QMIX 2in1 and NaOCI Precipitate: Documentation, Identification, and Exothermic Reaction

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QMIX 2in1 AND NaOCl PRECIPITATE: DOCUMENTATION, IDENTIFICATION, AND EXOTHERMIC REACTION

by

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ABSTRACT
QMIX 2in1 AND NaOCl PRECIPITATE: DOCUMENTATION, IDENTIFICATION, AND EXOTHERMIC REACTION

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Introduction: The aim of this study was to document and identify precipitate formation and exothermic reaction caused when mixing NaOCl and QMiX.

Methods: Photography captured time-dependent changes when mixing NaOCl and QMiX at; 0 seconds, 30 seconds, 1 minute, 30 minutes, 1 hour, and 24 hours. A differential scanning calorimeter (DSC) was utilized in determination of heat flow while analyzing temperature change (°C), enthalpy (mJ), and time (seconds) for NaOCl with QMiX and NaOCl with 2% chlorhexidine (CHX). 1H Nuclear Magnetic Resonance (NMR) and 2D NMR (DOSY) spectroscopy analyzed precipitation formed from NaOCl and QMiX.

Results: Digital photography documented immediate, intense color change (blue-green to orange-brown) with colorless, pungent gas production. 1-30 minutes - color change gradually lightened to a brownish-yellow and gas production lessened until cessation. 30 minutes - precipitation was noted throughout solution. 24 hours - precipitate coalesced from suspension to the bottom of the test tube. DSC analysis revealed endothermic reactions for both control groups (QMiX and QMiX, CHX and CHX). NaOCl and QMiX resulted in an exothermic reaction with an average temperature change of 4.60 +/- 0.20°C, enthalpy of 6266 +/- 608 mJ, and time to maximum peak of 3 +/- 1 seconds. NaOCl and CHX resulted in an exothermic reaction with an average temperature change of 0.40 +/- 0.20°C, enthalpy of 3045 +/- 384 mJ, and time to maximum peak of 52 +/- 7 seconds. All t-tests revealed a p value of $p<0.01$.1H NMR revealed trace amounts of aromatic ring compounds (CHX, PCU). 2D spectra indicated unidentified inorganic salt with a molecular weight of 500 g/mol.

Conclusions: Mixing 5.25% NaOCl and QMiX causes an immediate, intense reaction leading to color change, gas production, and a time-dependent precipitate formation (unidentified inorganic salt). Mixing creates an exothermic reaction and heat increase of 4.60°C +/- 0.20°C within 3 +/- 1 seconds. Intermediate irrigation protocols and drying techniques must be utilized to lessen precipitate and gas formation, helping to prevent occluded dentinal tubules before endodontic obturation. Further research is needed to identify the molecular structure of formed precipitate and evaluate potential in vivo effects

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DEDICATION

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This project is dedicated to my wife and best friend, Anna. I cannot thank you enough! Your continual love and support has made all things possible. Thank you for always allowing me to follow my dreams, and being there every step of the way. You will be the perfect mother, and I cannot wait to see what life has in store! Even with 5,569 yesterdays, each night I pray for just 1 more tomorrow.

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INTRODUCTION

The ultimate goal of endodontic treatment is either to prevent the development of apical periodontitis or, in cases where the disease is already present, to create adequate conditions for periradicular healing (1). This goal is defined with intent to repair and preserve natural tooth structure and associated periradicular bone. The cardinal principle of any health care profession is the thorough understanding of disease etiology and pathogenesis, which in turn, provides a framework for effective treatment.

The first recorded observation of bacteria in the root canal dates back to the 17th century when Antony van Leeuwenhoek reported "animalcules (2)."Almost 200 years later, Robert Koch confirmed a cause-and-effect relationship between bacteria and apical periodontitis. Kakehashi and colleagues further confirmed this concept in 1965, with the use gnotobiotic and conventional rats to prove causal relationships between endodontic infection and bacteria (3). The important role of bacteria as etiology was again documented in 1976 by Sundqvist et al, whom applied advanced anaerobic culturing techniques to the evaluation of bacteria occurring in the root canals of teeth with pulp that became necrotic after trauma (4**).** Moller and colleagues also provided strong evidence of microbiota causation of apical periodontitis. Using monkeys, Moller et al demonstrated that only devitalized pulps that were infected induced apical periodontitis lesions, whereas devitalized and noninfected pulps remained absent of significant pathologic changes in periradicular tissues (5).

Microbiota causing apical periodontitis are primary organized in biofilms colonizing the root canal system (6**).** Although Nair and colleagues were the first to observe this micro-environment, Riccucci and Siqueira revealed the high prevalence of

bacterial biofilms in association with primary and post-treatment apical periodontitis. Microbiota can breach dental hard-tissue barriers through several avenues such as dental carries, cracks, fractures, marginal leakage, and traumatic events (7). However irritants enter, most agree that a major biologic aim of root canal therapy is to address apical periodontitis by disinfection protocols through various cleaning and shaping techniques.

Primary objectives in a cleaning and shaping protocol consist of the following: removal of infected soft and hard tissue, allowing disinfecting irrigants access to the apical canal space, create space for the delivery of medicaments and subsequent obturation, and to retain the integrity of radicular structures (8). Cleaning and shaping encompasses the mechanical and chemical routes for removal of infectious and inflamed tissue. Peters suggested, at best, only 65% of canal walls are touched with instruments during root canal therapy due heavily to anatomic and morphologic complexities within the human tooth (9). An important mechanical objective is to retain as much cervical and radicular dentin as possible so as not to weaken the root structure, thereby preventing root fracture. To remove the untouched remaining tissues or fragments left from the mechanical removal, chemical irrigants and medicaments are introduced within the root canal system.

The objectives of irrigation in endodontics are mechanical, chemical, and biologic. The mechanical and chemical objectives are as follows: flush out debris, lubricate the canal, dissolve organic and inorganic tissue, and prevent the formation of a smear layer during instrumentation or to dissolve it once it has formed (10). Mechanical effectiveness will depend on the ability of irrigation to generate optimum streaming forces within the entire root canal system. The chemical effectiveness will depend on the

concentration of the antimicrobial irrigant, the area of contact, and the duration of interaction between the irrigant and infected material (11). The final efficiency of endodontic disinfection will depend on the chemical and mechanical effectiveness (12). The biologic function of irrigants is related to their antimicrobial effects. Irrigants should have a high efficacy against anaerobic and facultative microorganisms in their planktonic state and in biofilms, inactivate endotoxin, be nontoxic when they come in contact with vital tissues, and not cause an anaphylactic reaction (10). Efficiency of root canal irrigation in terms of debris removal and eradication of bacteria depends on several factors: penetration depth of the needle, diameter of the root canal, inner and outer diameter of the needle, irrigation pressure, viscosity of the irrigation, velocity of the irrigant at the needle tip, and type and orientation of the needle bevel (11).

Benefits of using irrigants in root canal therapy include: removal of particulate debris and wetting of the canal walls, destruction of microorganisms, dissolution of organic debris, opening of dentinal tubules by removal of the smear layer, and disinfection and cleaning of areas inaccessible to endodontic instruments. Properties for an ideal irrigant should include:

- Be an effective germicide and fungicide
- Be nonirritating to the periapical tissues
- Remain stable in solution
- Have a prolonged antimicrobial effect
- Be active in the presence of blood, serum, and protein derivatives in tissue
- Have low surface tension
- Not interfere with repair of periapical tissues

- Not stain tooth structure
- Be capable of inactivation in a culture medium
- Not induce a cell-mediated immune response
- Be able to completely remove the smear layer, and be able to disinfect the underlying dentin and its tubules
- Be nonantigenic, nontoxic, and noncarcinogenic to tissue cells surrounding the tooth
- Have no adverse effects on the sealing ability of filling materials
- Have a convenient application
- Be relatively inexpensive

An optimal irrigant would have all beneficial characteristics with no harmful or negative properties (13). Presently, no ideal solutions exist. However combined use of selected irrigation products and protocols greatly contribute to success and survival treatment outcomes. Today's model for effective and efficient endodontic diagnosis, prognosis, and treatment is through an evidence-based approach.

LITERATURE REVIEW

Sodium Hypochlorite – NaOCl

Sodium hypochlorite was first produced in 1789 for use as a hospital antiseptic in France. Coolidge introduced NaOCl to endodontics as an intracanal medicament in 1919 (14). Since, it has become the most commonly used irrigating solution (15) in root canal therapy for several reasons. NaOCl has antibacterial capacity, the ability to dissolve necrotic tissue, vital pulp tissue, and organic components of dentin and biofilms in a relatively fast manner (16). NaOCl also possesses the ability to inactivate endotoxins to some extent (17). When NaOCl contacts tissue proteins, nitrogen, formaldehyde, and acetaldehyde are formed. Peptide links are fragmented, proteins disintegrate, and chlorine atoms replace hydrogen on amino groups forming chloramines. Estrella and colleagues reported several factors that permits NaOCl to have such antimicrobial efficiency (18):

- Saponification reaction - as an organic and fat solvent that degrades fatty acid and transforms them into fatty acid salts and alcohol, thereby reducing surface tension of the remaining solution
- Neutralization reaction – through neutralizing amino acids by forming water and salt. With the exit of hydroxyl ions, the pH is reduced.
- Hypochlorus acid formation – formed once chlorine dissolves and comes in contact with organic matter. This weak acid acts as an oxidizer, which leads to amino acid degradation and hydrolysis.
- Solvent Action – releasing chlorine that combines with protein amino groups to form chloramines, which impedes cell metabolism. Chlorine is a strong

oxidant and inhibits essential bacterial enzymes by irreversible oxidation of SH groups.

- High pH – NaOCl is strong base with a pH > 11. The high pH interferes in cytoplasmic membrane integrity due to irreversible enzymatic inhibition, biosynthetic alterations in cellular metabolism, and phospholipid degradation observed in lipidic peroxidation.

NaOCl is used in concentration between 0.5% and 6% for irrigation during root canal therapy. This is due to controversy and contrasts within current and classic literature. While in vitro studies suggest higher concentration to be more effective in regards to antimicrobial properties (19), in vivo studies report similar effectiveness of low and high concentrations with ability to reduce bacteria from the root canal system. All concentrations lose antimicrobial abilities after two minutes, due to hydrolysis (20). The importance of volume is comprehended when using lower concentrations of NaOCl to ensure adequate efficiency (17). Spangberg and colleagues reported that toxicity rises with the use of higher concentrations compared to lower concentrations, however a low incidence of mishaps occurs due to confined anatomy with the root canal system (21). As toxicity to surrounding tissues is the major drawback of NaOCl, it is of utmost importance to ensure its use is maintained within the root canal space and the clinician does not extrude the irrigant out of the apex or a perforation (22).

Ethylenediamine Tetra-Acidic Acid – EDTA

EDTA was first described by Ferdinand Munz in 1935, and later into the endodontic specialty for its chelating properties in 1957 by Nygaard-Ostby (23). On direct exposure for extended time, EDTA has the ability to extract bacterial surface

proteins by combining with metal ions from the cell envelope, which can eventually lead to bacterial death (23). Chelators, such as EDTA, form a stable complex with calcium. When all available ions have ben bound, no further dissolution takes place, indicating that EDTA is self-limiting after roughly 7 hours (24).

EDTA's application in endodontics is widely used to remove the smear layer and inorganic portion of the root canal system. However, EDTA alone cannot normally remove the smear layer effectively. A proteolytic component, such as NaOCl, must be added to remove the organic components of the smear layer (25). The ability to remove the smear layer also incorporates the ability of EDTA to detach the adhered biofilm to canal walls, which suggest why EDTA proved to be highly superior to saline in reducing intracanal microbiota despite relatively low antiseptic capacity (26). The standard concentration for endodontic application is 17% and can remove the smear layer when in direct contact with root canal walls for less than one minute (27). EDTA can also act as a very effective lubricant while negotiating calcified, narrow, and complex apical anatomy. Also, it should be considered that heating of the EDTA is non-desirable, as calcium binding properties diminish as temperature rises (28).

Interaction of NaOCl and EDTA

Grawehr and colleagues studied the interactions of NaOCl and EDTA in 2003. They concluded that EDTA retained its calcium-complex ability when mixed with NaOCl, but EDTA negatively affected NaOCl. Antagonistic interactions included the loss of free available chlorine for NaOCl when in contact with chelators, which consequently reduced the tissue dissolution capability and to a lesser extent antimicrobial activities. This suggests clinically, that these two irrigants need to be used separately in an

alternating irrigating regime (29). In modern endodontics, EDTA is used at the completion of cleaning and shaping to reduce interaction of irrigants while maximizing smear layer removal potential.

Chlorhexidine – CHX

Chlorhexidine was developed in the United Kingdom and originally marketed as an antiseptic cream. Its general disinfection purposes include treatment for skin, eye, and throat infections in both humans and animals (30). It was introduced to endodontics and has been used as an irrigant for nearly two decades (15). CHX is a strongly basic molecule with a pH between 5.5 and 7. CHX digluconate salt is easily soluble in water and is very stable (31).

CHX is a broad-spectrum antimicrobial agent, with activity against gram-positive bacteria, gram-negative bacteria, and yeasts. Due to its cationic charges, CHX is capable of electrostatically binding to the negatively charged surfaces of bacteria, damaging the outer layers of the cell wall and rendering it permeable (32). Another advantage of this irrigant is its substantivity. The cationic nature of the CHX molecule allows anionic substrates, such as oral mucosa and tooth structure, to absorb the molecule. Studies have shown that the uptake of CHX onto teeth is reversible. This reversible reaction of uptake and release of CHX leads to substantive antimicrobial activity and is referred to as substantivity (33).

CHX is normally used at concentration between 0.12% and 2%. Loe and colleagues demonstrated that CHX, within these concentrations, exhibit low toxicity locally and systemically, and is slightly less toxic than NaOCl to surrounding periapical tissues (34). The antibacterial efficacy of CHX is also concentration dependent, as

2%CHX has demonstrated greater efficacy than 0.12%CHX in vitro (35). Allergic and anaphylactic reactions were reported in a few studies, but it is important to mention that patients that are allergic to NaOCl may also be allergic to CHX (36). The major drawback of CHX, compared to NaOCl, is the inability of tissue dissolution. Therefore, NaOCl maintains its role as the primary irrigant and workhorse during the chemo-mechanical debridement during root canal therapy.

Interaction of CHX with NaOCl

NaOCl and CHX produce a change of color and a precipitate. The reaction is dependent of the concentration of NaOCl. The higher the concentration, the more precipitate is formed. Basrani and colleagues reported the precipitate formation of 4-chloroaniline or PCA. Furthermore, a study using time of flight secondary ion mass spectrometry analysis shows the penetration of the precipitate inside dentinal tubules. The results were worrisome, as PCA had previously been reported to be toxic and humans with short-term exposure develop cyanosis, which is a manifestation of methemoglobin formation (35). Later work by Thomas and Sem, proved via NMR testing that no identifiable quantity of PCA was produced when mixing NaOCl and CHX (37). A follow up study to identify the molecular structure was published by Nowicki and Sem**.** They demonstrated that PCA was indeed not created, however two similar molecules; parachlorophenylurea (PCU) and parachlorophenylguanidyl-1,6-diguanidyl-hexane (PCGH) were formed (38). All studies speculated that the combination of CHX and NaOCl causes color changes and formation of a possibly toxic insoluble precipitate that may interfere with the seal of the root obturation. Therefore, conclusions were made that

intermediate irrigation and drying canal spaces with paper points should be used between NaOCl and CHX irrigation protocols (39).

Interaction of EDTA and CHX

The combination of EDTA and CHX was reported to produce a white precipitate. Rasimick and colleagues conducted a study to determine whether the precipitate involves the chemical degradation of CHX. The precipitate was produced and re-dissolved in a known amount of dilute trifluoroacetic acid. Results determined that CHX was found to form a salt with EDTA, rather than undergoing a chemical reaction (40). Although no interactions occur, intermediate rinsing and drying of canal spaces are suggested to reduce the potential for salt formation to reduce hindering of the seal during obturation.

QMiX 2in1

QMiX was introduced by Tulsa Dentsply in 2011 as a combination product for root canal irrigation. Suggested use is recommended to be at the end of instrumentations, after NaOCl irrigation. According to the patent (41), QMiX contains a CHX-analog, Triclosan (N-cetyl-N, N, N-trimethylammonium bromide), and EDTA-Na_2 as a decalcifying agent; it is intended as an antimicrobial irrigant as well as an agent to remove canal wall smear layers and debris. This product is a proprietary blend formula, so direct ingredients and concentrations are unavailable.

QMiX is suggested as a final rinse. Research conducted by Kolosowski et al (42) demonstrated PCA formation between regimes of NaOCl and CHX. They found that no PCA was found with the NaOCl and QMiX, however, methods between experimental groups were distinctly different and will be further detailed in the discussion section.

Grundling and colleagues evaluated the ability of QMiX to reduce lipopolysaccharide (LPS) levels in an in vitro model. Results demonstrated that QMiX was capable of reducing LPS levels better than 3%NaOCl, 2%CHX, and 17%EDTA (43). Liu and colleagues performed an in vitro comparison of the antimicrobial effectiveness of QMiX and other final irrigants in human root canals. Liu demonstrated that QMiX was more effective than that of EDTA with NaOCl against intracanal E. faecalis (44). Zhang and colleagues also documented QMiX as having greater antimicrobial properties compared to 2%CHX (45). Kalyoncuoglu and colleagues evaluated the antifungal efficacy of QMiX as a final irrigant. The study demonstrated that QMiX proved to be as effective as 5.25%NaOCl and 2%CHX and significantly superior than 17%EDTA against C. *albicans* (46).

Stojic and colleagues also assessed the efficacy of QMiX against *Enterococcus* faecalis and mixed plaque bacteria in planktonic phase and biofilms. QMiX and 1%NaOCl killed all planktonic E. *faecalis* and plaque bacteria in five seconds. QMiX and 2%NaOCl killed up to 12 times more bacteria than 1%NaOCl or 2%CHX (46). Wang and colleagues compared the antibacterial effects of different disinfecting solutions on young and old E. *faecalis* biofilms. 6%NaOCl and QMiX were the most effective disinfecting solutions again the young biofilm, whereas 6%NaOCl followed by QMiX were the best at disinfecting the older biofilm populations. Both were more effective than 2%NaOCl and 2%CHX (48**).** Morgental and colleagues showed that QMiX was less effective than 6%NaOCl and similar to 1%NaOCl in bactericidal action (49). Also, their study appeared to show that the presence of dentin slurry has the potential to inhibit most current antimicrobials in the root canal system.

Ordinola and colleagues found that several endodontic irrigants containing antimicrobial compounds such as CHX (QMiX) lacked an effective antibiofilm activity when the dentin was infected (50). They concluded that several chelating agents containing antimicrobials could not remove or kill significantly biofilms developed on infected dentin, with the exception NaOCl and 4% peracetic acid. Dissolution ability is therefore mandatory for an appropriate eradication of biofilms attached to dentin.

Stojic and colleagues investigated the effectiveness of smear layer removal by QMiX using scanning electron microscopy (47). QMiX was found to remove smear layer equally as well as EDTA. Elliot and colleagues found that QMiX outperformed EDTA's smear layer removal capabilities in the middle and apical thirds (51). Dai and colleagues examined the ability of two pH versions of QMiX to remove canal wall smear layers and debris using an open canal design. Within the limitations of the open-canal design, it was as effective as 17%EDTA in removing canal wall smear layers after the use of 5.25%NaOCl as the initial rinse (52). Aksel and colleagues showed that QMiX as a final solution showed less decalcification and erosion than 17%EDTA when used with 5%NaOCl as an initial irrigant (53). Souza and colleagues reported retention of both 2%CHX and QMiX in human dentin for up to 120 days (54). Zhang and colleagues reported QMiX antimicrobial properties, or substantivity, for up to 36 hours (45).

Tuncer and colleagues investigated the effect of QMiX on sealer penetration into the dentinal tubules. It was demonstrated that QMiX was as effective as EDTA plus CHX in allowing sealer to penetrate dentinal tubules in the apical third, and was significantly better in the coronal and middle thirds (55).

No current evidence has reported long term clinical outcomes for efficacy or biocompatibility of QMiX. Further clinical research from independent investigators in needed to corroborate findings.

Differential Scanning Calorimetry (DSC)

A Differential Scanning Calorimeter (DSC) is a thermodynamical tool for direct assessment of the heat energy uptake, which occurs in a sample within a regulated increase or decrease in temperature. The calorimetry is particularly applied to monitor the changes of phase transitions. During a DSC experiment, energy is introduced simultaneously into a sample cell (which contains a solution with the molecule of interest) and a reference cell (empty). Temperatures of both cells are raised identically over time. This test allows the most accurate measurement of endothermic and exothermic reactions that take place while two irrigants are mixed.

Nuclear Magnetic Resonance – NMR

Nuclear magnetic resonance spectroscopy (NMR) is a principal technique used to identify such precipitates. The technique is used to structurally characterize molecules based on chemical shift values and coupling between atoms. NMR is also able to determine purity of mixtures of molecules based on relative signal intensities. By exciting energy level transitions that are very low in energy, even the most fragile bonds remain intact. The presence or absence of specific molecules in a mixture can then be determined by comparing the mixture's NMR spectrum with spectra of pure compounds. NMR remains one of the most accurate ways to assess the identification of precipitate formation.

Purpose

The aim of this study is to document and identify the precipitate formed and exothermic reaction caused when mixing NaOCl and QMiX.

MATERIALS AND METHODS

Photographic Documentation

Digital photographs to document color and consistency changes over time were obtained on March 14, 2016 and February 3, 2017. Controls were prepared for both 5.25% NaOCl and QMiX. Samples of QMiX were also collected from a bottle previously opened but kept out of direct sunlight with the cap tightly fastened and a freshly opened bottle of QMiX to document changes with the proprietary solution over time.

On March 14, 2016 10mL of 5.25% NaOCl and 10mL of QMiX samples were collected from respective bottles into clear test tubes. Photos were captured immediately after sample collection. A volume of 5mL of 5.25% NaOCl was placed into a third clear test tube. Immediately after, 5mL of QMix was placed into the same test tube with 5mL of 5.25% NaOCl. Photo documentation of the solution was then recorded at 0 seconds, 30 seconds, 1 minute, 30 minutes, 1 hour, and 24 hours. Photography allowed capture of color and consistency change, precipitate formation, and duration between immediate changes and completion through visualization.

On February 3, 2017 two QMiX bottles were obtained. One bottle was previously opened in June 2016. When received from the supplier, this bottle had an airtight seal present. The white plastic container prevented exposure of direct sunlight to solution. The lid remained tightly fastened at all times other than when the solution was dispensed for clinical use. The bottle had an expiration date of May 2017. A second bottle was purchased and obtained from the supplier one week prior to February 3, 2017. This solution presented in a clear plastic container with a plastic seal wrapped rounds the outside of the cap to prevent tampering prior to delivery, but without an air-tight seal.

The clear plastic allowed light penetration and was stored in a cabinet away from direct sunlight prior to use. 10mL of QMiX from the previously opened container was dispensed from the bottle into a clear test tube. 10mL of QMiX from a freshly opened container was dispensed from the bottle into a second clear test tube. Digital photographs were immediately captured as solutions were placed into respective test tubes. Photographs of both bottles were also captured for documentation. Lastly, a photograph of the isolated precipitate after desiccation was obtained.

Differential Scanning Calorimeter

Samples of 5.25% NaOCl, QMiX, and 2% CHX at room temperature were collected for obtaining results on the Mettler-Toledo Inc. Columbus, Ohio DSC822^{e} differential scanning calorimeter. STARe software collected and interpreted sample data from DSC tests. All samples were collected at room temperature to mimic irrigant use within a clinical environment. Control tests were captured for QMiX and 2% CHX. To establish control, each DSC test was initiated to 35°C and began at 0:00 seconds. 0.05mL of QMiX was added to an empty crucible at 0:30 seconds and 0.05mL of QMiX was added at 5:00 minutes. The tests were allowed to continue for 30 minutes, for a complete run time of 35 minutes. Five QMiX control tests were obtained and analyzed. Five 2% CHX controls were completed and analyzed using an identical protocol in regards to time and volume of irrigant added. Five DSC tests were run to capture heat flow when combining 5.25% NaOCl with 2% CHX. Using the same protocol, 0.05mL of 5.25% NaOCl was added to an empty crucible at :30 seconds. .05mL of 2% CHX was added to the crucible at 5:00 minutes, and each test was completed at 35:00 minutes. Five tests were then run to capture heat flow when combining 5.25% NaOCl with QMiX. Again,

the protocol of adding 0.05mL 5.25% NaOCl to an empty crucible at 0:30 seconds was completed. 0.05mL of QMiX was added at 5:00 minutes, and each test had a total run time of 35:00 minutes. All data was collected, analyzed, and interpreted using STARe software. Data was then obtained and analyzed after experiment completion pertaining to; net temperature change, total enthalpy, and time of maximum heat generation using STARe software. To define points, data was collected at initial peaks, maximum peaks, and normalized integration numbers from each trial within the software. To define each peak, a right horizontal baseline was defined at the moment of the initial peak and one point was set at each peak's maximum height. For each figure, the normalized values on the top of each graph allowed for data collection in regards to temperature change and total enthalpy documentation for all 5.25% NaOCl – 2% CHX and 5.25% NaOCl – QMiX trials. Lower value sets on of each graph allowed for documentation of temperature change. For each data collection point, averages were all obtained. A two-tailed curve with differences in variance was realized after data point collection. T-tests were completed and analyzed for net temperature change, total enthalpy, and time for 5.25% NaOCl – 2% CHX and 5.25% NaOCl – QMiX trials. All data collected and analyzed can be found on tables and graphs within results. The Mettler-Toledo Inc. Columbus, Ohio DSC822^{e} differential scanning calorimeter machine and cell crucibles in which the experiment were completed are shown in figure 1.

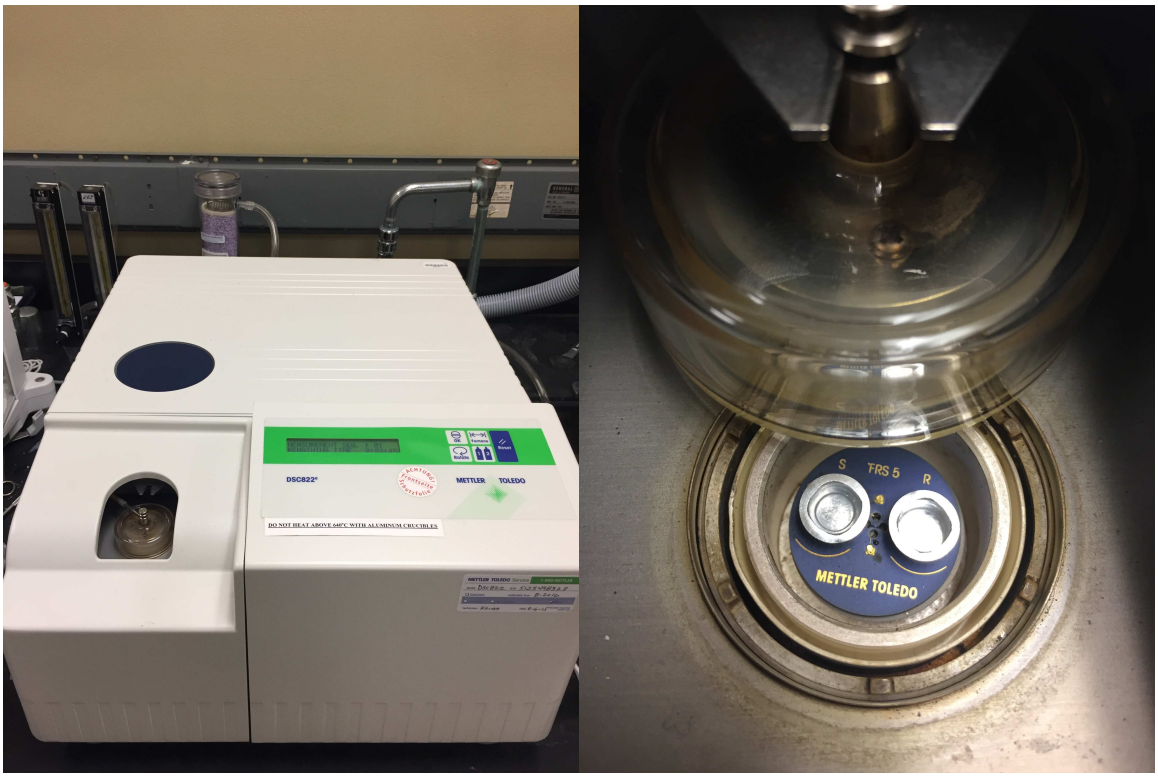

Figure 1 – Mettler-Toledo differential scanning calorimeter

Nuclear Magnetic Resonance

An Oxford NMR AS400 machine was used for completion of the 1D and 2D NMR experiments. Spectra analysis was completed with mNOVA software. Formation of precipitate was established using a 1:1 ratio of 5.25% NaOCl and QMiX. After 24 hours, excess solution was removed with a monoject syringe and remaining precipitate was desiccated for further isolation. Dry samples were allowed to desiccate under a heat lamp for 7 days. The precipitate was further isolated using trials of several solvents; acetone, chloroform, methanol, and deionized water. A 1 mL sample was taken and placed in 1.5 mL microfuge tubes and centrifuged at 14,000 rpm for 2.5 minutes. The precipitate solid was removed and dissolved in 1.0mL of dimethyl sulfoxide-d_6 or deuterated DMSO. One-dimensional (1H) and 2D (DOSY) NMR spectra were then collected for each of the

samples at 25°C. Resulting spectra were assigned in terms of the chemical shifts of all proton and carbon atoms in intact precipitate, which permitted identification of the 5.25%NaOCl-QMiX precipitation breakdown products. These products were then compared to samples and breakdown products by Dr. Sem and colleagues. (37, 38)

Figure 2 – Oxford NMR AS400

RESULTS

Photo documentation

All documented photographs of the color and consistency changes are shown in figures 3-7. Photographs were captured on March 14, 2016 and February 3, 2017. The initial photograph shows 10mL of 5.25% NaOCl with a slight yellowish hue as a control. The following photograph documents 10mL of a freshly opened bottle of QMiX, which appears as a clear solution. The following photographs document the changes when 5mL of QMiX is added to 5mL of 5.25% NaOCl with given times of 0 seconds, 30 seconds, 1 minute, 30 minutes, 1 hour, and 24 hours. The moment 5mL of QMiX is added to 5mL of 5.25% NaOCl, the color changes from a slight yellow to bluish-green. A colorless gas with a strong chlorine-based, foul odor was realized. 30 seconds after the solution is mixed, the color begins to change to a light orange-brown with further gas production. At 1 minute, the color continues to lighten and the bubbling gas begins to dissipate. By 30 minutes, evidence of an orange-brown precipitate begins to form within the lighter orange-yellow solution. Gas bubbles at 30 minutes are almost completely diminished. After one hour, the precipitate formation begins to solidify, and distinct clusters are visible. At 24 hours, the precipitate completely forms, and due to a density difference, drops to the bottom of the test tube. The precipitate after the 24-hour mark can be separated from the solution, however, the precipitate can mix back into solution if shaken vigorously. While mixing 5.25% NaOCl with QMiX, an immediate rise in temperature is noticed by touching the test tube. This temperature change can be felt by hand until the 30-minute mark. Please refer to DSC results for specification in heat produced. For identification purposes, the solution was extracted and the remaining precipitate was

desiccated and collected for NMR testing. The isolated, dried precipitate can be visualized in Figure 8.

Figure 9 shows two bottles of QMiX and differences in solution consistency with given time. A change in consistency of QMiX 2in1 solution was noted while providing root canal therapy for a patient within the Marquette University School of Dentistry Endodontic clinic on February 1, 2017. The left-most figure indicates a bottle of QMiX that was opened in June 2016. This bottle presents a white plastic that has impenetrable by sunlight. Upon removing the lid, an airtight factory seal was present. The expiration date for this bottle is May 2017. The figure on the right is a QMiX bottle opened in February 2017. Sunlight was able to penetrate the plastic and no airtight factory seal was present. There was plastic seal on the outside of the lid. The center picture shows the difference in new and older QMiX consistency. The new solution appears to be completely clear with no evidence of color or precipitate. The older solution, although clear in color, shows evidence of a white precipitate suspended in liquid. Within a matter of minutes, the white precipitate falls out of suspension to the bottom of the test tube.

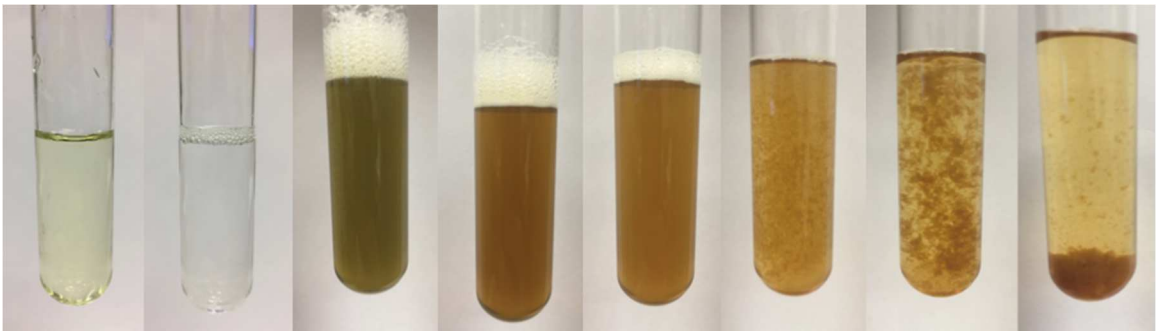

Figure 3 – 5.25% NaOCl and QMiX – time-dependent precipitate formation

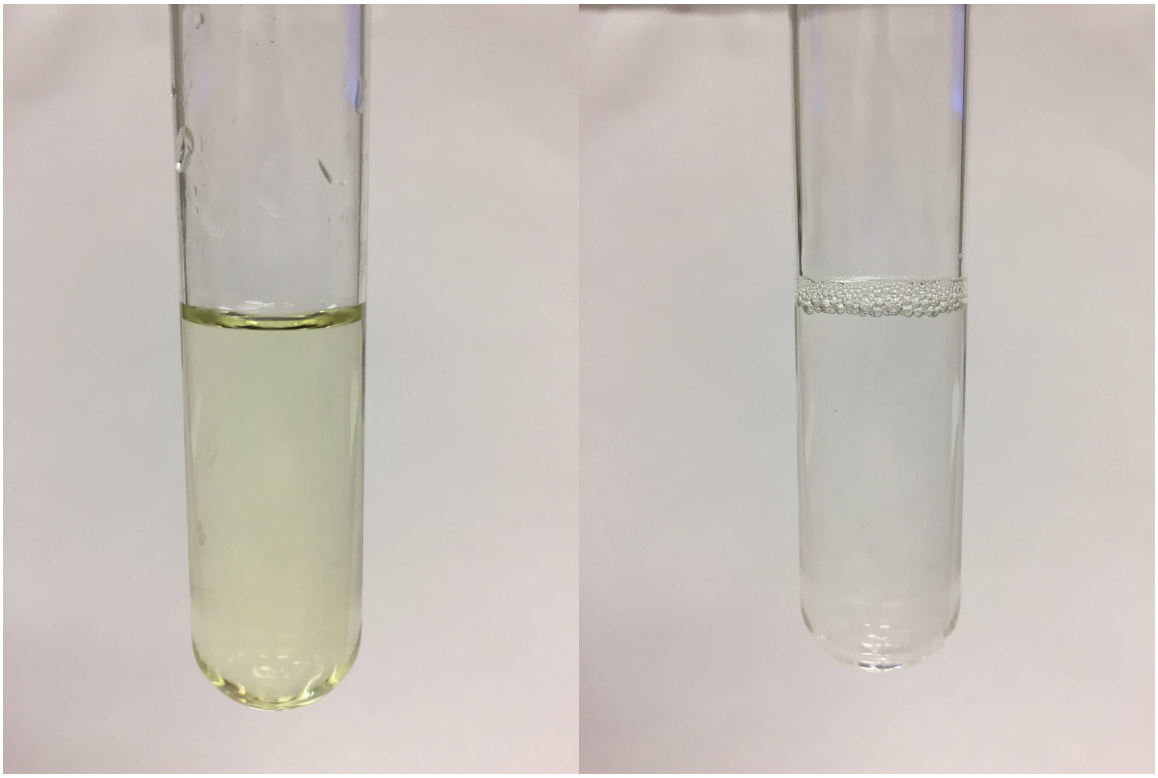

Figure 4 – 10 mL 5.25% NaOCl and 10mL QMiX

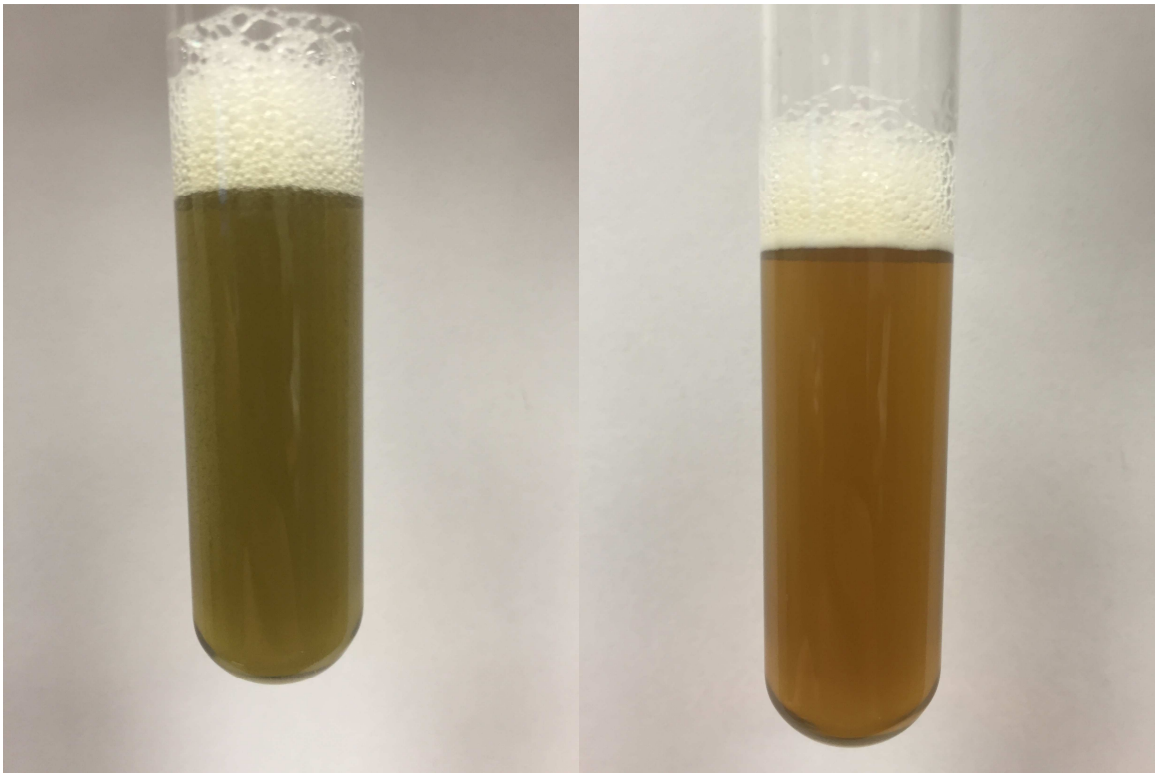

Figure 5 – 5.25% NaOCl and QMiX: 0 seconds and 30 seconds

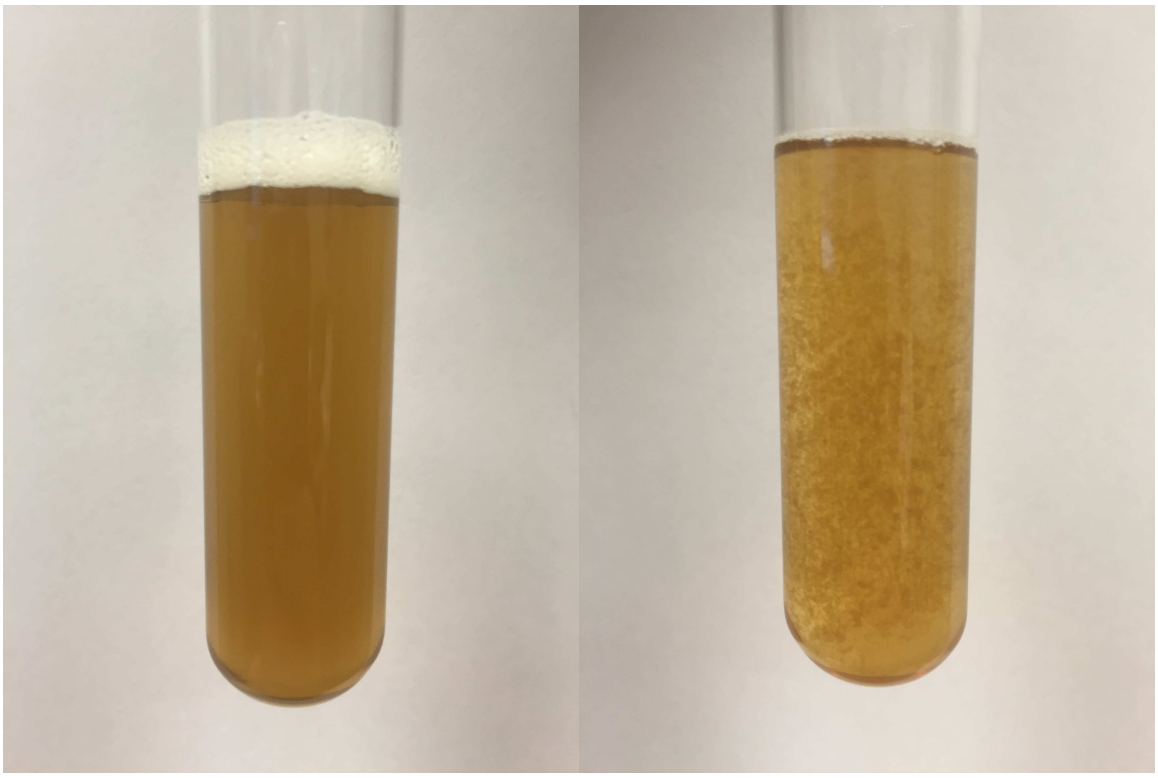

Figure 6 – 5.25% NaOCl and QMiX: 1 minute and 30 minutes

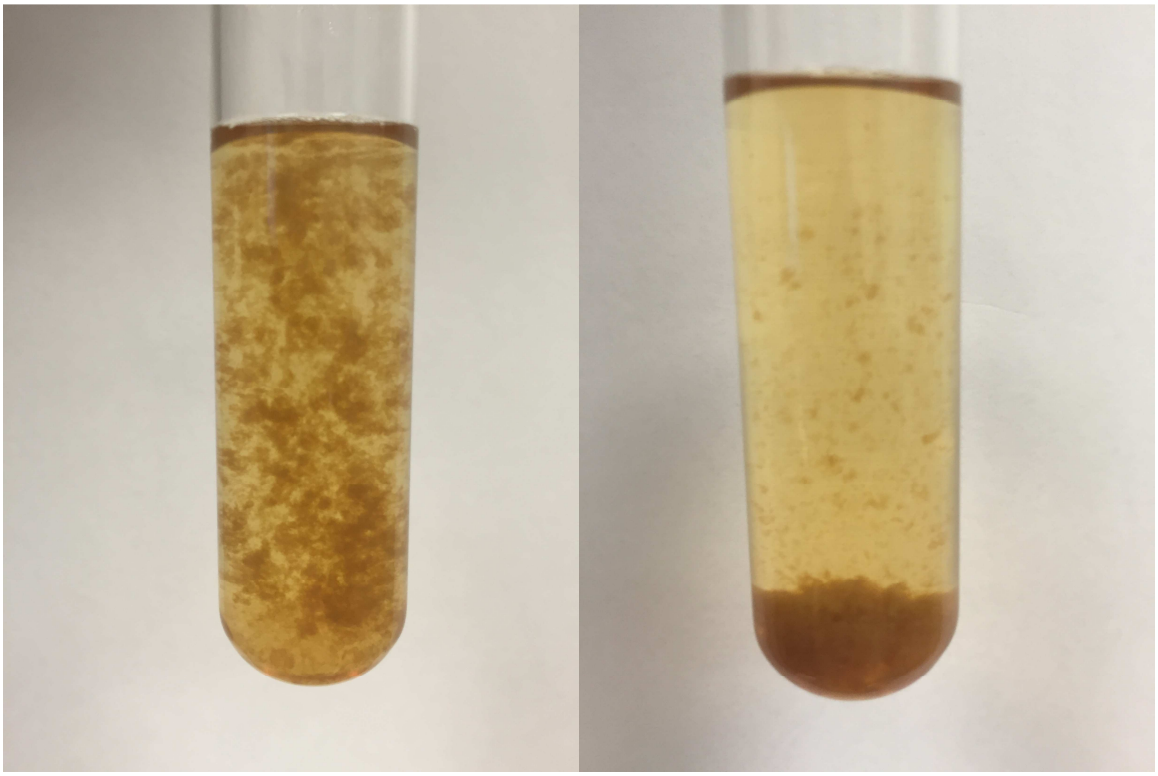

Figure 7 – 5.25% NaOCl and QMiX: 1 hour and 24 hours

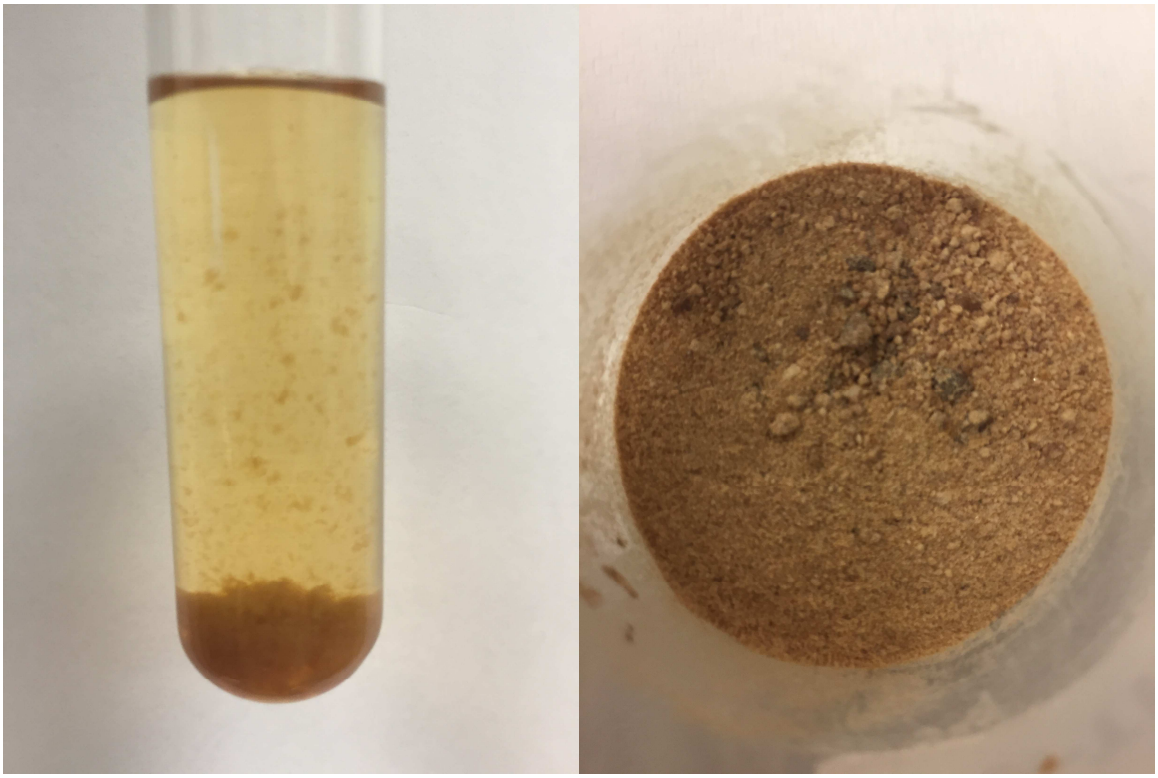

Figure 8 – 5.25% NaOCl and QMiX Precipitate Wet and Dry

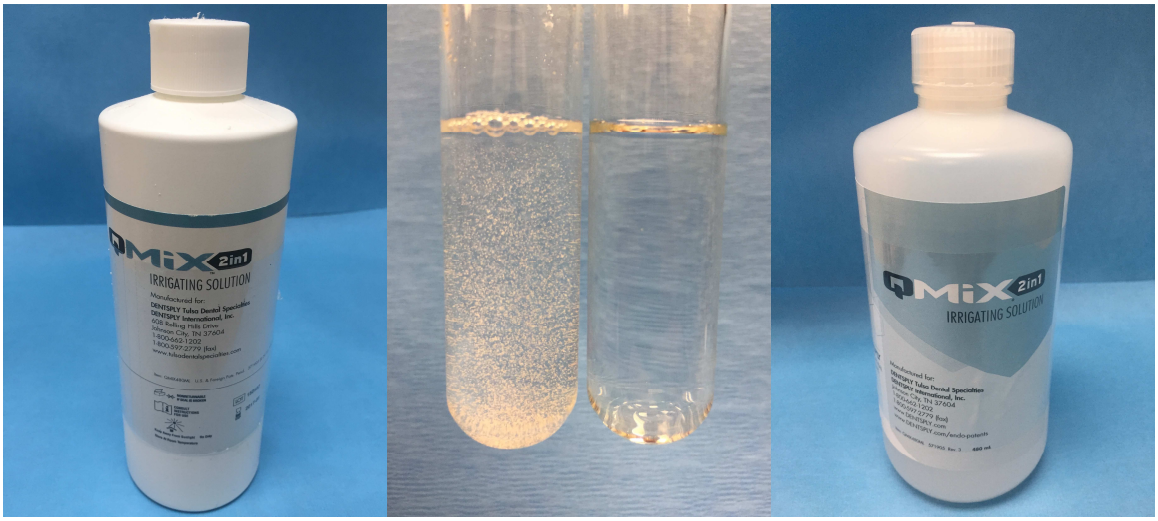

Figure 9 – QMiX Old Bottle (Exp 05/17), Collected Samples, New Bottle (Fresh)

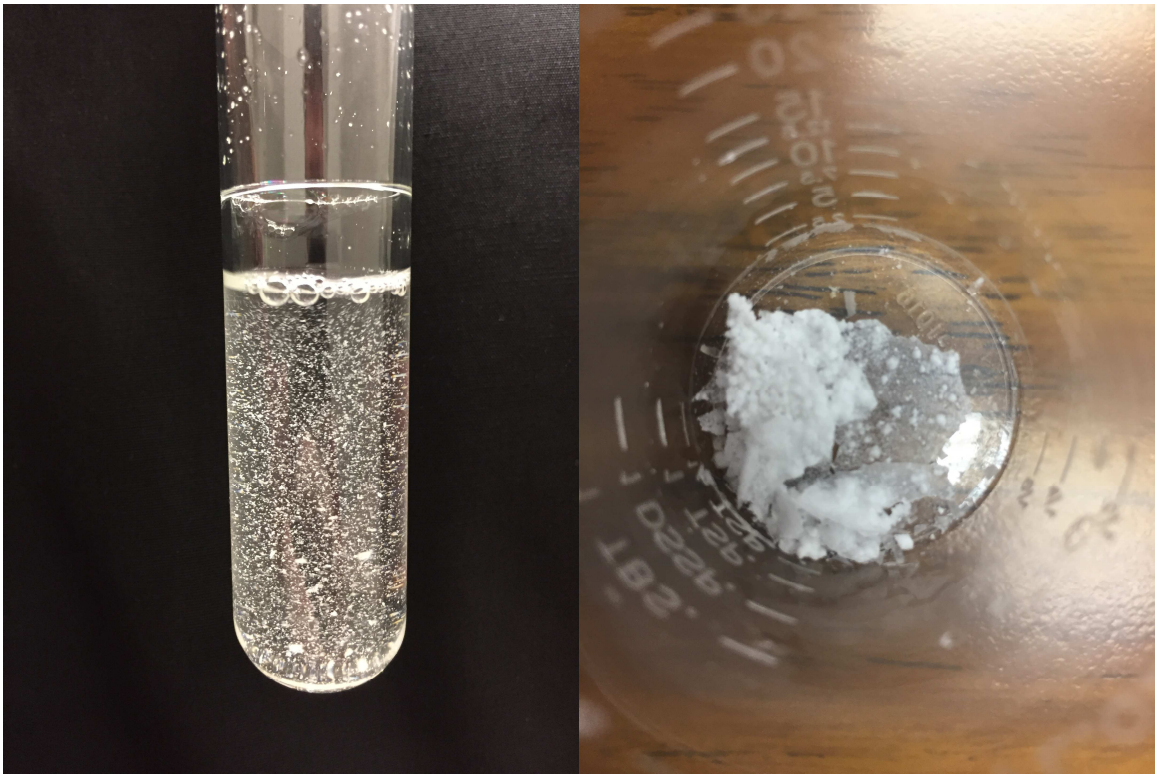

Figure 10 – Old sample (Exp. 05/17) of QMiX, Wet and Dry

Differential Scanning Calorimeter

Results for all DSC tests can be found in Table 1. DSC and statistical analysis was performed with results recorded for: *temperature reported in °C, enthalpy reports in mJ, and time in seconds* from solution addition to maximum peak. Averages and standard deviations were analyzed and recorded. Figure 11 exhibits exothermic and endothermic reaction results collected by STARe software.

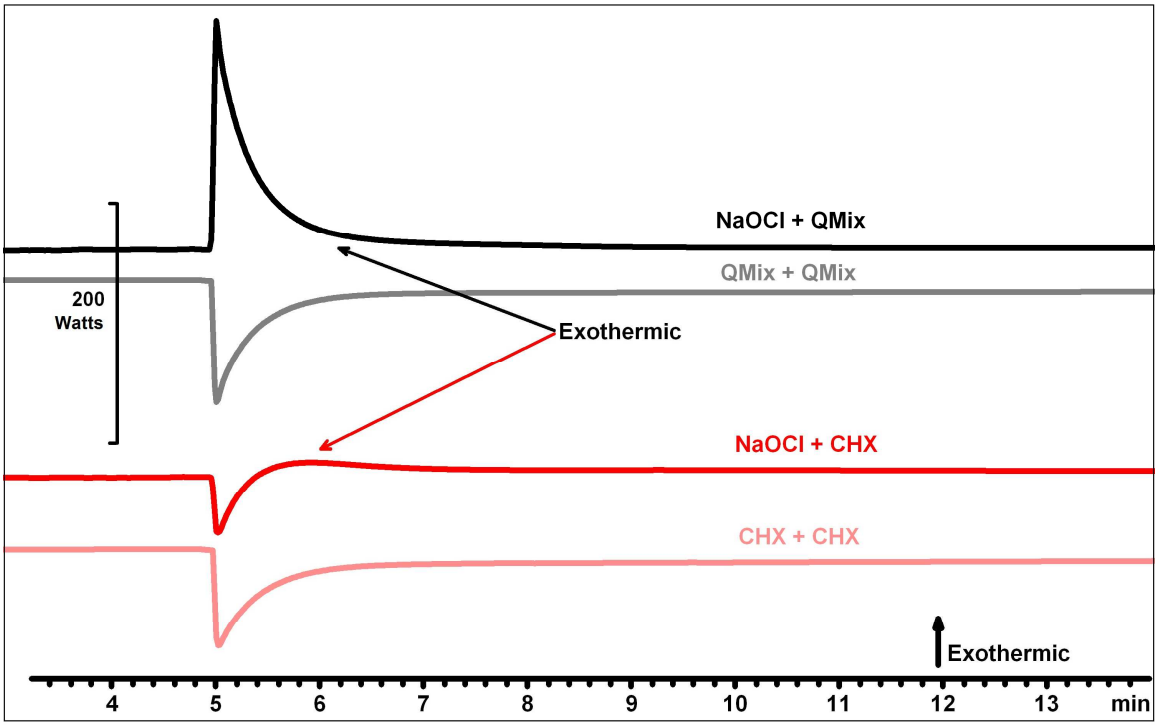

Figure 11 – DSC Endothermic and Exothermic Reactions

Temperature

QMiX and QMiX control runs revealed endothermic reactions and temperatures were not evaluated. CHX and CHX control runs also revealed endothermic reactions and temperatures were not evaluated.

Temperature was recorded in °C immediately before 0.05mL of 2% CHX was added to 0.05mL of 5.25% NaOCl and at the maximum peak during the exothermic reaction. Changes in temperature are as follows: 0.26°C, 0.67°C, 0.30°C, 0.49°C, and 0.27°C for an average temperature change of 0.40°C with a standard deviation of 0.20°C.

Temperature was recorded in °C immediately before 0.05mL of QMiX was added to 0.05mL of 5.25% NaOCl and at the maximum peak during the exothermic reaction. Changes in temperature are as follows: 4.83°C, 4.68°C, 4.03°C, 4.64°C, and 4.62°C for an average temperature change of 4.60°C with a standard deviation of 0.20°C.

Statistical analysis utilizing a t-test revealed a p value of 9E-08 or $p<0.01$.

Enthalpy

Controls were completed using 2% CHX and QMiX. 5 lots of 2% CHX were obtained for 5 runs. 5 tests resulted in the following endothermic enthalpy analysis when adding 0.05mL 2% CHX to 0.05mL 2% CHX: 1945.76mJ, 2075.25mJ, 1956.46mJ, 1900.72mJ, and 2014.63mJ for an average endothermic enthalpy of 1979mJ with a standard deviation of 68mJ. 2 lots of QMiX were obtained for 5 runs. 5 tests resulting in the following enthalpy when adding 0.05mL QMiX to 0.05mL of QMiX: 1771.25mJ, 1801.66mJ, 1966.08mJ, 1976.48mJ, and 2153.88mJ for an average endothermic enthalpy of 1934mJ with a standard deviation of 154mJ.

5 runs were completed by adding 0.05mL 2% CHX to 0.05mL 5.25% NaOCl. Exothermic enthalpy recordings are as follows: 1150.17mJ, 1415.65mJ, 1417.59mJ, 2063.66mJ, and 1245.53mJ. Endothermic enthalpy recordings are as follows: 526.62mJ, 309.49mJ, 421.02mJ, 389.12mJ, and 311.83mJ. Each run allowed the addition of the control enthalpy average of 1978.56mJ. Final total enthalpy for the 5.25% NaOCl – 2% CHX are as follows: 2602.11mJ, 3084.72mJ, 2975.13mJ, 3653.10mJ, and 2912.26mJ for an average total enthalpy of 3045mJ with a standard deviation of 384mJ.

5 runs were completed by adding 0.05mL QMiX to 0.05mL 5.25% NaOCl with recorded exothermic enthalpy as follows: 4198.19mJ, 3459.21mJ, 4243.39mJ, 4664.25mJ, and 5094.98mJ. An immediate exothermic reaction while adding QMiX to 5.25% NaOCl resulted in an endothermic enthalpy value of 0.00mJ for all 5 runs. Adding the control enthalpy of 1933.87mJ to each run, the final total exothermic enthalpy values are as follows: 6132.06mJ, 5393.08mJ, 6177.26mJ, 6598.12mJ, and 7028.85mJ for an average total enthalpy of 6266mJ with a standard deviation of 608mJ.

Statistical analysis utilizing a t-test revealed a p value of 2.70242E-05 or $p<0.01$.

Time

Time at insert was recorded in seconds when 0.05mL of 2% CHX was added to 5.25% NaOCl. Time was also recorded at the maximum peak of the exothermic reaction for each of the 5 runs. Time to peak exothermic reactions are as follows: 61.8 seconds, 49.8 seconds, 57.0 seconds, 48.6 seconds, and 43.8 seconds for an average of 52 seconds with a standard deviation of 7 seconds.

Time at insert was recorded in seconds when 0.05mL of QMiX was added to 5.25% NaOCl. Time was also recorded at the maximum peak of the exothermic reaction

for each of the 5 runs. Time to peak exothermic reactions are as follows: 4.2 seconds, 3.6 seconds, 3.0 seconds, 3.0 seconds, and 3.0 seconds for an average of 3 seconds with a standard deviation of 1 second.

Statistical analysis utilizing a t-test revealed a p value of 0.00101 or $p<0.01$.

Table 1 exhibits all data collected and analyzed utilizing STARe software within a Word Excel spreadsheet for interpretation.

Qmix/CHX added to NaOCL DSC Analysis

	CHX + CHX	Qmix + Qmix
1	1945.76	1771.25
2	2075.25	1801.66
3	1956.46	1966.08
4	1900.72	1976.48
5	2014.63	2153.88
Avg	1979	1934
St. Dev.	68	154

Report temp in °C
Report Enthalpy in mJ
Report time in seconds

NaOCL and Qmix

	Temp Before	Temp after	ΔT	Exo Enthalpy	Endo Enthalpy	Control Enthalpy	Final Total Enthalpy	Time at insert	Time at Exo Max	Time to Peak Exothermic Reaction Δtime	Seconds
1	34.67	39.5	4.83	4198.19	0	1933.87	6132.06	4.93	5	0.07	4.2
2	34.65	39.33	4.68	3459.21	0	1933.87	5393.08	4.94	5	0.06	3.6
3	34.67	38.7	4.03	4243.39	0	1933.87	6177.26	4.95	5	0.05	3
4	34.68	39.32	4.64	4664.25	0	1933.87	6598.12	4.95	5	0.05	3
5	34.65	39.27	4.62	5094.98	0	1933.87	7028.85	4.95	5	0.05	3
		Avg	4.6			Avg	6266			Avg	3
		St. Dev.	0.3			St. Dev.	608			St. Dev.	1

NaOCL and CHX

	Temp Before	Temp after	ΔT	Exo Enthalpy	Endo Enthalpy	Control Enthalpy	Final Total Enthalpy	Time at insert	Time at Exo Max	Time to Peak Exothermic Reaction Δtime	Seconds
1	34.63	34.89	0.26	1150.17	526.62	1978.56	2602.11	4.97	6	1.03	61.8
2	34.32	34.99	0.67	1415.65	309.49	1978.56	3084.72	4.97	5.8	0.83	49.8
3	34.64	34.94	0.3	1417.59	421.02	1978.56	2975.13	4.97	5.92	0.95	57
4	34.63	35.12	0.49	2063.66	389.12	1978.56	3653.10	4.97	5.78	0.81	48.6
5	34.7	34.97	0.27	1245.53	311.83	1978.56	2912.26	4.95	5.68	0.73	43.8
		Avg	0.4			Avg	3045			Avg	52
		St. Dev.	0.2			St. Dev.	384			St. Dev.	7
		T-test	9E-08			T-test	2.70242E-05			T-test	0.000101

Table 1 – DSC interpreted data results

Nuclear Magnetic Resonance

1H NMR spectra of 5.25% NaOCl and QMiX precipitate solution using DMSO-d_6 revealed several organic components. Precipitate collected from 5.25% NaOCl and QMiX, and precipitate collected from the older bottle of QMiX revealed relatively low

solubility in DMSO-d_6. Figure 12 depicts the initial 1H NMR experiment. Cross-reference to previous work by Sem and colleagues, revealed the presence of DMSO at 2.5 parts per million (ppm), and H_2O at 3.36 ppm. Trace amounts of unidentified aromatic compounds were noted in the 6.0-80 ppm. (37,38) Both precipitates collected revealed almost identical spectra on 1H NMR analysis. Further 2D NMR (DOSY) spectra analysis revealed a compound with a molecular weight around 500 g/mol. Figure 13 shows the DOSY spectra. Spectroscopic analysis concluded that all other peaks within the 1H NMR graphs were due to detergents and surfactants within the propriety blend of QMiX. Spectroscopist analysis reveals precipitation is caused by the ionic strength of sodium ions and color change is caused by the oxidation of unidentified detergents by NaOCl upon mixing.

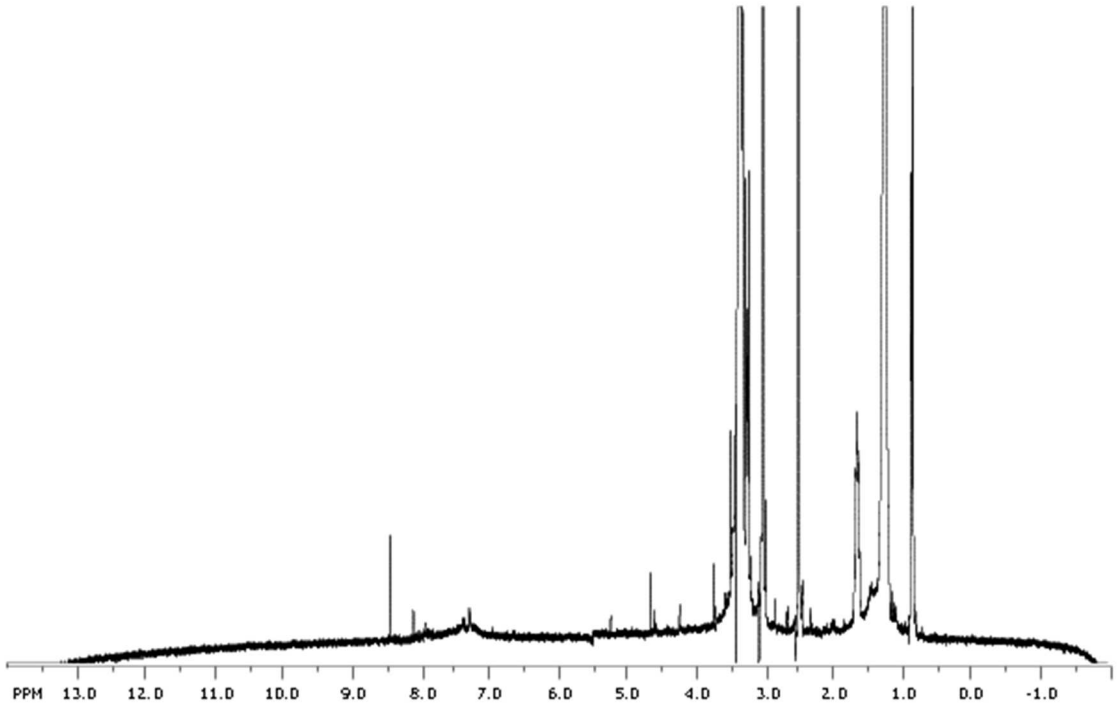

Figure 12 – 1H NMR Spectra Analysis 5.25% NaOCl and QMiX Precipitate

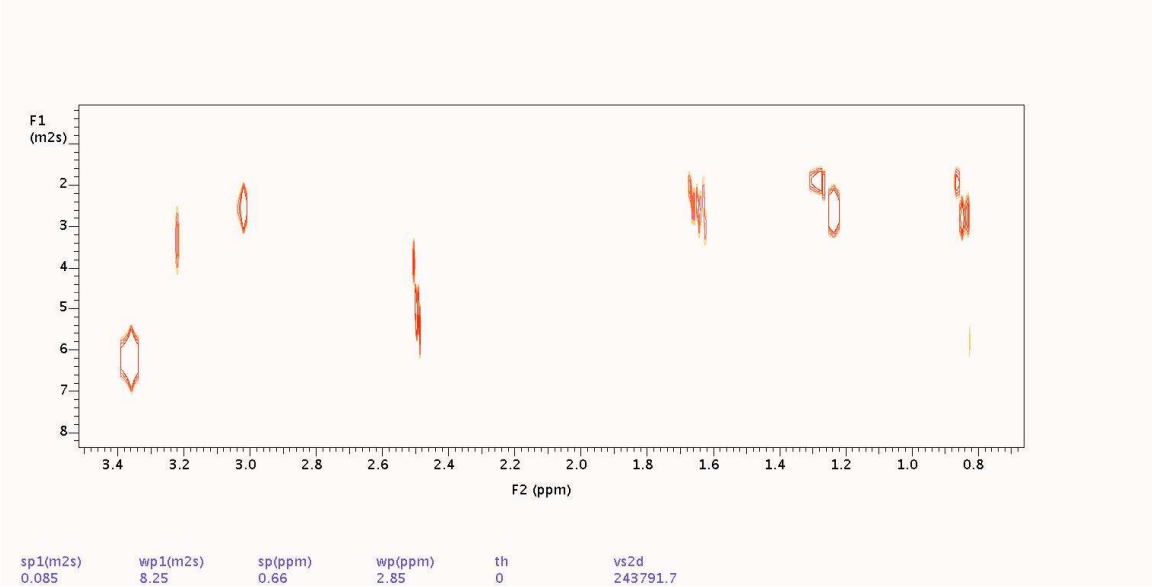

Figure 13 – 2D NMR (DOSY) Spectra Analysis 5.25% NaOCl and QMiX Precipitate

DISCUSSION

Precipitate formation, identification, and the heat production when mixing 5.25% NaOCl and QMiX has never before been documented within the literature. Current research states that color change exists but no precipitates form when combining NaOCl and QMiX (42). To the best of my knowledge, Tulsa Dentsply's current claim that no precipitation is formed when mixing QMiX with NaOCl is based upon Kolosowski's study with differing procedural protocols when comparing the interactions of NaOCl and CHX to NaOCl and QMiX. Kolosowski and colleagues obtained dentin blocks of human maxillary molars, and sectioned these blocks to expose dentin. In group 1, specimens were immersed in 2.5% NaOCl, followed by 17% EDTA, 2.5% NaOCl, and 2% CHX. It is assumed that most, if not all, U.S. trained practicing endodontists are aware of the interactions between NaOCl and CHX in thanks to works by Basrani et al (10) and Prado et al (56). Thus, the procedural flaw is evident in Group 1, as it is not the standard of care to rinse with NaOCl after EDTA if also using CHX as a final irrigant. In Group 2, specimens were immersed in 2.5% NaOCl, followed by saline and QMiX. Group 2 was not subjected to any additional rinse of NaOCl, as group 1 previously endured. One observation of this study is proof that the precipitate is both large enough to be visualized without magnification, yet small enough to penetrate and occlude dentinal tubules (42).

Photographic Documentation

As tests were not completed in the current study to identify the molecular structure of gas produced by mixing NaOCl with QMiX, Prado's study is cross referenced and cited (56).

Within Prado's study, in cases showing precipitate formation; a redox-reaction was observed for the precipitate formed in the reaction between NaOCl and CHX, as NaOCl is known to be an oxidating agent. This experiment suggests that the color change is due to oxidation, however, precipitation occurs due to sodium's ionic strength. The evaluation in the current study is that NaOCl is acting as an intense oxidizing agent, immediately evident with color change and as the time-dependent precipitate is formed between NaOCl and QMiX. The hypothesis is that the color change when mixing NaOCl with QMiX is directly related to NaOCl's oxidation capabilities of QMiX. Previously, mass spectroscopy analysis confirmed the presence of several products of chlorination from the oxidizing agent NaOCl, which was reported to occur at 1 to 6 guanidino nitrogens of CHX (57-60). These findings matched previous findings by Sem and colleagues (37-38), who did not find the presence of para-chloroaniline, and diverged from previous work published by Basrani and colleagues (61). Prado articulated the difference in findings as differences between sensitivity of tests utilized between authors. Prado also identified the lightened color of formed precipitate between NaOCl and CHX as orange-white when lesser concentrations of solutions were tested. This potentially suggests that the concentration of CHX is much less than 2%, which is the most commonly used concentration for utilization as a final irrigant for root canal therapy (63). Orhan and colleagues published findings in regards to the formation of PCA when mixing NaOCl and CHX while concluding, "this study will be cutoff proof that spectroscopic analysis does not contain free PCA (62)."

A milky-white precipitate previously documented in association of EDTA with CHX was analyzed by Prado and colleagues, and found the precipitate to be a product of

acid-base reactions. These findings correlated with Rasimick and colleagues, whom attributed the reaction to a salting-out process (40). The current study allowed the hypothesis of the particulate matter found in the previously opened bottle of QMiX. Furthermore, it is suggested that exposure of oxygen to an opened, yet capped, bottle of QMiX allows oxidation of the proprietary blend over several months, due the evidence of particulate matter shown in Figure 10.

QMiX directions for use specifically states not to inhale the solution and to use only in a well ventilated area. Grawehr et al and Mrvos et al previously provided literature pertaining to the immediate gas production and bubble formation when combining NaOCl and QMiX. These bubbles are expected to be mainly chlorine gas, a toxic compound (29,64). The bubble formation of chlorine gas (Cl_2) results from an increase in proton (H^+) concentration in the presence of chloride ions (Cl^-), which is the usual impurity of NaOCl solution, shifting the equilibrium toward the formation of Cl_2 (56). This would suggest the importance of four-handed dentistry to ensure suction is efficiently utilized when mixing such solutions during root canal therapy.

Further in vivo research must be conducted to determine the possibility of precipitate formation between 5.25% NaOCl and QMiX, while interacting with human cells and alike mediators confined to the pulpal and periradicular regions during root canal therapy.

The immediate color change remained even after solution extraction and weeks of desiccation and solvent washings. A strong consideration of coloration leaching and staining remaining dentin structures must also be realized.

Differential Scanning Calorimeter

Analyzed results from this experiment show that both 2% CHX and QMiX create exothermic reactions, or produce heat, when mixed with 5.25% NaOCl. Due to the incredible accuracy and sensitivity of DSC tests, minimum volume of solution are necessary. A total volume of 0.10mL of 5.25% NaOCl with 2% CHX allowed for an increase in temperature of 0.40°C after 52 seconds. A total volume of 0.10mL of 5.25% NaOCl with QMiX allowed for an increase in temperature of 4.60°C after only 3 seconds. Due to small amount of volume further studies would be necessary to see if temperature increases remain constant when greater volumes interact. Volumes would be suggested as total average volumes utilized during root canal therapy, or 10mL of 5.25% NaOCl and 60mL of QMiX. Furthermore, further in vivo research would be necessary to determine what effects, if any, the drastic increase in temperature when mixing NaOCl and QMiX would have on human cells and alike mediators in pupal and periradicular tissues during root canal therapy.

Nuclear Magnetic Resonance

NMR tests were utilized in the current study instead of destructive methods such as mass spectrometry and its combinations. This is due to the ability to keep chemical structure intact at the molecular level for greater sensitivity. However, all tests have limitations. One major limitation of NMR is the inability to identify unknown molecules within a given solution. Due to QMiX being a proprietary blend, exact names and chemical structures of surfactants/detergents are not readily available. If a further study were to be done to identify the molecular structure of the given precipitate with NMR testing, Tulsa Dentsply would have to specify all chemicals within QMiX's proprietary

blend. Elemental analysis could be utilized to identify the given precipitate's molecular structure without knowledge of specific QMiX ingredients; however, Marquette does not have this expensive and time intensive machine currently available. Thus, the current study reached the limit before the ability to identify the molecular structure of the precipitate was realized.

5.25% NaOCl and QMiX

As a primary goal of QMiX is to reduce or remove the smear layer and biofilm, it is of utmost important that caution is taken to reduce interaction and precipitate formation within the pulpal and periradicular space during root canal therapy. QMiX directions for use states that an intermediate irrigant is "desirable" but fails to mention that it is required or absolutely necessary. The understanding of this research is necessary for any clinician utilizing irrigants during root canal therapy, as by-products of commonly used irrigants maybe toxic to the periapical tissues (65). Furthermore, they have the ability to form precipitate, resulting in a chemical smear layer, which occludes the dentinal tubules and may interfere with the seal of root filling (66,67). Therefore, it is imperative that all efforts are made to remove the chance of by-product formation with the use of intermediate irrigants and/or drying protocols between irrigants that have documented and identified interactions. Lastly, without the ability to identify all proprietary compounds and alike precipitates, caution should be utilized at all times when using this irrigant in vivo.

Today's model for effective and efficient diagnosis, prognosis, and treatment is through an evidence-based approach. Ethical clinicians, practicing evidence-based-

endodontics should have full awareness of any/all armamentarium and interactions utilized while performing endodontic therapy.

CONCLUSIONS

Immediate color change, gas release, and precipitate formation occurs when mixing 5.25% NaOCl and QMiX. An unidentified inorganic salt precipitate forms within minutes. An elicited exothermic reaction produces an increase of 4.6°C +/- 0.4°C within 3 +/- 1 seconds. Intermediate irrigation protocols must be utilized when combining NaOCl and QMiX during endodontic therapies. Further research and release of information is necessary for identification of molecular structure and potential in vivo effects.

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