EFFECTS OF COPPER AND SILVER IONIC SOLUTIONS ON *E. COLI* BACTERIA AND TESTING OF A PROTOTYPE ION-GENERATION DEVICE

By

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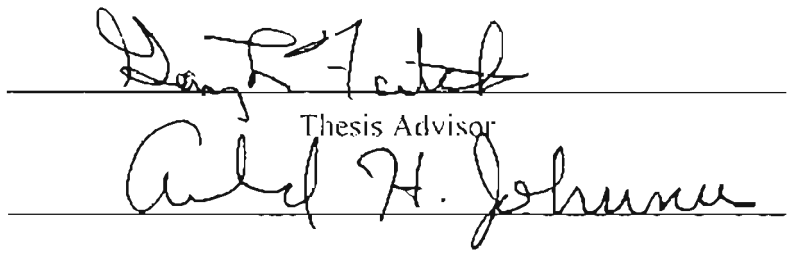

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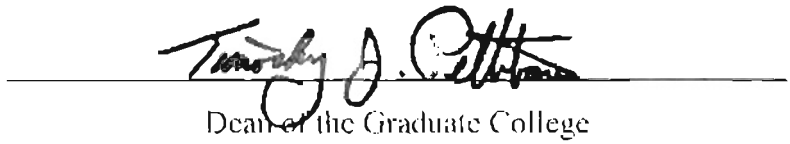

Dean of the Graduate College

PREFACE

In this work, the effectiveness of killing E. coli bacteria in potable water applications with combined copper and silver ionic solutions is tested. In addition, development and testing of a prototype portable ion generation device is continued from that of Mr. Michael Hicks.

I would like to sincerely thank Dr. Gary Foutch for serving as my major advisor. His guidance has been invaluable during the completion of my degree. I also wish to extend many thanks to Dr. James R. Whitlely and Dr. Arland H. Johannes for serving on my committee and for their dedication and support throughout my college career.

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NOMENCLATURE

C_a	absorbed concentration of an ionic species
C_c	complexed concentration of an ionic species
C_f	free concentration of an ionic species
C_t	total available concentration of an ionic species
DBP	disinfection by-product
F	Faraday's constant, 96,485 coulombs/mole of electrons
I	
HA	hydroxyapetite
IGD	prototype ion generation device
M	molarity
MCL	maximum contaminant level
n_{eq}	total number of equivalents
η_i	ionization efficiency of species *i*.
ppb	parts per billion, by weight
ppm	parts per million, by weight
SBF	simulated bio-fluid
SMCL	secondary maximum contaminant level
t	time

I. INTRODUCTION

Background

For the past century, chlorination has been the primary method of potable water disinfection. Although chlorination is reliable, it is not a perfect means of disinfection. The reaction of chlorine with organic compounds has been shown to form low levels of trihalomethanes and haloacetic acids. These compounds cause liver, kidney, and central nervous system damage, and increase the risk of cancer. In addition, chlorination alone does not provide a reliable residual disinfection action. After treatment, some pathogens remain viable, and can flourish in drinking water delivery systems. The addition of chloramines or chlorine dioxide as disinfectants can provide a level of residual disinfection, but these compounds can also cause health problems. Chloramines can cause mucous membrane irritation, stomach discomfort, and anemia. Chlorine dioxide can cause anemia and central nervous system damage, especially in infants and young children (US EPA, 2001)

In addition, most means of chlorination are not portable. Chlorine and chlorine-containing gasses and liquids are highly reactive and cannot typically be stored for long periods. In emergencies or natural disasters, reliable delivery of chlorine disinfection chemicals may not be possible, potentially leaving thousands without potable water. Isolated communities or developing countries may not have access to a reliable or

regulated municipal water supply, and are in danger from a wide variety of water-borne pathogens.

It has been shown that many water-borne bacteria can be effectively eliminated by exposure to solutions containing a combination of copper and silver ions. Some viruses, protozoa, fungi, and algae are also adversely affected by exposure to copper and silver ions. For these reasons, the alternative method of water purification by combined exposure to copper and silver ions has been investigated, and a prototype portable copper and silver ion generation device has been developed and tested.

Project Objectives and Overview

The objectives of this project were to develop and validate a prototype portable ion generation device (IGD). This IGD was designed to generate specific copper and silver ion concentrations in predetermined volumetric batches. The ionic solutions were tested to determine their ability to eliminate *E. coli* bacteria in potable water. The bacterial elimination performance of the solutions generated by the IGD was also compared to the performance of ionic solutions prepared by dilution of chemical reagents. Based on discussions in published literature, it was determined necessary to investigate the effects of light on bacterial kill rates and ion measurement in these metal-ion solutions. Because overexposure to copper and silver ions in drinking water may be a long-term health risk, it was necessary to carefully develop and test the IGD to insure accurate and repeatable generated concentrations near design criteria and below current US EPA limitations. During the testing stages, modifications were made to the IGD internal firmware to reduce variations between theoretically predicted and experimentally measured ionic concentrations and to enhance overall usability and effectiveness.

II. LITERATURE REVIEW

Obtaining water free from harmful bacteria, viruses, and protozoa is essential to maintaining good health. Large-scale drinking water disinfection has virtually eliminated water-borne illness in the civilized world. The purpose of this chapter is to review and compare current water disinfection alternatives as presented in available literature. Current water disinfection techniques will be reviewed and compared to past research and applications of copper and silver ions for potable water disinfection. In addition, alternative uses for copper and silver ionic solutions will be reviewed.

Current Disinfection Technologies

Chlorination

Chlorination serves as the principal drinking water disinfection method in most water utilities due to its high inactivation efficiency toward a broad spectrum of microorganisms, its low operation and maintenance costs, and its relatively simple application techniques (Pedahzur et al., 1995). A typical chlorine disinfection system is designed to provide efficient mixing of a chlorine-containing solution or gas with raw water for a contact time of at least 30 minutes. The chlorine solution can be in the form of elementary chlorine or as a chlorine-containing compound. Typical chlorine-containing compounds include sodium hypochlorite, calcium hypochlorite, chlorine dioxide, and chloramines (Yahya et al., 1993). The concentration of free chlorine in the reactor effluent is typically between 0.1 and 0.2 ppm.

Historically, a disinfection process was considered satisfactory if no coliform indicator was detected immediately following the disinfection process. Generally, chlorination is effective at eliminating a broad range of water-borne pathogens. However, as water-testing procedures have become more sophisticated and thorough, the detection of carcinogenic disinfection by products and chlorine-resistant organisms, including viruses and protozoa, has raised concern. Chlorine is highly reactive with organic materials in raw water. These interactions increase the chlorine demand to achieve acceptable levels of disinfection, since much of the chlorine goes toward oxidizing organic compounds. In addition, chlorine reactions with organic molecules lead to the formation of a wide variety of halogenated disinfection by-products (DBPs). There is a growing concern over these persistent halogenated by-products, which gradually accumulate and contaminate water sources. The DBPs of chlorination can include trihalomethanes, chloroform, chlorite, and haloacetic acids. Some of these compounds have been declared as probable human carcinogens, (e.g. chloroform, bromodichloromethane, and bromoform) and there is growing concern that the number of regulated compounds only reflects the current, limited state of knowledge, and not the real potential health risks (Pedahzur et al., 1995).

Another problem associated with water disinfection and distribution systems is the occurrence of high bacterial counts in previously disinfected water. This phenomenon is caused by the recovery of disinfectant-injured cells entering the distribution systems, referred to as "regrowth," or to the growth of native bacteria in the distribution system, referred to as "aftergrowth" (van der Wende and Characklis, 1990.) The regrowth and aftergrowth effects are caused by ineffective initial disinfection,

inadequate residual disinfection, the development of disinfectant-resistant bacterial strains, and the protection provided to enteric organisms by biofilms, which are difficult to control with ordinary disinfection techniques (LeChevallier et al., 1988). Although chlorination provides effective and rapid initial disinfection, it provides no substantial residual disinfection action. Regrowth and aftergrowth effects frequently occur in chlorinated disinfection systems.

Ultraviolet Light

Ultraviolet Light (UV) is emerging as a possible disinfection technique for small-scale applications. Ho et al. (1997) showed that a UV disinfection system had a higher initial viral elimination efficiency than a full-scale chlorination system, although water flowrates were significantly lower for the UV system. Tomowich (1998) tested the effectiveness of a UV device under simulated failure conditions. The tested device met initial disinfection requirements when subjected to a simulated partial lamp failure and flowrates in excess of the maximum design feed rate. UV treatment is environmentally benign, and produces almost no DBPs.

Although UV is an effective and clean method of disinfection, some shortcomings do exist. UV treatment can be very effective in clear water sources, but most untreated water sources are turbid. Sediments and algae present in the feed stream will scatter the UV rays, and reduce the overall effectiveness of a UV disinfection system (Burch et al., 1998). For UV treatment to be effective in turbid systems, prior filtration would be necessary. Because pathogens are only destroyed by direct exposure to high-intensity UV rays, UV treatment provides no residual effect. For this reason, regrowth effects are a significant concern if UV-treated water is to be stored for later distribution or

consumption. Finally, UV disinfection requires a reliable power source. Developing, remote, or disaster stricken areas may not have a reliable power infrastructure, making UV treatment unfeasible for those applications.

Biofilm Filtration

Filtration processes typically employ a coarse filter followed by a fine membrane filter. The coarse filter is used to remove larger particles and reduce the overall turbidity of the water, while the membrane filter is used for further purification (Burch et al., 1998). In a biofilm filter, the membrane is a synthetic or a naturally occurring biofilm. One type of biofilm filter is formed by biofilm growth on the surface of a slow-sand filter, and is often called a "greensand" filter. In large-scale sand filters, this surface film has been shown to remove up to 99% of all microbes present in untreated water (Burch et al., 1998). To function properly, greensand filters require a large amount of maintenance. The top layer of the biofilm must be raked periodically, while maintaining the integrity of the underlying film. Periodically, the top layer of sand must also be removed and replaced. The biofilm must then be allowed to reform before the filter is again effective. Slow sand biofilters provide adequate purification and produce no significant DBPs, but provide no residual disinfection action. In large-scale applications, regrowth potential is a significant concern. Slow sand filtration is also limited in the volume of water that can be treated. Maximum flowrates are on the order of 2 m^3/day for each square meter of biofilm surface (Burch et al., 1998). Despite their high maintenance requirements, low treatment capacity, and lack of residual disinfection effect, slow sand biofilters may be a feasible alternative for small-scale stationary applications in developing countries due to their simplicity and effectiveness.

Ozonation

Ozone is becoming increasingly popular as a potable water disinfectant, especially in Europe. Ozone exhibits high disinfection performance and rapid kill rates for a wide variety of species. Ozone behaves similarly to chlorine when it reacts with organic material in water. Organic material typically has a high oxidation capacity, and most of the ozone is consumed in these reactions. However, in these oxidation reactions, ozonation can produce low molecular weight ketones and aldehydes as DBPs. Because ozone is highly reactive, it can cause corrosion in disinfection and distribution loops. Ozone production is energy intensive and expensive, making it unpractical for emergencies and remote applications. Finally, ozone does not provide a residual disinfection effect, and regrowth is a concern in ozone-disinfected water distribution systems.

Iodination

Iodine is well known for its bactericidal properties, is chemically stable, and is simple and safe to apply without specialized equipment. However, the use of iodine in large-scale water purification is not economically feasible. Iodine is typically used in small-scale and personal water purification applications. Portable hiking and camping water purification systems often use iodine tablets or drops. The United States Army supplies field units with tincture of iodine for emergency water purification. NASA uses iodine as its main means of chemical water disinfection during space missions, although alternative methods are being investigated.

Not all of ionic forms of iodine exhibit bactericidal effects. This could result in higher applications of iodine to achieve necessary disinfection (Silverstein and Hurst,

1993). There is a lack of information about the long-term effects of consumption and exposure to iodinated water, possible DBPs, and bacterial resistance to iodine disinfection (Colombo and Sauer, 1987). Iodine appears to supply a residual disinfection effect, and can be removed at point-of-use by appropriate ion exchange techniques.

Summary

The above sections briefly summarize the current state of potable water disinfection technology. Each method has inherent strengths and weaknesses when applied, but none can be considered ideal alone. Combinations of methods (Ozone in combination with UV irradiation, for example) can lead to improved results and reduce the downsides to each method. In the following sections, metal ion disinfection will be discussed. In addition, alternative applications for metal ions solutions will be investigated.

History of Disinfection by Metal Ions

For thousands of years, metal ions have been used for disinfection. The ancient Greeks, Romans, and Egyptians stored water, wine, and oil in silver containers. Cyrus the Great, King of Persia (550-529 B.C.E.) had mule- drawn carts carrying water in silver vessels follow him at all times. Pliny the Elder (78 C.E.) noted that silver "slag" as an ingredient in plasters caused wounds to heal. Silver vessels in Mexico have been used for centuries to prevent the spoiling of milk. In 1884, Crede first applied a solution of 1% silver nitrate to the eyes of newborns as a protection against gonococcal opthalmia (gonorrheal infections of the eye). This practice was so effective that it became a state regulation in countries throughout the world. Ointments containing silver sulfadiazine

are used in the treatment of severe burns to promote healing and prevent infection. A 2500-year history of the use of silver for water purification and disease control has been established, without significant health impact or allergic reaction.

Copper is one of the best-known algaecides. Copper sulfate is frequently used to kill vegetation in freshwater lakes and ponds without harming aquatic animals. Copper compounds are incorporated into marine paints as algaecidal agents. Copper is also a potent fungicide.

Past Research of Copper and Silver Water Disinfection

Yahya et al. (1990) investigated the effects of combined copper and silver ionic solutions, with and without chlorination, on mixed bacterial systems. Solutions of copper and silver ions, at 400 and 40 parts per billion (ppb) concentrations, respectively, both with free chlorine at a concentration of 0.3 parts per million (ppm) and without, were evaluated over a four-week period in indoor and outdoor tests. Bacterial samples were isolated from bathwater and included human urine and mixed species of coliforms, pseudomonas, and staphylococci. These experiments showed that systems treated with copper/silver and reduced free chlorine (0.3 ppm) eliminated total coliforms, pseudomonas, and staphylococci as well as systems treated with 1 ppm free chlorine alone. No significant difference in bacterial numbers was observed between copper/silver/reduced chlorine disinfection and disinfection by higher levels of chlorine alone. However, copper and silver solutions without reduced chlorination were less effective than solutions that used chlorination alone. Copper/silver treatment alone yielded the slowest bacterial elimination kinetics with only a 0.015 $\log_{10}$ reduction per minute. Copper/silver ions in combination with reduced chlorination produced the fastest

elimination kinetics at 1.2 $\log_{10}$ reductions per minute. Chlorination alone produced a 0.75 $\log_{10}$ reductions per minute.

Landeen et al. (1989) investigated the effects of copper and silver ions on *Legionella pneumophila*. *L. pneumophila* is the bacteria responsible for legionnaire's disease, and has been isolated from cooling towers, air conditioning systems, evaporative condensers, hot water tanks, and whirlpools. These environments provide favorable growth conditions with elevated temperatures and mineral deposits. Inhalation of aerosols from infected water sources is believed to be the transmission vector for *L. pneumophila*. In Landeen's experiments, electrolytically generated copper/silver solutions in concentrations of 200/20, 400/40, and 800/80 ppb in combination with low levels of FC (0.1 to 0.4 ppm) were evaluated for their efficacy in eliminating *L. pneumophila*. These experiments were performed at room temperature (21 to 23°C) and elevated temperatures (40°C). At room temperature, a contact time of at least 24 hours was necessary for solutions of 400/40 ppb copper/silver to produce a 3 $\log_{10}$ reduction in viable bacterial colonies. The elimination rate at this concentration was 2.87×10^{-3} $\log_{10}$ reduction per minute. When the copper/silver concentration was increased to 800/80 ppb, the elimination rate increased to 7.50×10^{-3} $\log_{10}$ reduction per minute. Copper/silver solutions alone had significantly slower reduction kinetics than free chlorination. However, when copper/silver ions were combined with reduced levels of free chlorine, the rate kinetics were greater than those observed for comparable levels of free chlorine alone. This effect was only statistically significant when the concentration of free chlorine was greater than 0.4 ppm in 400/40 ppb copper/silver solutions.

Lin et al. (1996) investigated the individual and combined effects of copper and silver ions for the inactivation of *L. pneumophila*. In their experiments, *L. pneumophila* was completely eliminated (6 $\log_{10}$ reduction) within 2.5 hours at a copper ion concentration of 100 ppb. More than 24 hours were required to achieve a similar reduction in an 80 ppb silver ion solution. Combined solutions of copper/silver at 20/20, 20/40, 40/20, and 40/40 ppb required 8.2 hours, 5.6 hours, 3.6 hours, and 1.6 hours, respectively, to achieve complete inactivation of *L. pneumophila*. These combined disinfection rates were compared to the Gard Additive Model to determine whether the disinfection effects were additive or synergistic in nature. The Gard Additive Model compares the individual and combined disinfection effects of bacterial elimination experiments. If the effect of combined disinfection methods exceeds the summary disinfection performance of each individual method, then the combined method is said to have a synergistic effect. At copper/silver concentrations above 40/20 ppb, a synergistic effect was observed while simple additive effects were observed at lower copper concentrations. However, it was noted that these results might not be applicable in a real water distribution system because of the likelihood of multiple extraneous factors, including the presence of multiple bacterial and viral strains and complex water chemistry interferences.

Lin et al. (1998) later studied the inactivation *Myobacterium avium* by copper and silver ions. Copper and silver solutions were prepared by dilution of $CuCl_2$ and AgCl. At copper and silver concentrations of 800/80 ppb, respectively, 99.9% of the initial *M. avium* concentration was eliminated in 48 hours. Furthermore, after 7 days exposure to

combined copper and silver ions at all concentrations, no viable *M. avium* colonies could be recovered from the culture medium.

Cassells et al. (1989) investigated the efficacy of copper and silver solutions in combination with reduced levels of free chlorination for eliminating *Naegleria fowleri* amoebas in water. Copper and silver solutions with concentrations of 400/40 and 800/80 ppb copper and silver, respectively, were tested with and without 1 ppm free chlorine. After 72 hours exposure, the copper/silver solutions alone provided no significant effect against the *N. fowleri* amoebas. For copper/silver solutions alone, with concentrations of 400/40 and 800/80 ppb, respectively, the elimination constants were $k = 1.7 \times 10^{-4}$ $\log_{10}$ reductions per minute and 1.3×10^{-4} $\log_{10}$ reductions per minute, respectively. Free chlorine alone provided an elimination rate constant of $k = 0.33$ $\log_{10}$ reductions per minute. When 1 ppm free chlorine was added to the 400/40 and 800/80 ppb copper/silver solutions, rate constants of $k = 0.458$ $\log_{10}$ reductions per minute and 0.515 $\log_{10}$ reductions per minute, respectively, were observed. Copper/silver solutions of 800/80 ppb with 1 ppm free chlorine provided 99% elimination in 4 minutes, while chlorination alone required 6 minutes for comparable elimination. It was suggested that the combined effect of the different disinfection techniques provided a synergistic effect due to different disinfection mechanisms. Again, this experiment shows that the disinfection action of copper/silver solutions is greatly enhanced by the addition of reduced levels of free chlorine.

Abad et al. (1994) investigated the effects of copper and silver ions, in combination with low levels of free chlorine, on eliminating several human enteric viruses. The viruses investigated were Hepatitus A (HAV), Human Rotavirus (HRV),

Human Adenovirus (HAD), and Poliovirus (PV). Copper/silver solutions of 700/70 ppb were tested alone and with free chlorine at 0.5 and 0.2 ppm concentrations. PV was readily eliminated by the copper/silver solution combined with 0.5 and 0.2 ppm free chlorine. HAD was more resistant than PV, but was still readily eliminated. HAV and HRV were very resistant to the effects of the copper/silver solution, even when combined with 0.5 ppm free chlorine. Overall, it was observed that the addition of solutions containing 700/70 ppb copper/silver did not significantly improve the elimination of human enteric viruses after exposure to 0.2 or 0.5 ppm free chlorine. However, it was noted that the addition of copper and silver with low levels of free chlorine did provide a disinfection action comparable to higher levels of free chlorine alone. Abad et al. believed that the protein coating on enteric viruses might provide a measure of protection against copper and silver ions in disinfection.

Berger et al. (1976) investigated the effects of electrically generated silver ions on bacterial and mammalian cells. In their experiments, samples of several bacteria and mouse bone marrow were exposed to a 700 ppb silver ion solution, and to silver sulfadiazine. This study found that silver sulfadiazine was more effective for eliminating bacteria than the silver ion solution. The mouse bone marrow studies showed that silver ions caused no obvious detrimental effects such as cell aggregation, distortion, cellular lysis, or pH changes as compared to the control samples. However, there were indications that silver exposure may enhance the maturation sequence of certain types of cells. The authors concluded that anodic silver was an effective bactericidal agent at low concentrations without significant detrimental effect on mammalian cells.

Regulations Regarding Copper and Silver in Drinking Water

The United States Environmental Protection Agency (EPA) has established National Primary Drinking Water Standards that set mandatory water quality standards for drinking water contaminants. These primary standards set enforceable maximum containment levels (MCLs) for drinking water contaminants that pose a potential risk to public health. An MCL is the maximum allowable amount of a contaminant that can be delivered to a consumer. The primary MCL action level for copper concentration in drinking water is 1.3 mg/L (1300 ppb). Short-term exposure to concentrations above 1300 ppb can lead to gastrointestinal distress, while long-term exposure can lead to liver or kidney damage (US EPA, 2001). Silver concentration is not regulated by the National Primary Drinking Water Standards.

The EPA has also instituted National Secondary Drinking Water Standards. Secondary standards set non-mandatory water quality standards for 15 contaminants. The EPA does not enforce these secondary maximum contaminant levels (SMCLs). The SMCLs are established as guidelines to assist public water systems in managing their drinking water for aesthetic considerations such as color, odor, and taste. Secondary contaminants are not considered a risk to human health at the SMCL. The EPA SMCL for copper is currently 1000 ppb. Drinking water with copper concentrations above 1000 ppb may have a metallic taste or a noticeable blue-green color. The SMCL for silver is 100 ppb. Long-term consumption of silver ions above this concentration limit can cause a skin discoloration called Argyria. Persons with Argyria develop a permanent gray coloration in the skin. Argyria does not impair body function, and has never been to result from drinking water in the United States (US EPA, 1992). However, Argyria has been caused by a product known as "colloidal silver". Colloidal silver consists of silver

ions in water; either as electrolytically generated silver ions, protein-bonded silver, dissolved silver salts, or suspended powdered silver (which is probably silver oxide). These unregulated colloidal silver solutions can contain silver concentrations from 3 to 500 ppm, and can be purchased in health food stores or through internet vendors. Purveyors of colloidal silver often claim that Argyria is not caused by their product, or is caused by one of the alternate forms of colloidal silver. However, the US FDA requires that any product containing colloidal silver or silver salts labeled as an over-the-counter drug must meet the standards of new drug testing and approval as outlined in 21 CFR (21 CFR 310.548). Colloidal silver products may be sold as dietary supplements if certain disease prevention and curative claims are removed from advertisements and packaging.

Additional Uses for Metal Ion Generation Technology

Copper and silver ionic solutions have been investigated in applications other than drinking water disinfection. The following section discusses research and products that utilize copper and silver ions. These applications offer alternative product development opportunities for copper/silver ion technology.

Swimming Pool Disinfection

The largest application of copper and silver ion technology in the United States is swimming pool and whirlpool disinfection. There have been many studies in which copper and silver ionization systems were tested for their ability to adequately disinfect swimming pools. Landeen et al. (1989) investigated the use of electrolytically generated copper and silver ions in combination with reduced levels of free chlorine for swimming pool disinfection. Cultures of *L. pneumophila*, *Staphylococcus aureus*, *Pseudomonas*

aeruginosa, *E. coli*, and *Streptococcus faecalis* were individually tested in water containing 400/40 ppb copper/silver, 400/40 ppb copper/silver and 0.2 ppm free chlorine, and 1.0 ppm free chlorine alone. *L. pneumophila* showed a 5 $\log_{10}$ reduction after 7 minutes exposure to 400/40 ppb copper/silver in combination with 0.2 ppm free chlorine. Less than a 2 $\log_{10}$ reduction was seen after exposure to 1.0 ppm free chlorine alone. Similar results were observed for *E. coli*, where exposure to the copper/silver/reduced free chlorine produced a 4 $\log_{10}$ reduction in 1 minute, while exposure to 1.0 ppm free chlorine alone yielded a 3 $\log_{10}$ reduction per minute. These experiments also showed that *E. coli* exhibited about a 2 $\log_{10}$ reduction after 2 hours exposure to copper/silver at 400/40 ppb. The other organisms showed similar results in all test cases. In all cases, copper/silver in combination with reduced levels of free chlorine provided equivalent or superior disinfection to 1.0 ppm free chlorine alone.

Beer et al. (1999) performed a swimming pool disinfection study in which the disinfection characteristics of 0.4 ppm free chlorine combined with copper/silver ions were compared to 1.0 ppm free chlorine alone. Copper concentrations were maintained between 250 and 300 ppb. Silver concentrations were not monitored, but were believed to be about 1 percent of the copper ion concentration (2.5-3.0 ppb) by electrode design. The results of this study showed that copper and silver ions at about 300/3 ppb, respectively, in conjunction with 0.4 ppm free chlorine yielded statistically equivalent results for disinfection of coliform bacteria and heterotrophs when compared to 1.0 ppm free chlorine. A residual disinfection effect was also noted during a period between test phases for the systems using copper/silver ions in conjunction with chlorination. In addition, chlorine consumption was reduced, and algae and mold in and around the pool

were controlled. This study was directly compared to that of Landeen et al. (1989), and verified Landeen's results in a field application.

Cooling Tower Treatment

Wilsey (1997) suggested that a copper ion generation system could be used as an alternative to cooling tower water conditioning by other chemical means. Problems commonly found in cooling towers include scale formation, chemical corrosion, algae and bacterial growth, and growth-related fowling. To control these problems, chemicals such as acid, soda ash, sodium bicarbonate, algaecide, and bactericide are commonly used. Wilsey sites the disadvantages of cooling water treatment with chemicals alone as:

- Higher cost of initial materials of construction to combat the effects of harsh conditioning chemicals
- Higher maintenance and operating costs due to ongoing chemical testing and replacement
- Increased exposure to hazardous chemicals
- Increased expenses related to meeting EPA regulations and requirements

Wilsey states the advantages to using ionic technology in cooling towers as:

- Reduces the level of calcium carbonate scale in the cooling tower water, thus increasing heat transfer capability
- Reduces the amount of chlorine and other conditioning chemicals used in the cooling water, thus reducing chemical costs and environmental release
- Coagulates and separates particles allowing easier filtration and removal
- Inhibits the growth of algae and bacteria
- Create a more environmentally friendly system

Hot Water System Disinfection

L. pneumophila, the bacteria responsible for legionnaire's disease, thrives in hot-water distribution systems. Hospitals routinely disinfect their hot water systems in an attempt to reduce cases of legionnaire's disease in high-risk patients. Sporadic cases of legionnaire's disease have also occurred in apartment buildings, workplaces, and dormitories in which potable water has been shown to be the source. The traditional method of disinfection for these hot-water distribution systems is by periodic high doses of chlorination, or by the "superheat and flush" method. High levels of chlorination are effective, but are expensive, and can corrode plumbing. In the superheat and flush technique, the water in the system is brought to high temperatures (190-200 °F) and then flushed from the system. The superheat and flush method is time consuming, labor intensive, and has the potential to burn users if they are unaware of the maintenance. As an alternative method of *Legionella* control, copper and silver ions have been studied.

Liu et al. (1998) performed experiments in which one copper/silver ionization system was alternately installed into the hot-water recirculation lines of two hospital buildings colonized with *Legionella pneumophila*. A third building was used as a control. Four weeks after activation, no *Legionella* was detectable in the first test system. After 16 weeks of operation, the ionization system was disconnected and installed in the second building. Twelve weeks of operation eliminated detectable distal site *Legionella* in the second system. Recolonization did not occur in the first system for 6-12 weeks and 8-12 weeks in the second system following inactivation of the ionization system. The control building remained *Legionella* positive throughout the testing period. Copper concentrations measured in a sample of biofilm were much higher than those measured in

water samples. The accumulation of metal ions in biofilms and adsorbed to piping was given as a hypothesis for the residual disinfection effect following the removal of the ionization systems.

Stout et al. (1998) compared the performance of a copper/silver ionization system and the superheat and flush method for *Legionella* control in a hospital hot water system. In this research, a 36-month trial of a copper/silver ionization system was compared to the previous 13 years of using the superheat and flush method. Mean copper and silver concentrations were 290 and 54 ppb, respectively. The average number of cases of legionnaire's disease was 25 per year in the three years prior to instituting the superheat and flush method, six per year during the 13 years that the superheat and flush procedure was performed, and 2 per year in the three years after installing the copper/silver ionization system. This study concluded that copper and silver ions were superior to thermal treatment for control of *Legionella*. This study also showed that the costs of installation and maintenance for the copper-silver generation system was favorable to that of thermal treatment and were comparable to the cost of hyper-chlorination, but without corrosive effects or carcinogenic DBPs. A similar study by Meitzner et al. (1997) observed results supporting those reported by Stout et al., and concluded that copper/silver treatment was superior to thermal treatment for *Legionella* control.

Antibacterial Preservatives for Pharmaceuticals and Cosmetics

Another potential application for metal ion technology is as a preservative in pharmaceuticals and cosmetics. Simonetti et al. (1992) compared the effectiveness of electrochemically generated and chemically prepared silver solutions for eliminating the growth of several microbes. In these experiments, *E. coli* and *P. aeruginosa* bacteria,

Candida albicans yeast, and *Aspergillus niger* mold were tested. The results of this work showed that silver was effective at eliminating all tested species, although the *E. coli* was the most sensitive. Results also indicated that the electrolytically generated silver ions were more potent than those prepared by dilution of silver nitrate. It was proposed that nitrate ions might interfere with the function of silver ions in the disinfection process. The researchers concluded that silver was an effective antimicrobial agent in cosmetic and pharmaceutical applications.

Scalzo et al. (1996) continued Simonetti's work by investigating the effects of nonionic surfactants and botanical extracts on silver ions in the elimination of *C. albicans*. Nonionic surfactants and some botanical extracts are known to inhibit the effectiveness of many preserving agents, and they are often present in cosmetic products. Silver ions were electrolytically generated and the concentrations were adjusted to 10^{-6} M (about 100 ppb). Samples of silver solution were inoculated with bacterial and yeast samples and then a nonionic surfactant or botanical extract was added to determine if there was an inhibitory effect. These experiments showed that there was a slight reduction in kill rate when the surfactants and extracts were added, but that the silver solutions were still able to provide greater than 4 $\log_{10}$ reduction in viable *C. albicans* colonies within 24 hours of inoculation. Further investigation showed that electrolytically generated silver solutions at the concentrations tested were not a skin irritant. Because the added silver had the ability to maintain a low level of surviving organisms, even after multiple inoculations and in the presence of strongly interfering additives, Scalzo et al. concluded that silver ions would be an effective antimicrobial additive in cosmetic applications or multiple-dose pharmaceuticals.

Ingredients in Paints and Building Materials

The antimicrobial properties of copper have been known for centuries. Today, most seagoing ships use a paint containing powdered copper or copper sulfate to inhibit the growth of algae and barnacles. Rogers et al. (1995) investigated the effects of a paint incorporating silver to control mixed biofilms containing *L. pneumophila*. In this experiment, glass tiles were coated with silverized paint and suspended with uncoated control tiles in growth medium. This experiment showed that the paint was effective for controlling total surface colonization of microorganisms during the two-week experiment. Furthermore, closely adjacent control tiles were also protected by the silver paint. When the painted tiles were removed, rapid biofilm development was observed on the remaining untreated tiles. These tests show that a silver-containing paint could be useful for marine growth inhibition. Non-marine applications for a similar product may also exist.

Metal ions are also used to preserve wood products. In the United States, the most common form of pressure-treated lumber is labeled CCA. CCA stands for copper, chromium, and arsenic. Although CCA lumber is highly rot and insect resistant, studies indicate that high levels of arsenic are leached from the lumber over time. CCA lumber has been banned in Japan and Europe, where other copper-containing compounds are used to treat lumber. If CCA lumber is banned in the United States, there will be a strong demand for alternative methods of wood treatment.

Copper and silver ions have also been shown to prevent rot in very old wood products. A recent article in New Scientist (Coghlan, 1997) discusses the use of a sprinkler system, using water that contains copper and silver ions, to hydrate and prevent

microbial growth in a medieval coin mint. The mint was buried in mud nearly 500 years ago, and was rediscovered in 1991. The ion-generation system was developed by Roseland Hydronics. The ionic solutions are sprayed directly onto wooden surfaces for 100-second intervals, five times per day. Six months into the trial, no microbial growth or wood decay was observed.

3M Corporation has developed a line of roofing shingles that prevent roof algae. These algae produce dark streaks as they grow and decay. The algae can also damage the shingles by feeding on calcium carbonate filler in the shingles. Cleaning the shingles to remove the algae is only a temporary solution, and can damage the shingles. The 3M shingles contain replace 10% by weight of the standard ceramic granules with ceramic-coated copper granules. The copper leaches through the ceramic coating and coats the shingles, preventing algae growth. These shingles are guaranteed to prevent algae growth for ten years after installation.

Medical Applications

In 1969, Fox investigated the effects of silver ions paired with an antibiotic in the treatment of burn wounds. The combination of silver and sulfadiazine was at least 50 times more active than sulfadiazine alone. Silver sulfadiazine allows dermal structures to spontaneously heal burn wounds, reducing infections and decreasing the necessity of skin grafts in many situations. Silver sulfadiazine has become the treatment of choice for burn wound therapy. Silver sulfadiazine is also effective as a topical agent against viruses and sexually transmitted diseases. Silver sulfadiazine can also inhibit the growth of *Staphylococcus*, *Streptococcus*, and *Pseudomonas* bacteria (Davies et al. 1997).

In 1996, Edlich reported a complication with the use of copper and silver ionic solutions in burn therapy. A copper/silver ionization unit was used in conjunction with chlorination in a burn-treatment hydrotherapy pool for a period of 6 months. Although effective for water disinfection, Edlich reported a black, slimy layer of film had formed on the stainless steel handrails within the pool. This black film readily adhered to patients' skin, and was difficult to remove. Samples of the film were viewed with a scanning electron microscope, which revealed that the main constituent of this film was elemental silver. No solution to this problem could be reached other than to discontinue the use of the copper/silver disinfection system and to replace the fouled fittings.

More than 85,000 implant and device-related implants occur in the Unites States each year. Implant-associated infection following joint replacement surgery is a serious complication, and usually requires removal of the prosthesis. Oral antibiotics may not be effective because biofilm formation on the implant surface increases the resistance of the bacteria to antibiotic treatment. High doses of antibiotics at the bone-implant interface are required to prevent bacterial infections. Silver ions have been shown to prevent implant-associated infection following surgical procedures.

Shirkhanzadeh et al. (1995) investigated the effects silver ion technology on implant-associated infection, and its possible use as a substitute for high doses of antibiotics at the bone-tissue interface. In this experiment, microporous Hydroxyapatite (HA) coatings were electrochemically coated onto metal bone-implant material. By ion exchange methods, silver ions were introduced into the HA coating. After the HA coating was loaded with silver, the efflux rate of silver ions was tested in laboratory prepared Simulated Bio-Fluid (SBF) at 37 degrees C. The Ag-HA coatings were shown

to lose approximately 50% of their initial silver load within 24 hours. However, the mean silver concentration in the coatings remained above 1800 ppm (0.18% by weight) for the extent of the seven-day experiment. It is also noted that the release of silver ions *in vivo* would likely be much slower because the coating would be surrounded by host bone. This slower release rate would allow longer periods of antibiotic activity. It was also shown that the Ag-HA coating promoted an accelerated and strengthened bone-to-implant bonding process.

Data linking transmission of various diseases (including HIV) and reusable medical devices suggests that current disinfection techniques are inadequate. Sagripanti et al. (1994) investigated the effects of Cu(II) and Fe(III) ionic disinfection solutions on the stability of five surgical polymers. Sagripanti suggests that treatment of reusable devices with a Cu(II) solution, followed by a secondary disinfection will be effective in deactivating a variety of microorganisms. In his work, polyurethane, silicone rubber, polyamide, polyvinylchloride, and polyethylene were exposed to metal-based solutions and examined for signs of degradation. The results showed that only one form of PVC was damaged after repeated testing. Because ionic solutions have been shown to be effective at eliminating bacteria and have not been shown to harm some common reusable surgical materials, ionic solutions may be applicable as supplemental or primary disinfectants in medical applications.

III. BACTERIAL TESTING

Organisms Tested

The strain of bacteria tested in these experiments was *E. coli A* FPC, Microbiologics, St. Cloud, MN). The bacteria were delivere (freeze-dried) pellet, and kept refrigerated until needed. The benefit c from a lyophilized pellet is that this method allows for qualitativ bacterial preparations with minimal equipment and training. This p also eliminates the tedious task of preparing multiple serial dilut specified enumeration range. Detailed instructions for the preparation *E. coli* are presented in Appendix A.

Batch Disinfection Study

Plate Count and Kinetic Data

The treated and control samples were plated in triplicate procedure outlined in Appendix A. These plates were then incubat hours. Heterotrophic plate counts were performed on each plate to de of viable *E. coli* colony forming units at each sample time. The averaged for each sample time. Elimination kinetics ($\log_{10}$ reductio by application of the following equation:

$$\text{Log}_{10}\text{ Reduction} = \log_{10}(N_o/N_t)$$

Where N_o is the initial heterotrophic plate count, as taken at time = 0, and N_t is heterotrophic plate count taken at time = t. One $\log_{10}$ reduction is equivalent to a ter reduction in viable colony concentration. For example, if $N_o = 100$, and $N_t = 10$ at t minutes, then there had been 1 $\log_{10}$ reduction in ten minutes, or, 0.1 reductions/minute. A 1 $\log_{10}$ reduction corresponds to a 90% decrease in viable colo Greater than 2 $\log_{10}$ reductions (>99%) is considered complete elimination.

Determination of Initial Concentration

At t = 0, 1 ml samples were taken from the control system and serially dil These dilutions were plated in triplicate to more accurately determine the i concentration of *E. coli* bacteria in the control and test systems. The plates were all to incubate for 24 hours, and viable colonies were counted. The initial concentrati viable colonies in the control sample could then be back-calculated based on the nu of viable colonies and the number of serial dilutions. This initial concentration of v colonies was then used to calculate the $\log_{10}$ reduction.

Comparison of Chemically Prepared and Electrolytically Generated Solutions

In several experiments, 800/80 ppb copper/silver solutions were prepar dilution of Copper Nitrate and Silver Nitrate. The bactericidal performance of laboratory prepared solutions was compared to the bactericidal performance of generated solutions. It was determined that there was no significant difference performance of laboratory prepared and IGD generated copper/silver solutio eliminating *E. coli* bacteria at the concentrations tested.

Light Exposure and Disinfection Solution Effectiveness

Thurman and Gerba (1989) indicated that light exposure may reduce the bactericidal effectiveness of the copper/silver solution. Because the IGD has the potential to be used in direct sunlight, the effect of light on bacterial kill rates was investigated in this series of experiments. The investigated light source was simultaneous indirect sunlight and overhead fluorescent lighting. Unfortunately, the results of the lighted tests did not yield conclusive results. Empirically, tests performed in lighted conditions appeared to be less effective during the first 120 minutes of testing. However, the residual effect of the copper/silver solution was not affected by exposure to light. No viable *E. coli* colonies were observed after 24 hours of exposure to the copper/silver solution even after exposure to light.

IV. ION GENERATION DEVICE TESTING

Faraday's Law

The IGD was designed based on Faraday's Law. Faraday's law relates current flow through an electrochemical circuit to oxidation and reduction rates at the anode and cathode of that circuit. Faraday's Law can be written as:

$$n_{eq} = \frac{\int I \cdot dt}{F} \qquad \text{Equation IV-I}$$

Where n_{eq} is the number of equivalents of an ionic species that results from current flow I during a time period t. n_{eq} is also the number of moles of electrons required at the anode. Faraday's constant, F, is equal to 96,485 coulombs/mole of electrons. The integral form of Faraday's law accounts for changes in current flow over time. This equation was used to develop the IGD, which can monitor the total charge passed through the device, without having to hold the current constant. The IGD was originally designed to run on battery power and treat water with a wide conductivity range, so the ability to monitor accumulated charge is an important feature. However, the second prototype of the IGD is powered by 12V DC, transformed from 120V AC. This modification allowed for repeated generation testing without the possibility of current reduction to the IGD caused by battery drain.

For Faraday's law to be accurate, only oxidation of the anode metal can occur. However, competing reactions may also occur, reducing ionization efficiency. Side reactions depend on water chemistry, but common reactions might include the hydrolysis

of water molecules (Equation IV-II) or the oxidation of chloride ions to chlorine gas (Equation IV-III).

$$H_2O(l) + 2e^- \Leftrightarrow H_2(g) + 2OH^-(aq) \qquad \text{Equation IV-II}$$

$$2Cl^-(aq) \Leftrightarrow Cl_2(g) + 2e^- \qquad \text{Equation IV-III}$$

These side reactions compete for electrons with the metal electrode oxidation reaction, and reduce overall ionization efficiency.

Another potential problem is that copper ions can exist as either Cu(I) or Cu(II) oxidation states. The IGD was designed with the assumption that all copper ions would be generated as Cu(II). Standard electrode potentials show that copper metal is preferentially oxidized to Cu(II) rather than Cu(I). Any Cu(I) formed will be readily transformed to Cu(II), but this reaction requires energy to proceed, further reducing ionization efficiency.

Ion Specific Electrodes

In the IGD generation tests, concentration measurements were performed with Cole-Parmer copper (Model # 27502-15) and silver (Model # 27502-41) ion specific electrodes connected to a Cole-Parmer Benchtop Meter (Model # 59003-30). This setup allowed accurate concentration measurements to be performed in the laboratory on the day of experiment. Atomic absorption spectrophotometry is the most accurate method for ion concentration measurement, but the equipment was not readily available, samples must be removed from the lab, and results are delayed. One advantage of using ion-specific electrodes is that they yield relatively rapid and accurate (±2%) results when calibrated properly. One of the objectives of this project is to develop an IGD that

minimizes the risk of metal ion overexposure to the end user. By developing a correctly functioning IGD and implementing a method of online measurement and control, this objective can be achieved.

The main disadvantage of using ion-specific electrodes in a measurement and control strategy was discovered during the testing of the IGD. The settling time for the ion specific electrodes is much longer than stated in the electrode operating manuals. The manuals state that steady measurements can be achieved in 1-5 minutes. However, after unexpected results in some early tests, experiments were performed to determine the settling time required to obtain stable and repeatable measurements. Figures 1 and 2 show the results of these experiments. These experiments indicate that the settling time is approximately 35 minutes for generated copper solutions, and approximately 15 minutes for generated silver solutions. For this reason, concentration measurements were performed at 35 minutes for both copper and silver generated solutions. Obviously, 35 minutes is an excessive lag time when the entire generation process is from 30 seconds on the one-quart setting to 3 minutes on the five-gallon setting. For this reason, online feedback control is not feasible.

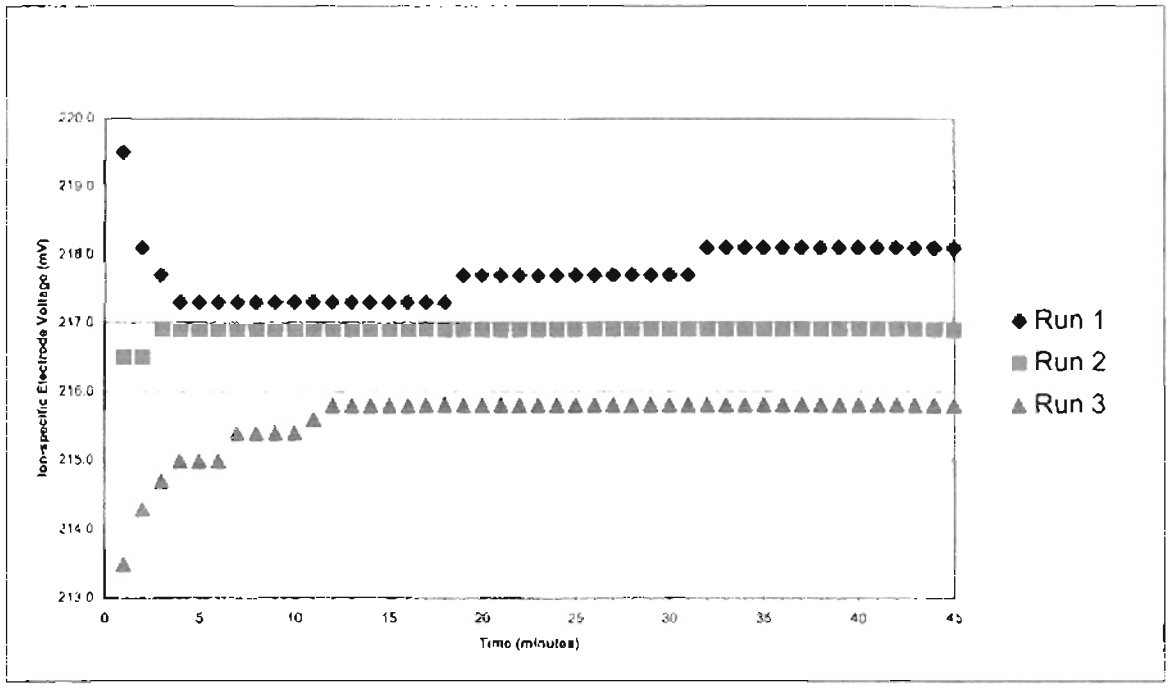

Figure 1: Millivolt reading as a function of time in generated silver ion solutions.

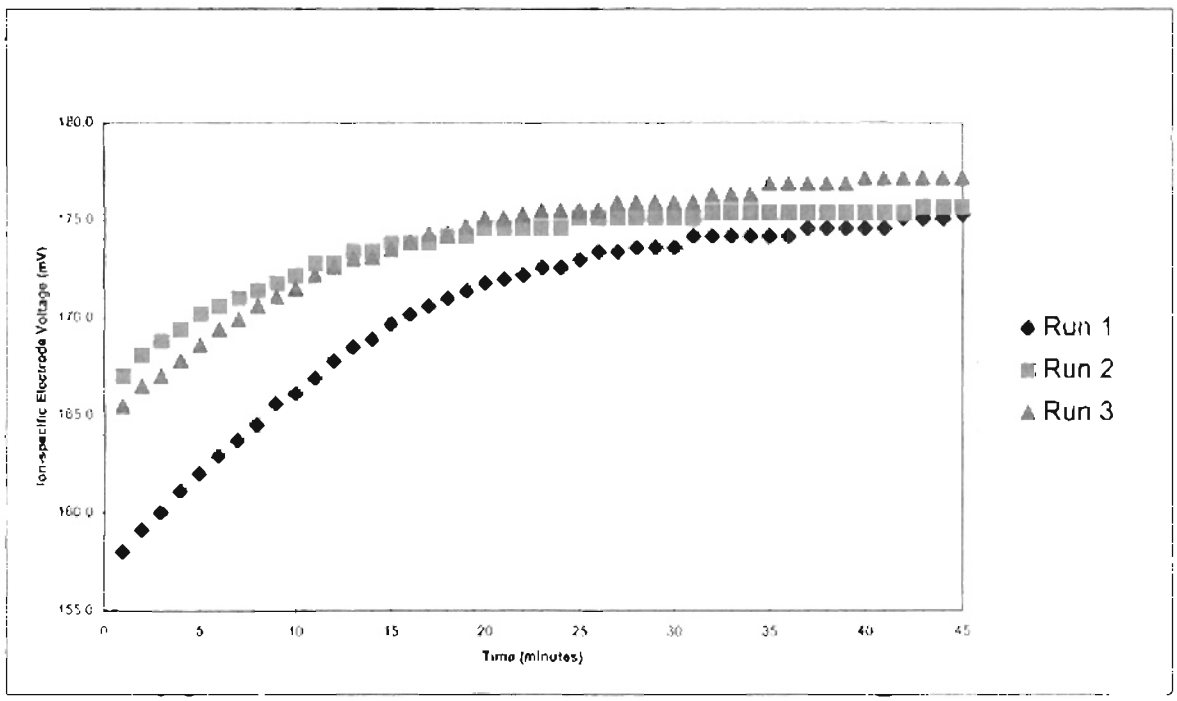

Figure 2: Millivolt reading as a function of time in generated copper ion solutions.

Background Ionic Strength

For the copper and silver ion-specific electrodes to function properly, the test samples and the calibration samples must have the same ionic strength. The electrode

manuals specify that calibration and test solutions contain 0.1 M sodium nitrate as a background ionic strength adjuster. However, the ISA also serves another purpose in this experiment. The IGD is designed the sense the conductivity of the generation liquid before the generation cycle begins. If the conductivity is not sufficient for generation to occur, then the IGD stops the generation cycle. For the IGD to function properly, solution conductivity and electrode spacing must be carefully adjusted. By using 0.1 M sodium nitrate solutions for generation, electrode spacing became the only variable. Thus, the 0.1 M sodium nitrate solution functions not only to allow the ion-specific electrodes to sense ionic concentrations correctly, but also allow the IGD to efficiently generate ions. All conductivity calibrations and generation experiments were performed in 0.1 M sodium nitrate solutions.

Measurement Interferences

The ion specific electrodes can be subject to interference by other ions in solution. In these generation and concentration experiments, the main ions present in a combined generation solution are Cu^{2+}, Ag^{+}, Na^{+}, and NO_3^{-}. The copper electrode manual states that silver ions in solution will poison the copper electrode. Other potential interferences for the copper electrode are mercury and high levels of ferric ion. The presence of mercury, sulfide, and proteins are listed as potential interferences for the silver ion-specific electrode. Work by Hicks (2000) indicated that copper concentration measurements were affected by low-levels of silver in solution, but could be approximated with a correction factor and multiple calibration curves. However, because of the specific reference to silver ions as an electrode poison, and the limited number of experimental measurements performed in Hicks's work, this researcher was hesitant to

use the copper ion-specific electrode in solutions containing silver ions. An alternative method of copper ion generation and measurement was developed. Appendix B gives detailed explanations of the ion generation and measurement techniques. Other potential sources of measurement error can be attributed to metal ion complexation, insoluble precipitate formation, temperature differences between calibration standards and test solutions, and ion adsorption onto the surface of generation containers.

The ion specific electrodes are only able to sense free ions in solution. However, the ions can complex or form precipitates. The total concentration of ions in solution is the sum of the free ions, complexed ions, and adsorbed ions, as shown by the following equation:

$$C_{T,i} = C_{f,i} + C_{c,i} + C_{a,i} \qquad \text{Equation IV-IV}$$

Where C_T is the total ionic concentration of species i, and C_f, C_c, and C_a are the free, complexed, and adsorbed concentrations of species i, respectively. Only free ions can be measured with the ion-specific electrodes, so any complexation or adsorption of free ions reduces the measurable free ion concentration. Copper ions can form complexes with acetate, ammonia, amino acids, citrate, cyanide, and chelating agents such as EDTA (Cole-Parmer, 27502). Silver ions can complex with cyanide, thiosulfate, ammonia, and EDTA. Because of the controlled nature of these experiments, complexation was not considered significant, but field applications could potentially show very different results.

Insoluble precipitates also reduce the measurable ion concentration. Copper ions form insoluble precipitates with sulfide, phosphate, and hydroxide. Silver ions mainly form insoluble precipitates with chloride ions. Precipitation equilibrium is controlled by

the concentration of ions in solution, solution pH, and temperature. Precipitation products were observed and isolated during copper ion generation, leading to reduced copper generation efficiency and lower copper ion concentration measurements. The precipitation product was hypothesized to be copper (II) hydroxide, formed by the following series of reactions at the copper anode.

$$2H_2O(l) + 2e^- \Leftrightarrow H_2(g) + 2OH^-(aq) \qquad \text{Equation IV-V}$$

$$Cu^{2+} + 2OH^- \rightarrow CuOH_2(s) \qquad \text{Equation IV-VI}$$

Temperature differences between calibration standards and measured solutions can also introduce error. The electrode manuals state that samples and standards should be within ±1°C to minimize temperature interferences. A 1°C temperature difference will introduce a 4% error at a 63.5 ppm copper ion concentration. All calibration and test solutions were prepared from the same source of distilled water. It was assumed that the water was at thermal equilibrium with the surroundings, and that the temperature of the surroundings remained approximately constant throughout the course of one set of experiments (about 8 hours). In addition, measurement containers were thermally isolated from the surface of the magnetic stir plate to eliminate heat transfer caused by the stirring motor. Temperature increase due to stirring and fluid friction during the measurement process was assumed negligible.

Chambers et al. (1962) cite the tendency for glassware to adsorb metal ions from solution. Silver adsorption is of greater concern than copper adsorption because adsorption rates for silver are higher (Hicks, 2000). Adsorption of ions onto glassware

continually removes ions from solution, making concentration measurements inaccurate over time. Landeen et al. (1989) showed a 15.4% decrease in silver ion concentration for solutions stored in Pyrex glass containers for 48 hours. Under the same test conditions, copper concentration was shown to decrease by 10%. Polyethylene containers only exhibited an 11.6% decrease in silver ion concentration and a 2.5% decrease in copper ion concentration over a 48-hour period. Landeen et al. (1989) also showed that most of the adsorption occurred in the first 24 hours. Adsorbed ions are difficult to effectively remove and can be released when equipment and glassware is used again. Polymer containers were shown to be superior to glassware for the storage of metal ion solutions because the rate of ion adsorption onto polymer surfaces was lower. For this reason, all bacterial elimination and ion generation experiments were performed in appropriately sized polypropylene containers. Polypropylene was chosen because it was able to withstand repeated autoclaving, while polyethylene was not. It is assumed that the adsorption effect is minimal because of choice of container material and minimal storage time for most copper /silver solutions.

Parallel Cathode Configuration

Due to concerns with ion interference affecting the accuracy and precision of the concentration measurements, copper and silver ions were generated and measured in separate containers. This approach eliminates the possibility of ionic interferences in the concentration measurements. Figure 3 shows a schematic diagram of the IGD arranged in the parallel cathode configuration. The parallel cathode configuration also allows for each set of electrodes to be made of either pure copper or silver. In the work performed by Hicks (2000), a 10:1 copper to silver by weight alloy electrode was designed and

tested. Hicks concluded that without absolute homogeneity in an alloy electrode, copper and silver ions will not be oxidized in the correct proportion. In a non-homogeneous alloy electrode, copper will be preferentially oxidized, and any non-uniformity in the electrode would lead to both over generation of copper and electrode pitting.

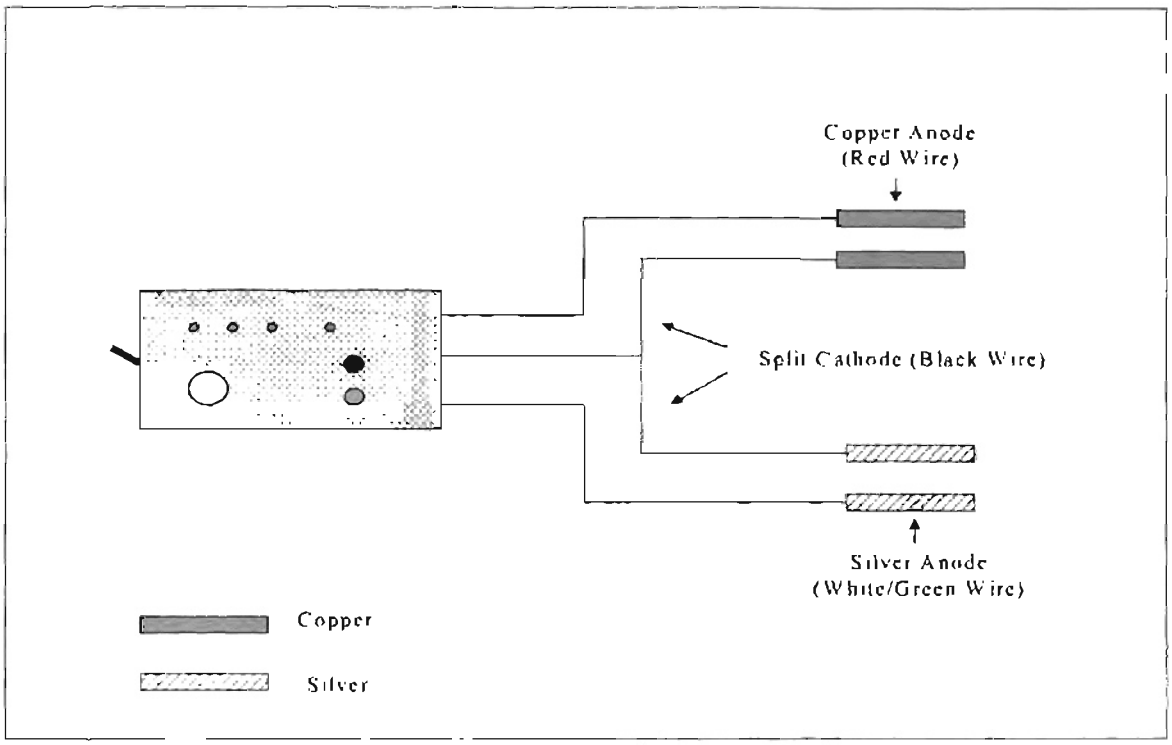

Figure 3: Schematic diagram of IGD in the parallel cathode configuration

Other Modifications to the Original IGD Design

Besides the implementation of a split cathode configuration, there have been several other modifications to the IGD. These modifications improve IGD usability and cause the IGD to function differently than the one used by Hicks.

One modification is the development of an electrode mounting fixture. The fixture provides a simple method of the changing the horizontal or vertical spacing of the metal generation electrodes. There is a fixture for each pair of metal electrodes. Each fixture consists of a 1" x 1" wooden bar, a photocopy of a cm ruler, two metal tubes

about 6" long, banana plugs, and a set of sliding clamps to hold the metal tubes in place on the wooden bar. Figure 4 shows a schematic diagram of the electrode mounting fixture. The fixture was held by a clamp affixed to a ring stand for ease of orientation during generation and measurement experiments.

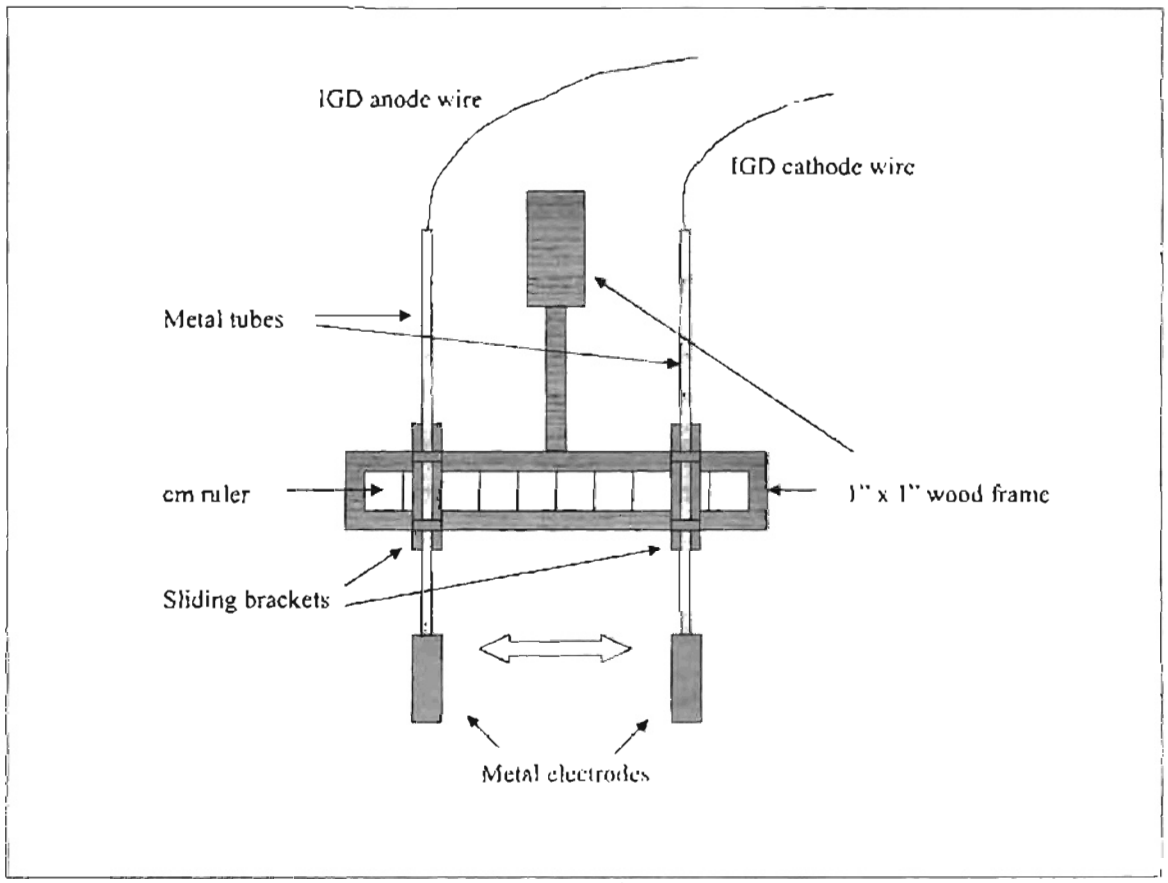

Figure 4: Schematic diagram of electrode mounting fixture

Ion Generation and Concentration Measurement Procedures

Preparation of Stock Solutions

Stock solutions of 5 M ISA, 1000 ppm Cu^{2+}, and 1000 ppm Ag^{+} were prepared as indicated in the Cole-Parmer electrode instruction manuals. Calibration standards were prepared by diluting these stock solutions with an appropriate dilution scheme. Appendix

B contains detailed instructions for preparation of stock solutions and calibration standards.

Preparation of Calibration Curves

Calibration curves were prepared by plotting electrode potential in mV against the log of the calibration standard concentration in ppb for a set of calibration standards. A typical calibration curve included 5-8 calibration points near the region of expected generated concentration. A least squares linear fit was generated and the linear equation was recorded. A sample calibration cure is shown in Figure 5.

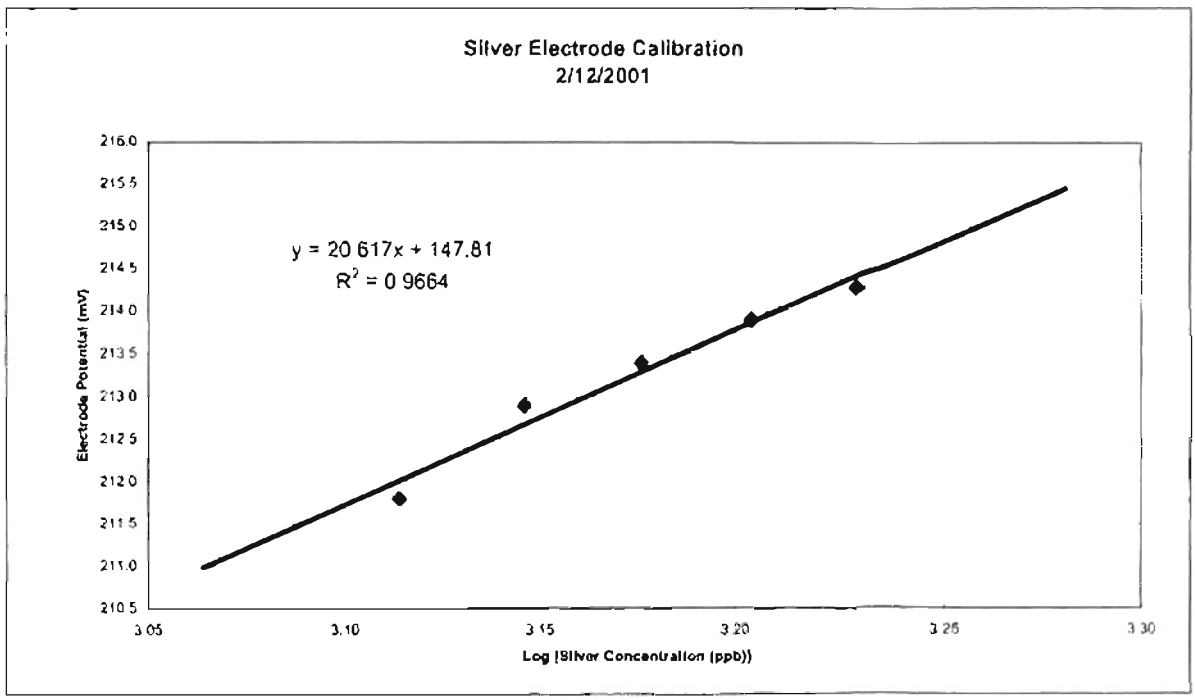

Figure 5: Sample silver ion-specific electrode calibration curve.

The correlation coefficient (R^2) of the linear fit always exceeded the 99% confidence level for (N-2) degrees of freedom (Volk, 1958). This linear relationship was then rearranged such that concentration measurements in generated solutions could be obtained. The form of the rearranged equation is:

$$C_i = 10^{\left[\frac{mV-b}{m}\right]}$$ Equation IV-VII

Where C_i is the concentration of ion *i* in a generated test solution in ppb, mV is the millivolt signal recorded by the benchtop meter, b is the y-intercept of the linear least squares trendline, and m is the slope of that same line. Through this equation, the concentration of an ion in a test solution could be calculated by measuring its corresponding millivolt signal with the correct ion-specific electrode. Appendix B contains detailed instructions for preparing calibration curves.

Ion Generation and Measurement

Figure 6 is a schematic diagram of the ion generation and testing setup.

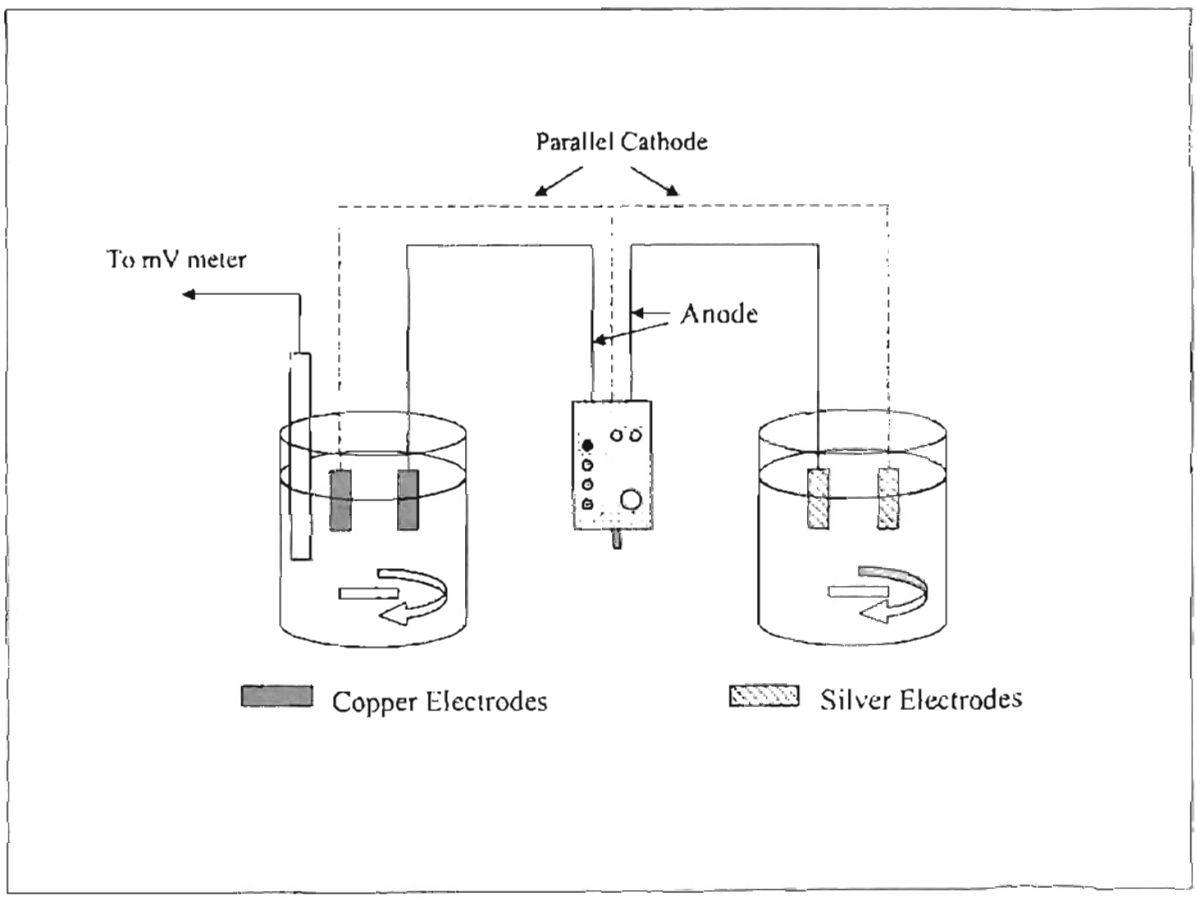

Figure 6: Schematic of ion generation and testing setup

Detailed instructions for IGD generation experiments can be found in Appendix B.

Precipitate Generation and Reduced Ion Concentration

During the copper ion generation cycle, the solution gradually acquired a blue-green tint, and small blue-green particles were observed suspended in the solution. The solution was filtered and the particles were isolated, dried, and examined. The substance exhibited physical characteristics of, and was believed to be Copper(II) Hydroxide ($Cu(OH)_2$). Chemical testing to verify the identity of this compound was not performed.

V. CONCLUSIONS, AND RECOMMENDATIONS

Conclusions

- Combined copper/silver ionic solutions at 800/80 ppb, respectively, yield between 1 and 3 $\log_{10}$ reductions in viable *E. coli* colonies during a 90 minute test period.
- Complete disinfection is reached some time after 90 minutes and before 24 hours of exposure to laboratory prepared and IGD generated copper/silver ionic solutions. The minimum exposure time to achieve complete elimination is unknown.
- Firmware Revision 4 properly generates silver ion concentration, but does not properly generate copper ion concentrations.
- Improper voltage regulation likely causes Copper Hydroxide precipitate formation. This precipitate formation reduces the copper ion generation efficiency.**Recommendations**
- Examine the effects of copper/silver solutions in conjunction with reduced chlorination. Published work indicates that combined treatment with copper/silver solutions and reduced chlorination can be more effective than either treatment alone. The potential added benefits of decreased chlorine consumption, reduced trihalomethane formation, and residual bactericidal effect from the copper/silver ions merit further study.

- Determine and attempt to eliminate the cause of precipitate formation in the copper generation cycle.
- Develop separate cathodes for both copper and silver. This will allow electrode polarity switching, and increased electrode life.
- Develop methods of copper/silver electrolytic ion generation in a flowing system. Application of ion generation in a flow system increases the potential applications of this technology.
- Investigate methods of online ion concentration measurement and feedback control. In a flow system, the risk of overexposure to copper/silver ions is increased without fast, reliable, and robust measurement and control techniques.
- Investigate methods of point-of-use copper/silver ion removal. Removal of copper/silver ions before consumption could allow higher concentrations of copper/silver to be employed without increasing the risk of overexposure. Higher ionic concentrations increase the rate of disinfection and provide increased disinfection results.
- Continue to develop and refine of the IGD. Specifically, further development of copper and silver electrode holder/applicator and refinement of IGD case will be necessary to prevent entry of environmental contaminants and to simplify use in the field.
- Explore alternative products for the application of copper/silver solutions for killing bacteria, viruses, algae, and fungi.

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APPENDICES

APPENDIX A: Biological Testing Procedures

This appendix contains detailed instructions for the growth and preparation of *E. coli* bacteria, instructions for preparation for intermediate solutions, inoculation of water samples, and plating and counting techniques.

Agar/Petri Dish Preparation Instructions

1. Dissolve 11.5 g of Nutrient Agar powder into 500 mL of distilled water in a 1 L straight-necked Erlenmeyer flask. Using a smaller flask will allow the mixture to boil over.
2. Place one stirring bar into the flask.
3. Heat/stir contents of flask until the mixture boils for approximately 1 minute (mixture will foam). Immediately remove flask with rubber-coated tongs and place in safe location.
4. Cover flask with plastic cap.
5. Autoclave for 15-20 minutes at 121 °C.
6. Allow Agar to cool for approximately 30 minutes.
7. Pour approximately 15-20 mL of Agar into sterile Petri dishes and cover.
8. Once Agar has cooled and become set, invert Petri dishes and place in original plastic bag until needed.
9. Allow plates to rest approximately 24 hours before use. Inspect each plate before use to determine if Agar has been contaminated.

Nutrient Broth/E.Coli Preparation Instructions

1. Dissolve 4 g of Nutrient Broth powder into 500 mL of distilled water in a 1 L straight-necked Erlenmeyer flask. Using a smaller flask will allow the mixture to boil over.
2. Cover with plastic cap and autoclave for 15-20 minutes at 121 °C.
3. Allow broth to cool overnight.
4. Dissolve one lyophilized E.Coli pellet into the cooled nutrient broth.
5. Mark time and date of start of incubation on flask.
6. Incubate at 37 °C for 24 hours.

Sodium Thiosulfate/Sodium Thioglycolate Preparation Instructions

1. Fill a 100 mL graduated flask approximately 1/2 full with deionized water. Pour the water into a 250 mL beaker.
2. Place the following amounts of dry chemicals into the beaker of water

   1.9364 g Sodium Thiosulfate

   1.3262 g Sodium Thioglycolate (the stinky stuff)

   Note: it is very difficult to weigh out the exact amount. If you can match to the third decimal place, that's good enough.
3. Stir the contents of the beaker with a glass stirring rod. Pour the beaker contents into the 100 mL graduated flask. Rinse the beaker walls with a small amount of deionized water from a dilution bottle and pour into the flask, being careful not to overfill. Fill the flask to the mark using the small deionized water dilution bottle (it works better for delicate jobs than the larger bottle). Stopper tightly and invert several times to mix thoroughly.

4. Moisten the filter cone with deionized water. Place the beaker under the filter funnel. Filter the TSTG mixture into the beaker. When finished, pour the filtered TSTG back into the graduated flask. Refrigerate until used.

Peptone/ Dilution Solution Preparation Instructions

Will yield a .1% peptone solution

1. Dissolve 1 gram of peptone powder in 1000 mL of distilled water
2. Autoclave 15 –20 minutes

Biological Testing Procedures

1. Prepare 1000 ml of metal ion solution in 0.1 M sodium nitrate either by IGD or by chemical dilution. Place on magnetic stir plate and begin gentle stir.
2. Prepare 1000 ml of 0.1 M sodium nitrate solution for the control. . Place on magnetic stir plate and begin gentle stir
3. Prepare and label sample containers and agar plates for triplicate samples of the control and test fluid at t = 0, 5, 10, 15, 20, 30, 45, 60, and 90 minutes. Also, prepare several additional sample containers and plates to take serial dilutions of the control solution at t = 0.
4. Add 4 ml of peptone and 100 µL of TSTG mixture to each sample container. Place in an ice bath.
5. Stir prepared *E. coli* broth well. Add 5 mL broth to sample and control containers. Allow stirring for about 5 seconds and then take multiple samples. These are the t = 0 samples. Plate 0.1 mL volumes of each sample in labeled agar plates and set aside. Serially dilute the control sample and plate triplicate samples from each dilution.

6. Take samples from test and control at previously described intervals. Plate in triplicate and set aside.
7. When test is concluded, place all plates in 27 °C incubator for 24 hours.
8. After 24 hours, count separate colonies on each plate and record.

APPENDIX B: IGD Testing Procedures

This appendix contains detailed procedures for calibration solution preparation, calibration curve development, generation solution preparation, ionic generation, and measurement.

Preparation of 5 Molar Ionic Strength Adjuster (ISA) Solution

1. Add 212.5 grams of reagent-grade sodium nitrate ($NaNO_3$) to a 500 ml volumetric flask containing about 250 ml of distilled water.
2. Swirl the flask to dissolve the solid $NaNO_3$.
3. Dilute to the mark with distilled water, cap, and upend several times to thoroughly mix the solution.
4. Store in a capped 500 ml Nalgene container until needed.

Preparation of 1000 ppm Cu^{2+} Copper Nitrate Standard Solution

1. Add 1.90 grams of reagent-grade copper nitrate trihydrate $Cu(NO_3)_2 \cdot 3H_2O$ to a 500 ml graduated flask containing about 250 ml of distilled water.
2. Swirl the flask to dissolve the solid $Cu(NO_3)_2 \cdot 3H_2O$.
3. Dilute to the mark with distilled water, cap, and upend several times to thoroughly mix the solution.
4. Store in a capped 500 ml Nalgene container until needed.

Preparation of 1000 ppm Ag^+ Silver Nitrate Standard Solution

1. Dry reagent-grade, pulverized silver nitrate ($AgNO_3$) in a laboratory oven at 150° C for one hour.
2. Add 0.785 grams of the dried $AgNO_3$ to a 500 ml volumetric flask containing about 250 ml of distilled water.
5. Swirl the flask to dissolve the solid $AgNO_3$.
6. Dilute to the mark with distilled water, cap, and upend several times to thoroughly mix the solution.
3. Store in a capped brown opaque 500 ml Nalgene container in a darkened cabinet until needed.

Preparation of 1000 ppb Calibration Base Solution (CBS) Standards

1. Using a micropipette, measure 1 ml of either the 1000 ppm Cu^{2+} standard solution, or the 1000 ppm Ag^+ standard solution.
2. Inject the standard solution into a 1000 ml volumetric flask about half full of distilled water and swirl several times.
3. Dilute to the mark with distilled water, cap, and upend several times to thoroughly mix.
4. Yield will be 1000 ml of 1000 ppb Cu^{2+} or Ag^+ CBS standard.
5. Cover the flask with aluminum foil or store in a dark location until needed. If the solution will not be used within 2 hours, store in a capped 1L Nalgene bottle.

Preparation of Calibration Solutions from 1000 ppb CBS Standards

1. Into a 100 ml graduated flask, use a micropipette to add 2 ml of 5 M $NaNO_3$.

2. Add corresponding volume of 1000 ppb Copper or Silver Calibration Base Standard (see tables below), dilute to mark, cap, and invert to mix.

Table B-I: Dilution volumes for preparation of copper electrode calibration solutions

To make 100 ml of copper calibration solution at these concentrations:	Add this volume of 1000 ppb copper CBS and dilute to 100 ml:
700 ppb	70 ml
750 ppb	75 ml
800 ppb	80 ml
850 ppb	85 ml
900 ppb	90 ml

Table B-II: Dilution volumes for preparation of silver electrode calibration solutions

To make 100 ml of silver calibration solution at these concentrations:	Add this volume of 1000 ppb silver CBS and dilute to 100 ml:
60 ppb	6 ml
70 ppb	7 ml
80 ppb	8 ml
90 ppb	9 ml
100 ppb	10 ml

Calibration Procedure

1. Connect copper (Cole-Parmer 27502-14) or silver (Cole-Parmer 27502-40) ion-specific electrode to Cole-Parmer Benchtop pH/mV/Ion/°C Meter (Cole-Parmer 59003-30)

2. Pour calibration standard into 250 ml polypropylene beaker and add small magnetic stir bar.
3. Place beaker on surface of magnetic stirrer, which has been thermally insulated with a thin sheet of Styrofoam or cardboard, and begin stirring motor.
4. Prepare ion specific electrode as described in accompanying manual.
5. Place ion specific electrode in a test tube holder and attach to a ring stand. Lower the tip of the ion specific electrode about 3 cm into the stirred calibration standard.
6. Record mV reading after 15 minutes.
7. Plot mV reading as a function of the log of the calibration standard concentration (in ppb).
8. Repeat steps 1-7 to gather 6 to 8 calibration points over the expected generation range (typically the design point ± 30%). After 5 to 6 calibration points have been plotted, use method of least squares to fit a linear trend line to the calibration points. The linear equation of this trend line will be used to calculate the concentration of generated ionic solutions in the following steps.

Ion Generation and Measurement Procedure

1. Prepare two separate 1-liter polypropylene bottles of 0.1 M $NaNO_3$. The 0.1 M $NaNO_3$ is prepared by adding 20 ml of 5 M $NaNO_3$ to a 1 L volumetric flask and diluting to the mark. Cap tightly and invert several times to mix thoroughly.
2. Remove 54 ml of 0.1 M $NaNO_3$ from each bottle and discard. The remaining 946 ml in each bottle equates to 1 quart.

3. Pour each quart of 0.1 M $NaNO_3$ into a 1000 ml polypropylene beaker. Add a small stirring bar to each beaker, set on an insulated stir plate, and begin stirring. Stir plates can be insulated with a thin sheet of Styrofoam or corrugated cardboard. Insulation minimizes heat transfer from the stir plate to the fluid.
4. Place each metal electrode bracket in a test tube holder affixed to a ring stand. Submerge the tips of the electrodes in the ISA filled beakers. Be certain that the copper electrodes are in one beaker, and the silver electrodes are in the other beaker, as shown in Figure 6.
5. Place the ion-specific electrode of the ion you want to measure into test tube holder affixed to a ring stand and position the electrode so that it may be submerged immediately following the generation cycle.
6. Use the yellow button on the IGD to select the 1-quart generation setting and then hold the yellow button until the generation cycle starts.
7. After the conclusion of the generation cycle, remove the metal electrodes from the solution and submerge the tip of the ion-specific electrode into the test solution.
8. Allow 35 minutes for settling time and record reading from benchtop meter.

APPENDIX C: Experimental Data for Bacterial Tests

Bacterial Test 1

Table C-I

Test Sample:	800ppb Cu / 80 ppb Ag / 0.1 M $NaNO_3$	
Control Sample:	Deionized Water	
Volume:	1 Quart	TMTC: Too many to count
Date:	6/10/00	TFTC: Too few to count
CFU Inoculation:	10 ml broth per quart	NA: Not applicable

Control Sample Colony Count					
Time (min)	A	B	C	Avg.	log Reduction
0	TMTC	TMTC	TMTC	TMTC	NA
5	TMTC	TMTC	TMTC	TMTC	NA
10	TMTC	TMTC	TMTC	TMTC	NA
15	TMTC	TMTC	TMTC	TMTC	NA
20	TMTC	TMTC	TMTC	TMTC	NA
30	TMTC	TMTC	TMTC	TMTC	NA
45	TMTC	TMTC	TMTC	TMTC	NA
60	TMTC	TMTC	TMTC	TMTC	NA
90	TMTC	TMTC	TMTC	TMTC	NA

Test Sample Colony Count					
Time (min)	A	B	C	Avg.	log Reduction
0	TMTC	TMTC	TMTC	300	0.0000
5	TMTC	TMTC	TMTC	TMTC	NA
10	TMTC	TMTC	TMTC	TMTC	NA
15	TMTC	115	174	145	-0.3173
20	97	59	295	150	-0.3001
30	60	48	56	55	-0.7394
45	30	0		15	-1.3010
60	48	30	84	54	-0.7447
90	0	0	0	0	NA

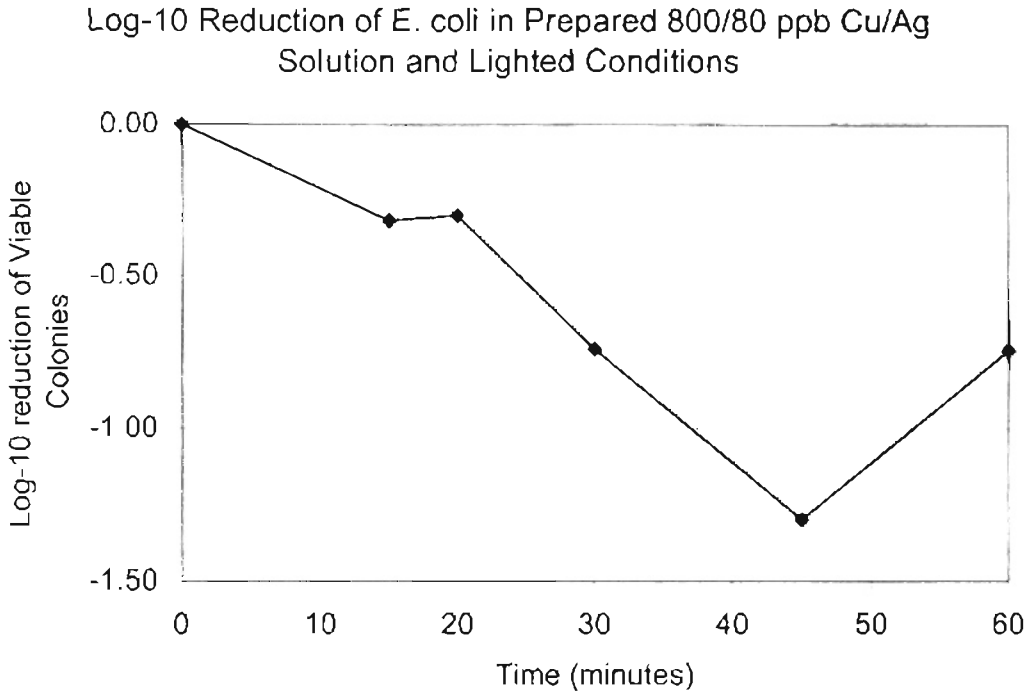

Figure C-1

Bacterial Test 2

Table C-II

Test Sample:	800ppb Cu / 80 ppb Ag / 0.1 M $NaNO_3$
Control Sample:	Deionized Water
Volume:	1 Quart
Date:	6/13/00
CFU Inoculation:	5 mL E.Coli broth per quart
Additional:	Test performed in darkroom conditions

Control Sample Colony Count				
Time (min)	A	B	C	Avg.
0 min				
1/5 dilution	TMTC	TMTC	TMTC	TMTC
1/50 dilution	350	318	301	323
90 min				
1/5 dilution	TMTC	TMTC	TMTC	TMTC
1/50 dilution	323	273	289	295

Test Sample Colony Count					
Time (min)	A	B	C	Avg.	log Reduction
0	227	224	90	180	0.0000
5	4	3	1	3	-1.8293
10	2	8	5	5	-1.5563
15	8	5	0	4	-1.6185
20	0	46	30	25	-0.8516
30	0	4	0	1	-2.1303
45	31	0	0	10	-1.2410
60	0	0	33	11	-1.2139
90	3	0	1	1	-2.1303

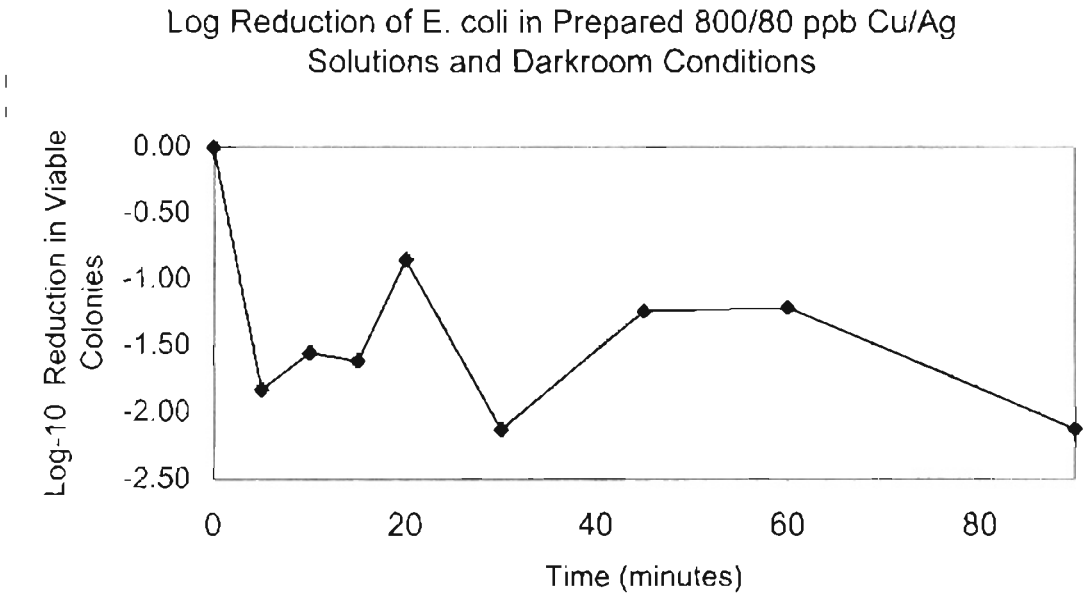

Figure C-2

Bacterial Test 3

Table C-III

Test Sample:	800ppb Cu / 80 ppb Ag / 0.1 M $NaNO_3$
Control Sample:	Deionized Water
Test Volume:	1 Quart
Date:	6/20/00
CFU Innoculation:	5 mL E.Coli broth per quart
Additional:	Tests performed in darkroom conditions

Control Sample Colony Count				
Time (min)	A	B	C	Avg.
0	TMTC	TMTC	TMTC	NA
5 mf	147	199	173	173
90	TMTC	TMTC	TMTC	NA
5 ml	157	214	290	220

Test Sample Colony Count					
Time (min)	A	B	C	Avg.	log Reduction
0	93	49	31	58	0.0000
5	1	1	2	1	-1.6360
10	TMTC	TMTC	2	NA	NA
15	51	28	15	31	-0.2649
20	0	16	0	5	-1.0339
30	0	0	0	0	NA
45	0	0	0	0	NA
60	0	3	0	1	-1.7609
90	57	TMTC	12	35	-0.2231

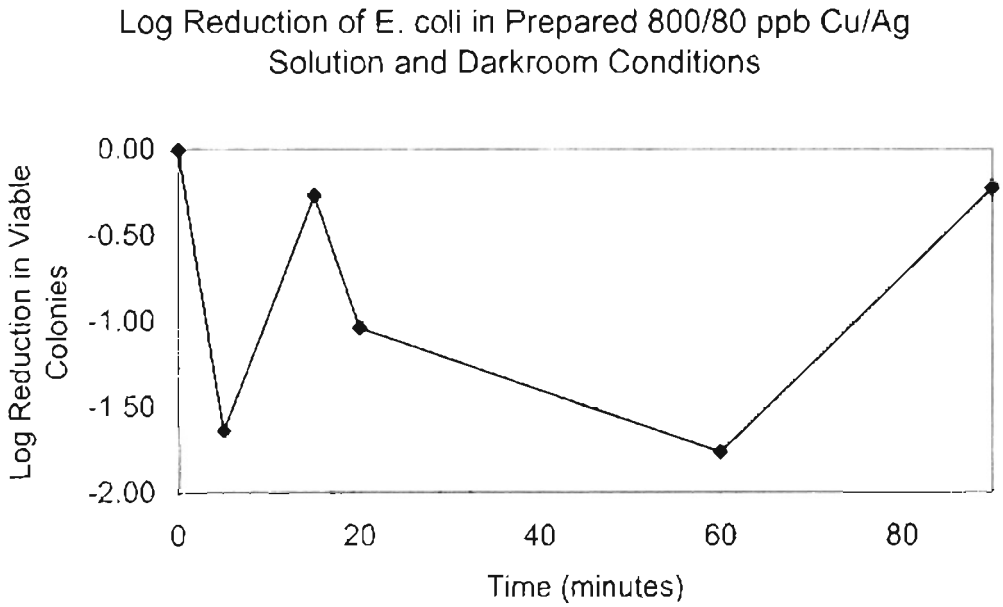

Figure C-3

Bacterial Test 4

Table C-IV

Test Sample:	800ppb Cu / 80 ppb Ag
Control Sample:	Deionized Water
Volume:	1 Quart
Date:	6/22/00
CFU Inoculation:	5 mL E.Coli broth per quart
Additional:	Test performed in lighted conditions

Control Sample Colony Count				
Time (min)	A	B	C	Avg.
0	TMTC	TMTC	TMTC	NA
1/5 dilution	TMTC	TMTC	148	148
90	TMTC	TMTC	TMTC	NA
1/5 dilution	172	154	TMTC	163

Test Sample Colony Count					
Time (min)	A	B	C	Avg.	log Reduction
0	TMTC	TMTC	TMTC	740	0.0000
5	134	124	132	130	-0.7553
10	45	77	107	76	-0.9865
15	100	53	76	76	-0.9865
20	56	45	68	56	-1.1185
30	47	63	34	48	-1.1880
45	36	37	41	38	-1.2894
60	78	49	65	64	-1.0631
90	45	41	30	39	-1.2819

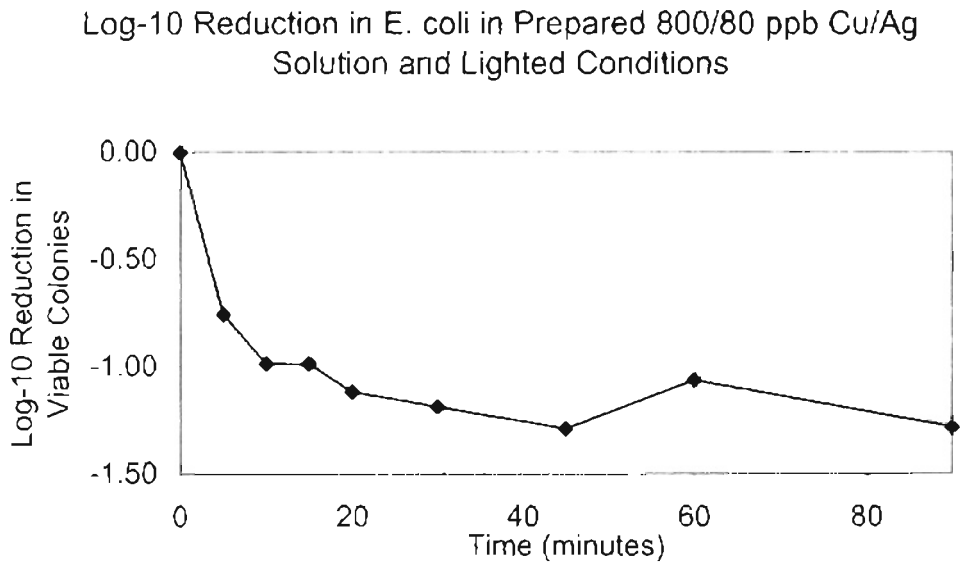

Figure C-4

Bacterial Test 5

Table C-IV

Test Sample:	800ppb Cu / 80 ppb Ag
Control Sample:	Deionized Water
Volume:	1 Quart
Date:	6/22/00
CFU Inoculation:	5 mL E.Coli broth per quart
Additional:	Test performed in darkroom conditions

Control Sample Colony Count				
Time (min)	A	B	C	Avg.
0	TMTC	TMTC	TMTC	NA
1/5 dilution	TMTC	TMTC	148	148
90	TMTC	TMTC	TMTC	NA
1/5 dilution	172	154	TMTC	163

Test Sample Colony Count					
Time (min)	A	B	C	Avg.	Inact. Rate
0	TMTC	TMTC	TMTC	740	0.0000
5	134	124	132	130	-0.7553
10	45	77	107	76	-0.9865
15	100	53	76	76	-0.9865
20	56	45	68	56	-1.1185
30	47	63	34	48	-1.1880
45	36	37	41	38	-1.2894
60	78	49	65	64	-1.0631
90	45	41	30	39	-1.2819

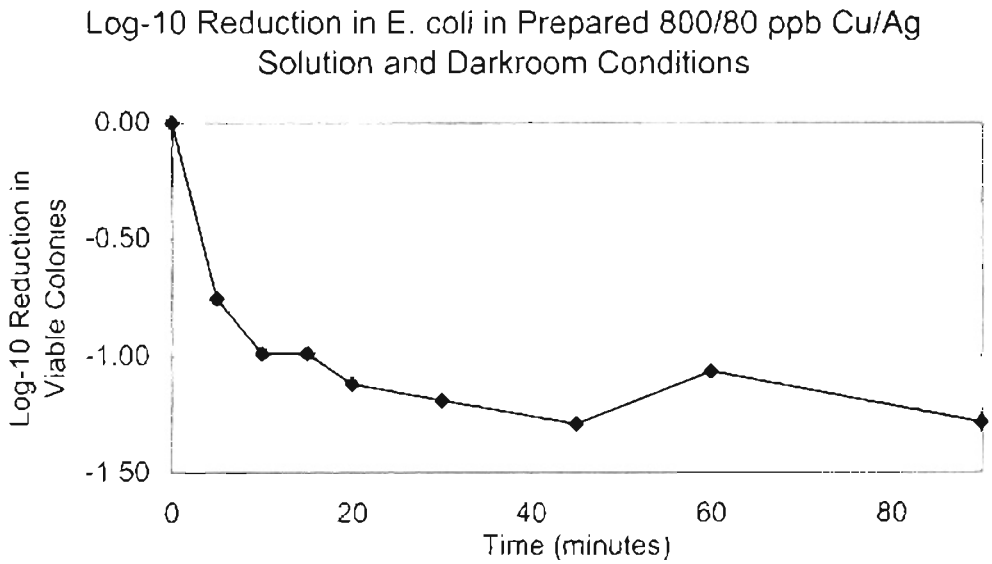

Figure C-5

Bacterial Test 6

Table C-VI

Test Sample:	Ionic concentration as generated by the IGD in 0.1M IS
Control Sample:	Deionized Water, 0.1 M ISA
Volume:	1 Quart
Date:	6/27/00 Test A
CFU Inoculation:	5 mL E Coli broth per quart
Additional:	Test performed in darkroom conditions

Control Sample Colony Count				
Time (min)	A	B	C	Avg
0	TMTC	TMTC	TMTC	TMTC
1/5 dilution	170	216	300	229
90	TMTC	TMTC	TMTC	NA
1/5 dilution	300	300	175	258
1 day	TMTC	TMTC	TMTC	NA

Test Sample Colony Count					
Time (min)	A	B	C	Avg.	Inact. Rate
0	TMTC	TMTC	TMTC	1143	0.0001
5	TMTC	TMTC	TMTC	TMTC	NA
10	TMTC	TMTC	16	16	-1.8539
15	9	TMTC	TMTC	9	-2.1038
20	TMTC	TMTC	TMTC	TMTC	NA
30	TMTC	TMTC	TMTC	TMTC	NA
45	TMTC	11	TMTC	11	-2.0167
60	6	TMTC	TMTC	6	-2.2799
90	NA	15	TMTC	15	-1.8820
24 hours	0	0	3	1	-3.0580

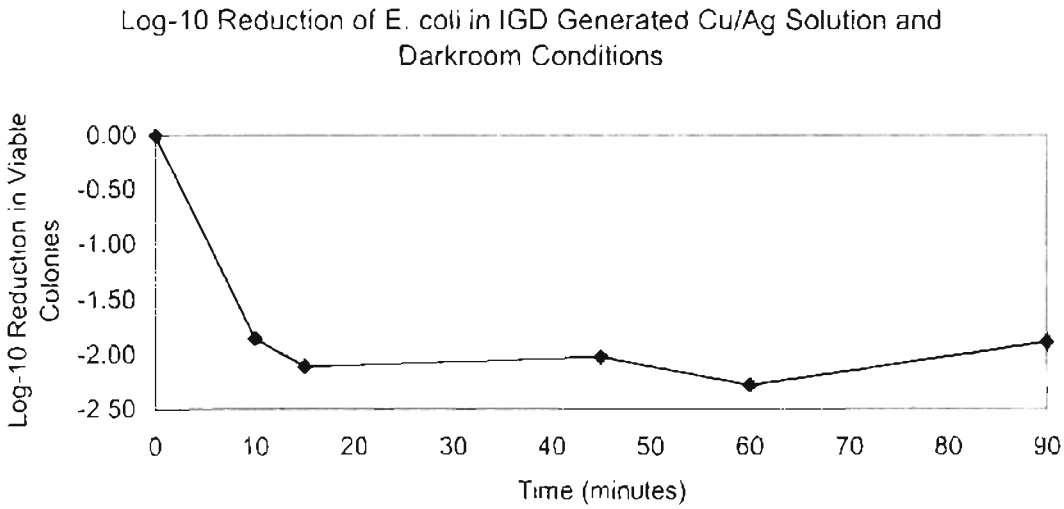

Figure C-6

Bacterial Test 7

Table C-VII

Test Sample:	620 ppb Cu / 62 ppb Ag / 0.1 M $NaNO_3$
Control Sample:	0.1 M $NaNO_3$
Volume:	1 Quart
Date:	7/11/00
CFU Inoculation:	5 mL E.Coli broth per quart
Additional:	Test performed in darkroom conditions
	Test A, Morning

Control Sample Colony Count				
Time (min)	A	B	C	Avg.
0	TMTC	TMTC	TMTC	
1/5 dilution	TMTC	TMTC	TMTC	NA
90	TMTC	TMTC	TMTC	
1/5 dilution	TMTC	TMTC	TMTC	NA

Test Sample Colony Count					
Time (min)	A	B	C	Avg.	log Reduction
0	TMTC	TMTC	TMTC	NA	NA
5	TMTC	TMTC	TMTC	NA	NA
10	TMTC	TMTC	TMTC	NA	NA
15	65	TMTC	TMTC	65	NA
20	TMTC	TMTC	TMTC	NA	NA
30	TMTC	TMTC	TMTC	NA	NA
45	7	TMTC	TMTC	7	NA
60	TMTC	TMTC	TMTC	NA	NA
90	TMTC	TMTC	TMTC	NA	NA
24 Hours	0	0	0	0	NA

Log reduction cannot be calculated because Control Sample dilutions yielded TMTC viable colonies

Bacterial Test 8

Table C-VIII

Test Sample:	620 ppb Cu / 62 ppb Ag / 0.1 M $NaNO_3$
Control Sample:	0.1 M $NaNO_3$
Volume:	1 Quart
Date:	7/11/00
CFU Inoculation:	5 mL E.Coli broth per quart
Additional:	Test performed in darkroom conditions Test B, Afternoon

Control Sample Colony Count				
Time (min)	A	B	C	Avg.
0	TMTC	TMTC	TMTC	
1/5 dilution	TMTC	TMTC	TMTC	NA
90	TMTC	TMTC	TMTC	
1/5 dilution	TMTC	TMTC	TMTC	NA

Test Sample Colony Count					
Time (min)	A	B	C	Avg.	log Reduction
0	TMTC	TMTC	TMTC	NA	NA
5	TMTC	TMTC	TMTC	NA	NA
10	TMTC	TMTC	TMTC	NA	NA
15	16	TMTC	TMTC	16	NA
20	TMTC	TMTC	TMTC	NA	NA
30	TMTC	TMTC	TMTC	NA	NA
45	1	TMTC	TMTC	1	NA
60	TMTC	TMTC	TMTC	NA	NA
90	109	42	TMTC	76	NA
24 Hours	0	0	0	0	NA

Log reduction cannot be calculated because Control Sample dilutions yielded TMTC viable colonies

Biological Experiment 9

Table C-IX

Test Sample: 800 ppb Cu / 80 ppb Ag / 0.1 M $NaNO_3$

Control Sample: 0.1 M $NaNO_3$

Volume: 1 Quart

Date: 7/20/00

CFU Inoculation: 2.5 mL E.Coli broth per quart

Additional: Test performed in lighted conditions
Test A, Morning

Control Sample Colony Count				
Time (min)	A	B	C	Avg.
0	TMTC	TMTC	TMTC	NA
1/5 dilution	TMTC	TMTC	TMTC	NA
90	TMTC	TMTC	TMTC	NA
1/5 dilution	TMTC	TMTC	TMTC	NA

Test Sample Colony Count					
Time (min)	A	B	C	Avg.	log Reduction
0	TMTC	TMTC	TMTC	NA	NA
5	TMTC	TMTC	TMTC	NA	NA
10	TMTC	TMTC	TMTC	NA	NA
15	TMTC	TMTC	TMTC	NA	NA
20	TMTC	TMTC	TMTC	NA	NA
30	TMTC	TMTC	TMTC	NA	NA
45	TMTC	TMTC	TMTC	NA	NA
60	TMTC	TMTC	TMTC	NA	NA
90	TMTC	TMTC	TMTC	NA	NA
24 Hours	0	0	0	0	NA

Initial concentration cannot be calculated because Control Sample dilutions yielded TMTC viable colonies

Bacterial Test 10

Table C-X

Test Sample:	800 ppb Cu / 80 ppb Ag / 0.1 M $NaNO_3$
Control Sample:	0.1 M $NaNO_3$
Volume:	1 Quart
Date:	7/20/00
CFU Inoculation:	2.5 mL E.Coli broth per quart
Additional:	Test performed in darkroom conditions
	Test B, Afternoon

Control Sample Colony Count				
Time (min)	A	B	C	Avg.
0	TMTC	TMTC	TMTC	NA
1/5 dilution	TMTC	TMTC	TMTC	NA
90	TMTC	TMTC	TMTC	NA
1/5 dilution	TMTC	TMTC	TMTC	NA

Test Sample Colony Count					
Time (min)	A	B	C	Avg.	log Reduction
0	TMTC	TMTC	TMTC	1500	0.0000
5	49	105	140	98	-1.1849
10	12	57	168	79	-1.2785
15	105	68	86	86	-1.2399
20	67	40	80	62	-1.3814
30	89	94	68	84	-1 2535
45	98	68	73	80	-1.2748
60	65	71	63	66	-1.3544
90	71	63	72	69	-1.3393
24 hours	0	0	0	0	NA

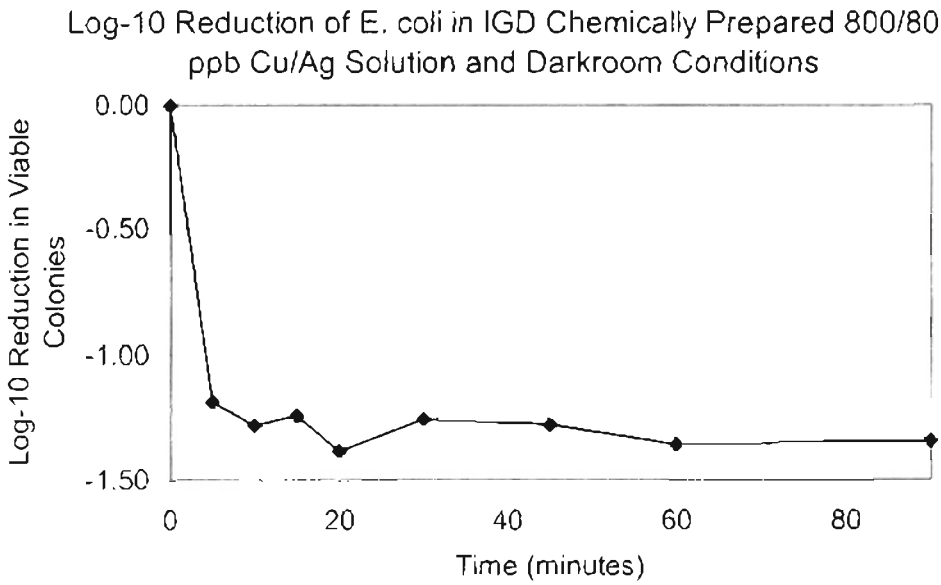

Figure C-10

APPENDIX D: Experimental Data for IGD Tests

IGD Test 1

Table D-1

Experiment: Copper electrode calibration
Date: 6/14/00
Performed by: RS
Additional: Calibration standards prepared in 0.1 M HSA

Copper Conc. (ppb)	Log Copper Conc	Meter mV Reading
550	2.740	161.3
600	2.778	162.1
650	2.813	162.9
700	2.845	163.2
750	2.875	164.6
800	2.903	165.3
850	2.929	167.1
900	2.954	167.8
950	2.978	168.8
1000	3.000	169.2

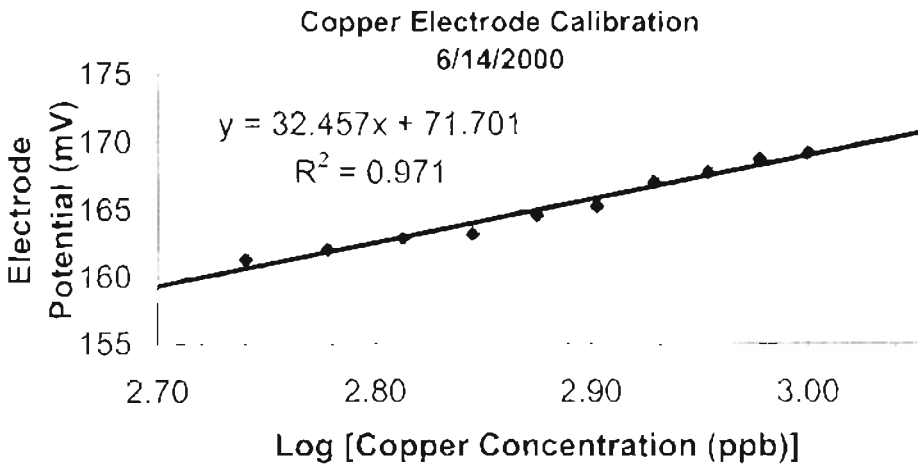

Figure D-1

Table D-II

Experiment: Repeat testing of isolated copper ion generation
Date: 6.14.00
Performed by: RS
Additional: Samples generated in 0.1 M HSA
Parallel electrode configuration

Test #	Electrode Potential (mV)	Cu Conc (ppb)	Desired Cu Conc. (ppb)	Ionization Efficiency
1	161.7	593	800	0.74
2	161.9	601	800	0.75
3	162.4	623	800	0.78
4	160.6	548	800	0.69
5	160.8	556	800	0.70
6	161.7	593	800	0.74
7	165.2	760	800	0.95
8	162.2	614	800	0.77
9	162.8	641	800	0.80
10	162.2	614	800	0.77

Avg. Ionization Efficiency	0.768

IGD Test 2

Table D-III

Experiment: Silver electrode calibration
Date: 6.20.00
Performed by RS
Comments: Meter readings performed at 10 minutes
Calibration standards prepared in 0.1 M HSA

Silver Conc. (ppb)	Log Silver Conc.	Meter mV Reading
40	1.602	190.9
50	1.699	205.6
60	1.778	208.8
70	1.845	213.5
80	1.903	216.1
90	1.954	220.2
100	2.000	224.4

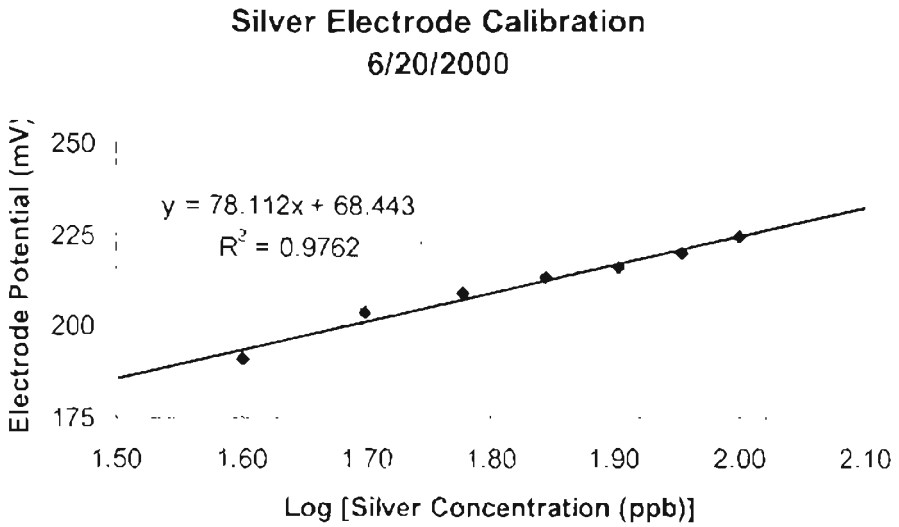

Figure D-2

Table D-IV

Experiment:	Repeat testing of isolated silver ion generation
Date	6/20/00
Performed by:	RS
Additional:	Samples generated in 0.1 M ISA
	Anode and cathode are pure silver
	Parallel electrode configuration
	Darkroom conditions
	Meter readings performed at 10 minutes

Test #	Electrode Potential (mV)	Ag (ppb)	Desired Ag Conc. (ppb)	Ionization Efficiency
1	208.0	61	80	0.76
2	206.9	59	80	0.74
3	206.4	58	80	0.73
4	207.4	60	80	0.75
5	208.2	62	80	0.77
6	206.7	59	80	0.74
7	207.8	61	80	0.76
8	211.9	69	80	0.86
9	212.0	69	80	0.86
10	211.0	67	80	0.84
			Avg. Ionization Efficiency	0.781

IGD Test 3

Table D-V

Experiment: Silver electrode calibration
Date: 7/6/00
Performed by: RS
Comments: Meter readings performed at 10 minutes
Calibration standards prepared in 0.1 M ISA

Silver Conc. (ppb)	Log Silver Conc.	Meter Reading (mV)
30	1.477	164.3
50	1.699	176.6
70	1.845	204.1
90	1.954	213.7
110	2.041	220.5

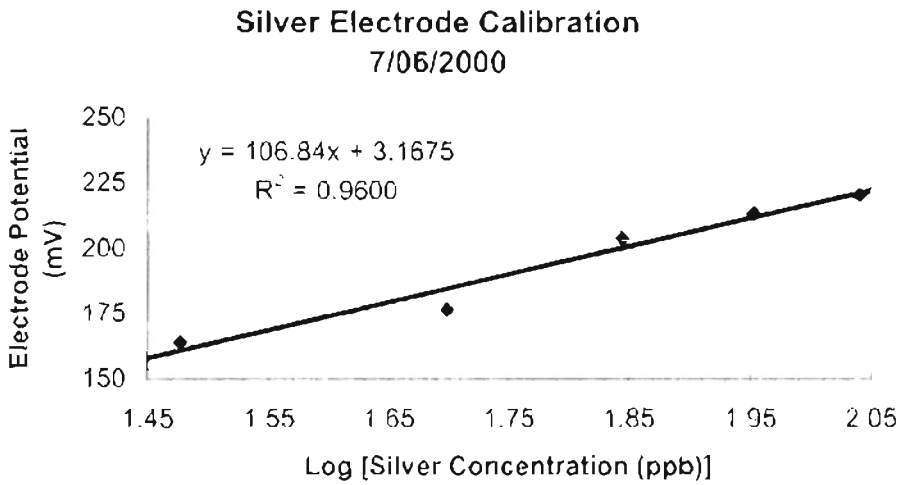

Figure D-3

Table D-VI

Experiment: Repeat testing of isolated silver ion generation
Date: 7/6/00
Performed by: RS
Additional: Samples generated in 0.1 M ISA
Anode and cathode are pure silver
Parallel electrode configuration
Darkroom conditions
Meter readings performed at 10 minutes

Test #	Electrode Potential (mV)	Ag Conc (ppb)	Desired Ag Conc. (ppb)	Ionization Efficiency
1	198.7	68	80	0.85
2	198.4	67	80	0.84
3	198.8	68	80	0.85
4	196.0	64	80	0.80
5	192.5	59	80	0.74
6	193.1	60	80	0.75
7	191.8	58	80	0.73
8	193.7	61	80	0.76
9	195.0	62	80	0.78
10	196.1	64	80	0.80

Avg. Ionization Efficiency	0.789

IGD Test 4

Table D-VII

Experiment: Copper electrode calibration
Date: 7/21/00
Performed by: RS
Additional: Calibration standards prepared in 0.1 M. ISA
Meter readings performed at 10 minutes

Copper Conc (ppb)	Log Copper Conc.	Meter mV Reading
550	2.740	162.4
600	2.778	165.8
650	2.813	167.3
700	2.845	168.1
750	2.875	169.0
800	2.903	169.3
850	2.929	171.1
900	2.954	172.7
950	2.978	174.1
1000	3.000	175.4

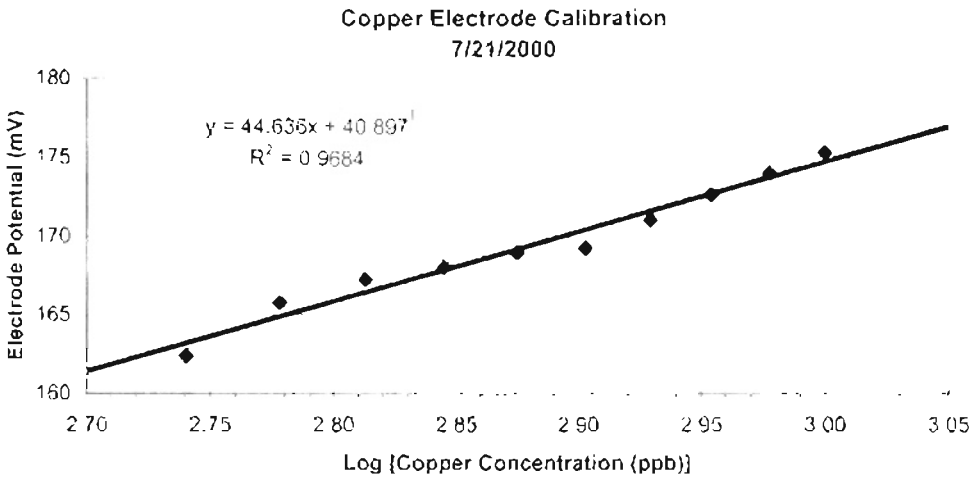

Figure D-4

Table D-VIII

Experiment: Repeat testing of isolated copper ion generation
Date: 7/21/00
Performed by: RS
Additional: Samples generated in 0.1 M HSA
Meter readings performed at 5 minutes
ICID firmware chip version 2

Test #	Electrode Potential (mV)	Cu Conc. (ppb)	Desired Cu Conc. (ppb)	Ionization Efficiency
1	162	517	800	0.65
2	159.8	461	800	0.58
3	161.6	506	800	0.63
4	159.8	461	800	0.58
5	159.9	464	800	0.58
6	156.9	397	800	0.50
7	159.6	456	800	0.57
8	161.5	503	800	0.63
9	162.5	530	800	0.66
10	163.5	558	800	0.70

Avg. Ionization Efficiency	0.607

IGD Test 5

Table D-IX

Experiment: Silver electrode calibration
Date: 7/24/00
Performed by: RS
Comments: Meter readings performed at 5 minutes
Calibration samples prepared in 0.1 M ISA

Silver Conc (ppb)	Log Silver Conc.	Meter Reading (mV)
20	1.301	195.9
30	1.477	208.2
40	1.602	215.5
50	1.699	222.4
60	1.778	226.8
70	1.845	230.7
80	1.903	233.2
90	1.954	236.1

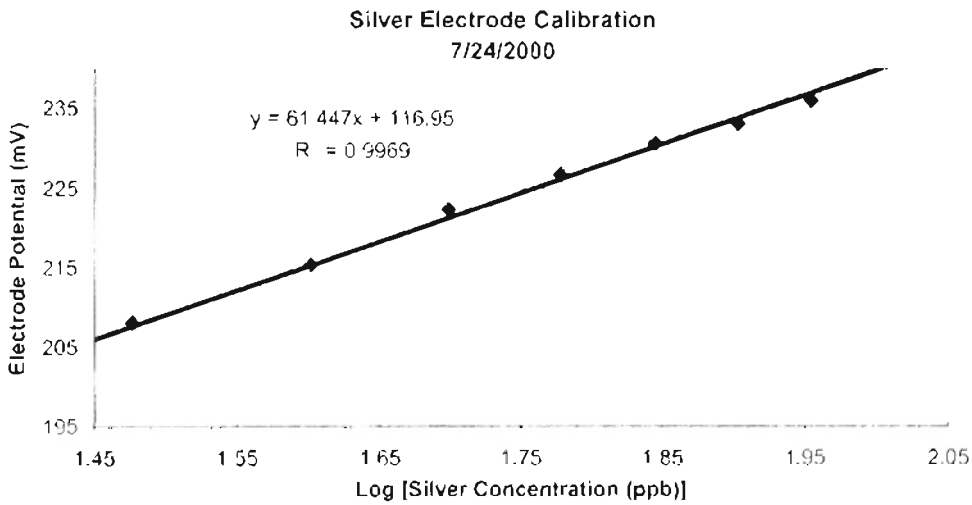

Figure D-5

Table D-X

Experiment: Repeat testing of isolated silver ion generation
Date: 7 24 00
Performed by: RS
Additional: Samples generated in 0.1 M HSA
Anode and cathode are pure silver
Parallel electrode configuration
Darkroom conditions
Measurements recorded after 5 minutes
IGD firmware chip version 2

Test #	Electrode Potential (mV)	Ag Conc. (ppb)	Desired Ag Conc. (ppb)	Ionization Efficiency
1	217.4	43	80	0.54
2	218.9	46	80	0.57
3	220.4	48	80	0.60
4	220.2	48	80	0.60
5	222.7	53	80	0.66
6	216.2	41	80	0.52
7	227.2	62	80	0.78
8	196.3	20	80	0.24
9	206.1	28	80	0.35
10	218.3	45	80	0.56

Avg. Ionization Efficiency	0.542

IGD Test 6

Table D-XI

Experiment: Copper electrode calibration
Date: 7 26 00
Performed by: RS
Additional: Calibration standards prepared in 0.1 M HSA
Meter readings performed at 5 minutes

Copper Conc (ppb)	Log Copper Conc	Meter mV Reading
500	2.699	159.5
600	2.778	164.6
700	2.845	165.6
800	2.903	168
900	2.954	170.2

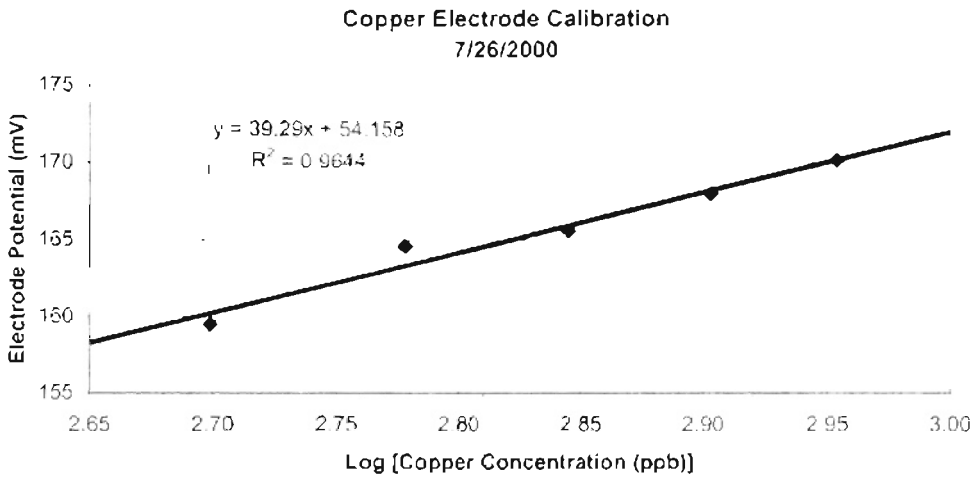

Figure D-6

Table D-XII

Experiment: Repeat testing of isolated copper ion generation
Date: 7/26/00
Performed by: RS
Additional Samples generated in 0.1 M HSA
Parallel electrode configuration
Meter readings performed at 5 minutes
IGD firmware chip 1 retest
Desired Cu concentration (ppb) 800

Test #	Electrode Potential (mV)	Cu Conc (ppb)	Ionization Efficiency	Generation Cycle Time (s)
1	161.3	535	0.67	34.5
2	160.5	509	0.64	30.40
3	161.9	552	0.69	32.70
4	162.4	569	0.71	32.8
5	163.2	596	0.75	33.1
6	162.8	582	0.73	33.6
		557	0.696	32.85
		Avg. Gen Conc. (ppb)	Avg. Ionization Efficiency	Avg. Cycle Time (seconds)

IGD Test 7

Table D-XIII

Experiment: Copper electrode calibration
Date: 7-27-00
Performed by: RS
Additional: Calibration samples prepared in 0.1 M ISA
Readings taken after 5 minutes

Copper Conc. (ppb)	Log Copper Conc.	Meter mV Reading
400	2.602	160.3
500	2.699	162.6
600	2.778	165
700	2.845	167.7
800	2.903	169
900	2.954	171.3

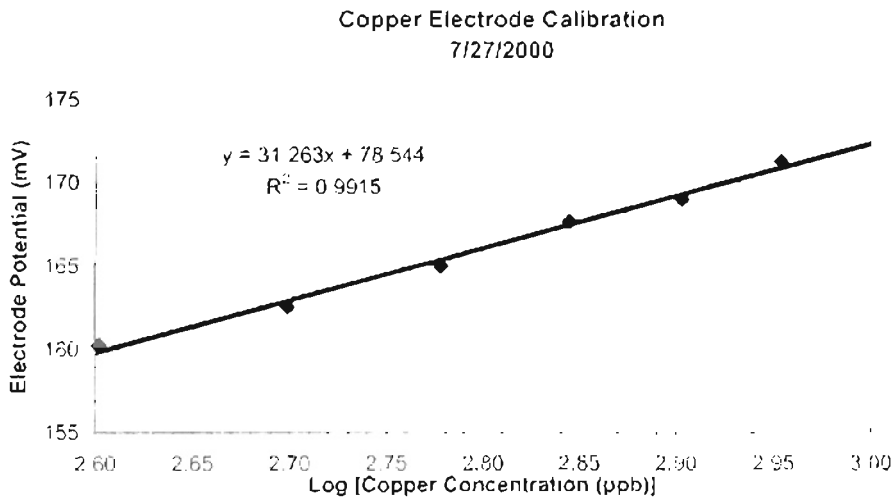

Figure D-7

Table D-XIV

Experiment:	Repeat testing of isolated copper ion generation
Date:	7/27/00
Performed by:	RS
Additional:	Samples generated in 0.1 M HSA
	Readings recorded at 10 minutes
	IGD firmware chip version 2 retest
	Desired Cu concentration (ppb) 800

Test #	Electrode Potential (mV)	Cu Conc. (ppb)	Ionization Efficiency	Generation Cycle Time (s)
1	172.3	998	1.25	42.5
2	171.2	920	1.15	42.60
3	172.4	1005	1.26	41.70
4	172.7	1027	1.28	42
5	172.6	1020	1.27	39.3
6	172.8	1035	1.29	39.1
		1001	1.251	41.20
		Avg. Gen. Conc. (ppb)	Avg. Ionization Efficiency	Avg. Cycle Time (seconds)

IGD Test 8

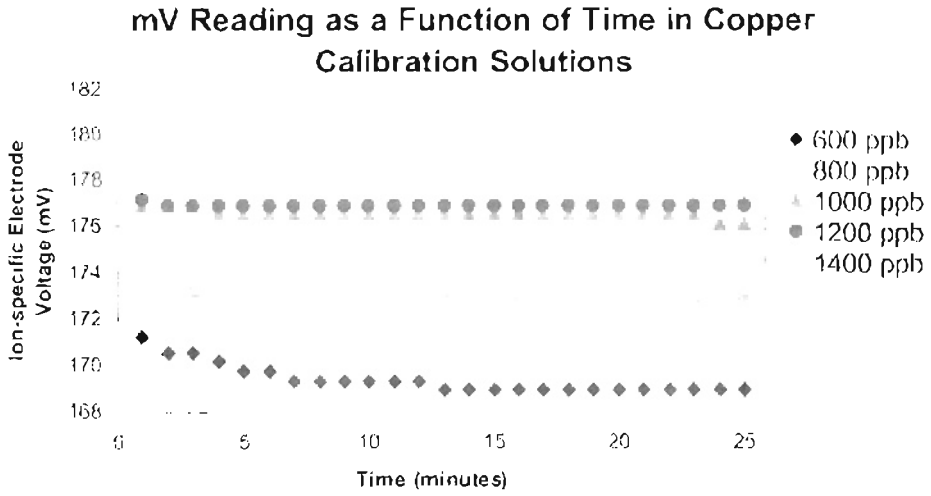

Figure D-8

Table D-XV

Experiment: Determination of correct reading time for copper calibration solutions
Date: 7-31-00
Additional: Solutions prepared in 0.1 M HSA

	600 ppb Cu	800 ppb Cu	1000 ppb Cu	12000 ppb Cu	14000 ppb Cu
Time (min)	mV Reading	mV Reading	mV Reading	mV Reading	mV Reading
1	171.2	173.6	176.9	177.2	180.1
2	170.5	173.6	176.9	176.9	180.1
3	170.5	173.2	176.9	176.9	179.7
4	170.1	173.2	176.5	176.9	179.7
5	169.7	173.2	176.5	176.9	179.7
6	169.7	173.2	176.5	176.9	179.7
7	169.3	173.2	176.5	176.9	179.7
8	169.3	173.2	176.5	176.9	179.7
9	169.3	173.2	176.5	176.9	179.7
10	169.3	173.2	176.5	176.9	179.7
11	169.3	173.2	176.5	176.9	179.7
12	169.3	173.2	176.5	176.9	179.7
13	168.9	172.9	176.5	176.9	179.4
14	168.9	172.9	176.5	176.9	179.4
15	168.9	172.9	176.5	176.9	179.4
16	168.9	172.9	176.5	176.9	179.4
17	168.9	172.9	176.5	176.9	179.4
18	168.9	172.9	176.5	176.9	179.4
19	168.9	172.9	176.5	176.9	179.4
20	168.9	172.9	176.5	176.9	179.4
21	168.9	172.9	176.5	176.9	179.4
22	168.9	172.9	176.5	176.9	179.4
23	168.9	172.9	176.5	176.9	179.4
24	168.9	172.9	176.1	176.9	179.4
25	168.9	172.9	176.1	176.9	179.4

IGD Test 16

Table D-XVI

Experiment: Determination of correct reading time for generated copper solutions
Date: 7/31/00
Additional: Generation in 0.1 M HSA
IGD firmware chip version 2

	Run 1	Run 2	Run 3
Time (min)	mV Reading	mV Reading	mV Reading
1	158.0	167.0	165.5
2	159.1	168.1	166.5
3	160.0	168.8	167.0
4	161.1	169.4	167.8
5	162.0	170.2	168.6
6	162.9	170.6	169.4
7	163.7	171.0	169.9
8	164.5	171.4	170.6
9	165.6	171.8	171.1
10	166.1	172.2	171.5
11	166.9	172.8	172.2
12	167.8	172.8	172.6
13	168.5	173.4	173.0
14	168.9	173.4	173.1
15	169.7	173.8	173.5
16	170.2	173.8	173.9
17	170.6	173.8	174.3
18	171.0	174.2	174.3
19	171.4	174.2	174.7
20	171.8	174.6	175.1
21	172.0	174.6	175.1
22	172.2	174.6	175.3
23	172.6	174.6	175.5
24	172.6	174.6	175.5
25	173.0	175.1	175.5
26	173.4	175.1	175.5
27	173.4	175.1	175.9
28	173.6	175.1	175.9
29	173.6	175.1	175.9
30	173.6	175.1	175.9
31	174.2	175.1	175.9
32	174.2	175.4	176.3
33	174.2	175.4	176.3
34	174.2	175.4	176.3
35	174.2	175.4	176.9
36	174.2	175.4	176.9
37	174.6	175.4	176.9
38	174.6	175.4	176.9
39	174.6	175.4	176.9
40	174.6	175.4	177.2

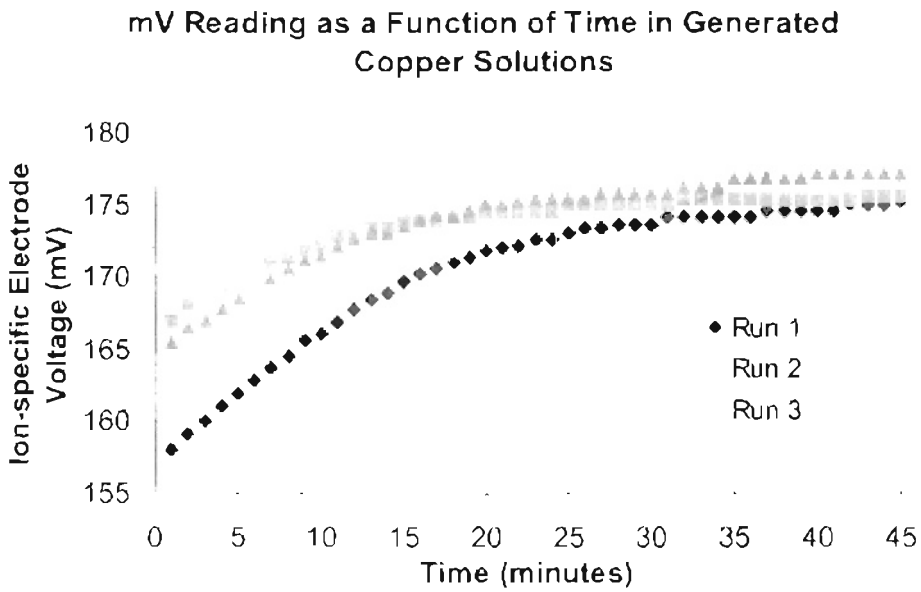

Figure D-9

IGD Test 10

Table D-XVII

Experiment:	Copper electrode calibration
Date:	08/08/00
Performed by:	RS
Additional	Calibration solutions prepared in 0.1 M ISA Meter readings performed at 10 minutes IGD firmware chip version 1 retest

Copper Conc (ppb)	Log Copper Conc	Meter mV Reading
500	2.699	164.8
600	2.778	166.7
700	2.845	169.5
800	2.903	170.9
900	2.954	172.7
1000	3.000	174.2

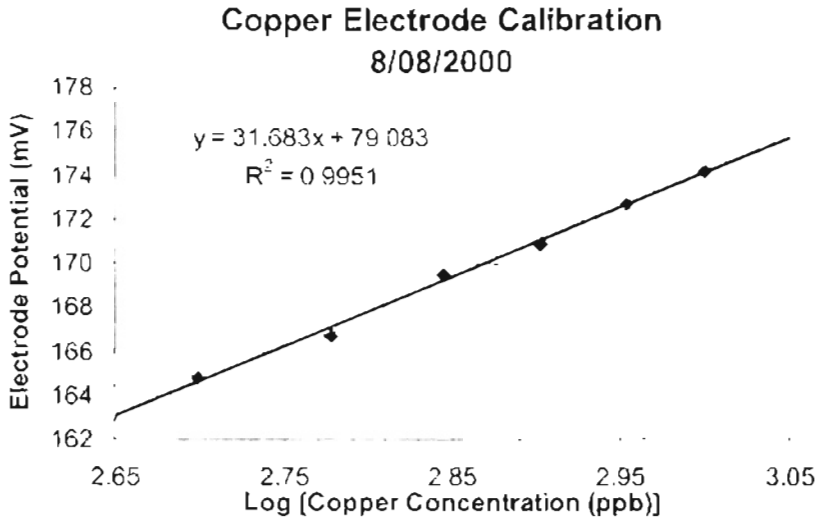

Figure D-10

Table D-XVIII

Experiment:	Repeat testing of isolated copper ion generation
Date:	8/8/00
Performed by:	RS
Additional	Samples generated in 0.1 M HSA
	Parallel electrode configuration
	Meter readings performed at 35 minutes
	IGD firmware chip version 1 retest
	Desired Cu concentration (ppb) 800

Test #	Electrode Potential (mV)	Cu Conc (ppb)	Ionization Efficiency	Generation Cycle Time (s)
1	172.3	875	1.094	32.0
2	172.4	882	1.102	32.2
3	172.3	875	1.094	31.2
4	173.4	948	1.185	31.4
5	172.5	888	1.110	32.0
6	172.8	908	1.135	32.6
7	171.9	850	1.063	31.4
8	169.6	719	0.899	34.3
		868	1.085	32.1
		Avg. Gen. Conc. (ppb)	Avg. Ionization Efficiency	Avg. Cycle Time (seconds)

IGD Test 11

Table D-XIX

Experiment: Determination of correct reading time for silver calibration solution
Date 8 23.00
Additional: Solutions prepared in 0.1 M HSA

	40 ppb Ag	60 ppb Ag+	80 ppb Ag+	100 ppb Ag+
Time (min)	mV Reading	mV Reading	mV Reading	mV Reading
1	201.2	206.5	213.0	223.1
2	199.8	206.3	213.0	223.1
3	199.0	206.3	213.0	223.1
4	198.5	206.3	213.4	223.1
5	198.2	206.3	213.4	223.4
6	197.8	206.3	213.4	223.4
7	197.8	206.3	213.4	223.4
8	197.8	206.4	213.4	223.4
9	197.4	206.4	213.8	223.4
10	197.4	206.4	213.8	223.4
11	197.4	206.4	213.8	223.4
12	197.4	206.4	213.8	223.4
13	197.4	206.4	213.8	223.4
14	197.4	206.4	213.8	223.4
15	197.4	206.4	213.8	223.4
16	197.4	206.4	213.8	223.4
17	197.4	206.4	213.8	223.4
18	197.4	206.4	213.8	223.4
19	197.4	206.4	213.8	223.4
20	197.0	206.4	213.8	223.4
21	197.0	206.4	213.8	223.4
22	197.0	206.4	213.8	223.4
23	197.0	206.4	213.8	223.4
24	197.0	206.4	213.4	223.4
25	197.0	206.4	213.4	223.4

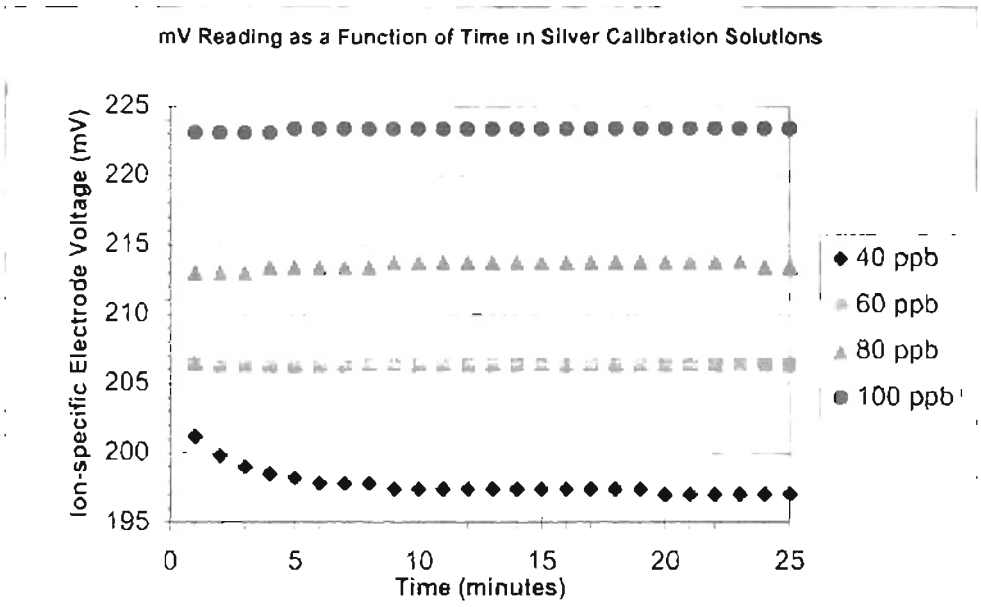
mV Reading as a Function of Time in Silver Calibration Solutions
Ion-specific Electrode Voltage (mV)
225
220
215
210
205
200
195
0
5
10
15
20
25
Time (minutes)
40 ppb
60 ppb
80 ppb
100 ppb

Figure D-11

IGD Test 12

Table D-XX

Experiment: Determination of correct reading time for generated silver sol
Date: 8-23-00
Additional: Solutions generated in 0.1 M HSA
IGD firmware chip version 1

	Run 1	Run 2	Run 3
Time (min)	mV Reading	mV Reading	mV Reading
1	219.5	216.5	213.5
2	218.1	216.5	214.3
3	217.7	216.9	214.7
4	217.3	216.9	215.0
5	217.3	216.9	215.0
6	217.3	216.9	215.0
7	217.3	216.9	215.4
8	217.3	216.9	215.4
9	217.3	216.9	215.4
10	217.3	216.9	215.4
11	217.3	216.9	215.6
12	217.3	216.9	215.8
13	217.3	216.9	215.8
14	217.3	216.9	215.8
15	217.3	216.9	215.8
16	217.3	216.9	215.8
17	217.3	216.9	215.8
18	217.3	216.9	215.8
19	217.7	216.9	215.8
20	217.7	216.9	215.8
21	217.7	216.9	215.8
22	217.7	216.9	215.8
23	217.7	216.9	215.8
24	217.7	216.9	215.8
25	217.7	216.9	215.8
26	217.7	216.9	215.8
27	217.7	216.9	215.8
28	217.7	216.9	215.8
29	217.7	216.9	215.8
30	217.7	216.9	215.8
31	217.7	216.9	215.8
32	218.1	216.9	215.8
33	218.1	216.9	215.8
34	218.1	216.9	215.8
35	218.1	216.9	215.8
36	218.1	216.9	215.8
37	218.1	216.9	215.8
38	218.1	216.9	215.8
39	218.1	216.9	215.8
40	218.1	216.9	215.8
41	218.1	216.9	215.8
42	218.1	216.9	215.8
43	218.1	216.9	215.8

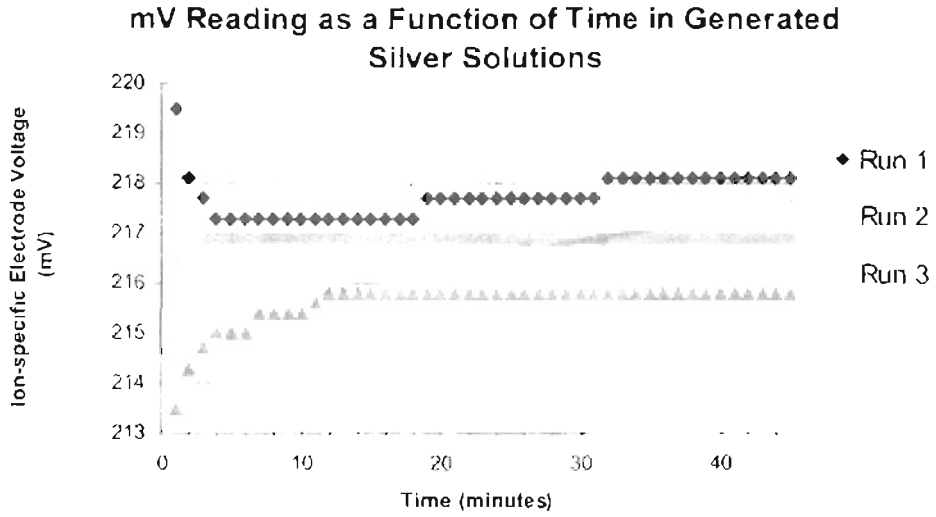

Figure D-12

IGD Test 13

Table D-XXI

Experiment	Silver electrode calibration
Date:	08/30/00
Performed by:	RS
Additional:	Calibration solutions prepared in 0.1 M ISA Meter readings performed at 15 minutes

Silver Conc. (ppb)	Log Silver Conc.	Meter mV Reading
70	1.845	209.5
80	1.903	213.2
85	1.929	214.1
90	1.954	216.4
95	1.978	216.9
100	2.000	218.8

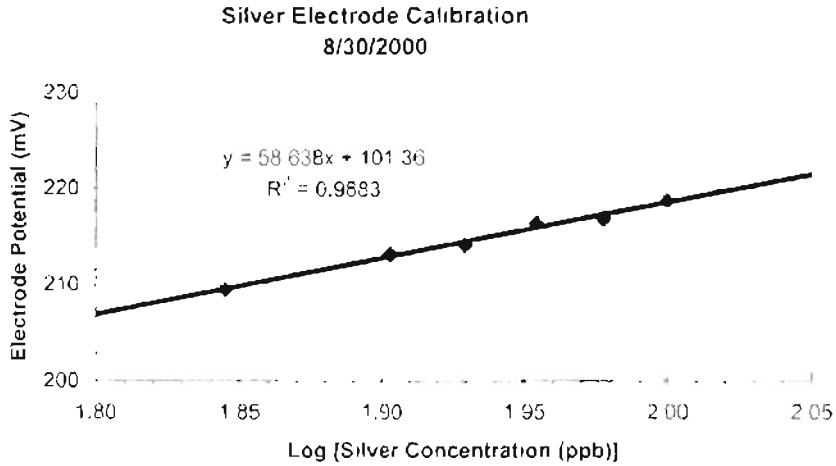

Figure D-13

Table D-XXII

Experiment: Repeat testing of isolated silver ion generation
Date: 8/30/00
Performed by: RS
Additional: Samples generated in 0.1 M HSA
Parallel electrode configuation
Meter readings performed at 35 minutes
IGD firmware chip version 1 retest

Test #	Electrode Potential (mV)	Ag Conc (ppb)	Desired Ag Conc. (ppb)	Ionization Efficiency
1	219.9	105	80	1.314
2	217.9	97	80	1.214
3	217.8	96	80	1.195
4	217.7	96	80	1.205
5	217.9	97	80	1.214
6	218.6	100	80	1.248
7	217.6	96	80	1.200
8	218.4	99	80	1.238
9	217.0	94	80	1.172
10	219.4	103	80	1.288
		98		1.229
		Avg. Gen. Conc. (ppb)		Avg. Ionization Efficiency

IGD Test 14

Table D-XXIII

Experiment: Copper electrode calibration
Date: 9/25/00
Performed by: RS
Additional: Calibration solutions prepared in 0.1 M HSA
IGD firmware chip version 3
Meter readings performed at 15 minutes

Copper Conc. (ppb)	Log Copper Conc	Meter mV Reading
750	2.875	169.7
800	2.903	170.1
850	2.929	171.1
900	2.954	171.9
950	2.978	172.8
1000	3.000	174.0

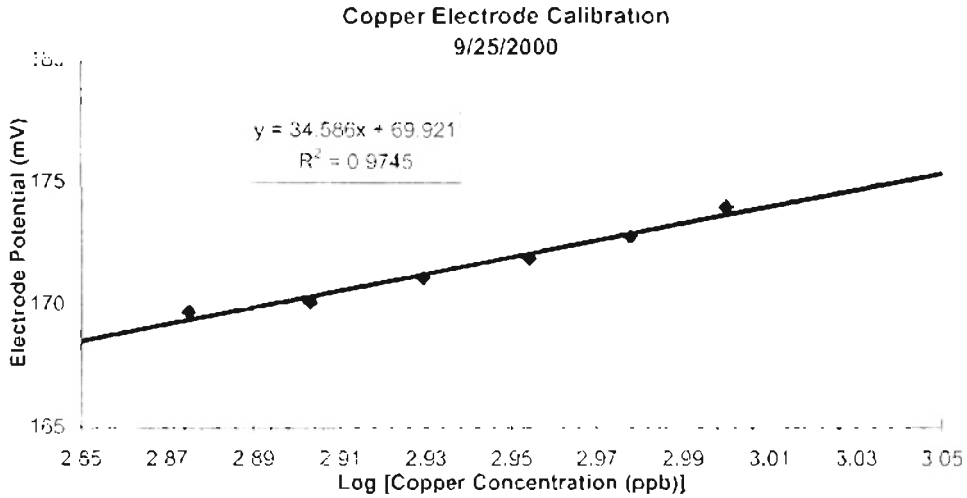

Figure D-14

Table D-XXIV

Experiment: Repeat testing of isolated copper ion generation
Date: 9.25.00
Performed by: RS
Additional: Samples generated in 0.1 M ISA
Parellel electrode configuration
Meter readings perfromed at 35 minutes
Chip I TLST
1 Quart Setup
Desired Cu concentration (ppb) 800

Test #	Electrode Potential (mV)	Cu Conc. (ppb)	Ionization Efficiency	Generation Cycle Time (s)
1	175.9	1159	1.449	30.0
2	174.3	1042	1.303	35.5
3	173.8	1008	1.260	34.3
4	174.0	1022	1.277	31.1
5	172.0	894	1.118	36.8
		1025	1.281	33.5
		Avg. Conc.	Avg. Ionization	Avg. Cycle Time

IGD Test 15

Table D-XXV

Experiment: Copper electrode calibration
Date: 11/10/00
Performed by: RS
Additional: Calibration solutions prepared in 0.1 M HSA
Meter readings perfromed at 15 minutes

Copper Conc (ppb)	Log Copper Conc	Meter mV Reading
600	2.778	166.4
800	2.903	170.2
1000	3.000	172.1
1200	3.079	174.5
1400	3.146	175.2

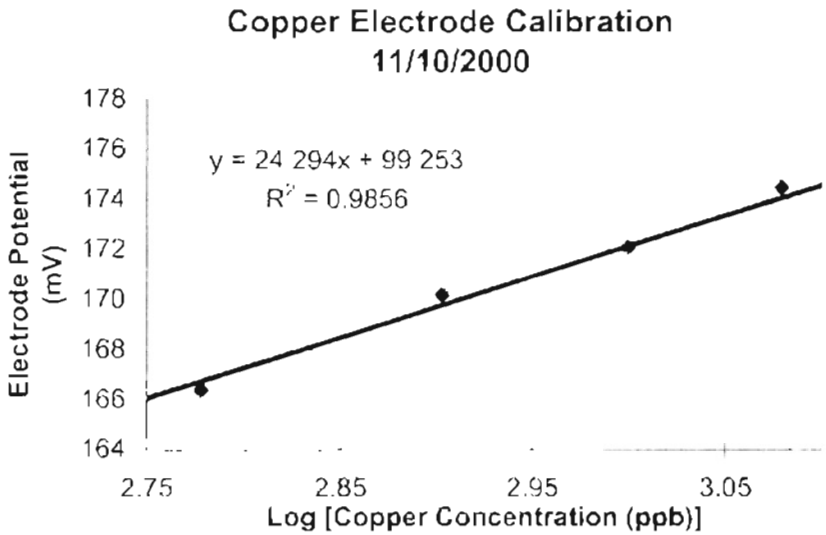

Figure D-15

Table D-XXVI

Experiment: Repeat testing of isolated copper ion generation
Date: 11 10 00
Performed by: RS
Additional: Samples generated in 0.1 M HSA
Parallel electrode configuration
Meter readings performed at 35 minutes
IGD firmware chip version 3
1 quart setup
Outlet voltage prior to test 121.5 - 0 1V
Desired Cu concentration (ppb) 800

Test #	Electrode Potential (mV)	Cu Conc. (ppb)	Ionization Efficiency	Generation Cycle Time (s)
1	171.8	969	1.211	131.0
2	172.1	997	1.246	135.0
3	172.0	987	1.234	129.0
4	171.8	969	1.211	131.0
5	172.0	987	1.234	127.0
		982	1.227	130.6
		Avg. Gen. Conc. (ppb)	Avg. Ionization Efficiency	Avg. Cycle Time (seconds)

IGD Test 16

Table D-XXVII

Experiment: Copper electrode calibration
Date: 12 20 00
Performed by: RS
Additional: Calibration solutions prepared in 0.1 M HSA
Meter readings performed at 15 minutes

Copper Conc (ppb)	Log Copper Conc.	Meter mV Reading
600	2.778	166.2
700	2.845	167.6
800	2.903	168.5
900	2.954	169.8
1000	3.000	171.2

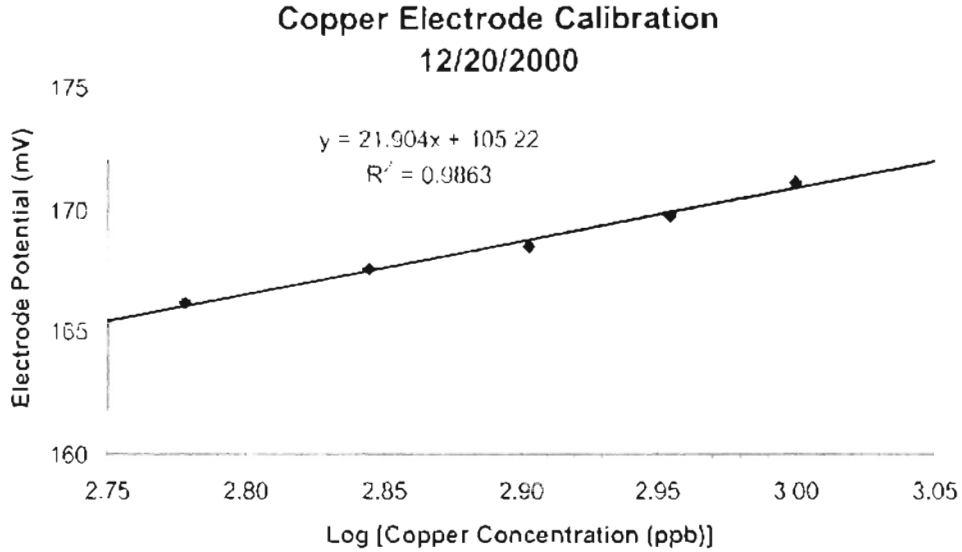

Figure D-16

Table D-XXVIII

Experiment:	Repeat testing of isolated copper ion generation
Date:	12.20.00
Performed by:	RS
Additional:	Samples generated in 0.1 M HSA
	Parallel electrode configuration
	Meter readings performed at 35 minutes
	IGD firmware chip version 4
	Liquant setting
	Outlet voltage prior to test: 121.5 +/- 0.1V
	Electrode Spacing 6cm
	Desired Cu concentration (ppb) 800

Test #	Electrode Potential (mV)	Cu Conc (ppb)	Ionization Efficiency	Generation Cycle Time (s)
1	167.0	661	0.827	17.5
2	165.7	577	0.721	17.2
3	166.0	595	0.744	17.8
4	165.6	571	0.714	17.9
5	165.0	536	0.670	17.8
		588	0.735	17.6
		Avg. Gen. Conc. (ppb)	Avg. Ionization Efficiency	Avg. Cycle Time (seconds)

IGD Test 17

Table D-XXIX

Test: Determination of the effect of light on generated silver ion measurement
Date: 1/9/01
Additional: Generation in 0 1 M ISA
Generated by Firmware ver. 4

Run 1Dark		Run 2 Dark		Run 3 Dark	
Time (min)	mV Reading	Time (min)	mV Reading	Time (min)	mV Reading
5	205.0	5	204 8	5	205.0
10	204 2	10	204 8	10	205.0
15	203.8	15	205 3	15	205 0
20	203 8	20	205.3	20	205 0
25	203.8	25	205.3	25	205 0
30	203 8	30	205.3	30	205.4

Run 1 Light		Run 2 Light		Run 3 Light	
Time (min)	mV Reading	Time (min)	mV Reading	Time (min)	mV Reading
5	205.5	5	203.9	5	204.7
10	205.5	10	204.2	10	204 7
15	205.5	15	204.5	15	204 3
20	205.1	20	204.9	20	204.3
25	204 6	25	205 3	25	203.9
30	204.6	30	205.7	30	203 9

IGD Test 18

Table D-XXX

Experiment:	Silver Electrode Calibration in 0.1 M HSA
Date:	1-12-01
Performed by	RS
Additional	Calibration solutions prepared in 0.1 M HSA Meter readings performed at 15 minutes

Silver Conc (ppb)	Log Silver Conc.	Meter mV Reading
50	1.699	193.3
60	1.778	199
70	1.845	202.1
80	1.903	205.9
90	1.954	208.1

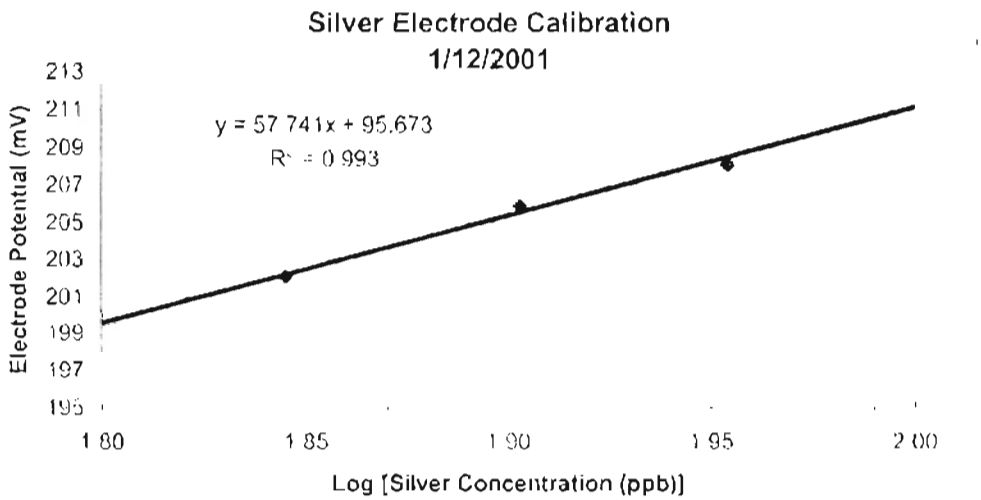

Figure D-17

Table D-XXXI

Experiment: Repeat testing of isolated silver ion generation
Date: 1-12-01
Performed by: RS
Additional: Samples generated in 0.1 M HSA
1 Quart volumetric setting
Parallel electrode configuration
Meter readings performed at 35 minutes
IGD firmware chip version 4
Electrode spacing 6 cm

Test #	Electrode Potential (mV)	Ag Conc (ppb)	Desired Ag Conc. (ppb)	Ionization Efficiency
1	206.9	84	80	1.055
2	206.8	84	80	1.051
3	205.6	80	80	1.002
4	205.2	79	80	0.986
5	204.4	76	80	0.955
6	203.9	75	80	0.936
		80		0.997
		Avg. Gen. Conc. (ppb)		Avg. Ionization Efficiency

IGD Test 19

Table D-XXXII

Experiment: Silver electrode calibration
Date: 1-29-01
Performed by: RS
Additional: Calibration solutions prepared in 0.1 M HSA
IGD firmware chip version 4
Meter readings performed at 15 minutes
Calibration standard concentrations increased for 1 gallon generation setting to 1 quart volume

Equivalent Ag Conc. (ppb)	Silver Conc. (ppb)	Log Silver Conc	Meter mV Reading
70	280	2.447	239.5
75	300	2.477	241.0
80	320	2.505	241.5
85	340	2.531	242.9
90	360	2.556	244.7

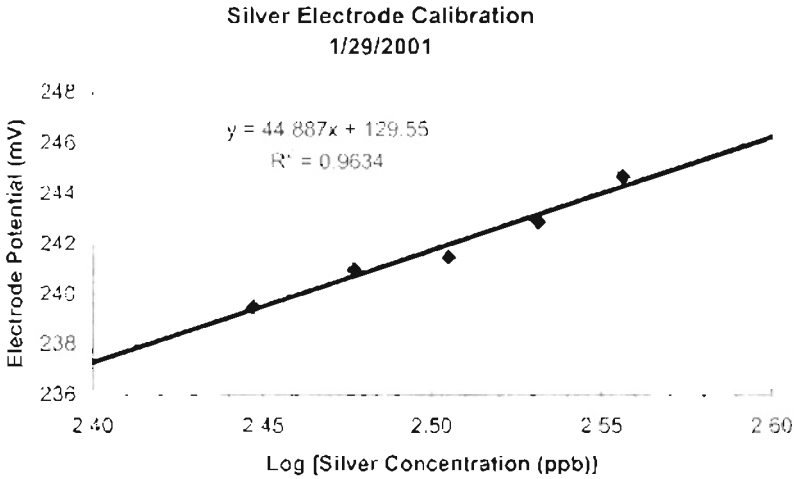

Figure D-18

Table D-XXXIII

Experiment: Repeat testing of isolated silver ion generation
Date: 1-29-01
Performed by: RS
Additional: Samples generated in 0.1 M HSA
1 gallon volumetric setting
Measurement electrode immersed at conclusion of generation cycle
Readings recorded at 35 minutes
Chipset 4-11-ST
Desired Ag concentration (ppb) 80

Test	Electrode Potential (mV)	Ag Conc. in quart (ppb)	Ag Conc. in gallon (ppb)	Ionization Efficiency
1	239.8	286	71	0.893
2	239.8	286	71	0.893
3	240.6	298	74	0.931
4	240.5	296	74	0.926
5	240.1	290	73	0.907
6	240.5	296	74	0.926
		292	73	0.913
		Avg. Qt. Conc. (ppb)	Avg. Gal. Conc. (ppb)	Avg. Ionization Efficiency

VITA

Randy M. Sharp

Candidate for the Degree of

Master of Science

Thesis: EFFECTS OF COPPER AND SILVER IONIC SOLUTIONS ON *E. COLI* BACTERIA AND TESTING OF A PROTOTYPE ION-GENERATION DEVICE

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Teaching Assistant, School of Chemical Engineering, Oklahoma State University, January 2001 to May 2001

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