Walsh University

The Remineralization Potential of Nano-Hydroxyapatite in Hydrogen Peroxide Whitening Mouthwash

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Abstract

The purpose of this experiment was to analyze the demineralizing effects of Colgate Optic White mouthwash on hydroxyapatite tooth enamel. Nano-hydroxyapatite particles were used to make 10% solutions with RO water, artificial saliva, 2% hydrogen peroxide, and Colgate Optic White mouthwash. Small samples from these solutions were centrifuged to create an artificial tooth that was examined for demineralization upon being treated RO water, artificial saliva, 2% hydrogen peroxide, or Colgate Optic White mouthwash. Demineralization of the samples was measured by UV-Vis spectroscopy and calcium concentration was measured to compare demineralization among the four different solutions. Calcium standards and validation samples were prepared to make a calibration curve at a wavelength of 652 nm. The results of this experiment showed that calcium concentration was determined to be greatest in samples treated with Colgate Optic White mouthwash and least in samples treated with artificial saliva. RO water and 2% hydrogen peroxide both showed calcium concentrations greater than artificial saliva, but less than Colgate Optic White mouthwash. Colgate Optic White mouthwash significantly demineralizes more than the other three solutions and there was not any significant difference between RO water and 2% hydrogen peroxide. Artificial saliva demineralized the least out of the four groups, which was expected. These results lead to the conclusion that 2% hydrogen peroxide and RO water can demineralize to the same extent and Colgate Optic White mouthwash must contain an ingredient other than 2% hydrogen peroxide that promotes demineralization. This is significant because whitening mouthwash is commonly used by Americans to whiten their teeth, however, they are unaware that their enamel is being damaged. Having the knowledge of this and investigating the products one uses is the key to maintaining one’s oral health and preventing enamel erosion.
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Introduction

Tooth enamel is composed of hydroxyapatite (Ca$_5$(OH)(PO$_4$)$_3$), which is a matrix of calcium and phosphate [8]. When bacteria such as Streptococcus mutans stick to the enamel, they form large masses, referred to as plaque, which create acid buildup. These acid attacks lower the pH of the oral environment and cause mineral loss, or the dissolving of calcium and phosphate, and lead to porous enamel [8]. Enamel that contains large pores due to acid-induced mineral loss, called demineralization, allows bacteria to easily infect the deeper portion of the enamel. This infection creates a lesion, which is referred to as a carie, and is the primary stage of cavity development [8]. As demineralization continues, the lesion grows, reaching deeper layers such as dentin and eventually forms a cavity, which can result in total loss of the tooth if not treated properly [8].

In dentistry, remineralization is defined as the process of gaining mineral content in the enamel [9]. Saliva serves as a source of calcium and phosphate ions, so it remineralizes tooth enamel naturally when the oral environment is at a neutral pH. However, under acidic conditions, saliva cannot overcome the acidity and demineralization occurs regardless because the rate of demineralization is much faster than the rate of remineralization [9]. Several factors including microhardness, lesion depth, surface roughness, and mineral composition can be measured to determine whether or not remineralization has occurred. Microhardness increases with remineralization and decreases with demineralization because enamel weakens with increased pore size. Lesion depth increases with demineralization and decreases with remineralization because as the lesion progresses, it reaches deeper layers in the enamel. Surface roughness is decreased with demineralization and increased with remineralization because as new mineral is formed on the enamel surface, the morphology is altered. Mineral content
increases with remineralization and decreases with demineralization because calcium and phosphate concentration are lowered when the pH decreases [9]. This process of remineralization and demineralization is constantly occurring in the mouth, which is why everyday oral hygiene must be performed to prevent caries formation.

**Literature Review**

**Hydrogen Peroxide Whitening Agents**

The use of whitening agents, or the “bleaching” of teeth, has increased in popularity recently due to its ability to reduce stains on the surface enamel, creating a brighter, more appealing smile. When hydrogen peroxide is applied to teeth, it diffuses into the enamel and decomposes into free radicals that react with pigment molecules. Pigment molecules are found in beverages, foods, and medications, and adhere more easily to rough surfaces, which are created on enamel after bleaching [3]. Pigment molecules appear as stains or yellowness in tooth enamel and when they react with hydrogen peroxide, they oxidize to hydrolyze and become more hydrophilic, allowing them to be easily removed with water [3]. Hydrogen peroxide is the most common whitening agent used because it is inexpensive and readily available, and its low pH enables it to easily enter the enamel [4]. However, studies have shown that bleaching via hydrogen peroxide can increase susceptibility to caries and cavities, enhance the progression of cavity formation, promote tooth sensitivity, and even cause further discoloration [4]. Hydrogen peroxide causes the enamel surface to weaken, allowing the formation of caries, which are lesions that eventually turn into cavities. Caries are caused by bacteria such as *Streptococcus mutans* that can easily adhere to the enamel and infect the tissue [3]. This easy adherence to enamel surface increases the rate of demineralization, or mineral loss, and allows for caries lesions to form.
There is often confusion regarding bleaching because dentists typically recommend hydrogen peroxide to promote gum health. However, high concentrations of hydrogen peroxide can cause negative side effects such as tooth sensitivity [4]. Healthy enamel reacts differently to hydrogen peroxide than demineralized enamel does because healthy enamel does not allow hydrogen peroxide to penetrate as deeply. Eroded or decaying enamel is very porous and weak, making it more susceptible to damage because hydrogen peroxide particles can enter deeper, more sensitive areas of the tooth [5]. A study by Briso involved the erosion of bovine tooth enamel to create caries lesions and then bleaching of the samples with hydrogen peroxide [5]. From this experiment, it was concluded that hydrogen peroxide from whitening treatments penetrates the enamel at a rapid rate and alters the tissue composition to induce caries lesion growth more effectively in demineralized samples than in sound enamel samples [5]. These findings are important because they show that once a caries lesion has developed, hydrogen peroxide from a bleaching agent can penetrate the enamel quicker and ultimately cause more damage than it can in healthy enamel.

Other than causing tooth sensitivity and an increase in susceptibility for caries lesions, hydrogen peroxide bleaching can cause mineral loss that leads to softening of the enamel. When enamel is weakened, surface microhardness is reduced because mineral content has been lost due to demineralization [6]. Borges performed a study to demonstrate that the use of hydrogen peroxide bleaching treatments can decrease microhardness [7]. This study determined that acidic bleach treatments reduced microhardness to a greater extent than neutral bleach treatments, meaning that the pH of the whitening treatment plays a key role in reducing microhardness [7].

To prevent the demineralizing effects of hydrogen peroxide in whitening regimens, remineralizing agents are often used as additives to promote enamel growth and strength.
Calcium and fluoride are the two most common substances added to bleaching treatments in order to remineralize enamel and to inhibit enamel degradation [8]. Several studies have been conducted to test the remineralization potential of different additives to whitening regimens. There are conflicting results in regards to evaluating the efficacy of calcium as an additive to bleaching treatments because some studies have shown that it is effective, while others conclude that it is ineffective [8, 9]. One reason this may be is due to the presence of artificial saliva during the treatment. Artificial saliva acts as a pH buffer similar to natural saliva and is used in research to create a more realistic oral environment during experimental treatment [8, 9].

An alternative to calcium as a remineralizing additive to whitening agents is fluoride, which is already very popular for its remineralizing effects in toothpastes, mouthwashes, and even chewing gum. Several studies have analyzed the effects of utilizing fluoride as an additive to hydrogen peroxide-based whitening agents under varying conditions [3, 10]. It was concluded that fluoride has greater remineralizing potential under neutral conditions than acidic conditions [3]. This shows that pH is an important factor that plays a role in whether or not enamel can be remineralized. Fluoride sources can vary, but the most common are sodium fluoride and titanium tetrafluoride [10]. Stannous fluoride has also been known to remineralize tooth enamel and also provide antibiotic effects [13]. When these sources were compared to one another, it was concluded that both sources are equally effective at preventing hydrogen peroxide-induced demineralization in enamel [10], meaning that although sources of fluoride can vary, the overall effect is generally equivalent.

**Nano-Hydroxyapatite**

Hydroxyapatite is the primary component of enamel that fills the small pores within a tooth’s structural matrix to provide protection and support. It is composed of calcium and
phosphate, which are the key minerals that build strong enamel [14]. Nano-hydroxyapatite is synthetic hydroxyapatite that is small in size, ranging between 50 and 1000 nanometers, allowing it to easily enter pores and penetrate as deep as the dentin layer [14]. Due to its ability to release calcium easily, nano-hydroxyapatite serves as a source of bioavailable calcium ions and has tremendous remineralizing potential [14]. Real enamel is organized by levels of hydroxyapatite rods, ranging from fine rods of hydroxyapatite as the fundamental basis to entire enamel rods that form a lattice-like structure over the dentin. Nano-Hydroxyapatite differs in its structure because it lacks the organization that enamel has and does not have a set orientation of its hydroxyapatite rods [15]. Nano-Hydroxyapatite is very similar to actual enamel in its components, but its ability to resist deformation under high friction is slightly less and its wear volume is slightly lower than that of true enamel. These differences are due to the specific orientation of the hydroxyapatite rods within the enamel that have not yet been replicated in nano-hydroxyapatite [15]. However, despite these differences, nano-hydroxyapatite is still the closest substance to actual enamel and researchers are steadily finding new ways to make nano-hydroxyapatite act more comparable to true enamel [15].

Many studies have been conducted to test the efficacy of nano-hydroxyapatite as a remineralizing agent in degraded enamel. Just as with fluoride, the pH of the environment when a treatment is being applied effects the remineralization potential of nano-hydroxyapatite. However, nano-hydroxyapatite is more effective under acidic conditions than under neutral conditions. This is a key difference between nano-hydroxyapatite and fluoride because demineralization is enhanced under acidic conditions, but since nano-hydroxyapatite also thrives in an acidic environment, it can prevent mineral loss more effectively than fluoride [16]. Huang’s study compared the effects of nano-hydroxyapatite to micro-hydroxyapatite, a slightly larger
version of the particle, and it was determined that nano-hydroxyapatite had an overall better remineralizing effect, which led to the conclusion that the size of the particle does matter and the smaller the particles of nano-hydroxyapatite, the more effective they are because smaller particles can penetrate pores more easily [16].

Despite the increased research of nano-hydroxyapatite, fluoride is still more commonly used as a cavity preventative. However, when nano-hydroxyapatite is compared to sodium fluoride, studies have shown that sodium fluoride forms globular structures on the enamel surface, while nano-hydroxyapatite forms rod-like structures. This difference can affect the ability of enamel to combat infection because rod-like structures are similar to the natural matrix of enamel, while globular structures can leave openings for bacteria to enter and cause damage [17, 18]. The shape of nano-hydroxyapatite is more natural to the enamel matrix and nano-hydroxyapatite can increase calcium and phosphate levels more than fluoride [17, 18].

Nano-hydroxyapatite has also been shown to reduce tooth sensitivity, which is a common problem for many individuals. Tooth sensitivity can be triggered by many different factors including cold air, hot beverages, sweet foods, pressure from brushing and/or eating, and chemical stimuli. The actual cause of tooth sensitivity is still being researched, but the common theory agreed upon by most scientists and dentists is that fluid within the branching channels of the dentin, called dentinal tubules, moves around and comes in contact with the dentin and the pulp, causing discomfort and pain [19]. Desensitizing toothpastes are common over-the-counter treatment options that are inexpensive and readily available to the general public. A recent clinical trial evaluated the efficacy of nano-hydroxyapatite desensitizing toothpaste and it was determined that nano-hydroxyapatite can effectively reduce tooth sensitivity [19]. These results
are relevant because nano-hydroxyapatite could be a potential option for individuals facing tooth sensitivity.

Several different options are available to incorporate nano-hydroxyapatite as an additive, but mouthwashes and toothpastes are the most common. When nano-hydroxyapatite is added to mouthwashes, it increases microhardness similarly to mouthwashes containing sodium fluoride [20]. Nano-hydroxyapatite is also just as effective as fluoride when it is incorporated into a dentifrice, or toothpaste, because it can equally increase mineral levels, reduce lesion size, and increase surface microhardness [201-23]. However, not all research agrees with these findings. One study compared fluoride toothpaste to nano-hydroxyapatite toothpaste and the results showed that surface hardness was greater and mineral loss was prevented to a greater extent after treatment with fluoride toothpaste than with nano-hydroxyapatite toothpaste [24]. The distinction between this study and other similar studies is the pH of the environment when treatments were applied. The majority of studies conducted that compare nano-hydroxyapatite to fluoride have concluded that nano-hydroxyapatite is just as effective as fluoride and has equal, if not greater, remineralization potential [21-23].

**Nano-Hydroxyapatite and Hydrogen Peroxide Whitening Agents**

Due to the increased use of hydrogen peroxide whitening agents and research findings concluding that hydrogen peroxide has the ability to deteriorate enamel, several studies have been conducted to determine whether or not applying a remineralizing regimen post-bleaching could prevent tooth sensitivity. Tooth sensitivity is a common side effect of bleaching and is associated with enamel damage [25, 26]. A short-term clinical trial determined that using nano-hydroxyapatite toothpaste after bleaching can significantly reduce tooth sensitivity [25]. A long-term clinical trial that incorporated nano-hydroxyapatite into its whitening agent also concluded
that the use of nano-hydroxyapatite significantly reduced tooth sensitivity [26]. This means that nano-hydroxyapatite, whether applied after or incorporated into the whitening treatment, can prevent demineralization in enamel.
Materials and Methods

Treatment Solution Preparation

All treatment solutions were prepared prior to experimentation and made in stock amounts. One liter of artificial saliva was prepared using the recipe found in Appendix 1. Nano-Hydroxyapatite was used in powder form and ordered from Sigma Aldrich Supply Company. All treatment solutions containing nano-hydroxyapatite were at a concentration of 10% nano-hydroxyapatite because there is evidence that shows this concentration is optimal for remineralization to occur [12]. Four different stock solutions were prepared of 10% nano-hydroxyapatite in a solvent. The solvents included RO water, artificial saliva, 2% hydrogen peroxide, and Colgate Optic White mouthwash. The hydrogen peroxide treatment was at a 2% concentration because this is equivalent to the concentration of hydrogen peroxide within the whitening mouthwash that was used in the test group [27].

The negative control was artificial saliva because this substance represents natural saliva, which does not demineralize enamel. It was predicted that tooth enamel would not demineralize when treated with artificial saliva. The positive controls were RO water and 2% hydrogen peroxide because previous research has shown that these substances will naturally demineralize tooth enamel. It was predicted that tooth enamel would demineralize when treated with RO water or 2% hydrogen peroxide. The final treatment was Colgate Optic White mouthwash, which served as the test group. This specific brand of mouthwash was selected because it contained hydrogen peroxide as its active ingredient, but also lacked any remineralizing agent such as fluoride or calcium [27]. It was predicted that this substance would demineralize tooth enamel, but the extent and how this substance compares to the positive and negative controls was unknown.
Artificial Teeth

An in vitro system was used by preparing artificial teeth before using real enamel. Human teeth are difficult to experiment on within people’s mouths and obtaining extracted teeth in large quantities is difficult. Preparing artificial teeth was the most logical solution for experimenting on enamel without running out of samples to test. The artificial teeth used in this experiment were prepared by pipetting 500 microliter samples of the prepared 10% nano-hydroxyapatite solutions into centrifuge tubes. The tubes were then placed in a centrifuge and spun for approximately 30 seconds to separate the nano-hydroxyapatite from its solvent. The result after centrifuging can be seen below:

Image 1. Artificial tooth produced by centrifugation.

The separated nano-hydroxyapatite forms a hard precipitate at the bottom of the centrifuge tube and acts as an artificial tooth because it contains the same components as true enamel. The
solvent, varying from RO water, artificial saliva, 2% hydrogen peroxide, and Colgate Optic White mouthwash, serves as a treatment solution being applied to the artificial tooth.

Samples were then collected from the top layer of the solvent and demineralization of the artificial tooth can be measured. If the solvent that is in contact with the artificial tooth causes demineralization, calcium ions will be present in the solvent and demineralization potential can be measured. The greater the concentration of calcium ions, the more demineralization occurred and the lower the concentration of calcium ions, the less demineralization occurred. The centrifuge tubes were prepared in time increments of 1 day, 2 days, 7 days, and 14 days from the original stock solutions prepared on day 1.

**Ultraviolet-Visible Spectroscopy**

A Jasco UV-Vis 650 spectrophotometer was used to measure demineralization of the samples drawn from the treatment solvent applied to the artificial teeth. This instrument can be seen below:

![Image 2. Jasco UV-Vis 650 spectrophotometer](image)

A spectrophotometer contains a light source that contains many wavelengths, or colors, of light that are sent through a monochromator, which separates those different wavelengths and selects
one specific wavelength that a sample will absorb. The absorbance of that specific wavelength is then used to determine the concentration of a specific ion present in the sample [28].

In this experiment, the samples used were drawn from the solvents that were used to treat artificial teeth and the ion concentration measured was calcium. Arsenazo dye was added to each sample because this dye binds to free calcium ions and ranges from magenta in low calcium concentration to dark indigo in high calcium concentration. This dye acts a chromophore, or color molecule, that absorbs specific wavelengths of light. A calibration curve was made for each wavelength by preparing different calcium solutions in artificial solution, ranging from 1.0 to 5.0 ppm of calcium. Validation samples were also prepared to validate the calibration curve before testing samples and their concentrations included 0.5, 1.5, 3.5, and 4.5 ppm of calcium in artificial saliva.

**Microhardness Testing**

Microhardness is a measurement of tooth enamel strength that can be used to determine whether or not an enamel sample has undergone remineralization or demineralization. There are a variety of tests that can be utilized in order to measure microhardness, including abrasion, indentation, and scratch tests. Instruments that are commonly used to obtain microhardness measurements include the Knoop diamond indenter and Vickers microhardness tester, along with scanning electron microscopy and atomic force microscopy. The Knoop diamond indenter provides a Knoop Hardness Number (KHN), while the Vickers microhardness tester determines a Vickers Hardness Number (VHN), but they are essentially equivalent. The Vickers microhardness tester can make a deeper indentation and is less sensitive to surface conditions than the Knoop microhardness tester [29]. The average hardness for enamel is within the range
of 250-360 KHN/VHN, however, there is some deviation that has been reported due to varying parameters [30].

This experiment used a Vickers Microhardness Tester to measure VHN values of bovine tooth enamel. A Shimadzu type M was the model used throughout this experiment, which is shown below:

![Image 3. Shimadzu type M Vickers microhardness tester](image)

The Vickers microhardness tester has a diamond indenter with a square base and a pyramidal shape to allow penetration into samples. A weight is placed on top of the diamond indenter to force the tip into the surface of the substance, which is shown below:
The resulting impression is then measured using a high-power microscope with a scale to determine the diameter. A detailed diagram is shown below to explain the indentation made by the microhardness tester.

The force from the weight and the diameter measurement are then put into an equation, which can be viewed in Appendix 2 to calculate the hardness value. This hardness value serves as a Vickers Microhardness Number (VHN) that ranges between 100 and 1000 [29].
This instrument was essential to this experiment because demineralized enamel is weaker than normal enamel and is not as hard, decreasing its hardness value. Normal enamel or remineralized enamel has a higher hardness value than demineralized enamel and can indicate whether or not the enamel is being demineralized. Before testing true enamel, aluminum was tested in order to calibrate the instrument and not damage any specimens during practice. The artificial teeth could not be measured for hardness, however, true enamel could be tested. Due to recent government restrictions on the use of human teeth for research, bovine teeth have become a popular substitute because of the convenience to obtain them and their similar microstructure. The primary reason bovine teeth are preferred over human teeth is they have increased uniformity and flatness, which reduces the risk of caries and cavities [31, 32]. Bovine teeth were selected to use for true enamel experimentation in this study due to the evidence previously stated and the availability from a generous donor.

The solution in which an enamel sample is stored, in addition to how long it is stored within a solution can greatly affect the mineral content of the sample, which can in turn influence microhardness. Storage solutions are important to any experiment because they prevent dehydration and bacterial contamination of a sample. Some commonly used storage solutions include distilled water, thymol, ethanol, saline, glutaraldehyde, and artificial saliva. There is evidence to support the idea that the amount of time a specimen spends in a solution does not cause any effects on the mineral content of the enamel [33]. Distilled water is one of the more common storage solutions used for short-term use and in between treatments, while thymol is preferred for long-term storage before experimentation because of its ability to preserve microhardness [33]. Based on the findings from several studies, this experiment used 70%
ethanol as the storage solution for bovine teeth and was changed each month prior to the experiment.
Results

Upon analyzing the spectra, which can be seen below, two peaks were chosen, resulting in the selection of 415 nm and 652 nm as the specific wavelengths.

![Figure 1. Spectra of calcium assay](image)

The calibration curves at each wavelength and the validation samples against a calibration curve are shown below:

![Figure 2. Calibration curve at 415 nm](image)
Upon completing this experiment, a trend was determined throughout the four solution groups and their demineralization potentials, which can be seen on the following page:
Figure 5. Comparison of demineralization potential among test groups

The negative control group, which contained artificial saliva as the solvent in contact with the artificial tooth, showed the lowest calcium concentration. The positive control groups, which included 2% hydrogen peroxide and RO water, showed greater calcium concentration than artificial saliva. However, what was not expected was that RO water and 2% hydrogen peroxide showed no significant difference between each other. The most noticeable of the four groups was the test group, which contained Colgate Optic White mouthwash. This solution showed significance among all of the other test groups and had the greatest calcium concentration.

There did not appear to be any trend between the time increments chosen. This may be due to the lack of an extended period of time as the experiment was stopped after 14 days. However, there is not a current prediction of the appearance of a trend had the experiment been extended for a longer period of time.
An Anova test was performed on all the data collected from UV-Vis spectroscopy through Microsoft Excel. A t-test was also performed on the positive control groups, which were 2% hydrogen peroxide and RO water, to analyze for significance. More information regarding the statistical analysis of the data collected can be seen below:

<table>
<thead>
<tr>
<th>Groups</th>
<th>Count</th>
<th>Sum</th>
<th>Average</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>RO Water</td>
<td>3</td>
<td>4.50536</td>
<td>1.50178</td>
<td>0.052847996</td>
</tr>
<tr>
<td>Artificial Saliva</td>
<td>3</td>
<td>3.25129</td>
<td>1.08376</td>
<td>0.012722338</td>
</tr>
<tr>
<td>2% Hydrogen Peroxide</td>
<td>3</td>
<td>4.65397</td>
<td>1.55132</td>
<td>0.028026088</td>
</tr>
<tr>
<td>Mouthwash</td>
<td>3</td>
<td>23.64089</td>
<td>7.88029</td>
<td>0.269690329</td>
</tr>
</tbody>
</table>

Table 1. Summary

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F</th>
<th>P-Value</th>
<th>F-Crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>0.395808561</td>
<td>2</td>
<td>0.197904281</td>
<td>6.343328367</td>
<td>0.033102</td>
<td>5.143253</td>
</tr>
<tr>
<td>Within Groups</td>
<td>0.187192845</td>
<td>6</td>
<td>0.031198808</td>
<td></td>
<td>96.68976</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.583001406</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Anova

<table>
<thead>
<tr>
<th></th>
<th>RO Water</th>
<th>2% Hydrogen Peroxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>1.501786667</td>
<td>1.551323333</td>
</tr>
<tr>
<td>Variance</td>
<td>0.052847996</td>
<td>0.028026088</td>
</tr>
<tr>
<td>Observations</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Hypothesized Mean Difference</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Df</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>T stat</td>
<td>-0.301705146</td>
<td></td>
</tr>
<tr>
<td>P One-Tail</td>
<td>0.388956072</td>
<td></td>
</tr>
<tr>
<td>T Crit One Tail</td>
<td>2.131846786</td>
<td></td>
</tr>
<tr>
<td>P Two Tail</td>
<td>0.777912144</td>
<td></td>
</tr>
<tr>
<td>T Crit Two Tail</td>
<td>2.776445105</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. T-test
Discussion

The results of this experiment show that Colgate Optic White mouthwash demineralizes more than RO water, artificial saliva, and 2% hydrogen peroxide. Although it was expected for this test group to demineralize the artificial tooth more than RO water and artificial saliva, it was not predicted to demineralize more than 2% hydrogen peroxide. The Colgate Optic White mouthwash contains 2% hydrogen peroxide as its active ingredient and was expected to demineralize to generally the same extent as 2% hydrogen peroxide. This is interesting data because it leads to the conclusion that something else in the mouthwash is causing demineralization to occur in addition to the demineralizing effect of hydrogen peroxide. The other ingredients listed for this mouthwash include water, glycerin, propylene glycol, sorbitol, polysorbate 20, phosphoric acid, citric acid, flavor, sodium saccharin, and several copolymers [27]. Ingredients such as copolymers and “flavor” do not explain their components, so until that information is released, it is unknown whether those substances contribute to demineralization.

The results also show that RO water and 2% hydrogen peroxide demineralize to the same extent without any significant difference. This means that 2% hydrogen peroxide is not as harmful as it seems, however, it does still contribute to demineralization. These conclusions should be considered when choosing a whitening product because even the smallest enamel loss over time can steadily lead to tooth sensitivity, a cavity, weakened enamel, and other oral issues. The artificial saliva solution showed the least demineralization, which was expected and showed that the model developed in this study was realistic.

Additionally, from these results it can be predicted that true enamel would follow these trends if introduced to these test solutions. Due to the evident similarities between nanohydroxyapatite and true enamel, it can be expected that true enamel would behave the same way.
if RO water, artificial saliva, 2% hydrogen peroxide, and Colgate Optic White mouthwash were applied to a sample.

**Limitations**

This experiment was limited in time, which contributed to setbacks with the calcium assay and progression with microhardness testing. Making a calibration curve with a strong $R^2$ value takes practice and can be difficult because every time there is a slight change in the instrument, a new calibration curve must be made. Also, the calcium assay used Arsenazo dye, which is very sensitive to pH changes. If the pH is slightly off balance, the dye does not properly bind to calcium ions in the sample and the color immediately turns very dark blue. The color of the samples is important because the dye is what absorbs the wavelength of light from the UV-Vis and provides a concentration value for the ion in question. This occurred in the beginning of performing the calcium assay and took a good portion of the time allotted to the calcium assay away.

Additionally, when statistical analysis was performed on the data from the calcium assay, it was determined that an error had occurred when measurements from the 7 day samples were taken. This caused all data from day 7 to be discarded, minimizing the data pool to three time increments, instead of the original four.

Lastly, when the microhardness tester arrived, it was discovered that the calibration sample provided did not fit the vise on the instrument. This was problematic in that the instrument was useless until it was calibrated because any measurements taken on the instrument would not be considered valid until the instrument was calibrated. Once this issue was resolved, there was not any time left to take hardness measurements on real enamel.

**Future Research**
Future studies should examine the hardness of real enamel and how it is influenced by treatment of various solutions. Different whitening mouthwash brands is also suggested because in this study, only one specific mouthwash brand was tested. There is also a multitude of different types of whitening products, including toothpastes, strips, trays, pens, and in-office treatments. These various types of whitening products could also serve as great future research projects for students to look into. Additionally, looking into the time intervals further and extending beyond 14 days would be a very interesting study. This experiment only examined up to 14 days with very little trend shown, but there may be a possibility of a trend appearing over a greater time period.
Appendices

Appendix 1: Artificial Saliva Recipe

Materials: 10 g/L carboxymethyl cellulose

1.0 g/L methyl p-hydroxybenzoate

0.8 g/L K$_2$HPO$_4$

0.3 g/L KH$_2$PO$_4$

0.624 g/L KCl

0.06 g/L MgCl$_2$

0.0044 g/L NaF

Instructions: Add carboxymethyl cellulose to a volumetric flask and fill it to approximately 1/3 with distilled water. Pour into a beaker and apply medium to low heat. Allow it to stir rapidly for at least twelve hours with parafilm over the top to prevent contamination. Once twelve hours has passed, all other chemicals can be added and distilled water can be used to fill up to the 1 L mark. Stir again to combine the contents until all is dissolved.

Appendix 2: Calculating Hardness Values

$$HV = \frac{2F \cdot \sin \Theta}{D^2}$$

$HV =$ Hardness Value (Kgf/mm$^2$)  $F =$ Force from Weight (Kgf)  $D =$ Diameter of Indentation (mm)
References


