# THE DIFFERENCE IN PRODUCTION METHODS ON THE PROPERTIES, STABILITY, CORROSIVITY, AND ANTIMICROBIAL ACTIVITY OF CHLORINE-BASED SANITIZERS

by

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(Under the Direction of Yen-Con Hung)

## ABSTRACT

Chlorine-based sanitizers, such as electrolyzed-oxidizing (EO) water, are complex solutions that play a large role in food safety applications. Properties, such as free chlorine stability, corrosivity and antimicrobial activity, of chlorine-based sanitizers are influenced chiefly by the chemical makeup of the solution. In this study, methods were developed to accurately estimate the free chlorine, total chlorine and residual chloride of various chlorine-based sanitizers. After these methods were developed, they were then used to record property changes in chlorine-based sanitizers under various conditions. It was discovered that various organic macromolecules (amino acids, phenolic compounds and lipids) affected the free and total chlorine concentrations of chlorine-based sanitizers differently. Based on these interactions, a model of free chlorine loss was developed that helps to predict how much free chlorine will be lost in a given food environment. In addition to environmental stability, the constituents of chlorine-based sanitizers have specific effects on metal surfaces that are commonly found in food processing environments. It was discovered that the pH and chloride concentration of chlorine based sanitizers each affected the corrosion rate. A solution's pH was generally inversely proportional to the corrosion rate of metal surfaces while chloride concentration was directly proportional. Additionally, corrosion rates were found to be greater in metal samples that were not submerged in chlorine-based sanitizers, but were instead just above the liquid surface. As for the antimicrobial activity of chlorine-based sanitizers, both pH and chloride played significant roles. Reduction of viable cell numbers in a culture of *Escherichia coli* O157:H7 was indirectly proportional to pH and also indirectly proportional to residual chloride concentration, as determined by using fresh and artificially aged samples of chlorinated water and EO water. This research helps to highlight the importance of several factors intrinsic to chlorine-based sanitizers to how these chlorine-based sanitizers interact with their environments. Additionally, this research helps to provide manufacturers of chlorine-based sanitizers practical information on how to generate safer and more effective sanitizers.

INDEX WORDS: chlorine, electrolyzed water, stability, corrosion, antimicrobial

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# CHAPTER 1

# INTRODUCTION AND LITERATURE REVIEW

## Introduction

An increase in world population has lead food manufacturers to seek rapid and efficient methods for producing and distributing large amounts of food, often with little to no processing, given the current consumer demand for fresh foodstuffs. Because of all this, the threat of foodborne illness is of great concern in today's world. A 2011 report by the Centers for Disease Control (CDC) estimates that 1 in 6 Americans get sick, 128,000 are hospitalized and 3000 die every year of foodborne illness. Because of this, effort is undertaken to devise strategies to help prevent the spread of foodborne illness. One such strategy is the use of chlorine-based sanitizers, which have a history of use in food processing. One type of chlorine-based sanitizer, called electrolyzed-oxidizing (EO) water, is seeing increasing use in the United States. Electrolyzed oxidizing (EO) water, or electro-chemically activated water (ECA or ECAW), is a solution generated by passing a dilute salt solution ($NaCl$ and $KCl$ are commonly used) through an electrolytic cell. The anode side of an electrolytic cell, from which acidic EO water is obtained, produces various chlorine compounds and ions such as hypochlorous acid ($HOCl$), hypochlorite ion ($OCl^-$) and chlorine gas ($Cl_2$), which are all collectively known as free chlorine. $HOCl$ is the main antimicrobial agent present in EO water. EO water generally has a low pH (2.3 – 2.7) and a high oxidation – reduction potential (ORP) (> 1000 mV). The low pH and low concentration of free chlorine (typically around 40-50 mg/L) in EO water make it an effective and safe antimicrobial agent. Despite this, there are several problems with EO water and chlorine-based sanitizers in specific. First, chlorine species are inherently unstable, meaning the free chlorine present in EO water can be lost over time or in reaction with other chemical species (such as several types of organic compounds). Chlorine-based sanitizers are also notorious corrosion agents, and many food processers will not use chlorine-based sanitizers in certain situations. Of larger concern however, is that there are a variety of different methods for producing chlorine-based sanitizers like EO water, and these different methods cause changes in key properties that affect the chemical activity and biological activity of EO water.

The overall purpose of this study was to investigate EO water generation methods and compare the EO water produced by different generation methods based on their environmental stability, corrosivity, and antimicrobial activity. This dissertation consists of five chapters. The first chapter presents an introduction and literature review. The second chapter explores methods useful in determining key parameters (free chlorine, total chlorine, hypochlorus acid , hypochlorite, and chloride) of chlorine-based sanitizers. The third chapter seeks to determine how chlorine-based sanitizers with different properties react with several classes of organic compounds and what are some likely products. The fourth chapter investigates the ability of different types of EO and chlorinated water to corrode metals commonly found in food processing environments. The fifth chapter presents a study of the antimicrobial activity of several types of EO and chlorinated water. The works cited are included in each chapter, and some chapters are formatted in accordance with the publishing journal’s style

## Literature Review

### *Chemical description of chlorine-based sanitizers*

Chlorine-based sanitizers, which are defined as sanitizers that contain chlorine as the main antimicrobial agent, have seen extensive use in commercial and residential settings as an effective means of sanitization. Because of this, there are several different types of chlorine–based sanitizers developed for use in different situations. For the purposes of this literature review, the focus will be on chlorine-based sanitizers that use hypochlorous acid (HOCl) and hypochlorite (OCl-) (together known as free chlorine) as the main antimicrobial compounds. The chlorine-based sanitizers in question include diluted bleach and electrolyzed-oxidizing (EO) water.

Diluted bleach is the most widely used chlorine-based sanitizer in food processing environment because of its low cost and effectiveness. Diluted bleach is typically bleach solution (up to 12% in food processing environments diluted to 50-100 mg/L free chlorine, depending on what purpose the sanitizer is being used for. Because of this, the pH of diluted bleach tends to be alkaline (~9.3 pH) and have an ORP of ~ 600 mV. Additionally, the form of free chlorine present in diluted bleach is almost all $OCl^-$ The pH is typically not modified because doing so would increase the risk of corrosion as well as present a health hazard due to dangerous chlorine gas ($Cl_2$).

EO water is different from diluted bleach in several ways. First, EO water is generated as needed by means of a special type of electrolytic cell called an EO water generator. The solution collected from an EO water generator ranges from acidic (~ pH 2.5) to slightly acidic (~ pH 6.0), resulting in HOCl being the main form of free chlorine. Because of this, the ORP is higher as well (> 1100 mV at pH 2.5 and 900 mV at pH 6.0).

There are a great many factors associated with EO water generators that affect the properties of EO water. Since there are many factors to discuss, a detailed literature of EO water and EO water generators is presented in Chapter 2.

### *Reactions of chlorine species with organic compounds*

Chlorine is a highly-reactive element that readily forms compounds with many other compounds. Free chlorine readily oxidizes phenolic compounds, amines and metal ions to form chlorinated byproducts, some of which are a human health hazard.

Phenolic compounds, mostly present in humic and fulvic acids that are ubiquitous in soil, react with chlorine compounds to produce trihalomethane compounds such as chloroform. The pH-dependent process occurs by a series of chlorination reactions followed by a ring cleavage.

Amines, found mostly in proteins in a food environment, react with chlorine compounds to produce chloramines. Like in the case of phenolic compounds, the process is pH-dependent. Amines are most commonly found in high protein surfaces, such as the surface of meat.

Although they aren't organic, metal ions, such as ferrous ($Fe^{2+}$) ions react with chlorine compounds as well. In particular, ferrous ions react with free chlorine to form ferric chlorides ($FeCl_3$), among other compounds. Although iron is not present in significant amounts in food environments compared to the previously-mentioned compounds, it is important to note that iron is present in soil, so its contribution to free chlorine loss when chlorine-based sanitizers are used as wash water cannot be discounted.

A detailed literature review of reactions of chlorine-based sanitizers with organic compounds is presented in Chapter 3.

### *Corrosion of metal surfaces by chlorine species*

It is well known that chlorine solutions cause corrosion in susceptible materials (Bohner and Bradley, 1991; Daufin *et al.*, 1988; McCafferty, 2003). Corrosion is defined as the process between a material and its environment that results in the degradation of the material. As such, corrosion is not so much a material's property as it is a response to the environment. The rate of corrosion depends on several environmental variables, such as pH, electrochemical potential, temperature, and the concentration, as well as the identity, of chemical species within the material's environment. Since there are a variety of different environmental conditions that a material can be subjected to, there are also several different corrosion processes that can occur. The main form of corrosion concerning EO water, as well as most other sanitizers used in the food processing environment, is aqueous corrosion (both uniform and localized) (Fontana and Green, 1986).

Uniform corrosion, as the name implies, deals with non-localized corrosion over the entire surface of a material. There are two types of uniform corrosion, based on environment: aqueous corrosion and gaseous corrosion. Uniform gaseous corrosion is usually only a concern at higher temperatures. Additionally, uniform corrosion (at least the concept *in toto*) is not a concern with EO water in food processing (Ayebah and Hung, 2005).

Of a greater concern in food processing environments in localized corrosion occurring at uncoated (i.e. not anodized or galvanized) surfaces that are exposed to standing EO water for extended periods of time. Areas such as welds, joints, bolts and edges are particularly susceptible to localized corrosion. In addition to the surface condition, localized aqueous corrosion is a factor of pH (namely, acidity), oxidizing power, temperature and heat transfer, fluid movement and solution components.

The pH of a solution plays a rather complex role in the corrosion process. For example, pH's role in corrosion appears to be much more dominant when the $pH < 5$ as opposed to $5 < pH < 9$. However, some metals (namely aluminum and zinc) experience a large increase in corrosion rate when $pH > 9$ (Tomashov, 1966) (Vujicic and Lovrecek, 1985). One of the reasons why there is complexity surrounding the role of pH in corrosion is because of counter ions. A counter ion (sometimes referred to as a conjugate base), can either slow down (i.e. $SO_4^{2-}$ from sulfuric acid) or speed up ($Cl^-$ from hydrochloric acid) corrosion at certain pH levels (Chin and Nobe, 1972) (Ellison and Schmeal, 1978).

Oxidizing power also plays a part in determining corrosion rates. Due to the polarization within electrolytic cells, for instance, corrosion is often a problem unless steps are taken protect the electrodes. Anodes can be protected by applying a surface oxide coating, as discussed in relation to titanium oxides by Suffredini *et al.* (2000). Additionally, concentrated $H_2SO_4$, $HNO_3$ and NaOH can be added externally to the anode in an electrolytic cell to help mitigate corrosion (Fontana and Green, 1986).

The role of temperature in corrosion, like pH, is also complex. Temperature should be discussed in terms of other variables because temperature is a factor which increases or decreases the activity of other variables involved in corrosion. For example, temperature has an effect on the solubility of many chemical species in aqueous solutions. Corrosion rates for iron in the presence of oxygen are known to increase as temperatures increase. This increase happens until a certain temperature is reached, then the corrosion rate decreases above that temperature. The reason for this is that oxygen solubility reaches a point that allows for too little oxygen in solution to promote faster corrosion rates (Tomashov, 1966).

The influence of fluid flow rate on corrosion is dependent on the metal, fluid components, fluid physical properties, the shape of the container the fluid is help in and the corrosion mechanism at work. Generally, greater fluid flow rates mean less localized corrosion (i.e. pitting) due to a high mass transfer rate (Copson, 1960).

Solution components (and their concentrations) should be considered in relation to the variables previously mentioned, as all of those variables directly influence solution components and concentration. In terms of chloride, its concentration actively depends on pH, temperature and fluid flow. In turn, the concentration and identity of chlorine in solution will influence the oxidizing power of the solution.

The rate of corrosion of metals in the presence of solutions such as EO water is a product of several closely-associated variables. Therefore, any attempt at corrosion research must account for all of the previously-discussed variables.

Several studies have been performed specifically on the effect EO water has on metal surfaces common in food processing environments. One study by Ayebah and Hung (2005) discovered that pH was a significant factor in the mass loss of aluminum, carbon steel, and copper when the metals were exposed to chlorine-based sanitizers of different pHs. Lower pH values lead to greater degrees of mass loss in all metal types. Additionally, that study and one by Tanaka *et al.* (1999) noted that stainless steel is greatly resistant to corrosion by acidic EO water. In addition to changes in mass, changes in surface roughness (as measured by a diamond-tipped surface roughness tester) were apparent as well. Another study by Dong *et al.* (2003) showed similar pH trends in terms of corrosion of alloys used in dental fillings. Also, the corrosion rate was significantly decreased in the dental alloys when near neutral electrolyzed water was used. Despite these 2 studies providing evidence of the corrosive nature of EO water, other authors

(Tanaka *et al.,* 1999) have concluded that the amount of corrosion, at least in hemodialysis equipment, is not severe. It remains to be seen if this applies in all circumstances. It also remains to be seen what the role of chloride ions present in EO water is in corrosion.

*Antimicrobial activity of EO water*

Chlorine-based sanitizers have been used to effectively kill microorganisms that cause foodborne illness for years. Since chlorine compounds are strong oxidants, they are able to effectively oxidize a large array of cell compounds. Additionally, HOCl, as a weak acid, is able to move inside bacterial cells in order to oxidize internal cell components such as DNA and internal proteins.

Chlorine-based sanitizers are very effective in killing a wide variety of microorganisms. Some organisms, such as *E. coli* O157:H7 are more affected by chlorine-based sanitizers than organisms like *Listeria monocytogenes*. Also, the efficacy of chlorine-based sanitizers is dependent on the amount of organic soil present as well as the properties of the chlorine-based sanitizer itself. A more detailed literature review of EO water food safety applications is in Chapter 4.

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# CHAPTER 2

# CHLORINE SPECIES AND THEIR REACTIONS WITH ORGANIC COMPOUNDS

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Chlorine-based sanitizers have seen widespread use in food sanitation. Chlorine is commonly added to drinking water in many countries around the world. Also, chlorine has been used on food preparation surfaces as well as food itself. Fruits, vegetables and meats can be rinsed with chlorine to help control microbial load (Wei, Cook & Kirk, 1985).

Different forms of chlorine are used in food sanitation. Chlorine dioxide ($ClO_2$) is one such form of chlorine and is an effective antimicrobial agent. Chlorine dioxide is less reactive with organic matter than chlorine, so chlorine dioxide tends to not produce as many disinfections byproducts (DBPs) as chlorine does. However, there are issues that limit the feasibility of chlorine dioxide. Since chlorine dioxide is less stable than chlorine, it must be generated on-site and used as soon as it is made. For that reason, there is very little residual antimicrobial activity when chlorine dioxide is used. Additionally, there is some concern about the safety of chlorine dioxide. Chlorine dioxide vapors have been shown to increase respiratory problems, such as bronchitis, in exposed workers. Because of these issues, the use of chlorine is favored in food processing settings.

When chlorine is added to water, HOCl and its ionized form hypochlorite ($OCl^-$) are formed. HOCl is a strong oxidant, and it freely oxidizes many organic compounds (Folkes, Candeias & Wardman., 1995) (Hawkins, Pattison & Davies, 2003) (Whyman, 1996) (Winterbourn & Brennan, 1997). Also, as a weak acid, HOCl is able to diffuse through cell membranes and acidify the interior of cells (Gutknecht & Tosteson, 1973**.** Cell membranes are mostly impermeable to charged substances. The reason why is because ions have a high affinity to water molecules, which develop dipoles by nature. The hydrophobic portion of the cell membrane contains no water, so charged particles cannot pass through it. HOCl is a weak acid, so it does not dissociate to any great extent. Being both uncharged and small, HOCl is free to diffuse across the cell membrane. In addition to hydrolysis of saccharide and peptide bonds, hypochlorite can also interact with disulfide bonds. Disulfide bonds are formed by sulfur-containing amino acids such as cysteine and methionine, and they play an important role in determining a proteins folded structure. Hypochlorite readily oxidizes these bonds, resulting in their cleavage (Whyman, 1996). These properties help to make HOCl an effective antimicrobial agent.

Although HOCl is effective at reducing microbial numbers, its effectiveness is negatively impacted by the presence of organic compounds. In particular, antioxidant compounds such as ascorbate, glutathione and taurine are able to rapidly react with any available HOCl (Folkes, Candeias & Wardman., 1995) (Hawkins, Pattison & Davies, 2003) (Winterbourn & Brennan, 1997) . It was also found that HOCl was consumed to varying degrees when mixed with milk, minced meat and chopped cabbage (Oomori, Oka, Inuta & Arata, 2000) (Udompijitkul, Daeschel, & Zhao, 2007).

Solutions of sodium hypochlorite (bleach) are commonly-used sanitizers in commercial and domestic settings. Typically, bleach solutions of 3-6% sodium hypochlorite are used in home applications, and this is often diluted by the user prior to use. Industry follows stricter guidelines where bleach use is concerned. One industry that makes use of bleach solutions for sanitation is the poultry industry. The USDA, Food Safety and Inspection Service (FSIS) allows up to 50 mg/L in poultry chiller water. The FSIS also requires that chlorinated water containing a minimum of 20 mg/L available chlorine be used to sanitize surfaces that carcasses have come in contact with. Another industry that makes use of hypochlorite solutions is the produce industry, where the federally-mandated limit of chlorine in wash water is 2000 mg/L (21 CFR, 2003).

While bleach solutions are widely used as sanitizers, they are not without drawbacks. The efficacy of bleach solutions are dependent on pH.

**Chlorine Chemistry**. Chlorine is an extremely reactive nonmetal, as evidenced by the wide variety of chlorine compounds in nature (Öberg, 2002). Chlorine, in its pure form, is a poisonous, yellow-green gas. Most chlorine reactions are second order, and the kinetics are pH dependent as chlorine has a pH-dependent aqueous chemistry, causing several different chlorine species (such as HOCl, OCl-, $Cl_2$, etc.) to be present in water (Doré, 1989). These different chlorine species react with substances differently. If the pH is from 5-7, the following reaction predominates:

$$Cl_2 + H_2O \rightarrow HOCl + Cl^- + H^+ \qquad (1)$$

HOCl is the predominant species at pH 3-7 (Gordon & Tachiyashiki, 1991). Above pH 8, the hypochlorite ion ($OCl^-$), predominates. The pKa of HOCl is 7.46 at room temperature. HOCl readily decomposes upon exposure to light. In the process of decomposition, formation of $Cl_2$ is possible if the pH is sufficiently low ($pH < 4$). This change of chlorine species with pH are shown on the Figure 2-1 (Deborde & von Gunten, 2008).

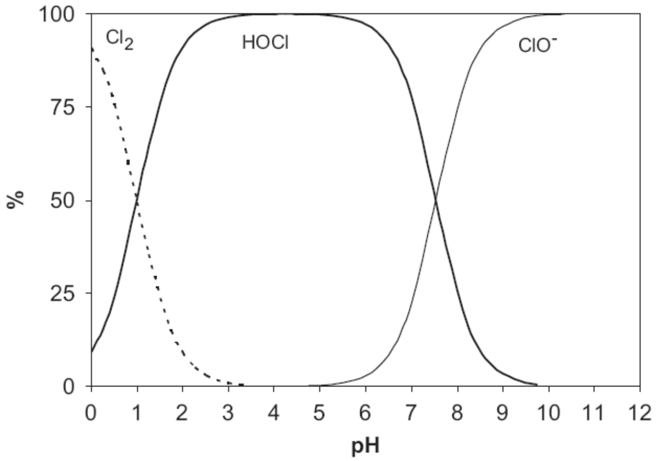

**Figure 2-1 - The relative percentages of three different chlorine species in solution as a function of pH (Deborde and von Gunten, 2008).**

**Properties of HOCl and OCl$^-$.** OCl$^-$ is more stable than HOCl, so OCl$^-$ has also been frequently used as a disinfecting agent. OCl$^-$ is most commonly found in the form of sodium hypochlorite (NaOCl) or calcium hypochlorite ($Ca(OCl)_2$). Since compounds such as NaOCl readily dissociate to OCl$^-$ in aqueous solutions, NaOCl's is not as an effective bacteriocidal agent as HOCl, because biological membranes are relatively impermeable to ions (Wyman, 1996). Despite this, OCl$^-$ is still an effective oxidant.

Standard reduction potential is a measure of how easily a chemical species acquires electrons, becoming reduced in the process and oxidizing the species the electrons were acquired from. The larger and more positive the number is, the greater ability as an oxidant the species has. However, OCl$^-$ has a standard reduction potential of 0.9 V, and HOCl has a standard reduction potential of 1.49 V, making HOCl a stronger oxidant than OCl$^-$ is (Dowd, 1994).

HOCl is a strong oxidant, and it freely oxidizes many organic compounds. Also, as a weak acid, HOCl is able to diffuse through cell membranes and acidify the interior of cells. This reaction increases significantly when the pH is lowered to the point that HOCl predominates because HOCl has greater penetration power due to its uncharged nature (Wyman, 1996). Cell membranes are mostly impermeable to charged substances. The reason why is because ions have a high affinity to water molecules, which develop dipoles by nature. HOCl is a weak acid, so it does not dissociate to any great extent. Being both uncharged and small, HOCl is free to diffuse across the cell membrane. These properties help to make HOCl an effective antimicrobial agent.

**Chlorine and Organic Compounds.** The reaction of chlorine species with organic matter is a concern for two reasons. Available chlorine can be "used up" by organic compounds not associated with microorganisms, resulting in a lower amount of chlorine available for disinfections. This may be alleviated by prewashing a food product to remove extraneous organic matter on the surface, or a greater concentration of chlorine can be used to help insure that more chlorine is available for disinfection. Another point of concern is that some forms of chlorine can react with some organic compounds to form toxic halogenated disinfections byproducts (DBPs).

A variety of chlorinated compounds can be formed, depending on the substrates present. The DBPs formed include trihalomethanes (THMs), chloramines and haloacetic acids (HAAs). Of all these, THMs are the prevalent in drinking water, followed by HAAs (Shin, Chung, Choi, Kim, Park & Kum, 1999). The US EPA had previously set a limit of 0.08 mg/L for total THMs in water and a goal for the total removal of chloroform from drinking water (U.S. Environmental Protection Agency, 1994). However, the total removal mandate for chloroform was removed as of 2000 (U.S. Environmental Protection Agency, 2000). HAAs have an even lower limit at 0.06 mg/L. However, HAAs are not thought to be very common DBPs. Chloramines have a limit of

4 mg/L. The reason for this is that chloramines are themselves used as disinfection agents and are not know to produce toxic DBPs.

**Formation and Chemistry of Chlorinated Disinfection Byproducts.** Trihalomethanes are the most prevalent DBPs in drinking water (Boorman, Dellarco, Dunnick, Chapin, Hunter, Hauchman, Gardner, Cox & Sills, 1999). Chloroform ($CHCl_3$) is the chief THM formed by HOCl. THMs, especially chloroform, are the most studied of all DBPs due to their prevalence. Formation of chloroform has been studied in humic substances (Gallard & Gunten, 2002) (Iriarte-Velasco, Álvarez-Uriarte & González-Velasco, 2006) triclosan (Rule, Ebbett & Vikesland, 2005), citric acid (Larson & Rockwell, 1979) and resorcinol (Özbelge, 2001). When in contact with chlorine components, aldehydes and ketones are converted to chloroform via a base-catalyzed reaction pattern (Deborde & von Gunten, 2008). These reactions start with a rate-limiting enolization step that converts ketones to enols. There are exceptions to this, however, 3-ketoglutaric acid is rapidly converted into an enol because of the increased enol stability conferred by carboxylate groups within the compound (Larson & Rockwell, 1979). After enolization, rapid chlorination steps yield a trichloromethyl ketone. Finally, hydroxide nucleophilic substitution in the compound results in chloroform and a carboxylic acid. For compounds such as acetylacetone, the conversion to THM process is very similar.

The main difference is the early cleavage of the compound to form acetate and a chlorinated compound, which is further chlorinated before undergoing nucleophilic substitution to yield acetate and chloroform. In aromatic compounds such as humic acids, enolization is followed by chlorination. In most cases 2-chloro, 2,4-dichloro, and 2,4,6-trichloro phenolic and resorcinol compounds are among the first compounds formed when humic compounds are the substrates (Deborde & vonGunten, 2008), (Gallard & vonGunten, 2002), (Rule, Ebbett & Vikesland, 2005). The chlorination continues until the methyl group is trichlorinated. Finally, hydroxide attacks the resulting trihaloketone to yield chloroform and a carboxylic acid. Alcohols are other potential substrates that will react with chlorine to form chloroform. It is suggested that chloroform formation can occur through the mixing of bleach and alcohols such as isopropanol and ethanol. Because the reactions are very slow, only a few studies on the chlorine reactivity with alcohols have been undertaken (Deborde & vonGunten, 2008).

Chloramines are another class of disinfection byproducts formed by halides. HOCl transfers a chloride to nitrogen to form mono-, di- and trichloramines (Abia, Armesto, Canle, Garcia & Santabella, 1997). Chloramine (monochloramine), is the most common haloamine. Many animals are sensitive to chloramine, and chloramine is toxic to a few animals, such as fish.

The formation of chloramines involves the addition of chlorine to amine groups or ammonia to form monochloramine, dichloramine or trichloramine. The particular chloramine yielded from the reaction is dependent on chlorine-to-nitrogen ratio, pH, temperature and contact time (Donnermair & Blatchley III, 2003). If there is significantly more nitrogen than chlorine, monochloramine ($NH_2Cl$) will be the dominant product (White, 1999). HOCl reacts with both organic and inorganic amines in solution, but the distribution depends on affinity for the organic and inorganic nitrogen compounds, distribution of the nitrogen compounds and pH (Yoon & Jensen, 1993). Chlorine reactivity with $NH_4^+$ (species present at acidic pHs) is negligible. Most of the chlorination happens at pH 8-9 ($k_{app} > 10000\ M^{-1}s^{-1}$) (Deborde & von Gunten, 2008).

HAAs are classes of organic halides that are formed when halides such as chlorine take the place of methyl hydrogens in acetic acid. The formation of haloacetic acids seems to be pH-dependent. More is known about THMs than HAAs, but of the nine HAAs known, only five of them are regulated in drinking water. They are monochloro-, monobromo-, dichloro-, and trichloroacetic acid (Liang & Singer, 2003). Haloacetic acids, in particular chloroacetic acid, dichloroacetic acid and trichloroacetic acid, have been receiving more attention recently from the scientific community. Like THMs, HAAs can be produced from humic and fulvic subtances. Dichloro- and trichloroacetic acids account for the greater majority of HAAs formed, with dichloroacetic acid being the most common (Fahimi, Keppler & Schöler, 2003) (Shin, Chung, Choi, Kim, Park & Kum, 1999). The rates of these reactions are pH dependent. In the presence of resorcinolic compounds, pHs below 7 yield mostly HAAs while pHs above 7 yield mostly THMs. Therefore, pH appears to play a large role in the determination of DBP species formed.

**Health Concerns of Chlorinated Disinfection Byproducts.** THMs, the most common of which is chloroform but also include bromoform, dibromochloromethane and dichlorobromomethane, have been reported as a possible health threat in dishwashers (Olson and Corsi, 2004), indoor swimming pools (Aggazzotti, Fantuzzi, Righi & Predieri, 1995) and from the use of bleach-containing household products (Odabasi, 2008) as well as being prevalent in chlorinated drinking water. Chloroform causes carcinogenesis by nongenotoxic methods, namely decreased methylation which leads to overexpression of proto-oncogene (Coffin, Ge, Yang, Kramer, Tao, & Pereira, 2000). The authors speculate that THMs in general cause a decreased methylation of the c-*myc* proto-oncogene, which leads to the gene’s subsequent uncontrolled expression. It has also been speculated that THMs present in disinfected drinking water lead to an increase in bladder and colorectal cancers (Villanueva, Cantor, Grimalt, Castaňo-Vinyals, Malats, Silverman, Tardon, Garcia-Closas, Serra, Carrato, Rothman, Real, Dosemeci & Kogevinas, 2006). Despite this, it is uncertain if the health risks are serious enough to warrant stricter regulation. Although direct contact by oral gavage with $CHCl_3$ has been shown to cause tumorigenesis in mice, $CHCl_3$ administered in drinking water has not (Coffin, Ge, Yang, Kramer, Tao, & Pereira, 2000). The reason for this is the incremental ingestion of chloroform through water does not tax the liver's detoxification mechanisms as opposed to oral gavage, which provides more chloroform at once. This is the reason why chloroform has been classified as a threshold carcinogen (U.S. EPA, 1994). A threshold carcinogen is defined as a substance that is only carcinogenic after a certain level, or threshold, is reached. Since this is the case, it is unlikely that food, which is not expected to contain a substantial amount of chloroform, would be a major route of chloroform exposure.

There are many more types of HAAs than there are THMs or chloramines. Two such HAAs, trichloroacetate and dichloroacetate, have been shown to cause liver tumors in rats and mice (Boorman, Dellarco, Dunnick, Chapin, Hunter, Hauchman, Gardner, Cox & Sills, 1999). It is speculated that these two compounds have different modes of carcinogenicity. Dichloroacetate is thought to modify intracellular signaling pathways (U.S. Environmental Protection Agency. 1998), while trichloroacetate is thought to be related to peroxisome proliferation (Bull, Sanchez, Nelson, Larson & Lansing, 1990).

Chloramines, as stated previously, are useful as disinfection agents. However, chloramines lack the effectiveness of other chlorine-based disinfectants, as they showed little to no effect on *E. coli* (Donnermair & Blatchley, 2003). There is not much information on the toxicity or carcinogenicity of the chloroamines, except for anecdotal evidence that trichloroamine may be linked to an increase in asthma. Chlormine toxicity is mostly a concern in water rather than food.

This has led some people to explore alternatives to disinfection by chlorination, such as ozonation, disinfection by chlorine dioxide and UV irradiation of drinking water. All of these methods have drawbacks. For example, chlorine dioxide has less reactivity with organic matter than chlorine does, but it is less stable. Some iodine-based sanitizers can stain and corrode equipment. Quaternary ammonium compounds are not approved for direct food contact. UV irradiation is only useful in killing organisms at the surface due to its weak ability to penetrate water. Because of all these issues, chlorination remains the most cost effective method of treating drinking water.

Acute toxicities of chlorinated compounds and government control limits on these compounds are given in Table 2-1. The government control limits information help illustrate the relative toxicity of each compound.

### Table 2-1 – Toxicity and regulatory information for several chlorine compounds

| **Compound** | **$LD_{50}$ (rat, oral)** | **US government control?** |
|---|---|---|
| hypochlorous acid | > 3000 mg/kg | yes, MRDL = 4 mg/L |
| sodium hypochlorite | 5000 mg/kg (at 12.5%) | no |
| chloroform | 908 mg/kg | yes, MCL = 0.08 mg/L for **all** trihalomethanes |
| bromodichloromethane | 916 mg/kg | yes, MCL = 0.08 mg/L for **all** trihalomethanes |
| chlorodibromomethane | 1186 mg/kg | yes, MCL = 0.08 mg/L for **all** trihalomethanes |
| monochloroacetic acid | 76 mg/kg | yes, MCL = 0.06 mg/L for **all** haloacetic acids |
| dichloroacetic acid | 2820 mg/kg | yes, MCL = 0.06 mg/L for **all** haloacetic acids |
| trichloroacetic acid | 5000 mg/kg | yes, MCL = 0.06 mg/L for **all** haloacetic acids |
| chloramine | 935 mg/kg | yes, MRDL = 4 mg/L for **all** chloramines |
| dichloramine | no data available | yes, MRDL = 4 mg/L for **all** chloramines |
| trichloramine | no data available | yes, MRDL = 4 mg/L for **all** chloramines |
| chlorine dioxide | 292 mg/kg | yes, MRDL = 0.8 mg/L |
| chlorite | 292 mg/kg | yes, MCL = 1 mg/L |
| chlorate | 1200-7000 mg/kg | not specifically |

MCL = maximum contaminant level; MRDL = maximum residual disinfectant level; LD50 = lethal dose, 50%

**Rates of reaction and DBP yields involving HOCl and $OCl^-$** Several works have attempted to elucidate the role of HOCl and $OCl^-$ in the production of DBPs with a particular interest in the production of THMs such as chloroform. Table 2-2 offers a summary of information in the literature that has explored the role of different species of chlorine as affected by pH in the formation of DBPs with different substrates:

**Table 2-2 – Concentrations and reaction rates for the formation of various chlorinated compounds using a variety of substrates as a function of pH**

| **Substrate** | **Concentration of product (mg/l) or rate of reaction ($M^{-1}s^{-1}$)** | | | **Product formed** | **Reference** |
|---|---|---|---|---|---|
| | **HOCl (pH ≤ 7)** | | **OCl- (pH ≥ 8)** | | |
| hexane | 0.15 | mg/l | 0.1 | chloroform | Chaidou *et al.*, 1999 |
| benzene | 0.1 | mg/l | 0.12 | chloroform | Chaidou *et al.*, 1999 |
| toluene | 0.11 | mg/l | 0.2 | chloroform | Chaidou *et al.*, 1999 |
| ethylbenzene | 0.3 | mg/l | 0.3 | chloroform | Chaidou *et al.*, 1999 |
| styrene | 0.35 | mg/l | 0.4 | chloroform | Chaidou *et al.*, 1999 |
| m-xylene | 0.55 | mg/l | 0.55 | chloroform | Chaidou *et al.*, 1999 |
| 2-ethyltoluene | 0.1 | mg/l | 0.12 | chloroform | Chaidou *et al.*, 1999 |
| naphthalene | 0.23 | mg/l | 0.25 | chloroform | Chaidou *et al.*, 1999 |
| acetone | 2.2 | mg/l | 3.6 | chloroform | Chaidou *et al.*, 1999 |

| | | | | | |
|---|---|---|---|---|---|
| isopropanol | 0.7 | mg/l | 1 | chloroform | Chaidou *et al.*, 1999 |
| 1,2-dichlorobenzene | 0 | mg/l | 0.1 | chloroform | Chaidou *et al.*, 1999 |
| 1,4-dichlorobenzene | 0 | mg/l | 0.1 | chloroform | Chaidou *et al.*, 1999 |
| aniline | 3 | mg/l | 3.2 | chloroform | Chaidou *et al.*, 1999 |
| nitrobenzene | 0.2 | mg/l | 0.3 | chloroform | Chaidou *et al.*, 1999 |
| 2-chloroanaline | 3 | mg/l | 3.2 | chloroform | Chaidou *et al.*, 1999 |
| 3-chloroanaline | 1 | mg/l | 1.3 | chloroform | Chaidou *et al.*, 1999 |
| 4-chloroanaline | 3 | mg/l | 3 | chloroform | Chaidou *et al.*, 1999 |
| phenol | 0.7 | mg/l | 1 | chloroform | Chaidou *et al.*, 1999 |
| hydroquinone | 0 | mg/l | 1.7 | chloroform | Chaidou *et al.*, 1999 |
| resorcinol | 11 | mg/l | 11.2 | chloroform | Chaidou *et al.*, 1999 |
| catechol | 0 | mg/l | 0.2 | chloroform | Chaidou *et al.*, 1999 |
| pyrogallol | 0 | mg/l | 0.2 | chloroform | Chaidou *et al.*, 1999 |
| phloroglucinol | 7.5 | mg/l | 13 | chloroform | Chaidou *et al.*, |

| | | | | | |
|---|---|---|---|---|---|
| | | | | | 1999 |
| 2-chlorophenol | 2 | mg/l | 2.2 | chloroform | Chaidou *et al.*, 1999 |
| 3-chlorophenol | 2.2 | mg/l | 2.4 | chloroform | Chaidou *et al.*, 1999 |
| 4-chlorophenol | 1 | mg/l | 1.2 | chloroform | Chaidou *et al.*, 1999 |
| o-cresol | 1 | mg/l | 1.8 | chloroform | Chaidou *et al.*, 1999 |
| m-cresol | 1 | mg/l | 1.5 | chloroform | Chaidou *et al.*, 1999 |
| p-cresol | 0 | mg/l | 0.4 | chloroform | Chaidou *et al.*, 1999 |
| humic acid | 0.074 | mg/l | 0.095 | chloroform | Dowd, 1994 |
| bromide | 1.55 x $10^{3}$ | $M^{-1}s^{-1}$ | 9 x $10^{-4}$ | $OBr^{-}$ | Kumar & Margerum, 1987 |
| sulfite | 7.6(±0.4) x $10^{8}$ | $M^{-1}s^{-1}$ | 2.3(±0.2) x $10^{4}$ | $ClSO_3^{-}$ | Fogelman *et al.*, 1989 |
| cyanide | 1.22(±0.03)x$10^{9}$ | $M^{-1}s^{-1}$ | 2.3(±0.2) x $10^{4}$ | ClCN | Fogelman *et al.*, 1989 |
| cystiene | 1.2 x $10^{9}$ | $M^{-1}s^{-1}$ | 1.9 x $10^{5}$ | cystiene disulfide | Armesto *et al.*, 2000 |
| methionine | 3.3 x $10^{8}$ | $M^{-1}s^{-1}$ | 5.5 x $10^{5}$ | sulfoxides | Pattison & Davies, 2001 |
| N-methylformamide | 1.70 x $10^{-3}$ | $M^{-1}s^{-1}$ | 1.82 x $10^{-2}$ | chlorinated amide | Thomm & Wayman, 1969 |
| N-methylacetamide | 1.70 x $10^{-2}$ | $M^{-1}s^{-1}$ | 9.20 x $10^{-3}$ | chlorinated amide | Thomm & |

| | | | | | |
|---|---|---|---|---|---|
| | | | | | Wayman, 1969 |
| (N,N)-dimethylurea | 0.82 | $M^{-1}s^{-1}$ | 0.0083 | chlorinated amide | Thomm & Wayman, 1969 |
| gemfibrozil | $10^{0}$ | $M^{-1}s^{-1}$ | $10^{-0.75}$ | chloramines, chloroform | Deborde & Gunten, 2008 |
| naproxen | $10^{0.5}$ | $M^{-1}s^{-1}$ | $10^{0}$ | chloramines, chloroform | Deborde & Gunten, 2008 |
| trimethoprim | $10^{1.75}$ | $M^{-1}s^{-1}$ | $10^{1.5}$ | chloramines, chloroform | Deborde & Gunten, 2008 |
| indometacine | $10^{1.75}$ | $M^{-1}s^{-1}$ | $10^{1}$ | chloramines, chloroform | Deborde & Gunten, 2008 |
| enrofloxacin | $10^{2.5}$ | $M^{-1}s^{-1}$ | $10^{2.5}$ | chloramines, chloroform | Deborde & Gunten, 2008 |
| sulfamethoxazole | $10^{3}$ | $M^{-1}s^{-1}$ | $10^{2.75}$ | chloramines, chloroform | Deborde & Gunten, 2008 |
| sulfamedimethoxine | $10^{4}$ | $M^{-1}s^{-1}$ | $10^{3.75}$ | chloramines, chloroform | Deborde & Gunten, 2008 |
| ciprofloxacin | $10^{5.75}$ | $M^{-1}s^{-1}$ | $10^{6}$ | chloramines, chloroform | Deborde & Gunten, 2008 |
| acetaminophen | $10^{1}$ | $M^{-1}s^{-1}$ | $10^{1.25}$ | chloramines, chloroform | Deborde & Gunten, 2008 |
| 4-n-nonylphenol | $10^{1}$ | $M^{-1}s^{-1}$ | $10^{1.25}$ | chloramines, chloroform | Deborde & Gunten, 2008 |
| triclosan | $10^{2.5}$ | $M^{-1}s^{-1}$ | $10^{3}$ | chloramines, chloroform | Deborde & Gunten, 2008 |
| bisphenol A | $10^{1.75}$ | $M^{-1}s^{-1}$ | $10^{2}$ | chloramines, chloroform | Deborde & Gunten, 2008 |

| | | | | | |
|---|---|---|---|---|---|
| estrogenic steroid hormones | $10^2$ | $M^{-1}s^{-1}$ | $10^{2.5}$ | chloramines, chloroform | Deborde & Gunten, 2008 |
| p-iodophenol | 8 | $M^{-1}s^{-1}$ | 30 | chloroform | Gallard & Gunten, 2002 |
| p-methylphenol | 10 | $M^{-1}s^{-1}$ | 50 | chloroform | Gallard & Gunten, 2002 |
| p-cyanophenol | 8 | $M^{-1}s^{-1}$ | 10 | chloroform | Gallard & Gunten, 2002 |
| phenol | 10 | $M^{-1}s^{-1}$ | 50 | chloroform | Gallard & Gunten, 2002 |
| p-chlorophenol | 8 | $M^{-1}s^{-1}$ | 20 | chloroform | Gallard & Gunten, 2002 |
| ammonia | $8.9 \times 10^3$ | $M^{-1}s^{-1}$ | $2.6 \times 10^4$ | monochloramine | Qiang & Adams, 2004 |

The use of aromatic compounds in the study by Chaidou, Georgakilas, Stalikas, Saraci & Lahaniatis (1999) is significant because these compounds approximate humic substances, which are ubiquitous in water. Also, the decision to use chlorinated phenols as reactants in some of the tests is probably to approximate partially-chlorinated intermediates. All of these results show a definite relationship between pH and chlorination or oxidation of compounds, and most of them show that more DBPs, such as chloroform, are formed at pHs ≥ 8 than at pHs ≤ 7. In many cases, the rate of DBP decreases as pH decreases, as indicated in the literature.

Work done by Özbelge (2001) indicates the same trend of reduced chloroform formation as pH decreases. In the study, the pH of hypochlorite was adjusted to pH 4, 7 and 10. The hypochlorite was then added to resorcinol in a ratio of 1:1 (resorcinol:chlorine), 1:3 and 1:10. No chloroform was formed at pH 4 for the 1:3 ratio, but 50% of the chlorine was converted to chloroform and 95% of the chlorine was converted to chloroform at pH 7 and 10, respectively. At the 1:10 ratio, 32% and 82% of the chlorine was converted to chloroform at pHs 4 and 7, respectively. Chloroform formation at pH 10 was not studied for this ratio.

## Summary

With a rise in the incidence of foodborne pathogens in food, it becomes increasingly important for consumers to adopt measures to ensure food safety. Chlorine-based sanitizers are still the most popular chemicals to ensure food safety. There is a concern that the production of toxic byproducts will negate the health benefits of treating drinking water. Based on the information in the literature, pH plays an important role in the determination of the type and amount of DBPs formed, with lower, more acidic, pHs resulting in the formation of less chloroform. This review also stresses the need for more research on the link between pH and THM, as well as HAA, production.

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# CHAPTER 3

# ELECTROLYZED OXIDIZING WATER GENERATION METHODS

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Waters, B. W., Amazonas, M., and Y.-C. Hung. 2012. Electrolyzed oxidizing water generation methods. *J Func Water*. 7(1):1-13. Reprinted here with permission of the publisher

Electrolyzed oxidizing (EO) water, also known as Denkaisui in Japan, or electro-chemically activated water (ECA or ECAW), is a solution generated by passing a dilute salt solution (NaCl and KCl are commonly used) through an electrolytic cell. The anode side of an electrolytic cell, from which acidic EO water is obtained, produces various chlorine compounds and ions such as hypochlorous acid (HOCl), hypochlorite ion ($OCl^-$) and chlorine gas ($Cl_2$), which are all collectively known as free chlorine. HOCl is the main antimicrobial agent present in EO water. EO water generally has a low pH (2.3 – 2.7) and a high oxidation – reduction potential (ORP) (> 1000 mV).

On the cathode side of the electrolytic cell, a dilute solution of NaOH is generated, and this is sometimes referred to as electrolyzed reducing (ER) water. Unlike EO water, the principal use of ER water is not in the reduction of microbial numbers. Instead, ER water is mainly used as a degreaser and cleanser1). Additionally, work has been done on the health benefits of ER water in terms of cancer prevention, wound care, skin care and also as drinking water for improving digestion and cow milk production2). Chemically, ER water is characterized as having a high pH (10-11.5) and a low ORP (-800 to -900 mV)3).

## Properties of EO water

EO water is primarily a solution consisting of HOCl, $OCl^-$, and dissolved $Cl_2$, that when in contact with organic materials, variable amounts of organic chlorides, such as trihalomethanes (THMs), chloramines and haloacetic acids (HAAs), all often referred to as bound (or combined) chlorine, are formed. The sum total of of HOCl, OCl-, $Cl_2$, and chloramines is known as the total chlorine concentration of an EO water solution. Ultimately, the concentration and ratio of the species of free chlorine, combined chlorine and chloride ion are dependent on method of generation, and pH of EO water.

In its pure form, chlorine ($Cl_2$) is a poisonous yellow-green gas. However, chlorine exhibits a pH-dependent chemistry. In other words, the pH of the solution that chlorine is dissolved in will play a dominant role in what compound chlorine will assume. From pH 3 to 7, HOCl is the dominant chlorine species. HOCl is a strong oxidant (ORP > 900 mV), and is the preferred free chlorine species for disinfection. HOCl is generated by the hydrolysis of dissolved $Cl_2$, as demonstrated in the following equation:

$$Cl_2 + H_2O \rightleftharpoons HOCl + Cl^- + H^+ \qquad (1)$$

Due to the high molar concentration of water in aqueous solutions, this reaction proceeds via 1st order kinetics ($k_1 = 22.3 s^{-1}$) 4). Disregarding the effects of all other factors (i.e. product concentrations, temperature, catalysts), the concentration of $Cl_2$ is the most important factor in determining forward reaction rate in equation 1.

Below pH 3, $Cl_2$ becomes increasingly dominant. One major cause of free chlorine loss from water at low pH (< 4) is due to off-gassing of $Cl_2$. The solubility of chlorine gas at 20ºC is about 7g/l pure water, and it is reasonable to assume that this solubility can be altered by temperature changes and changes in partial pressure over the surface of the liquid (as Henry's Law states). At low pHs, the reverse reaction of equation 1 predominates, and it is a 3rd order reaction ($k_{-1} = 4.3 \times 10^4 M^{-2}s^{-1}$) 4). As evident, this reaction proceeds at a much greater rate than the forward reaction described previously. However, it is important to understand that since this reaction relies on the availability of free protons and hypochlorous acid, a low pH is necessary to shift the equilibrium to the left in equation 1. A low pH is necessary for this because both hypochlorous acid and free protons exist in greater quantities at low pH, thereby increasing the likelihood of the simultaneous collision of the three species required for the reverse reaction in equation 1 to predominate.

Above pH 7.54 (the pKa of HOCl at room temperature), $OCl^-$ is the dominant free chlorine species. $OCl^-$ is not as strong an oxidant as HOCl is (ORP ~ 600-700 mV), but it is more chemically stable. The pKa of HOCl can be determined experimentally using a variety of methods and the Henderson-Hasselbalch equation below:

$$pH = pKa + \log ([OCl^-] / [HOCl]) \qquad (2)$$

At pH 7.54 (at 25ºC), 50% of HOCl is deprotonated to $OCl^-$. Therefore, above pH 7.54, the dominant chlorine species is $OCl^-$.

The relative distribution of the three forms of free chlorine as they relate to pH can be visualized on Figure 3-1$^{5)}$:

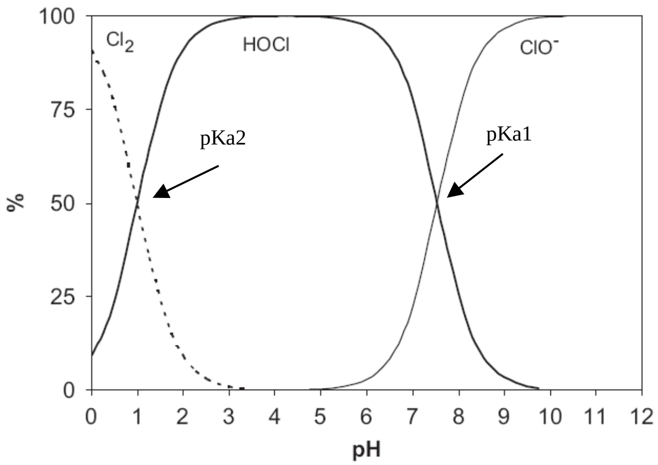

**Figure 3-1: Distribution of chlorine species from pH 0 to 12**

Adapted from Deborde and vonGunten's review (2008).

Figure 3-1 assumes a chloride concentration of 5 mM. The label "pKa1" on the graph denotes the pKa of the $OCl^-$ / HOCl couple. This pKa does not appear to be concentration dependent, but it is pH dependent. The "pKa2" label denotes the apparent pKa of the $Cl_2$ / HOCl couple. Unlike pKa1, pKa2 appears to shift based on chloride concentration. To be more specific, pKa2 will increase with increasing chloride concentration6). According to the graph, the dominant free chlorine species below pH 1 is $Cl_2$. The dominant species between pH 1 and 7.5 is HOCl, and the dominant species above pH 7.5 is $ClO^-$.

Standard reduction potential is a measure of how easily a chemical species acquires electrons, becoming reduced in the process and oxidizing the species the electrons were acquired from. The larger and more positive the number is, the greater ability as an oxidant the species has. However, $OCl^-$ has a standard reduction potential of 0.9 V, and HOCl has a standard reduction potential of 1.49 V, making HOCl a stronger oxidant than $OCl^-$ is7).

Electrochemical reactions within an EO water generator cause the formation of the chlorine species observed in EO water. The reactions in an EO water generator can be divided into 2 different categories: electrode localized reaction and reactions in solution.

## Principle of EO water generation

### *Electrode localized reactions*

Electrode localized reactions are simply reactions that occur in the double layer region surrounding electrodes. The double layer region consists of specifically-adsorbed ions (the inner Helmholtz plane) followed by a layer of solvated ions (the outer Helmholtz plane). In terms of EO water generators, the reactions can be divided into two different types of reactions: an oxidation of chloride ion at the anode and a reduction of water at the cathode.

The anode side of an electrolytic cell, from which EO water is obtained, carries out the following reaction:

$$2Cl^- \rightleftharpoons Cl_2 + 2e^- \qquad (3)$$

Electrons are abstracted from chloride ions ($Cl^-$) in water by the anode to form chlorine gas.

As mentioned previously, a dilute solution of NaOH is generated on the cathode side of an electrolytic cell used in EO water production. The following reactions occur in the cathode side:

$$2H_2O + 2e^- \rightleftharpoons 2OH^- + H_2 \qquad (4)$$

Water is reduced at the cathode to form hydrogen gas and hydroxide ions in (4). In contrast to EO water, ER water is characterized by a high pH (10-11.5) and a low ORP (-800 to -900 mV)3).

*Reactions in solution*

Besides the solvation of chloride compounds in the feed solution, there are other chemical reactions occurring in solution which play an important role in the function of an EO water generator. The solution reactions in EO water are dependent on the electrode-localized reactions.

$$Cl_2 + H_2O \rightleftharpoons HOCl + Cl^- + H^+ \quad (5)$$

$$HOCl \rightleftharpoons OCl^- + H^+ \quad (6)$$

Dissolved chlorine gas either leaves solution or undergoes a rapid reaction with water to form hypochlorous acid (HOCl), protons and more chloride ions, as shown in (5). In (6), HOCl can lose a proton to form its conjugate base hypochlorite ($OCl^-$) in a pH – dependent equilibrium.

Other reactions also occur in solution, and these reactions may contribute to the unique properties of EO water, despite being low in concentration. Like the primary reactions, these reactions are pH-dependent as well. Many of these reactions are involved in the slow decomposition of HOCl. For example, in the pH range of 5-8, the mechanism proposed by Adam *et al.*8) involves the synthesis of chlorate ($ClO_3^-$) from HOCl. These findings support much earlier work done by Lister 9). The decomposition of HOCl to $ClO_3^-$ is third order, and the values of ΔH* and ΔS* are 64.0 ± 0.6 kJ / mol and -67.0 ± 2 J / mol K respectively. Another paper by Adam and Gordon10) claims that $Cl^-$ catalyzes the decomposition of $OCl^-$ in the pH 9-10 range. Decomposition of HOCl at lower pH values is difficult to measure because any decomposition is overshadowed by the off-gassing of $Cl_2$ at these pHs.

Also of importance in EO water generation is the concept of conversion efficiency. Conversion efficiency, or the percentage of salt that can be converted into HOCl, is an important concern in EO water generators. Conversion efficiency is defined as the following:

$$\% \text{ conversion efficiency} = [\text{free chlorine}] / [\text{initial salt}] \times 100 \qquad (7)$$

Conversion efficiency is important for a number of reasons. Obviously, greater conversion efficiency leads to a higher concentration of free chlorine in EO water while using a lower concentration of electrolyte in the feed solution. Another important concern is corrosion. $Cl^-$ is a significant catalyst of corrosion[11)]. Therefore, lowering the concentration of $Cl^-$ in EO water through use of generation technologies with greater conversion efficiency is one possible strategy to limit corrosion of EO water.

## EO water generators

In theory, an EO water generator is a type of electrochemical cell that is used for the separation and oxidation state change of $Cl^-$ and its counter ion. A generalized sketch of an electrolytic cell is shown in Figure 3-2 below:

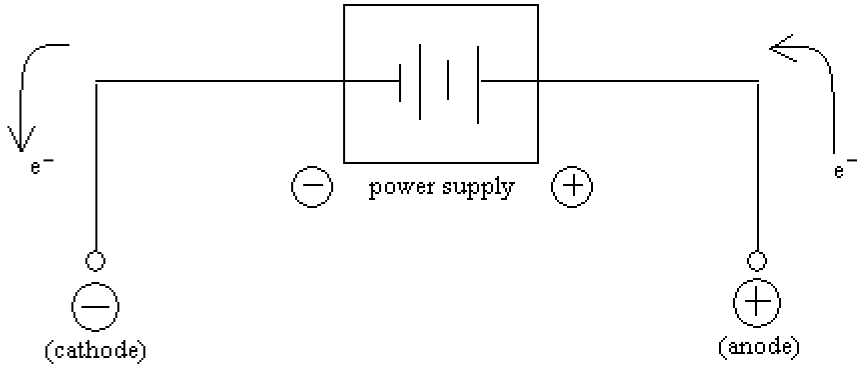

**Figure 3-2: Typical electrolytic cell**

adapted from Bard and Faulkner[12)]

An electrolytic cell requires two electrodes and a power supply. The polarity of electron flow from the power source in Figure 3-3 indicates that the electrode on the left is a cathode, and the electrode on the right is an anode. The cathode and anode (which are negatively and positively charged in the figure, respectively) are best described by the types of redox reactions that happen around them. Reduction reactions happen at the cathode, and oxidation reactions happen at the anode. Both the power supply and electrodes will be covered in greater detail later in this review.

As mentioned previously, an EO water generator functions as an electrolytic cell. Due to the variation in EO water generator design, there is a great deal of difference in the operating parameters normally used by a generator. As such, the most essential parameters are the chemical requirements need for the desired reactions to occur. The functioning of the cell will depend largely on conditions such as amount and direction of the electrical current and electrolyte concentration[13)].

In the operation of an EO water generator, a power source provides the electron flow that allows an electrical potential to develop in the cell. The size and direction of the potential will determine the nature of the reactions that happen at the electrodes. A relationship between potential and current is usually best described by a current-potential curve. A current-potential curve shows the potential needed to drive a reaction at a certain rate, which is known as the overpotential. In EO water generators, a potential of 2 V or greater is sufficient to cause the oxidation of chloride at the anode because the potential (vs. standard hydrogen electrode) of equation (3) above is 1.36[12)]. Cell potentials in an EO water generator are set dependent on the electrolyte concentration, for the purpose of avoiding arcing[13)].

Several studies have confirmed that the amount of chloride present in the feed solution of an EO water generator will relate to the amount of free chlorine produced by the generator [1,14)]. The consensus is that an increase in chloride concentration in solution will lead to an increase in free chlorine concentration. However, there are other factors, such as hydraulic regime and solution residence time, that could have an impact on the value of $Cl^-$ concentration as a variable for free chlorine formation.

### *Electrodes in EO water generation*

Since electrodes are the sites where significant reduction of $Cl^-$ to $Cl_2$ takes place, the electrodes are arguably the most important factors in EO water generators, as well as electolytic cells in general. Electrolytic cells in general have 2 electrodes made from metal, typically titanium and stainless steel for the anode and cathode, respectively [15,16)] or semiconductors. In addition to a metal core construction, electrodes are often coated with oxides[16)]. Oxides made from iridium and ruthenium are the most commonly used, although studies have been undertaken with mixed oxide coatings, such as tin and iridium oxide [17)] as well as nickel and cobalt oxides [18)]. Also, precious metals such as platinum are sometimes added as part of the surface coating as well [19)]. There are several reasons for coating electrodes. Coating an electrode helps to protect it from corrosion as well as increases the activity of the electrode.

Pure metal electrodes are not often used in applications involving chlorine solutions due to the risk of decreased output over time due to electrode surface corrosion. Oxide coatings reduce the oxidation of the metal electrode core by forming a passive barrier. The coatings also increase the interfacial area of the electrode, allowing for greater reaction rates.

### *Separatory membranes in EO water generation*

Membranes are other factors which can affect the function of an EO water generator. The purpose of membranes in EO water generators is to divide the water to anode and cathode chambers and/or allow the selective movement of ions into the anode and cathode compartments of the electrolytic cell.

There are several different membrane types to consider: diaphragms, monopolar membranes and bipolar membranes. A typical diaphragm cell is pictured in Figure 3-3:

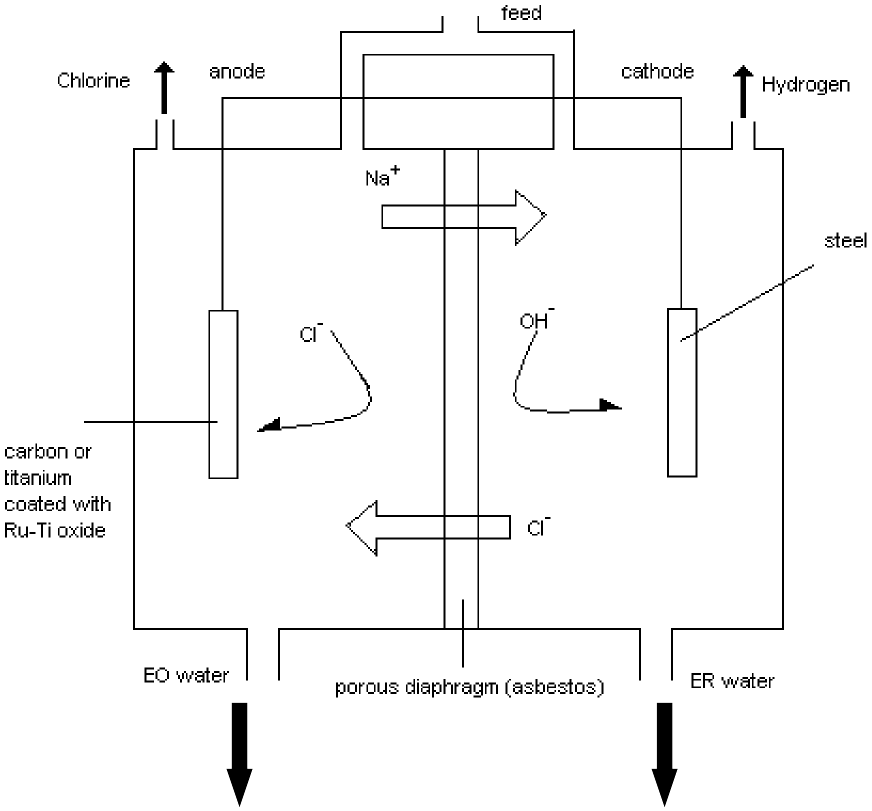

**Figure 3-3: Diaphragm Cell Model**

Diaphragm cells make use of an asbestos “diaphragm” that resists the flow of hydroxide ions to the anode. Sodium ions are able to freely pass through the diaphragm. Additionally, some 7 chloride ions are able to pass through the diaphragm as well. In a sense, the diaphragm functions not as an impermeable wall (as the name diaphragm implies), but as a selectively-permeable membrane. Solvent is able to freely pass through the diaphragm but certain charged species are not. This method of separation of the cathode and anode compartments of the electrolytic cell is being replaced by monopolar and bipolar membranes, mainly due to environmental concerns associated with the asbestos diaphragm found in diaphragm cells.

Monopolar and bipolar membranes both have a standard design. The difference between a diaphragm cell and a membrane cell lies in the behavior of the partition (membrane) separating the anode and cathode chambers. Another difference lies in the composition of the cathode, which is usually nickel in membrane cells. Ion exchange membranes allow the flow of ions in a specific direction, dependent on the nature of the membrane, but not vice-versa. Generally, there are cation exchange membranes, which permit the flow of positive ions, and anion exchange membranes, which permit the flow of negative ions. Figure 3-4 is a model of an anion exchange membrane.

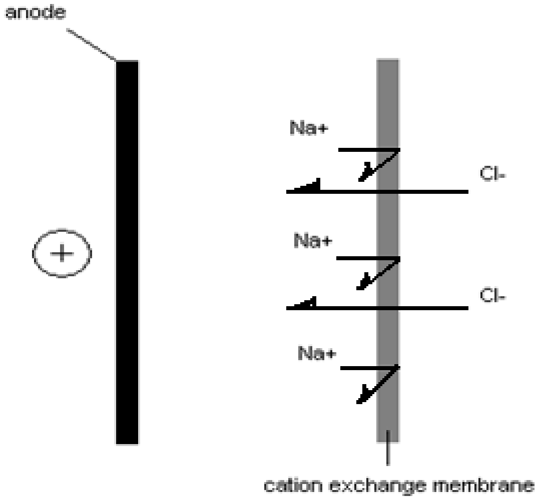

**Figure 3-4: Anion Exchange Membrane**

adapted from Pabby *et al.* 20)

Monopolar membranes are single-layered membranes that allow the selective transport of ions in the direction of charge. Specifically, anion exchange membranes only allow the transport of negatively-charged cations while prohibiting the transport of positively-charged anions. In general, exchange membranes must have the following properties in order to be effective in EO water generation 21):

1) A membrane must have the physical and chemical ability to withstand NaOH and $Cl_2$.
2) A membrane must have low electrical resistance.

3) A membrane must allow transport only $Cl^-$ from the catholyte to the anolyte.
4) A membrane must operate at high current density.
5) A membrane must be immune to impurities (i.e. heavy metals) in the solution.

In contrast to the single layer design of monopolar membranes, dipolar membranes employ two different membranes with a hydrophilic layer sandwiched between them. An example of a dipolar membrane is shown in Figure 3-5:

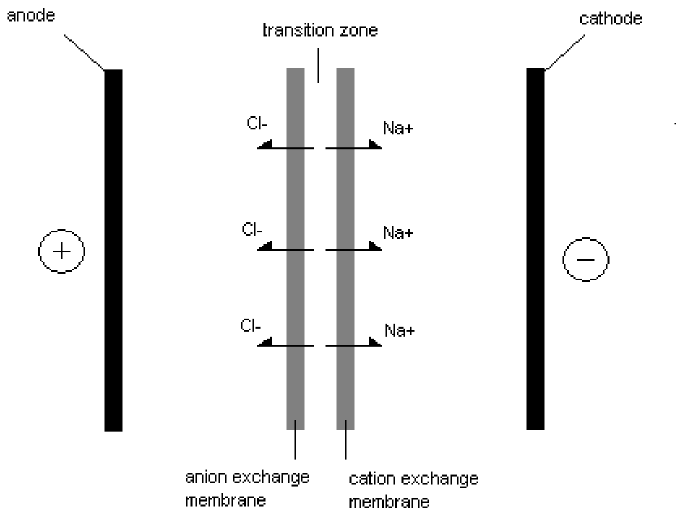

**Figure 3-5: Dipolar membrane**

adapted from Pabby *et al.* 20)

Figure 3-5 depicts a dipolar membrane that consists of a cation and anion selective membrane. Generally, ions in the transition region between the two membranes travel through the membranes in the direction of their charges 22). A special type of EO water generator with three chamber cells has electrolyte flowing through the middle chamber between the cation and anion membranes. Pure EO water can be produced at the anode chamber. The membranes separate

$Na^+$ and $Cl^-$ in the direction of current flow towards the cathode and anode, respectively. This design is considered economical because of low power consumption, the ability to operate at high current densities; high acid and base concentrations can be produced, a high current efficiency and low maintenance costs[54)].

### *Generator parts in EO water generation*

The power source of an EO water generator is one of the most basic parts of the generator that is not subject to modification. The power source provides an external voltage that is greater than the open circuit potential of the cell. In practice, a 120V power supply is commonly used. Some models of generators with adjustable voltage can range from 0-18V[23)].

The body of an EO water cell must be constructed with several considerations. First, the body must be water-tight to prevent the leakage of electrolyte solution onto open circuitry. Because of the corrosive nature of the solutions being used and produced, EO water generator parts are often made of plastics such as PVC.

In addition to concerns about the solutions being handled by the EO water generator, movement of different fluids through the generator is also important. The body must be constructed to allow the efficient import of electrolyte solution and the efficient export of EO water, ER water (if there is a separation between the anode and cathode), and gases such as oxygen and hydrogen. The accumulation of these gases in the generator could lead to an explosion hazard.

## Factors affecting EO water generation

There are many factors both internal and external to an EO water generator that affect performance of the cell. One of the most important sets of factors are those relating to the electrodes. Electrode material, surface area, electrode geometry and surface condition must all be considered when addressing the contribution of the electrodes to an EO water generator's performance.

Electrode material has been the focus of several studies in EO water generation, such as the development of metal oxide coated metal 16,24), mixed metal oxides coating metal18,25) and doped carbon-based electrodes26,27). Electrode material was found to influence material yield (referred to as conversion efficiency), which is the fraction of the starting material converted into the desired product15). As mentioned previously, electrodes involved in chlorine production by salt electrolysis are mostly titanium coated with oxides from the platinum group. Ruthenium oxide, the most common coating, is often used with oxides of titanium, tin and iridium 28-30). For chlorine production involving hydrolysis of hydrochloric acid (HCl), carbon anodes are preferred. Electrode material will also influence other aspects of the process, such as overpotential, selectivity, lifetime as well as behavior under nonoptimal conditions15).

The surface area and geometry of an electrode directly influence the function of EO water generators in several ways. First, the geometry of an electrode can influence energy consumption in the electrolytic cell. Both electrolysis and the movement of solution through the cell represent most of the energy consumption in an electrolytic cell15). The energy cost of pumping solution can be minimized by the electrode having an open, porous construction. This type of electrode construction imparts less turbulence to solution flow. In addition to energy consumption, electrode geometry can influence reaction rate. A porous electrode has a higher mass transfer coefficient, allowing for a greater rate of reaction[15)]. Of particular note is that both desirable and undesirable reactions can have their rates influenced by electrode geometry. For example, cylindrical and coaxial configuration of electrodes are claimed to have a higher salt conversion rate than parallel plates. Also, coaxial cells use a ceramic membrane, which has the disadvantage of being fragile during transportation, but with a longer life span in use, compared with "plastic" membranes or diaphragms.

Surface condition, in this case, refers to corrosion, scaling or some other type of fouling of the electrode surface. These surface conditions of an electrode can be negatively affected by the presence of impurities in solution.

### *Internal factors affecting EO water generation*

Mass transfer factors refer to the movement of molecules in bulk solution to the electrode surface region. Figure 3-6 below illustrates the concepts of the electrode surface region and the bulk solution:

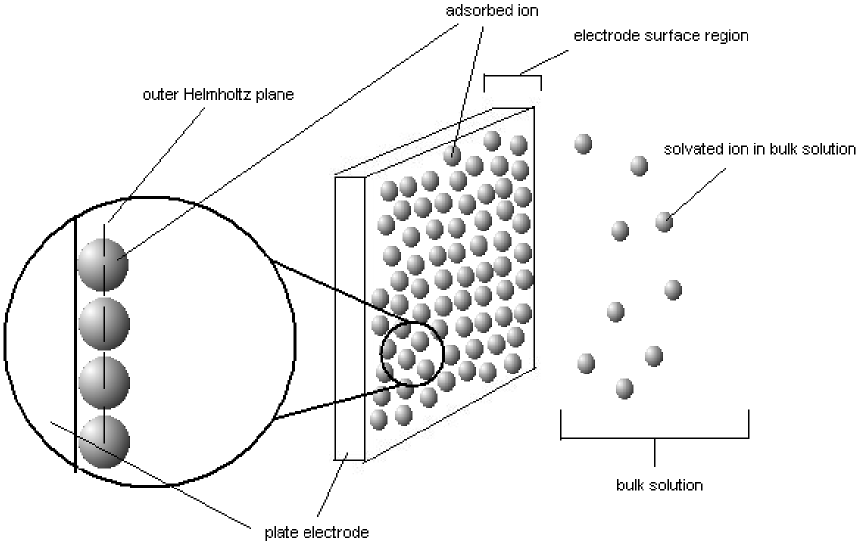

**Figure 3-6: Electrode surface region and bulk solution**

adapted from Bard and Faulkner [12)]

The greater the rate of mass transfer, the more efficient the electrolytic process is. There are several factors of mass transfer to be considered: mode of transfer, surface concentrations and adsorption.

Mode of transfer refers to the method by which solvated ions move from the bulk solution phase to the electrode surface region. Diffusion and convection are two examples of how transfer of solvated ions could occur. The mode of transfer applicable to particular electrolytic generator is usually dependent on factors relating to construction of the generator. Surface concentration refers to the density of solvated ions in the electrode surface region, and adsorption is defined as the method and rate which solvated ions adhere to the electrode surface. Both surface concentration and adsorption are influenced by electrode-specific factors (as discussed previously in this review) as well as factors relating to the solution itself.

Solution variables, such as bulk concentration of electroactive species, concentrations of other species as well as the nature of the solvent, all influence an EO water generator's function[12)]. The concentration of electroactive species (in this case, the concentrations of chloride ion and its oxidized electrolysis product, $Cl_2$) will influence the rate of electrolysis based on simple equilibrium mechanics. Low concentrations of chloride ion will cause a decreased production of $Cl_2$ (which is subsequently converted to HOCl). Likewise, increasing the concentration of chloride ion will increase production of HOCl under many circumstances[13,14)]. Other factors worthy of consideration are so-called “loss” reactions in electrolytic generators. Depending on the generator design, the following reactions could occur to a greater or lesser degree [31)]:

$$6ClO^- + 3H_2O \rightleftharpoons 2ClO_3^- + 4Cl^- + 6H^+ + 3/2\ O_2 + 6e^- \qquad (8)$$

$$ClO^- + H_2O + 2e^- \rightleftharpoons Cl^- + 2OH^- \qquad (9)$$

$$2HOCl + ClO^- \rightleftharpoons ClO_3^- + 2Cl^- + H^+ \qquad (10)$$

$$2ClO^- \rightleftharpoons O_2 + 2Cl^- \qquad (11)$$

Reaction (8) takes place at the anode. $OCl^-$ is oxidized along with water to produce chlorate ($ClO_3^-$) ions. At the cathode, $ClO^-$ is reduced to $Cl^-$, as shown in (9). Reactions (10) and (11) both take place in solution. Overall, these reactions are said to be more prevalent when solutions from the cathode and anode are allowed to mix, as is the case for some EO water generation technologies.

Electrical factors, such as potential, current and quantity of electricity, also affect the rate of electrolysis[12)]. As mentioned previously, electrical potential is supplied by a 120V power supply in many cases. Work by Ezeike and Hung[13)] concluded that voltage was an important contributor in the production of free chlorine. Generally, increases in voltage led to corresponding increases in free chlorine.

### *External factors affecting EO water generation*

In addition to factors within the generator itself, variables external to the generator, such as temperature, pressure and time, can also influence the efficiency of the electrolysis process. The role of temperature in EO water generators has been explored previously [1,14)]. It was determined that temperature was significant in the determination of the magnitude of DC current and reaction rates. However, when compared with other variables such as salt concentration, the role of temperature was determined as not significant. For this reason, temperature is not regarded as an important factor in generator function[1)].

In addition to temperature, pressure has been determined to play a role in function of a EO water generator[32,33)]. The role of fluid pressure in EO water generators is on gas solubility and concentration of $Cl_2$ in solution. In particular, increases in pressure lead to greater solubility of $Cl_2$ in solution. Decreases in the solubility of chlorine gas from a sample of EO water lead to decreases the level of free chlorine in the solution[34)]. The effect of pressure on $Cl_2$ concentration of EO water is only positive to a point, however. It is expected that eventually the $Cl_2$ concentration will provide a concentration barrier to the production of more $Cl_{2.}$

The discussion of time as a variable is limited to residence time of feed solution. It is expected that residence time of feed solution will be directly proportional to the concentration of free chlorine produced through electrolysis.

### Common types of EO water generators

There are a wide variety of EO water generators available today. In Table 3-1 below, several EO water generation technologies are compared by type of feed solution used, membrane type, the ability for a user to control pH and/or current, and the need for dilution before use.

**Table 3-1: Comparison of Common EO water generation technologies**

| Generator | System 1 | System 2 | System 3 | System 4 | System 5 |
|---|---|---|---|---|---|
| **Feed solution** | NaCl | NaCl | HCl | NaCl | NaCl |
| **Membrane arrangement** | single non-selective | single non-selective | none | single ion selective | none |
| **pH control** | yes | no | no | yes | no |
| **Current control** | yes | yes | no | no | no |
| **Need for dilution** | no | no | no | yes | no |

*All systems are available in Dr. Hung's lab at the University of Georgia

#### *Differences in feed solution among common generator types*

Brine solutions are the most common types of feed solutions used in EO water generation. The chlor-alkali process in industry utilizes an estimated 90 million tons of salt per year$^{35)}$, and the manufacture of chlorine is the largest single use of salt. Of the salts that can be used in EO water production, NaCl is the most commonly-used salt$^{36-38)}$. Other salts, such as KCl can also be used, but NaCl is more readily available and more economical. Since both the salts are readily soluble in water (both over 30g/100g of water at room temperature) and the potentials required for $Cl^-$ oxidation at the anode are the same for both salts (-1.36 V), there is essentially no electrochemical difference between the two. EO water generators that use brine feed solutions are therefore the most common types of generators available, and the function of these types of generators is the most studied.

In contrast to salt, some EO water generators use HCl as a source of $Cl^-$ ions. A few EO water generators are commercially available, especially for producing near neutral pH EO water. Outside of EO water generation, studies have been conducted to convert gaseous HCl waste into chlorine in order to address environmental concerns$^{39)}$. One large difference that exists between NaCl and HCl is solubility. At room temperature, the solubility of NaCl is about 38 g / 100 g of water. In contrast, the solubility of HCl is approximately 70 g /100 g of water at room temperature. Theoretically, the higher solubility of HCl can lead to more $Cl^-$ ions in solution for electrolysis. Also, EO and ER water generated using HCl lacks $Na^+$ ions. This is beneficial for operation that caustic soda (NaOH) is not used. NaOH may also contribute to the production of oxygeb radicals, like $OH^\cdot$ and $O^\cdot$, which represent a more rapid path to corrosion than $Cl^-$.

### *Differences in pH control among common generator types*

In cases where pH control is needed, one method of pH control involves the restriction of ER water exit from the generator. Some generators are constructed in a way such that the back pressure created from doing this allows ER water to mix with EO water inside the cell. This is outlined in Figure 3-7 below:

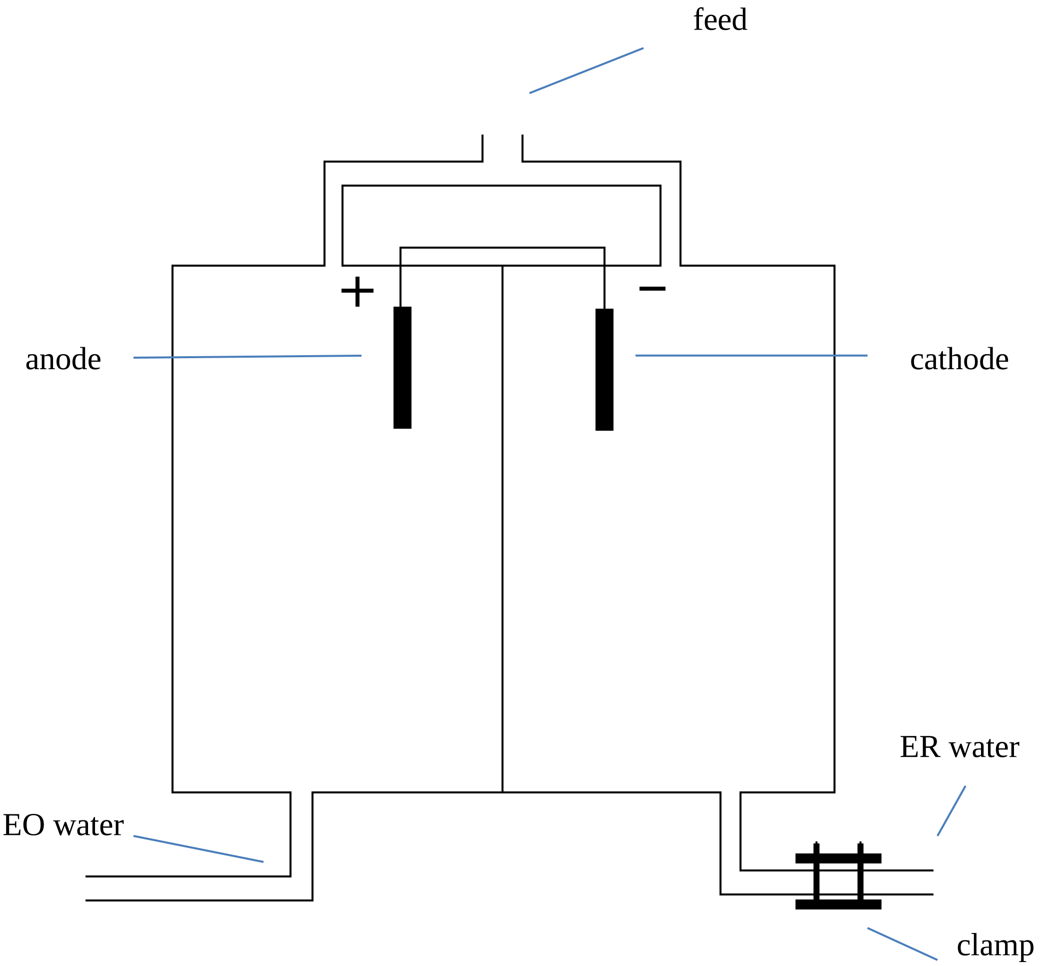

**Figure 3-7: pH control by back pressure**

In Figure 3-7, feed solution enters the generator, and the electrochemical reactions occur in the same way as other EO water generators. The difference is that ER water flow is restricted as it exits the machine (in the case of Figure 3-7, by a clamp). This causes a buildup of pressure which forces some ER water into the anode chamber to mix with EO water. The near neutral pH EO water exits the machine from the EO water outlet. Essentially, the pH of the EO water can be adjusted by controlling the back pressure at the ER side.

Another method of pH control involves a recycling mechanism that allows alkaline water to be mixed with EO water, as illustrated by Figure 3-8:

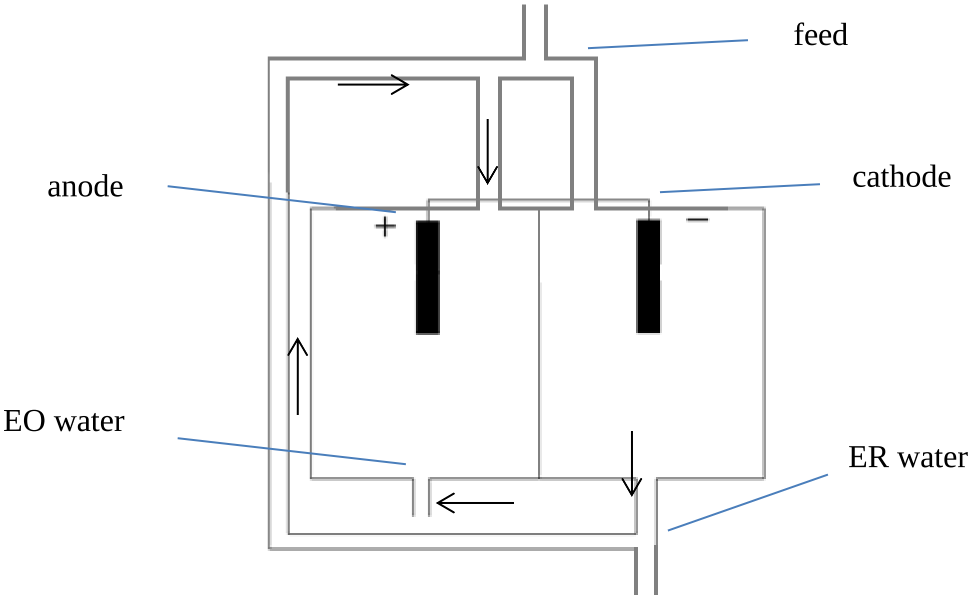

Figure 3-8: pH control by alkaline water recycling

The arrows in Figure 3-8 represent the flow of alkaline water from the cathode to the anode, which results in an increase in sodium and hydroxide ions to produce an EO water solution of a higher pH.

Generators that produce acidic EO water have a division between the anode and cathode regions of the cell. This is done in order to prevent the anolyte from mixing with the catholyte. Another way to generate near neutral EO water is to not have a division between the anode and cathode [42)]. This type of EO water has a pH typically between 5 and 7 and a lower ORP (~850 mV at pH 5) [40,41)]. Table 3-2 summarizes the properties of acidic EO water and near neutral EO water.

**Table 3-2: Comparison of acidic EO water and near neutral EO water properties**

| | **Acidic EO** | **Near Neutral EO** |
|---|---|---|
| ***General chemical properties*** | | |
| **pH** | **2.3-2.7** [3,59)] | **5.0-6.5** [42,43)] |
| **ORP** | **> 1000 mV** [3)] | **800-200 mV** [40,41,43)] |
| **Primary chlorine species** | **HOCl, $Cl_2$** [10)] | **HOCl, $OCl^-$** [8,58)] |

The properties of acidic and near neutral EO water contribute to their stability and antimicrobial activity. In terms of stability, near neutral EO water is much more stable due to no loss of $Cl_2$ through evaporation and the relatively slow rate of $ClO^-$ decomposition than acidic EO water [43,44,45)], which is more prevalent at lower pH values [44,45)]. On the antimicrobial property, near neutral EO water has a high percentage of chlorine in the form of hypochlorous acid and can compensate for its low ORP values.

### *Differences in current control and membrane technology among common generator types*

Some EO water generation technologies allow the user to control the magnitude of the current through the electrolytic cell. As explained previously, increases in electrical current in an EO water generator result in increased concentrations of free chlorine. Besides this, current control can also allow control the rate of undesired reactions [46)]. Current control allows for the prediction of energy consumption in a specific process[47)] as well as the efficiency of the process[48)]. There are a few options available for current control. One such option is the use of amplifier instruments to regulate changes in potential or current[49)].

Membrane technology is not just limited to the specificity of the membrane. Often, the number of membranes and their arrangement can influence the efficiency of an EO water generator. Semipermeable, or selectively permeable, ion exchange membranes have already been introduced in this review. Cationic membranes, in particular, have been studied in some detail either alone50,51) or in comparison to other types of membranes, such as diaphragms52). In the chlor-alkali industry, diaphragm cells are being phased out in favor of ion exchange membranes. Ion exchange membranes carry an advantage over diaphragms because the membranes have less of an environmental impact in terms of construction materials used in membrane production as well as power consumption. There are two types of ion exchange membrane configurations to consider: single and bipolar.

Single ion exchange membranes, as discussed earlier, allow a specific charge of ion to pass through while excluding counter charged ions. The membrane is used to physically separate the cathode and anode in an electrolytic cell. In the chlor-alkali industry, cation exchange membranes are most often used53). Cationic membranes are constructed of perfluorinated polymers (Nafion® polymers). The original Nafion® polymers utilized sulfonic groups, however problems at high NaOH concentrations led to modifications53). Eventually, the single cation exchange membranes evolved into composite membranes. Composite membranes have a thin layer of carboxylate polymer on the cathode side of the membrane. The layer prevented the back migration of hydroxide ($OH^-$) ion, and it was still sufficiently thin to keep membrane resistance low53).

Bipolar membranes (Figure 3-10) are cation and anion exchange membranes joined closely together. In the chlor-alkali industry, bipolar membranes are manufactured to be immersed in an electrolyte flow that runs perpendicular to the membranes below

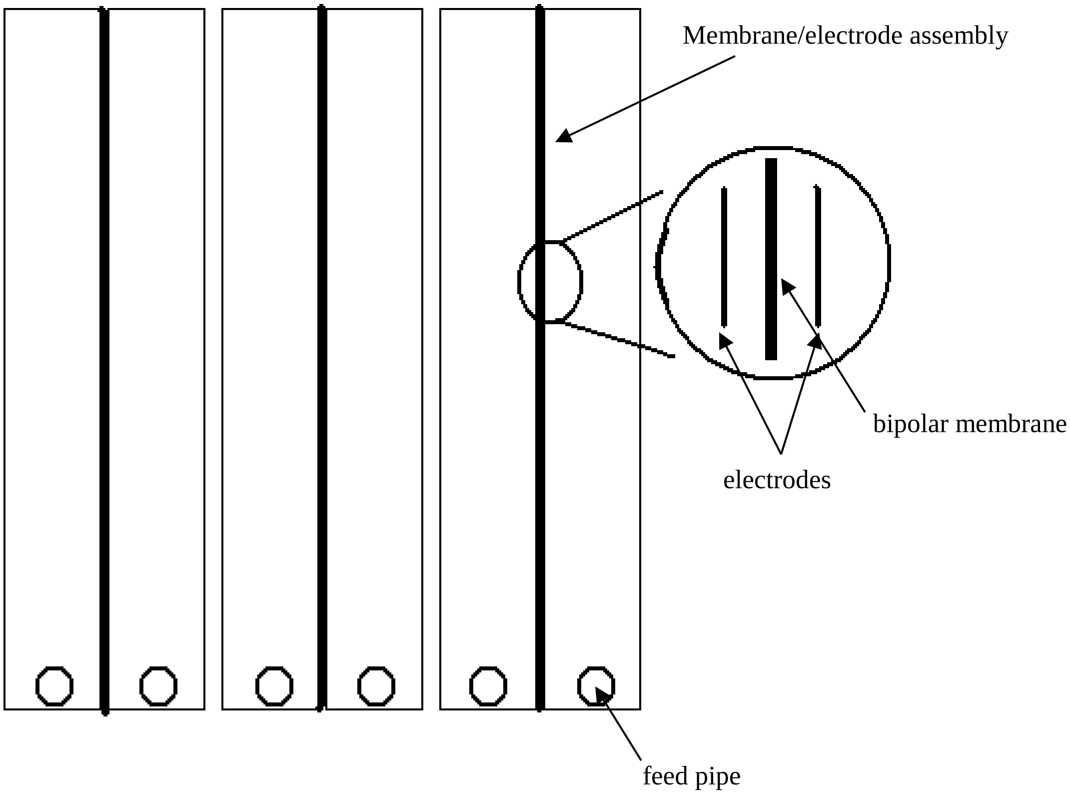

**Figure 3-9: Bipolar membrane unit configuration**

adapted from Schniders 57)

The design in Figure 3-9 enables single units of bipolar membranes to be used modularly as components in a rack. This design saves space and allows the individual units to be connected electrically in a series. Feed pipes carry brine to a distributor (not pictured) in a direction perpendicular to plane of view.

*Differences in solution flow among common generator types*

The flow of feed solution and product solution in an EO water generator is another factor worthy of consideration. For the chlorine manufacture industry, there is a compelling need to increase variables, such as mass transfer, that lead to higher product yields55). Previous work by Hsu 1) indicated that increases in water flow rate increased electrolysis current. Other work by Hsu14) as well as Ezeike and Hung13) indicated that increases in water flow rate decreased the level of free chlorine in EO water. Both groups concluded that lower residence time of electrolyte within the cell led to a decreased level of free chlorine. However, generator design changes to increase residence time must not reduce the production rate/capacity. Increased residence time can be achieved through modification of speed that electrolyte passes through the cell or configuring the flow pattern in the cell to allow for multiple passes. Another way to modify residence time is to induce turbulence. Subbaiah and coworkers56) reported that increasing turbulence enhances the mass transfer coefficient at the membrane surface, leading to higher yields in product.

## Conclusions

There is a variety of EO water generation technologies commercially available. Due to differences in construction and operation, the exact definition and quality of EO water can be significantly different. Understanding EO water generators, in particular their design, components and factors effecting EO water generator performance can lead to the research and manufacture of safer and more effective EO water generators.

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# CHAPTER 4

# ELECTROLYZED WATER: FOOD SAFETY APPLICATIONS

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Waters, B. W. and Y.-C. Hung. 2012. Electrolyzed water in food safety. from *Encyclopedia of Agricultural, Food, and Biological Engineering* (http://www.tandfonline.com/doi/abs/10.1081/E-EAFE2-120048421). Reprinted here with permission of the publisher

## Introduction

Electrolyzed oxidizing (EO) water is a solution generated from passing a dilute salt solution (NaCl and KCl are commonly used) through an electrolytic cell separated by a membrane. The anode side produces hypochlorous acid (HOCl) as the antimicrobial. HOCl can lose a proton to form its conjugate base hypochlorite ($OCl^-$) in a pH – dependent equilibrium. HOCl is the main antimicrobial factor present in EO water. EO water generally has a low pH (2.3 – 2.7) and a high oxidation – reduction potential (ORP) (> 1000 mV). However, slightly acidic or near neutral EO water has also become popular.

EO water has been studied as a possible intervention in the spread of foodborne illness. The mode of action, applications and challenges to implementation of EO water in food safety have all been explored.

## Mode of action of EO water

HOCl is a strong oxidant, and it freely oxidizes many organic compounds. Also, as a weak acid, HOCl is able to diffuse through cell membranes and acidify the interior of cells. This reaction increases significantly when the pH is lowered to the point that HOCl predominates because HOCl has greater penetration power due to its uncharged nature (1). Cell membranes are mostly impermeable to charged substances. The reason why is because ions have a high affinity to water molecules, which develop dipoles by nature. The hydrophobic portion of the cell membrane contains no water, so charged particles cannot pass through it. HOCl is a weak acid, so it does not dissociate to any great extent. Being both uncharged and small, HOCl is free to diffuse across the cell membrane. These properties help to make HOCl an effective antimicrobial agent.

Standard reduction potential is a measure of how easily a chemical species acquires electrons, becoming reduced in the process and oxidizing the species the electrons were acquired from. The larger and more positive the number is, the greater ability as an oxidant the species has. However, $OCl^-$ has a standard reduction potential of 0.9 V, and HOCl has a standard reduction potential of 1.49 V, making HOCl a stronger oxidant than $OCl^-$ is (2).

## Applications of EO water in the food industry

### *Produce*

Studies focused on the inactivation of microbes such as *E. coli* O157:H7, *Listeria moncytogenes*, *Salmonella typhimurium* and *Staphylococcus aureus* on leafy greens like lettuce and spinach (3-5), *E. coli* O157:H7, *L. moncytogenes* and *Salmonella enteritidis* on tomatoes (6-7), *E. coli* O157:H7 and *L. moncytogenes* on strawberries (8-9) , *E. coli* O157:H7 on broccoli (9), norovirus on raspberries (10), and *Salmonella* spp. on sprouts (11) have been reported.

In most studies, acidic EO water (pH 2.3 – 2.7) was used as the antimicrobial agent. In a few cases, EO water with a near neutral pH (6.3-6.5) was used as the antimicrobial agent (12). In comparison to acidic EO water, near neutral EO water is not as an effective antimicrobial treatment. For example, Guentzel *et al.* (12) found that near neutral pH (6.3-6.5) EO water solutions that had a free chlorine concentration of 120 mg/L were only able to reduce *E. coli* O157:H7 numbers on lettuce by 0.25 log, following a 10 minute treatment.

In addition to its efficacy as an antimicrobial, the effect of EO water on the quality of produce has been explored. Hung *et al.* (13) found that EO water did not affect and (in the case of strawberries) sometimes improved the quality of strawberries and broccoli. Li *et al.* (14), Park *et al.* (5) and Lee *et al.* (15) found similar positive results with fresh cut potatoes, lettuce, and yams, respectively

### *Grain*

Despite the lower water activity, the grain and milling industries are concerned with bacteria such as *Bacillus cereus, S. aureus* and *Salmonella* spp. (16). EO water has been explored as an intervention, along with citric acid, in various rice cereal grains against *B. cereus* spores (17) and achieved a 1 log reduction in *B. cereus* spores and a 2 log reduction in vegetative cells. A different study using germinated brown rice showed aerobic plate count reductions of almost 5 log after a 12 hour rinsing treatment with EO water (18).

### *Beef*

In beef processing, the organisms of greatest concern are various Shiga toxin-producing *E. coli* (STEC). Numerous STEC outbreaks over the past 30 years have highlighted the need for a solution designed to prevent fecal contamination of beef (particularly comminuted beef products). Presently, EO water is being investigated as a means to wash animal hides before slaughter in an attempt to reduce microbial load. One such study (19) reported a 65% reduction in *E. coli* O157:H7 numbers on hide treated with EO water. Similarly, EO water reduced *E. coli* O157:H7 numbers by 0.76 log CFU/ml in 26 seconds exposure time on bovine cheek meat (20).

In addition to its use as a hide sanitizer, EO water has been explored as a general sanitizer in abattoirs (21). The results of this study indicated that EO water use resulted in roughly 1 log lower total aerobic counts in abattoirs than iodophor treatment, which is the currently approved treatment by Canadian regulatory authorities (21). Similarly, work by Cutter *et al*. (22) found a roughly 1 log reduction of *E. coli* O157:H7 on beef carcass tissue treated with hypochlorite solutions.

### ***Poultry***

In poultry processing, the organisms of concern are *Salmonella* spp. and *Campylobacter jejuni*. Several studies (23-27) have shown that EO water is effective at killing *Salmonella* spp. and *C. jejuni* on both broiler carcasses and shell eggs. Park *et al.* (26) reported a roughly 2 log reduction in *C. jejuni* cell numbers on chicken using EO water versus deionized water. Also, no *C. jejuni* cells were detected in the wash water. Because of this, EO water can be used in poultry chiller tanks to prevent cross contamination (24). Other studies on poultry meat have also reported a similar 2 log reduction in *C. jejuni* and *Salmonella* spp. numbers (28).

EO water is also used as a wash for shell eggs. Washing shell eggs is important because microorganisms can pass through micropores in the shell surface, reducing hatch rates and contaminating the egg (29). One study by Bialka *et al*. (23) found a 3 log reduction in *E. coli* K12 cell numbers when EO water was used as an intervention on the pilot scale. Park *et al.* (25) reported a 4 log reduction in Salmonella and *L.* monocytogenes on shell eggs treated with acidic and alkaline electrolyzed water. A study by Fasenko *et al.* (30) found that EO water sprayed on hatchling eggs does not affect chick health.

### ***Seafood***

In seafood, the pathogens of greatest concern are *Vibrio vulnificans* and *Vibrio parahaemolyticus*. Near neutral pH EO water has been shown to be an effective antimicrobial agent against pure cultures of both organisms, resulting in no detectable growth after 30 seconds exposure. EO water washes have been proven to be effective at reducing *V. parahaemolyticus* numbers on tilapia (31). Another study by Huang (32) reported that aerobic plate counts and volatile basic nitrogen (an indicator of seafood spoilage) increased the least in yellow fin tuna samples treated with EO water.

In addition to reducing pathogen cell numbers on seafood, EO water has been used to reduce the numbers of histamine-producing bacteria on fish (33). Work has also been done on preserving freshness of seafood and preventing cross contamination by incorporating near neutral pH EO water into ice. Results indicated that EO water used in such a way would be an effective way of controlling the spread of pathogenic bacteria in Pacific saury (34).

### ***Food processing and service***

Another area of great importance in the food industry is surface sanitation. Inadequate surface sanitation is routinely cited as a contributing factor to the spread of foodborne illness. EO water has been explored as a food contact surface sanitizer in a variety of situations, and it has measured up favorably against other, more established, methods of tableware and utensil sanitation (35). Additional work by Park *et* al. (36) indicated that EO water could be an effective antimicrobial agent when used on glass, stainless steel, china and ceramic surfaces while work by Venkitanarayanan *et al.* (37) showed a 5 log reduction of *E. coli* O157:H7 on plastic cutting boards treated with EO water.

In industry, the clean in place (CIP) method of cleaning food processing machinery provides a convenient and time-saving alternative to disassembly. EO water has, in particular, been explored as a CIP agent by the beverage industry, and it has been studied as a means to remove apple juice fouling from processing machinery (38).

EO water has been explored in the inactivation of *L. monocytogenes* biofilms (39-41). In addition to the activity of EO water against biofilms, EO water was found to not contribute to the corrosion of materials common in food processing (42). *L. monocytogenes* is arguably the organism of greatest concern where biofilm formation is expected, so many studies have focused on *L. monocytogenes* as the target organism. For example, a study by Liu *et al.* (43) indicated that EO water was effective at killing *L. monocytogenes* cells on stainless steel and ceramic tile containing seafood residue. In another study by Liu and Su (44), EO water was shown to be an effective sanitizer for seafood processing gloves. A 5 minute treatment of gloves contaminated with *L. monocytogenes* yielded no survivors.

## Challenges and role of EO water in food safety

Although many studies have focused on the disinfection of surfaces with EO water, the prevention of cross contamination during washing is perhaps the area of greatest impact. Several factors limit the efficacy of EO water as an antimicrobial treatment. One factor is surface roughness. Grooves and pits on a food’s surface can entrap microorganisms, leading to protection for the cells from washing treatments (45). Another factor is the presence of organic materials. Generally, the presence of contaminants in chlorine-based sanitizers leads to the loss of free chlorine, which is responsible for most of the antimicrobial activity of chlorine solutions (46).

In addition to its effectiveness for microbial inactivation on various food products, EO water has the greatest value as a factor inhibiting cross contamination. In one study (4), it was discovered that, despite a < 2 log reduction of *E. coli* O157:H7 numbers on produce, wash water remained free of pathogens when EO water was used. This was not the case with tap water, where pathogens were detected in the wash water.

## Conclusions

The industry's need for an effective, convenient and safe means of foodborne illness intervention will necessitate the need for alternative methods of controlling contamination. EO water is an example of one such intervention that is being explored and used in a variety of food systems for the control of foodborne illness.

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# CHAPTER 5

# EVALUATION OF DIFFERENT METHODS IN THE DETERMINATION OF PROPERTIES OF CHLORINE-BASED SANITIZERS

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## ABSTRACT

Chlorine-based sanitizers, such as electrolyzed oxidizing (EO) water, are used for the sanitation of food contact surfaces and the prevention of cross contamination in various food processing settings. Several different chlorine species are present in EO water, and these chlorine species are important to the antimicrobial efficacy. Measurements of free and total chlorine, hypochlorous acid (HOCl), hypochlorite ion ($OCl^-$) and chloride were conducted on chlorinated water and EO water solutions using a variety of commercially available methods. The chlorinated and EO water solutions differed by pH, initial free chlorine concentration, chloride concentration and organic compound concentration. It was discovered that these factors influenced the methods used to measure the various chlorine species by differing amounts. The data from this study will help in determining the appropriate methods of measuring free chlorine, total chlorine and chloride in chlorine-based sanitizers under various conditions.

**KEYWORDS:** electrolyzed water, chloride, free chlorine, total chlorine, measurement

## Introduction

Chlorine-based sanitizers, such as electrolyzed oxidizing (EO) water, have been used in a variety of applications to control the spread of pathogenic organisms. Due to the reactive nature of chlorine, the nature of the chlorine species in solution could be different in one chlorine-based sanitizer compared to another. In order to gain a more complete understanding of the quality of EO water, it is important to know all significant forms of chlorine in solution. Forms of chlorine that are significant in EO water include free chlorine, bound chlorine, total chlorine, chloride ion and hypochlorous acid to hypochlorite ratio.

Free chlorine refers to the chlorine that is available for disinfection and is not bound to organic compounds (Nakajima, Nakano, Harada, Taniguchi, Yokoyama, Hirose, Daikoku, & Sano, 2004). In this case, free chlorine could refer to hypochlorous acid (HOCl), hypochlorite ($OCl^-$) and chlorine ($Cl_2$). There are several methods useful for estimating free chlorine, and one of the frequently used methods is a titration involving N,N-diethyl-p-phenylenediamine (DPD) and ferrous ethylenediammonium sulfate (FEAS). The DPD-FEAS method has been used in several studies (Guentzel, Lam, Callan, Emmons, & Dunham, 2008) (Kim, Hung, & Brackett, 2000) (Kim, Hung, Brackett, & Frank, 2001) (Len, Hung, Erickson, & Kim, 2000) as the method of free chlorine determination. The method has a range of 0 - 3.5 ppm, so it is necessary to dilute solutions with high chlorine content to fit in this titration range. In the assay, free chlorine oxidizes DPD to form a magenta color, which is the result of a free radical known as a Würster dye (Zarei & Sovizi, 2011). The magenta species is then reduced back to DPD by FEAS upon titration. In addition to titration with FEAS, free chlorine can be quantified spectrophotometrically using a DPD color change standard curve. This method has a similar range to the titrimetric method, with the added benefit of being quicker and more objective.

Bound chlorine refers to chlorine that has reacted with organic compounds, forming an organohalide. One of the most common organohalides formed in the presence of proteins and amino acids are chloramines. Chloramines can be estimated by a variety of methods. One such method is the so-called indophenol method developed by the Hach Company. This method is a colorimetric method in which monochloramine reacts with a substituted phenol to form a monoamine intermediate, which then reacts with excess substituted phenol to form green-colored indophenol. Other methods for detection include methods that utilize chromatographic separation followed by various detection schemes, such as flame ionization (Steverink & Steunenberg, 1979) and amperometric detection (Ge, Wallace, & O'Halloran, 1990). Total chlorine refers to the sum of bound chlorine and free chlorine, and can be calculated using the DPD-FEAS method as well as the DPD spectrophotometric method.

Despite the usefulness of the DPD-FEAS method in determining free chlorine, the assay cannot differentiate one component of free chlorine from another. It has been shown that both HOCl and $OCl^-$ can be determined quantitatively by UV spectroscopy (Morris, 1966). HOCl absorbs UV light at 234 nm best, and $OCl^-$ absorbs UV light at 292 nm best. The HOCl and $OCl^-$ concentrations can then be calculated from the absorbance readings using the Beer's Law equation below:

$$A = \varepsilon bc \qquad (3)$$

Where "A" is absorbance. "b" and "c" are light path length (cm) and concentration (in M), respectively, and "ε" is molar absorptivity ($M^{-1}\ cm^{-1}$). The molar absorptivities of HOCl and $OCl_-$ are 100 and 350, respectively (Morris, 1966). The main weakness to the UV method is that the method is prone to interference from other species. For example, the presence of ferrous sulfate results in much higher than expected absorbance readings for HOCl and $OCl^-$.

Chloride ion ($Cl^-$) concentrations are an important consideration in EO water for several reasons. The conversion efficiency of an EO water generator is determined by the concentration of chloride ions andcan be measured by means of a titration involving silver nitrate. Silver nitrate reacts with chloride as follows:

$$AgNO_3 + Cl^- \rightleftharpoons AgCl + NO_3^- \qquad (4)$$

The titration endpoint is marked when all the $Cl^-$ has reacted with silver. After that, silver reacts with chromate to form a red-colored compound as follows:

$$AgNO_{3\,(aq)} + NaCrO_{4\,(aq)} \rightleftharpoons AgCrO_{4\,(s)} + NaNO_{3\,(aq)} \qquad (5)$$

An alternate method of chloride determination is a reaction with mercuric thiocyanate ($Hg(SCN)_2$) in the presence of ferric ions. Chloride reacts with $Hg(SCN)_2$ to form mercuric chloride, and iron reacts with thiocyanate to form ferric thiocyanate, which can be detected at 254 nm (Zall, Fisher & Garner, 1956).

The purpose of this study is to identify best methods of chlorine species determination using sodium hypochlorite solution as the standard (with initial pH at alkaline range) and evaluate the effect of pH, chloride content, initial chlorine concentration and organic addition on chlorine species determination. Methods identified were then used to evaluate whether they are also appropriate for EO water with an initial pH at acidic range.

## Materials and Methods

### *Sodium hypochlorite sample preparation*

Twenty four different 1 L 20 mg/kg and 40 mg/kg initial free chlorine samples were made by adding 0.4 ml/L and 0.8 ml/L, respectively, of 5% NaOCl (Ricca Chemical, Arlington, TX, U.S.A.) into deionized water. The pH of the samples was adjusted to 2.5, 6.0 and 9.3 using

1 N and 0.1 N HCl. Known concentrations of chloride (200 mg/kg and 600 mg/kg) were added to the chlorinated water samples in the form of NaCl. The NaCl was added in addition to the chloride already present in solution. Organic materials were added in the form of 20 mg peptone to the 40 mg/kg initial chlorine sample and 10 mg peptone to the 20 mg/kg initial chlorine sample. Samples containing peptone were capped and placed in a lightless environment without agitation at room temperature for 1 hour before measurements were conducted. Samples without organics were measured immediately after preparation.

*Treatment conditions and parameter measurement*

Total chlorine, free chlorine, HOCl and $OCl^-$ and chloride measurements were performed on each sample within 30 minutes after preparation. Chlorine loss on each sample was minimized as the tests are performed by sealing the containers and placing them in a dark environment while they were awaiting measurement.

Free chlorine and total chlorine were measured by the DPD-FEAS titrimetric method (Hach Company, Loveland, CO, U.S.A.). For the DPD-FEAS method, a 5 ml sample was taken from each sample, and the volume was adjusted to 100 ml with deionized water. The volume was split into four 25 ml samples. Two of the 25 ml samples were used in free chlorine tests while the other two were used in total chlorine tests. The contents of a DPD free or total chlorine reagent packet (Hach Company, Loveland, CO, U.S.A.) were added to each sample under agitation. The free chlorine tests were measured immediately by titrating with a cartridge of 0.00564N ferrous ethylenediammonium sulfate (FEAS) (Hach Company, Loveland, CO, U.S.A.) via digital titrator. The total chlorine tests were allowed to sit for 3 minutes before being titrated with FEAS. The result on the digital titrator was multiplied by 0.2 to yield a result in mg/L.

Free chlorine and total chlorine were also measured by the DPD colorimetric method (Hach Company, Loveland, CO, U.S.A.). For this method, 5 ml of sample was added to 495 ml of deionized water. The diluted sample was distributed to 4 beakers (25 ml/beaker) so that 2 total chlorine and 2 free chlorine tests could be performed. A portion of the diluted sample was also used as a blank for a DR/890 colorimeter measurement (Hach Company, Loveland, CO, U.S.A.). DPD total and free chlorine ACCUVAC ampules (Hach Company, Loveland, CO, U.S.A.) were used to collect portions of the diluted sample in the beakers for testing. Free chlorine tests were read in the colorimeter immediately after mixing while the total chlorine tests were allowed to sit for 3 minutes. The results were reported as mg/L.

Chloride was measured by the silver nitrate titrimetric method (Hach Company, Loveland, CO, U.S.A.). Each sample was diluted 1:5 using deionized water. The contents of a chloride 2 indicator powder pillow (Hach Company, Loveland, CO, U.S.A.) were added to 100 ml of diluted sample and allowed to mix. The samples were then titrated to an endpoint (brown color) using 1.128N silver nitrate cartridges (Hach Company, Loveland, CO, U.S.A.) dispensed using a digital titrator. The number on the titrator was multiplied by 2.5 to yield a concentration measurement in mg/L.

Chloride was also measured by the mercuric thiocyanate spectrophotometric method (Hach Company, Loveland, CO, U.S.A.). One ml of sample was diluted 1:100 with deionized water. Two ml of mercuric thiocyanate solution (Hach Company, Loveland, CO, U.S.A.) and 1 ml of ferric ion solution were added to 25 ml of the diluted samples. After 2 minutes, each mixture was read using a DU 520 UV/Vis spectrophotometer (Beckman Coulter Inc., Brea, CA, U.S.A.) at 455 nm. Deionized water was used as a blank, and the absorbance readings for the samples were related to concentrations using a standard curve constructed using samples of salt water with known chloride concentrations. Chloride was also measured by a $Cl^-$ ion probe. An Orion 9617BNWP Chloride Combination Electrode (Thermo Fisher Scientific Inc., Waltham, MA, U.S.A.) was used in the chloride measurements. The probe output was read on an Accumet® AR50 Dual Channel pH/ion/conductivity meter (Thermo Fisher Scientific Inc., Waltham, MA, U.S.A.). Results were displayed in ppm.

HOCl and $OCl^-$ were measured spectrophotometrically. The concentration of HOCl and $OCl^-$ was determined using the Beer's Law relationship mentioned previously. The same UV/Vis spectrophotometer mentioned above was used to measure HOCl at 234 nm and $OCl^-$ at 292 nm. Quartz cuvettes were used, and the path length was measured to be 1 cm. The molar absorptivities of HOCl and $OCl^-$ were 100 and 350, respectively. The results were reported in mg/L.

### *EO water sample preparation*

In the second part of this experiment, 20 mg/kg and 40 mg/kg initial chlorine concentration EO water samples were generated using a Hoshizaki ROX20-TA (Hoshizaki Electric Inc. Toyoake, Aichi, Japan) EO water generator by setting the generator amperage at 7 and 12, respectively. EO water samples were collected and dispensed into screw cap bottles. The initial pH of the EO water was adjusted to 2.5, 6.0 and 9.3 using 1 N and 0.1 N NaOH. Organics and chloride ions were added as described in the chlorinated water procedure above.

### *Statistical analysis*

Data was subjected to an analysis of variance with a completely randomized factorial design. Statistical analysis was performed using SAS (2008) General Linear Model procedure performed with SAS Software Release 9.2 (SAS Institute). T-tests were used for pairwise comparisons. Least significant difference of means tests were done for multiple comparisons, and all tests were performed with a level of significance of 0.05. Two replications of each experiment (the chlorinated water and EO water experiments) were done, and two measurements were taken for each sample on each test.

## Results and Discussion

### *Methods of free and total chlorine determination*

Table 5-1 shows the results of free chlorine determination by two different methods in chlorinated water samples under various conditions. In terms of free chlorine measurements, the DPD and DPD-FEAS methods are comparable. For most samples, there is no significant difference between the two methods. This is an expected outcome because both methods rely on the same way of measuring free chlorine (the oxidation of DPD). The methods only differ in how the oxidation of DPD is interpreted. The concentration of chloride did not have a significant effect on either of the measurements for free chlorine concentration (Table 5-1). The reason for this is that neither of the two methods for free chlorine determination measure chloride as free chlorine. Although chloride does participate in the chemistry of free chlorine production and stability, such reactions require energy (to remove valence electrons in order to form chlorine radicals, which spontaneously react to form free chlorine) or time (Lifshitz & Perlmutter-Hayman, 1960). Due to the thermodynamic barriers involved, the participation of chloride in free chlorine chemistry is minor in comparison to other factors explored in this study.

The addition of peptone to chlorinated water had a large effect on the free chlorine measurements. The samples without organic addition are closer to the initial chlorine concentration values than the samples with organic addition. Both methods of free chlorine measurement reveal this trend. The amounts of peptone used in this study were chosen in order to provide a roughly 50% reduction in free chlorine after one hour incubation at room temperature in the dark. In terms of the effect on the precision of the DPD and DPD-FEAS methods of free and total chlorine determination, organic addition does not seem to be a factor that affects measurement precision. Examples of this on Table 5-1 can be seen by comparing 2 samples with the same pH, initial chlorine concentration and organic addition. In all cases, the samples differ by no more than 4 ppm free or total chlorine for either the DPD or DPD-FEAS measurements. Therefore, it is unlikely that organic addition affects the precision of free or total chlorine measurements among similar samples.

The effect of pH on free chlorine measurements is evident on Table 5-1. There is a general trend of lower free chlorine readings at higher pH values when organics are present. For example, the free chlorine reading using the DPD method at 20 mg/kg initial chlorine, pH 2.5, 10 mg/kg peptone and 200 mg chloride addition is 8.45 mg/kg. For 20 mg/kg initial chlorine, pH 9.3, 10 mg/kg peptone and 200 mg chloride addition, the free chlorine by the DPD method is 5.75 mg/kg. The reason for this is that hypochlorite reacts more readily with proteins at higher pH values due to more favorable amino acid oxidation states (Armesto, Canle & Santabella, 1993).

Another way that pH can affect the free chlorine readings is by speciation changes in the chlorine itself. As mentioned previously, chlorine has a pH-dependent chemistry in aqueous solutions. At very low pH values (< 1.5), most of the free chlorine is available as chlorine gas. Chlorine gas, like all gases, has a temperature-dependent solubility. Generally, lower temperatures are required for increased gas solubility. In Table 5-1, even though there is no statistically-significant difference between the DPD and DPD-FEAS measurements in most cases, a comparison between the two methods shows a greater degree of difference between the free chlorine measurements at pH 2.5 as opposed to pH 9.3. One example of this includes the comparison between DPD and DPD-FEAS at 20 mg/L initial chlorine, pH 2.5, no organics, and 600 mg chloride addition (20.25 mg/L for DPD and 17.6 mg/L for DPD-FEAS) versus a similar sample (initial chlorine, organic addition and chloride addition the same) at pH 9.3 (17 mg/L DPD and 17.55 mg/L DPD-FEAS). A possible explanation of this is that more free chlorine is present at pH 2.5 as $Cl_2$ than at pH 9.3. Because of this, the DPD-FEAS method, which involves agitation of solutions in open containers, has generally lower readings (more pronounced at lower pH values). The agitation in the method may contribute to the loss of chlorine to the atmosphere.

Table 5-1 also shows the results of total chlorine measurements and similar with the free chlorine measurements, the two methods compare reasonably well. For most samples, (with the possible exception of the results for pH 6, 20 mg/L initial chlorine and pH 2.5, 20 mg/L initial chlorine with no added organic) there is no significant difference between the two methods. This is an expected outcome because both methods rely on the same way of measuring total chlorine (the oxidation of DPD). The methods only differ in how the oxidation of DPD is interpreted. The previously mentioned samples that did show a significant difference between DPD and DPD-FEAS total chlorine measurements appear to not be indicative of any particular trend.

A comparison between the free chlorine and total chlorine measurements for a sample that has no organic addition reveals the same or slightly higher readings for total chlorine versus free chlorine. One possible explanation for higher total chlorine readings than free chlorine readings include a small amount of bound chlorine contamination in the 5% NaOCl reagent being read as a part of total chlorine. The concentration of non-free chlorine in such solutions increases over time. As a result, strategies that slow the decay of free chlorine in NaOCl solutions (such as packaging in opaque containers and including warnings to close containers between use) are often employed.

The largest difference between free and total chlorine measurements lies in the samples that had organic addition. In all of those samples, the measurements for free chlorine were lower than for total chlorine. This is different from the samples that did not have organic addition, in which the free and total chlorine measurements were much closer together. The reason for the difference between the free and total chlorine measurements in samples with organic addition is that chlorine reacts with peptone and converts a portion of the free chlorine to bound chlorine. Bound chlorine is a part of total chlorine but not free chlorine. As a result, the free chlorine measurements decrease at a much faster rate than the total chlorine measurements. There is a question why the total chlorine measurements for the samples with added organics do not equal the total chlorine measurements on similar samples without added organics. An explanation for this may be due to the variety of oxidation and chlorination products that are produced in many reactions. The chloramines (which are detected as total chlorine) that are expected to be formed as end products in amino acid oxidation reactions may be intermediates instead. For example, Hawkins, Pattison and Davies (2003) have suggested that sulfur-containing amino acids, heterocyclic amino acids and α-amino group amino acids undergo further oxidation reactions in aqueous environments to yield a variety of oxidized products and chloride, which are not detectable as free or total chlorine.

Given the similarity in precision and accuracy of the DPD and DPD-FEAS methods of free and total chlorine determination, it is the opinion of the paper authors that either method is appropriate for the determination of free or total chlorine. Precision and accuracy aside, the DPD method may be a more appealing method because it is more subjective and requires less training to use than the DPD-FEAS method.

*Spectrophotometric determination of HOCl and $OCl^-$ in chlorinated water*

The results of HOCl and $OCl^-$ determination by UV spectroscopy in chlorinated water are shown in Table 2-2. In addition to concentrations of HOCl and $OCl^-$, sums of HOCl and $OCl^-$, as well as ratios of HOCl to $OCl^-$, are displayed on Table 5-2. Like free and total chlorine, the concentrations of HOCl and $OCl^-$ can be affected by pH, initial chlorine, chloride (added as NaCl) and organic (added as peptone) addition. As with the free and total chlorine measurements, the measurements of HOCl and $OCl^-$ are not affected by chloride concentration. HOCl and $OCl^-$ concentrations are similar between any two samples that differ only by chloride concentration. This is not the case with organic addition, pH and initial chlorine concentration.

One of the greatest sources of variation among samples can be seen when two samples that differ only by organic addition are compared. The means calculated HOCl is at least 4 times greater in samples where peptone is added, as compared to similar samples without peptone. For example, the calculated concentration of HOCl in the pH 2.5, 20 mg/L initial chlorine, 200 mg added chloride sample with organic addition is 50.4 mg/kg, which is roughly 4 times greater than a similar sample without organic addition (12.7 mg/L). Calculated $OCl^-$ also differs by the same factor of 4 between samples that have peptone versus samples that do not. Based on these results, it is apparent that peptone interferes with the spectrophotometric method of HOCl and $OCl^-$ concentration determination. The nature of the interfering compound(s) is not understood, but appears to increase with an increase in organic addition.

The pH of chlorinated water also plays a large role in the HOCl and $OCl^-$ measurements. Generally, the concentration of HOCl decreases with increasing pH while the concentration of $OCl^-$ shows an opposite trend. For example, the concentrations of HOCl in chlorinate water samples with an initial chlorine concentration of 20 mg/L, 200 mg added chloride and no organics at pH 2.5, 6.0 and 9.3 are 12.7, 10.8 and 0.5 mg/L, respectively. For the concentration of $OCl^-$ in the same samples at pH 2.5, 6.0 and 9.3, the concentrations are 0.7, 0.88 and 13.53 mg/L, respectively. At pH 2.5 and 6, the majority of free chlorine is in the form of HOCl. At pH 9.5, the majority of free chlorine is in the form of $OCl^-$, as evidenced by the near 0 ratios (0.07 to 0.01) of $HOCl/OCl^-$ at all pH 9.3 samples without peptone as shown on Table 5-2. In the samples with peptone, HOCl is similar at pH 9.3 and 6, while being greater at pH 2.5. $OCl^-$ in samples with peptone is higher than similar samples without peptone at pH 2.5, equivalent in similar samples without peptone at pH 6, and lower than similar samples without peptone at pH 9.3. This data suggests that the pH effect on HOCl and $OCl^-$ measurements in samples with peptone is more because amino acids in peptone react with free chlorine with a rate dependent on solution pH (Armesto, Canle & Santabella, 1993). This could result in the disproportionate ratios of HOCl / $OCl^-$ at different pHs.

The initial chlorine concentrations used in this study provided a means to check for proportionality in the UV spectroscopic method of HOCl and $OCl^-$ determination. Generally speaking, the calculated concentrations of HOCl and $OCl^-$ for all samples with 20 mg/kg initial chlorine were half of what similar samples at 40 mg/kg initial chlorine were. These results help to validate the usefulness of the method as a means to compare relative HOCl and $OCl^-$ concentrations in different chlorinated water samples. A conversion factor of some experimentally-determined value is required to make reasonable estimations on the absolute HOCl and $OCl^-$ concentrations.

The sum of HOCl and $OCl^-$ is displayed to see how the result relates to total chlorine concentrations presented in Table 5-1. The sums show a clear division between samples that have no organic versus those that do. At 20 mg/L and 40 mg/L in the samples without added organics, the sums of HOCl and $OCl^-$ are not significantly different than total chlorine. To contrast, the sums of HOCl and $OCl^-$ for samples with added organics are significantly different than total chlorine and, in a few cases, different from samples at different pHs as well. The reason for this disparity between pH could be the difference in reaction rates among free chlorine and peptone at the different pHs. Different levels of interfering products could be produced, and this would cause a shift in peak intensity among pHs. The fact that this phenomenon is seen in the 40 mg/L initial chlorine but not in 20 mg/L may be due to higher free chlorine concentrations leading to faster reaction rates and, as a result, more interfering compounds.

Since the sum of HOCl and $OCl^-$ is a derived value, all of the discussion about the role of pH, initial chlorine, chloride (added as NaCl) and organic (added as peptone) addition on HOCl and $OCl^-$ applies here. If all free chlorine is accounted for by the sum of HOCl and $OCl^-$, then the result should match the initial chlorine reading in the samples without the addition of organic material (peptone). In earlier works (Chen, 1967) (Len, Hung, Erickson, & Kim, 2000) (Mishalanie, Rutkowski, Hutte, & Birks, 1986), various methods of UV spectroscopy were used to detect and quantify HOCl and $OCl^-$ in water and other solvents. Len *et al.* (2000) found concentrations of HOCl and $OCl^-$ were measured to be comparable to the concentrations of free chlorine as determined by DPD method. In this study, the concentrations of HOCl and $OCl^-$ are not comparable to the free chlorine concentrations in any of the treatments. Possible reasons for this include differences in equipment (spectrophotometers and cuvettes) used as well as temperature effects on absorbance (Morris, 1966).

The ratios of HOCl to $OCl^-$ pH 2.5 was 18.2 for 20 mg/L initial chlorine and no chloride or organic added. The Henderson-Hasselbalch equation predicts a ratio of $1 \times 10^5$, but the equation does not apply at this pH as it does for higher pHs due to the evolution of chlorine gas. At pH 6 and 9.3, the ratio of HOCl / $OCl^-$ for 20 mg/L initial chlorine and no chloride or organic added was 12.1 and 0.04, respectively. These are more closely-aligned with the expected values at those pHs, as predicted by the equation (31.6 and 0.02, respectively). The ratio data on Table 5-2 also suggests that minor differences exist between samples at pH 2.5 and 6. There is a significant degree of overlap in the means of similar samples at pH 2.5 and 6. For example, the two treatments at 20 mg/L initial chlorine, pH 2.5 and no organic addition (18.2 and 15.1) overlap the two treatments under the same conditions, but at pH 6 (12.2 and 10.3). This trend holds true for the samples with 40 mg/L initial chlorine as well. However, the samples at pH 9.3 are dissimilar from samples at other pHs in terms of HOCl / $OCl^-$ . This result is to be expected because most free chlorine below pH 7.5 is in the form of HOCl, while most free chlorine above 7.5 is in the form of $OCl^-$ (Deborde & Gunten, 2008).

The UV spectroscopic determination of HOCl and $OCl^-$ is useful in the determination of both the concentration and ratio of free chlorine species. However, the presence of peptone and other chlorine species limits the usefulness of this method. Therefore, this method is most useful to determine free chlorine of chlorine solutions and EO water that lack interfering compounds.

### *Methods of chloride determination*

The means plus/minus standard deviations for chloride ion measurements in chlorinated water are given in Figure 5-1. Initial chlorine concentration and organic addition were determined not to be significant factors in the chloride measurements. The most significant single factors affecting the chloride measurements were method of measurement (probe, HgSCN method, and $AgNO_3$ titration), pH, and initial chloride concentration. Significant combination factors included measurement method x pH and measurement method x initial chloride concentration. The initial levels of chloride were 600 mg/kg and 200 mg/kg, and they changed very little during the course of the experiment. Therefore, the initial levels of chloride were significant factors in the chloride measurements because they remained far apart throughout the experiment.

The pH of chlorinated water has a significant effect on the measurement of chloride ions. Generally, all 3 methods of chloride determination report higher concentrations of $Cl^-$ at pH 2.5 than at pH 6.0 or 9.3. The reason for this is different depending on the method used. The probe is susceptible to interference by excess ions (in this case, protons). Therefore, it is a good strategy to control pH when using a probe. The HgSCN and $AgNO_3$ methods can also be affected by excess ions. In the case of these two tests, interference arises from oxidation state changes in reagents, producing a greater than expected color change. All three methods produce similar results of chlorine concentration at pH 6 and 9.3, so hydrogen ion concentration appears to play an important role in chloride ion determination only when the hydrogen ion concentration exceeds a certain value.

The concentration of chloride ions also has an effect on the chloride ion measurements by all three methods. However, a 400 mg/L increase in chloride ion concentration (from 200 mg/kg to 600 mg/kg chloride) addition only produced a 300 mg/L increase in the chloride ion measurement for any of the methods. The figure shows further evidence that the measurements at pH 2.5 are obviously different from measurements using the same methods at other pHs. The chloride probe seems to be more accurate at pH 2.5, 600 mg/kg chloride while the $AgNO_3$ titration seems to be more accurate at pH 2.5, 200 mg/kg chloride. The HgSCN method does not seem to be accurate at any chloride concentration at pH 2.5. At pH 6.0 and 9.3, the chloride probe is more accurate than the other 2 methods at a chloride concentration of 200 mg/kg. However, the HgSCN method is more accurate at the higher chloride concentration at the same pHs. A potential solution to chloride measurements using the probe would be to adjust the pH of the sample to pH 6 using compounds that doesn’t produce interfering counterions.

### *Chlorine species determination in EO water*

Since EO water is a chlorine-based sanitizer that is gaining general acceptance in commercial and household environments, we felt it was beneficial to test the methods of chlorine-species determination in EO water. A comparison of all the recommended methods of chlorine species determination on chlorinated and EO water is shown in Table 5-3. All the samples contained an initial chlorine concentration of 20 mg/L, and the comparisons between chlorinated and EO water samples with 40 mg/L initial chlorine (data not shown) are comparable. The DPD-FEAS titration methods of free and total chlorine yield consistently similar results between chlorinated water and EO water samples possessing the same treatment characteristics (i.e. pH, chloride and organic addition). There are a few cases where statistically-significant ($p < 0.05$) differences exist. A comparison of HOCl and $OCl^-$ concentrations among these samples yields inconsistent results.

Based on comparisons of the chlorinated water and EO water in Table 5-3, the measured HOCl and $OCl^-$ concentrations appear to be similar. Table 5-3 also shows the chloride probe readings in EO water samples read about 200-400 mg/L higher chloride than the chlorinated water samples due to incomplete conversion of $Cl^-$ to HOCl or OCl- during electrolysis.

The data on Table 2-3 provide supporting evidence that final solution pH is more important than initial pH of the solution (pH 2.5 for EO water and pH 11 for sodium hypochlorite). All methods of free and total chlorine determination, as well as determination of HOCl and $OCl^-$, work at the various conditions used in this study regardless of the initial pH. The data also highlights the complexity of EO water in terms of residual chloride. The selected methods of chlorine species determination worked for the chlorine-based solutions at different pH regardless of the initial pH of the solution

## Conclusions

This study has shown that various factors found in EO and chlorinated water can have an effect on the outcome of parameter measurements commonly used in chlorine-based solutions. For free and total chlorine determination, either the DPD or DPD-FEAS method of species determination can be used reliably at the ranges of pH, chloride, chlorine and peptone concentration used in this study. For the determination of HOCl and $OCl^-$, the UV spectroscopic method did not give accurate answers, and the method appeared to be sensitive to the addition of contaminants such as peptone. For chloride concentration determination, pH seems to play a significant role in the accuracy of readings obtained by each of the three methods. Care must be taken to use the most accurate method, dependent on the levels of the variables present in a sample, in order to obtain reliable data.

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## Table 5-1: Free and total chlorine determination in chlorinated water samples at various levels of chlorine, chloride and pH*

| | | | | Free chlorine (mg/L) | | Total chlorine (mg/L) | |
|---|---|---|---|---|---|---|---|
| Initial chlorine (mg/kg) | pH | Organic addition | Chloride addition (mg) | DPD | DPD-FEAS | DPD | DPD-FEAS |
| 20 (1) | 2.5 | no | 200 | 18.15 a | 16.85 a | 20 a | 18.65 a |
| | | | 600 | 20.25 a | 17.6 a | 22a | 19.1b |
| | | yes | 200 | 8.5 a | 10.65 a | 14.75 a | 14.1a |
| | | | 600 | 9 a | 8.4 a | 16 a | 13.45 a |
| | 6.0 | no | 200 | 19 a | 19.05 a | 23.75 a | 19.3 a |
| | | | 600 | 15.25 a | 19.38 a | 23.75 a | 19.55 b |
| | | yes | 200 | 13.25 a | 11.3 a | 16.5 a | 14.65 b |
| | | | 600 | 13.75 a | 13.95 a | 18.75 a | 16.7 a |
| | 9.3 | no | 200 | 19.25 a | 18.45 a | 21.5 a | 19.45 a |
| | | | 600 | 17 a | 17.55 a | 20 a | 19.15 a |
| | | yes | 200 | 5.75 a | 8.45 a | 11.25 a | 13.65 a |
| | | | 600 | 6.75 a | 8.7 a | 13 a | 13.3 a |
| 40 (2) | 2.5 | no | 200 | 40 a | 35.95 a | 38 a | 37.2 a |
| | | | 600 | 38 a | 34.25 a | 41 a | 37 a |
| | | yes | 200 | 18.5 a | 19.5 a | 30.25 a | 29.4 a |
| | | | 600 | 19.5 a | 18.15 a | 31.5 a | 27.5 a |
| | 6.0 | no | 200 | 40.25 a | 37.5 a | 42.5 a | 36.6 a |
| | | | 600 | 40.75 a | 37.65 a | 40.75 a | 40.35 a |
| | | yes | 200 | 23.5 a | 20.85 a | 33.25 a | 28.2 a |
| | | | 600 | 21.25 a | 22.95 a | 30.5 a | 28.45 a |
| | 9.3 | no | 200 | 40.75 a | 38.95 a | 44.5 a | 40.4 a |
| | | | 600 | 39.25 a | 39 a | 41.75a | 39.3 a |
| | | yes | 200 | 10.75 a | 14.95 b | 20.75 a | 20.95 a |
| | | | 600 | 18.5 a | 12.9 a | 21 a | 22.95 a |

1. 10 mg/kg peptone was added as the organic addition

2. 20 mg/kg peptone was added as the organic addition

* Each value is an average of 4 samples and mean values not followed by the same letter in a row for a particular measurement (free and total chlorine) are significantly different

($p \leq 0.05$)

## Table 5-2: Spectrophotometric determination of HOCl and OCl- in samples of chlorinated water*

| Initial chlorine (mg/L) | pH | Organic addition | Chloride addition (mg) | HOCl (mg/L) | OCl$^-$ (mg/L) | HOCl + OCl$^-$ | HOCl / OCl$^-$ |
|---|---|---|---|---|---|---|---|
| 20 $_{(1)}$ | 2.5 | no | 200 | 12.7 | 0.70 | 13.4 b | 18.2 bc |
| | | | 600 | 11.7 | 0.77 | 12.5 b | 15.1 bcd |
| | | yes | 200 | 50.4 | 2.35 | 52.8 a | 21.4 ab |
| | | | 600 | 52.7 | 2.65 | 55.4 a | 19.9 ab |
| | 6.0 | no | 200 | 10.8 | 0.88 | 11.7b | 12.2 cd |
| | | | 600 | 13.6 | 1.32 | 14.9 b | 10.3 de |
| | | yes | 200 | 44.6 | 1.80 | 46.4 a | 24.8 a |
| | | | 600 | 42.0 | 1.98 | 44.0 a | 21.1 ab |
| | 9.3 | no | 200 | 0.50 | 13.53 | 14.0 b | 0.04 f |
| | | | 600 | 0.40 | 13.71 | 14.1 b | 0.03 f |
| | | yes | 200 | 40.9 | 7.65 | 48.6 a | 5.35 ef |
| | | | 600 | 41.4 | 7.13 | 48.5 a | 5.81 ef |
| 40 $_{(2)}$ | 2.5 | no | 200 | 25.6 | 1.80 | 27.4 f | 14.2 c |
| | | | 600 | 27.1 | 2.10 | 29.2 f | 13.0 cd |
| | | yes | 200 | 108 | 4.85 | 113b | 22.2 ab |
| | | | 600 | 115 | 5.73 | 120 a | 20.0 b |
| | 6.0 | no | 200 | 24.8 | 2.43 | 27.2 f | 10.2 de |
| | | | 600 | 27.3 | 2.35 | 29.7 f | 11.6 cd |
| | | yes | 200 | 87.0 | 3.68 | 90.7 e | 23.7 a |
| | | | 600 | 93.4 | 4.19 | 97.6 d | 22.3 ab |
| | 9.3 | no | 200 | 2.10 | 28.3 | 30.4 f | 0.07 f |
| | | | 600 | 0.30 | 27.7 | 28.0 f | 0.01 f |
| | | yes | 200 | 95.0 | 10.7 | 106 c | 8.85 e |
| | | | 600 | 95.7 | 11.8 | 108 bc | 8.09 e |

1. 10 mg/kg peptone was added as the organic addition

2. 20 mg/kg peptone was added as the organic addition

* Each value is an average of 4 samples and mean values not followed by the same letter in a column for a particular grouping (i.e. 20 mg/L initial chlorine and 40 mg/L initial chlorine) are significantly different ($p \leq 0.05$)

## Table 5-3: Comparison of recommended chlorine species determination methods in chlorinated water and EO water at 20 mg/L initial chlorine*

| Initial chlorine (mg/L) | pH | Organic addition | Chloride addition (mg) | Water type | Free chlorine (DPD-FEAS) (mg/L) | Total chlorine (DPD-FEAS)(mg/L) | HOCl | OCl- | Cl- (probe) (mg/L) |
|---|---|---|---|---|---|---|---|---|---|
| 20 | 2.5 | no | 200 | Cl water | 16.85 a | 18.65 a | 12.7 b | 0.70 a | 359 |
| | | | | EO water | 23.7 a | 21.15 a | 13.6 a | 0.70 a | 735 |
| | | | 600 | Cl water | 17.6 a | 19.1 a | 11.7 a | 0.77 a | 621 |
| | | | | EO water | 21.55 a | 24.45 a | 14.0 a | 2.02 a | 1170 |
| | | yes | 200 | Cl water | 10.65 a | 14.1 a | 50.4 a | 2.35 a | 372 |
| | | | | EO water | 10.45 a | 15.95a | 51.9 a | 2.35 a | 710 |
| | | | 600 | Cl water | 8.4 a | 13.45 a | 52.7 a | 2.65 a | 644 |
| | | | | EO water | 12.15 a | 16.8 b | 56.5 a | 2.46 b | 1122 |
| | 6.0 | no | 200 | Cl water | 19.05 a | 19.3 a | 10.8 a | 0.88 a | 172 |
| | | | | EO water | 16.9 b | 20.75 a | 11.3 a | 1.98 a | 431 |
| | | | 600 | Cl water | 19.38 a | 19.55 a | 13.6 a | 1.32 a | 503 |
| | | | | EO water | 15.5 a | 19.75 a | 12.3 a | 2.35 a | 703 |
| | | yes | 200 | Cl water | 11.3 a | 14.65 a | 44.6 a | 1.80 a | 160 |
| | | | | EO water | 11.25 a | 15.65 a | 44.3 a | 1.69 a | 491 |
| | | | 600 | Cl water | 13.95 a | 16.7 a | 42.0 a | 1.98 a | 375 |
| | | | | EO water | 13.8 a | 17.65 a | 44.6 a | 2.13 a | 769 |
| | 9.3 | no | 200 | Cl water | 18.45 a | 19.45 a | 0.50 ab | 13.53 a | 157 |
| | | | | EO water | 26.55 a | 26 a | 4.85 a | 16.5 a | 330 |
| | | | 600 | Cl water | 17.55 a | 19.15 a | 0.40 b | 13.71 a | 488 |
| | | | | EO water | 21.9 a | 23.4 a | 6.56 a | 15.6 a | 721 |
| | | yes | 200 | Cl water | 8.45 a | 13.65 a | 40.9 a | 7.65 a | 202 |
| | | | | EO water | 10.4 a | 13.3 a | 38.8 b | 8.09 a | 418 |
| | | | 600 | Cl water | 8.7 a | 13.3 a | 41.4 a | 7.13 a | 374 |
| | | | | EO water | 13.8 a | 16.7 a | 52.3 a | 9.48 a | 741 |

* Each value is an average of 4 samples and mean values not followed by the same letter in a column for a particular grouping (i.e. Cl water and EO water directly below it) are significantly different (p ≤ 0.05)

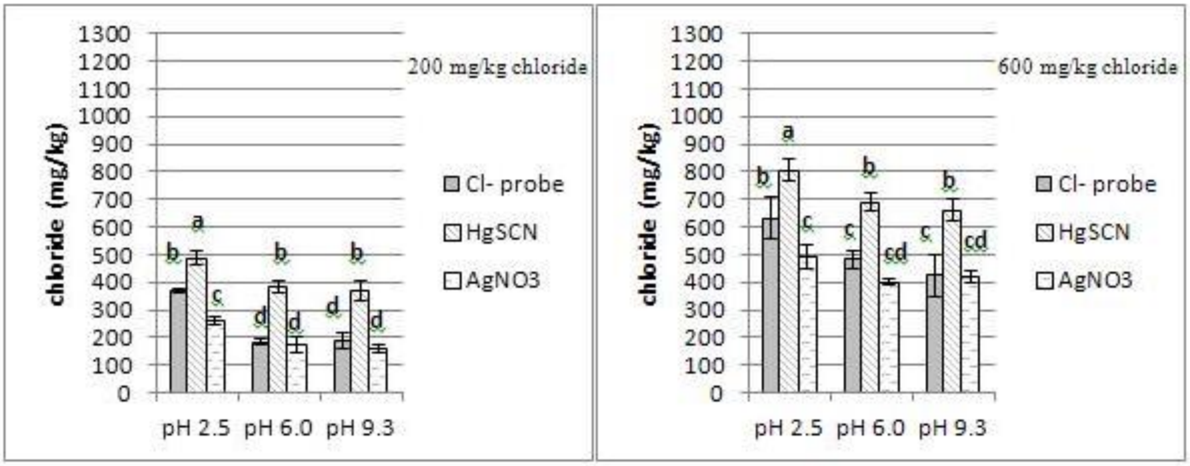

Figure 5-1: Determination of chloride ion in samples of chlorinated water

# CHAPTER 6

# THE EFFECT OF ORGANIC LOADS ON STABILITY OF VARIOUS CHLORINE-BASED SANITIZERS

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## Abstract

This study examined the effect of pH of chlorine-based sanitizers on the reaction of free chlorine with compounds present in a variety of foods. A model food system was first used to determine chlorine reactions with individual organic compounds. Different classes of organic compounds were added to chlorinated water at three different pHs (2.5, 6.0, and 9.3). Free and total chlorine concentrations were recorded in each sample by titrimetric assay. The results show that the level of free chlorine loss is both organic species-dependent, and pH-dependent. Wash water from 6 different food sources was added to chlorinated water at three different pHs. The peptone plus catechol model appeared to be the best general predictor of free chlorine loss across all the treatments. GC-MS analysis of samples with resorcinol added indicated that chloroform is produced as a primary trihalomethane product at higher pH values. The findings of this study indicate that solution pH, along with types of organic compound, play a significant role on free chlorine loss, and chlorine loss can be predicted by the protein and phenolic content of the wash solution.

**Keywords**

Chlorine, modeling, pH, stability

## Introduction

Chlorine-based sanitizers, such as sodium hypochlorite solutions (NaOCl), have long been considered an inexpensive and effective method for sanitization in food processing environments (EPA, 1992). Because of this, chlorine-based sanitizers have been studied not only in food washing applications but also as treatments of food contact surfaces. Chlorine forms several compounds in water, such as hypochlorous acid, chlorine ($Cl_2$) (HOCl) and hypochlorite ($OCl^-$), all of which are effective antimicrobial agents due to the reactivity of both chlorinated compounds.Free chlorine (HOCl, $Cl_2$, and $OCl^-$) combined with organic chlorine compounds such as chloramine comprise total chlorine. Free chlorine species are much more effective antimicrobial agents than chloramines are, so the measurement of free chlorine is more important in terms of a sanitizers antimicrobial activity. One of the main reasons that chlorine-based sanitizers are effective antimicrobial agents is because of their high oxidation reduction potential (also known as redox potential) (ORP > 1100 mV) (Kim *et al.*, 2000) for hypochlorous acid (HOCl). Since HOCl is a strong oxidant, it freely oxidizes many organic compounds. Also, as a weak acid, HOCl is able to diffuse through cell membranes and acidify the interior of cells (Gutknecht & Tosteson, 1973). Also, HOCl has more penetration power than $OCl^-$ due to its uncharged nature (Wyman, 1996). These properties help to make HOCl an effective antimicrobial agent. As a result, chlorine-based sanitizers have seen widespread use in a variety of food processing and service applications.

Chlorine-based sanitizers are commonly-used in most food processing industries (Walker & LaGrange, 1991; Cherry, 1999). The USDA FSIS recommends a 50 mg/L chlorine limit on most meat applications with no post treatment rinse. Although diluted NaOCl solutions account for the majority of chlorine-based sanitizer usage, electrolyzed (EO) water with a predominant

HOCl component has been studied for use in processing equipment (Venkitanarayanan *et al.*, 1999; Park *et al.*, 2002), beef (Cutter *et al.*, 1995; Bach *et al.*, 2006), poultry and egg (Bialka *et al.*, 2004; Park *et al.*, 2005), seafood (Huang *et al.*, 2006a, 2006b), and produce (Kim *et al.*, 2003; Pangloli *et al.*, 2009; Hung *et al.*, 2010; Pangloli *et al.*, 2011). Additionally, EO water has been approved by the US Environmental Protection Agency (EPA) for food industry use (Park *et al.*, 2002).

Despite the widespread use and antimicrobial efficacy of chlorine-based sanitizers, there are several limitations to chlorine-based sanitizers. One of the main concerns with using chlorine-based sanitizers in food processing environments is the reactivity of chlorine with organic substances in the environment. HOCl specifically reacts with amines, reduced sulfur and activated aromatics (Deborde & Gunten, 2008). Amines, like those contained in the backbones of amino acids, react rapidly with HOCl. Higher rates of reaction are typically found in sulfur-containing amino acids, which are the preferential targets of HOCl reactions (Armesto *et al.*, 2000; Pattison & Davies, 2001). Amino acids with aromatic side chains, such as tryptophan and tyrosine, produce halogenated acetates (Hong *et al.*, 2009). As for activated aromatics, HOCl reacts with these compounds (on the order of 3-4 logs slower, compared to amino acids and peptides) to produce various chlorinated organic compounds, mostly chlorinated phenols (Gallard & Gunten, 2002). Resorcinol, on the other hand, can quickly produce chloroform (Jackson *et al.*, 1987). Another important factor contributing to the antimicrobial efficacy and stability of chlorine-based sanitizers is the presence of metal ions. For ferrous iron (Fe(II)), the apparent rate of reaction is rapid and similar in magnitude to the reaction rate of HOCl with phenolic compounds (Folkes *et al.*, 1995).

The rate of free chlorine loss (defined in this study as the concentration change in free chlorine, which includes chlorine species such as $OCl^-$, HOCl and $Cl_2$) in the environment is not dependent on just the concentration and type of organic materials present. Free chlorine loss is also affected by pH. In compounds containing amine groups, such as amino acids, maximal reaction rates were recorded at pH~8.5 (Armesto *et al.*, 1993; Antelo *et al.*, 1995). Higher reaction rates at more alkaline pHs are also observed in materials other than amino acids. At a higher pH (when a greater amount of $OCl^-$ is expected to be present, pka HOCl = 7.5), the rate constant is expected to increase due to the iron speciation (Deborde & von Gunten, 2008).

Organic compounds in water react with chlorine and affect the antimicrobial activity of the chlorine-based sanitizer being used. In order to save on production costs while ensuring adequate treatment, it is important to know the reactivity of organic compounds with free chlorine. For those reasons, it is important to understand the stability of free chlorine when it is used to treat different food products with different compositions (i.e. carbohydrates, protein, fat, minerals, and phenolics) The purpose of this study was to evaluate the effect of different food compositions on the stability of free chlorine at different pHs.

## Materials and Methods

### *Preparation of sodium hypochlorite solutions for single organic compound experiments*

Forty mg/L initial free chlorine samples were made by adding 0.8 ml/L of 5% NaOCl (Ricca Chemical, Arlington, TX, U.S.A.) into deionized water in glass screw cap bottles. The pH of the samples was measured by an Accumet AR50 pH meter (Thermo Fisher Scientific Inc., Waltham, MA, U.S.A.) and then adjusted to 2.5, 6.0 and 9.3 using phosphoric acid and sodium hydroxide with 10 mM phosphate buffers. The chlorinated water samples were used within 2 hours of creation, and the bottles were sealed and held at room temperature until use. Free and total chlorine was quantified using DPD-FEAS titrimetric tests for free and total chlorine (Hach Company, Loveland, CO, U.S.A.).

*Single organic compound experiments*

Seventeen different treatments were monitored for free and total chlorine loss. Stock solutions of peptone (Becton Dickson Co., Sparks, MD, USA), catechol (Sigma Aldrich Inc., St. Louis, MO, USA) , soluble starch (Fisher Scientific Co., Fair Lawn, NJ, USA), and iron (II) sulfate ($FeSO_4$) (Sigma Aldrich Inc., St. Louis, MO, USA), used as model compounds for protein, phenolics, carbohydrates and minerals in food, respectively, were made by dissolving 250 mg of peptone, catechol, resorcinol, soluble starch, or $FeSO_4$ in 100 ml deionized water ($dH_2O$). A corn oil stock solution was made by adding 0.36 ml of corn oil purchased from a local supermarket to 99.64 ml $dH_2O$ in a 1.5L Whirl-Pak® bag (Nasco, Fort Atkinson, WI, USA). The stock was homogenized for 10 sec at 260 rpm using a Stomacher 400 Circulator (Seward Ltd., West Sussex, UK). After mixing, 0.25 ml of corn oil stock was added to 249.75 ml chlorinated water in a 1.5L Whirl-Pak® and homogenized in the same manner as the stock. After the free and total chlorine concentrations of a chlorinated water sample were quantified to give a t=0 reading, 1 ml of an organic compound stock (or $dH_2O$) was added to 999 ml of chlorinated water at different pHs. The combined solutions were sealed and incubated at room temperature (~ 20ºC) in the dark. After 60 minutes, two free and total chlorine measurements were taken for each sample. The experiment was repeated twice.

### *Effect of pH on the production of chlorine byproducts*

Three 500 ml samples of 40 mg/L free chlorine were made by adding 0.4 ml of 5% NaOCl to 499.6 ml dH2O. The pH of the samples were adjusted to 2.5, 6.0 and 9.3 using phosphoric acid and sodium hydroxide with 10 mM phosphate buffers. A stock solution of resorcinol was made by dissolving 16 mg of resorcinol (MP Biomedicals Inc., Solon, OH, USA) in 10 ml dH2O, and used immediately. A half milliliter of resorcinol stock was added to 99.5 ml of pH-adjusted chlorinated water to reach a final resorcinol concentration in each treated sample of 8 mg/L. The samples were poured into small bottles so that no headspace remained. The bottles were then capped with caps fitted with rubber septa and incubated at room temperature for 30 minutes before trihalomethane (THM) analysis was conducted. A total of 6 treatments (pH 2.5, 6.0, and 9.3 chlorinated water with and without resorcinol) were used.

### *Analysis of THM by GC-MS*

Analysis of the THM reaction products from chlorinated water samples with added resorcinol were determined by the Lab for Environmental Analysis, Department of Crop and Soil Sciences, UGA, Athens, GA ,U.S.A. The analytical unit used consisted of a HP 5890 Series II GC oven and a HP 5971 MSD and was equipped with a purge and trap sample introduction that consisted of a Teckmar 3000 concentrator and a Teckmar 2016 auto sampler. In addition to a blank and standard, 5 ml of each of the 6 different treatments was injected by plastic syringe into purge trap unit. The inlet temperature of the unit was set at 250°C, and the samples were injected into a HP-624 column (30 m x 0.32 mm inner diameter x 1.8 µm thickness). The initial oven temperature was set at 32° C for 2 minutes, then increased at a rate of 2°C/min to reach a final temperature of 80° C for 2 minutes. After 2 minutes, the temperature was increased at a rate of

20°C/min to 180°C, where the column was held for 1 minute. The total run time per sample was *34 min.* The set-up was also equipped with data processing and automatic liquid sampling units. Fragmentation patterns were analyzed by comparison to external standards.

### *Food product preparation*

Bunch romaine lettuce, precut and washed bagged romaine lettuce, strawberries, cherry tomatoes, beef and turkey breasts were purchased from 3 local supermarkets in Griffin, GA, U.S.A. All food products were stored at 4ºC until use. Both types of lettuce were used fresh and any wilted or discolored leaves were discarded. The lettuce was cut with clean scissors to approximately 1 $in^2$ pieces. 10 g of lettuce from each lot was placed in Whirl Pak® bags. An equal volume of $dH_2O$ was added to the bags. Three strawberries or three tomatoes were placed in a Whirl Pak® bag, and an equal volume of $dH_2O$ was added to the bags. Strawberries and tomatoes that appeared soft, discolored or non-intact were not used. Beef and turkey breasts were cut to yield portions of 40-50 g and placed in a Whirl Pak® bag. An equal volume of $dH_2O$ was added to the bags.

All the sample bags were placed on a rotary shaker for 20 minutes at 140 rpm. Portions of the wash water were tested for total phenolic concentration and total protein concentration as described in the following sections. Three replicate samples from 3 different lots of each food product (purchased from different locations) were used. Two measurements were made on each sample, and the experiments were done twice.

### *Test for total phenolics*

Total phenolic compounds were quantified using the Folin-Ciocalteu assay as outlined by Singleton and Rossi (1965). 1 ml of sample was added to 70 ml $dH_20$ in a 125 ml screw cap bottle then 5 ml Folin-Ciocalteu's phenol reagent (Sigma Aldrich Co., St Louis, MO, U.S.A.) was added to the solution. After mixing, 15 ml of a 20% (w/v) solution of sodium carbonate was added followed by enough water to bring the total volume to 100 ml. The mixtures were sealed and incubated for at least 2 hr at room temperature. The samples were then read at 750 nm in a 1 cm quartz cuvette using a DU 520 UV/Vis spectrophotometer (Beckman Coulter Inc., Brea, CA, U.S.A.). The total phenolic concentration of a test sample was calculated using catechol as a standard and reported as mg/L.

### *Test for total protein*

Total protein was quantified using the Bradford assay (Bradford, 1976). 0.1 ml of sample was mixed with 5 ml Bradford's reagent (Sigma Aldrich Co.). The samples were then read at 595 nm in a 1 cm quartz cuvette using a DU 520 Uv/Vis spectrophotometer (Beckman Coulter Inc.). The total protein concentration of a test sample was calculated using bovine serum albumen as a standard and reported as mg/L.

### *Effect of food wash water on free chlorine*

The effect of food wash water on free chlorine demand was tested by adding 1 ml of food wash water to 99 ml chlorinated water (40 mg/L free chlorine) at pH 2.5, 6.0 or 9.3. The chlorinated water solutions were made as described above. Free chlorine concentration before addition of food wash water and after 10 minutes incubation with food wash solution at room temperature was measured as described above.

### *Chlorine loss standards for model formation*

Dilutions of peptone and bovine serum albumen (BSA) were prepared in order to form standard curves of free chlorine loss versus protein concentration. Eight different stock concentrations (50, 100, 250, 500, 1000, 2000, 3000, and 4000 mg/L) of peptone or BSA (Sigma Aldrich Inc., St. Louis, MO, USA) were made by dissolving the reagents in $dH_2O$ and vortexing until suspended. One ml of a stock was added to 99 ml of chlorinated water at pH 2.5, 6.0 or 9.3 buffered with 10 mm phosphate buffer. After addition of the protein stock, the chlorinated water was incubated at room temperature for 10 minutes in sealed 250 ml screw top glass bottles. Free chlorine was quantified using the DPD-FEAS titrimetric test for free chlorine mentioned above. Free chlorine loss was recorded as the difference between the time = 0 and time = 10 min free chlorine readings.

Dilutions of catechol and resorcinol were prepared in order to form standard curves of free chlorine loss versus phenolic concentration. Six different stock concentrations (10, 20, 50, 100, 200, 300 mg/L) of catechol or resorcinol were made by dissolving the reagents in $dH_2O$ and vortexing until suspended. Free chlorine loss was determined as in the peptone and BSA protocol above.

### *Statistical analysis*

Data was subjected to an analysis of variance with a completely randomized factorial design. Statistical analysis was performed using SAS (2008) General Linear Model procedure performed with SAS Software Release 9.2 (SAS Institute, Cary, NC, USA). Regression models were generated for the food wash data using the PROC RSREG command in SAS, using backwards regression to find the model with the highest correlation coefficient and F value. Least significant difference of means tests were done for multiple comparisons, and all tests were performed with a level of significance of 0.05.

## Results and Discussion

### *Single organic compound*

Figure 6-1 shows the results of 3 organic sources (catechol, peptone and resorcinol) on free and total chlorine concentrations of chlorinated water. The direct comparison of free and total chlorine loss caused by the different treatment combinations (3 levels of pH and 3 types of organic compounds) reveals differences in disinfection byproduct production. For catechol, the loss of free chlorine was comparable to the loss of total chlorine at a particular pH. For example, the mean free chlorine and total chlorine loss (defined in this study as the concentration change in total chlorine, which includes chemical species such as $OCl^-$, HOCl ,$Cl_2$, and chloramine compounds) caused by catechol at pH 6.0 (34.3 mg/L and 35.8 mg/L, respectively) were not significantly different ($p > 0.05$). A possible explanation for this is that the main products of reactions involving free chlorine and catechol are phenol and substituted catechols (Michalowicz *et al.*, 2007), which may not be detected by the titrimetric method used to quantify total chlorine. Therefore, total chlorine losses will be similar to free chlorine losses.

A comparison of the free and total chlorine loss caused by resorcinol at each pH (Figure 6-1) reveals a pattern similar to catechol. In other words, the free chlorine loss at each pH (except for 2.5) is not significantly different from the total chlorine loss at the same pH. For example, the mean free and total chlorine loss caused by resorcinol at pH 9.3 was 11.7 mg/L and 10.7 mg/L., respectively. Like catechol, the reaction products of resorcinol (which are compounds such as chloroform and various other substituted aromatics and carboxyl compounds) (Gallard & von Gunten, 2002) may not be detected by the titrimetric method used to quantify total chlorine.

Peptone is the only one of the organic sources that shows a significant difference between the free and total chlorine losses for each treatment. At all 3 pH levels, the free chlorine loss exceeds the total chlorine loss (Figure 6-1). This is expected because the prevalent products of chlorine reactions with amino acids are chloramines, which are the result of nucleophilic attack of hypochlorite on the α amines (Pattison & Davies, 2001). Chloramines are readily detected as total chlorine and not free chlorine, so total chlorine losses will lag behind free chlorine losses.

Figure 6-1 shows that pH is a significant factor affecting the loss of free and total chlorine in solution. The mean loss of free chlorine caused by catechol at pH 2.5, 6.0, and 9.3 was 24, 34.3, and 38.1 mg/L, respectively. The loss of free chlorine caused by catechol is not significantly different between pH 6.0 and 9.3. This may be because catechol reacted with almost all chlorine at pH 6.0 and 9.3. The mean loss of free chlorine caused by peptone at pH 2.5, 6.0, and 9.3 was 9.5, 14.0, and 23.1 mg/L, respectively. Unlike catechol, all 3 pH treatments were significantly different from each other in terms of free chlorine loss. Due to low demand of peptone on chlorine, free chlorine was remaining at the end of the reaction time, so a better degree of differentiation between all the peptone treatments was possible.

The chlorinated water solutions treated with resorcinol do not follow the same pattern of free chlorine and total chlorine loss with respect to pH that catechol and peptone do. According to Figure 3-1, the free chlorine loss at pH 2.5, 6.0, and 9.3 is 10.3 mg/L, 12.8 mg/L, and 12.4

mg/L, respectively. The lack of oxidation reaction rate increase with increases in pH was noted by other researchers (Cimetiere, 2010). The lack of pH effect on free chlorine loss in this instance is possibly due to the site of the initial chlorination. In resorcinol, reactions with free chlorine are initiated by the electrophilic halogenation of resorcinol at the 2 carbon position. There is no indication that this reaction is affected by pH, although subsequent cleavage and haloform reactions after this chlorination are (Oliver & Lawrence, 1979).

The effect of pH on reaction rates observed in this study is consistent for the results obtained by other, previously mentioned, works for amino acids and phenolic compounds. The reason why pH is a factor in free chlorine loss in the instance of this study is that compounds at higher pHs are more easily oxidized in a more electron-rich (i.e. reduced) state.

In contrast to the chemical species on Figure 6-1, the food components in Figure 6-2 do not show a practically significant change in free chlorine concentration at the given conditions. The corn oil treatment at pH 2.5 shows the greatest free chlorine loss (1.9 mg/L), but the chlorine loss is minimal in comparison to the organic treatments in Figure 6-1. Despite the minimal free chlorine loss, there is a statistically significant difference between the 2 pH treatments using corn oil, with the free chlorine loss at pH 2.5 being greater than at 9.3. Hypochlorite is known to form chlorohydrins when reacted with unsaturated fatty acids (Winterbourn *et al.*, 1992), but it is unclear what role pH plays in the rate of chlorine incorporation into the fatty acid chain.

The chlorinated water samples treated with iron sulfate and starch do not show a significant loss of free chlorine in Figure 6-2. Previous works (Folkes *et al.*, 1995) have indicated that ferrous iron ions (from 3.8 mg/L iron sulfate) react with free chlorine rapidly. The lack of substantial free chlorine loss when iron sulfate is used as a treatment can be explained by the fact that the concentration of iron used in the experiment (2.5 mg/L) is insufficient to cause significant free chlorine loss, resulting in an excess of chlorine versus iron. Starch was previously indicated to not cause free chlorine loss (Oomori *et al.*, 2000), and our findings confirm this.

### *GC-MS analysis of disinfection byproducts formed from resorcinol*

Table 6-1 shows the partial results of a GC-MS analysis of trihalomethanes (THMs). As expected from previous studies (Norwood *et al.*, 1980), chloroform accounts for the largest percentage of THMs detected in all samples, indicating that it is the main THM product when chlorine reacts with organic materials. The data indicates that the pH 6 and 9.3 chlorinated water when reacting with resorcinol produce 10 times chloroform (9.54 mg/L and 12.4 mg/L, respectively) than chlorinated water at pH 2.5 (1.1 mg/L). While both of these numbers are beyond the theoretical yield of 8 mg/L (by the starting resorcinol concentration), it must be noted that machine reading error and column residue could contribute to the readings. All other THM products (dichloromethane, dibromochloromethane, dibromomethane, bromoform) and haloacetic acids (chloroacetic acid, bromoacetic acid, dichloroacetic acid, trichloroacetic acid, bromochloroacetic acid, dibromoacetic acid) were at µg/L level and were about 100 times less than chloroform.

According to the literature, pH is an important factor in the formation of THMs in chlorine solutions. In most cases, chlorination of resorcinol yields 2-chloro, 2,4-dichloro, and 2,4,6-trichloro resorcinolic compounds (Gallard & vonGunten, 2002; Rule *et al.*, 2005; Deborde & vonGunten, 2008). Hydroxide ions attack the resulting trihaloketone to yield chloroform and a halogenated carboxylic acid (Rook, 1979). The presence of hydroxide ions allows for aromatic ring cleavage and subsequent chloroform formation, so increases in hydroxide concentration (as seen when pH increases) would result in a quicker rate of chloroform formation. The results in this study appear to follow that trend, so it can be concluded that increases in pH increase the rate of chloroform formation from resorcinol.

### *Modeling free chlorine loss*

Figure 6-3 shows the free chlorine loss in chlorinated water at 3 pHs (2.5, 6.0, and 9.3) treated with strawberry wash water. The chlorine loss appears to follow the same trend in regards to pH that catechol and peptone showed in Figure 6-1. In contrast, the beef washes in Figure 6-4 do not show a discernible pH effect on free chlorine loss. Specifically, the mean free chlorine loss was 0, 3.3, and 6.5 mg/L at pH 2.5, 6.0, and 9.3, respectively for the strawberry wash solutions. The mean free chlorine loss in pH 2.5, 6.0 and 9.3 chlorinated water treated with beef wash was 32, 33 and 33 mg/L, respectively. The reason for this is likely explained by reaction kinetics related to concentration. The beef washes represented a higher chlorine demand (around 30 mg/L total phenolic and protein concentration) than the strawberry washes (less than 10 mg/L total phenolic and protein concentration), and the initial free chlorine concentration of the chlorinated water samples was 40 mg/L. Free chlorine may have been limiting in the chlorinated water samples treated with beef wash water, causing the free chlorine loss at the three pH levels to appear to be similar. Similarily, the free chlorine loss in the chlorinated water samples treated with turkey wash water was at 30, 37, and 36 mg/L for pH 2.5, 6.0 and 9.3, respectively (data not shown).

*Regression equations*

To model the effect of composition of different food wash solutions on chlorine loss, the two significant components (protein and phenolics) were used. These substances were used because they were determined in the individual organic experiments above to have the greatest impact on free chlorine. Two protein standards (peptone and bovine serum albumen (BSA)) and 2 phenolic standards (catechol and peptone) concentration and chlorine loss were calculated. The prediction equations are shown below:

$$\text{free Cl loss by BSA}=-4.67+0.43x+1.78z \qquad (1)$$

$$\text{free Cl loss by peptone}=0.92+0.38x-0.0008x^2 \qquad (2)$$

$$\text{free Cl loss by catechol}=-4.7+0.94y+2.93z+0.15yz-0.02y^2-0.27z^2 \qquad (3)$$

$$\text{free Cl loss by resorcinol}=-1.14+5.3y-0.16y^2 \qquad (4)$$

"X" is the concentration (in mg/L) of total protein in solution. "Y" is the concentration (in mg/L) of total phenolic compounds in solution, and "Z" is the pH (2.5, 6.0, or 9.3) of the solution. The R-square values for equations (1), (2), (3), and (4) were 0.97, 0.94, 0.94, and 0.95, respectively.

Table 6-2 is a comparison of the observed free chlorine loss as compared to the predicted chlorine loss of turkey wash solution using the 4 possible standard combinations for protein and phenolic standards as an example. The results in Table 6-2 are typical of the other food wash treatments, with a few exceptions. Given the range of the standard deviation for the measured values and all of the combination models, the standard that is the closest to the measures values is the peptone plus catechol standards.

Table 6-3 shows the comparison between observed free chlorine loss and predicted free chlorine loss using the peptone plus catechol model in all the food wash treatments. The table also reports the phenolic and protein concentrations as measured by the Folin-Ciocalteu and Bradford assays, respectively. The “diluted beef” treatment on Table 6-3 is the beef wash solution diluted by a 1:2 factor of beef wash solution to deionized water. This was done in order to provide a “middle” level of free chlorine loss because all the wash solutions used provided either very low (all the produce washes) or very high (all the meat wash solutions) free chlorine loss.

Table 6-3 demonstrates that the predicted values for free chlorine loss are higher than expected in instances of low free chlorine demand treatments. In the case of the bagged lettuce, the free chlorine loss is insignificant in the practical sense. For example, the observed free chlorine loss in the bagged lettuce samples at pH 6.0 was 2.38 mg/L while the predicted free chlorine loss was 4.45 mg/L. Since this trend does not appear to be linear (i.e. there is not a 2 fold difference between observed versus predicted free chlorine loss in samples with higher free chlorine demand treatments, such as beef and turkey), the peptone plus catechol model should be useful to predict chlorine loss when in contact of food materials.

In conclusion, this study has shown that model food components (peptone, catechol, resorcinol) and food wash water have an effect on the free chlorine concentration of chlorine solutions. The level of free chlorine loss in a chlorine solution is based on both the pH of the chlorine solution as well as the concentration and constituents (i.e. types of compounds present) of food wash water. Additionally, pH affects the rate of chlorine byproduct formation, as well as the type of chlorine byproduct formed. Increases in pH lead to the formation of chloroform as a chief THM product.

Generally, higher pH values of chlorine solutions correlate with greater free chlorine loss when exposed to organic matter. In addition to pH, the constituent compounds in food wash water are an important factor in free chlorine loss. Protein (as modeled by peptone) and phenolics (as modeled by catechol and resorcinol) caused significant chlorine loss of chlorinated water whereas carbohydrates (modeled by starch), fat (modeled by corn oil) and mineral (modeled by iron sulfate) had no significant effect on chlorine loss.

Modeling chlorine loss in systems with many factors, such as food washes, is not an exact process, but modeling can be useful to help predict free chlorine loss based on the main factors of chlorine loss in wash solutions. Also, modeling chlorine loss provides a means for industry to gauge the stability of a chlorine based sanitizer in different levels of chlorine demand. This study has demonstrated that it is possible to arrive at an estimate for free chlorine loss based on the protein and phenolic content of a wash solution. The combination model of peptone plus catechol seems to provide the closest prediction to measured free chlorine loss.

## Acknowledgments

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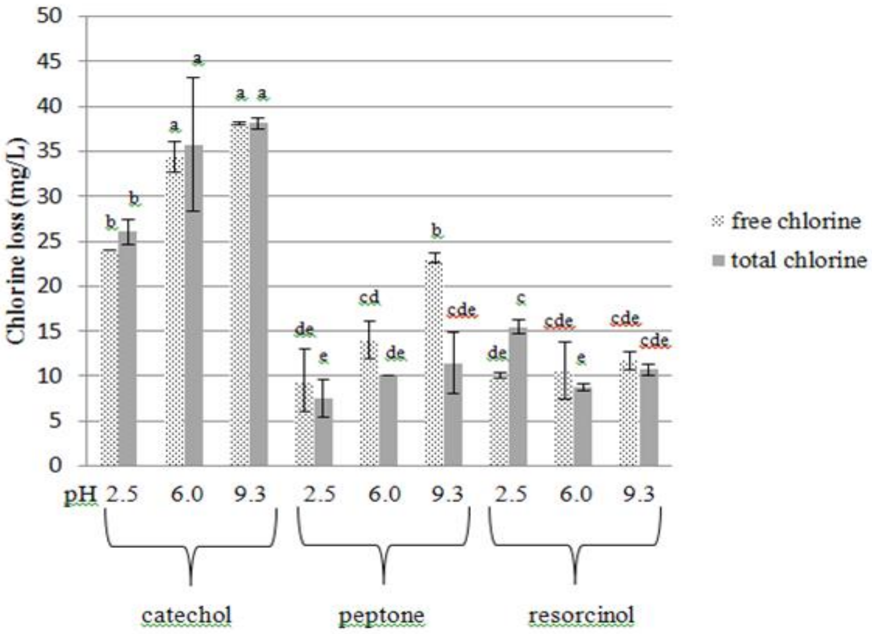

**Figure 6-1: Effect of catechol, peptone and resorcinol on free and total chlorine loss in chlorinated water at pH 2.5, 6.0 and 9.3.**

Error bars represent +/- standard deviation of 4 measurements

Means not labeled with the same letter are significantly different ($p \leq 0.05$)

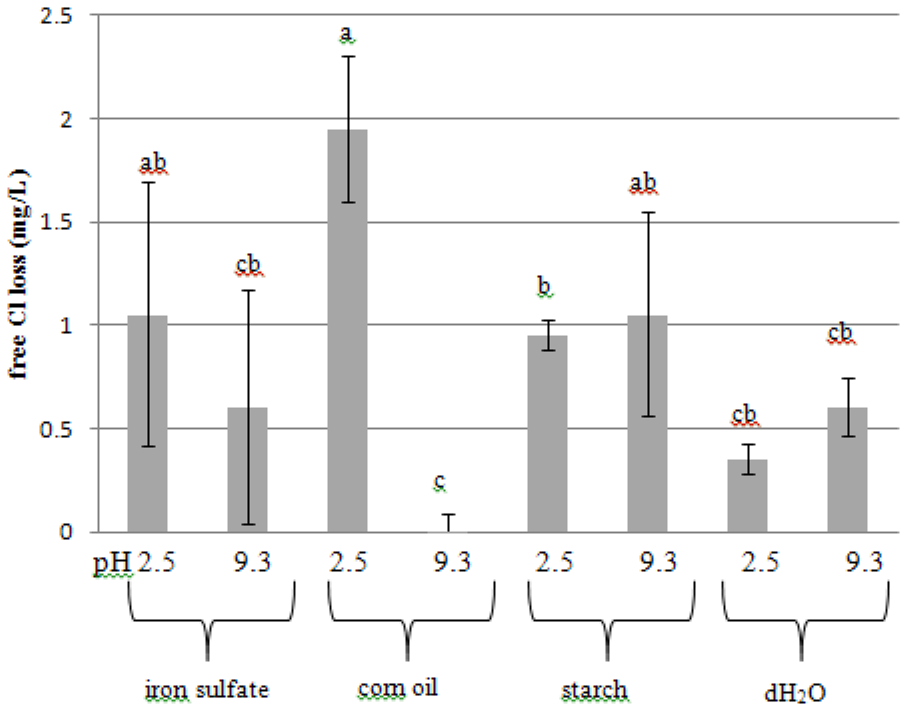

Figure 6-2: Effect of iron sulfate, corn oil, starch, and deionized water on free chlorine loss in chlorinated water at pH 2.5 and 9.3.

Error bars represent +/- standard deviation of 2 measurements

Means not labeled with the same letter are significantly different in terms of free Cl loss ($p \leq 0.05$)

# Table 6-1: GC-MS analysis of trihalomethanes (THMs) formed from resorcinol

| | Chloroform | Dichloromethane | Bromodichloromethane |
|---|---|---|---|
| | concentration (in mg/L) | | |
| **pH 2.5 chlorinated water** | 0.43 ± 0.51 b | 0 ± 0 a | 0.50 ± 0 b |
| **pH 6.0 chlorinated water** | 0.77 ± 0.57 b | 0.052 ± 0.067 a | 0.012 ± 0.002 b |
| **pH 9.3 chlorinated water** | 1.46 ± 0.83 b | 0.041 ± 0.058 a | 0.033 ± 0.016 a |
| **pH 2.5 chlorinated water with 8 mg/L resorcinol** | 1.10 ± 1.24 b | 0 ± 0 a | 0 ± 0 b |
| **pH 6.0 chlorinated water with 8 mg/L resorcinol** | 12.4 ± 8.35 a | 0.004 ± 0.004 a | 0.006 ± 0.004 b |
| **pH 9.3 chlorinated water with 8 mg/L resorcinol** | 9.54 ± 6.21 ab | 0 ± 0a | 0 ± 00 b |

Data is presented as a mean ± standard deviation of 2 measurements

Means not labeled with the same letter in a column are significantly different ($p \leq 0.05$)

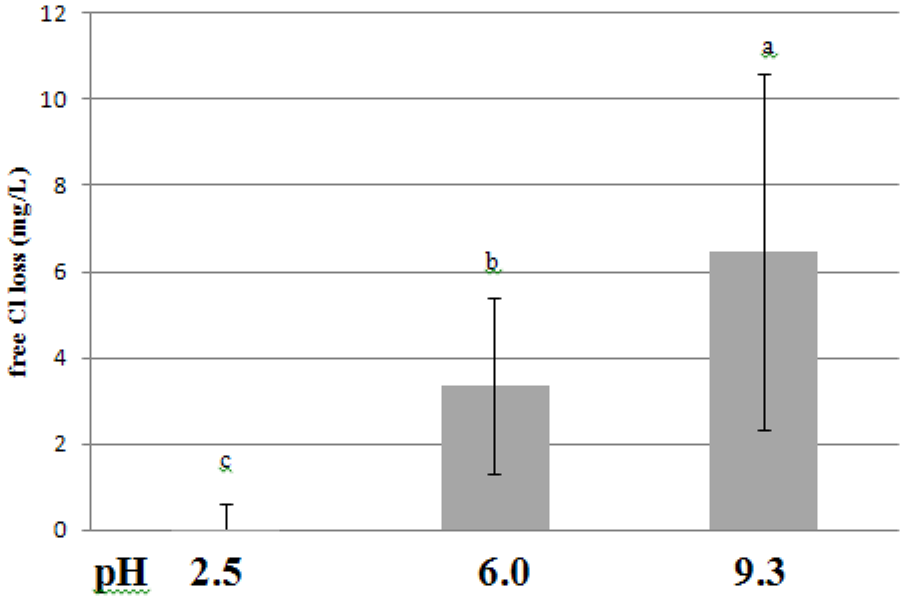

**Figure 6-3: Free chlorine loss of chlorinated water solutions at different pH when reacted with strawberry wash solution**

Error bars represent +/- standard deviation of 6 measurements

Means not labeled with the same letter are significantly different ($p \leq 0.05$)

Initial chlorine water concentration 40 mg/L

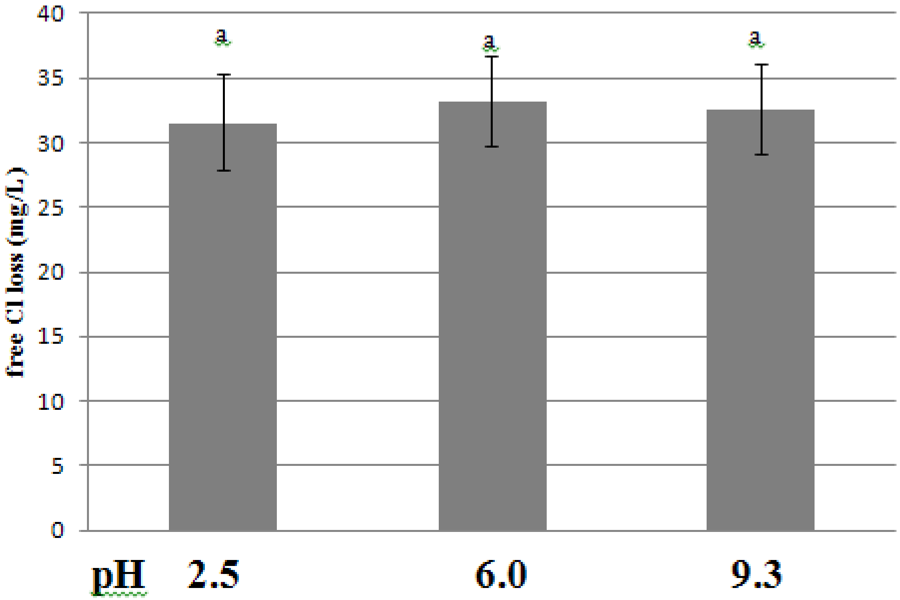

**Figure 6-4: Free chlorine loss of chlorinated water solutions at different pH when reacted with beef wash solution**

Error bars represent +/- standard deviation of 6 measurements

Means not labeled with the same letter are significantly different ($p \leq 0.05$)

Initial chlorine water concentration 40 mg/L

## Table 6-2: Observed free chlorine loss as compared to the predicted chlorine loss of turkey wash solutions

| pH | measured | pep + cat | pep + res | BSA + cat | BSA + res |
|---|---|---|---|---|---|
| 2.5 | 30.7 ± 2.51 b | 25.7 ± 5.34 c | 48.1 ± 7.08 a | 26.8 ± 5.59 bc | 49.1 ± 7.33 a |
| 6 | 36.7 ± 2.12 d | 36.0 ± 7.34 d | 49.9 ± 6.07 b | 43.3 ± 7.61 c | 57.2 ± 6.30 a |
| 9.3 | 36.0 ± 1.54 d | 37.2 ± 11.1 c | 48.1 ± 7.08 b | 50.4 ± 11.3 b | 61.2 ± 7.33 a |

Data reported as means +/- standard deviation of 6 measurements

Means not labeled with the same letter in each row are significantly different ($p \leq 0.05$)

Initial chlorine water concentration 40 mg/L

pep + cat = peptone and catechol combination model

pep + res = peptone and resorcinol combination model

BSA + cat = BSA and catechol combination model

BSA + res = BSA and resorcinol combination model

## Table 6-3: Observed free chlorine loss and predicted free chlorine loss using the peptone plus catechol model in all the food wash treatments

| Sample | pH | protein (mg/L) | phenol (mg/L) | measured chlorine loss (mg/L) | predicted chlorine loss (mg/L) |
|---|---|---|---|---|---|
| bagged lettuce | 2.5 | 0.40 ± 0.30 | 0.12 ± 0.09 | -0.40 ± 1.61 b | 2.16 ± 0.11 a |
| | 6 | | | 2.38 ± 1.00 b | 4.45 ± 0.13 a |
| | 9.3 | | | 1.36 ± 1.18 a | 0.54 ± 0.17 b |
| bunch lettuce | 2.5 | 0.45 ± 0.42 | 0.53 ± 0.12 | 0.06 ± 1.65 b | 2.66 ± 0.18 a |
| | 6 | | | 3.72 ± 1.32 b | 5.22 ± 0.22 a |
| | 9.3 | | | 3.38 ± 1.36 a | 1.52 ± 0.27 b |
| tomato | 2.5 | 0.17 ± 0.19 | 0.02 ± 0.02 | 0.03 ± 2.05 b | 1.98 ± 0.06 a |
| | 6 | | | 2.69 ± 2.22 b | 4.18 ± 0.07 a |
| | 9.3 | | | 0.82 ± 1.24 a | 0.23 ± 0.08 a |
| strawberry | 2.5 | 1.28 ± 1.98 | 1.42 ± 1.39 | -0.48 ± 1.08 b | 4.13 ± 2.12 a |
| | 6 | | | 3.34 ± 2.05 b | 6.90 ± 3.04 a |
| | 9.3 | | | 6.46 ± 4.14 a | 3.83 ± 3.53 a |
| beef | 2.5 | 23.2 ± 2.97 | 8.71 ± 2.11 | 31.5 ± 3.77 a | 20.1 ± 2.94 b |
| | 6 | | | 33.2 ± 3.49 a | 26.9 ± 4.04 b |
| | 9.3 | | | 32.6 ± 3.49 a | 27.2 ± 5.08 b |
| turkey | 2.5 | 30.0 ± 2.88 | 13.0 ± 5.64 | 30.7 ± 2.51a | 25.7 ± 5.31b |
| | 6 | | | 36.7 ± 2.12 a | 36.0 ± 7.34 a |
| | 9.3 | | | 36.0 ± 1.54 b | 37.2 ± 11.1b |
| diluted beef | 2.5 | 11.3 ± 2.21 | 3.72 ± 0.61 | 12.1 ± 2.58 a | 10.7 ± 1.36 a |
| | 6 | | | 10.9 ± 4.02 b | 15.5 ± 2.34 a |
| | 9.3 | | | 16.0 ± 2.24 a | 12.7 ± 1.94 b |

Data reported as means +/- standard deviation of 6 measurements

Means not labeled with the same letter in each row are significantly different ($p \leq 0.05$)

Initial chlorine water concentration 40 mg/L

# CHAPTER 7

# EFFECT OF CHLORINE-BASED SANITIZERS PROPERTIES ON CORROSION OF METALS COMMONLY FOUND IN FOOD PROCESSING ENVIRONMENT

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## Abstract

Corrosion of metal surfaces is a major concern when using chlorine-based sanitizers in a food processing environment. In order to gauge the effect of pH and chloride concentration on the corrosion of metal surfaces commonly found in a food processing environment, different metal samples (stainless steel, carbon steel, aluminum, and copper) were exposed to chlorinated water (CW) and electrolyzed oxidizing (EO) water. The samples were suspended in the CW and EO solutions in such a fashion as to observe corrosion on the metals completely submerged in the solution as well as above the solution’s surface. It was discovered that the pH and, to a lesser degree, the chloride of the CW and EO water samples played a significant role in mass loss for all the metal samples. Also, increases in surface roughness were linked to pH and chloride concentrations as well. Additionally, metal surfaces left suspended above the solution surfaces tended to show greater increases in surface roughness compared to the metal surfaces completely submerged in the solutions. This data demonstrates the pressing need for care when selecting a chlorine-based sanitizer for use in food processing environments.

## Introduction

Electrolyzed-oxidizing (EO) water is a low (~ 2.5) pH, high ORP (~ 1100 mV) chlorine-based sanitizer that contains hypochlorous acid as its primary antimicrobial compound (Kim et al., 2000; Venkitanarayanan et al., 1999). Additionally, EO water can be modified by mixing electrolyzed-reducing (primarily a sodium hydroxide solution) water with the EO water to produce slightly acidic EO water, which usually has a pH of 6.0 and an ORP of 900 mV (Cao et al., 2009). Despite the effectiveness of EO water in killing many types of foodborne pathogens, it is well known that chlorine-based sanitizers such as EO water may cause corrosion in susceptible metals.

Corrosion is defined as the process between a material and its environment that results in the degradation of the material. As such, corrosion is not so much a material's property as it is a response to the environment. The rate of corrosion depends on several environmental variables, such as pH and the concentration, as well as the identity, of chemical species within the material's environment (Fontana and Green, 1986).

The pH of a solution plays a rather complex role in the corrosion process. For example, pH's role in corrosion appears to be much more dominant when the $pH < 5$ as opposed to $5 < pH < 9$. However, some metals (namely aluminum and zinc) experience a large increase in corrosion rate when $pH > 9$ (Tomashov, 1966; Vujicic and Lovrecek, 1985). One of the reasons why there is complexity surrounding the role of pH in corrosion is because of counter ions. A counter ion (sometimes referred to as a conjugate base), can either slow down (i.e. $SO_4^{2-}$ from sulfuric acid) or speed up ($Cl^-$ from hydrochloric acid) corrosion at certain pH levels (Chin and Nobe, 1972; Ellison and Schmeal, 1978).

In terms of EO water in previous works (Ayebah and Hung, 2005; Dong et al., 2003), pH was discovered to play a large role in corrosion rate. All the metals (stainless steel, carbon steel, aluminum, copper) and dental alloys (Au-Ag-Pd and silver) tested in the above two 2 studies showed a higher rate of corrosion (as measured by mass loss and surfaces roughness changes) in EO water samples that had a low (< 3.0) pH.

Solution components such as chloride ions are also large factors that determine corrosion rates in chlorine-based sanitizers. Many studies have explored the role of chloride in the corrosion of various metals, especially stainless steel, and all seem to agree that increased chloride concentrations lead to higher rates of corrosion (Fang et al., 2011; Prawoto et al., 2009). It must be noted however, that these studies occurred in solutions other than chlorine-based sanitizers, so the role of chloride in EO water on corrosion still needs clarification.

The rate of corrosion of metals in the presence of chlorine-based sanitizers including EO water is a product of several closely-associated variables discussed above. Therefore, any attempt at corrosion research must account for all of these variables. The purpose of this study was to evaluate the corrosive effect of chlorine-based sanitizers with different properties on several metal surfaces commonly found in food processing environments.

## Materials and Methods

### *Metal sample preparation and cleaning*

Four different metals cut into strips (coupons) were used: 316 stainless steel (SS), ASTM A-36 medium carbon steel (CS), 3003-H14 aluminum (Al) and 110 copper (Cu) (University of Georgia Instrument Design and Fabrication Shop, Athens, GA, USA). All coupons were cut and polished (120 grit) in the same place they were purchased from so that they had a food grade finish. The coupons for each material were cut to be $^{1}/_{16}$” thick, ½” wide, and 3” long. Additionally, all coupons had a 1/4” hole drilled in the center, ¼” away from one end. Prior to use, the specimens were degreased by scrubbing each coupon with a bleach-free detergent powder (Alconox®, Alconox Inc., White Plains, NY, USA) using a brush with soft nylon bristles. After being rinsed in deionized water, the specimens were soaked in acetone and kept in a dessicator before use. The degreasing method is described in American Society of Testing and Materials (ASTM) standard G31-72 (ASTM, 2004).

### *Solutions used for corrosion testing*

Six types of chlorinated water were used in this study: pH 2.5 with and without 600 mg/L chloride added, pH 6.0 with and without 600 mg/L chloride added, and pH 9.3 with and without 600 mg/L chloride added. The chlorinated water samples were made by diluting 5% NaOCl in deionized water to yield a 40 mg/L free chlorine concentration. The pH for each chlorinated water sample was adjusted by the addition of 1N HCl. Chloride concentrations were adjusted by the addition of NaCl.

In addition to the 6 types of chlorinated water in this study, electrolyzed oxidizing (EO) water from 3 different generators were also used as treatment solutions in this study. The properties of the 5 different EO water solutions produced from the 3 different generators are described in Table 4-1. Samples “EO1” and “EO2” were produced from the first EO water generator with low chloride ion concentration and diluted with deionized water to yield a free chlorine concentration of 40 mg/L (from about 60 mg/L). “EO2” was produced from a second EO water generator and was used as is. “EO4” and “EO5” were produced from the third EO water generator with high chloride ion and chlorine concentration (160 mg/L free chlorine) and diluted with deionized water to yield a free chlorine concentration of 40 mg/L.

### *Measurement of solution properties*

Solution pH was measured using a digital pH/ORP meter (Accumet AR50, Fisher Scientific Co., Fair Lawn, NJ). Free chlorine was determined by the DPD-FEAS titrimetric method (Hach Scientific, Loveland, CO). Chloride ions were measured by a chloride ion probe. An Orion 9617BNWP Chloride Combination Electrode (Thermo Fisher Scientific Inc., Waltham, MA, U.S.A.) was used in the chloride measurements. These properties were measured and recorded after each solution was made.

### *Immersion tests*

A total of 77 coupons (11 treatment solutions for 7 weeks) for each metal per rep were used for the experiment. The coupons were immersed individually in Mason jars containing 400 ml of each test solution so that 25% of the surface area of each coupon is above the solution surface, leaving 75% submerged. The coupons were suspended by means of fluorocarbon string tied through the hole in each coupon. The ends of the string were anchored on the outsides of the jar.

After recording solution and coupon properties (weight and surface roughness), the experiment was started by suspending each coupon in test solution as described previously. Test solutions were replaced with freshly prepared solutions every 72 hours. After each week of exposure (and every week thereafter until no coupons are remaining), 11 coupons (one from each of the 11 different treatment solutions) from each of the 4 metal groups were removed from their solutions. Removed samples were visually inspected for signs of corrosion damage. Following the visual inspection, bulk corrosion products were cleaned off the coupons using a mild nonionic detergent (Alconox®) and a soft bristled brush. SS, CS, and Cu coupons were further cleaned by immersion in 10% HCl, and Al was cleaned by immersion in 10% $HNO_3$ according to the recommendations outlined in ASTM standard G1-03 (ASTM, 2013). After cleaning, the coupons were rinsed, dried, and surface roughness and mass were recorded. This experiment was repeated twice.

### *Measurement of coupon properties*

The mass, surface roughness and appearance of each coupon were evaluated both before the testing began and at the conclusion of the testing period. Mass was measured using an analytical balance and recorded to the nearest 0.0001 g. Surface roughness was measured using a Hommel T1000 surface roughness tester (Hommel, New Britain, CT, USA) with a diamond tip. The surface tester works by dragging a diamond-tipped probe across the metal surface. Surface roughness changes are read by up and down movements of the tip on the surface roughness tester’s probe arm. The up and down movements are converted to electric pulses, which are interpreted by the machine’s program.

There were 3 areas of interest on each coupon: the submerged area, the air/water interface, and the unsubmerged area. Three different parameters were used to represent surface roughness. The average of the absolute value of all peaks and pits in the measurement are referred to as the “Ra”, while the “Rmax” is the absolute value of the highest peak or deepest pit. The “Rz” value refers to the absolute value average of the largest 5 peak and valley measurements. The appearance of each coupon was also evaluated by microscopy, and micrograph images were taken to compare to images of the coupons that were taken prior to testing.

### *Statistical analysis*

Data was subjected to an analysis of variance with a completely randomized factorial design. Statistical analysis was performed using SAS (2008) General Linear Model procedure performed with SAS Software Release 9.2 (SAS Institute, Cary, NC, USA). Least significant difference of means tests were done for multiple comparisons, and all tests were performed with a level of significance of 0.05.

## Results and Discussion

### *Mass loss*

Mass loss on target materials is a widely-accepted measure of corrosion damage (ASTM, 2004). Overall, CS and Cu showed the greatest mass loss over a 7 week period (Table 7-2). The mass loss in Al is less, but roughly proportional to the CS and Cu mass loss. SS showed little to no mass loss during the testing period, and this was expected due to the degree of passivation that SS surfaces possess.

Mass loss in Al appears to be affected by chlorine solution pH predominantly. The 4 treatments with the largest mass loss were EO3, EO4, CW1, and CW1S (0.087, 0.074, 0.085, and 0.076g, respectively), and they were all around pH 2.5. The mass loss for all other treatments was significantly less. As a general trend, the mass loss follows the treatment pattern of significantly greater mass loss for solutions at pH 2.5 than at pH 6.0 or 9.3. The mass loss for solutions at pH 6.0 was not significantly different than for solutions at pH 9.3. This is expected because previous studies have shown that Al is more vulnerable to corrosion at low pH (Chatalov, 1952). Except EO1, the mass losses for EO3, EO4, CW1, and CW1S were at least 12 times greater than other treatment solutions. For EO1, although the pH was at 2.5, the mass loss compared to the 4 highest cases is only about half. The reason for EO1, having a much lower mass loss than EO3, EO4, CW1, and CW1S may be due to a much lower chloride ion concentration for EO1 than the other pH 2.5 solutions. Table 7-1 shows that the chloride concentration as measured by $Cl^-$ probe for EO1 (90 mg/L) is much less than the 4 highest mass loss treatments (912, 280, 357, and 871 mg/L for EO3, EO4, CW1, and CW1S, respectively). It is reasonable to assume that chloride concentration plays a role in mass loss here because other works (Zaida *et al.*, 2008) have demonstrated similar corrosion trends in aluminum.

Cu shows a similar pattern of mass loss to Al, but the differences of mass loss between acidic vs. near neutral and basic pH solutions are less defined. Like Al, the 5 largest mass losses were seen in the coupons treated with EO1, EO3, EO4, CW1, and CW1S (0.138, 0.415, 0.156, 0.337, and 0.316g, respectively). Unlike in Al, the mass loss in Cu is the greatest in EO3. This may be due to the increased chloride concentration present in EO3 (912 mg/L) versus CW1 (357 mg/L) and CW1S (871 mg/L). The differences in mass loss, and the implied differences in corrosion rates, among solutions with different chloride concentrations has some support in the work of Hong and Macauley (1997), which agrees that chloride concentration plays a role in corrosion rates.

CS shows similar mass loss patterns to Al and Cu because the 5 largest mass losses were seen in coupons treated with EO3, EO4, CW1, and CW1S (0.352, 0.447, 0.307, 0.419, and 0.417 g, respectively). These treatment solutions were all near pH 2.5. As a whole, the mass losses for CS are much greater than either Al or Cu, even when treatments that are above pH 2.5 are considered.  This fact suggests that there is not a great deal of practical difference between all the different treatments, even if there is a statistical difference.  The corrosion potential of carbon steel has been explored in other works with regards to pH and chloride concentration (Boucherit and Tebib, 2005).  It was found in that study chloride concentration was a more important factor in pitting corrosion potential than pH was.  The mass loss data collected in the present study suggests that chloride concentration and pH are both important factors in corrosion.  However, mass loss data is not a good gauge of the level of pitting corrosion, so further study on carbon steel is required to elucidate the mechanism of corrosion in the presence of chlorine-based sanitizers.

A similar study by Ayebah and Hung (2005) reached similar conclusions concerning mass loss in SS, CS, Al, and Cu.  More mass was lost in all cases (except for SS) at acidic pHs than at other, more alkaline pHs.  However, their study did not seek to elucidate the role of chloride in corrosion caused by chlorine-based sanitizers, whereas the effort to do this was made in this current study.

As stated previously, mass loss generally follows changes in pH and chloride concentration.  However mass loss doesn’t conform to pH and chloride levels the same for each metal.  For CS, one would expect the mass losses for EO 3, EO 4, and EO 1 to be wider apart than what they are (0.447, 0.307 and 0.352 g, respectively), given the differences in chloride concentration for the 3 solutions listed on Table 7-1.  The same situation is seen in Cu for EO 3, EO 4, and EO 1 as well (0.415, 0.156, and 0.138 g, respectively).  A possible explanation is EO4 was diluted before use, and this dilution changed the pH (from 2.5 to 3) and chloride concentration.  The higher pH and lower chloride ion concentration (280 vs 912 mg/L) may explain why the EO 4 solution caused less mass loss than EO 3 on Cu and CS.  In addition, this mass loss difference helps to illustrate that chloride concentration for some metals (such as Cu) needs to reach a certain concentration in order for the corrosion rate to increase rapidly. Evidence of this is in a study by Hong and Macauley (1997) wherein the leaching rate of copper increased from near 0 (at a chloride concentration of 177 mg/L) to 0.8 parts per million (ppm) (when the chloride concentration was 344 mg/L) in 2 hours. It is possible that a similar mechanism was occurring in both CS and Cu in this study.

The reason for observing mass loss in corrosion studies is that mass loss remains a good general indicator of corrosion. However, this study seeks to understand corrosion of metal surfaces exposed to chlorine-based sanitizers beyond the general sense. For that reason, surface roughness measurements were utilized as a method for gauging corrosion at specific, different regions on metal surfaces.

### *Surface roughness*

Surface roughness was used as a means of gauging corrosion amongst metal surfaces that experience constant chlorine-based sanitizer exposure (called submerged in this study) versus surfaces that receive intermittent exposure (interphase) and surfaces that are exposed to air above a sanitizer's surface (top). Table 7-3 gives a comparison of Ra, Rz, and Rmax change for aluminum over 7 weeks. Despite being different values, all 3 measures of surface roughness show the same general trends. The largest values for the 3 different measurements of surface roughness can be found in the order EO3 > CW 1S > EO 4 > CW 1 > EO 1. Also, the top and interphase values are greater than the sub values of surface roughness, which is the consensus for not only aluminum but the other 3 metals as well. Because there was no readily observable difference between the 3 measures of surface roughness and since Ra was considered a more comprehensive measure, the Ra value was used in this study for the analysis of surface roughness.

As implied by the mass loss data, SS appears to be unaffected by the CW and EO treatments over the course of the study. Table 7-4 shows that the Ra values for a specific treatment across the 3 regions of measurement (submerged, interphase, and top) are not significantly different for any of the 11 different treatments, as observed by reading the data by row. Also, there is no consistent difference in surface roughness at any of the 3 regions amongst the 11 treatments, as observed by reading the data in each column. In short, the data between treatments does not show a definite trend indicative of influence by treatment type. The surface roughness data for the stainless steel is in agreement with the mass loss data, in that both sets of data show little to no change, leading to the assumption that stainless steel is resistant to corrosion under the conditions set forth in the experiment.

Table 7-5 displays the surface roughness data for CS. In every treatment except for CW 3 and CW 3S, the Ra value for the top region is greater than the submerged region. In most cases, the Ra of the top region is higher than both the interphase and submerged regions. However, there are some instances where the top and interphase regions are not statistically different (CW 1 and CW 2), yet in every treatment the Ra of the top region is higher than the submerged region. The reason for this can be explained in 2 different ways. It was observed during the study that there was a buildup of corrosion products above the water line. These corrosion products are likely caused by “wet” chlorine gas in the humid environment of the container headspace. Wet chlorine gas can readily corrode steel (Craig and Anderson, 1995). Additionally, the corrosion products are found in deep pits and elevated peaks, so complete removal is difficult without using harsh treatments that can substantially change the base metal topography and composition.

As for the interphase measurements, there is a degree of fluidity in that in some circumstances the interphase measurements are more similar to the top measurements than the submerged measurements, and in other cases the interphase measurements have more in common with the submerged measurements than the top measurements. This can be explained by variations in filling volume (i.e. deviations from 400 ml) coupled with the extremely narrow range of measurement at the interphase region. This phenomenon could help to explain the variation not only in the instance of CS, but Al and Cu as well.

Like in the mass loss measurements, the surface roughness measurements for Al (Table 7-6) show well defined trends. There is a significant difference between the top Ra measurements versus the submerged Ra measurements for CW 1(2.344 µm), CW 1S (3.754 µm), EO 3 (4.731 µm), and EO 4 (2.786 µm). All other treatments do not appear to show a difference among the 3 Ra measurement regions. In addition to this, the Ra measurements for the treatments at each region show CW 1, CW 1S, EO 3, and EO 4 to be the 4 largest values. This data supports the mass loss data in suggesting that corrosion happens mostly in chlorine solutions at lower pH and higher chloride concentration. EO 1, despite having a low pH, has a low chloride concentration. This low chloride concentration likely resulted in the lower surface roughness values (0.747, 0.756, and 0.534 for the top, interphase and submerged positions, respectively). The surface roughness data for the aluminum is in agreement with the mass loss data, in that both sets of data show the exact trend in pH leading to changes in the aluminum, leading to the assumption that aluminum is resistant to corrosion under some of the higher pH conditions set forth in the experiment.

The difference in Ra between the 3 measured regions in copper (Table 7-7) does not appear to follow the trend of top > interphase > submerged present in CS and Al. This suggests that the treatments do not cause the pitting corrosion that appears to be present in CS and Al. In terms of pH, there is a general pattern of lower pH solutions resulting in coupons with greater changes in surface roughness (CW 1, CW 1S, EO 3, and EO 4 have the largest Ra changes out of all treatments). However, chloride concentration appears to play an important role in surface roughness changes in copper as well. The order of chloride concentration amongst the pH 2.5 treatments is EO 3 (912 ppm) > CW 1S (871 ppm) >> CW 1 (357ppm) > EO 4 (280 ppm) >EO 1 (90 ppm). The surface roughness changes at the top portion of the copper coupons is EO 3 (1.624 µm) > CW 1S (1.392 µm) > CW 1 (1.099 µm) > EO 4 (0.906 µm) > EO1 (0.770 µm). This similarity suggests that there is a positive correlation between chloride concentration and surface roughness changes in copper exposed to chlorine-based sanitizers at low pH. The surface roughness data for the copper is in agreement with the mass loss data, in that within both measurements, similar changes based on pH and chloride concentration of the solutions occur. To be more specific, the larger mass changes that occur in coupons exposed to low pH and high chloride samples are mirrored by changes in surface roughness at all 3 areas measured.

## Conclusions

As confirmed in the study, the pH of a chlorine-based sanitizer is a significant factor in determining the corrosion rate of different types of metals used in a food processing environment. To a lesser degree, chloride concentration plays an important part as well. Given this information, food processors must weigh the antimicrobial benefits of low pH chlorine-based sanitizers versus the potential of these solutions to damage metal surfaces. A possible solution would involve the use of near neutral (pH ~ 6) chlorine-based sanitizers, which show a decreased rate of corrosion versus more acidic chlorine solutions while still containing a significant amount of HOCl, the main antimicrobial compound in chlorine-based sanitizers such as EO water. Another possible solution would be to use an EO or chlorine solution with low chloride ions in order to limit the effect of chloride on corrosion of food processing equipment

Also important is the fact that the top portion (unsubmerged area) in the surface roughness measurements of the metal coupons showed a greater degree of roughness. This would suggest that surfaces above liquid levels are at greater risk of corrosion. A suggestion here would be to use a submerge treatment when using chlorine solutions and to keep head space to a minimum.

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## Table 7-1: Properties of chlorinated and EO water samples

| Sample | Salt addition | [free Cl] (mg/L) | pH | ORP | $[Cl^-]$(mg/L) |
|---|---|---|---|---|---|
| CW 1 | N | 40 | 2.5 | 1150 | 360 |
| CW 1S | Y | 40 | 2.5 | 1150 | 870 |
| CW 2 | N | 40 | 6.0 | 940 | 50 |
| CW 2S | Y | 40 | 6.0 | 940 | 400 |
| CW 3 | N | 40 | 9.3 | 690 | 30 |
| CW 3S | Y | 40 | 9.3 | 690 | 460 |
| EO 1 | N | 60, diluted to 40 | 2.5 | 1150 | 90 |
| EO 2 | N | 60, diluted to 40 | 6.0 | 950 | 80 |
| EO 3 | N | 40 | 2.5 | 1160 | 910 |
| EO 4 | N | 250, diluted to 40 | 3.0 | 1160 | 280 |
| EO 5 | N | 250, diluted to 40 | 7.2 | 690 | 500 |

Data presented in this table are representative values of samples used throughout the experiment and is not an average value

CW = chlorinated water

EO1&2 are EO water prepared from EO generator #1 at pH 2.5 and 6.0, respectively

EO3is EO water prepared from EO generator #2 at pH 2.5

EO4&5 are EO water prepared from EO generator #3 at pH 2.5 and 6.0, respectively

**Table 7-2: Mass loss over 7 weeks in different chlorinated and EO water solutions**

|  |  |  | **Mass loss (g)** |  |  |  |
|---|---|---|---|---|---|---|
| **Sample** | **Salt addition** | **pH** | **SS** | **CS** | **Al** | **Cu** |
| CW 1 | N | 2.5 | 2.0 x $10^{-4}$b | 0.419a | 0.085a | 0.337b |
| CW 1S | Y | 2.5 | 0.002b | 0.417a | 0.076a | 0.316b |
| CW 2 | N | 6.0 | 2.0 x $10^{-4}$b | 0.280cd | 0.001c | 0.084de |
| CW 2S | Y | 6.0 | 1.0 x $10^{-4}$b | 0.306bc | 0.001c | 0.046e |
| CW 3 | N | 9.3 | 1.0 x $10^{-4}$b | 0.231de | 0.006bc | 0.046e |
| CW 3S | Y | 9.3 | 3.0 x $10^{-4}$b | 0.225e | 0.004bc | 0.054e |
| EO 1 | N | 2.5 | 1.0 x $10^{-4}$b | 0.352b | 0.033b | 0.138cd |
| EO 2 | N | 6.0 | 1.0 x $10^{-4}$b | 0.304bc | 0.002bc | 0.083de |
| EO 3 | N | 2.5 | 0.008a | 0.447a | 0.087a | 0.415a |
| EO 4 | N | 3.0 | 0.003b | 0.307bc | 0.074a | 0.156c |
| EO 5 | N | 7.2 | 3.0 x $10^{-4}$b | 0.314bc | 0.003bc | 0.073e |

Data reported as means of 2 measurements

SS = stainless steel, Al = aluminum, CS = carbon steel, Cu = copper

CW = chlorinated water

EO1&2 are EO water prepared from EO generator #1 at pH 2.5 and 6.0, respectively

EO3is EO water prepared from EO generator #2 at pH 2.5

EO4&5 are EO water prepared from EO generator #3 at pH 2.5 and 6.0, respectively

Means not labeled with the same lowercase letter in each column are significantly different ($p \leq 0.05$)

## Table 7-3: Surface roughness changes (Ra, Rz, and Rmax) on aluminum over 7 weeks

| | | | Ra | | | Rz | | | Rmax | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sample** | **Salt addition** | **pH** | **sub** | **inter** | **top** | **sub** | **inter** | **top** | **sub** | **inter** | **top** |
| CW 1 | N | 2.5 | 1.45 | 2.35 | 2.34 | 12.6 | 23.75 | 21.2 | 23.0 | 37.1 | 30.5 |
| CW 1S | Y | 2.5 | 2.06 | 2.17 | 3.75 | 15.9 | 16.95 | 24.0 | 21.9 | 25.0 | 33.2 |
| CW 2 | N | 6.0 | 0.301 | 0.369 | 0.384 | 1.97 | 3.369 | 5.21 | 2.35 | 6.38 | 7.51 |
| CW 2S | Y | 6.0 | 0.336 | 0.356 | 0.415 | 2.45 | 2.879 | 3.65 | 3.52 | 6.01 | 5.28 |
| CW 3 | N | 9.3 | 0.662 | 0.410 | 0.349 | 5.54 | 3.166 | 2.86 | 6.59 | 4.22 | 4.20 |
| CW 3S | Y | 9.3 | 0.571 | 0.401 | 0.379 | 5.19 | 2.470 | 3.57 | 7.28 | 3.34 | 4.60 |
| EO 1 | N | 2.5 | 0.534 | 0.756 | 0.747 | 4.74 | 9.557 | 7.09 | 6.42 | 15.6 | 13.8 |
| EO 2 | N | 6.0 | 0.321 | 0.394 | 0.474 | 2.77 | 3.216 | 3.74 | 5.48 | 6.30 | 4.97 |
| EO 3 | N | 2.5 | 1.95 | 3.32 | 4.73 | 14.8 | 27.33 | 29.8 | 22.9 | 47.4 | 46.4 |
| EO 4 | N | 3.0 | 0.714 | 1.49 | 2.79 | 7.24 | 11.96 | 22.0 | 13.2 | 19.2 | 33.0 |
| EO 5 | N | 7.2 | 0.407 | 0.455 | 0.459 | 2.98 | 3.784 | 5.44 | 4.25 | 7.28 | 6.04 |

Ra = average surface roughness; Rz = top average surface roughness; Rmax= highest surface roughness value

sub = submerged, inter = interphase, top = top

CW = chlorinated water

EO1&2 are EO water prepared from EO generator #1 at pH 2.5 and 6.0, respectively

EO3is EO water prepared from EO generator #2 at pH 2.5

EO4&5 are EO water prepared from EO generator #3 at pH 2.5 and 6.0, respectively

all values are the results of 1 experiment

## Table 7-4: Average surface roughness (Ra) changes on stainless steel over 7 weeks

| Sample | Salt addition | pH | Ra (µm) submerged | Ra (µm) interphase | Ra (µm) top |
|---|---|---|---|---|---|
| CW 1 | N | 2.5 | A0.216bc | A0.238a | A0.214b |
| CW 1S | Y | 2.5 | A0.222bc | A0.215ab | A0.224b |
| CW 2 | N | 6.0 | A0.215bc | A0.188ab | A0.209b |
| CW 2S | Y | 6.0 | A0.256ab | A0.213ab | A0.206b |
| CW 3 | N | 9.3 | A0.241abc | A0.249a | A0.241ab |
| CW 3S | Y | 9.3 | A0.207c | A0.212ab | A0.194b |
| EO 1 | N | 2.5 | A0.205c | A0.205ab | A0.199b |
| EO 2 | N | 6.0 | A0.236abc | A0.224ab | A0.219b |
| EO 3 | N | 2.5 | A0.271a | A0.237a | A0.311a |
| EO 4 | N | 3.0 | A0.220bc | A0.207ab | A0.246ab |
| EO 5 | N | 7.2 | A0.199c | A0.216ab | A0.250ab |

Data reported as means of 2 measurements

CW = chlorinated water

EO1&2 are EO water prepared from EO generator #1 at pH 2.5 and 6.0, respectively

EO3is EO water prepared from EO generator #2 at pH 2.5

EO4&5 are EO water prepared from EO generator #3 at pH 2.5 and 6.0, respectively

Means not labeled with the same uppercase letter to the left of the number in each row are significantly different ($p \leq 0.05$)

Means not labeled with the same lowercase letter to the right of the number in each column are significantly different ($p \leq 0.05$)

## Table 7-5: Average surface roughness (Ra) changes on carbon steel over 7 weeks

| Sample | Salt addition | pH | Ra (µm) submerged | Ra (µm) interphase | Ra (µm) top |
|---|---|---|---|---|---|
| CW 1 | N | 2.5 | B1.598bcd | A2.279abc | A2.299cd |
| CW 1S | Y | 2.5 | B1.531bcde | B1.922bcd | A3.042bc |
| CW 2 | N | 6.0 | B1.304de | A2.885a | A3.181abc |
| CW 2S | Y | 6.0 | B2.280a | B1.611cd | A3.389abc |
| CW 3 | N | 9.3 | A1.774bc | A1.897bcd | A2.579bcd |
| CW 3S | Y | 9.3 | A1.906ab | B1.108de | AB1.475d |
| EO 1 | N | 2.5 | C1.324de | B2.432ab | A3.445abc |
| EO 2 | N | 6.0 | C1.194e | B2.289abc | A4.319a |
| EO 3 | N | 2.5 | B1.890b | AB2.261bc | A2.829bc |
| EO 4 | N | 3.0 | C1.360de | B2.282abc | A3.474ab |
| EO 5 | N | 7.2 | B1.471cde | B1.108e | A3.526ab |

Data reported as means of 2 measurements

CW = chlorinated water

EO1&2 are EO water prepared from EO generator #1 at pH 2.5 and 6.0, respectively

EO3is EO water prepared from EO generator #2 at pH 2.5

EO4&5 are EO water prepared from EO generator #3 at pH 2.5 and 6.0, respectively

Means not labeled with the same uppercase letter to the left of the number in each row are significantly different ($p \leq 0.05$)

Means not labeled with the same lowercase letter to the right of the number in each column are significantly different ($p \leq 0.05$)

## Table 7-6: Average surface roughness (Ra) changes on aluminum over 7 weeks

| Sample | Salt addition | pH | Ra (μm) submerged | Ra (μm) interphase | Ra (μm) top |
|---|---|---|---|---|---|
| CW 1 | N | 2.5 | B1.453b | A2.345b | A2.344c |
| CW 1S | Y | 2.5 | B2.060a | B2.174bc | A3.754ab |
| CW 2 | N | 6.0 | A0.301c | A0.369e | A0.384d |
| CW 2S | Y | 6.0 | A0.336c | A0.356e | A0.415d |
| CW 3 | N | 9.3 | A0.662c | B0.410e | B0.349d |
| CW 3S | Y | 9.3 | A0.571c | A0.401e | A0.379d |
| EO 1 | N | 2.5 | A0.534c | A0.756de | A0.747d |
| EO 2 | N | 6.0 | A0.321c | A0.394e | A0.474d |
| EO 3 | N | 2.5 | B1.948a | AB3.321a | A4.731a |
| EO 4 | N | 3.0 | C0.714c | B1.485cd | A2.786bc |
| EO 5 | N | 7.2 | A0.407c | A0.455e | A0.459d |

Data reported as means of 2 measurements

CW = chlorinated water

EO1&2 are EO water prepared from EO generator #1 at pH 2.5 and 6.0, respectively

EO3is EO water prepared from EO generator #2 at pH 2.5

EO4&5 are EO water prepared from EO generator #3 at pH 2.5 and 6.0, respectively

Means not labeled with the same uppercase letter to the left of the number in each row are significantly different ($p \leq 0.05$)

Means not labeled with the same lowercase letter to the right of the number in each column are significantly different ($p \leq 0.05$)

## Table 7-7: Average surface roughness (Ra) changes on copper over 7 weeks

| Sample | Salt addition | pH | Ra (μm) submerged | Ra (μm) interphase | Ra (μm) top |
|---|---|---|---|---|---|
| CW 1 | N | 2.5 | B0.779cde | A1.055abcd | A1.099bc |
| CW 1S | Y | 2.5 | A1.125bc | A1.262abc | A1.392ab |
| CW 2 | N | 6.0 | A1.041bcd | A1.279abc | A1.080bc |
| CW 2S | Y | 6.0 | B0.566e | B0.469f | A0.848c |
| CW 3 | N | 9.3 | A0.783cde | A0.853cdef | A0.832c |
| CW 3S | Y | 9.3 | A0.783cde | A0.782def | A0.694c |
| EO 1 | N | 2.5 | A0.839bcde | A0.968bcde | A0.770c |
| EO 2 | N | 6.0 | AB1.252b | A1.329ab | B0.805c |
| EO 3 | N | 2.5 | A1.749a | A1.422a | A1.624a |
| EO 4 | N | 3.0 | AB0.703de | B0.625ef | A0.906c |
| EO 5 | N | 7.2 | A0.614e | A0.799def | A0.811c |

Data reported as means of 2 measurements

CW = chlorinated water

EO1&2 are EO water prepared from EO generator #1 at pH 2.5 and 6.0, respectively

EO3is EO water prepared from EO generator #2 at pH 2.5

EO4&5 are EO water prepared from EO generator #3 at pH 2.5 and 6.0, respectively

Means not labeled with the same uppercase letter to the left of the number in each row are significantly different ($p \leq 0.05$)

Means not labeled with the same lowercase letter to the right of the number in each column are significantly different ($p \leq 0.05$)

# CHAPTER 8

# THE EFFECT OF PH AND CHLORIDE CONCENTRATION ON THE STABILITY AND ANTIMICROBIAL ACTIVITY OF CHLORINE-BASED SANITIZERS

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## Abstract

Chlorinated water and electrolyzed oxidizing (EO) water solutions were made to compare the free chlorine stability and microbicidal efficacy of chlorine-containing solutions with different properties. It was discovered that among chlorinated and EO water solutions at low pH (~2.5), the rate of free chlorine loss in samples stored under "open" conditions (i.e. exposed to air) was directly correlated with chloride concentration. In a similar experiment, the microbial log reduction of freshly made versus open stored (24 hr) of chlorinated and EO water to be influenced by pH and chloride concentration. Lower pH values resulted in a greater degree of free chlorine loss and, depending on the amount of free chlorine remaining in aged samples, greater numbers of surviving microbial cells after treatment. Higher pH values (~6.0) did not appear to have a significant effect on free chlorine loss or numbers of surviving microbial cells when fresh and aged samples were compared. This study found chloride levels in the chlorinated and EO water solutions had a significant effect on both free chlorine stability and its microbicidal efficacy in the low pH solutions. Greater concentrations of chloride in pH 2.5 samples resulted in decreased free chlorine stability and lower microbicidal efficacy.

**KEYWORDS:** electrolyzed water, free chlorine, antimicrobial,

## Introduction

Foodborne illness caused by microorganisms such as *Escherichia coli* O157:H7 is a significant problem in today's world. A variety of foods such as fresh produce (Beuchat, 1995) and meat (Doyle *et al.*, 1997) have been implicated in outbreaks associated with this organism. Therefore, it is considered a priority to find effective interventions to limit the threat of foodborne illness posed by *Esherichia coli* O157:H7.

Electrolyzed oxidizing (EO) water has been explored as a possible antimicrobial treatment in several studies. In EO water, free chlorine (HOCl, $OCl^-$ and $Cl_2$) is generated from the electrolysis of salt water solutions. EO water is characterized by its low pH (2.3 – 2.7) and a high oxidation – reduction potential (ORP) (> 1000 mV) (Len *et al*., 2000). EO water has been shown be effective in reducing the numbers of *E. coli* O157:H7, *Salmonella enteritidis*, *Listeria monocytogenes* and *Bacillus cereus* F4431/73 (Len *et al.*, 2000; Park *et al.*, 2004; Venkitanarayanan *et al.,* 1999). EO water has been used to treat a wide variety of foods such as vegetables (Hung *et al.*, 2010; Kim *et al*., 2003; Koseki *et al.*, 2004; Pangloli and Hung, 2011), fruit (Abassi and Lazarovits, 2006; Kim and Hung, 2012; Udompijitkul *et al*., 2007), meat (Northcutt *et al.,* 2007; Rahman *et al*., 2012), and seafood (Huang *et al.*, 2006; Ozer and Demirici, 2006; Xie *et al.,* 2012).

In addition to acidic EO water, so-called slightly acidic (or near neutral) EO water has also been explored as a possible microbicidal treatment. Slightly acidic EO water typically has a pH 6.0-6.5 and an ORP of >900 mV (Rahman *et al.*, 2010), and it is usually made by mixing acidic EO water with electrolyzed reducing water generated in the same EO water generator (Deza *et al*, 2003). The higher pH of slightly acidic EO water helps to decrease the risk of corrosion (Ayebah and Hung, 2005) and enhance the stability (Len *et al.*, 2000) while at the same time providing adequate microbicidal activity (Deza *et al*, 2003).

Despite the effectiveness of EO water, there remains concern about the efficacy of EO water when used in less than ideal situations. Several previous studies have investigated the stability of EO water (Rutala *et al.*, 1998) as it relates to antimicrobial activity. Park *et al.* (2004) studied the effect of pH and chlorine on EO water's antimicrobial activity and Len *et al.* (2002) studied the effect of pH on both chlorine loss and microbicidal efficacy. Earlier work (Waters and Hung, 2013) has shown that pH plays a significant role in free chlorine stability, but the role of chloride in stability and its effect on the microbicidal efficacy of chlorine-based sanitizers is not fully understood. These studies only focused on the role of pH in free chlorine stability and microbicidal activity, and there has been no work on what other factors (or combination of factors) in EO water affect its stability and, as a result, its antimicrobial activity.

Often, it is necessary to dilute EO water solutions or slightly acidic EO water solutions before use because the typical concentration for use on food is 50-200 ppm free chlorine (Cherry, 1999). It is speculated that diluting EO water can have an impact on its microbicidal efficacy beyond the reduction in free chlorine concentration, but there is little evidence in support or against that.

There are many EO water studies reported in the literature. However they were produced based on different equipment and sometimes different principles. Some EO water was generated at low pH (pH < 3.0) and some at slightly acidic (pH about 6). Some EO water samples contain low or high chloride concentrations; some produce at the chlorine concentration ready to use and some need to be diluted further before use. It is understood that both the pH and chloride concentration of EO and chlorinated water solutions can differ from one solution to another depending on how the solutions are made. Hence, the purpose of this stud is to examine how pH, dilution, and chloride concentration of chlorine-based sanitizers affect the solution stability and antimicrobial activity.

## Materials and Methods

### *Sample preparation for stability study*

To determine stability of free chlorine, chlorinated water was made by diluting 5% NaOCl (Ricca Chemical, Arlington, TX, U.S.A.) in 2 L deionized water. Electrolyzed-oxidizing (EO) water samples were generated from 3 different EO water generators. EO-1 and EO-2 were made using different settings on the same generator (generator #1) to yield acidic and near neutral EO water samples, respectively. Sample EO-3 was made using a different generator (generator #2) to produce acidic EO water, and samples EO-4 and EO-5 were made using a third generator (generator #3) to yield acidic and near neutral EO water samples, respectively. The pH of the chlorinated water samples (CW-1 and CW-2 on Table 8-1) was adjusted by the addition of 1N HCl and 1N NaOH, and the pHs (as well as ORP) of all samples were recorded by probes attached to an Accumet® AR50 Dual Channel pH/ion/conductivity meter (Thermo Fisher Scientific Inc., Waltham, MA, U.S.A.). An Orion 9617BNWP chloride combination electrode (Thermo Fisher Scientific Inc., Waltham, MA, U.S.A.) was used for the chloride measurements. Free chlorine was measured by the DPD-FEAS titrimetric method (Hach Company, Loveland, CO, U.S.A.). All samples were used within one hour.

### *Stability study*

Two liters of all the chlorinated and EO water samples on Table 8-1 were prepared as described above. The 2 L samples were placed in 2 L screw cap bottles and magnetically stirred constantly for 48 hr under open conditions (cap off). The pH, ORP and free chlorine concentrations of each sample were measured every 1, 4, 8, 24, and 48 hours as described in the previous section. This experiment was repeated twice.

### *Culture preparation*

A 5 strain cocktail of *E. coli* O157:H7 which consisted of CDC-658 (human feces isolate from a cantaloupe-associated outbreak), E-19 (calf feces isolate), F-4546 (human feces, alfalfa associated outbreak), H-1730 (human feces, lettuce associated outbreak), and LJH-557(apple cider outbreak) was made by growing 10 ml cultures of each strain in tryptic soy broth (TSB) over 24 hr at 37°C. Before the experiments, at least 2 successive 24h transfers of each *E. coli* O157:H7 isolate were prepared in TSB at 37°C. After growth, the cultures were sedimented by centrifugation at 2000 x g at 22°C for 15 min. Following centrifugation, the supernatant fluids in each culture were discarded, and the cells were resuspended in 10 ml of 0.1% peptone. Equal volumes of each isolate were combined to make the cocktail.

### *Preparation of chlorinated water and EO water for antimicrobial study*

Five hundred ml of each chlorinated and EO sample as presented on Table 5-1 were created. The pHs of the chlorinated water samples (CW-1, CW-1S, CW-2, and CW-2S) were adjusted by the addition of phosphoric acid to avoid the addition of chloride. The chloride ion concentrations of samples CW-1S and CW-2S were modified by the addition of 0.3 g NaCl per

500 ml of chlorinated water. EO water samples were generated using 3 different EO water generators. EO-6 and EO-7 were generated by EO water generator #4 (an upgraded version of generator #1) and used as is. EO-6D and EO-7D were made using generator #4 at a chlorine concentration of 120 mg/L and diluted with deionized water to 40 mg/L. EO-3 was made using generator #2 and used as is. EO-4D and EO-5D were made using generator #3 at a free chlorine concentration of 140 mg/L and then diluted to 40 mg/L using deionized water. All properties listed on Table 5-2 (pH, free chlorine and chloride) were measured as described in the stability study sample preparation above. Two hundred fifty ml of the prepared chlorinated and EO water solutions was used "fresh" while the other half was "aged" artificially (i.e. at an accelerated rate) by agitating a 500 ml screw cap bottle (with the cap left off) containing the solution for 24 hr at 120 rpm using an orbital platform shaker (C10 platform shaker, New Brunswick Scientific, Edison, NJ, USA).

### *Antimicrobial properties*

To determine the effects of storage, aged EO water and chlorinated water samples treated as described above were used to treat *E. coli* O157:H7 cocktails as described in this section. A volume of 1 ml of the mixed *E. coli* O157:H7 culture was added to screw cap tubes containing 9 ml EO water or chlorinated water. The culture solution in the tubes was mixed by vortex for 5 sec, and incubated at room temperature for 1 min. After 1 min, the culture solutions were treated with 2X D/E broth in a Whirl Pak bag (Nasco, Fort Atkinson, WI, USA). The culture solutions were serially diluted in 0.1% peptone water and plated on TSA supplemented with 0.1% sodium pyruvate. The plates were incubated at 37°C for 24 hr prior to counting colonies.

### *Statistical analysis*

Statistical analysis was performed using SAS (2008) General Linear Model procedure performed with SAS Software Release 9.2 (SAS Institute). T-tests were used for pairwise comparisons. Least significant difference of means tests were done for multiple comparisons, and all tests were performed with a level of significance of 0.05. Two replications of each experiment (the stability and antimicrobial activity experiment) were done, and two measurements were taken for each sample on each test.

## Results and Discussion

### *Solution stability*

The chlorinated and EO water solutions chosen for this part of the study all had pH values that were near 2.5 or 6.0 (Table 8-1). Besides this, the solutions differed most in terms of chloride concentration. Figure 8-1 shows the results of a study monitoring free chlorine loss over time in solutions at different pH and containing different levels of chloride ions. All of the pH 6.0 samples of chlorinated and EO water (CW-2, EO-2, and EO-5D) showed a constant free chlorine concentration over 48 hours, signifying little to no loss of free chlorine. This is expected because at least one previous study (Len *et al.*, 2002) has noted that chlorine loss is reduced at pH > 6. It is known that chlorine solutions at pH > 3.0 do not contain any free chlorine as $Cl_2$. Since most free chlorine is lost through $Cl_2$, it is reasonable to assume that the main reason why so little free chlorine is lost at pH 6 is due to the relative stability of HOCl and $OCl^-$ versus $Cl_2$.

The pH 2.5 samples can be divided into 2 general groups: a "high" chloride group (EO-3 and EO-4D, with chloride concentrations of 460 and 390 mg/L, respectively) and a "low" chloride group (CW-1 and EO-1, both with chloride concentrations of approximately 150 mg/L). The high chloride group shows a greater degree of free chlorine instability over 48 hours as compared to the low chloride group. In both of the high chloride samples, the free chlorine was almost gone after 48 hours. In contrast, there was more than 20 mg/L of free chlorine remaining after 48 hours for both of the low chloride samples. At pH 9-14, this loss of free chlorine could be explained by $Cl^-$ serving as a catalyst in free chlorine decomposition (Adam and Gordon, 1999). This is unlikely in this study due to both the pH differences (2.5 in this study versus 9-14) and time (48 hours in this study versus > 100 days). The most likely explanation of the higher rate of free chlorine loss in the solutions with higher $Cl^-$ is described by the reaction:

$$HOCl + H_3O^+ + Cl^- \rightarrow Cl_2 + 2H_2O \qquad (1)$$

Equation (1) models the decomposition of HOCl in strongly acidic ($pH < 3.0$) conditions (Lister ,1952). In this study, the case as described by (1) can lead to free chlorine loss because the increased levels of $Cl^-$ in the high chloride solutions can lead to increased production of $Cl_2$, which in turn leaves solution (Whitney and Vivian, 1941) due to open container and the solution is agitated. It is unknown how much free chlorine is expected to be lost given standardized conditions and varying concentrations of $Cl^-$. More work needs to be done in order to determine the details of the loss relationship.

### *Antimicrobial activity*

Table 8-3 shows the log reduction data for the *E. coli* O157:H7 cocktails in the chlorinated and EO water solutions described in Table 5-2. The chlorinated and EO water solutions described in Table 8-2 can fit in several different categories, based on pH and $Cl^-$ concentration: low pH/low $Cl^-$ (CW-1, EO-6, EO-6D, EO-4D), low pH/high $Cl^-$ (CW-1S, EO-3), near neutral pH/low $Cl^-$ (CW-2, EO-7, EO-7D), and near neutral pH/high $Cl^-$ (CW-2S, EO-5D).

It should be noted that the levels of free chlorine recorded in “aged” versus “fresh” samples showed the same trend in terms of residual chloride (Table 8-2) as it relates to free chlorine loss as reported on Table 8-1. In other words, the free chlorine loss was greater in the chlorinated and EO water solutions that contained the most residual chloride and at low pH. In EO-3 and CW-1S, no free chlorine was measured after 24 hr. This contrasts with CW-1 and EO-4D wherein free chlorine was still detected (6.25 and 9.25 mg/L, respectively). Even more free chlorine was detected in EO-6 and EO-6D (15.0 and 20.3 mg/L, respectively), which contained the lowest concentration of chloride ions of all EO water samples near pH 2.5. Also, like in the stability study, little to no free chlorine was lost in all the chlorinated and EO water samples at or near pH 6.0 (Table 8-2).

The data on Table 8-3 demonstrate that both pH and $Cl^-$ concentration determine antimicrobial efficacy in fresh and aged EO and chlorinated water solutions. All but one of the low pH/low $Cl^-$ solutions show 8-9 log reductions in cell numbers in both the aged and fresh solutions, with aged EO-4D being the sole exception. In EO-4D, the log reduction drops from 9.12 to 5.20 from the fresh to aged samples. One possible explanation of the differences in aged EO-4D versus the other low pH/low $Cl^-$ solutions is that the $Cl^-$ concentration is high in EO-4D, according to Table 8-3 (262 ppm versus < 70 ppm for the others). However, this difference does not seem to have a practically significant effect on free chlorine loss versus other similar solutions. For example, the free chlorine concentration of aged CW-6 was 6.25 mg/L (Table 8-2), while the free chlorine of aged EO-4D was 9.25 mg/L. Despite this difference, log reductions in CW-6 and EO-4D were 9.08 and 5.20, respectively. Given this data, it is unlikely that $Cl^-$ as it relates to free chlorine stability was the main factor here. Another possible explanation is that EO-4D was diluted (approximately 3-4 times) to adjust the free chlorine from 140 mg/L to 40 mg/L. Diluting samples of EO water does not only reduce the levels of free chlorine but also its properties other antimicrobial agents produced in the electrolysis process. For example, oxidation reduction potential (ORP) is a value that translates into the ratio of oxidized to reduced species in solution. Dilution may not affect the absolute ORP values, but dilution can reduce the concentration of oxidants in solution. EO-6D was diluted from 120 mg/L free chlorine to 40 mg/L free chlorine while EO-4D was diluted from 140 mg/L free chlorine to 40 mg/L free chlorine, so a reduction of oxidant concentration is possible in these two cases. Other studies (Jeong *et al.*, 2006) have investigated the presence of reactive oxygen species (ROS) in EO water, so dilution of EO water may reduce the ROS in the solution.

In contrast to the aged low pH/low $Cl^-$ solutions, the aged low pH/high $Cl^-$ solutions (CW-1S and EO-3) showed next to no reduction in cell numbers (Table 8-3). It is easy to see why this is the case because Table 8-2 shows that CW-1S and EO-3 have no detectable free chlorine after being aged for 24 hr. This data is in agreement with other data in this study that reports higher reductions in free chlorine amongst solutions with high $Cl^-$ versus solutions with low $Cl^-$ concentrations (Figure 8-1). Thus, it is apparent by these data that $Cl^-$ concentration in any free chlorine-containing solution regardless of origin (i.e. CW or EO water) is an important consideration in stability and hence antimicrobial activity because higher levels of $Cl^-$ in solution lead to greater degrees of instability of free chlorine in solution. However, this observation only seems to apply to free chlorine solutions at strongly acidic pH (i.e. $\leq 3.0$).

The near neutral pH/low $Cl^-$ and near neutral pH/high $Cl^-$ solutions do not appear to differ greatly from each other in terms of log reduction of cell numbers. With the exception of EO-7, all the near neutral pH/low $Cl^-$ and near neutral pH/high $Cl^-$ solutions (CW-2, EO-7D, CW-2S, EO-5D) show similar fresh and aged sample log reductions of *E. coli* O157:H7 (between 4.61 and 5.4 log reductions, Table 8-3). Also, the near neutral pH/low $Cl^-$ and near neutral pH/high $Cl^-$ solutions listed above contain similar levels of chlorine in the fresh solutions (between 37.5 and 41.5 mg/L free chlorine) as in all the acidic pH solutions (between 38.5 and 43.8 mg/L). Despite this, there is 3-4 log less reduction in the near neutral samples compared to the acidic samples. The reason for this is that chlorine solutions at pH > 6 contain more $OCl^-$, which is not as effective a microbicidal agent as HOCl (Rudolph and Levine, 1941. For the aged solutions, it is reasonable to assume that these solutions all have similar microbicidal efficacy to the fresh solutions due to the fact that both the fresh and aged samples show little reduction in free chlorine (< 12 mg/L free chlorine loss in aged versus fresh, Table 8-2). Also noteworthy is that all the near neutral treatments mentioned previously contained a broad range of $Cl^-$ concentrations (51 to 749 mg/L, Table 8-2) but showed similar *E.* coli O157:H7 cell log reductions. These results provide evidence that $Cl^-$ is not directly related to the microbicidal efficacy of chlorine-based sanitizers and increased pH leads to decreased antimicrobial activity in chlorine solutions.

The reason why EO-6 is different is likely due to its pH. In comparison, the pH of EO-6 is much lower (4.38 fresh and 4.43 aged according to Table 8-2) than the other near neutral pH/low $Cl^-$ and near neutral pH/high $Cl^-$ solutions (> 6 for both fresh and aged samples). At such a low pH there is significantly more HOCl in comparison to free chlorine-containing solutions that have a pH greater than 6 (more $OCl^-$). A higher ratio of HOCl versus $OCl^-$ leads to higher numbers of cell death in cultures treated with EO and chlorinated water solutions as reported in the literature (Len *et al.*, 2000; Park *et al.*, 2004; Xiong *et al.*, 2010). Additionally, there is predicted to be very little $Cl_2$ in free chlorine-containing solutions at pH ~4.3, so there is greater free chlorine stability, as evidenced by the 37.5 and 31.5 free chlorine concentrations in the fresh and aged samples, respectively. Given these 2 facts, it is reasonable to assume that the EO-6 solution has both higher free chlorine stability and antimicrobial efficacy than the other near neutral pH/low $Cl^-$ and near neutral pH/high $Cl^-$ solutions.

## Conclusions

From the data, it can be concluded that low pH chlorine-based sanitizers have stronger microbicidal properties than near neutral pH chlorine-based sanitizers. Also, low pH chlorine-based sanitizers are unstable and can lose a significant amount of chlorine during storage. The level of chlorine loss in low pH chlorine-based sanitizers is dependent on the level of chloride ions in solution. Diluting chlorine based sanitizers can change properties such as oxidant concentration and pH, and this could directly affect microbicidal efficacy. In working with chlorine-based sanitizers that use species of free chlorine as the primary antimicrobial agents, both pH and $Cl^-$ are important considerations. While pH directly affects the antimicrobial activity of free chlorine, $Cl^-$ affects the chlorine stability and indirectly affects the antimicrobial activity. Since chlorine based sanitizers are sometimes prepared ahead of time and used as needed in food processing environments, it is important to understand that the level of residual $Cl^-$ in such a sanitizer could reduce its antimicrobial efficacy over time. Depending on how the CW or EO water is prepared, there could be a great difference in pH, whether dilution is needed and amount of chloride in solution. Therefore, one way to maximize the effectiveness of chlorine based sanitizers is to ensure that the pH of the solution is low when no storage is involved. When EO water needs to be stored for a brief period, the solution should be kept at low pH and low residual chloride. When long term storage is needed, the pH of the solution should be maintained at near neutral pH, but a higher amount of EO water should be used due to lower microbicidal properties than the lower pH solutions.

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### Table 8-1: Chlorinated and electrolyzed oxidizing water solutions used in stability experiments

| **Sample** | **pH** | **ORP** | **[free Cl] (mg/L)** | **[Cl-] (mg/L)** |
|---|---|---|---|---|
| CW-1 | 2.80 | 990 | 37 | 150 |
| CW-2 | 6.05 | 960 | 36 | 50 |
| EO-1 | 2.75 | 1070 | 36 | 150 |
| EO-2 | 6.40 | 730 | 20 | 80 |
| EO-3 | 2.60 | 1140 | 34 | 460 |
| EO-4D | 2.80 | 1070 | 50 | 390 |
| EO-5D | 5.8 | 950 | 30 | 500 |

Chlorinated water samples = CW-1 and CW-2

EO-1 and EO-2 are acidic and near neutral (respectively) EO water made using generator #1

EO-3is acidic EO water made using generator #2

EO-4D and EO-5D are acidic and near neutral (respectively) EO water made using generator #3 at 140 mg/L and diluted with deionized water

Data is representative of all samples made throughout the course of the experiments and is not an average

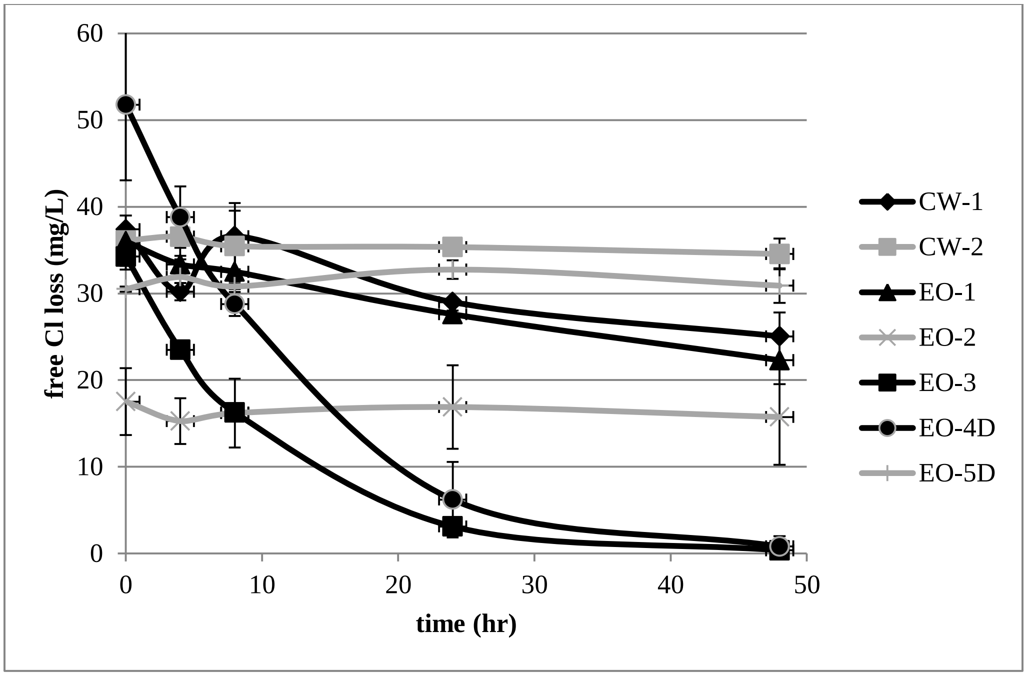

**Figure 8-1: Changes in artificially aged free chlorine concentration in chlorinated water and EO water samples over time**

Chlorinated water samples = CW-1 and CW-2

EO-1 and EO-2 are acidic and near neutral (respectively) EO water made using generator #1

EO-3is acidic EO water made using generator #2

EO-4D and EO-5D are acidic and near neutral (respectively) EO water made using generator #3 at 140 mg/L and diluted with deionized water

Error bars represent standard deviation

## Table 8-2: Chlorinated and electrolyzed oxidizing water solutions

| Sample | pH | | ORP | [free Cl] (mg/L) | | [Cl-] (mg/L) | |
|---|---|---|---|---|---|---|---|
| | fresh | aged | fresh | fresh | aged | fresh | aged |
| CW-1 | 2.50 ± 0.17 | 2.57 ± 0.21 | 1150 | 43.8 ± 1.06 | 6.25 ± 1.06 | 62.4 ± 18.8 | 36.1 ± 14.9 |
| CW-1S | 2.50 ± 0.17 | 2.49 ± 0.20 | 1150 | 43.8 ± 1.06 | 0.00 ± 0.00 | 729 ± 178 | 702 ± 46.0 |
| CW-2 | 6.28 ± 0.05 | 6.17 ± 0.03 | 945 | 41.5 ± 11.3 | 31.5 ± 3.54 | 39.5 ± 29.2 | 51.5 ± 7.21 |
| CW-2S | 6.28 ± 0.05 | 6.2 ± 0.16 | 945 | 41.5 ± 11.3 | 29.8 ± 5.30 | 699 ± 231 | 740 ± 21.1 |
| EO-3 | 2.78 ± 0.09 | 2.84 ± 0.11 | 1130 | 43.0 ± 4.24 | 0.00 ± 0.00 | 1215 ± 191 | 1200 ± 70.7 |
| EO-4D | 3.10 ± 0.02 | 3.41 ± 0.00 | 1100 | 40.0 ± 0.00 | 9.25 ± 0.35 | 294 ± 57.3 | 263± 24.8 |
| EO-5D | 7.17 ± 0.30 | 6.95 ± 0.18 | 900 | 40.0 ± 0.00 | 34.0 ± 8.49 | 801 ± 381 | 749 ± 294 |
| EO-6 | 3.09 ± 0.01 | 3.18 ± 0.01 | 1110 | 38.5 ± 7.07 | 15.0 ± 1.41 | 76.6 ± 30.8 | 78.1 ± 12.8 |
| EO-7 | 4.38 ± 0.66 | 4.43 ± 0.30 | 1000 | 37.5 ± 2.83 | 31.5 ± 2.83 | 63.9 ± 14.0 | 87.1 ± 2.97 |
| EO-6D | 3.20 ± 0.02 | 3.28 ± 0.01 | 1100 | 40.0 ± 0.00 | 20.3 ± 1.06 | 52.5 ± 23.0 | 56.8 ± 3.89 |
| EO-7D | 6.82 ± 1.07 | 6.60 ± 0.75 | 900 | 40.0 ± 0.00 | 31.8 ± 6.72 | 48.1 ± 17.9 | 62.5 ± 2.55 |

ORP values are representative of free chlorine solutions at the pH listed

Chlorinated water samples = CW-1, CW-1S, CW -2, CW -2S

EO-3 is EO water prepared from EO generator #2 at pH 2.5

EO-4D & EO-5D are EO water prepared from EO generator #3 at pH 2.5 and 6.0, respectively, at 140 mg/L chlorine concentration and diluted with deionized water

EO-6 & EO-7 are EO water prepared from EO generator #4 at pH 2.5 and 6.0, respectively

EO-6D & EO-7D are prepared from EO generator #4 at pH 2.5 and 6.0, respectively, at 120 mg/L chlorine concentration and diluted with deionized water

Data is presented as mean of 2 repetitions ± standard deviation

## Table 8-3: Reduction of E. coli O157:H7 in fresh and aged CW and EO water

| **Sample** | **log reduction (CFU/ml)** | |
|---|---|---|
| | **fresh** | **aged** |
| CW-1 | 9.12a | 9.08a |
| CW-1S | 9.12a | 0.24c |
| CW-2 | 4.96b | 4.61b |
| CW-2S | 5.40b | 5.27b |
| EO-3 | 9.12a | 0.29c |
| EO-4D | 9.12a | 5.20b |
| EO-5D | 4.91b | 4.81b |
| EO-6 | 9.07a | 9.07a |
| EO-7 | 9.12a | 9.08a |
| EO-6D | 9.12a | 9.08a |
| EO-7D | 4.86b | 5.08b |

Chlorinated water samples = CW-1, CW-1S, CW -2, CW -2S

EO-3 is EO water prepared from EO generator #2 at pH 2.5

EO-4D & EO-5D are EO water prepared from EO generator #3 at pH 2.5 and 6.0, respectively, at 140 mg/L chlorine concentration and diluted with deionized water

EO-6 & EO-7 are EO water prepared from EO generator #4 at pH 2.5 and 6.0, respectively

EO-6D & EO-7D are prepared from EO generator #4 at pH 2.5 and 6.0, respectively, at 120 mg/L chlorine concentration and diluted with deionized water

Data reported as means of 2 measurements

Means not labeled with the same lowercase letter to the right of the number in each column are significantly different ($p \leq 0.05$)

# CHAPTER 9

# SUMMARY AND CONCLUSIONS

EO water and other types of chlorine-based sanitizers must be described not just in terms of free chlorine content but also pH and residual chloride content as well. The pH of a sample of EO or chlorinated water will determine what form of free chlorine is prevalent. The prevalent form of free chlorine will directly affect a chlorine-based sanitizer's interactions with organic compounds in its environment, corrosivity, shelf stability, and antimicrobial activity. Acidic EO water and chlorinated water show a greater degree of corrosivity and antimicrobial activity while showing reduced shelf stability and interactions with organic compounds. Near neutral EO and chlorinated water show a greater degree of shelf stability and interactions with organic compounds while showing decreased corrosivity and antimicrobial activity.

Residual chloride will directly affect a chlorine-based sanitizer's corrosivity and shelf stability. Higher levels of residual chloride lead to higher degrees of corrosion and decreased shelf life in acidic chlorine-based sanitizers (which may impact antimicrobial activity if the sanitizer is not used in a timely manner), while lower levels of residual chloride lead to lower corrosion levels and increased stability in acidic chlorine-based sanitizers.