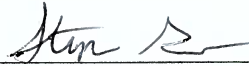


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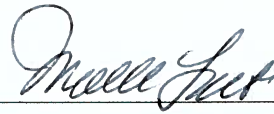
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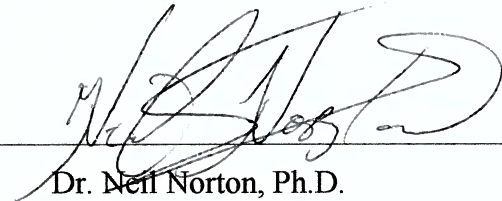
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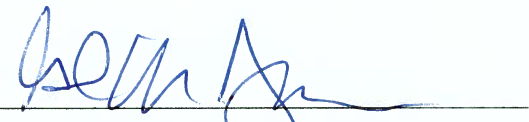
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AVAILALABLE FLUORIDE ION IN SOLUTION AND ONE MINUTE FLUORIDE
RELEASE RATE OF A TOOTHPASTE FORMULATION

By

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A THESIS

Submitted to the faculty of the Graduate School of Creighton University in Partial
Fulfillment of the Requirements for the Degree of Master of Science in the Department of
Oral Biology

Omaha, NE

April 10, 2014

ABSTRACT

Damaged enamel of an early carious lesion can be repaired through the body's natural repair process known as remineralization. For remineralization to occur, bioavailable calcium, phosphate, and fluoride ions must be present at the surface of the tooth. Toothpaste products supply bioavailable fluoride ions to the oral cavity. Calcium and phosphate ions are supplied primarily through the saliva, but in limited quantity. In this research, calcium nitrate salt and potassium phosphate dibasic salt solutions were encapsulated in microcapsules and formulated with toothpaste. The microcapsules rupture upon brushing, releasing bioavailable calcium, phosphate, and fluoride ions for remineralization. Total available fluoride and one minute fluoride release rates tests were performed to ensure that these new formulations will meet the requirements for the FDA monograph for over the counter anticaries products and the ADA Seal of Acceptance. Results for these two tests indicate that these formulations meet the requirements for the FDA and ADA. The microcapsule approach shows promising potential to become a novel delivery platform for bioavailable calcium and phosphate ions that aid in remineralization.

ACKNOWLEDGEMENTS

I would like to thank Creighton University and the School of Dentistry, specifically Dr. Stephen Gross, Dr. Mark Latta, Dr. Neil Norton, Dr. Wayne Barkmeier, and Dr. Margaret Jergenson for their guidance and support during this research and the opportunity to contribute to the field of dentistry. My colleagues, Michael Slater, Ryan Cooper, Joshua Fulton, Jennifer Hartjes, and James Doyle also have my deepest gratitude for their assistance and efforts in the progression of this research. I would also like to thank Premier Dental Products and Creighton University their previous, current, and continued support, without which this research would not be possible.

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CHAPTER 1

INTRODUCTION

1.1 Oral Hygiene and Preventative Practices

Dental caries is the most prevalent chronic disease worldwide that affects children and adults. According to the most recent National Health and Nutrition Examination Survey (NHANES, data collected from 1999 – 2004), 42% of children age 2 to 11 have suffered from dental caries in primary teeth, while 59% of adolescents age 12 to 19 and 92% of adults age 20 to 64 have suffered from dental caries in permanent teeth.¹ Dental caries is largely preventable through proper oral hygiene and healthy diet. In the early stages of the carious progression, the disease is reversible with prompt treatment. However, once the disease has progressed too far, the treatment options are limited to operative means, such as restorative fillings, root canals, or extractions.² Operative treatment for caries costs patients billions of dollars each year and can be invasive, painful, prone to failure or infection, and can leave the patient susceptible to secondary caries.³

A shift in the clinical focus has gone from operative treatments to preventative/non-operative approaches. Preventative approaches include fluoridated varnishes, water supplies, and toothpaste. Sealants and regular dental cleanings also target caries prevention.³ The addition of fluoride into oral products aids the remineralization process, which is the oral cavity's natural repair process for carious damage. Prior to full cavitation, the initial carious lesion can be repaired through this natural process. Remineralization occurs when calcium and phosphate ions, primarily from saliva, recrystallize on the damaged surface of enamel and replace lost structure. Fluoride ions can also participate in the precipitation of mineral on the tooth surface, but fluoride ions are not naturally occurring in saliva. Remineralized enamel is more resistant to demineralization than natural enamel, especially if fluoride ions are incorporated into the

new mineral structure.⁴ Therefore, significant research has explored innovative biomaterials and delivery systems that increase the availability of these essential remineralizing ions.

1.2 Tooth Structure

Every tooth has three calcified tissues that are vulnerable to demineralization: enamel, dentin, and cementum. The crown portion of tooth is visible in the oral cavity, while the root portion is situated in the gingiva. Enamel is the outer protective layer of the crown and it covers the inner layer of dentin. Cementum is the outer protective layer for the root and it covers an inner layer of dentin as well. Enamel is comprised of 96 wt% hydroxyapatite (HAP) mineral with various ion substitutions. The chemical formula of HAP is $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. The chemical formula of enamel is represented by $\text{Ca}_{10-x}(\text{Na})_x(\text{PO}_4)_{6-y}(\text{CO}_3)_z(\text{OH})_{2-u}(\text{F})_u$. Although enamel is more soluble in acids than HAP due to ion substitutions, it is still simply referred to as HAP since the two structures are similar.^{5,8,10} However, the major contribution to why enamel is more soluble than pure HAP is due to carbonate ion lattice substitutions that replace phosphate ions. Carbonate and phosphate cannot be interchanged on a one to one stoichiometric ratio. Therefore, carbonate substitutions tend to disrupt the crystalline lattice structure of enamel, making carbonate sites much more susceptible to acid attack.^{9,10}

The HAP mineral forms tiny crystalline rods (or prisms), about 4 μm in diameter, that run from the anatomical surface of the tooth to the dentino-enamel junction. The spaces between the prisms are filled with water and organic material that makes up the remaining 4 wt% of enamel composition. The spaces also form diffusion pathways for

mineral components, acids, and other ions, which serves as the mechanism of the remineralization and demineralization cycle of the enamel surface.⁵

1.3 Dental Caries

Dental caries is a disease defined as “the localized destruction of susceptible dental hard tissues by acidic by-products from bacterial fermentation of dietary carbohydrates.”³ Dental caries begins with the colonization of bacteria on the surface of the tooth. The bacteria secrete organic substrates which serve as a protective shield from desiccation and various host defenses. Since the teeth are a non-shedding surface, the bacterial colonies accumulate on the tooth and form dental plaque or biofilm, which can only be removed by manually cleaning the surface. The bacteria in the biofilm generate acid by-products through fermentation of carbohydrates from the patient’s diet. These weak organic acids reduce the pH which creates an acidic environment in the tissues surrounding the biofilm and result in mineral loss of the tooth.² Bacterial groups that are most often involved in the development of caries are the *mutans streptococci* and the *lactobacilli* species.^{2,6,8}

The organic acids produced by the bacteria diffuse into the enamel structure, where they dissociate in the presence of water and produce hydronium ions, lowering the local pH. The hydronium ions have an affinity to the carbonate “weak spots” as well as phosphate and hydroxyl groups in the enamel, attracting these ions out of the lattice and releasing surrounding ions from the crystal structure, initiating the demineralization process. The critical pH is when the solution is just saturated with respect to the mineral. When the pH of the saliva is below the critical value, the mineral in enamel will dissolve due to the abundance of hydronium ions in solution.^{7,8,9,10} The critical pH for an

individual is dependent upon the concentrations of calcium ions and phosphate ions in the saliva. With normal concentrations of calcium ions and phosphate ions in the saliva, the critical pH of enamel or HAP is around 5.5.⁷

The enamel pellicle offers some protection against acid-induced enamel demineralization. The pellicle is a biofilm formed on the surface of enamel consisting of proteins, carbohydrates, and lipids. The pellicle formation initiates when salivary proteins adsorb onto the enamel surface, in as little as one minute after exposure to the oral environment.^{19,20} Bacterial organisms colonize on the pellicle, initiating plaque formation. However, bacterial adherence directly to enamel mineral is possible as well. Although the pellicle acts as a substrate for disease-inducing bacterial colonies, the selective adsorption properties of the pellicle favor harmless bacteria, decreasing the amount of detrimental bacteria. The salivary pellicle protects enamel from demineralization by decreasing the rate of dissolution of calcium and phosphate constituents in the hydroxyapatite. The pellicle also minimizes dental abrasion and friction between teeth during oral functions such as mastication.¹⁹ The pellicle may also inhibit surface precipitation of calcium, phosphate, and fluoride ions, thereby promoting subsurface remineralization.²⁰

1.4 Remineralization/Demineralization Balance

Remineralization is the body's natural repair process to prevent caries progression.⁴ Carious demineralization initially presents as a white spot lesion on the tooth, which is characterized by subsurface demineralization with the surface layer still intact. The loss of mineral in the enamel causes the lesion to appear white due to the change in its

refractive index compared to the unaffected enamel surrounding the void.⁹ The lesion can be repaired during the white spot “phase” because there is a suitable matrix for the deposition of ions to rebuild the lost crystalline structure, while fully cavitated lesions require restorative procedures to replace lost hard tissues.^{2,4,7,11} Remineralization requires bioavailable calcium and phosphate ions, primarily supplied through the saliva.^{4,9}

There is a constant flux between conditions that favor demineralization or remineralization within the oral cavity every day. These conditions will determine if a lesion is repairable or if it progresses into an irreparable cavitation.² This balance between mineral loss and mineral gain is dependent upon the critical pH, ion product (I_p) of the solution in contact with the mineral (in this case, the saliva or plaque fluid), and the solubility product (K_{sp}) of the enamel mineral.⁷ The K_{sp} of a mineral with the ability to dissociate into separate ions is defined as the product of the concentrations (mol/L) of component ions, raised to the appropriate power, in a saturated solution. The K_{sp} for HAP (in a saturated solution) is approximately 10^{-55} and represented by $[Ca]^{10}[PO_4]^6[OH]^2$. While the value of K_{sp} remains constant, the concentrations of the three components can vary, provided their products always equal the K_{sp} .^{7,11,17} The I_p is similar to the K_{sp} , but is calculated based on the concentrations of calcium, phosphate, and hydroxide ions in the saliva or plaque fluid. When $I_p = K_{sp}$, the solution is just saturated with respect to HAP, when $I_p > K_{sp}$, the solution is supersaturated, and when $I_p < K_{sp}$, the solution is unsaturated. In the natural oral environment, $I_p > K_{sp}$, and enamel will not dissolve. However, when the pH is below the critical pH, hydronium ions will bind with hydroxide ions to form water in solution. When the hydroxide ion concentration is reduced, an

increase in calcium ion and phosphate ion concentrations must occur to maintain saturation and thus surface enamel will dissolve below the critical pH.⁷

Fluoride has been utilized in a vast array of dental products in the past 4 or 5 decades to combat carious lesions and promote remineralization. Fluoride has been incorporated into dentifrices, mouth rinses, topical gels, dental varnishes, and drinking water supplies.⁹ Topical fluoride has the ability to prevent, arrest, and even reverse the carious process in three ways: inhibiting bacterial metabolism, inhibiting demineralization, and enhancing remineralization. Bacterial metabolism is inhibited by fluoride when the pH in the oral environment falls, allowing hydrogen fluoride (HF) to form from dissociated H^+ and F^- ions. While fluoride ions are unable to pass into the bacterial cell, hydrogen fluoride is able to diffuse through the cell wall and membrane of the bacteria. Once inside the cell, hydrogen fluoride dissociates once more, releasing fluoride ions causing disruptions in enzyme activity. For example, the enzyme enolase, which is vital for carbohydrate metabolism, is inhibited by fluoride ions. Fluoride inhibits demineralization by directly replacing lost carbonate or hydroxyl ions within the mineral structure, creating a fluorapatite structure. Fluorapatite is more resistant to acid attack than the naturally occurring carbonated hydroxyapatite (enamel), thus inhibiting the dissolution of mineral during an acid challenge. Fluoride also enhances the remineralization process. When fluoride ions are dissolved in solution (the saliva or plaque fluid), the ions are able to adsorb onto the existing demineralized crystal surface, which act as “nucleating” sites for remineralization. The fluoride ions on the enamel surface attract calcium ions, which in turn attract phosphate ions, to the nucleating sites, promoting new crystal formation.^{5,8,9}

1.5 Other Remineralizing Technologies

While fluoride can help in the remineralization process, calcium and phosphate ions are vital to rebuilding lost mineral. To create fluorapatite during remineralization, 10 calcium ions and six phosphate ions are required for every two fluoride ions. This means that net mineral gain may be limited by the lack of calcium and phosphate ions. Thus, there is a need for a delivery platform that is able to provide bioavailable calcium, phosphate, and fluoride ions to the oral cavity. One issue facing the clinical use of calcium and phosphate ion delivery systems is the low solubility of calcium phosphates in the oral cavity, particularly when fluoride ions are introduced as well. Insoluble calcium phosphates do not localize at the damaged tooth surface, are difficult to implement, and require an acidic environment to dissociate into ions that can diffuse into the enamel and repair subsurface lesions. Soluble calcium and phosphate delivery systems must be utilized in low concentrations to prevent the ions from becoming insoluble. Soluble calcium and phosphate ion delivery systems are also unable to localize at the tooth surface, which does not allow adequate concentration gradients to form to favor subsurface remineralization.^{5,12} However, referring to this type of platform as “soluble” salt delivery is arguably a misnomer, since salts at such low concentrations would be considered insoluble according to one commonly accepted definition: “a salt with a K_{sp} of less than 10^{-5} .”¹⁸ These limitations have resulted in the development of innovative delivery systems that use high concentrations of calcium phosphate to aid in remineralization. These commercially available products claim to overcome the limited bioavailability of calcium and phosphate ions by using specific forms of calcium

phosphates. These systems can be categorized into three types, depending on which form of calcium phosphate being utilized: crystalline, unstabilized amorphous, or stabilized amorphous delivery systems.^{11,12}

The first delivery system uses the numerous crystalline phases (and corresponding solubility products) of calcium phosphate to deliver bioactive calcium and phosphate ions to subsurface enamel lesions for remineralization. Theoretically, the ions should dissociate from the dental product when it comes in contact with the saliva, allowing the ions to travel within the enamel and remineralize the subsurface lesion. This would require the saliva to be undersaturated with respect to the calcium phosphate crystalline phase, which is not the case when the pH of the saliva is within the normal range. Ions provided through crystalline delivery systems also cannot localize on the tooth surface, which limits their ability to promote remineralization in subsurface lesions. Examples of this delivery platform are dentifrice additives such as Brushite (dicalcium phosphate dihydrate) or tricalcium phosphate (TCP). NovaMin™ is a bioactive glass product that uses calcium sodium phosphosilicate solids to supply ions for the remineralization process. However, there is minimal evidence supporting subsurface lesion remineralization *in vivo* for any of these products.^{11,12}

The second type of delivery system uses unstabilized amorphous calcium phosphate (ACP) technology and a dual chamber delivery device. Calcium and phosphate salts are kept in separate chambers of the delivery device until the product is administered in the oral cavity. The salts dissolve in the saliva, releasing calcium and phosphate ions, which very quickly precipitate as ACP or amorphous calcium fluoride phosphate (ACFP) when fluoride ions are also present. These phases are unstable in the oral environment, and

often transform into more stable crystalline phases, hydroxyapatite or fluorhydroxyapatite. However, before phase transformation, calcium and phosphate ions can theoretically be briefly available for remineralization of subsurface lesions. Enamelon™ is one such technology used with fluoride containing dentifrices; however studies are showing contradicting efficacy results in independent rat caries trials when compared to regular fluoride dentifrices.^{11,12}

The third type of delivery system uses stabilized amorphous calcium phosphate to supply calcium and phosphate ions used in remineralization. The solubility of calcium and phosphate ions in biological systems is regulated by proteins. This delivery platform is a biomimetic approach that uses casein, which is the stabilizing protein in milk, to deliver calcium and phosphate ions to the oral environment. Casein phosphopeptide-stabilized amorphous calcium phosphate (CPP-ACP) and casein phosphopeptide-stabilized amorphous calcium fluoride phosphate (CPP-ACFP) systems use peptide chains to stabilize calcium and phosphate ion clusters that can be slowly released near the tooth surface. The dissociation of the calcium, fluoride, and phosphate ions from the peptide chains is determined by the equilibrium of free and CPP-bound ions in the saliva, providing a consistently available supply of ions. Recaldent™ is one such product that is added to dentifrices and sugar free gum to aid in remineralization. Evidence has shown that CPP-ACP and CPP-ACFP can promote remineralization of subsurface lesions *in situ* and these complexes have an affinity for plaque, localizing the bioavailable ions near the demineralized surface. However, enzymes in the plaque fluid degrade the phosphopeptide chains, reducing the capacity for calcium and phosphate ion stabilization. These systems

have also been shown to have greater efficacy in active white spot lesions when compared to inactive lesions, likely due to the greater porosity of active lesions.^{11,12}

1.6 Microcapsule Research

A new approach for a remineralizing technology has recently been proposed. Davidson et al. showed that ions can be slowly released from microcapsules containing aqueous salt solutions.¹³ The rate of release can be controlled through variations of the microcapsule chemical structure, initial concentration of salt in solution, and the identity of the salt dissolved in the solution. This approach, when added to dentifrices and other dental products, has the potential to release ions at a long term, sustainable rate.¹³ In this study, we incorporated aqueous solutions containing calcium ions in microcapsules and aqueous solutions containing phosphate ions in microcapsules into a dentifrice. This method keeps the salt solutions separate until the microcapsules are ruptured in the oral cavity by manual brushing. The separation of salt solutions prevents calcium phosphate and calcium fluoride precipitates from forming in the continuous toothpaste phase, which would otherwise render them unusable for remineralization processes.

Toothpaste containing fluoride is marketed as an over the counter anticaries product. The U.S. Food and Drug Administration (FDA) has regulations on the ingredients used in over-the-counter drug products. Products that meet these requirements are awarded the FDA monograph, indicating that the product is effective, yet safe for use by the general population. The acceptable amount of fluoride incorporated is dependent upon the active ingredient in a particular dentifrice. Crest® Cavity Protection uses 0.243 w/w% sodium fluoride as the active ingredient. The FDA approves the use of 0.188

w/w% to 0.254 w/w% sodium fluoride in toothpaste formulations, the total fluoride ion not exceeding 0.15 w/v%. The available fluoride ion concentration of dentifrices using sodium fluoride as the active ingredient must be at least 650 part per million (ppm). The anticaries product must also meet the biological test requirements for the animal caries reduction and one of the following tests: enamel solubility reduction or fluoride enamel uptake.¹⁴ The American Dental Association (ADA) also endorses dental products by awarding the ADA Seal of Acceptance, which further validates the safety and efficacy of a marketed product. The Seal of Acceptance is awarded to anticaries dentifrices when the following efficacy data is supplied for each of the following tests: total fluoride, available fluoride in fresh and aged samples, one minute fluoride release rate in fresh and aged samples, and bioavailability in demineralized enamel.¹⁵ In this research, toothpaste that was formulated with microcapsules containing either or both aqueous solutions of calcium nitrate or potassium phosphate dibasic were tested for the total fluoride release (TFR) in fresh samples, which satisfies the available fluoride ion tests, and the one minute fluoride release rate in fresh samples.¹⁵ This was done to determine if the inclusion of microcapsules effects these test results and if the presence of bioavailable calcium ions and phosphate ions can increase the enamel fluoride uptake.

CHAPTER 2

MATERIALS & METHODS

2.1 Materials

All of the materials used during this research were purchased from Buehler, Sigma-Aldrich, Fisher Scientific, Pharmaco-AAPER, Acros Organics, MP Biomedicals, or Alfa Aesar. Materials purchased from Sigma-Aldrich: L-Ascorbic acid, ammonium heptamolybdate tetrahydrate, potassium antimonyl tartrate trihydrate, ethylene glycol, and cyclohexanone. From Fisher Scientific: potassium chloride and potassium phosphate dibasic. Hexanes were purchased from Pharmaco-AAPER. Methyl benzoate and isophorone diisocyanate were from Acros Organics. Calcium nitrate tetrahydrate was purchased through Alfa Aesar. Sodium fluoride was purchased from MP Biomedicals. A Thermo Scientific Barnstead filtration system was used to generate nanopure water. A Scientific Industries Vortex-Genie 2 was used to mix samples. Samples were stirred on a Thermix® Stirring Hot Plate Model 210T. Commercially available Crest® Cavity Protection Cool Mint Gel toothpaste was also used in this study.

2.2 Pre-polymer Synthesis

Polyurethane pre-polymers were synthesized via solution polymerization using isophorone diisocyanate and ethylene glycol to create the urethane linkages.

A clean 500 mL, three neck round bottom flask with 24/40 joints was fitted with two septa, on the left and right joints and secured with copper wire. A clean stir bar was added to the flask and a greased 24/40 flow adapter was placed in the center joint and secured with a clip. The round bottom flask was secured with a ring clamp and the vacuum tubing from the manifold was attached to the flow adapter. The vacuum tube was secured with a hose clamp. To evacuate the flask, the vacuum pump was turned on, then

the vacuum port on the manifold was opened, and lastly the valve on the flow adapter was opened. The flask was allowed to evacuate for at least 5 minutes. Next, adventitious water was removed from the flask using a Bunsen burner to flame dry the glassware for approximately two minutes. The flask was allowed to cool to room temperature and then the flow adapter valve was closed. The manifold port was closed next, followed by turning off the vacuum pump. Next, the valve on the inert gas tank, containing either nitrogen or argon, was opened. The port on the manifold was opened next. The valve on the flow adapter was opened and closed quickly to prevent the septa or adapter from forcefully dislodging as the inert gas was introduced to the flask. The valve was then left open, to allow the continual flow of inert gas into the flask, and one of the septa was removed. Using a graduated cylinder, 150 mL of the cyclohexanone solvent was poured into the flask. Then 27.8 grams of isophorone diisocyanate was added to the flask using a syringe. The septum was replaced and secured once again with copper wire. The solution was stirred for 30 minutes. A septum was once again removed and 0.055 mol (3.41 grams) of ethylene glycol was slowly added using a syringe. The septum was replaced and secured with copper wire. The reaction flask was then submerged in an oil bath. The flask was purged with nitrogen gas by inserting a degassing needle connected to the manifold into the available septum. A syringe needle was inserted into the other septum to increase the flow of inert gas into the reaction solution during purging. Care was used to ensure the tip of the degassing needle was completely submerged in the solution. The valve on the flow adapter was closed, followed by the inert gas port on the manifold. The manifold port corresponding to the degassing needle was then opened, and the appearance of bubbles in the solution indicated the inert gas was flowing. The solution

was stirred for 30 minutes at room temperature while it was purged. Once the reaction was purged, the degassing needle port on the manifold was closed first, and then the valve on the inert gas tank was closed. The thermocouple tip was inserted into the oil bath, making certain that the tip was completely submerged. The temperature controller was set to 80 °C and the syringe needle was removed, followed by the degassing needle. The reaction was allowed to run over night.

The next day, the flask was removed from the oil bath and cooled to room temperature. One of the septa was removed and both the solution and stir bar were transferred to a clean, 500 mL one neck round bottom flask with a 24/40 joint. A 24/40 flow adapter was lubricated using vacuum grease and inserted in the joint of the flask and secured with a clip. The flask was secured to a ring stand and the flow adapter was fitted with the vacuum line from the cold trap and secured with a hose clamp. The Dewar was filled about one third of the way full with isopropanol. The cold trap was well lubricated with vacuum grease to prevent the two components from seizing and placed together. The cold trap was lowered into the Dewar and secured with a ring clamp. Dry ice was slowly added (small pieces added over a 5-10 minute period) to the Dewar to cool the isopropanol. The round bottom flask was submerged in the oil bath and the temperature was set to 100 °C, ensuring that the thermocouple tip was completely submerged in the oil. Glass wool encased in aluminum foil was used to insulate the exposed glassware, including the flow adapter, the reaction flask, and the top portion of the cold trap. The vacuum pump was turned on, followed by opening the flow adapter valve. The cyclohexanone solvent evaporates during this step, and then travels through the cold trap as a gas, where it cools and condenses back into a liquid and is collected. Initially, the

cold trap is emptied about every ten minutes, with increasing time intervals as there is less solvent being pulled from the reaction. This was done by closing the flow adapter valve on the round bottom flask, then turning off the vacuum pump. The vacuum seal was broken by removing the tubing from the flow adapter on the cold trap. The cold trap was carefully lifted out of the Dewar and the two pieces were separated. The solvent was collected in a beaker (a heat gun may be required if the solvent has frozen in the cold trap). The cold trap was greased once again, put back together, fitted with the vacuum line, and placed back in the Dewar. A couple more pieces of dry ice were added to the Dewar, and the vacuum pump was turned on, then the flow adapter valve on the flask was opened. This process was repeated until all of the solvent had been recovered and the pre-polymer product appeared dry. The flow adapter was closed, and then vacuum pump turned off. The insulation was removed and the flask was lifted out of the oil bath. The flask was allowed to cool over night to ensure there was no remaining solvent.

When the pre-polymer was determined to be dry, the flow adapter was removed from the flask. A Kimwipe saturated with hexanes was used to clean the neck of the flask to prevent grease contamination in the product. The flask was secured to a ring stand, with the mouth pointed towards the floor, at about a 45 degree angle. A 20 mL scintillation vial was placed directly under the mouth of the flask, and the mouth of the flask was lowered until it was about an inch away from the mouth of the vial. The pre-polymer was heated using a heat gun, which allowed the material to flow towards the mouth of the flask and eventually flow into the vial. The vial was properly labeled and stored at room temperature until use.

2.3 Oil Solution

The oil solution was made at least 24 hours prior to being used for microcapsule syntheses. Methyl benzoate, an emulsifying agent, and polyurethane pre-polymer were added to an Erlenmeyer flask with a stir bar. The solution was stirred for 24 hours to disperse the polyurethane.

2.4 Salt Solution

Salt solutions were made to be encapsulated in the microcapsules. A 0.5 M calcium nitrate salt solution and a 0.5 M potassium phosphate dibasic salt solution were prepared for encapsulation. Salt solutions were prepared by weighing the appropriate mass of salt for the desired molarity in a weigh boat. The salt was transferred sequentially to an Erlenmeyer flask, rinsing the weigh boat with nanopure water to ensure all the salt was transferred. Additional nanopure water was added to the Erlenmeyer flask to dissolve the salt (less than the desired final volume). The solution was allowed to stir until the salt was completely dissolved. The aqueous salt was transferred to a volumetric flask using a glass funnel (the Erlenmeyer flask and funnel were washed with nanopure water to transfer any residual salt) and diluted with the proper amount of nanopure water to reach the target molarity. The solution was capped and mixed by inverting the flask about 15 times. More nanopure water was added if a drop in volume occurred and the flask was mixed again. This process was repeated until the volume in the flask remained constant after mixing. The prepared salt solutions were stored at room temperature in labeled Nalgene bottles.

2.5 Microcapsule Synthesis

A reverse emulsion was used to encapsulate salt solutions in a polyurethane shell. With the addition of kinetic energy, aqueous salt solutions form tiny droplets within a continuous phase of oil. The pre-polymer in the oil solution encases these droplets, forming microcapsules that contain the salt solution. The synthesis takes place in a custom made IKA® LR 1000 dispersing vessel with the IKA® LR 1000 control base. The apparatus includes a glass reactor vessel with a lid, IKA® T25 digital ULTRA TURAXX® dispersion motor, dispersion shaft, stir paddles, and a temperature probe.

The reactor vessel was placed on the control base and turned to lock the vessel into place. Correct placement of the vessel has the vertical notch facing forward, while the grey block is situated toward the rear. The lid of the vessel was removed and the premade oil phase was poured into the reactor unit. The lid was replaced, securely latching the clamps on either side of the vessel. The glass stopper was inserted in the opening on the lid used for adding reactants and secured with a clip. Next the dispersion shaft was inserted into the lid through the opening on the left side and screwed into place, ensuring that the O-ring on the shaft was correctly positioned. The dispersion shaft should be submerged in the oil phase, but above the height of the stir paddles. The “cheese head” screws are loosened to adjust the height of the dispersion shaft, if necessary, and retightened to hold the shaft at the proper height. A metal rod is used to tighten the dispersion shaft one quarter of a turn more to secure placement. Next, the dispersion motor was placed on the dispersion shaft. The grey knob on the right side of the motor was turned until it was “finger tight”. The motor was also secured by tightening the silver levers on the backside of the motor. The thermocouple tip was inserted through the designated opening on the right side of the reactor lid. The tip should be submerged in the

oil phase, but not too deep as to interfere with the paddle rotation. The thermocouple tip was locked in place by tightening the “cheese head” screws. The metal rod was again used to tighten the thermocouple tip one quarter turn more.

The power button was pressed to turn the instrument on and the paddle mixing control knob was turned to 10 rpm, and pushed to begin rotation. This low rotation setting was used to ensure that the paddles freely rotate without colliding with the thermocouple tip or dispersing element. If the paddles collide with the thermocouple tip, the mixer should be stopped, and the thermocouple tip or dispersing shaft should be adjusted accordingly. If the paddles turned without collision, the paddle speed was increased to 150 rpm. Then, the temperature control was set to 70 °C. The dispersing motor was then set to 4,000 rpm (note: the display on the motor will read 4.0 for 4,000 rpm). The plexiglass safety shield was positioned in front of the entire apparatus as a safety measure, and the solution was premixed at these settings for 30 minutes while the oil phase was heated to the proper temperature.

After 30 minutes, the mixing speed can be increased to the desired speed, typically between 8,000 and 16,000 rpm. The clip and glass stopper on the reactor lid were removed. Using a Pasteur pipette, 200 mL of the desired salt solution (either 0.5 M calcium nitrate or 0.5 M potassium phosphate dibasic) were added slowly through the opening over a fifteen minute period. Care was taken to ensure the salt solution was deposited in the oil solution, not on the walls or dispersing shaft. The glass stopper and clip were replaced.

After 30 minutes, the clip and glass stopper were removed again and 4.75 grams of ethylene glycol were added via syringe and needle. The addition of ethylene glycol

quenches the solution of any remaining isocyanate groups in the pre-polymer and extends to molecular weight of the polyurethane. The glass stopper and clip were replaced and the reaction was mixed for an additional 3 ½ hours (which equates to 4 total hours of reaction time after the addition of the aqueous solution).

Upon completion of the reaction, the mixing motor was slowly turned down to 500 rpm, then turned off. Then the temperature control was turned off, followed by the paddle mixer being turned down to 0 rpm. The instrument was turned off. The motor was then removed from the dispersion shaft by releasing the silver levers and grey knob. The thermocouple tip was released using the metal rod and unscrewed, then removed from the reaction vessel. The dispersion shaft was removed with the same method as the thermocouple. The reactor vessel was turned counter clockwise to release the locking mechanism, and lifted from the base. The lid clamps were released, and the solution was poured into a clean beaker. Using a syringe, the contents of the beaker were transferred to 15 mL centrifuge tubes. The tubes were centrifuged for several minutes at 3,400 rpm. If the microcapsules did not completely pelletize, the supernatant (methyl benzoate) was poured off and replaced with hexanes, then centrifuged again to produce a microcapsule pellet in the bottom of the centrifuge tube. The microcapsules were stored in centrifuge tubes (in methyl benzoate or hexanes) at room temperature until use.

2.6 Total Fluoride Release (TFR)

This test is used to determine the total available fluoride in a particular toothpaste formulation and ensure that the fluoride concentration is within the FDA and ADA guidelines. The FDA requires at least 650 ppm fluoride ion be available in solution. The

ADA requires 90% of the labeled fluoride amount be available in solution. Fluoride and calcium ion concentrations were determined using potentiometry, and phosphate ion concentrations were measured using the molybdenum blue method (see section 2.8).

2.6a Toothpaste Formulations

Three different toothpaste formulations were tested under TFR conditions: (a) 100 w/w% toothpaste (control), (b) 97 w/w% toothpaste with 3 w/w% microcapsules containing 0.5M calcium nitrate, and (c) 94 w/w% toothpaste with 3 w/w% microcapsules containing 0.5M calcium nitrate and 3 w/w% microcapsules containing 0.5M potassium phosphate dibasic. Five grams of each formulation was prepared.

The targeted amount of toothpaste was weighed out in a 125 mL Erlenmeyer flask using a spatula. For the samples with microcapsules incorporated: microcapsules were centrifuged for several minutes to form a pellet and the supernatant was poured off. Using a Pasteur pipette, the proper mass of microcapsules was transferred to the flask. If both kinds of microcapsules were being incorporated, they were stored in separate centrifuge tubes before adding them to the toothpaste sample. Care was used to deposit the toothpaste and microcapsules in the bottom third of the flask, to ensure the entire sample was incorporated into the solution. Using a 12 mL syringe, approximately 50 mL of nanopure water was added to the flask, followed by a stir bar. The flask was parafilm and allowed to mix on a Thermix® Stirring Hot Plate Model 210T on level 6 until the toothpaste completely dispersed, usually about 10 minutes. After the sample is completely dispersed, the contents of the flask were transferred to a 100 mL volumetric flask and washed of any residual sample with nanopure water. The flask was diluted with

nanopure water to the correct volume. The flask was capped and inverted 10 to 15 times to mix. Using a 12 mL syringe, a 0.45 μm GHP ACRODISC filter, and a needle, about 12 mL of the slurry was pulled through the filter and stored in centrifuge tubes. The pulled sample will be used to determine fluoride and calcium ion concentrations using ion specific electrodes. The molybdenum blue method will be used to determine phosphate ion concentrations of samples that contain phosphate ion containing microcapsules.

2.7 One Minute Fluoride Release Rate (OMFRR)

This test is used to predict that adequate fluoride is will be released during brushing. The ADA requires that 80% of the labeled fluoride amount in a toothpaste formulation be available in the solution after this experimental procedure is performed. Calcium ion and fluoride ion concentrations were determined using potentiometry and phosphate ion concentrations were determined using the molybdenum blue method (see section 2.8).

2.7a Toothpaste Formulations

Three different toothpaste formulations were tested under OMFRR conditions: (a) 100 w/w% toothpaste (control), (b) 97 w/w% toothpaste with 3 w/w% microcapsules containing 0.5M calcium nitrate, and (c) 94 w/w% toothpaste with 3 w/w% microcapsules containing 0.5M calcium nitrate and 3 w/w% microcapsules containing 0.5M potassium phosphate dibasic. Two grams of each formulation was prepared.

The targeted amount of toothpaste was weighed out in a 20 mL scintillation vial using a spatula. For the samples with microcapsules incorporated: microcapsules were

centrifuged for several minutes to form a pellet and the supernatant was poured off. Using a Pasteur pipette, the proper mass of microcapsules was transferred to the vial. If both kinds of microcapsules were being incorporated, they were stored in separate centrifuge tubes before adding them to the toothpaste sample. Care was used to deposit the toothpaste and microcapsules in the bottom half of the vial, to ensure the entire sample was incorporated into the solution. Using a micropipette, 6 mL of nanopure water was added to each sample vial. The vial cap was screwed on tightly and the samples were vortex mixed (speed 10) for exactly one minute, creating toothpaste slurries. The slurries were allowed to rest for one minute. Using a 12 mL syringe, a 0.45 μm GHP ACRODISC filter, and a needle, about 1.5 mL of the slurry was pulled through the filter and stored in microcentrifuge tubes. The pulled sample will be used to determine fluoride and calcium ion concentrations using ion specific electrodes. The molybdenum blue method will be used to determine phosphate ion concentrations of samples that contain phosphate ion containing microcapsules.

2.8 Ion Release Measurements

2.8a Calcium Ion and Fluoride Measurements

Potentiometry was used to determine calcium ion and fluoride concentrations of samples tested under OMFRR conditions and TFR conditions. Ion specific electrodes (ISE) and reference electrodes were used for release measurements. For calcium ion measurements, an ELIT 2673 calcium specific electrode was used. For fluoride measurements, an ELIT 2736 fluoride specific electrode was used. An ELIT 001N silver chloride reference electrode was used for both calcium and fluoride measurements. The

ion specific electrodes were conditioned before measurements were taken by soaking in 1,000 ppm of calcium ion or fluoride containing (respectively) solutions for at least 30 minutes. The standards used ranged from 0.1 ppm to 5.0 ppm. About 10 mL of standard was added to a 30 mL beaker with a small stir flea. The system was first calibrated with the widest standard range (0.1 ppm to 5.0 ppm), since the concentration of the samples is unknown. The concentration range can be narrowed to increase measurement accuracy once the calculated ppm of a sample is determined. Fluoride measurements for TFR samples use specific standards that are made the same day the measurements are taken. These will be discussed later in this section. The standard with the lower concentration will be generically referred to as standard A, while the standard with the higher concentration will be referred to as standard B.

After conditioning, the ISE was rinsed thoroughly with nanopure water and blotted with a Kimwipe. The ISE was inserted in the ELIT 201 dual head BNC connector of the instrument. The cap to the reference electrode was removed and the electrode was rinsed with nanopure water, blotted dry, and inserted in the ELIT 201 dual head BNC connector. A Thermix® Stirring Hot Plate Model 210T was below the dual head connector and the stir setting was set to five. Standard A was placed on the stir plate, and the dual head connector was lowered until the reference electrode and ISE were submerged in the standard solution, but not touching the stir bar or the bottom of the beaker. Air bubbles that may have formed at the electrode membrane were removed by gently flicking the electrodes a few times. The lower of the two standards is always measured first during calibration.

To begin the calibration curve, the program icon was selected on the computer screen. The ISE being used was selected in the drop box menu ('Ca' for calcium measurements or 'F' for fluoride measurements). The calibrate button was selected, and the concentration of standard A was entered in the first box (i.e. 1.0 was entered if using the 1.0 ppm standard). The read button was selected and the millivolt reading was recorded after two minutes by pressing the record icon. The electrodes were raised out of the first standard solution and blotted dry with a Kimwipe to remove any excess solution on the electrodes. Standard A was replaced with standard B on the stir plate and the electrodes lowered into solution. The process was repeated to measure standard B. Once the second standard was measured, the finish button was selected and the computer calculated the slope, which was recorded. For calcium measurements, the acceptable slope range was 26 ± 3 mV/decade. For fluoride measurements, the acceptable slope range was -54 ± 5 mV/decade. If the slope was not in the acceptable range, the electrodes were allowed to soak for a longer period of time, up to several days.

Once the slope was within the acceptable range, the unknown samples could be prepared for measurements. For OMFRR fluoride and calcium measurements and TFR calcium measurements, dilutions were made in 10 mL volumetric flasks to allow the sample readings to fall within the calibration curve. Aliquots of the pulled slurries were added to the flasks ranging from 100 μ L to 2 mL, depending on the desired dilution factor. An ionic strength adjustment buffer was added and the volume increased to the demarcation using nanopure water. The flasks were capped and inverted several times to mix, then transferred to 30 mL beakers with stir fleas for testing. OMFRR samples were stirred on a Thermix® Stirring Hot Plate Model 210T for five minutes on a setting of 5

before testing for fluoride or calcium. TFR samples do not require this mixing step. For calcium measurements, 2%v/v (200 μ L) of 4 M potassium chloride was added as an ionic strength adjustment buffer (ISAB). For fluoride measurements, 50%v/v (5 mL) of a total ionic strength adjustment buffer (TISAB) was added. The TISAB included 57 mL of acetic acid, 45 grams of sodium chloride, and 45 grams of CDTA (1,2-diamino cyclohexan N,N,N,N-tetraacetic acid) in 500 mL of distilled water. The pH of the TISAB solution was adjusted to 5.5 via drop wise addition of 5.0 M NaOH. The volume of the solution was increased to 1 liter with nanopure water.

To make a TFR sample for fluoride measurements, 10 mL of the toothpaste slurry and 1 mL of TISAB are added to a 20 mL scintillation vial using a micropipette, making the overall dilution 1 in 22. The cap is screwed on the vial, and the vial is vortex mixed for five minutes (speed 10). The samples were allowed to rest for one hour. The samples are poured into 30 mL beakers with stir fleas and are ready to test for fluoride concentration. Standards used for TFR fluoride measurements were 9.09 ppm sodium fluoride and 90.9 ppm sodium fluoride. These standards must be made the same day the measurements are taken. To make the 9.09 ppm standard, a micropipette is used to add 1 mL of TISAB and 10 mL of 10 ppm sodium fluoride to a 30 mL beaker with a stir flea. To make the 90.9 ppm standard, a micropipette is used to add 1 mL TISAB and 10 mL of 100 ppm sodium fluoride to a 30 mL beaker with a stir flea.

To measure the prepared samples, the system was calibrated in the same manner as before by running the two selected standards. If the slope was satisfactory, the electrodes were blotted then placed in the unknown sample. Air bubbles were removed by gently flicking the electrodes. The measure button on the computer screen was pressed and the

sample was measured for two minutes. The millivolt reading and calculated ppm of the sample were recorded. For each calibration, two unknown samples were measured and then the system was recalibrated with the standards. Each sample was read in triplicate. The electrodes were blotted with a Kimwipe before they were submerged in the next solution.

The millivolt readings and the slope of the calibration curve were used with the Nernst equation, $E_{\text{cell}} = E_{\text{cell}}^{\ominus} - (RT/nF)\ln Q$, to calculate the actual ppm of a given sample. The equation can be rewritten as $E_{\text{cell}} = E_{\text{cell}}^{\ominus} - (2.303 \cdot RT/nF)\log Q$. The cell potential is represented by E_{cell} while the standard cell potential is represented by $E_{\text{cell}}^{\ominus}$. Q is the ion concentration, R represents the universal gas constant ($8.314 \text{ J K}^{-1} \text{ mol}^{-1}$), T is the absolute temperature (298 K), n refers to the number of moles of electrons transferred in the cell reduction, and F denotes Faraday's constant ($9.648 \times 10^4 \text{ C mol}^{-1}$).

Rewriting the Nernst equation to fit the $y=mx+b$ model of a straight line reads as $E_{\text{cell}} = (RT/nF)\log[\text{ion}] + E_{\text{cell}}^{\ominus}$. Where E_{cell} (y) is the sample reading in millivolts, (RT/nF) is the slope (m), $\log[\text{ion}]$ (x) is the log of the concentration of F^- or Ca^{2+} and $E_{\text{cell}}^{\ominus}$ is the y-intercept (b). In other words, $\text{mV} = \text{y-intercept} + (\text{slope})(\log[\text{ion}])$. The concentration and mV reading from one of the standards was chosen and the y-intercept was calculated using the mV reading, the slope, and the $\log[\text{ion}]$ from that standard. The calculated concentration of a sample was calculated from the mV reading from that sample, the slope from the calibration curve, and the y-intercept. The calculated ppm of the sample is multiplied by the dilution factor used to determine the actual ppm of the sample.

2.8b Phosphate Ion Measurements

Phosphate ion concentrations were measured for samples with microcapsules containing phosphate ions using uv-vis spectroscopy and the molybdenum blue method.¹⁶ Absorbance values of the molybdenum complex were measured at 882 nm on a Tecan Infinite M200 spectrophotometer. The method selected to measure the unknown samples was developed for use with the Corning 48 well plate and included a 20 minute kinetic cycle that agitated at an amplitude of 6 mm for 60 seconds between absorbance readings.

The mixed reagent was prepared in a 50 mL Erlenmeyer flask from 10 mL of 2.5 M sulfuric acid, 6 mL ascorbic acid, 3 mL ammonium heptamolybdate, and 1 mL potassium antimonyl tartrate trihydrate. The flask was covered with aluminum foil until it was added to the wells. Using a micropipette, 750 μ L of nanopure water were added to the wells as blanks, followed by 750 μ L of each standard, and a volume of unknown sample and nanopure water determined by the desired dilution factor. For example, if a 1 in 10 dilution is desired, 75 μ L of the sample and 675 μ L of nanopure water are added. Each unknown sample dilution was performed in triplicate. Next, 150 μ L of the mixed reagent were added to each well. The well plate cover was placed back on the plate and the Magellan program was opened on the computer. The well plate was placed in the instrument and the start icon was selected. The previously created method and sample ID were selected from the menu and a workspace name was created to save the data. Measurements began by selecting the run icon. If the standard curve produced an R-value greater than 0.999 the data was considered accurate and exported to Excel to calculate the concentration of each unknown sample.

CHAPTER 3

RESULTS

Bioavailable calcium, phosphate, and fluoride ions are required for remineralization of enamel to occur. Commercially available dentifrices must meet certain Food and Drug Administration (FDA) regulations regarding fluoride content to be marketed as a safe and effective anticaries product. The American Dental Association (ADA) also provides a Seal of Acceptance that further validates the safety and effectiveness of an anticaries dentifrice provided the product meets certain testing criteria. The following tests were conducted to determine if toothpaste formulations that contain encapsulated aqueous salt solutions will meet the FDA and ADA requirements to be marketed as a safe and effective anticaries product.

Reverse emulsion was used to encapsulate calcium nitrate aqueous salt solutions and potassium phosphate dibasic aqueous salt solutions in polyurethane based microcapsules. The microcapsules containing various salt solutions were mixed into Crest® Cavity Protection toothpaste, which uses 0.243% sodium fluoride as its active ingredient, which falls within the acceptable range outlined by the FDA. The following tests were performed to provide efficacy data for our novel toothpaste formulation: total fluoride release (TFR), which satisfies available fluoride ion tests, and the one minute fluoride release rate (OMFRR).

3.1 Total Fluoride Release (TFR)

Potentiometry was used to study the release of available calcium and fluoride ions using the TFR sampling procedure. Phosphate ion concentrations were also measured for the samples that included phosphate containing microcapsules using the molybdenum blue method. Ion concentrations are reported in part per million.

The formulations tested contained: (a) 100 w/w% toothpaste, (b) 97 w/w% toothpaste with 3 w/w% 0.5M calcium nitrate-containing microcapsules, and (c) 94 w/w% toothpaste with 3 w/w% 0.5M calcium nitrate-containing microcapsules and 3 w/w% 0.5M potassium phosphate dibasic-containing microcapsules. Twelve samples of each formulation were measured in triplicate and the average ion release measurements and standard deviations are reported in Table 1.

Ion Measured	Toothpaste Only (Control)		Toothpaste and 3wt% 0.5M Ca		Toothpaste and 3wt% 0.5M Ca and 3wt% 0.5M PO4		
	Ca (ppm)	F (ppm)	Ca (ppm)	F (ppm)	Ca (ppm)	F (ppm)	PO4 (ppm)
Average	3.1	1105	7.7	1073	6.11	1029	7047
Standard Deviation	0.37	20	1.77	22	4.2	21	529

Table 1: Total Fluoride Release samples. Calcium, fluoride, and phosphate ion measurements are reported for three toothpaste/microcapsule formulations prepared using the TFR procedure. Formulations tested were (a) 100 w/w% toothpaste, (b) 97 w/w% toothpaste with 3 w/w% 0.5M calcium nitrate-containing microcapsules, and (c) 94 w/w% toothpaste with 3 w/w% 0.5M calcium nitrate-containing microcapsules and 3 w/w% 0.5M potassium phosphate dibasic-containing microcapsules.

Table 1 shows the ion release measurements for samples prepared using the TFR procedure. Toothpaste only released 3.1 ppm calcium ions and 1105 ppm fluoride ions. Toothpaste formulated with 3 w/w% 0.5M calcium nitrate-containing microcapsules released 7.7 ppm calcium ions and 1073 ppm fluoride ions. Toothpaste formulated with 3 w/w% 0.5M calcium nitrate-containing microcapsules and 3 w/w% 0.5M potassium phosphate dibasic-containing microcapsules released 6.11 ppm calcium ions, 1029 ppm fluoride ions, and 7047 ppm phosphate ions.

3.2 One Minute Fluoride Release Rate (OMFRR)

Potentiometry was used to study the release of available calcium and fluoride ions using the OMFRR sampling procedure. Phosphate ion concentrations were also measured for the samples that included phosphate containing microcapsules using the molybdenum blue method. Ion concentrations are reported in part per million.

The formulations tested contained: (a) 100 w/w% toothpaste, (b) 97 w/w% toothpaste with 3 w/w% 0.5M calcium nitrate-containing microcapsules, and (c) 94 w/w% toothpaste with 3 w/w% 0.5M calcium nitrate-containing microcapsules and 3 w/w% 0.5M potassium phosphate dibasic-containing microcapsules. Twelve samples of each formulation were measured in triplicate (with the exception of fluoride ion measurements for 100 w/w% toothpaste, in which 30 samples were measured in triplicate) and the average ion release measurements and standard deviations are reported in Table 2.

Ion Measured	Toothpaste Only (Control)		Toothpaste and 3wt% 0.5M Ca		Toothpaste and 3wt% 0.5M Ca and 3wt% 0.5M PO4		
	Ca (ppm)	F (ppm)	Ca (ppm)	F (ppm)	Ca (ppm)	F (ppm)	PO4 (ppm)
Average	0.18	1007	1.5	962	1.7	891	2.4
Standard Deviation	0.05	24.5	0.43	114	0.86	40.7	1.94

Table 2: One minute fluoride release rate samples. Calcium, fluoride, and phosphate ion measurements are reported for three toothpaste/microcapsule formulations prepared using the OMFRR procedure. Formulations tested were (a) 100 w/w% toothpaste, (b) 97 w/w% toothpaste with 3 w/w% 0.5M calcium nitrate-containing microcapsules, and (c) 94 w/w% toothpaste with 3 w/w% 0.5M calcium nitrate-containing microcapsules and 3 w/w% 0.5M potassium phosphate dibasic-containing microcapsules.

Table 2 shows ion release measurements for samples prepared using the OMFRR procedure. Toothpaste only released 0.18 ppm calcium ions and 1007 ppm fluoride ions. Toothpaste formulated with 3 w/w% 0.5M calcium nitrate-containing microcapsules released 1.5 ppm calcium ions and 962 ppm fluoride ions. Toothpaste formulated with 3 w/w% 0.5M calcium nitrate-containing microcapsules and 3 w/w% 0.5M potassium phosphate dibasic-containing microcapsules released 1.7 ppm calcium ions, 891 ppm fluoride ions, and 2.4 ppm phosphate ions.

CHAPTER 4

DISCUSSION

Remineralization requires bioavailable calcium, phosphate, and fluoride ions in the oral environment. Commercially available anticaries toothpaste use fluoride salts as the active ingredient to supply bioavailable fluoride ions. However, fluoride ions cannot remineralize damaged enamel without bioavailable calcium and phosphate ions. Our goal is to provide bioavailable calcium and phosphate ions through the addition of microcapsules containing aqueous salt solutions to toothpaste. The microcapsules should, in theory, keep the fluoride, calcium, and phosphate ions separate and prevent undesirable precipitates from forming within the packaging. The microcapsules will rupture during brushing, providing the necessary bioavailable calcium, phosphate, and fluoride ions for remineralization. If precipitation does occur, the fluoride ions will not be available to the oral environment and the product will no longer be marketable as an anticaries product. The FDA and ADA have testing requirements that must be met in order to receive the FDA monograph for an over the counter anticaries drug and the ADA Seal of Acceptance. Here, we tested different toothpaste and microcapsule formulations for total fluoride release (TFR), which satisfies the available fluoride ion tests, and the one minute fluoride release rate (OMFRR).

Microcapsules were synthesized using reverse emulsion to encapsulate aqueous salt solutions in a polyurethane shell. Microcapsules used in this work contained the following aqueous salt solutions: 0.5M calcium nitrate and 0.5M potassium phosphate dibasic. Crest® Cavity Protection Cool Mint Gel toothpaste was used in these tests as well.

The first test performed was the total fluoride release (TFR) test which shows the concentration of available fluoride ion in solution. Crest® Cavity Protection Cool Mint

Gel is labeled as having 1100 ppm fluoride ion. The FDA requires that at least 650 ppm fluoride ion be available in solution, while the ADA requires that 90% of the labeled fluoride amount be available in solution, which equates to 990 ppm fluoride ion. Sample formulations tested were: (a) 100 w/w% toothpaste (control), (b) 97 w/w% toothpaste with 3 w/w% microcapsules containing 0.5M calcium nitrate, and (c) 94 w/w% toothpaste with 3 w/w% microcapsules containing 0.5M calcium nitrate and 3 w/w% microcapsules containing 0.5M potassium phosphate dibasic. Potentiometry was used to determine the calcium and fluoride ion concentrations. The molybdenum blue method was used to determine the phosphate ion concentration in formulations that contained encapsulated aqueous phosphate salt solutions.

The TFR control samples consisted of 100 w/w% toothpaste. The control samples showed 3.1 ppm calcium ions and 1105 ppm fluoride ions available in solution. Since the toothpaste does not include calcium-containing microcapsules, the calcium ions seen here could be due to another ingredient in the toothpaste, such as the water source. It could also be possible that the ion specific electrodes were not wiped well enough in between measurements and the standard solutions contaminated the samples during the measuring procedure. The available fluoride ion in solution showed adequate amounts of fluoride released to receive FDA and ADA approval. Although the average fluoride ion concentration in solution was 1105 ppm, which is larger than the labeled amount of fluoride (1100 ppm), the excess 5 ppm most likely is due to small variations in the homogeneity of the toothpaste samples. This amount of fluoride is considered negligible.

The second formulation tested for TFR consisted of 97 w/w% toothpaste and 3 w/w% microcapsules containing 0.5M calcium nitrate. These samples released 7.7 ppm

calcium ions and 1073 ppm fluoride ions. The increase in calcium ions in solution compared to the control is expected with the addition of calcium-containing microcapsules. The decrease in fluoride ion in solution is expected due to the 3 w/w% substitution of microcapsules for toothpaste. These samples are expected to release 3% less fluoride ions than the control samples, which is 1067 ppm fluoride. To be accepted by the ADA for the Seal of Acceptance, these formulations must release at least 960 ppm fluoride and the FDA requires 650 ppm fluoride ion in solution, both of which are exceeded. Again, the average fluoride ion concentration is slightly above what should be expected according to the active ingredient label, most likely due to variations in homogeneity of the samples.

The third formulation tested for total fluoride release consisted of 94 w/w% toothpaste with 3 w/w% 0.5M calcium nitrate-containing microcapsules and 3 w/w% 0.5M potassium phosphate dibasic-containing microcapsules. These samples showed 6.1 ppm calcium ions in solution and 1029 ppm fluoride ions in solution. Phosphate measurements showed 7047 ppm in solution. The increase in calcium ions available in solution is expected when compared to the control due to the addition of calcium nitrate-containing microcapsules. The slight decrease in calcium ions compared to toothpaste formulations with calcium-containing microcapsules could be the result of calcium phosphate salts precipitating out of solution, or simple sampling variation. The labeled amount of fluoride when adjusting for the 6% of toothpaste lost due to the substitution of microcapsules is 1034 ppm. For the ADA Seal of Acceptance, these formulations must release at least 930 ppm fluoride ion and the FDA requires at least 650 ppm, both of which are exceeded. The incorporation of phosphate-containing microcapsules shows that

large quantities of bioavailable phosphate ions can be supplied to the oral environment to be used in remineralization processes. This formulation shows promising results for supplying bioavailable calcium, phosphate, and fluoride ions for remineralization.

This test was performed to determine if the addition of calcium-containing and phosphate-containing microcapsules could supply bioavailable calcium and phosphate ions in solution, without affecting the available fluoride ion in solution so the product can still be marketed as an anticaries product. These results indicate that the microcapsule approach offers a novel effective delivery platform for bioavailable calcium and phosphate ions. Further research into this approach is required to increase the validity of these findings. This test should also be performed on aged toothpaste samples, which must meet the same requirements as fresh samples on the date of expiry. Testing should also be done to determine the caries reduction efficacy, which is another requirement of the ADA and the FDA.

The second test performed was the one-minute fluoride release rate (OMFRR) which shows the concentration of available fluoride ion in solution after one minute. Crest® Cavity Protection Cool Mint Gel is labeled as having 1100 ppm fluoride ion. The ADA requires that 80% of the labeled fluoride amount be available in solution, which equates to 880 ppm fluoride ion. Sample formulations tested were: (a) 100 w/w% toothpaste (control), (b) 97 w/w% toothpaste with 3 w/w% microcapsules containing 0.5M calcium nitrate, and (c) 94 w/w% toothpaste with 3 w/w% microcapsules containing 0.5M calcium nitrate and 3 w/w% microcapsules containing 0.5M potassium phosphate dibasic. Potentiometry was used to determine the calcium and fluoride ion concentrations. The molybdenum blue method was used to determine the phosphate ion

concentration in formulations that contained encapsulated aqueous phosphate salt solutions.

The OMFRR control samples for consisting of 100 w/w% toothpaste showed 0.18 ppm calcium ion in solution and 1007 ppm fluoride ion in solution after one minute. The low concentration of calcium in control samples is to be expected since no calcium-containing microcapsules are incorporated. The calcium ions that are present could be attributed to a water source or another ingredient in the toothpaste. The fluoride ion in solution exceeds what the ADA requires for the Seal of Acceptance, which is 880 ppm.

The second formulation tested for the OMFRR consisted of 97 w/w% toothpaste with 3 w/w% 0.5M calcium nitrate-containing microcapsules. These samples showed 1.5 ppm calcium ion in solution and 962 ppm fluoride ion in solution. The increased calcium concentration is attributed to the incorporation of calcium-containing microcapsules. However, it was noted that the one minute vortex mixing step did not always thoroughly mix all the microcapsules into the toothpaste solution. If a method to minimize microcapsule clumping is utilized, this formulation has the potential to release more calcium ions into solution than indicated from these results. To qualify for the ADA Seal of Acceptance, this formulation needs to show 854 ppm fluoride ion in solution, which is 80% of the labeled fluoride amount when accounting for the 3% of toothpaste that was substituted for microcapsules. These results confirm that this formulation exceeds the required amount of fluoride ion in solution.

The third formulation tested for the OMFRR consisted of 94 w/w% toothpaste with 3 w/w% 0.5M calcium-nitrate containing microcapsules and 3 w/w% 0.5M potassium phosphate dibasic containing microcapsules. These samples showed 1.7 ppm calcium

ions in solution and 891 ppm fluoride ions in solution. Phosphate measurements showed 2.4 ppm in solution. The increased calcium concentration in solution is attributed to the addition of calcium-containing microcapsules. However, as stated previously, there was microcapsule clumping seen after these samples were vortex mixed. This could be causing less calcium ions to be released in solution than would be possible if all of the microcapsules were incorporated into the toothpaste solution. For the ADA Seal of Acceptance, 80% of the labeled amount of fluoride must be released into solution. Accounting for the 6% of toothpaste that is substituted for microcapsules, the labeled amount of fluoride in this formulation is 1034 ppm. This formulation must release at least 827 ppm fluoride ion to meet the 80% requirement, which is exceeded. This formulation shows significantly less available phosphate ions in solution. Again, this is most likely due to the clumping of microcapsules that are not ruptured during the vortex mixing step. Modification to this procedure is necessary to minimize the clumping effect observed.

This test was performed to determine if the addition of calcium-containing and phosphate-containing microcapsules could supply bioavailable calcium and phosphate ions in solution, without affecting the available fluoride ion in solution so the product can still be marketed as an anticaries product. These results indicate that the microcapsule approach offers a novel effective delivery platform for bioavailable calcium and phosphate ions. Further research into this approach is required to increase the validity of these findings. This test should also be performed on aged toothpaste samples, which must meet the same requirements as fresh samples on the date of expiry. There should also be refinements to the formulation and mixing procedures that can produce more homogenous samples to be tested for OMFRR and reduce microcapsule clumping.

This study has demonstrated that calcium-containing and phosphate-containing microcapsules can offer a novel delivery platform for supplying bioavailable calcium and phosphate ions in the oral cavity that can aid in remineralization, while still maintaining the fluoride content guidelines set by the FDA and ADA for anticaries products. Further research examining the concentration of aqueous salt solutions encapsulated in the microcapsules would optimize the amount of calcium and phosphate ions released in solution and the remineralization efficacy of this approach.

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