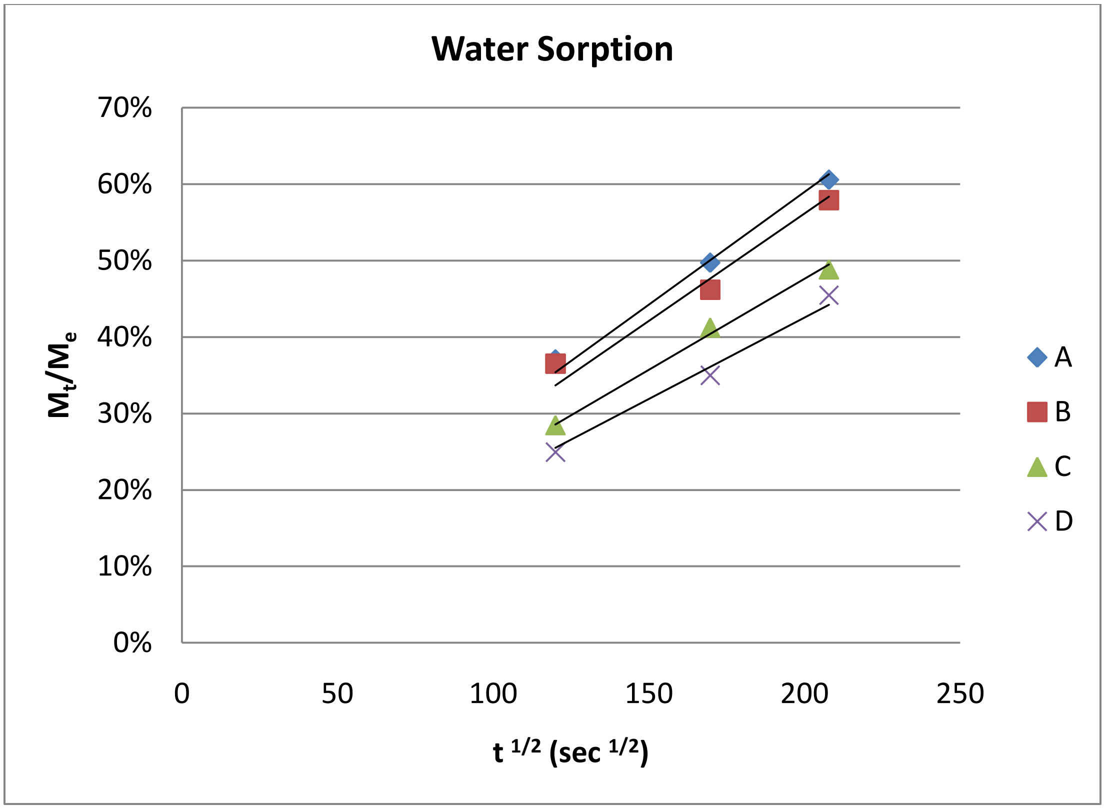


FIGURE 19. Diffusion coefficient of WS data.

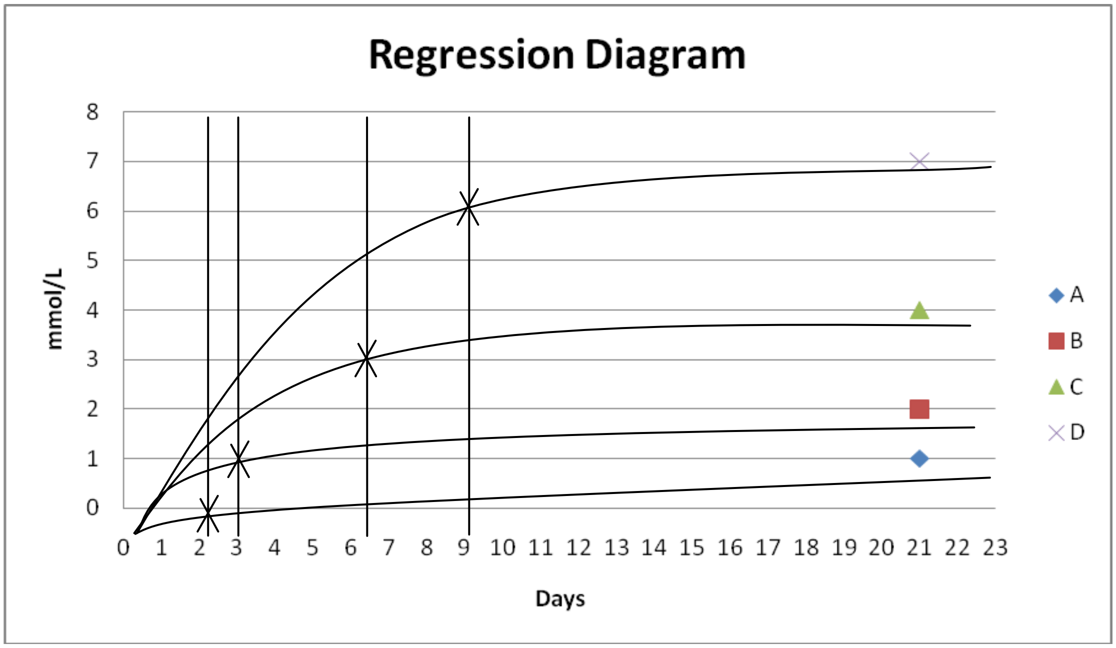


FIGURE 20. Simplified diagram for log and linear regression relation.

DISCUSSION

CALCIUM AND PHOSPHATE RELEASE

As mentioned in the literature review, there are several factors determining the amount of Ca and PO_4 release. They are:

1. The nature of the polymer network structure[21, 37]and composition of the monomer system (major effect on the release).[19]
2. Filler level[21] and type (much lesser effect on the release).[19]
3. Material permeability to water diffusion rate.[11, 21, 37]
4. Internal pH.[37]
5. Internal rate of conversion to a thermodynamically stable apatite.[11, 21, 37]

From Figure 9 and Figure 13, we can see that there is a similar trend in Ca and PO_4 ion release, which is an initial burst of Ca and PO_4. In the first 24 hours, it followed a power relation first and then turned into a linear release relation from 1 day to 21 days. However, a plateau was not reached yet after 21 days for both Ca and PO_4.

The mean and SD of the Ca and PO_4 ion release are revealed in Table III and Table VI. In general, the ion release increased with increasing immersion time and was least for group A with the least filler concentration; however, the ion release increased as the filler concentration increased.

Although both Ca and PO_4 have the same release trend, PO_4 release concentrations were 20 times more than Ca release. This trend is because of the chelating effect of FA on the calcium ions. Therefore, more PO_4 is released, although the filler used is TCP, which contains 3 Ca ions to 2 PO_4 ions. The Ca and PO_4 release trend is the reverse of studies in the literature.[3, 9, 11, 19-21, 37-39]

Two-way ANOVA testing revealed a statistically significant interaction between time and filler level percent ($p = <0.001$) on the Ca released.

One-way ANOVA test for only 21-day data revealed a statistically significant difference in filler level percent ($p = <0.001$).

As previously mentioned in the literature review, it was explained in Skrtic's study that EBPADMA matrix has a more open network structure because of its less cross-linked density, which enhances the diffusion of ions. In addition, it was reported that HmDMA absorbed 29-percent less water compared with composites without HmDMA.[3]. Moreover, it is believed that HEMA has the potential for promoting mineral saturation by allowing more water uptake or by enabling better access of the filler to water.[3, 11] For these reasons, Skrtic achieved higher Ca and PO_4 levels in HEMA/EBPADMA-containing polymers.[3, 10] However, this might not be the case in our TCP-filled composite when comparing our results with Skrtic's results.

The TCP filler is between 1 μm to 4 μm in size and is FA-treated. When handling the TCP filler, the particles demonstrated a tendency to agglomerate with each other causing an uneven dispersion of filler in the composite matrix.

This uneven dispersion of large TCP agglomerates throughout the composite,[21] especially in groups C and D, and the absence of coupling agent between the TCP filler and matrix might be contributing factors to the increased amount of Ca and PO_4 released.[25]

Another reason could be that samples in our current study were soaked in deionized water, while samples in the other studies were soaked in buffered saline solution[3, 9, 11, 20-21] or saliva-like solution.[9, 27] We soaked the samples in deionized water was to set absolute measurement for the ion levels in this initial study.

When the results were compared with results in the study done by Skrtic and Antonucci[3] in 2007, we can see that the Ca release concentration in our study is lower than the concentrations in the Skrtic and Antonucci[3] study with 40-percent ACP-filled composite with EBPADMA matrix after soaking samples for 9 days. From Table III in our study, we can see that group B that contains 40-percent TCP filler released less Ca after 7 days compared with 3.4 mmol/L Ca release after 9 days from ACP-filled composite in Skrtic and Antonucci's study. From Figure VI, PO_4 release in our study was very high after 7 days compared with Skrtic and Antonucci's study, where the PO_4 release was 0.8 mmol/L after 9 days.[3]

Furthermore, when comparing the Ca and PO_4 release results in our study with a study done by O'Donnell et al.[21] in 2008, we can observe the dissimilarity in the results. They found that EBPADMA matrix composite filled with 30-percent, 35-percent and 40-percent ACP filler concentration had lower WS, while maintaining adequate mechanical strength and ion release concentrations. Disk samples were soaked in saline solution for 1 month, 3 months and 6 months. After 1 month, the release of Ca ion concentration was approximately 0.01 mmol/L, 0.28 mmol/L, 0.52 mmol/L, and PO_4 ion release was 0.09 mmol/L, 0.12 mmol/L, 0.14 mmol/L for 30-percent, 35-percent and 40-percent ACP filler concentrations respectively. Table III and Table VI of our current study shows Ca and PO_4 concentration for group A (30-percent filler level) and B (40-percent filler level) after 21 days. After comparing the results of both studies, it is clear to us that Ca concentration is 17 times more at 30-percent filler level and almost similar to each other at 40-percent filler concentration. However, PO_4 concentration is 38 times more at 30-percent filler level and 43 times more at 40-percent filler level than the concentrations in O'Donnell's study.[21] As it was stated earlier, this trend is because of the chelating effect of FA on the calcium ions. Therefore, more PO_4 than Ca is released.

REGRESSION ANALYSIS FOR CALCIUM AND PHOSPHATE

The regression analysis of the short-term data showed that the initial burst reaction followed a logarithmic pattern that continued to a linear pattern. The transition from a logarithmic to a linear pattern happened earlier in group A, followed by group B, then group C and lastly group D. This regression relation is simplified in a diagram presented in Figure 20. This diagram is not accurate to the Ca and PO_4 regression data; it is only to help simplify the explanation of this relation. The vertical lines in the diagram represent the points of transformation from a logarithmic pattern to a linear regression pattern. The diagram shows that all groups had a logarithmic regression in the beginning. However, this logarithmic regression pattern transformed to a linear regression in group A rather early; group B behaved in the same manner but the logarithmic pattern was seen over a longer period followed by a linear regression pattern; group C had a longer logarithmic pattern than group B, and lastly, group D behaved for a longer period of time in a logarithmic regression pattern that transformed into a linear pattern. However, this trend was more evident in PO_4 release than Ca release data.

The data indicated that the Ca and PO_4 ion release increased with increasing filler levels at a rate faster than being linear. This release trend is similar to other studies in the literature.[24, 40]

This release trend is probably due to the following reasons:

- The amount of releasing filler particles increase with increasing filler level.[40]
- The interface between the filler and matrix is also increased with increasing filler level, which most likely serves as an easier pathway for the diffusion of water in and ions out of the TCP-filled resin composite.[40]
- Therefore, it could be concluded that the previous factors are most likely contributing to the increase in ion release rate faster than being linearly proportional to the filler level.

WATER SORPTION AND DIFFUSION COEFFICENT

Water sorption is an important property that can affect the mechanical properties of the resin composite,[41-44] having an influence on the strength, abrasion resistance, volume and color stability.[41, 43-47] When water penetrates the polymer, it results in breaking of secondary bonds (van Der Wals forces) between the polymer chains. In turn, water will have a plasticizing effect on the polymer consequently reducing the bond strength,[42] the mechanical properties,[41-44, 48] and the longevity of the material.[41, 43-44, 48]

Diffusion coefficient is the proportionality constant representing the amount of a substance diffusing across a unit area and a unit thickness through concentration gradient at a given time.[49]

According to ISO 4049 standards regarding WS, the WS values obtained of all materials should be less than or equal to 40 $\mu g/mm^3$.

It has been reported that the WS and the diffusion coefficient of resin based-composite materials are dependent on:

- The chemistry of the monomers and extent of polymerization of the polymer matrix.[11, 39, 41, 50-52]
- Filler size and distribution.[41, 51-53]
- The interfacial properties between the filler and the resin matrix.[51, 54]

Looking at the WS results in Table X, it is clear that the TCP-filled composite material did not meet the ISO 4049 requirement. It is also evident from Figure 17 that there was an initial high WS in the first 3 days that reached a plateau after this time interval.

Moreover, two-way ANOVA test showed a statistically significant difference and showed that the time and concentration are contributing factors in the WS of the TCP-filled composite. Also, one-way ANOVA test revealed a statistically significant difference in the last (21 days) time interval and filler level concentration.

The WS results contradict a study conducted by Berger et al.[55] in 2009 in which they soaked samples for 10 days in water. Water sorption was almost 18 $\mu g/mm^3$ for Esthet-X (minifill) and about 21 $\mu g/mm^3$ for Renamel (microfill).

These results also contradict and are much higher than the results in a study done by Plain et al.[36] in 2005, after soaking samples of the Z100 and Filtek Z250 resin composite samples for 30 days. The WS in Plain’s study was between 13 $\mu g/mm^3$ to 16 $\mu g/mm^3$.

When comparing the diffusion coefficient results in Figure 19 and Table XI with the study done by Plain et al.,[36] it is shown that their diffusion coefficient is a little less (6.98×10^{-13} and 4.10×10^{-13} m^2/s) than the results of our study.

One explanation for the dissimilarity in the results in WS and diffusion coefficient could be attributed to the difference in monomer chemistry and difference in filler size of resin composites.[11, 36] Since HEMA is very hydrophilic, that might be one of the reasons for the high WS values.[38]

The high WS could be attributed to the unhindered sorption of water by the TCP agglomerates with increasing time.[3] It is possible that TCP filler has a high affinity for water and that the filler/matrix interface has an influence on the water diffusion process,[11, 21] because there was no coupling agent between the filler and the matrix.[25] The lack of the intimate contact between the filler and the matrix may result in the existence of voids that also may be a contributing factor to the increased WS.[11]

Finally, the timing of plateau in WS is close to the time of release behavior transitioning from power relation to linear relation. This may indicate a transition in the ion release mechanism. Also, the release may be related to the rate of WS. When the samples are saturated with water, the Ca and PO_4 ions are released. Therefore, the release rate is determined by dissolution. And, the transition is an indicator of this change in mechanism.

SUMMARY AND CONCLUSION

The results can be summarized as follows:

1. Calcium released from the novel TCP-filled composite is similar to or lower than the calcium released from other calcium phosphate materials reported in the literature.
2. Phosphate released from the novel TCP-filled composite is greater than the phosphate released from other calcium phosphate materials reported in the literature.
3. Phosphate released from the novel TCP-filled composite is 20 times more than the calcium released.
4. The results indicate that the Ca and PO_4 ion release increased with increasing filler level at a rate faster than being linear.
5. The novel TCP-filled composite had very high water sorption and failed to meet the ISO 4049 specification requirements.
6. The novel TCP-filled composite had a high diffusion coefficient.
7. Two-way ANOVA test revealed that there is a statistically significant interaction between time and filler level percent ($p = <0.001$) on the calcium, phosphate released and water sorption.
8. One-way ANOVA test for only 21-day data revealed that there is a statistically significant difference in filler level percent ($p = <0.001$) for calcium, phosphate released and water sorption.

From the results, it can be concluded that the concentrations of calcium and phosphate released and water sorption are highly affected by the chemistry, the nature of the polymer network structure, and the composition of the monomer system. Moreover, the release is affected by the filler level and type, the dispersion, and the absence of coupling agent. Soaking media may also be contributing factors to the ion release and water sorption.

Therefore, although this novel TCP-filled restorative material may release calcium and phosphate, it cannot serve as a restorative material at this time due to high water sorption values that adversely affect the mechanical properties of the material. Further study is needed to improve the material and to evaluate its ability in promoting remineralization of the tooth structure in order for it to serve its purpose.

REFERENCES

1. Zero DT. Dental caries process. Dent Clin North Am 1999;43(4):635-64.

2. Adday M EG, Edgar M, Orchardson R. Tooth wear and sensitivity. Chemistry of demineralization and remineralization of enamel and dentine. London: Martin Dunitz Ltd; 2000.

3. Skrtic D, Antonucci JM. Dental composites based on amorphous calcium phosphate - resin composition/physicochemical properties study. J Biomater Appl 2007;21(4):375-93.

4. ten Cate JM. Current concepts on the theories of the mechanism of action of fluoride. Acta Odontol Scand 1999;57(6):325-9.

5. Whelton H. Introduction: the anatomy and physiology of salivary glands. Saliva and oral health. London: British Dental Association; 2004.

6. Reynolds EC. Calcium phosphate-based remineralization systems: scientific evidence? Aust Dent J 2008;53(3):268-73.

7. Karlinsey RL, Mackey AC, Walker ER, Frederick KE. Preparation, characterization and in vitro efficacy of an acid-modified beta-TCP material for dental hard-tissue remineralization. Acta Biomater 2009.

8. Skrtic D, Hailer AW, Takagi S, Antonucci JM, Eanes ED. Quantitative assessment of the efficacy of amorphous calcium phosphate/methacrylate composites in remineralizing caries-like lesions artificially produced in bovine enamel. J Dent Res 1996;75(9):1679-86.

9. Dickens SH, Flaim GM, Takagi S. Mechanical properties and biochemical activity of remineralizing resin-based Ca-PO4 cements. Dent Mater 2003;19(6):558-66.

10. O'Donnell JN, Antonucci JM, Skrtic D. Amorphous calcium phosphate composites with improved mechanical properties. J Bioact Compat Polym 2006;21(3):169-84.

11. Regnault WF, Icenogle TB, Antonucci JM, Skrtic D. Amorphous calcium phosphate/urethane methacrylate resin composites. (Pt 1). Physicochemical characterization. J Mater Sci Mater Med 2008;19(2):507-15.

12. Magalhaes AC, Furlani Tde A, Italiani Fde M, Iano FG, Delbem AC, Buzalaf MA. Effect of calcium pre-rinse and fluoride dentifrice on remineralisation of artificially demineralised enamel and on the composition of the dental biofilm formed in situ. Arch Oral Biol 2007;52(12):1155-60.

13. Lippert F, Parker DM, Jandt KD. In vitro demineralization/remineralization cycles at human tooth enamel surfaces investigated by AFM and nanoindentation. J Colloid Interface Sci 2004;280(2):442-8.

14. Yanagisawa T, Miake Y. High-resolution electron microscopy of enamel-crystal demineralization and remineralization in carious lesions. J Electron Microsc (Tokyo) 2003;52(6):605-13.

15. Yli-Urpo H, Narhi M, Narhi T. Compound changes and tooth mineralization effects of glass ionomer cements containing bioactive glass (S53P4), an in vivo study. Biomaterials 2005;26(30):5934-41.

16. Reynolds EC, Cain CJ, Webber FL, et al. Anticariogenicity of calcium phosphate complexes of tryptic casein phosphopeptides in the rat. J Dent Res 1995;74(6):1272-9.

17. Azarpazhooh A, Limeback H. Clinical efficacy of casein derivatives: a systematic review of the literature. J Am Dent Assoc 2008;139(7):915-24; quiz 94-5.

18. Skrtic D, Stansbury JW, Antonucci JM. Volumetric contraction and methacrylate conversion in photo-polymerized amorphous calcium phosphate/methacrylate composites. Biomaterials 2003;24(14):2443-9.

19. Skrtic D, Antonucci JM, Eanes ED, Eichmiller FC, Schumacher GE. Physicochemical evaluation of bioactive polymeric composites based on hybrid amorphous calcium phosphates. J Biomed Mater Res 2000;53(4):381-91.

20. Skrtic D, Antonucci JM, Eanes ED. Improved properties of amorphous calcium phosphate fillers in remineralizing resin composites. Dent Mater 1996;12(5):295-301.

21. O'Donnell JN, Langhorst SE, Fow MD, Antonucci JM, Skrtic D. Light-cured dimethacrylate-based resins and their composites: comparative study of mechanical strength, water sorption and ion release. J Bioact Compat Polym 2008;23(3):207-26

.

22. Nancollas G. The involvement of calcium phosphates in biological mineralization and demineralization. Pure Appl Chem 1992;64:1673-8.

23. Schirrmeister JF, Seger RK, Altenburger MJ, Lussi A, Hellwig E. Effects of various forms of calcium added to chewing gum on initial enamel carious lesions in situ. Caries Res 2007;41(2):108-14.

24. Xu HH, Weir MD, Sun L, et al. Strong nanocomposites with Ca, PO(4), and F release for caries inhibition. J Dent Res;89(1):19-28.

25. Dickens-Venz SH, Takagi S, Chow LC, Bowen RL, Johnston AD, Dickens B. Physical and chemical properties of resin-reinforced calcium phosphate cements. Dent Mater 1994;10(2):100-6.

26. Chow LC, Hirayama S, Takagi S, Parry E. Diametral tensile strength and compressive strength of a calcium phosphate cement: effect of applied pressure. J Biomed Mater Res 2000;53(5):511-7.

27. Dickens SH, Flaim GM. Effect of a bonding agent on in vitro biochemical activities of remineralizing resin-based calcium phosphate cements. Dent Mater 2008;24(9):1273-80.

28. Nakagawa A, Matsuya S, Takeuchi A, Ishikawa K. Comparison of the effects of added alpha- and beta- tricalcium phosphate on the basic properties of apatite cement. Dent Mater J 2007;26(3):342-7.

29. Tung M. Calcium phosphates: structures, composition, solubility and stability. Calcium phosphates in biological and industrial systems: Norwell, MA: Springer; 1998.

30. Ghosh SK, Nandi SK, Kundu B, et al. In vivo response of porous hydroxyapatite and beta-tricalcium phosphate prepared by aqueous solution combustion method and comparison with bioglass scaffolds. J Biomed Mater Res B Appl Biomater 2008;86(1):217-27.

31. Zhang F, Chang J, Lu J, Lin K, Ning C. Bioinspired structure of bioceramics for bone regeneration in load-bearing sites. Acta Biomater 2007;3(6):896-904.

32. Miao S, Cheng K, Weng W, et al. Fabrication and evaluation of Zn containing fluoridated hydroxyapatite layer with Zn release ability. Acta Biomater 2008;4:441-6.

33. Goldberg I, Peleg Y, Rokem JS. Citric, fumaric, and malic acids. Biotechnol Food Ingred 1991;74:349-74.

34. Sideridou I, Achilias DS, Spyroudi C, Karabela M. Water sorption characteristics of light-cured dental resins and composites based on Bis-EMA/PCDMA. Biomaterials 2004;25(2):367-76.

35. Santos C, Clarke RL, Braden M, Guitian F, Davy KW. Water absorption characteristics of dental composites incorporating hydroxyapatite filler. Biomaterials 2002;23(8):1897-904.

36. Palin WM, Fleming GJ, Burke FJ, Marquis PM, Randall RC. The influence of short and medium-term water immersion on the hydrolytic stability of novel low-shrink dental composites. Dent Mater 2005;21(9):852-63.

37. Skrtic D, Antonucci JM, Liu DW. Ethoxylated bisphenol dimethacrylate-based amorphous calcium phosphate composites. Acta Biomater 2006;2(1):85-94.

38. Antonucci JM, Fowler BO, Weir MD, Skrtic D, Stansbury JW. Effect of ethyl-alpha-hydroxymethylacrylate on selected properties of copolymers and ACP resin composites. J Mater Sci Mater Med 2008;19(10):3263-71.

39. O'Donnell JN, Antonucci JM, Skrtic D. Illuminating the role of agglomerates on critical physiocemical properties of amorphous calcium phosphate composites. J Compos Mater 2008;42(21):2231-46.

40. Xu HH. Dental composite resins containing silica-fused ceramic single-crystalline whiskers with various filler levels. J Dent Res 1999;78(7):1304-11.

41. Sideridou I, Tserki V, Papanastasiou G. Study of water sorption, solubility and modulus of elasticity of light-cured dimethacrylate-based dental resins. Biomaterials 2003;24(4):655-65.

42. Indrani DJ, Cook WD, Televantos F, Tyas MJ, Harcourt JK. Fracture toughness of water-aged resin composite restorative materials. Dent Mater 1995;11(3):201-7.

43. Gohring TN, Besek MJ, Schmidlin PR. Attritional wear and abrasive surface alterations of composite resin materials in vitro. J Dent 2002;30(2-3):119-27.

44. Musanje L, Shu M, Darvell BW. Water sorption and mechanical behaviour of cosmetic direct restorative materials in artificial saliva. Dent Mater 2001;17(5):394-401.

45. Shintani H, Satou N, Yukihiro A, et al.. Water sorption, solubility and staining properties of microfilled resins polished by various methods. Dent Mater J 1985;4(1):54-62.

46. Martin N, Jedynakiewicz NM, Fisher AC. Hygroscopic expansion and solubility of composite restoratives. Dent Mater 2003;19(2):77-86.

47. Nagem Filho H, D'Azevedo MT, Nagem HD, Marsola FP. Surface roughness of composite resins after finishing and polishing. Braz Dent J 2003;14(1):37-41.

48. Soderholm KJ, Roberts MJ. Influence of water exposure on the tensile strength of composites. J Dent Res 1990;69(12):1812-6.

49. Anusavice KJ. Phillips' science of dental materials. 11th ed. St. Louis, Missouri: Saunders; 2003.

50. Ferracane JL. Elution of leachable components from composites. J Oral Rehabil 1994;21(4):441-52.

51. Beatty MW, Swartz ML, Moore BK, Phillips RW, Roberts TA. Effect of microfiller fraction and silane treatment on resin composite properties. J Biomed Mater Res 1998;40(1):12-23.

52. Venz S, Dickens B. NIR-spectroscopic investigation of water sorption characteristics of dental resins and composites. J Biomed Mater Res 1991;25(10):1231-48.

53. Calais JG, Soderholm KJ. Influence of filler type and water exposure on flexural strength of experimental composite resins. J Dent Res 1988;67(5):836-40.

54. Oysaed H, Ruyter IE. Composites for use in posterior teeth: mechanical properties tested under dry and wet conditions. J Biomed Mater Res 1986;20(2):261-71.

55. Berger SB, Palialol AR, Cavalli V, Giannini M. Characterization of water sorption, solubility and filler particles of light-cured composite resins. Braz Dent J 2009;20(4):314-8.

ABSTRACT

THE ION RELEASE BEHAVIORS AND WATER SORPTION OF NOVEL RESIN-BASED CALCIUM PHOSPHATE CEMENT

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Calcium phosphate-filled restorative materials were developed to provide calcium (Ca) and phosphate (PO_4) ions, which have been proposed to enhance remineralization of demineralized tooth structure. Recently, tricalcium phosphate (TCP)-filled restorative materials were introduced as an alternative to amorphous calcium phosphate. The TCP filler has a more crystalline structure than ACP and is therefore potentially stronger. The aim of the present study was to examine TCP-filled restorative resins at different concentration levels at different time intervals to characterize the concentrations of Ca and PO_4 ions released, and to measure the water sorption (WS) of these resins.

An *in vitro* study was conducted by formulating resin composite using TCP as the filler mixed with EBPADMA, HmDMA, and HEMA as the resin matrix. One-hundred-sixty samples were prepared, 40 samples of each filler concentration (30 percent, 40 percent, 50 percent, and 60 percent) by weight. From each filler concentration, 5 samples

of each of the 8 time points (time intervals of 4 h, 8 h, 12 h, 24 h, 3 d, 7 d, 14 d, and 21 d) were immersed in 100-ml deionized water. Calcium and PO_4 ions were measured using atomic absorption spectroscopy and light spectroscopy, respectively. Water sorption (WS) was measured according to ISO 4049 specification and then the WS and the diffusion coefficient were calculated. The significance level was set at $p = <0.001$.

The results indicated that Ca and PO_4 ion release increased with increasing filler level at a rate faster than being linear. In addition, WS results were very high and failed to meet the ISO 4049 specification requirement. Diffusion coefficient results were also high. One-way ANOVA test for only 21-day data revealed that there is a statistically significant difference in filler level percent, and two-way ANOVA testing revealed that there is a statistically significant interaction between time and filler level percent on the Ca, PO_4 released and WS.

It can be concluded that the concentrations of Ca and PO_4 released and WS were affected by composition of the monomers, filler level and type, dispersion, and the absence of coupling agent.

Although this TCP-filled restorative material may release Ca and PO_4, it cannot serve as a restorative material due to high WS values. Further study is needed to improve the material and evaluate its ability in promoting remineralization of the tooth structure in order for it to serve its purpose.

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