ANTIMICROBIAL COPPER IODIDE MATERIALS

by

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DEDICATION

To Mom.

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ABSTRACT

Environmental microorganisms are implicated as the causative agents in a significant portion of healthcare associated infections (HAI) and antimicrobial resistant infections (AMR), which result in increased costs and suffering around the world. Furthermore, common environmental microorganisms participate in microbiological degradation of materials and the bio-fouling of various systems. This also results in a tremendous amount of damage in many different materials and many different sectors.

The focus of this dissertation was the development of an additive that could be easily added to common materials to make them self-disinfecting and to protect them from microbial damage. The ultimate goal was to develop an additive that could be added using standard techniques and without adversely affecting the final material.

Cuprous iodide (CuI) was determined to be an ideal starting material for the development of improved antimicrobial materials because of its neutral appearance and high antimicrobial activity as compared to other silver and copper materials. It was found that the antimicrobial efficacy of CuI could be amplified if prepared as a small particle and especially in the presence of vinylpyrrolidone polymers. A comminution process was then developed to produce these small particles. By using select copolymers, various CuI small particle formulations were developed to be compatible with a variety of different matrices.

The efficacy of these CuI containing matrices was dependent on the compatibility of the CuI formulation with the matrix. A variety of applications were demonstrated with good antimicrobial efficacy where the particles were easily added to the finished material with minimal or no change in appearance.

Chapter 1. Introduction

1.1. Motivations

1.1.1. Summary

Microbes, including bacteria, fungi, viruses, and protozoa, are responsible for a tremendous amount of monetary and nonmonetary costs due to the degradation of materials and infectious diseases. Although these issues are dealt with in very different ways, they are both inextricably linked and it has increasingly been found that the contamination of one leads to the contamination of the other. Microbial contamination of materials is typically treated using biocides designed only to protect the material from degradation; however, a rapidly growing class of materials are those that are designed to prevent the transfer of these microbes between humans via surfaces.

The actual number of infections that are acquired through contaminated materials is unknown, however, it is accepted to be significant. Especially in healthcare/hospital settings where infection has been increasingly associated with environmental contamination, there is a clear linkage between contaminated materials and diseased patients. The high cost of hospital acquired infections and the threat of antimicrobial drug resistant pathogens has encouraged many materials solutions dedicated towards reducing the spread of pathogenic microorganisms. One approach has been through the development of materials with antimicrobial self-disinfecting surfaces.

Currently there are two types of antimicrobial surfaces approved by the US Environmental Protection Agency (US EPA) to make public health claims. In 2008, the Copper Development Association first registered five copper metal alloys through the US Environmental Protection Agency (EPA) to make public health claims[1]. Since then, 282

copper alloys have been registered to make similar public health claims[2]. In 2012, a 16 wt% cuprous oxide containing material was approved for Cupron Technologies to make similar claims. These materials have been proven to be very effective at reducing the rate of patient acquisition of certain infectious diseases, however, they suffer from various cosmetic and processing limitations.

It is believed that the development of antimicrobial additives to produce cosmetically attractive and easily processed, self-disinfecting surfaces will accelerate the implementation of this technology. While many antimicrobial additives (e.g., 10,10-oxybisphenoxyarsine [OBPA]) exist for the protection of the material from microbial degradation (e.g. preservatives/biocides), these have not passed the US EPA criteria for making public health claims. Many of these materials are too toxic, thermally unstable, or expensive, which precludes them from being incorporated in typical matrices using traditional processing at sufficient levels to enable a meaningful self-disinfecting effect.

Thus, there is a need for an antimicrobial additive that is compatible with typical materials using traditional manufacturing processes (e.g., melt blending of thermoplastics), which is also minimally toxic, thermally stable, and inexpensive for creating cosmetically appealing and effective self-disinfecting surfaces.

1.1.2. Role of Surfaces in Infectious Diseases

Many pathogens are shed from patients and contaminate the surrounding environment. Many of these pathogens can survive and remain infective on environmental surfaces for months. These include most gram-positive bacteria (e.g. *Staphylococcus aureus*, *Clostridium difficile*), many gram-negative bacteria (e.g.,

Pseudomonas aeruginosa), many mycobacteria (e.g. *Mycobacterium tuberculosis*), and many gastrointestinal viruses (e.g. *Poliovirus*)[3]. Although it has been established that surfaces are commonly contaminated with these pathogenic microorganisms, it is difficult to prove that they are the direct origin of any particular infection.

After several decades of very strict hygiene practices, several studies in the 1970s and 1980s suggested that the effects of contaminated environmental surfaces had a negligible effect on infection rates. As a result of these studies, routine sampling of hospital environmental surfaces was reduced in the US from 75% of hospitals in 1975 to almost none today[4]. A growing body of more recent evidence, however, suggests that environmental surfaces are a substantial reservoir for a variety of pathogens to infect humans[5].

Many studies have reported that the rate of infection in hospital patients correlates strongly with the infective condition of the previous occupant of the room. If a hospital room was previously occupied by a patient who was colonized with a pathogenic bacterium, then the likelihood of that same bacterium colonizing the next patient in that room is significantly higher. This has been demonstrated for methicillin-resistant *Staphylococcus aureus* (MRSA)[6], vancomycin-resistant enterococci (VRE)[7] [8], multi-drug-resistant *P. aeruginosa*[9], multi-drug-resistant *Acinetobacter baumannii* [10], and *Clostridium difficile*[11]. This increased risk of infection is often quite substantial. For instance, a study by Datta et al. on 10 intensive care units found that admitted patients had a 40% increased risk of acquiring VRE or MRSA if their room had previously been occupied by a VRE or MRSA infected patient[12].

While healthcare environments appear to be the most studied, there is ample evidence that all surfaces can harbor and spread pathogens. Device surfaces, such as phones[13], have been indicated as pathogen reservoirs for many years. Many reports have established that home[14] and public surfaces[15] are commonly contaminated with pathogenic microorganisms. Furthermore, many case studies indicate that pathogens survive on surfaces in an infectious state for long times and are commonly not eliminated using standard cleaning practices. Some of these organisms and case studies that demonstrate the contribution of environmental surfaces to infection are described below.

Norovirus is the most common cause of gastroenteritis, which causes inflammation in the digestive system, nausea, vomiting, and diarrhea. It is known to persist on many environmental surfaces[16] and can survive for weeks on stainless steel[17]. Gastroenteritis is responsible for 19-21 million sicknesses, 56,000-71,000 hospitalizations, and 570-800 deaths per year in the US[18].

A Centers for Disease Control and Prevention (CDC) report on five acute gastroenteritis outbreaks occurring on US cruise ships from July to December of 2002 found that three of the cruise ships suffered from repeated gastroenteritis outbreaks on subsequent voyages despite adherence to standard CDC disinfection protocol in between voyages[19]. The original organism was found to be responsible for the repeated outbreaks on each ship. Outbreaks continued on the three ships until they were taken out of service for 7-10 days for aggressive cleaning and sanitization. Many similar situations have been reported in hotels and hospitals.

These viruses can remain infective on a variety of surfaces for extended periods of time and in many cases, are not removed or disinfected using typical

cleaning/disinfection routines[20] [21]. Self-disinfecting surfaces may, however, offer a solution. A study by Warnes et al showed that norovirus was fully (>99.999%) killed within 2 hours on a variety of copper alloys, but remained active for weeks on stainless steel, PVC, and glass[22]. In particular, it was found that released cuprous ions were especially responsible for inactivation of the virus in another study by the same authors[23].

Clostridium difficile is an anaerobic gram-positive bacterium and was responsible for hundreds of thousands healthcare associated infections in 2011 with 29,000 reported deaths occurring within the first 30 days of diagnosis[24]. It has been shown to survive on various hospital surfaces for up to five months[25], and is known to form dormant, hard-to-kill spores. Kato et al. found a positive correlation between *C. difficile* contamination levels on environmental surfaces and healthcare workers hands across three hospitals in Japan[26]. Furthermore, a single strain of *C. difficile* was found to be dominant on both the surfaces and in infected patients. Fawley et al. similarly found a significant correlation between levels of *C. difficile* on environmental surfaces, healthcare worker hands, and infection rates in a hospital ward[27]. In these cases there was a single dominant strain on the hands, the environment, and in infected patients. These studies reinforce the suggestion that the environment can harbor and spread the specific infective pathogens that infect patients.

The impact of the environment on the spread of *C. difficile* is further supported by the fact that enhanced cleaning has been shown to reduce the incidence of infection by *C. difficile*[28] and is recommended by the US CDC[29]. The complete elimination of vegetative *C. difficile* cells on copper surfaces has been reported to occur within 30 minutes[30]; however, complete elimination of spores has been reported in the range of 2 days[31].

Many other organisms survive on surfaces and likely colonize patients or are transmitted between healthcare workers and other patients. These are discussed more in the following sections describing the importance of surfaces in hospital acquired infections (1.1.2.1) and antimicrobial resistant pathogens (1.1.2.2). Many of these organisms are also eliminated by copper based surfaces, which have recently been shown to provide significant health and cost savings as discussed in section 1.1.4.

It is now generally believed that environmental surfaces substantially contribute to the spread of pathogenic microorganisms. Although microbial contamination of surfaces has been well correlated with infection rates in many studies, such correlations are often confounded by other factors. Nevertheless, it is accepted that environmental surfaces harbor and spread pathogenic microorganisms. In healthcare settings there has recently been significant effort dedicated to understanding the role of surface contamination in the spread of infectious disease.

1.1.2.1.Healthcare Associated Infections

Healthcare associated infections or hospital-acquired infections, HAIs, have become recognized as a major drain on the healthcare system as well as having a large impact on mortality and morbidity. Most reports suggest that there is approximately a 1-in-25 chance of a patient acquiring an HAI during their healthcare visit. HAIs include infections occurring during treatment, but possibly not presenting until after discharge; as well as occupational infections of healthcare workers.

Comprehensive HAI surveillance systems have not yet been implemented in many countries and HAI rates are mainly estimated by extrapolation of smaller studies

rather than by direct tracking. Furthermore, lack of uniform diagnostic criteria for HAIs, as well as the convoluted methods of monitoring infection rates and determination of origination, results in inherently approximate values of HAI rates. Nevertheless, nearly every study has reported HAI rates in the range of large percentages of the patient population.

According to the World Health Organization (WHO), 7% of all hospital patients in high-income countries and 10% of all patients in developing countries will develop an HAI. In the US, the risk is reportedly slightly less, and around 4 to 5% of US hospital patients are expected to acquire an infection as a result of their stay[32]. This results in millions of additional sicknesses and hundreds of thousands of deaths attributed to HAIs each year. In the US alone HAIs are reported to account for nearly 100,000 deaths per year (US CDC[33]).

The monetary cost of HAIs is similarly staggering. A survey of 1,355,347 admissions from 2001 to 2006 in 55 US hospitals by Kilgore et al. found that each nosocomial infection increased medical costs by $12,197 on average[34]. The WHO has reported similar figures, demonstrating the average cost per HAI in Mexico was $12,155 USD. The WHO reports the direct cost of HAIs to amount to €7 billion/yr in Europe and $6.5 billion/yr in the US. The US CDC, however, has reported the costs to be significantly higher, ranging from $28-45 billion/yr[35].

Despite conflicts and inaccuracies in reporting, both the human and fiscal costs of HAIs are sufficiently large that the prevention of even a small fraction of these infections would result in a substantial economic benefit and meaningful public health improvement. Furthermore, many HAIs are also associated with antimicrobial resistance

and thus prevention of HAIs is also important as a precaution against antimicrobial resistant organisms.

It is believed that contaminated surfaces are involved in the cross-transmission of microbes between patients and health care personnel and are responsible for at least some portion of HAI occurrences. A large number of case studies have associated contaminated surfaces with HAI outbreaks or directly tracked the transfer of unique organisms from infected patients to the environment. Generally, bacterial contamination of hospital surfaces is strongly associated with patient proximity[36]. The transmission efficiency of microbes from surface to hand and hand to mouth is high[37] and many studies have demonstrated the importance of hand cleanliness in infection control. Thus, although it is difficult to provide a direct link to HAI rates, there is sufficient evidence and rationale to suggest that environmental surfaces are at least partly to blame.

As such, self-disinfecting surfaces represent a useful tool in combatting HAIs. To this end, a U.S. Department of Defense funded study found that the implementation of self-disinfecting copper surfaces resulted in a 50% reduction in the incidence of HAIs across 3 separate hospital intensive care unit (ICU) wards (see section 1.1.4). Even if this figure is used with cautiously, it represents a potential tremendous savings of cost and human health.

1.1.2.2.Antimicrobial Resistance

HAIs are commonly associated with antimicrobial resistant organisms. In fact, about 70% of HAI mortalities are associated with an antimicrobial resistant pathogen[38]. Thus, these two major issues are inseparable and must be dealt with together.

Antimicrobial resistance (AMR) refers to the ability of a microorganism to resist being killed or inactivated by an antimicrobial. This term has especially been used to refer to resistance against antibacterial drugs. For instance, although bacteria, parasites, viruses and fungi are included in the WHO report on AMR, antibacterial drug resistance accounts for the majority of the report[39]. A brief review of several aspects of AMR and the differences between AMR drug resistance and resistance to other AM substances is presented.

In his Nobel Prize speech for discovering penicillin, Alexander Flemming warned of antibacterial drug resistance. Even before penicillin was publicly introduced, resistance by staphylococcus species had been observed. Since then, antibiotics have been used and produced at enormous levels; >23 million kg/year in the US with 50% going to humans and 50% going to agriculture[40]. A lack of stewardship programs, regulation, and misunderstanding of the consequences of AMR has rendered a large fraction of once useful drugs, useless today.

In 1975, Stuart Levy demonstrated that the use of tetracycline in a farm directly led to tetracycline resistant bacteria in humans who lived on and around the farm. Since then many other studies have shown that antimicrobial resistance, once developed, rapidly spreads throughout the surrounding community. Further, it has been shown that antibiotic resistance is accelerated near wastewater sites, farm effluent discharge, and other areas where antibiotic containing wastes are released. In 2013 the FDA issued voluntary guidance for phasing out medically important antibiotics from agriculture and some nations have already established more rigorous AMR surveillance and regulations.

Despite these efforts, it is not appropriate to assume that this will impact current AMR levels to make these drugs useful again.

Although humans have accelerated AMR by irresponsible use, antibacterial drug resistance is a natural phenomenon that results from the selective pressure caused by the use of antibacterial drugs themselves. Wherever antimicrobial drugs are used resistance is likely to eventually develop. As these drugs are used, surviving resistant bacteria are selected for, become dominant, and spread; and eventually clinically relevant levels of the drug are not effective. Once these resistant microbes colonize and infect humans, another drug is needed to eliminate the bacteria. As this cycle repeats, however, multidrug resistant organisms (MDROs) are spawned, some of which are untreatable because they are resistant to all of the available drugs.

Commonly these resistance characteristics are carried on transferable genes, such as plasmids and transposons, which can transfer resistance between different bacterial species through horizontal gene transfer. Thus, non-resistant bacteria may become resistant by encountering resistant bacteria in the environment[41] and without ever encountering the antimicrobial drug. Similarly, MDROs can also form in this fashion. Thus, it is expected to be especially beneficial to prevent intermingling of various AMR organisms in high-risk areas such as in hospital environments.

It is worth considering the impact that antimicrobial drugs, and antibiotics in particular, have had on human health over the last 100 years. In the US 100 years ago infectious disease accounted for more than 30% of all deaths with tuberculosis, pneumonia, and diarrhea being the three most prevalent causes of death. Today, infectious disease accounts for less than 5% of mortality in the US.

Additionally, over the last 100 years, the mortality rate for specific infectious diseases has been dramatically reduced. For instance, the mortality rates for skin infections have decreased from 11% to 0.5% according the US CDC. These improvements are the result of improved public health measures (e.g. sanitation, clean water) and the development of antimicrobial drugs, especially antibiotics/antibacterials. As MDROs develop, however, we are becoming unable to cure or prevent many infections and run the risk of returning to pre-antibiotic era mortality rates for certain infectious diseases.

Although bacterial cells were first observed in 1675, up until the 19th century infectious disease was believed to result from contaminated air (miasma), religious origins (e.g. demons), or imbalances of the humors (earth, fire, water, air). Treatments routinely involved religious intervention, the use of minerals (commonly mercury and arsenic), herbal remedies, and other folk medicines.

Pioneering work by Robert Koch and Louis Pasteur in the late 1800s brought germ theory to the forefront and opened the door to systematic methods for combatting infectious disease. Antiseptics were immediately developed by Joseph Lister (for whom Listerine mouthwash was named), which resulted in immediate and substantial reductions in surgical site infections. The search for an internal antiseptic led to the development in 1908 of the first antibacterial drug, arsphenamine; an arsenic based agent only useful against treponema (e.g., syphilis). This was followed by the development of sulfonamides in the 1930s and the commercialization of penicillin in the 1940s. By the 1970s most of the fifteen antibacterial drug classes used today had been developed. Since the 1970s only

one new class of antibacterial has been discovered and antibacterial drug development has nearly slowed to a crawl compared to the activity of the 20th century.

As reports of MDROs increase, our arsenal of available antimicrobial drugs does not. The antibacterial drug development pipeline is nearly empty. This is partially due to the poor return on investment for the development of antibiotic drugs compared to drugs that treat chronic disorders. Drug R/D is very expensive and time-consuming and many other antimicrobial drugs already exist on the market to compete with any newcomers. Further, the possibility of AMR rapidly rendering new drugs obsolete is a major concern. Nevertheless, there are an increasing number of MDRO infections being reported, with rising mortality rates.

Since the 2000s, a number of national and international programs and agencies have formed in order to report/monitor/combat AMR as the number of untreatable MDRO pathogens increases. In response to growing concerns about AMR, the CDC has established four core actions for combatting antimicrobial resistance, including improved infection and transmission prevention. The WHO has also suggested "emphasis should be placed on prevention, including strengthening hygiene and infection prevention and control measures, improving sanitation…" Thus, self-disinfecting surfaces appear to be potential tools for the fundamentals tasks suggested by both the CDC and WHO for combatting AMR.

A substantial body of evidence suggests that environmental surfaces act as infection reservoirs for AMR pathogens[42]. It has been demonstrated that the use of self-disinfecting copper alloys can reduce the clinical infection rates of various resistant and

multi-drug resistant organisms. Thus, it is believed that self-disinfecting surfaces are a useful preventative measure in the war against AMR.

1.1.3. Traditional Disinfection Solutions

It is useful to explore why current cleaning strategies have not been sufficient. While the use of disinfectants on non-critical surfaces has been widely promoted for the reduction of surface microbial contamination, these products are typically applied by hand and suffer from poor adherence to application protocol. The application of disinfectants beyond simple surfaces (e.g. floors, counters, handrails, etc.) and on the intricate touch-surfaces (e.g. keyboards, telephones) is often inconvenient and less likely to be routinely and diligently carried out. Furthermore, most EPA registered disinfectants require a 10-minute exposure period, which must be followed according to federal law. This presents a gross burden of time to properly disinfect any substantial area.

Poor adherence to disinfection guidelines and irresponsible cleaning practices may also contribute to spread of disease causing pathogens. Contaminated disinfectants have been responsible for the creation of several pathogenic outbreaks. Pseudomonas species have been found in 80% of reported contaminated disinfectants, including commonly used chlorohexidrine, quaternary ammonium compounds, and phenolic-based disinfectants. Cleaning/disinfecting solutions must be changed or recharged every 3 to 4 rooms to prevent the accidental spread of pathogens. Mops and other reusable cleaning supplies can also spread microbial contaminants if they are not properly disinfected themselves between cleanings.

Many studies have found that if enhanced cleaning practices are adhered to, they can significantly reduce the rates of HAI and AMR. However, many studies have also found that surfaces are often not properly cleaned because adherence to good cleaning practice is often poor. The majority of hospital rooms (cleaned by hospital staff) are not appropriately cleaned[43]. A study by Carling et al. of 20,646 environmental surfaces at 36 US hospitals found that only 48% of surfaces were regularly cleaned to baseline, using empty rooms after terminal discharge as baseline[44]. The same study also found that improved cleaning was well correlated with increased hospital spending on environmental service personnel ($p=0.02$). Thus, improved cleaning is a timely and costly tool.

Surface cleanliness standards do not currently exist for the microbial hygiene of hospital environmental surfaces and hospital cleaning has not until quite recently been perceived or investigated a science[45]. Nevertheless, various agencies have suggested 2.5 aerobic cfu/cm^2 as an acceptable baseline for hospital microbial hygiene[46]. Using traditional cleaning methods, a large number of hospital surfaces have been reported above this baseline.

Six hundred and twenty hand washing stations were randomly selected within four UK hospitals and monitored for microbial contamination by Griffith et al. in 2003. It was found that 20% of faucet handles, soap dispensers, and paper towels dispensers were above the 2.5 aerobic cfu/cm^2 baseline. Touch surfaces in two different surgical wards were surveyed over 52 weeks and found to be above the baseline for 64% of the beds and hoists, 62% of the bedside lockers, and 44% over-bed tables. It is believed that these contaminated surfaces contribute to surgical site infections.

Justifying the use of disinfectants routinely or on all surfaces has been controversial with many reports conflicted on whether the disinfection of all noncritical surfaces is clinically and financially justifiable.[47] A randomized crossover study by Wilson et al. on the effects of cleaning hospital rooms three times per day using disinfectants compared to once per day, did not find enhanced cleaning to be cost or clinically justified[48]. A significant reduction of pathogens on healthcare workers' hands and environmental surfaces during the three cleanings per day time-period was reported, but no significant reduction in the rate of patient acquisition of MRSA was observed. This may possibly be due to the proliferation of bacteria originating from an untreated pathogenic reservoir.

Another study by Dancer et al., however, compared two hospital wards over the same time period wherein one received enhanced cleanings and the other did not. In this study, it was found that enhanced cleaning methods significantly reduced patient acquisition of MRSA by 26.6%[49]. The estimated cost savings of enhanced cleaning in the Dancer study were estimated between 30,000 to 70,000 GBP.

Despite the lack of direct clinical evidence, disinfection of noncritical surfaces is generally believed to be a useful practice and is recommended by the CDC (and most other national/global health agencies) for preventing the spread of pathogenic organisms. Nevertheless, routine disinfection of surfaces is inherently a timely and costly operation. The addition of enhanced disinfection cleaning practices may reduce the spread of HAI and AMR pathogens, but the cost/benefit justification has not been accepted. Self-disinfecting materials, however, may provide a low cost supplement to current cleaning practices.

1.1.4. Evidence of the Usefulness of Self-Disinfecting Copper Surfaces

Although a large number of studies demonstrate the efficacy of self-disinfecting surfaces in reducing surface contamination, only a handful of data exist demonstrating that the use of implementation of these self-disinfecting materials truly results in improved human health. In particular, copper surfaces are the only surfaces that have been proven to have real-world public-health benefits.

A 2010-2011 study by Salgado et al. supported by the US Department of Defense and US Army found that the use of copper metals in place of traditional touch surfaces resulted in significant reduction of HAIs and patient acquisition of AMR pathogens[50]. Six objects were replaced with copper replicates in three separate hospitals. Copper was used to fabricate bed rails, over bed tables, intravenous poles, and arms of the visitor chairs in the rooms. Two additional objects were fabricated from copper and used appropriately at each hospital (e.g. computer mouse, palm rest of laptops, television bezel). Patients were randomly assigned to each room over the course of one year.

In the copper treated rooms the rate of HAI was reduced more than 50%. The incidence of MRSA and VRE colonization was also dramatically reduced for patients in the copper rooms. An additional finding of this study was the association of HAI incidence and microbial burden of the rooms. Rooms having >5 CFU/cm^2 were responsible for 89% of the reported HAIs. Many other studies have demonstrated that copper metal is effective at continuously reducing the microbial burden on its surface, but this is the only study demonstrating actual human health benefits.

This study was the first of its kind and only explored the use of copper alloys, which were the only materials available at the time to make such claims during the time

that the study was conducted. Nevertheless, it provides direct clinical evidence for the usefulness of self-disinfecting surface technology by demonstrating a reduction in HAIs and AMR colonization, two of the most pressing problems in healthcare today.

1.1.5. Arguments Against Self-Disinfecting Surfaces

Despite the observed and proposed benefits of self-disinfecting surfaces, there are several arguments against their use. The reduction of non-AMR organisms and nonpathogenic microbes along with HAI and AMR associated pathogens may contribute to the severity of other diseases. For instance, the "hygiene hypothesis" suggests that the association between lower infection rates and higher levels of autoimmune and allergic disorders in western countries is a result of a reduced infection burden in these countries[51] [52] [53] [54]. However, this concern may be alleviated by only using self-disinfecting materials in high-risk areas (e.g., hospitals).

The use of self-disinfecting surfaces might warrant further and separate consideration on the basis of modification of commensal flora (e.g. reduction of skin bacteria from antimicrobial textiles); however, various studies have demonstrated that the non-specific reduction of microbial life results in a measureable human benefit in hospital settings. For instance, in a study of 7727 patients, daily bathing using chlorohexidrine impregnated wipes resulted in a 23% and 28% reduction in MDRO acquisition and HAI acquisition, respectively[55].

It is also important to consider the potential environmental and lifecycle consequences caused by the use of antimicrobial materials. For instance, silver-based antimicrobial materials were shown by Yang et al. to inhibit bioreactor landfill

operations[56]. Similarly, Choi et al. found that silver antimicrobials could have detrimental affects on microorganisms important for wastewater treatment[57].

1.2. Materials Solutions in Infection Control

1.2.1. Summary

Material solutions designed to prevent microbial transmission and infection fall into three major categories[58 59]. Any of these three approaches can be combined, and some technologies do not fit well into any individual category. Nevertheless, it is useful to consider them here as separate tactics as part of a overall strategy of reducing pathogen transmission on materials surfaces:

(1) Repulsive and Anti-Adherent Surfaces

(2) Contact-Active Antimicrobial Surfaces

(3) Antimicrobial Pesticide Releasing Surfaces

1.2.2. Repulsive or Anti-Adherent Surfaces

Surfaces that are repulsive or anti-adherent towards microbes are used in many applications where the attachment of microbes or biofilms can be problematic. These are often used in the biomaterials sector for the prevention of infections related to implantable devices. Similar tactics are also used in some non-stick marine coatings to prevent micro and macro biofouling of boat hulls. In the marine environment these anti-adherent (PTFE) coatings tend to become fouled when boats are at rest. Shear forces during travel remove the organisms, which would otherwise remain anchored on traditional coatings.

Repulsive and anti-adherent coatings usually rely on use of low energy surfaces (e.g. PTFE) to prevent strong microbial sorption from occurring and/or incorporate specific chemical moieties that inhibit the adherence, or repel, bacterial structures. Since most microorganisms are negatively charged, negatively charged chemical groups (e.g. COO^-) on the surface are effective at repelling microorganisms. Uncharged chemical groups can also be used to inhibit the adherence of bacterial structures (e.g. PEGylated surfaces, heparin).

While repulsive or anti-adherent coatings are promising for many applications, they are not useful (on their own) for self-disinfecting environmental surfaces because they do not actively reduce microbial populations. Although they may be useful for enabling easily cleanable surfaces through preventing the attachment of biofilms, they are not solutions on their own because they do not improve cleanliness without an external stimulus (e.g. liquid flow, wiping).

1.2.3. Non-Leaching Antimicrobial Surfaces

Contact-active antimicrobial surfaces rely on interactions between surface features and the microbe, which results in cell death. This is typically achieved through the chemical attachment of contact-active antimicrobial groups, surfaces with special antimicrobial topography, or through photoactive surfaces.

Quaternary ammonium compounds (QACs) are a common class of molecules that are grafted on to surfaces to yield non-leaching, antimicrobial surfaces (e.g. silanated quats). Other cationics and cationic polymers (e.g. chitosan) can be attached or incorporated and commonly exhibit antimicrobial properties resulting from the

interaction between the cationic surface and the negatively charged surface of most microorganisms.

Other contact-active coatings are based on the use of unique surface topography that physically ruptures the cell wall of contacted microbe. These are often nano structures with very high aspect rations, which are capable of piercing the cell (e.g. dragon-fly wings, black-silicon).

A major drawback of using contact-active antimicrobial coatings in environmental surfaces is the potential for deactivation due to abrasion and other physical and chemical damage. A further concern is the deactivation of a contact active surface to become upon exposure to soil, cleaning products, other chemicals, or solar radiation. These concerns may be alleviated if the antimicrobial agent is incorporated throughout the bulk of the material, such that it is regenerated as the surface is worn down.

Apart from attached physical and chemical antimicrobial agents, another major group of contact-active antimicrobial surfaces is based on photocatalytic surfaces. Coatings such as anatase, TiO_2, are capable of generating reactive oxygen species (ROS) when exposed to light. These ROS may also migrate away from their source, however, and thus not necessarily result in true contact killing. Nevertheless, because these surfaces do not release antimicrobial compounds stored from the material itself, these are not usually considered antimicrobial releasing surface.

These less traditional materials (e.g. photocatalytic agents, high-aspect surface structures) may face additional regulatory challenges, as they may not easily fit in to defined regulatory categories. Instead, these materials may be considered devices since they require a specific external stimulus to become active.

1.2.4. Leaching Antimicrobial Surfaces

Antimicrobial releasing surfaces are designed to release an antimicrobial agent. This may occur continuously, as a result of ablation, or as a response of an environmental trigger. Traditional antibiotics can be incorporated in materials designed for implantation or intimate contact with the body, but they are not suitable for environmental surfaces because of AMR concerns.

Common releasing antimicrobial agents used in environmental antimicrobial surface technologies are based on materials that release hypochloride (e.g. N-halamine), iodine, quaternary ammonium compounds, other organics (e.g. triclosan, furanones), or metal ions (e.g. silver, copper, zinc). Some of these agents are discussed separately in section 1.4.

This approach has been in use for many years in the prevention of biofouling of worlds shipping fleet predominantly using copper oxide based coatings. In these coatings the release rates are tailored to afford continuous protection from microbiological (e.g. bacteria, algae) and macrobiological (e.g. barnacles, crustaceans) fouling for many years through the release of copper and other biocides (e.g. zinc pyrithione). Likewise, in both the medical and consumer sectors various antimicrobial surface technologies using antimicrobial metals have been also used for many years. Silver has found especially large amounts of use in the medical sector, however, as mentioned previously, currently only copper is used for antimicrobial materials with public health surfaces.

These added antimicrobial additives are usually distributed fully through the material or coating, so that these surfaces tend to be continuously renewed through

normal abrasion. For similar reasons, however, these materials tend to face greater environmental scrutiny and human toxicity concerns. Nevertheless, this class of antimicrobial surface has the most promise for use in self-disinfecting touch surfaces and is by far the most successfully implemented.

This class of antimicrobial surface also benefits from the ability to use the arsenal of well-known antimicrobial agents. If sufficiently low amounts of antimicrobial agent are added the original properties of the matrix may be retained. The use of standard processing/manufacturing techniques is possible if the additives can be compatibly added. For these reasons inorganic-based antimicrobials agents are often benefited by their ability to withstand high processing temperatures (e.g. in melt blending operations).

1.3. Antimicrobial Agents

1.3.1. Summary

In the US, antimicrobial agents are classified as either pesticides or as drugs. Antimicrobial drugs, as traditionally imagined, operate through highly specific pathways (e.g. inactivation of a particular bacterial enzyme), which make them safe for use in humans. This specificity, however, enables antimicrobial resistance to develop relatively rapidly compared to most antimicrobial pesticides, which are typically very non-specific in their mode of action (e.g. generation of toxic reactive species), and also makes them not useful in the body. Antimicrobial agents used in materials (e.g. silver) are considered pesticides and regulated as such by the US EPA with guidance from the US FDA depending on the application. The EPA further classifies antimicrobial pesticides as a preservative or as making public health claims. Most materials as currently treated cannot

be readily made in to self-disinfecting surfaces using their current preservatives because of high human toxicity.

1.3.2. Regulatory Aspects of Antimicrobials

In addition to the physical and mechanistic characteristics that govern the properties of antimicrobial agents, the regulatory aspects of antimicrobials technologies dictate how and where they can be used. Thus it is a necessary burden to understand the regulatory landscape of antimicrobial products in order to design or implement new antimicrobial solutions. That is to say, the research conducted in this dissertation has been done with the recognition of the factors that influence the regulatory perspective of antimicrobials technologies.

In the United States, antimicrobial agents are classified drugs or pesticides based on their intended end-use application:

(1) Antimicrobial drugs such as those used in antimicrobial soaps, anti-dandruff shampoos, medications, or otherwise active in or on humans or animals are generally subject to the regulations of the US FDA under the Federal Food, Drug, and Cosmetic Act (FFDCA).

(2) Antimicrobial pesticides such as those used to protect food crops, prevent microbial fouling of materials, or used otherwise in or on nonliving surfaces are generally regulated by the US EPA under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Pesticide Registration Improvement Acts (PRIA) of 2004, 2007, and 2012.

These generalizations are not strictly true, however, and additional regulatory classifications based on the setting of the antimicrobial product may also dictate which agency is responsible for oversight. Inanimate surfaces in contact with food and other applications may also be subject to FDA restrictions. Antimicrobial textiles may be subject to EPA regulation generally, however, the FDA may regulate certain textiles when they are used in hospitals[60].

As such, the US EPA is the predominant regulating body for materials containing or consisting of antimicrobial materials, with or without public health claims. These materials are considered pesticides under FIFRA. The EPA designates antimicrobials used in surface disinfectants, self-disinfecting surfaces, or materials preservation as antimicrobial pesticides, a distinctly regulated subclass of pesticides.

Antimicrobial pesticides are further classified as products that make public health claims and as those that do not. Non-public health claims include the prevention of algae, odor and stain causing bacteria, microbes that are only infectious to animals, and bacteria that can cause spoilage or fouling of materials. Public health claims include claims to prevent any disease or disease-causing organism.

Products that make public health claims are then further distinguished as sterilizers/sporicides, disinfectants, and sanitizers. Sterilizers/sporicides must destroy all microbial life including fungi, viruses, bacteria, and their spores. Since, spores are considered the hardest to kill; sporicide is usually used synonymously with sterilizer. Disinfectants must inactivate bacteria and fungi. Further, sanitizers are only required to reduce the microbial contamination to safe levels but are not required to eliminate it.

Thus, sterilizers/sporicides, disinfectants, and followed by sanitizers are listed in decreasing order of antimicrobial strength.

Products which do not make public health claims use silver, copper, and other antimicrobial agents (e.g. triclosan) currently exist in the market without direct antimicrobial registration. These products fall under the EPAs treated article exemption, which allows for antimicrobial claims to be made for a product without EPA registration so long as (A) that public health claims are not made and that (B) a known EPA registered antimicrobial pesticide active ingredient is used in the product.

An added AM ingredient (i.e. antimicrobial pesticide) of any non-FDA product must always be approved by the EPA whether or not the product itself makes AM claims. Certain minimum risk active ingredients are exempted from certain registration requirements for certain applications; however, these are limited to natural substances such as cinnamon oil and garlic.

As mentioned previously, the only touch surfaces currently approved by the EPA to make public-health claims are copper based metal alloys and copper oxide based materials. As a result of this, the public health benefits of antimicrobial touch surfaces have only been evaluated for copper based materials. Since these materials make public health claims, both the finished product (end-use) and antimicrobial active ingredient must be EPA registered for these products, and they cannot use the treated article exception.

Many other antimicrobial agents are registered for use as preservatives in many materials systems. These preserved materials are also commonly marketed with public heath claims; however, the manufactures of such materials can be subject to large fines.

The use of ulterior terminology such as "anti-odor" claims is increasingly being used to avoid the requirement of regulatory approval surrounding antimicrobial claims.

1.3.3. Definitions and Terminology

Antimicrobial agents are used to some degree in nearly every industry, home, and workplace, thus it is not surprising that the terminology surrounding antimicrobials is commonly muddled. *Antimicrobial* is the general term for any compound that kills, hinders, or slows the growth of microorganisms. This includes all of the compounds with antimicrobial properties including the antibiotics, antibacterials, antifungals, antivirals, antiprotozoals (antimalarials) drugs, and antimicrobial pesticides. Each of these terms is uniquely defined.

Antibiotics are a specific class of antibacterial drug, which are distinguished as natural products produced by other microorganisms. The term antibiotic, however, tends to be used colloquially to include any antibacterials drug. Although there is some ambiguity surrounding the designation of fully synthetic compounds that are chemically identical to naturally produced antibiotics; or the designation of naturally produced antibiotics that are subsequently chemically modified (semi-synthetics), generally these are lumped together as part of the antibiotic class of antibacterial drugs.

It seems to have become common practice, however, for the specific term antibiotic to be used even less specifically, and is even commonly used to refer to antimicrobials in general. The reverse has also become a common occurrence and the broad term antimicrobial is often used to for describing specific antibacterial drug phenomena. For instance, antibacterial drug resistance is the most reported and most

heavily monitored form of AMR. For example, the 2014 W.H.O. report, "Antimicrobial Resistance Global Report on Surveillance," focuses mostly on antibacterial resistance and AMR tends to almost always refer to bacterial AMR. It should be noted that viruses account for the majority of infectious diseases and AMR is also concern in treatment of viral disease. However, the rate at which AMR is affecting our ability to treat bacterial infections makes it an especially alarming cause for concern.

Microbes also have a major impact on materials degradation and reduced process efficiency in industrial processes and antimicrobial preservatives are routinely used. In this context the term antimicrobial is interpreted as referring to products with antibacterial properties, while other additives are referred to separately with reference to their intended target organism (e.g. algaecides, fungicide). This usage likely also contributes to the skewed perception that the term antimicrobial refers strictly to bacterial inhibition.

Despite these terminological incongruences, the observation of the major regulatory, usage, mechanistic distinctions among various antimicrobial technologies can aid in practically navigating the subject.

1.3.4. Mechanistic Considerations in Antimicrobials

An important distinction between the many antimicrobial materials is in the specificity in their mode of action. In general, antimicrobial drugs and antimicrobial pesticides can be distinguished according to some general mechanistic considerations. Like their regulatory definitions, these mechanistic generalizations are overly broad and riddled with exceptions.

Antimicrobial drugs have historically been naturally sourced materials, which have very specific mechanisms that only target specific parts of particular microbes. This property has made them useful and safe for treating infections in the human body.

Antimicrobial pesticides, on the other hand, have historically been synthesized or inorganically sourced, and have non-specific modes of action that make them useful and cost-effective solutions for protecting materials, food crops, and materials from microbial damage.

Many naturally sourced antimicrobial pesticides and many inorganic antimicrobial drugs exist, however, it is useful to consider a few representative examples of antimicrobial drugs and antimicrobial pesticide.

Penicillin, for example, specifically inhibits penicillin-binding-proteins (PBPs) involved in crosslinking of the bacterial cell wall and typically does not adversely affect other parts of the bacterial cell or human cells. This makes penicillin exceptionally useful and safe for human drug use, however, it also allows for bacteria to develop resistance through any number of different mechanisms rather quickly. These resistance mechanisms can include producing excess targets (e.g. additional PBPs), enzymatically degrading the antimicrobial (e.g. penillinase), and modifying the target to not be affected by the antimicrobial. Thus, antimicrobial drugs such as penicillin that target a specific pathway are not suitable for widespread use because of high probability of AMR developing.

In areas where antimicrobial drugs have been used excessively, antimicrobial resistance to those drugs has been observed. For instance, in his now famous studies Stewart Levy measured the increased incidence of humans and other animals become

colonized with AMR pathogens surrounding farms in which those antimicrobial drugs had been used[61 62 63].

Antimicrobial pesticides, on the other hand, tend to operate through a variety of non-specific mechanisms. This reduces the probability of resistance occurring. For example, silver has been used since antiquity to prevent spoilage of water and foodstuffs with minimal resistance reported compared to the antimicrobial drugs. Silver acts in a very non-specific manner through the binding of silver ions with enzymes, proteins, nucleic acids, and through the production of reactive oxygen species (ROS). Preventing all of these lethal events is less likely compared to the prevention of one of them as is needed for specific antimicrobials like penicillin.

Nevertheless, some bacteria have developed resistance to silver and other heavy metals through high efflux capacity and the ability to precipitate the metals as insoluble salts. These bacteria are especially found naturally in mining environment and these microbes are even exploited to remediate heavy metal contaminated environments. Often, a reduced efficacy of these non-specific antimicrobial pesticides may occur through some change in the environment caused by the bacteria (e.g. low pH inactivation of iodine[64]), which presents as resistance but is not true resistance as experienced with the antibiotics (e.g. enzymatic attack of antibiotic or modification of target site).

In many cases, the antimicrobial mechanisms and related resistance affects may depend on factors such as the concentration and environment for a given organism. For example, triclosan has been used as an antimicrobial pesticide and antimicrobial drug in many consumer products (e.g. dish drying racks, toothpaste) due to its useful non-specific efficacy. Recently, however, it has been reported that at low use levels triclosan acts

specifically to inhibit fatty-acid synthesis[65]. The implication of this is alarming due to the possibility of cross-resistance with medically important fatty-acid inhibiting antimicrobial drugs as a result of exposure to triclosan in the environment.

1.4. Select Antimicrobial Pesticides

Several of the common antimicrobial pesticides used as additives in plastics and coatings are reviewed below. Additionally some other antimicrobial pesticides with greater potential for use in producing self-disinfecting surfaces are also introduced. A useful quantifiable index of the antimicrobial additives does not exist due to the strong dependence on testing methodology, organism strain specificity, and influence of seemingly innocuous variables that have a strong influence on the results. Nevertheless, many studies have been conducted in which groups of antimicrobial compounds are tested following standard minimum inhibitory concentration type tests.

1.4.1. Common Antimicrobial Pesticides in Materials Preservation

Microbes can degrade nearly any material through either direct metabolism or modification of the environment via the release of metabolites and formation of superstructures such as biofilms, which create unfavorable microenvironments. Although the direct metabolism of some materials, such as polymers, wood, as well as engineering fluids, foodstuffs, pharmaceuticals, and cosmetics by microbes has been well known and documented since antiquity, only in the last several decades has microbiological induced corrosion (MBIC) been regarded as a significant source of damage to metals and ceramics.

The suppression of microbial degradation in any of these materials is typically treated by application of an antimicrobial agent as a preservative. The microbiological preservatives used also require regulatory, technical, and cost considerations which results in a number of different antimicrobial agents available for use in many various applications.

A major challenge faced by preservative users and producers is the increasing concern over the consequences of preservative use from ecological and human toxicity. As described by HW Rossmoore in his handbook on biocide use[66], these concerns extend throughout every area in which preservatives are use. Rossmoore articulated this concern via quotation of the United Auto Workers on concerns of preservative use in metalworking fluid: "*The worker is caught between disease-producing microbes and cancer causing biocides*."

Thus, it is apparent that a major objection to increasing the level of currently used preservatives in plastics and coatings to achieve meaningful surface decontamination stems from toxicity concerns. For instance, OBPA, 10,10-oxybisphenoxarsine, an arsenic-based compound, is a common plastic preservative. Around half of the plastics biocide market worldwide is accounted for by OMPA use (circa 2004)[67].

Other limitations that also preclude this approach include thermal instability, low density, low AM biocidal activity, and high cost. Nevertheless, it is useful to review the treatment of materials degradation in plastics and coatings: an additive capable of preventing the transfer of pathogenic microbiological contamination should also be capable of preserving the material itself from microbiological attack; and the methods used for treatment provide both teaching and framework for the creation of self-

disinfecting materials. That is, it would be best to have a self-disinfecting additive that could be simply added to current processes.

Although some polymers are inherently resistant to microbial attack, the plasticizers, fillers, and pigments added to formulate complete plastics are often susceptible to microbial attack. Polyolefins, polyesters, ABS, PTFE, epoxies, silicones, and phenolic resins have an inherent resistance to microbiological attack, whereas, cellulose based polymers, polyurethanes, and polyvinyl acetate are readily attacked[68].

The additives (e.g. plasticizers, fillers, pigments) used in any of these materials may make them liable to attack. Conversely, some of these additives may coincidently have antimicrobial properties themselves (e.g. tin organometallics added as thermal and photo stabilizers, also provides protection from biological attack[69]). Generally, however, the addition of a suitable biocide at a rate of 0.1-5% is needed to prevent degradation.

Some processes incorporate a biocide during polymerization (e.g. during production of polyurethane foam); however, biocides are usually added during compounding. Topical treatments are rarely employed because they are susceptible to abrasion and cracking. In compounding, it is preferred to add the biocide as a masterbatch (i.e. additive-polymer concentrate) to reduce dusting and worker exposure.

As with plastics, coatings almost always require the addition of some form of biocide, although they have different technical requirements. Both, "*in-can*" preservation an "*in-film*" is required to protect components from being colonized. Thus, liquid coating preservatives must remain sufficiently suspended or dissolved and provide protect to any secondary phases (e.g. emulsion systems).

Antifouling coatings should be considered separately as their performance requirements are significantly greater than coating preservation. Antifouling coatings must prevent the micro and macro fouling (attachment of slime, barnacles, algae, etc.) and typically incorporate >40% Cu_2O in the coating. Other specialty pesticide coatings (e.g. insecticidal coatings are used to kill cockroaches in ships[70]) should also be considered separately and depend on the use of different types of pesticides.

Many other materials besides common plastics and coatings undergo microbiologically induced degradation. In fact, very few systems do not require the use of a biocide in some form to prevent microbiologically induced degradation. These biocides may be added to the operating environment or to the materials itself in the case of preservatives. Often the same biocides may be used in the materials and in the operating environment. For example, quaternary ammonium compounds (QUATs) may be used as a materials preservative or in the operating environment of the material. Other biocides are only sensible for use in the operating environment (e.g. glutaraldehyde, [GLUT]). Nevertheless, the selection of an appropriate biocide is dependent upon economic, safety, regulatory, and efficacy considerations; and often results in the use of blends of multiple biocides (e.g. GLUT-QUAT mixtures are commonly used in servicing of oil wells).

In some cases, mixtures of different biocides can result in a synergistic or antagonistic effect with respect to their stability and their efficacy. Thus, the proper selection of a biocide package can become quite complicated and it is useful to have at least some understanding of how the biocides used are effective in the materials and systems in which they are being applied.

1.4.2. Antimicrobial Metals

The oligodynamic effect is used to describe the ability of certain metals to exert toxic effects against microorganisms. These metals are largely accepted to function through a variety of pathways by binding to and inactivating various proteins, nucleic acids, enzymes, and by generating reactive oxygen species. Resistance mechanisms to oligodynamic metals largely stem from the increased expression of genes responsible for metal ion efflux pumps, although certain microbes have an innate resistance towards metals due to their ability to precipitate metals as insoluble salts (e.g. metal sulfides in desulfvibrio species). The latter, however, are less commonly associated with human disease compared to the former.

Among the metals with antimicrobial properties, silver and copper are the most commonly used commercially, most researched, best understood, and generally the most effective. Silver based antimicrobial surface technologies have a long history in both consumer products, medical devices (e.g. catheters), and also in antimicrobial drug formulations. Many other antimicrobial metals are also known including zinc, titanium, and selenium. These tend to be relatively less effective compared to silver and copper, however. Many other antimicrobial metal compounds based on mercury, arsenic, lead, and other metals have been used as antimicrobial pesticides. However, their toxicity profiles prohibit their use in forming useful antimicrobial surfaces. Similarly, other metals including gold and platinum have known oligodynamic effects, but are cost prohibitive compared to silver and copper.

1.4.2.1.Silver

Anecdotal evidence suggests that humans have used silver compounds and silver metal for their antimicrobial properties for thousands of years. Today, silver is used in metallic from, as a salt, in zeolites, in other carriers (e.g. silver ion exchanged glasses), and in nanoparticle form. It is believed that the antimicrobial action of silver is a result of released Ag^+ silver ions. This belief is supported by experiments demonstrating that if the oxidization of silver metal is inhibited then its antimicrobial properties are neutralized[71] [72]. The antimicrobial action of silver ions can be neutralized by addition of thiol containing compounds such as cysteine and sodium thioglycolate[73] (i.e. Dey-Engley neutralizing broth), which suggests that silver can also interact with thiol groups inside the cell (e.g. proteins, enzymes, nucleic acids).

A wide variety of antimicrobial mechanisms have been suggested for the antimicrobial efficacy of silver. The inhibition of respiration by silver in E. coli was observed by Holt to result in the generation of toxic reactive oxygen species[74]. A number of morphological changed caused by silver in *E. coli* and *S. aureus* were observed by Feng et al. including observable damage to the DNA and to the cell walls and membrane of both organisms[75].

1.4.2.2.Copper

Like silver, copper has been used since ancient times to preserve food, water, and elsewhere to protect humans from disease causing microorganisms, and in various forms (e.g. metal, salts, etc.). Again, the release of metal ions is responsible for the antimicrobial efficacy. Although the antimicrobial properties of copper are less well

researched or understood compared to silver, they are also believed to occur through a variety of pathways including the binding of metal ions to proteins and nucleic acids and the generation of reactive oxygen species.

Unlike silver, which only forms monovalent (Ag^{+}) cations, copper can form both cuprous (Cu^{+}) and cupric (Cu^{++}) cations. The cuprous ions have superior antimicrobial properties compared to cupric ions[76]. Bestwick et al. showed that increased toxicity of Cu^{++} in anoxic conditions for *E. coli* was the result of increased Cu^{+} formation; and that Cu^{+} was generally 5-10x more effective than Cu^{++}. Thus, although compounds that release more copper are generally more effective[77], compounds that specifically release more cuprous ions are believed to have superior antimicrobial properties at equivalent available ion concentrations.

For instance, copper alloy surfaces have been shown to operate through the release of copper ions[78] and antimicrobial efficacy has been shown to be a function of the copper content and the corrosion rate[79]. Copper metals can be expected to have an oxide layer on their surface composed of a mixture of cupric and cuprous oxides. In wet conditions the cupric oxide dominates[80] and in dry conditions the cuprous oxide dominates[81]. Hans et al. examined the affects of oxide speciation on the antimicrobial activity of copper metal surfaces. It was found that the development of a cupric oxide layer hindered the antimicrobial properties of copper, whereas the build-up of a cuprous oxide layer did not impair the antimicrobial efficacy. Furthermore, it was also demonstrated that the killing power of copper metal and cuprous oxide was similar against *E. hirae* and *S. aureus*.

Sunada et al. compared the antiviral efficacy of a variety of cuprous, cupric, and silver compounds. The compounds were dispersed in ethanol and dried on to glass substrates at equal metal concentrations (mole basis). Suspensions of *QB bacteriophage* (model of human influenza virus) were inoculated on to the substrates and incubated for 30 minutes before being harvested. It was found that the cuprous compounds were significantly more active than the cupric or silver compounds. Furthermore protein adsorption and enzyme deactivation by cuprous oxide were found to be significantly greater compared to cupric oxide and silver metal, indicating that Cu_2O is more active with these biomolecules.

Table 1.1 – Cu or Ag Compounds against *QB bacteriophages* [Sunada et al.]

Cuprous compounds (Log_{10} Reduction)		Cupric compounds (Log_{10} Reduction)		Silver compounds (Log_{10} Reduction)	
Cu2O	5.8	CuO	0.012	Ag	0.69
Cu2S	4.6	CuS	0.59	Ag2S	0.26
CuCl	6.6	CuCl2	0.25	AgI	0.25
CuI	6.0				

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of an AM agent are useful metrics for quantifying the efficacy of the AM and refer to the minimum concentrations required to prevent growth and to actually kill the microbe, respectively. Ruparelia et al compared the MIC and MBC for silver nanoparticles of 3 nm and for copper nanoparticles of 9 nm[82]. The copper particles also had an oxide layer (composition not reported). The nanoparticles were dispersed in nutrient broth, inoculated with bacteria, and monitored for increased turbidity as a measure of bacterial growth. The results (Table 1.2) indicated that copper was more effective against *B. subtilis*, equivalent against *S. aureus*, and inferior against *E coli. B. subtilis* has an increased number of amine and carboxyl groups on its surface compared to

other organisms, which have a greater affinity for copper and may account for the higher copper susceptibility[83]. The speciation of copper at the surface (i.e., oxide layer) has a dramatic impact on the antimicrobial efficacy and the tendency is for less effective cupric oxide to form in wet (e.g. dispersed) conditions[84].

Table 1.2 – MIC and MBC for silver or copper nanoparticles [Ruparelia et al.]

Bacteria	MIC (ppm)		MBC (ppm)	
	Ag	Cu	Ag	Cu
Escherichia coli (Average of 4 strains)	120	210	155	235
Bacillus subtilis (Single strain)	40	20	60	40
Staphylococcus aureus (Average of 3 strains)	120	140	160	160

Vargas-Reus et al.[85] compared the antimicrobial efficacy of 10-50 nm nanoparticles of silver, cuprous oxide, cupric oxide, zinc oxide, titanium dioxide, and tungsten oxide. The MIC and MBC for the particles was determined against four bacteria commonly associated with peri-implantitis (a disease caused by infection of the gums and bone surrounding dental implants). With the exclusion of the *Aggregatibacter actinomycetemcomitans*, cuprous oxide had the lowest MIC and MBC. *Aggregatibacter actinomycetemcomitans* had a 10x greater resistance against copper than the other bacteria, and for this bacterium the silver materials had the lowest MIC and MBC, followed by copper materials (Table 1.3).

Table 1.3 – MIC/MBC of various metals against some oral bacteria [Vargas-Reus et al]

Peri-implantis Bacteria	10-50 nm Metal Particles (ppm)											
	Ag		CuO		Cu2O		ZnO		WO3		TiO2	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Porphyromonas gingivalis	250	250	500	2500	<100	<100	250	250	2500	2500	2500	>2500
Prevotella intermedia	100	100	250	250	<100	<100	1000	1000	2500	>2500	1000	>2500
Fusobacterium nucleatum	100	100	250	250	<100	<100	250	500	2500	>2500	1000	>2500
Aggregatibacter actinomycetemcomitans	100	100	250	250	1000	1000	250	250	2500	>2500	250	>2500

These studies indicate that cuprous ions are responsible for the antimicrobial efficacy of copper. It appears that cuprous-based materials are generally more effective than silver materials, however, there are some organisms, which exhibit significant resistance to copper.

1.5. Testing of Antimicrobial Pesticides and Materials

1.5.1. Testing of Antimicrobial Agents

Many different tests exist for the testing of antimicrobial additives and materials. Testing of the additives themselves can be similar to the in-vitro evaluation of common antimicrobial drugs and is usually less involved than evaluation of finished materials. In both cases, however, care must be taken to ensure that testing reagents do not adulterate the sample, and that the materials are being tested in relevant fashion.

The testing of antimicrobial additives themselves is usually carried out using planktonic bacteria through exposure to the antimicrobial for a given time, followed by neutralization and enumeration. Neutralization of metal ions can be achieved, for

instance, by the addition of strong chelating agents, so that the efficacy of the material at different time points can be examined. Enumeration of surviving bacteria is carried out by serial dilution of neutralized suspensions in a growth medium wherein the microbial concentration can be directly counted. Alternative, indirect methods, such as optical density measurements can also be used as a measure of bacterial growth and are usually less cumbersome than traditional plating type methodologies.

Other more convenient but qualitative tests are also very commonly performed. For instance, a swatch of antimicrobial material may be placed on to an inoculated agar plate. After some time a "zone of inhibition" may develop around the swatch where bacteria are absent. This zone can be measured and compared to other swatches to rate the relative efficacy of various antimicrobial agents.

Care must be taken, however, to ensure that the testing media does not interfere with the antimicrobial agent. In the case of copper, for instance, proteinaceous test media have a propensity to neutralize the antimicrobial efficacy of copper compounds, which can result in poor efficacy using "zone of inhibition" type tests.

1.5.2. Common Antimicrobial Surface Tests

Antimicrobial evaluation of a material must be conducted in such a way that the test performed mimics the conditions of environment in which the antimicrobial might be used. Although quantitative bacterial reductions may vary from one test to another, close control of testing conditions can help ensure that the essential results are preserved upon repetition. To this end, a number of standard tests for antimicrobial surfaces have been devised, however, two are particularly common.

1.5.2.1.JIS Z-2801

The Japanese Industrial Standards Committee designed the JIS Z-2801 test for testing the antibacterial activity of surfaces with the exception of textiles. A similar test methodology has been adopted by ISO (ISO 22196)[86], but it is not identical, and the JIS method is the dominant method used.

The JIS Z-2801 test involves the inoculation of a surface with bacterial suspension, which is then covered by a sterile film and stored for 24 hours in >90% humidity. The inoculum is then collected, neutralized, and the bacteria are enumerated. In this way, the sample does not dry out and the conditions resemble that of a wet sock.

The JIS Z-2801, despite its popularity has several shortcomings. One obvious issue is that under use conditions most environmental surfaces are not maintained at >90% humidity. Further, during the test period any adherent bacteria (e.g., biofilm) may not be collected when the inoculum is collected.

1.5.2.2.EPA Protocol for Copper/Copper-Alloy Surfaces

In order to address the public health claims put forth by the Copper Development Association, the EPA developed a series of significantly more challenging tests than the JIS Z-2801, which correct the above mentioned shortcomings.

The “Protocol for the Evaluation of Bactericidal Activity of Hard, Non-porous Copper/Copper-Alloy Surfaces” by the US EPA was designed to test the self-disinfecting properties of copper and copper alloys, however, it has since been used in the evaluation

of copper oxide loaded materials. The test has recently been updated and a new version remains in draft guidance.

The new test involves inoculating the surface with a bacterial suspension and allowing the sample to stand uncovered for 1 hour under ambient conditions, which may or may not result in drying of the surface. In addition to the bacteria, a soil load is also present during inoculation. After 1 hour, the bacteria are recovered in a neutralizing broth using ultrasonication to collect adherent organisms. The bacteria are enumerated and compared to stainless steel standards. This protocol additionally calls for repeated exposure to physical wear, cleaning agents, and repeated inoculations as a simulation of real world conditions.

1.6. Objectives and Structuring of this Dissertation

This objective of this research has been to develop an antimicrobial additive for common plastic and coating materials to allow them to achieve self-disinfecting properties without the cosmetic limitations introduced by the use of current copper metal or copper oxide solutions. More specifically, this research describes the development of antimicrobial copper iodide small particles, whose surfaces have been modified to make them compatible with different matrices. Attention has been focused on two main areas, production of particles and the impact of particle surface chemistry. Attention is given towards assessing the importance of particle surface chemistry and the compatibility with different matrices on dispersibility and resultant antimicrobial performance. To this end, it has been important that the additive be incorporated using traditional processing routes

under normal processing conditions. Additional attention is given to the development of economically viable, scalable processing routes for these particles.

While the initial motivation for this research stems from recognized needs in healthcare settings, the development and application of self-disinfecting surfaces and antimicrobial agents are very important in many other fields. Self-disinfecting food contact surfaces, packaging and processing equipment may potentially aid in reducing the number of food borne illnesses, which are responsible for thousands of deaths per year. Furthermore, antimicrobial additives are widely used in a variety of industrial processes for the prevention of microbiological fouling, for example, in heat exchangers. Self-disinfecting materials may have the potential to maintain improved processing conditions in these fields.

Furthermore, with the exception of some metals and ceramics, nearly every material requires an antimicrobial additive to preserve the integrity of the material from direct microbiological attack. And still, many metals and ceramics remain susceptible to microbial induced corrosion and require antimicrobial treatment of their operating environments to prevent microbial attack (e.g. oil and gas wells). Thus, in addition to developing a self-disinfecting surface, the implementation of copper iodide as an antimicrobial preservative to protect materials was also addressed.

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Chapter 2. Copper Iodide Small Particles as an Antimicrobial Agent

This chapter describes the selection of surface modified small particles of copper iodide as a preferred antimicrobial agent. First, the selection of copper iodide as a prospective material to be used in the development of an antimicrobial additive is presented. Second, the motivation for using surface modified small particles of copper iodide compared to commercially available copper iodide is presented.

2.1. Comparative Antimicrobial Properties of Several Metal Compounds

This section summarizes the motivation for the use of CuI as an antimicrobial (AM) additive. Several silver and copper compounds were comparatively screened for the AM efficacy against a gram-positive and gram-negative bacterium. The compounds were selected based on their known use in medical and consumer products and information available in the literature.

Silver chloride, silver bromide, silver iodide, cuprous chloride, cuprous bromide, cuprous iodide, and cuprous oxide (AgCl, AgBr, AgI, CuCl, CuBr, CuI, and Cu2O, respectively) were evaluated for their efficacy at 60 ppm metal concentration as dispersions in saline against planktonic forms of *S. aureus* and *P. aeruginosa*. Antimicrobial activity was assessed for the compounds immediately after they were dispersed, after 3 months of aging in aerated DI-water, and after an oxidative treatment with hydrogen peroxide.

The cuprous compounds performed better than the silver compounds in all three tests. After aging or exposure to hydrogen peroxide, however, copper iodide was the only compound that fully retained its original antimicrobial efficacy. This result is attributed to

the generation of a secondary antimicrobial agent, iodine, which does not occur for the other materials.

2.1.1. Introduction

In 2008, five copper metal alloys were the first surfaces with antimicrobial public health claims registered with the US EPA as antimicrobial by the Copper Development Association[1]. Since then, a total of 282 copper metal alloys have been registered to make similar antimicrobial public health claims[2]. In 2012, a 16% cuprous oxide containing material of Cupron Technologies was approved by the EPA to make antimicrobial surfaces with similar public health claims. Currently these two antimicrobial surfaces approved by the US EPA to make public health claims both have a distinct copper or reddish-brown appearance.

Copper metal surface have been demonstrated as effective in reducing the microbial burden on environmental surfaces and their use has directly resulted in significant reductions in the occurrence of healthcare associated infections and acquisition of multi-drug-resistant organisms in real-word clinical trials of three US hospitals. Thus it is believed that the widespread implementation of these materials would represent a cost-saving and life-saving measure. The implementation of these surfaces, however, is limited by the high cost of manufacturing with copper alloys and the cosmetic limitations of the 16% cuprous oxide containing materials.

The present study was undertaken as a preliminary screening of candidate materials which could be used as additives for plastics and coatings to create self-disinfecting surfaces. These plastics and coatings are envisioned as replacements for

current EPA, approved antimicrobial surfaces, copper metal and cuprous oxide based materials without the aforementioned cosmetic and manufacturing limitations.

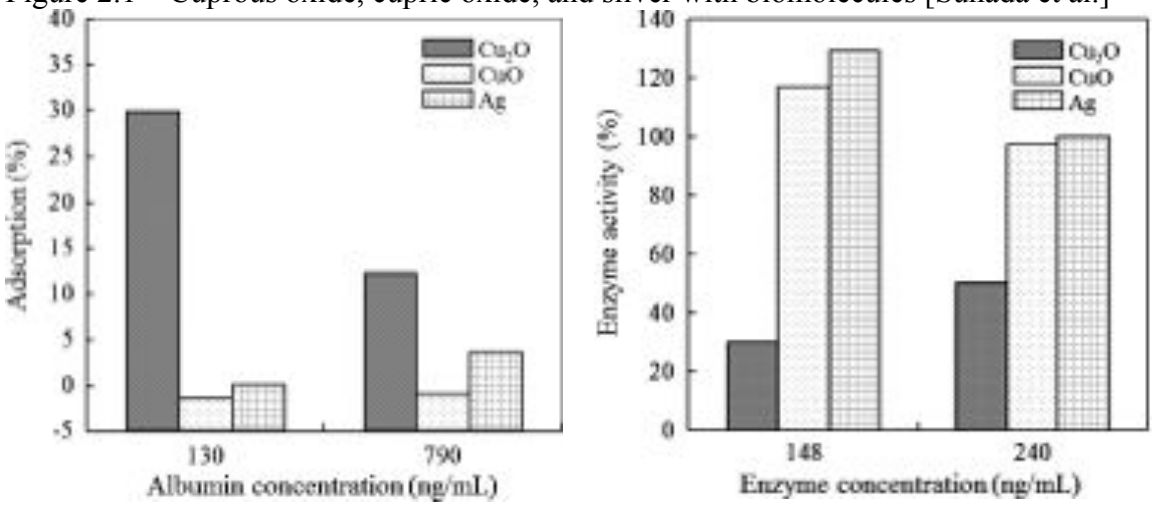
Silver-based and copper-based compounds were chosen as candidate materials because of their established non-specific antimicrobial efficacy, generally high melting/decomposition points, and safety considerations. Silver and copper are well known antimicrobial metals, which have both been used since antiquity for antimicrobial applications in various forms (e.g., metals, metal salts, loaded zeolites, nanoparticles, etc.). Both silver and copper based antimicrobials are believed to exert bactericidal effects through the release of their metal ions, which then disrupt a wide variety of metabolic and reproductive pathways in microbes.

Under biologically relevant environmental conditions, silver is known to release monovalent silver ions (Ag^{+}) while copper typically releases both cuprous (Cu^{+}) and cupric (Cu^{++}) ions. In water the ratio of cupric to cuprous ions released from copper metal, however, is on the order of 1,000,000 to 1. It is known, however, that the cuprous ion (Cu^{+}) is a significantly more potent antimicrobial than the cupric ion (Cu^{++})[3].

Sunada et al.[4] compared the antiviral efficacy of several cuprous, cupric, and silver compounds. The compounds were dispersed in ethanol and 0.13 g of copper compound or 0.23 g of silver compound was dried on to glass substrates. Suspensions of *QB bacteriophage* (a model for human influenza virus) were inoculated on to the substrates and incubated for 30 minutes before being harvested. It was found that the cuprous compounds were significantly more effective at destroying the virus than the cupric or silver compounds (Table 2.1). Furthermore, a mechanistic comparison of

cuprous oxide, cupric oxide, and silver metal showed that cuprous oxide had significantly more protein adsorption and enzyme deactivation activity as shown in Figure 2.1.

Figure 2.1 – Cuprous oxide, cupric oxide, and silver with biomolecules [Sunada et al.]

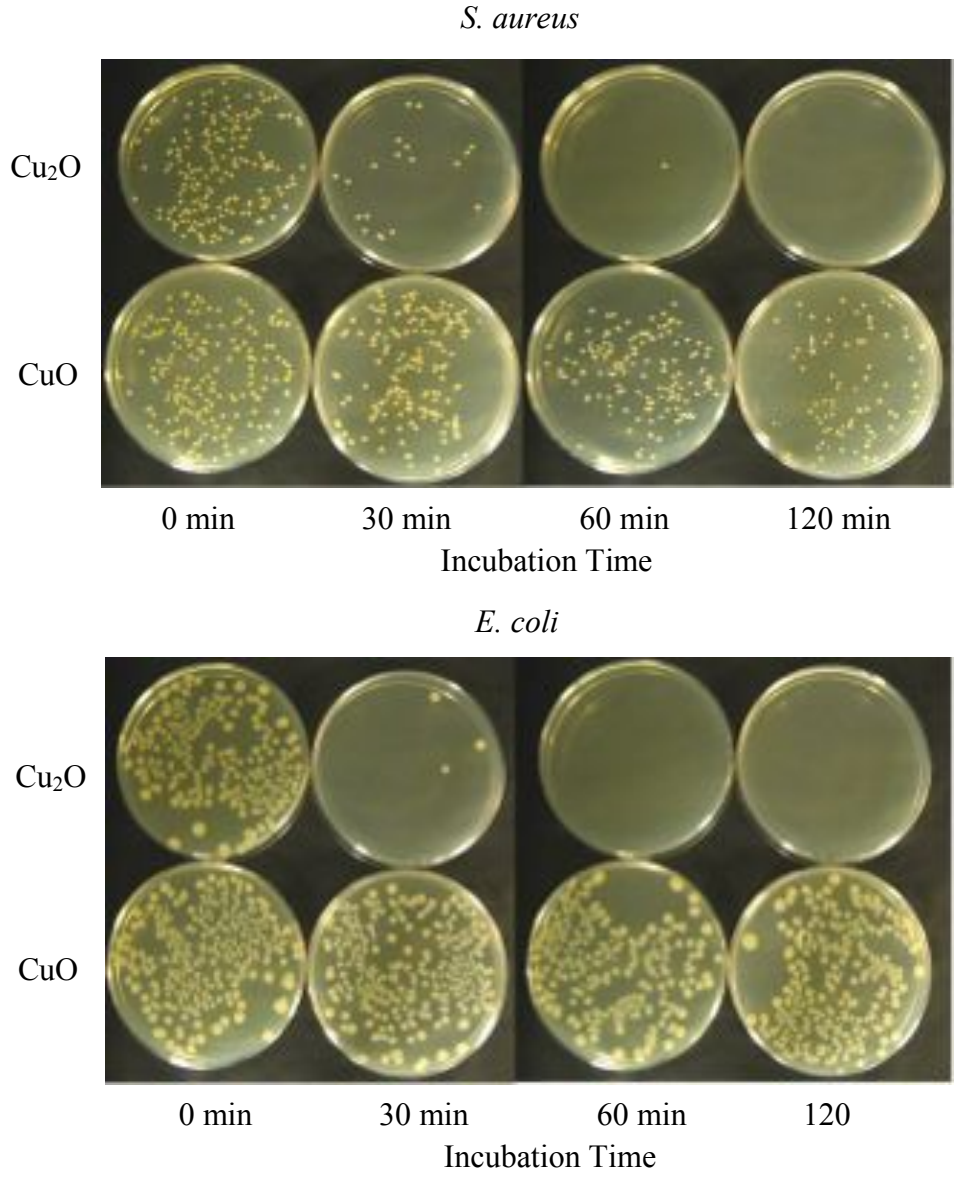


Additional testing by Sunada was also performed to compare the bactericidal properties of cuprous oxide, cupric oxide, and silver metal against *E. coli* and *S. aureus*. The results of this test were similar to the antiviral results as shown in Figure 2.2 and demonstrated that cuprous materials may have much greater antimicrobial efficacy than cupric or silver (not shown) compounds.

Table 2.1 – Cu or Ag Compounds against *QB bacteriophages* [Sunada et al.]

Cuprous compounds (Log_{10} Reduction)		Cupric compounds (Log_{10} Reduction)		Silver compounds (Log_{10} Reduction)	
Cu2O	5.8	CuO	0.012	Ag	0.69
Cu2S	4.6	CuS	0.59	Ag2S	0.26
CuCl	6.6	CuCl2	0.25	AgI	0.25
CuI	6.0				

Figure 2.2 – Antibacterial Activity of Cu_2O and CuO [Sunada et al.]



Although there are a number of studies dedicated to the evaluation of a single antimicrobial compound, there are few studies similar to that of Sunada that directly compare the efficacy of different antimicrobial materials side-by-side. Additionally, results of microbiological studies can differ depending on the test methods and specific organism strain used. Thus, a practical comparative index of these compounds does not

exist and it is difficult to compare accurately results from different publications on individual compounds.

Nevertheless, the use of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) testing of AM agents provides useful metrics for quantifying the efficacy of the AM agent and refer to the minimum concentrations required to prevent growth and to kill the microbe, respectively. Largely, however, these tests are useful for determining if a particular AM would be appropriate for preventing infection by a particular pathogen, but are often not meaningful enough to make reliable comparisons between how the materials will behave in different environments.

Based on the results of Sunada and others, various cuprous compounds appear to share comparable antimicrobial efficacy. In the case of a materials additive, however, it is important to consider the long-term stability of the additive. Since cuprous ions are known to be particularly unstable and silver ions are more stable, the present study was undertaken to explore the antimicrobial efficacy of several cuprous and silver materials before and after different various aging conditions.

A study by Zakharova on the effects of storage time of 50 nm copper metal particles in water found that its efficacy against *E. coli* was completely eliminated after 24-hour storage period in water[5]. This was attributed to the formation of a cupric layer on the surface. Larger, 100 nm particles did not demonstrate the same drop in efficacy; however, longer-term storage studies were not carried out.

As presented in the next section, initially antimicrobial efficacy of a variety of copper and silver compounds was screened against a gram-positive and a gram-negative bacterium, such that further and more thorough development could be dedicated to a

single preferred antimicrobial compound. In this study, AM efficacy studies were conducted following a modified ASTM E 640 – 78 test using a high viscosity gel as a model system, rather than formulating finished plastics/coatings containing each additive. This was determined to be a practical test because of its minimal equipment and reagent requirements, as well as minimal microbiological preparatory requirements compared to other test methods.

2.1.2. Materials and Methods

Chemicals

Silver chloride, silver bromide, silver iodide, copper metal, cuprous oxide, cuprous chloride, cuprous bromide, and cuprous iodide (AgCl, AgBr, AgI, Cu_2O, CuCl, CuBr, CuI); sodium carboxymethylcellulose (NaCMC, MW=700,000) and sodium chloride (NaCl) were used as received from Sigma Aldrich (St Louis, MO USA) for preparation of test formulations.

Tryptic soy broth (TSB), tryptic soy agar (TSA), and Dey-Engley Neutralization Media (DE-Broth) were used for antimicrobial evaluations as received from MO BIO Laboratories Inc., (San Diego, CA USA).

Organism and culture media

S. aureus (ATCC #25923) and *P. aeruginosa* (ATCC #9027) were acquired from the University of Arizona (Gerba Environmental Lab). The bacteria were cultured on tryptic soy agar (TSA). Test inoculum were grown for 16 hours in tryptic soy broth (TSB) at 37°C and concentrated to 5 McFarland.

Sample Preparation

Samples were prepared by dispensing each silver or copper compound in DI-water to form 2000 ppm silver or copper mixtures (10 mL each). While agitating with ultrasonication (VWR Sonicator 75D), 300 µL of each mixture was combined with 9.7 mL of aqueous 5% NaCMC solution with aqueous 0.9% NaCl solution to form 60 ppm metal containing gels. Standard gel samples without any metal were also prepared.

In order to accelerate oxidative aging, 5 mL of the 2000 ppm metal mixtures were combined with 34 µL of 30% H_2O_2 and aged for 1 week at 50°C. Aging at 50° C was carried out in order to decompose any residual H_2O_2 to negligible levels[6]. Another set of gel samples was prepared as before at 60 ppm metal using these hydrogen peroxide treated and aged mixtures. Standard gel samples containing aged hydrogen peroxide treated DI-water without any metal salts were also prepared.

The remaining portion of the 2000 ppm metal mixtures were stored in sealed 10 mL glass vials. These were stored under ambient laboratory conditions and continuously rotated using a test tube rocker for 3 months. After aging, another set of gels was prepared at 60 ppm metal concentration using these aged mixtures. Standard gel samples containing similarly stored DI-water were also prepared.

Antimicrobial Efficacy Screening

The prepared 60 ppm metal gel samples were inoculated with 10 µL of bacterial inoculum and mixed using a sterile glass rod. After 24 hours of exposure, approximately 100 µL of each inoculated sample was added to 900 µL Dey-Engley neutralization broth (DE-broth) contained in a 1.5 mL microcentrifuge tube. To accomplish this, 100µL of gel was suctioned into a positive displacement pipette and repeated rinsed with the 900 µL

DE-broth until the sample was fully diluted into DE-Broth. These neutralized samples were serial diluted with TSB to 10^1, 10^2, 10^3, 10^4, 10^5, 10^6, 10^7, and 10^8 dilutions in microtiter well plate.

Another dilution set was prepared as a positive control to determine the initial bacterial load by inoculating a standard saline sample and immediately neutralizing and diluting.

All samples were prepared in triplicate such that there were 3 replicates of each sample (24 wells per sample). These TSB dilutions were incubated at 37°C and monitored for increased turbidity as an indicator of positive bacterial growth for 24 hours.

The most probable number (MPN) technique was used to estimate the bacterial concentration according to the number of replicates exhibiting positive growth in each dilution series. The MPN technique has been demonstrated as an effective method for estimating the concentration of bacteria according to the number of dilutions observed with positive growth and is well described by the FDA's Bacteriological Analytical Manual (BAM)[7]. Briefly, analysis of the dilution samples was carried out by iteratively solving Equation 2.1 for the bacterial concentration, λ, which makes the number of observed positive samples most probable – the most probably number, MPN, λ.

Equation 2.1 – Most Probable Number (as described in FDA's BAM)

$$\sum_{i-1}^{D} \frac{p_i s_i}{1 - \exp(-\lambda s_i)} = \sum_{i-1}^{D} t_i s_i$$

$D = number\ of\ dilutions$
$p_i = number\ of\ positive\ samples\ in\ the\ i^{th}\ dilution$
$s = amount\ of\ sample\ remainging\ in\ the\ i^{th}\ dilution$
$t = numer\ of\ replicates\ in\ the\ i^{th}\ dilution$
$\lambda = most\ probable\ number\ of\ bacteria\ in\ sample$

All antimicrobial activities are reported relative to the positive control as $\log_{10}$ reductions according to Equation 2.2. In the event that no bacteria were recovered from the test sample, the log reduction was reported as greater than the log(MPN) of the positive control to indicate that at least this concentration would have been eliminated.

Equation 2.2 – Antimicrobial Activity

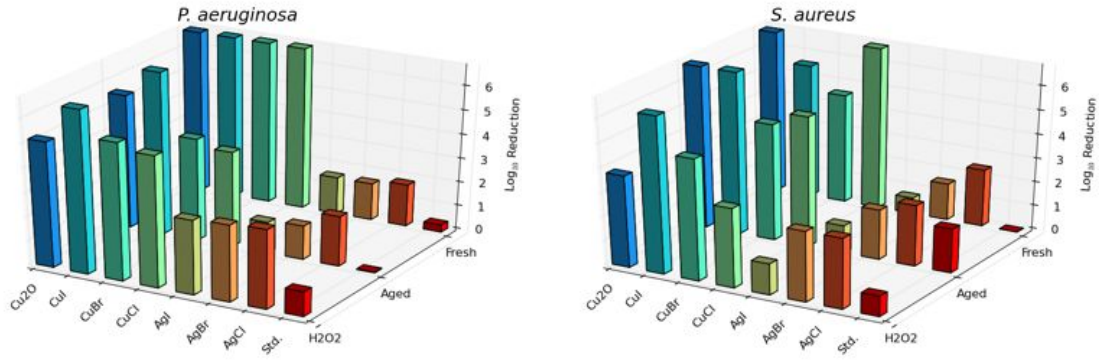
$$\log_{10}(Reduction) = \log_{10}(\frac{\lambda_{control}}{\lambda_{sample}})$$

2.1.3. Results

The antimicrobial activities for the metal compounds tested without aging, with 6 months of aging, and with hydrogen peroxide treatment are shown graphically in Figure 2.3. In all cases the silver compounds demonstrated significantly less antimicrobial activity in this test against both *S. aureus* and *P. aeruginosa* than the cuprous materials.

The cuprous compounds without aging resulted in complete reductions of *P. aeruginosa*, however, improved performance was noted against *S. aureus* for copper iodide. The performance of most of the copper materials degraded with aging or after exposure to hydrogen peroxide. Copper iodide, however, fully retained its antimicrobial activity in all tests against both *S. aureus* and *P. aeruginosa*.

Figure 2.3 – Antimicrobial Efficacy of Various Cu and Ag Compounds



CuI demonstrates superior antimicrobial efficacy after exposure to H_2O_2 or aging as compared to the other silver and copper compounds tested.

2.1.4. Discussion of Results

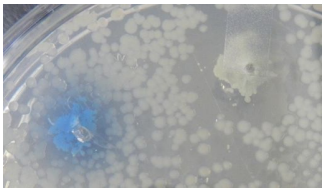
The comparative AM performance of the cuprous and silver compounds agrees with the results obtained by Sunada et al. Chloro-cuprous complexes are formed much faster and at lower chloride concentrations compared to chloro-silver complexes. With reference to the data of Fritz[8], Aherland[9], and Fritz[10] on chloro-cuprous and chloro-silver complex formation it is found that cuprous chloride is around 34,000 times more soluble than silver-chloride in 0.1 M NaCl. For instance, At 0.1 M NaCl (0.6 wt% NaCl), CuCl is soluble to 102 mM Cu (6.5 ppm Cu) while AgCl is soluble to 0.003 mM Ag (324 ppt Ag) due to chloro-metal complex formation. Thus, cuprous ions are expected to have significantly greater availability in biologically relevant (i.e. chloride containing) solutions as compared to silver than would be predicted without consideration of complex ion formation.

A brief study on the impact of various testing media on the antimicrobial performance of the several of the antimicrobial compounds presented here was performed prior to conducting this study. It was found that the silver compounds performed equivalently effectively in various test medias (Butterfield's buffer, phosphate buffered

saline, tryptic soy broth, tryptic soy agar, saline); whereas, the cuprous compounds performed poorly in heavily proteinaceous or phosphate-containing media. Generally, the AM performance of the cuprous materials degraded quickest in tryptic soy agar and least in saline. Similarly, blue products readily formed in the tryptic soy based test media but much less so in saline. Thus, a saline gel was used in this study rather than other media types.

It should be emphasized that different testing media can result in significant differences in efficacy. For instance, several reports indicate poor AM efficacy of copper compounds compared to silver by performing a Kirby-Bauer disk diffusion type assays using tryptic soy agar that contains digested proteinaceous constituents. This test is commonly used to asses the susceptibility of a microbe to an AM drug, but in this test the AMs may be exposed to proteinaceous agars that have a tendency to form complexes or promote the oxidation of cuprous ions. This is evidenced by the formation of blue/green colors. In these tests, the silver compounds may outperform the cuprous species while in other tests (i.e., this study) the results are reversed. Figure 2.4 demonstrates the result of a Kirby-Bauer type test using cuprous chloride and silver chloride powders on to an *E. coli* inoculated Mueller-Hinton Agar. A zone of inhibition formed around the silver material but not around the cuprous material. This demonstrates that silver appears preferable in this type of test because of the strong interactions of the test media with the cuprous ions.

Figure 2.4 – CuCl and AgCl on an *E. coli* inoculated Mueller Hinton Agar Plate



CuCl (left) becomes inactivated by the growth media and does not exhibit a zone of inhibition. AgCl (right) remains active and demonstrates a distinct zone of inhibition.

The decrease in antimicrobial activity for most of the cuprous compounds after aging agrees with studies conducted by Zakharova demonstrating that the AM activity of copper is reduced after aging in water due to the formation of cupric species[11]. These aging studies also agree well with the results of Hans who demonstrated that the antimicrobial activity of copper metal is a function of the oxide species on the surface, which is further dependent on environmental conditions. The oxidative treatments can similarly be interpreted as accelerated aging experiments and again agree with the common theme that cupric ions have inferior antimicrobial efficacy as compared with cuprous ions[12].

The improved performance of cuprous iodide compared to the other cuprous compounds in both the aging and oxidative treatments can be explained by consideration of the degradation mechanisms of cuprous iodide. Cuprous ions tend to undergo autocatalytic disproportionation to form cuprous species and metallic copper (Reaction 2.1), which is then further oxidized (Reaction 2.2). After prolonged aging in aerated water or direct exposure to oxidizing agents, the vast majority of cuprous ions will be converted to less effective cupric ions.

Reaction 2.1 – Disproportionation of Monovalent Cu^{+}

$$2Cu^{+} \rightarrow Cu^{0} + Cu^{++} \qquad E^{0} = 0.368 \text{ V}$$ [13]

Reaction 2.2 – Oxidation of Cuprous Ions in Aerated Water

$$4Cu^{+} + O_2 + 4H^{+} \rightarrow 4Cu^{++} + 2H_2O \qquad E^{0} = 1.076 \text{ V}$$ [14]

It is probable that the rate of degradation would proceed more slowly for CuI than for the other cuprous compounds because of its greater water insolubility compared to the other cuprous species explored. However, this does not explain the retained AM efficacy of CuI after exposure to H_2O_2.

In the case of CuI, dissolved iodide ions are capable of reducing cupric ions, which results in the regeneration of CuI as well as the production of molecular iodine (Reaction 2.3). This was confirmed using a starch indicator to demonstrate the presence of free iodine after aging of H_2O_2 exposure, but not of the fresh material.

This formation of iodine as CuI degrades explains the retained AM efficacy of CuI after oxidation. Although halide anions are not particularly antimicrobial, the molecular halogens are very effective antimicrobial agents. For instance, molecular chlorine and bromine are commonly used to disinfect water while molecular iodine is a common surgical antiseptic.

Reaction 2.3 – Favorable Regeneration of CuI and Generation of Iodine

$$2Cu^{++} + 4I^{-} \rightarrow 2CuI + I_2 \qquad E^0 = 0.34 \text{ V}$$ [15]

Unlike CuI, however, the formation of bromine and chlorine does not occur with CuBr or CuCl (Reaction 2.4 and Reaction 2.5) since neither bromide nor chloride are capable of reducing the cupric ion to reform cuprous species.

Reaction 2.4 – Unfavorable Regeneration of CuBr and Generation of Bromine

$$2Cu^{++} + 4Br^{-} \rightarrow 2CuBr + Br_2 \qquad E^0 = -0.45 \text{ V}$$ [16]

Reaction 2.5 – Unfavorable Regeneration of CuCl and Generation of Chlorine

$$2Cu^{++} + 4Cl^{-} \rightarrow 2CuCl + Cl_2 \qquad E^0 = -0.82 \text{ V}$$ [17]

It should be noted that H_2O_2 is itself capable of oxidizing iodide, chloride, or bromide to iodine, chlorine, or bromine, respectively. Aqueous iodine, however, is substantially more stable than dissolved chlorine or bromine. Thus, under the conditions in this experiment for the H_2O_2 treated cuprous halides that the generated chlorine and bromine were negligible.

Although the cuprous materials appeared similar in testing of freshly dispersed material, after aging or oxidative treatment it seems that CuI would be a more appropriate

candidate material for use in an environmental surface. In this case, the use of CuI might allow for less material to be used and remain more effective after times compared to the other cuprous materials. While the silver materials demonstrated insignificant degradation of their AM activity, they were discouraged due to their very high cost and anticipated higher loadings required to make self-disinfecting claims.

Several antimicrobial producers have recently published similar findings to those presented here, which indicate that CuI is a preferred AM agent compared with other cuprous materials. For instance, in a World Patent Application WO2014193875 A1 by Cupron (2014), a 3% Cu_2O containing polyester yarn, a 3% CuI containing polyester yarn, and a 1% CuI containing polyester yarn were evaluated for copper release and antimicrobial efficacy. 2 g of each yarn was place in water for two hours, after which the copper ion release was assed. Furthermore, each yarn was tested for AM efficacy using against several bacteria after a 24-hour exposure.

Table 2.2 – Data on CuI Yarn adapted from WO2014193875A1 (Cupron Patent App.)

Yarn Composition	Cu Release (ppm)	AM Efficacy (Log_{10} Reduction)*				
		MRSA	VRE	PA	EC	EE
3% Cu_2O (2.66% Cu)	30	1.40	2.21	2.64	2.80	1.25
4% CuI (1.33% Cu)	10	4.00	4.00	4.00	4.00	4.00
1% CuI (0.33% Cu)	3	N/T	N/T	N/T	N/T	2.15

* MRSA=Methicillin resistant *Staphylococcus aureus*; VRE=vancomycin resistant enterococci; PA= *Pseudomonas aeruginosa*; EC = *Escherichia coli*, EE = *Enterobactera erogenes*; N/T=Not tested.

In addition to these data, the WO2014193875 patent application also discloses the impact of "heat-drawing" of CuI loaded PET fibers, which resulted in the improved dispersion of CuI particles and improved antimicrobial efficacy. The improved sample resulted in the achievement of >3 log reduction, while the undrawn sample resulted in

0.93 $\log_{10}$ reduction for *S. aureus*. It should be noted that the present research was conducted prior to the publication of this patent application.

It is important also to note, that the particle size of these materials was not controlled in this experiment. These results are reinforced by section 2.4, which demonstrates that small particles of some silver and copper materials, with controlled particle sizes, follow similar trend as shown here. Thus, any effects due to mismatch in particle size are likely minimal.

2.1.5. Further Discussion on the Selection of Cuprous Iodide

In addition to improved retention of antimicrobial efficacy after aging and oxidative treatments, several other characteristics of cuprous iodide favored its selection as a material for further development.

Pure copper iodide is a colorless white solid with water solubility approximately an order of magnitude less than that of cuprous oxide[18]. Despite the white appearance of pure cuprous halides, they tend to appear somewhat colored due to oxidation in air. Cuprous bromide and cuprous chloride often appear green due to the formation of cupric halides on the surface. Cupric iodide, however, does not exist and copper iodide is often found as a light tan colored material as oxygen from air displaces iodide to form CuO on the surface along with molecular iodine[19].

Among the cuprous halides, CuI does not appreciably change color when stored in either DI-water or distilled water for up to two years, whereas CuCl and CuBr rapidly oxidize to become a murky green. This corresponds with the above results, demonstrating the prolonged efficacy of CuI compared with other materials. Thus it was expected that

CuI could provide antimicrobial surfaces with improved cosmetics and retained efficacy as compared with copper metal and cuprous oxide based surfaces.

Apart from these data, additional knowledge of CuI indicates that it would also be a practical material for further development as an AM additive.

CuI is included in the FDA's Generally Recognized As Safe (GRAS) list for compounds that may be added as an iodine source in table salt, for human consumption. Additionally, copper and iodine are both micronutrients for humans. Cuprous bromide, cuprous chloride, and cuprous oxide are not included in the GRAS list. Furthermore, copper iodide is commonly used to produce heat-stabilized thermoplastics approved for use in food contact applications. Taken together, these aspects indicated that a CuI based antimicrobial additive would be expected to be a safe, low-risk material.

A note on the cost of copper iodide should be mentioned. Although, CuI is markedly less expensive than silver it is notably more expensive than other copper compounds due to the cost of iodine. At present the cost of CuI is around 5× the cost of Cu_2O, but still around 10× less than the cost of silver.

2.2. Antimicrobial Efficacy of Variously Sized CuI Small Particles

This section is presented as an experimental report and summarizes the motivation for the use of small particles of CuI for further AM development. Attention is given to the comparative AM efficacy of differently sized CuI particles.

2.2.1. Introduction

It was demonstrated previously in Section 2.1 that CuI is an effective antimicrobial agent with activity against both gram-positive and a gram-negative bacteria and has several advantages compared to other silver and copper compounds. Similarly, CuI is an effective AM agent against nearly every class of pathogenic microorganism including various bacteria, viruses, yeast, and molds. It has been shown in the literature that nanoparticles of CuI also have useful AM properties, but there have been no studies comparing the efficacy of variously sized CuI particles and of unprocessed, bulk CuI.

It is known that the antimicrobial activity of some antimicrobials may be improved when they are used as small particles. In these cases less AM material can be used to achieve equivalent microbial reductions in a given time; or, equivalent reductions may be achieved with equivalent amounts of active ingredient in a shorter time[20]. Much of the focus on improved performance from small particle AMs has been dedicated to nanoparticles (NPs) and especially to nanoparticles of silver and nanosized pharmaceutical antibacterials.

NPs have received a large amount of attention in the last several decades owing to their unique properties, which can diverge dramatically from their bulk counterparts. NPs of water insoluble AM drugs, for instance, have been employed to improve their

bioavailability in humans. Similarly, NP AM pesticides are commonly incorporated into a variety of consumer products; especially through the use of NP silver additives. The use of NP silver as a drug is also common in many medical devices including catheters and wound dressings as an infection control measure.

Additionally, the use of small particles can dramatically change the appearance of a material compared to the use of larger particles. For instance, micron sized zinc oxide can be replaced with NPs of zinc oxide in sunscreen lotion to improved the cosmetic appearance of the lotion. In traditional sunscreen formulations, micron-sized zinc oxide intensely scatters light and the resultant lotion is chalky and opaque. The use of zinc oxide NPs instead, however, results in transparent lotions.

Despite the recent popularity of NPs, however, small particles have been appreciably produced and utilized in similar applications for hundreds of years. For example the optimum size of rutile titania, common white pigment, is around 200-220 nm. Nevertheless, several agencies have suggested that particles up to large sizes should also be regulated as nanotechnologies owing to their exploitation of size-dependent properties. For example, particles up to 1μ may be considered nano according to current draft FDA nanotechnology guidance[21].

Despite these ambiguities, however, most regulatory bodies define NPs as having a dimension between 1 and 100 nm[22]. In particular the US EPA, the governing body of antimicrobials in environmental surfaces, abides by the 1-100nm nanoparticle definition. Thus, it was prudent to explore whether the AM efficacy of CuI could be improved at smaller sizes inside and outside of this nanoparticle size range.

CuI is typically supplied as a fine powder with a particle size between 1 and 100μ. Thus comparisons were made between nanoparticles (NPs, 1-100nm), small microparticles (SMPs, 100-1000nm), medium microparticles (MMPs, 1-10 μm), and large microparticles (LMPs, 10μ-100μ) of CuI. These were expected to have particle sizes sufficiently separated to elucidate differences in AM efficacies, if such differences exist.

In the production of small particles, a capping agent or peptizing agent is may be needed to stabilize the particles sterically or electrostatically, respectively. This material in addition to size effects, may also contribute to modulation of antimicrobial performance. For instance, Daima et al. demonstrated that surface modification of gold nanoparticles with tyrosine or lysine resulted in dramatically different AM efficacy rates[23]. This was attributed to the cationic nature of lysine that would be attracted to bacterial cells, which are typically slightly negatively charged. In contrast to this, however, it was found by Murthy et al. that anionic-carboxyl (dodecanedioic acid) surface modified gallium oxide nanoparticles were more effective than cationic-amine (lysine) modified gallium oxide nanoparticles at inhibiting bacterial adhesion and biofilm formation[24]. Similarly, in the case of silver particles mixed results have been reported for the impact of different protecting agents on the antimicrobial efficacy of different organisms[25].

In the case of CuI, it was decided to focus attention on polyvinylpyrrolidone (PVP) for several reasons. PVP has been used in the production of stable CuI nanoparticles. PVP is a mild reducing agent and is a well-known iodophor used in the preparation of iodine based surgical scrubs. Additionally, PVP is also commonly used as

a binder in pharmaceutical preparations and is a very safe material, which as expected from this use could result in pelleted non-dusting materials.

2.2.2. Materials and methods

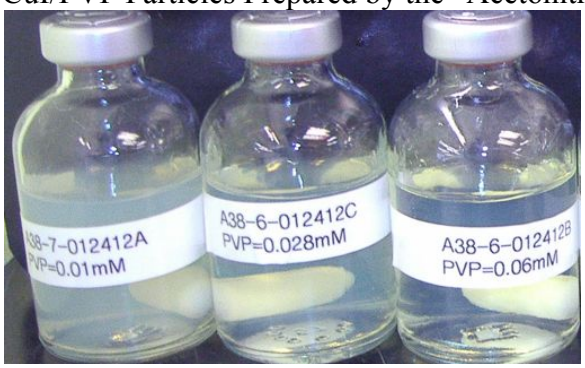
Chemicals

Copper iodide (CuI), Acetonitrile, polyvinylpyrrolidone MW=10,000 (PVP), and polyethylenimine MW=800 (PEI), were used as received from Sigma Aldrich (St. Louis, MO USA). Ethycellulose was acquired from Dow Chemical. Tryptic soy broth (TSB) and tryptic soy agar (TSA) were acquired from MO BIO Laboratories Inc. (San Diego, CA USA). 18Ω deionized water was used throughout. Copper iodide was acquired from separate manufactures with nominal particles sizes of 1 micron and 10 microns for preparation of MMPs and LMPs.

Chemical Preparation of Polyvinylpyrrolidone Modified CuI Small Particles

PVP modified copper iodide small particles were prepared by adapting a procedure described by Yang et al[26]. To a round bottom flask would be added a quantity of PVP, CuI, and a volume of acetonitrile capable of fully dissolving the CuI and PVP. As the acetonitrile was removed under vacuum, CuI would precipitate out as to form particles. The size of these particles could be controlled by adjustment of the amount PVP in the mixtures. The dry material was then capable of being redispersed in water to form stable dispersions. As an example of this process, Figure 2.5 shows three preparations with increasing levels of PVP. As the PVP level was increased, the particle size decreased, and the turbidity of the particles dispersed in water was reduced.

Figure 2.5 – Example CuI/PVP Particles Prepared by the "Acetonitrile Method"



Polyvinylpyrrolidone modified copper iodide nanoparticles, CuI-PVP-NPs, were prepared using a relatively large amount of PVP. Briefly, to a round bottom flask was added 4.05 g of PVP, 0.041 g of CuI, and 300mL acetonitrile. This was stirred to form a translucent yellow solution. The acetonitrile was removed under reduced pressure on a rotary evaporator to form a pale yellow solid with 1% CuI in solids. This material was redispersed in DI-water to form a translucent dispersion for particle size and AM analysis.

In order to elucidate if PVP contributed to the AM efficacy of the small particle formulations a similar formulation was prepared using PEI rather than PVP in a similar fashion as was used in the CuI-PVP-NP preparation. Briefly, to a round bottom flask was added 4.05 g of PEI, 0.041 g of CuI, and 300mL acetonitrile. This was stirred to form a translucent yellow solution. The acetonitrile was removed under reduced pressure on a rotary evaporator to form a pale yellow solid with 1% CuI in solids. This material was redispersed in DI-water to form a translucent dispersion for particle size and AM analysis.

Polyvinylpyrrolidone modified copper iodide small microparticles, CuI-PVP-SMPs, were prepared by reducing the amount of PVP in the CuI NP synthesis. Briefly, to a round bottom flask was added 4.05 g of PVP, 4.05 g of CuI, and 600mL acetonitrile. This was stirred to form a translucent yellow solution. The acetonitrile was removed under reduced pressure on a rotary evaporator to form a pale green solid with 50% CuI in solids. This material was redispersed in DI-water to form a milky dispersion for particle size and AM analysis.

Copper iodide medium microparticles, CuI- PVP-MMPs, were prepared through the selection of a commercial CuI powder supplied as 1 μ particles. 1 g of this CuI powder was mixed with 1 g PVP, and 100 mL DI-water in order to eliminate differences that might occur due to the mild reducing nature of PVP. This mixture was then dried under reduced pressure to form a white solid with 50% CuI in the solids. This material was redispersed in DI-water to form a milky dispersion for AM analysis.

Copper iodide large microparticles, CuI- PVP-LMPs, were prepared through the selection of a commercial CuI powder supplied as 10 μ particles. 1 g of this CuI was mixed with 1 g PVP, and 100 mL DI-water in order to eliminate differences that might occur due to the mild reducing nature of PVP. This mixture was then dried under reduced pressure to form a white solid with 50% CuI in solids. This material was redispersed in DI-water to form a milky dispersion for AM analysis.

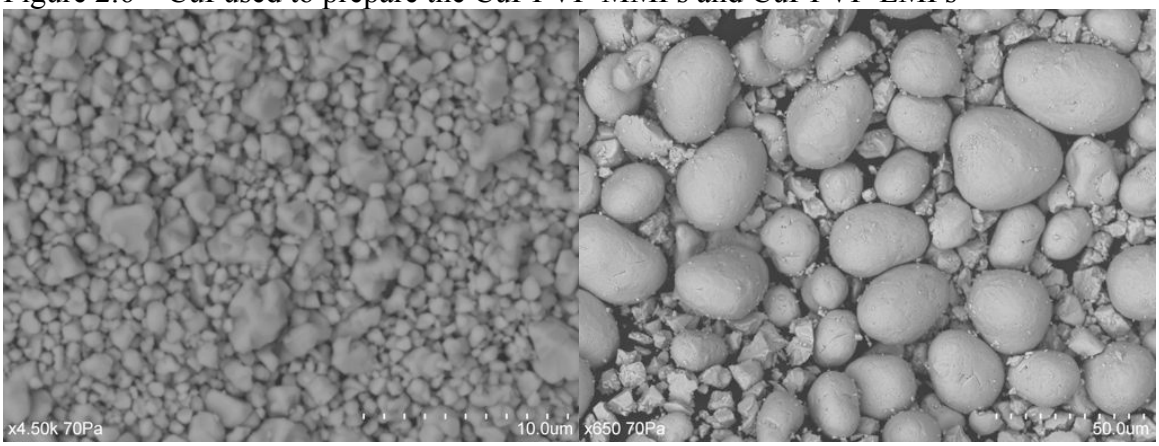
Particle Size Analysis

The hydrodynamic radius of the CuI-PVP-NPs and CuI-PVP-SMPs were measured using a DynoPro Nanostar dynamic light scattering system. The CuI-PVP-NPs were determined to be monodisperse with a radius of 8 nm and 32% polydispersity. The

CuI-PVP-SMPs were determined to be monodisperse with a radius of 122 nm and 82% polydispersity.

The CuI particles used to prepare the CuI-PVP-MMPs and CuI-PVP-LMPs were examined before addition of PVP using a Hitachi S4800 scanning electron microscope. The nominal radius of the MMPs was taken as approximately 1 micron. The nominal radius of the LMPs was taken as approximately 10 microns.

Figure 2.6 – CuI used to prepare the CuI-PVP-MMPs and CuI-PVP-LMPs



Left, CuI used for CuI-PVP-MMPs. Right, CuI used for CuI-PVP-LMPs.

Antimicrobial Suspension Testing

S. aureus (ATCC #25923) and *P. aeruginosa* (ATCC #9027) were cultured on Trypic Soy Agar. Test cultures were grown for 16 hours (overnight) in Trypic Soy Broth at 37°C. These overnight cultures were used as the inoculum without washing or dilution.

Each CuI particle formulation (NPs, SMPs, MMPs, and LMPs) was diluted to 60 ppm Cu in sterile saline. To 10 mL of each 60 ppm Cu dispersion was added 0.5 g NaCMC (MW 700,000). These were agitated with heating until the NaCMC fully dissolved and viscous mixtures were formed. These were used as the test formulations. Standards were similarly prepared without any CuI.

Test suspensions were inoculated with 100 µL of bacterial suspension and mixed using a sterile glass rod. After approximately 0, 30, 60, 120, 180 minutes and 24 hours, approximately 100 µL of this suspension was directly streaked on to a TSA plate. These streaked plates were incubated at 37°C for 24 hours and qualitatively evaluated for growth.

Antimicrobial Coatings Test

The SMPs, MMPs, LMPs CuI particles were each incorporated into ethylcellulose coatings to determine if they would exhibit similar efficacy patterns as observed during suspension testing. Briefly, 0.022 g of each 50wt% CuI/PVP mixture was dispersed in 1.5 g ethanol. This dispersion was then combined with 3.5 g Ethycellulose and 6 mL ethanol with 0.05 g Novec FC4430 Fluorosurfactant . These coating mixtures contained 0.1% Cu in the solids. The above procedure was followed for each CuI particle formulation (SMPs, MMPs, and LMPs) as well as without CuI to form standard coatings. The CuI NPs contained too much PVP to form robust coatings and were not tested in a coating formulation.

Each coating formulation was coated on to 2”×2” aluminum substrates by hand using a #18 wire wound Mayer rod. These were subsequently dried in a vacuum oven to remove entrapped air bubbles. The coated aluminum substrates were then cut into 1”×1” pieces for AM evaluation. The coatings had a nominal thickness of approximately 5-15µ as measured using a Tencor Alpha Step surface profilometer.

Antimicrobial evaluation of the coatings was conducted using a modified version of the Japanese Industrial Standard (JIS) method Z 2801 (Japanese Standards Association 2000). *P. aeruginosa* (ATCC #9027) was cultured on Trypic Soy Agar. Test cultures

were grown for 16 hours (overnight) in Trypic Soy Broth at 37°C. The culture was subsequently centrifuged, washed, and redispersed to approximately 0.5 McFarland in sterile saline (0.9% NaCl) before antimicrobial testing.

The coatings were assumed as sterilize from production due to ethanol and stored under dry heat (~50°C) until testing. The substrates were allowed to cool to room temperature and each substrate was inoculated with 10 µL of inoculum and placed in an empty 50mL sterile specimen jar. After 24 hours, 10 mL DE broth was added to each jar. Each jar was then sonicated for 2 minutes to collect any bacteria adhered to the coating. This broth was then diluted and enumerated using the MPN technique. Each sample was tested in triplicate for each organism.

2.2.3. Results

Figure 2.7 – Qualitative Measure of Growth in Antimicrobial Suspension Test

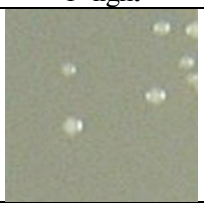
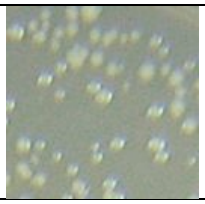
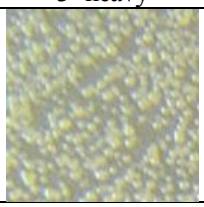
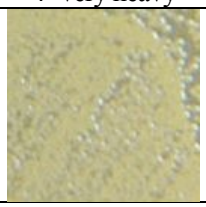
0=none	1=light	2=moderate	3=heavy	4=very heavy
				
None	1-4 CFU/cm^2	5-10 CFU/cm^2	10+ CFU/cm^2	Not Separable

Table 2.3 – Antimicrobial Suspension Test for CuI Particles against *P. aeruginosa*

Sample	Qualitative growth (n=3) at Exposure Time (min)					
	0	30	60	120	180	24 Hours
CuI-PVP-NP	3.7	2.3	0.7	0.7	0.0	0.0
CuI-PVP-SMP	4.0	3.0	1.0	0.0	0.0	0.0
CuI-PVP-MMP	4.0	4.0	2.7	2.3	2.0	0.0
CuI-PVP-LMP	4.0	4.0	3.3	3.3	3.0	0.0
CuI-PEI-NP	4.0	4.0	4.0	4.0	2.7	0.0
Standard	4.0	4.0	4.0	4.0	4.0	4.0

0=none, 1=light, 2=moderate, 3=heavy, 4=very heavy growth

Table 2.4 – Antimicrobial Suspension Test for CuI Particles against *S. aureus*

Sample	Qualitative growth (n=3) at Exposure Time (min)					
	0	30	60	120	180	24 Hours
CuI-PVP-NP	4.0	3.3	1.0	0.0	0.0	0.0
CuI-PVP-SMP	4.0	3.7	1.0	0.0	0.0	0.0
CuI-PVP-MMP	4.0	4.0	3.7	3.0	2.3	0.0
CuI-PVP-LMP	4.0	4.0	4.0	4.0	3.7	0.0
CuI-PEI-NP	4.0	4.0	4.0	4.0	3.7	0.0
Standard	4.0	4.0	4.0	4.0	4.0	4.0

0=none, 1=light, 2=moderate, 3=heavy, 4=very heavy growth

Table 2.5 – CuI-Ethylcellulose Coatings versus *S. aureus* and *P. aeruginosa*

Coating	Organism	Log_{10} Reduction
SMP (t=24 hr)	PA	>3.67 ± 0.00
	SA	>3.61 ± 0.00
MMP (t=24 hr)	PA	1.69 ± 0.03
	SA	0.98 ± 1.02
LMP (t=24 hr)	PA	0.50 ± 0.24
	SA	0.64 ± 0.09
Standard Coating (t=24 hr)	PA	-0.05 ± 0.12
	SA	0.20 ± 0.01

> Indicates that no microbes were recovered. Log_{10} reduction was at least this value.

2.2.4. Discussion of Results

These results demonstrate the improved AM efficacy of CuI when used as a PVP modified small particle. Table 2.3 and Table 2.4 show that the CuI SMPs and NPs were more effective than commercially available CuI particles against gram-negative bacteria or gram-positive bacteria. Both the MMP and LMP copper iodide particles had negligible efficacy after 3 hours exposure for both bacteria, but achieved a complete reduction in bacteria after 24 hours. Thus it appeared that small particles CuI would be favorable for the creation of self-disinfecting surfaces.

The PVP modified small particles performed substantially more effectively than the PEI modified small particles. This is likely due to the slight reducing nature of PVP,

which is capably of maintaining the cuprous state; whereas the aminic nature of PEI likely the formation of cupric species.

The incorporation of the CuI particles into ethylcellulose coatings showed a similar trend as was observed in the planktonic tests. The CuI-PVP-SMP demonstrated dramatically superior antimicrobial efficacy compared to either of the larger CuI-PVP-MMP or CuI-PVP-LMP formulations. The CuI-PVP-NP formulation was not incorporated into a coating due to the high PVP loading and loss of integrity of the final coating.

2.3. Confirmatory Experiments on the Usefulness of CuI Small Particles

This section is presented as an experimental report of several confirmatory experiments on the efficacy of small particles of CuI. Attention is given to the use of traditional microbiological techniques in confirming the antimicrobial efficacy of the CuI-PVP-SMP particles.

2.3.1. Introduction

Section 2.2 demonstrated that small particles (NPs and SMPs) of CuI were preferable as antimicrobial agents compared to larger particles; however, these tests were conducted using test procedures adapted from industrial microbiology test methods, which were found useful for experimental development owing to their reduced time and supplies needed. Traditional microbiological assays, however, are useful for providing more precise measurements.

Thus, similar suspensions and coatings as presented in Section 2.2 using the CuI-PVP-SMPs and CuI-NPs were tested under collaboration with Jason Torrey (Gerba Lab, University of Arizona) using standard microbiological techniques. Furthermore, the comparative efficacy of large non-functionalized CuI particles was assessed.

In addition to testing of the CuI-PVP-SMPs and CuI-PVP-NPs, however, it was also important to confirm that PVP was not exerting an antimicrobial effect itself or was contributing to the AM efficacy of the CuI based small particles.

2.3.2. Materials and Methods

Materials

Copper iodide (CuI), acetonitrile, and polyvinylpyrrolidone MW=10,000 (PVP), and N-ethyl-pyrrolidone (NEP) were used as received from Sigma Aldrich (St. Louis, MO USA).18Ω deionized water was used throughout. Copper iodide was acquired from two manufactures for the preparation of MMPs and LMPs. An aliphatic polycarbonate–diol based polyurethane resin, Esacote PU 71, was acquired from Lamberti (Italy). A trifunctional polyazidrine crosslinking agent PZ-28 was acquired from PolyAziridne (NJ, USA).

Preparation of Copper Iodide Samples for AM Evaluation

Polyvinylpyrrolidone modified copper iodide nanoparticles, CuI-PVP-NPs; and polyvinylpyrrolidone modified copper iodide small microparticles, CuI-PVP-SMPs were prepared as described in the previous section (2.2). Two batches of these SMP CuI particles were prepared CuI-PVP-SMP1 and CuI-PVP-SMP2. These particles were monodisperse with a particle diameter of 241 & 215 nm and polydispersity of 36.5 & 42.4% measured using dynamic light scattering.

In the previous section, a similar preparation resulted in particles of 122 nm. This large size fluctuation tended to result from lack of control during precipitation and especially for larger sized particles. Particle of 5-10 nm tended to provide much more reproducible dispersions, however, at the expense of increased PVP. This contributed to the decision to move towards an alternative processing route as described in Chapter 3.

Copper iodide large microparticles were prepared through the selection of a commercial CuI powder supplied as nominal particle size of 10 μ. This CuI was tested with and without the addition of PVP, CuI-PVP-LMPs and CuI-LMPs, in order to

explore the differences that might occur due to the mild reducing nature of PVP. In the case where PVP was added, it was added to account for 90% of the solids in an aqueous mixture.

In order to elucidate if PVP contributed to the AM efficacy of the small particle formulations a similar formulation was prepared using PEI rather than PVP in a similar fashion as was used in the CuI-PVP-NP preparation. Briefly, to a round bottom flask was added 4.05 g of PEI, 0.041 g of CuI, and 300mL acetonitrile. This was stirred to form a translucent yellow solution. The acetonitrile was removed under reduced pressure on a rotary evaporator to form a pale yellow solid with 1% CuI in solids. This material was redispersed in DI-water to form a translucent dispersion for particle size and AM analysis.

Preparation of Copper Iodide Containing Coatings for AM Evaluation

Several polyurethane coatings were prepared at various copper concentrations using the CuI-PVP-SMPs. Briefly; the CuI-PVP-SMPs were dispersed in 7wt% N-ethyl-pyrrolidone (NEP). This dispersion was added to a mixture of Esacote PU 71 polyurethane resin (Lamberti, Italy) and combined with 3% PZ-28 trifunctional polyazidrine crosslinking agent (PolyAziridne NJ, USA). After stirring for 30 minutes the coatings was brush coated by hand on to 2-inch by 2-inch aluminum substrates. CuI was added at 0.1, 0.3, 0.5, 1.0, 3.0, and 5.0 wt% as copper in the dry coatings.

Several additional polyurethane coatings were similarly prepared with the addition of PVP alone for the purposes of evaluating the impact of added PVP on the swelling behavior of these coatings. PVP was added at comparable levels at which they were added resultant from the addition of the CuI-PVP-SMP formulation, namely between 0.3-15% PVP. These coatings were soaked in water or ethanol for 5 or 30

minutes to determine the solvent uptake change relative to standard coatings without added PVP.

Antimicrobial Analysis

Antimicrobial analysis was conducted in collaboration with Jason Torrey (Gerba Lab, University of Arizona) using standard microbiological techniques. Additional discussion of the techniques used here, although not these results in particular, can be referenced through his PhD dissertation[27]. Briefly, the particles were diluted in phosphate buffered saline to 60 ppm copper, inoculated with bacteria. After a given time period, samples were neutralized in DE-broth and enumerated with respect to positive controls.

The CuI SMP and bulk particles were evaluated to confirm that the AM efficacy of the SMPs, and to confirm their superiority to non-functionalized, larger CuI particles. The CuI-PVP-SMPs particles were tested against *P. aeruginosa*, *S. aureus*, *E. coli*, and *Salmonella typhimurium*. The CuI-PVP-LMPs and CuI-LMPs were only tested against *P. aeruginosa*. The CuI-PVP-NPs and CuI-PEI-NPs were tested against *Streptococcus mutans.* Additionally, PVP was tested alone against *P. aeruginosa* at up to 8wt% PVP in order to confirm that PVP did not exert an antimicrobial effect itself.

Prior to conducting AM efficacy experiments on the CuI containing coatings, the survivability of the *P. aeruginosa* strain used was evaluated on standard PU coated aluminum substrates without CuI. Reduction of *P. aeruginosa* was evaluated using the JIS Z 2801 method in humidity chambers and at environmental humidity. All substrates were sprayed with 70% ethanol and allowed to dry twice before testing. Bacteria was inoculated onto sample coupons in triplicate and covered with a sterile polyethylene cover slip. Triplicate samples were placed in humidity or chambers or left at room

humidity for the duration of the exposure period. Bacteria was recovered by swabbing the surface after the desired contact times. All tests conducted at room temperature.

AM evaluations of the CuI coatings were conducted in a similar fashion to the *P. aeruginosa* survivability tests, however, coating were contained in controlled humidity chambers since this method resulted in higher survival of *P. aeruginosa* in the aforementioned survivability assay.

2.3.3. Results

Table 2.6 through Table 2.9 demonstrates the very high efficacy of the CuI-PVP-SMP formulation against a variety of common pathogenic bacteria.

Table 2.6 – CuI SMP AM Efficacy in Suspension against *S. aureus*

Time	PBS Control	CuI-PVP-SMP1	CuI-PVP-SMP2
	Log_{10} Reduction	Log_{10} Reduction	Log_{10} Reduction
15 min	-0.05 ± 0.05	3.55 ± 1.07	2.70 ± 0.11
2 hr	-0.13 ± 0.10	> 4.16 ± 0.21	> 4.31 ± 0.00
6 hr	-0.03 ± 0.01	> 4.31 ± 0.00	> 4.31 ± 0.00

Original titer = 1.02E+06 cfu / mL

Table 2.7 – CuI SMP AM Efficacy in Suspension against *E. coli*

Time	PBS Control	CuI-PVP-SMP1	CuI-PVP-SMP2
	Log_{10} Reduction	Log_{10} Reduction	Log_{10} Reduction
15 min	-0.46 ± 0.13	> 4.09 ± 0.00	> 3.94 ± 0.21
2 hr	0.06 ± 0.11	> 4.09 ± 0.00	4.09 ± 0.00
6 hr	0.01 ± 0.06	> 4.09 ± 0.00	> 4.09 ± 0.00

Original titer = 6.12E+05 cfu / mL

Table 2.8 – CuI SMP AM Efficacy in Suspension against *Salmonella typhimurium*

Time	PBS Control	CuI-PVP-SMP1	CuI-PVP-SMP2
	Log_{10} Reduction	Log_{10} Reduction	Log_{10} Reduction
15 min	-0.13 ± 0.06	3.24 ± 0.74	> 4.37 ± 0.00
2 hr	-0.08 ± 0.02	> 4.37 ± 0.00	4.13 ± 0.34
6 hr	0.23 ± 0.07	> 4.37 ± 0.00	> 4.37 ± 0.00

Original titer = 1.16E+06 cfu / mL

Table 2.9 – CuI SMP AM Efficacy in Suspension against *P. aeruginosa*

Time	PBS Control	CuI-PVP-SMP1	CuI-PVP-SMP2
	Log_{10} Reduction	Log_{10} Reduction	Log_{10} Reduction
15 min	0.17 ± 0.07	> 5.23 ± 0.00	> 5.23 ± 0.00
2 hr	0.06 ± 0.45	> 5.23 ± 0.00	> 5.23 ± 0.00
6 hr	0.45 ± 0.01	> 5.23 ± 0.00	> 5.23 ± 0.00

Original titer = 8.40E+06 cfu / mL

Table 2.10 demonstrates the dramatically reduced efficacy of the large microparticles of CuI. Compare to Table 2.9 for CuI-PVP-SMP.

Table 2.10 – Suspended large CuI microparticles against *P. aeruginosa*

Time	PBS Control	CuI-LMP	CuI-PVP-LMP
	Log_{10} Reduction	Log_{10} Reduction	Log_{10} Reduction
30 min	0.26 ± 0.01	0.43 ± 0.04	0.13 ± 0.11
3 hours	1.55 ± 0.02	2.34 ± 1.94	0.67 ± 0.05

Original titer = 2.57E+06 cfu / mL

Table 2.11 demonstrates that the CuI-PVP-NP formulation has dramatically improved efficacy as compared to the CuI-PEI-NP formulation. PEI has a high number of amine groups that may act to sequester and/or oxidize copper ions, whereas PVP has some reducing capability that may help stabilize the cuprous state. Interestingly, however, the addition of PVP to the CuI-LMP formulation (Table 2.10) reduced the AM efficacy of the material, perhaps by sequestering any formed iodine.

Table 2.11 – CuI-PEI-NPs and CuI-PVP-NPs against *Streptococcus mutans*

Time	PBS Control	CuI-PEI-NP	CuI-PVP-NP
	Log_{10} Reduction	Log_{10} Reduction	Log_{10} Reduction
15 min	0.19 ± 0.00	-0.04 ± 0.26	> 4.75 ± 0.00
2 hr	0.81 ± 0.10	0.29 ± 0.10	> 4.60 ± 0.21
6 hr	1.68 ± 0.41	0.65 ± 0.02	> 4.75 ± 0.00
24 hr	> 4.75 ± 0.00	3.38 ± 0.04	> 4.75 ± 0.00

Original titer = 2.83E+06 cfu / mL

Table 2.12 demonstrates that *P. aeruginosa* has an improved survival rate on polyurethane coatings when contained in a 100% humidity chamber. Based on these results, subsequent coatings tests were conducted using a humidity chamber.

Table 2.12 – Survivability of *P. aeruginosa* on Standard Polyurethane Coatings

Time	No Humidity Chamber	Humidity Chamber
	Log_{10} Reduction	Log_{10} Reduction
6 hr	-0.06 ± 0.03	0.00 ± 0.09
24 hr	0.37 ± 0.24	-0.56 ± 0.08

Original Titer = 1.96+06 cfu/mL

Table 2.13 and Table 2.14 demonstrate the utility of these small particles in a polyurethane coating. As expected with increasing amounts of CuI additive an increase in efficacy at shorter times.

Table 2.13 – Polyurethane Coatings with CuI-SMP (0.1-0.5% Cu) vs. *P. aeruginosa*

Time	Polyurethane Control	Cu in PU (Cu 0.1%)	Cu in PU (Cu 0.3%)	Cu in PU (Cu 0.5%)
	Log_{10} Reduction	Log_{10} Reduction	Log_{10} Reduction	Log_{10} Reduction
1 hr	-0.08 ± 0.16	-0.09 ± 0.03	-0.05 ± 0.15	-0.16 ± 0.07
6 hr	-0.38 ± 0.06	0.64 ± 0.65	3.21 ± 0.68	3.97 ± 0.43

Original Titer = 1.15E+06 cfu/mL

Table 2.14 – Polyurethane Coatings with CuI-SMP (1-5% Cu) vs. *P. aeruginosa*

Time	Polyurethane Control	Cu in PU (Cu 1.0%)	Cu in PU (Cu 3.0%)	Cu in PU (Cu 5.0%)
	Log_{10} Reduction	Log_{10} Reduction	Log_{10} Reduction	Log_{10} Reduction
1 hr	0.42 ± 0.10	0.52 ± 0.04	> 5.28 ± 0.64	> 5.65 ± 0.00
6 hr	0.17 ± 0.02	1.77 ± 0.17	> 5.50 ± 0.28	> 5.55 ± 0.17

Original Titer = 2.26 E+06 cfu/mL

Table 2.15 demonstrates that PVP is not an active antimicrobial agent. This test was designed to ensure that the particular PVP used in these experiments did not contain any adulterants that might exert an antimicrobial effect. No antimicrobial efficacy was observed for PVP.

Table 2.15 – Confirmation of Non-AM Activity of PVP using *P. aeruginosa*

Time	PBS Control	0.09% PVP	8.1% PVP
	Log10 Reduction	Log10 Reduction	Log10 Reduction
1 hr	-0.50 ± 0.00	-0.42 ± 0.02	-0.39 ± 0.06
6 hr	-0.12 ± 0.00	-0.15 ± 0.08	-0.05 ± 0.02

Original titer = 9.83E+05 cfu / mL

Table 2.16 demonstrates that the addition of a large amount of PVP to the CuI-PVP-NPs resulted in minimal change in the AM performance of the material. The slight improvement may have resulted from the reducing nature of PVP. As noted, this is the opposite as seen for larger CuI particles (Table 2.10).

Table 2.16 – Efficacy of CuI-PVP-NPs with Excess PVP versus *P. aeruginosa*

Time	PBS Control	CuI-PVP-NP 0.09% PVP	CuI-PVP-NP 8.1% PVP
	Log_{10} Reduction	Log_{10} Reduction	Log_{10} Reduction
1 hr	-0.50 ± 0.00	3.55 ± 0.37	> 4.06 ± 0.34
6 hr	-0.12 ± 0.00	> 4.29 ± 0.00	> 4.29 ± 0.00

Original titer = 9.83E+05 cfu / mL

2.3.4. Discussion

The results of this study supplement sections 2.1 and 2.2 in demonstrating the broad spectrum and AM efficacy of CuI-PVP-SMP formulations. Furthermore, Table 2.6 through Table 2.9 show that these CuI-PVP-SMPs were more effective against several Gram-negative bacteria than against the Gram-positive *S. aureus*. In particular, the particles were found to be most effective against *P. aeruginosa*, achieving greater than 5 log reduction (>99.999% reduction) in 15 minutes. By comparison, the 10μ copper iodide particles had negligible efficacy against *P. aeruginosa* after 30 minutes and moderate efficacy after 2 hours exposure.

Table 2.15 demonstrates that PVP is not an active antimicrobial agent. This test was designed to ensure that the particular PVP used in these experiments did not contain any adulterants that might exert an antimicrobial effect. No antimicrobial efficacy was observed for PVP.

Table 2.15 demonstrates that PVP is not antimicrobial at up to 8% PVP in solution. At 60 ppm Cu PVP would have produced approximately 180 ppm PVP in solution for the CuI-PVP-SMP formulation. Furthermore, the addition of PVP to the CuI-LMPs resulted in reduced AM efficacy. This was likely due to the chelation of any free iodine resulting from oxidation of the material. In contrast, the increased amount of PVP in the CuI NP formulations did not result in reduced efficacy compared to the CuI SMP material. This suggests that when CuI is intimately associated with PVP it experiences an improvement in it's antimicrobial efficacy, in stark contrast to the reduced efficacy observed in bulk CuI by the addition of PVP.

For silver nanoparticles it has been reported that different capping agents can dramatically affect the AM efficacy[28] and the amount of particulate matter capable of penetrating through cellular structures[29].

In further support of the role that PVP plays in the PVP-CuI system, it was observed that replacement of PVP with PEI resulted in a dramatic loss of antimicrobial efficacy. When CuI-NPs were stabilized with PEI and tested under identical conditional as the PVP formulated material they demonstrated markedly less activity.

On the basis of these results, which supported the use of a PVP modified CuI small particle, several polyurethane coatings were prepared and tested. The efficacy of the coatings increased with increasing copper concentration, however, dramatic

reductions were not achieved until large quantities of CuI were added to the coatings. At these high levels of CuI, however, the quality of these coatings was reduced due to the high levels of PVP added.

2.4. CuI Small Particles Versus Small Particles of Other Materials

This section is presented as an experimental report of several experiments on the superior efficacy of small particles of CuI as compared with small particles of other materials. Attention is given to the use of traditional microbiological techniques in determining the antimicrobial efficacy of CuI nanoparticles with silver based nanoparticles.

2.4.1. Introduction

Section 2.1 suggested that CuI is a preferred antimicrobial material among various silver or copper based materials because of it's improved efficacy or retained antimicrobial properties, respectively. Furthermore, sections 2.2-2.3 suggest that polyvinylpyrrolidone modified CuI small particles are more effective compared with raw CuI.

To this end, several silver based small particles were prepared and compared with CuI in order to determine if the trends observed in 2.1 would apply to the materials when prepared as small particles. From a practical point of view however, because silver is a significantly more expensive material the silver particles were evaluated at a reduced concentration.

In addition, several CuI and silver compounds were prepared as simple mixtures of particles and as solid solution particles in order to determine if any synergy existed between silver and CuI, as has been reported elsewhere for Ag^{+} and cupric (Cu^{++}) ions[30].

2.4.2. Materials and Methods

Polyvinylpyrrolidone modified CuI nanoparticles were prepared by combining 15.75 g PVP with 175 mL acetonitrile and 0.167 g CuI. This was stirred to form a pale green solution. This solution was then dried under reduced pressure to form a pale green solid. This solid was then dispersed in DI-water to form a dispersion of 647 ppm Cu. This was then diluted to 59.07 ppm Cu in sterile PBS for AM evaluation.

Polyvinylpyrrolidone modified Ag metal nanoparticles were prepared by first dissolving 20.0 g PVP in 50 mL DI-water to act a both a reducing and capping agent. To this solution was added 0.0493 g $AgNO_3$. This solution was heated to 70°C with stirring for 7 hours. This was then diluted to 10 ppm Ag in sterile PBS for AM evaluation.

Polyvinylpyrrolidone modified AgBr nanoparticles were prepared by first dissolving 1.52 g PVP in 23 mL DI-water to act a both a reducing and capping agent. To this solution was added 0.0246 g $AgNO_3$. To this solution was drop wise added solution of 0.0179 g KBr in 10 mL DI-water at a rate of 0.34mL/min.

Polyvinylpyrrolidone modified mixed metal particles were prepared by dissolving 10 g PVP, 0.0246 g $AgNO_3$, 0.0350 $Cu(NO_3)_2$ in 40 mL DI-water. To this was drop wise added a solution of 0.0481 g KI in 10 mL DI-water while stirring at a rate of 0.34mL/min.

Particle sizes for all particles were determined by dynamic light scattering to be in the range of 5-15 nm. All particle suspensions were tested for antimicrobial efficacy shortly after preparation.

Antimicrobial analysis was conducted in collaboration with Jason Torrey (Gerba Lab, University of Arizona) using standard microbiological techniques. Additional

discussion of the techniques used here, although not these results in particular, can be referenced through his PhD dissertation.

2.4.3. Results

Table 2.17 through Table 2.19 reinforce the belief that CuI is a more potent antimicrobial agent than various silver compounds against *P. aeruginosa*. Table 2.17 demonstrates that at a reasonably cost equivalent basis (see Section 2.1.5), CuI is much more effective than silver. 2.18 and 2.19 show that mixtures of Ag and CuI as mixtures of separate nanoparticles or mixed-metal halide nanoparticles, respectively against *P. aeruginosa*; CuI alone is still the preferred material.

Table 2.17 – Efficacy of Ag^0, AgBr, or CuI Nanoparticles against *P. aeruginosa*

Time	Control	Ag^0 (10 ppm Ag)	AgBr (10 ppm Ag)	Ag^0 + AgBr (10 ppm Ag from each)	CuI (59.07 ppm Cu)
2 hr	0.30 ± 0.08	0.94 ± 0.06	0.95 ± 0.03	0.92 ± 0.06	>5.38 ± 0.00
6 hr	0.84 ± 0.00	1.11 ± 0.03	1.07 ± 0.03	1.08 ± 0.07	>5.38 ± 0.00
24 hr	1.29 ± 1.29	>5.38 ± 0.00	>5.38 ± 0.00	>5.38 ± 0.00	>5.38 ± 0.00

Original Titer = 1.08 E07cfu/mL

Table 2.18 – Mixtures of Ag^0 or AgBr and CuI Nanoparticles vs. *P. aeruginosa*

Time	Control	CuI (12 ppm Cu)	CuI + Ag^0 (12 ppm Cu, 2 ppm Ag)	CuI+AgBr (12 ppm Cu, 2 ppm Ag)
2 hr	0.11 ± 0.03	0.42 ± 0.02	0.46 ± 0.00	0.41 ± 0.02
6 hr	0.07 ± 0.05	1.22 ± 0.07	1.32 ± 0.15	1.13 ± 0.03
24 hr	0.06 ± 0.03	>4.41 ± 0.00	>4.41 ± 0.00	>4.41 ± 0.00

Original Titer = 1.27 E06 cfu/mL

Table 2.19 – Efficacy of CuI or AgCuI Nanoparticles against *P. aeruginosa*

Time	Control	CuI (12 ppm Cu)	AgCuI (10 ppm Ag, 6 ppm Cu)
30 min	0.07 ± 0.05	0.33 ± 0.02	0.07 ± 0.02
1 hr	0.06 ± 0.03	0.33 ± 0.03	0.08 ± 0.04
2 hr	0.13 ± 0.01	0.42 ± 0.02	0.20 ± 0.02

Original Titer = 1.27 E06 cfu/mL

Table 2.20 through Table 2.22 reinforce the belief that CuI is a more potent antimicrobial agent than various silver compounds against *S. aureus*. Table 2.20 demonstrates that at a reasonably cost equivalent basis (see Section 2.1.5), CuI is much more effective than silver. Table 2.21 and Table 2.22 show that mixtures of Ag and CuI as mixtures of separate nanoparticles or mixed-metal halide nanoparticles, respectively against *S. aureus*; CuI alone is still the preferred material.

Table 2.20 – Efficacy of Ag^0, AgBr, or CuI Nanoparticles against *S. aureus*

Time	Control	Ag^0 (10 ppm Ag)	AgBr (10 ppm Ag)	Ag^0 + AgBr (10 ppm Ag from each)	CuI (59.07 ppm Cu)
2 hr	0.05 ± 0.00	0.08 ± 0.02	0.46 ± 0.43	0.02 ± 0.00	>4.44 ± 0.00
6 hr	0.10 ± 0.07	0.22 ± 0.02	0.39 ± 0.02	0.22 ± 0.03	>4.44 ± 0.00
24 hr	0.26 ± 0.07	4.29 ± 0.21	>4.44 ± 0.00	>4.44 ± 0.00	>4.44 ± 0.00

Original Titer = 1.27 E06 cfu/mL

Table 2.21 – Mixtures of Ag^0 or AgBr and CuI Nanoparticles vs. *S. aureus*

Time	Control	CuI (12 ppm Cu)	CuI + Ag^0 (12 ppm Cu, 2 ppm Ag)	CuI+AgBr (12 ppm Cu, 2 ppm Ag)
2	0.11 ± 0.10	1.81 ± 0.00	1.66 ± 0.01	1.71 ± 0.04
6	0.16 ± 0.06	2.96 ± 0.00	3.16 ± 0.02	3.03 ± 0.21
24	0.59 ± 0.18	>4.34 ± 0.00	>4.34 ± 0.00	>4.34 ± 0.00

Original Titer = 1.06 E06 cfu/mL

Table 2.22 – Efficacy of CuI or AgCuI Nanoparticles against *S. aureus*

Time	Control	CuI (12 ppm Cu)	AgCuI (10 ppm Ag, 6 ppm Cu)
0.5	0.07 ± 0.05	0.95 ± 0.02	0.04 ± 0.07
1	0.06 ± 0.03	1.35 ± 0.01	0.06 ± 0.07
2	0.13 ± 0.01	1.81 ± 0.00	0.09 ± 0.01

Original Titer = 1.06 E06 cfu/mL

2.4.4. Discussion

These results demonstrate that polyvinylpyrrolidone modified CuI nanoparticles are superior to polyvinylpyrrolidone modified nanoparticles of silver or silver bromide. Based on these results along with the findings of sections 2.1-2.3 it was decided that CuI was a preferred antimicrobial material and particularly that surface modified small particles of CuI would be a preferred material for incorporation into various matrices for AM activity. From a materials viewpoint, however, these materials were unsatisfactory because of their high cost to manufacture, low scalability, and negative effects on materials to which they were incorporated (e.g. swelling). As such, further efforts were dedicated to the development of more practical preparations and formulations of CuI-based small particles, which is the subject of the next chapter.

2.5. Chapter 2 Conclusions

2.5.1. Conclusions

In light of these findings it was decided to develop a process for producing improved small particle CuI formulations as an AM additive. To this end several problems with the materials described in this chapter needed to be addressed. In particular, an improved method of production and an improved formulation that would not result in dramatic water uptake was needed. Furthermore, it was realized that these materials needed to be prepared with different surface chemistries to facilitate their incorporation into many various materials. These challenges are addressed in next chapters, Chapter 3 and Chapter 4.

2.6. Literature Cited in Chapter 2

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Chapter 3. CuI Small Particle Production Through Wet Media Milling

The previous chapter described the selection of CuI, and more specifically of small particles of CuI as a preferred antimicrobial agent. These particles, however, were of low practical value because of their large manufacturing footprint, solvent demand, and negative impact on materials to which they were added. The negative impact as an additive was caused by a high amount of surface modifier required in relation to the active material.

This chapter is dedicated to the development of a process to produce useful CuI small particles through the use of a wet media milling comminution process. The process developed has a small footprint, high efficiency, and is readily scalable using commercially available equipment with no waste stream. Using this process, highly antimicrobial and practical CuI particulate formulations were produced such that they could be tailored for incorporation in different matrices. Chapters 4 and 5 will present results on the tailoring of these particles for different materials and their application in various systems, respectively. This chapter focuses on the production process itself.

First, the basics of comminution processing and small particle stabilization are reviewed. Second, a series of preliminary experiments confirming the usefulness of wet media milled CuI small particles, and the selection of stabilizing agents for this process are presented. Third, a series of experiments designed to optimize the production of these small particles is presented.

While the focus of this chapter is on producing small particles of CuI for AM applications, many of its teachings are applicable to the development of improved small particle production of other materials for other applications.

3.1. Motivation and Wet Media Milling

3.1.1. Motivation

The CuI particles demonstrated in Chapter 2, although very effective, were not exceptionally useful as materials additives because they tended to affect negatively the properties of materials to which they were added. Most commonly, increased swelling of the finished material was observed when useful amounts of CuI were added using the particles described in Chapter 2. Thus, although these particles were very effective, they were not useful as a materials additive.

Several other methods of production of CuI small particles were investigated. These mainly included alternative chemical syntheses of CuI. All of the wet chemical methods explored, however, were found to require large amounts of stabilizing polymer in order to form powders that could be redispersed without aggregation and without the use of specialized dispersing equipment. Typically, these synthesis methods also required large amounts of solvents and produced very dilute suspensions of materials. Thus, not only did these production methods yield disappointing products, but also their scalability and production efficiencies were very poor.

In addition to technical requirements, regulatory considerations were taken in to account as a further guide to the development of a production method. As outlined in Chapter 1, the regulations of antimicrobials in the US falls under the Federal Insecticide,

Fungicide, and Rodenticide Act (FIFRA) and the Pesticide Registration Improvement Acts (PRIA) of 2004, 2007, and 2012. In short, the chemical identity of the active ingredient must be very well characterized, particularly with regard to byproducts and contaminants. Thus, the chemical preparation of CuI particles (as in Chapter 2) was not a viable option because each separate formulation would require a separate characterization. Since it was expected that various CuI formulations would be required for different materials matrices, this presented a dramatic limitation. For instance, formulations useful in aqueous coating systems were not expected to be useful in polyolefin based thermoplastics or solvent-borne coatings.

This required the development of a process using well-characterized, vendor-supplied CuI, which could be used to produce different small particle formulations for different matrices in a modular fashion. To this end, a bottom-down, comminution process was developed using high-energy-ball-milling, also known as wet media milling.

The use of commercial CuI in a comminution process allows for small particles to be prepared while still using a chemically well-defined active ingredient. In this fashion, small particles of CuI were prepared with controlled particles sizes and controlled surface chemistries such that the resultant particles could be readily dispersed in many various materials matrices to make them antimicrobial.

3.1.2. Comminution

Comminution is carried out in a variety of industries in order to meet different requirements. In mining, for instance, ore may be comminuted to 10-100μ in order to liberate valuable mineral from gangue. Recently, the pharmaceutical industry has focused on the comminution of water insoluble drugs to nanoparticle sizes to improve their

bioavailability in the body[1]. Still other industries, such as technical ceramics, commonly comminute materials to the 0.1-1μ size range to improve the their performance. While the feed materials and produced particles vary over several orders of magnitude between these different industries, the fundamental goal is the same: to produce smaller particles from a larger feed.

The differences in the technical requirements of produced particles and the economic impact of processing in these various industries has resulted in different comminution goals within each industry. In some industries, the cost of comminution may be trivial while in other it may represent the dominant processing cost. A review of the literature on comminution in these respective industries demonstrates the very marked differences in the goals and research focus of different groups, and thus the teachings of these industries also differ widely.

In minerals processing, comminution often accounts for more than 50% of the total operating costs; thus the mining industry has made significant contributions towards the development efficient and continuous processing schemes to handle very large quantities of ore. In minerals processing, however, great lengths are often taken to avoid the production of small particles below tens or hundred of microns (fines) because they negatively impact the recovery of valuable minerals during floatation processes.

Comminution in some pharmaceutical production, on the other hand may operate on a much smaller scale. Furthermore, the cost of comminuting a drug is trivial compared to the other costs associated with drug development. The industry has made significant contributions towards the production of stable and well-dispersed particulate matter in the range of nanometers to microns; but minimal literature exists on the development on

efficient and optimized processing for these materials. In fact, much of the literature commonly reports high-energy input comminution is carried out for times on the order of 24 hours for very small amounts of material.

Although several industries are heavily involved in the comminution of inorganic small particles, minimal literature exists on the development of optimized grinding methodology for the production of dryable and redispersible stabilized small particles on the order of 10-200 nm.

Perhaps the most comparable industry to that of AM additives with respect to comminution processing is that of inks and pigments. It was in fact the need for better methods of grinding and dispersing of pigments that gave way to today's high-speed wet media mills. Pigments are considered crude even in their chemically pure form until various grinding processes are applied to produce small particles. Until they are comminuted they often do not have the correct color or strength.

Commonly these tinctorially crude pigments are comminuted from particle sizes in the range of 10s of microns to finished sizes of 10s or 100s of nm^2. The finished pigments may then be concentrated as a liquid, press cake, or fully dried. Redispersion of these concentrated pigments in the final product is of critical to achieving proper color strength, and another stage of wet media milling or high shear mixing is typically used to wet, deagglomerate, and disperse the pigment particles. Thus, the formation of auto-redispersible material is largely not of importance during pigment comminution. Nevertheless, many of the requirements of pigment production are the same for the production of antimicrobial additives.

In this development of a CuI AM additive, it was decided that the finished material needed to be auto-redispersible when added as a dry powder to any desired matrix since many processing systems may not be amendable to additional processing steps required to disperse the particles as is commonly is done for the redispersion of pigments. A methodology for optimized production of dryable and auto-redispersible small particles with controlled sizes through grinding appears to be lacking in the literature. The production of an auto-redispersible small particle formulation is not as straightforward as it may appear and is dependent on both the specific formulation and grinding parameters.

3.1.2.1.Crushing and Grinding

Comminution is typically separated into two stages, crushing and grinding. Crushing shatters large particles through the impact of rigid surfaces[3] to produce several moderately sized fragments. Grinding occurs through many various chipping, impact, abrasion, and fracture events, resulting in the formation of much smaller particles compared to crushing. Grinding, however, consumes significantly more energy and is a much slower and stochastic process compared with crushing. In a mineral processing operations, ore is first processed through crushers and then processed through grinders (i.e. ball/rod mills) until the desired size is achieved. These different energy requirements and stages of comminution can be understood through consideration of some basic principles of the fracture of materials.

In 1921, A.A. Griffith published his now-famous work describing why glass fibers tend to fracture well below the strength that would be predicted considering the

strength of atomic bonds. The stress required to fracture glass is often several orders of magnitude less than the theoretical stress required to break atomic bonds in the material. This was reconciled by the consideration of flaws in the fiber.

Griffith postulated that fracture would occur when the decrease in elastic strain energy (of the applied stress field) balanced the increase in surface energy of the new surface area produced in fracture. For brittle material, this led to the expression:

Equation 3.1 – Fracture stress for brittle materials

$$\sigma_f = \sqrt{\frac{2E\gamma}{\pi a}}$$

a = flaw length, E = Youngs modulus, σ_f = fracture stress,
γ = surface energy

For ductile materials, however, Griffith's theory is modified to also account for the development of a plastic zone at the crack tip as well as other modes of energy dissipation. Physically, the loading and unloading of a plastic region results in the dissipation of heat energy and an overall increase in the energy required to cause fracture. Largely motivated to apply Griffith's theory to describing fracture of steel, Irwin consolidated the surface energy with additional dissipative energy (i.e. plastic and heat).

Equation 3.2 – Fracture stress for ductile materials

$$\sigma_f = \sqrt{\frac{2EG}{\pi a}}$$

a = flaw length, E = Youngs modulus, σ = fracture stress
G = surface energy + dissipative energy

The transition from crushing to grinding can be thought of as the transition between comminution of flawed brittle material to less flawed more ductile material. In practice, however, crushing and grinding operations are defined by the energy required to achieve some reduction ratio, the product size relative to their feed size.

3.1.2.2.Energy Considerations

The differences between crushing and grinding are further understood by considering the energy input required for size reduction. Several "grinding laws" are commonly used to describe the energy required to reduce a feed particle to some product size. These laws, although originating from coal and mineral processing, have been successfully applied across industries.

In 1857 Rittinger proposed that the energy required to comminute a particle was proportional to the new surface area created – typically resulting in an overestimate.

In 1883 Kick proposed that the energy required to comminute a particle was directly proportional to the size reduction (i.e. independent of feed size) – typically resulting in an underestimate.

In 1952 Bond suggested that the energy required was proportional to the new crack length created. Bond's law has been used most in practice and in particular for minerals processing applications during grinding.

These three proposed laws appear to be in direct conflict; however, they are three different solutions to the same integrated grinding rate equation,

Equation 3.3 – Integrated grinding rate equation

$$E_m = \frac{C}{n-1}\left(\frac{1}{X_P^{n-1}} - \frac{1}{X_F^{n-1}}\right)$$

E_m = Specific Energy Requirement (J/kg)
X_P = Product Size (m)
X_F = Feed Size (m)
C = Constant
n = Constant

When n=1, Kick's law applies. When n=1.5, Bond's law applies and when n=2, Rittinger's Law applies. Furthermore, it is often noted that as particle feed size decreases, the energy required to achieve reduction becomes increasingly dependent on the size of the feed. Accordingly, the observed grinding law is expected to shift from Kick's to Bond's to Rittinger's as feed size decreases. This phenomenological occurrence is easily rationalized by consideration of Griffth's theory; since smaller particles will tend to have fewer flaws. Thus, as the size of the material is reduced, the theoretical strength of the material is approached and comminution becomes increasingly energy intensive.

Physically, in the early stages of grinding the progeny particles are sufficiently flawed that they are as easily fractured as their parent particles. As grinding progresses, however, the flaws in the progeny fragments have fewer and fewer flaws, and more and more energy is required to cause breakage.

The actual energy required for a comminution operation, however, is much greater than the energy required for material fracture. Grinding is necessarily an energy intensive process. A large portion of input energy is lost to frictional heat generation and fluid drag in the mill itself. As grinding is essentially a random process, a number of

particles undergoing impact receive insufficient force to cause fracture and a number receive excess force, which for smaller particles is increasingly released at heat. Furthermore, a significant amount of energy is used to transport material through the mill and to prevent over-heating through cooling.

Typically, only a small fraction (4-8%) of the energy supplied is actually used to comminute the material[4]. This energy expenditure, however, can be reduced through optimization of operating conditions and the use of chemical additives or grinding aids[5].

Grinding aids are added during comminution to reduce the surface energy and soften the comminuted materials, making comminution easer. This is known as the Rehbinder effect[6]. To this end, grinding in water tends to reduce the energy input required for comminution by approximately 30% compared to dry grinding for many materials. Other grinding aids, that reduce the tendency for agglomeration and thus reduce the energy expenditure on deagglomeration of the product during milling, as well as other aids are used.

The use of classification equipment is especially useful for reducing energy use and increasing throughput. The output material from the mill can be continually classified and separated such that the undersized material is collected as the product and the oversized material can be recirculated through the mill again. This can be achieved through screening (for large particles) or various wet classification systems (e.g. hydrocyclones)[7]. In this fashion, additional energy is not wasted on particles that have already been appropriately comminuted and only appropriately sized material goes on to the next processing step. In the case of suspended small particles, however, useful separation and classification equipment is less widely available and is complicated by the

use of dispersing agents since many classification systems depend on differential settling velocities to achieve separation. Some hydrocyclones may be effective in separating microns from even nanometer-sized materials, enabling this process.

Additionally, appropriate selection of grinder type, milling media, and operating conditions dramatically impacts the energy required to achieve a given size reduction. A crusher designed to comminute raw ore down to inch-sized pieces will not effectively comminute inch-sized pieces down to micron size particles. Similarly, a grinder optimized to produce micron-sized particles will not be effective in preparing nanometer-sized particles. Largely, the differences between the uses of different equipment can be considered on basis of how and the rate at which energy is supplied to the material.

Single particle fracture experiments have demonstrated several important characteristics of brittle fracture, which directly relate to the selection of processing equipment. Importantly, energy efficiency (defined as energy needed to produce new surface) is maximized under slow compressive loading compared to high-energy ballistic loading[8]. Furthermore, in the case of slow compressive loading, energy utilization is further maximized as the rate of energy input is reduced. A lower limit on the rate of energy input exists, however, below which Griffith's criterion is not satisfied and fracture will not occur.

These single particle fracture experiments have traditionally been limited to micron-sized particles due to the inherent difficulty in both controlling and measuring individual sub-micron particles. Recently, however, transmission electron microscopy coupled with nanoindentation methods have been used to observe the fracture of individual nanoparticles during compression. In studies by Nowak and Mook, e.g., it was

reported that the fracture toughness of silicon nanoparticles began increasing dramatically at sizes below 110 nm[9] [10]. The energy release rates upon fracture were also increased and dependent upon the loading history of the particles, implying that defects are more readily decreased as particle size decreases compared to larger particles. An important caveat of this is that energy that is accumulated as defects is more rapidly dissipated upon fracture in nanoparticles compared to larger materials; where this defect energy may contribute to the subsequent fracture of a progeny particle.

Similar in scope to the results of Mook and Nowak, a study by Volkert[11] found that the reduction in diameter of single gold columns ranging from 8μ to 180nm resulted in dramatic increases in the yield stress and strain hardening rate.

While single-particle studies are important for improving our fundamental understanding of nanoparticles, in practice attention must be paid to the systems of interest holistically. Here the system of focus is a wet media mill used to produce nanoparticles of cuprous iodide.

3.1.3. Wet Media Milling

Typical tumbling ball mills operate by rotating a drum or canister filled with grinding media. As this drum rotates, the media is moved upwards and then cascades down providing impact energy for the material to be comminuted[12]. Rotating the drum faster increases the energy input rate as the balls are lifted to higher positions, however, this energy input rate is limited by the critical speed at which centrifuging occurs and the grinding media becomes stuck to the sides of the mill. When a ball mill is operated at lower energy inputs (e.g. lower speed), cascading occurs more often, and more attrition

type events occur, which results in larger number of fines being generated albeit slowly. Thus the production of very fine materials in these mills requires very long grinding times.

In many comminution operations, however, the production of fines is intentionally avoided, and ball mills are operated at high speeds where production of fines is reduced and high throughput can be achieved. For the high throughput production and dispersal another solution is needed.

The solution to this problem is credited to Szegvari[13], who in 1922 used a paint can, pebbles, and a drill press to create the first stirred media mill. Szegvari needed a fine dispersion of sulfur to be used in the vulcanization of a new latex process for making rubber[14]. Using his creation fine sulfur particles were produced in minutes, which would have otherwise taken days to prepare with a traditional ball mill. Today this machine would be called an attritor (or low-speed stirred media mill) because of its relatively low speed.

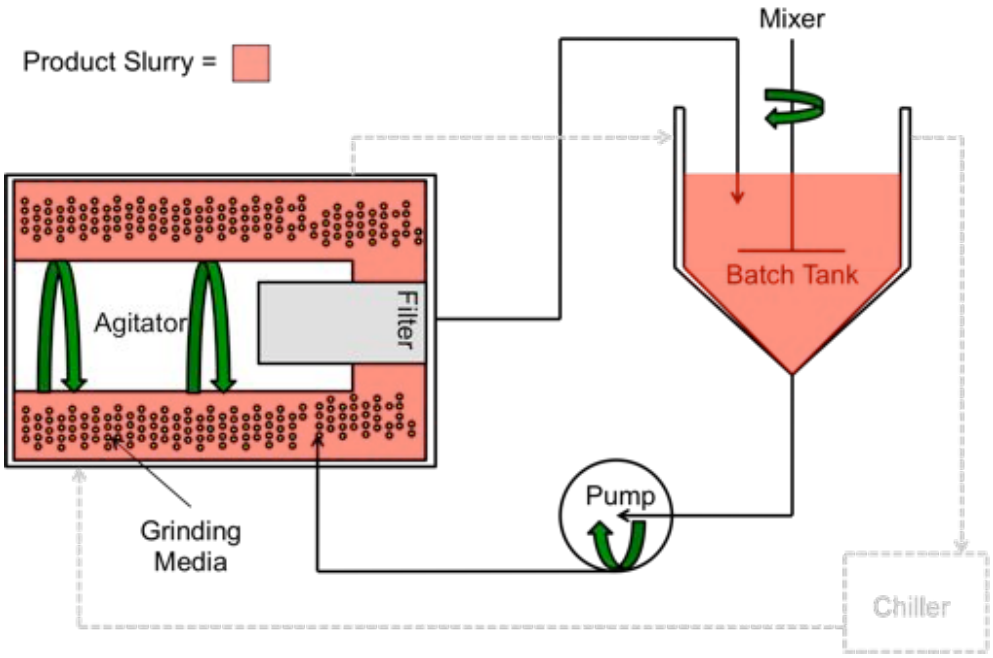
In 1948, DuPont implemented high-speed media mills for preparing paint pigments by applying high-speed agitation to an open, sand filled, vertical, grinding chamber. The applied speed, however, was limited by vortex formation, which resulted in the inclusion of air and emulsification. Thus, the grinding chamber was reconstructed as a closed chamber in order to allow high-speed agitation to be applied. Material to be comminuted was pumped up from the bottom and removed from the top using a screen to prevent media from escaping with the product.

Since DuPont's sand mill, many different high-speed stirred media mills have been produced. They all share the same basic features of an agitated media filled grinding

chamber and an outlet with some means of preventing media escape. Otherwise, however, they vary in almost every aspect. The grinding chamber and agitator geometry and construction may be varied. The grinding media size and composition as well as operating conditions of these mills may also vary widely.

Traditionally, vertical disk media mills have been used in the preparation of fine particle sized pigments. Recently however, horizontal media mills have been shown to be more efficient[15]. A schematic of a horizontal wet media mill is shown in Figure 3.1.

Figure 3.1 – Horizontal Wet-Media Mill Schematic



3.1.3.1.Grinding Media

Media size, cost, and physicochemical characteristics especially density, hardness, and reactivity play significant roles in the selection of grinding media. The literature provides general guidance on media selection, but media must be evaluated holistically in the grinding application; there is no definitive answer to the question of which media to use.

Typical guidance from literature regarding size is that media should be around 100-1000 times the desired product size[16] and around 10 times the feed size[17]. Thus, for preparing particles in the sub-micron size domain, media in the range of 10s to 100s of microns is appropriate.

Zirconia based grinding media are the most common grinding media and are available in sizes of 10s-100s of microns. Zirconia itself, however, is not used alone; it is stabilized with yttria or magnesia to improve fracture toughness. Yttria stabilized zirconia (YZT) is probably the most commonly referenced in the literature because it has the best wear resistance and highest grinding efficiency. Various zirconia silicates, alumina based, and other media are available; however, all have less wear resistance than YZT. A lanthium based glass ceramic material produced by 3M (ZGC, 3M Advanced Materials Division [experimental product]) is another grinding media with comparable properties to YZT.

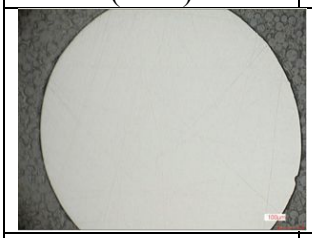
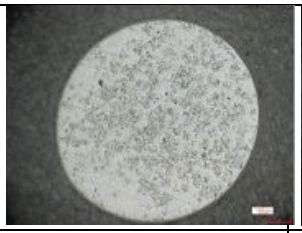
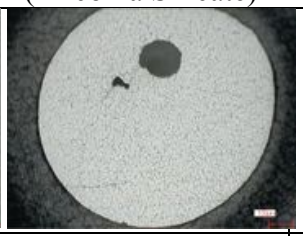
Hard and dense media will impact the particles with more energy and hence improve grind efficiency. Care must be taken, however, that the mill interior is not damaged. Tungsten carbide media are available, but are considered exotic media and are used far less than the zirconias. Other, non-ceramic, small grinding medias, such as metals and plastics[18], are also produced and used for various applications (e.g. microbial cell disruption), but comminution processing of inorganic material typically requires hard, dense, ceramics. Furthermore, contamination issues arise with the use of metal medias.

Media quality is an additional factor that must be considered. Ceramic grinding media are typically produced by two methods, the sintering process or fusion process.

Sintering involves the forming shaped media followed by densification at high temperature. The fusion process involves dropping molten material through a cooled gas atmosphere, during which time the surface tension shapes the materials in to a spherical form as it solidifies or into a book mold in which it solidifies.

During the fusion process the material may phase-separate, air can become entrapped inside the media, or particles may stick together forming irregular shapes. These flaws result in a weak media that can contaminate the grind and result in inefficient grinding as energy is consumed in fracturing the media rather than grinding the product. Thus, sintered media are preferred because their more homogenous, dense structure results in stronger media with better wear resistance.

Figure 3.2 – Good and poor quality grinding media [Adapted from Weber et al.[19]]

Sintered Process (YZT)	Fusion Process (Zirconia Silicate)		
			
Dense, uniform	Phase Separation	Air Entrapment	Irregular shape

Although high quality media can last for many production runs, it represents a high cost of operations and a large capital expenditure. For instance, a laboratory scale wet media mill with a 1 L grinding chamber 80% filled with media would require approximately 3 kg of YZT media. As a rule of thumb, for every half reduction in size beyond around 300µ, the cost of media tends to double. According to current costs, to fill the grinding chamber would cost $1000 using 300µ YZT media or nearly $10,000 using

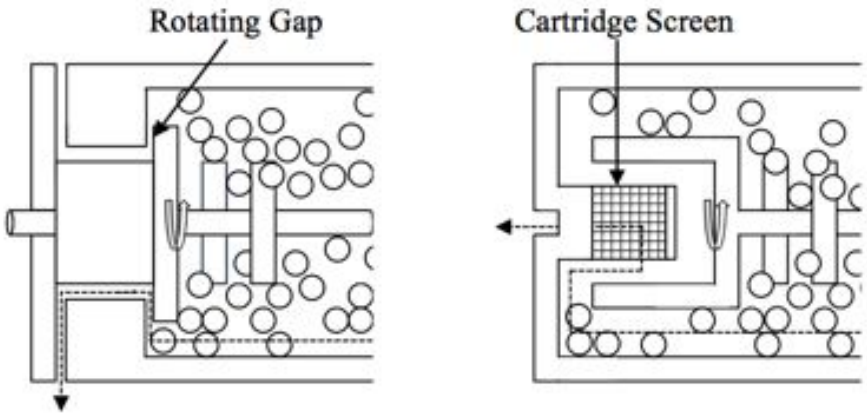
30μ YZT media. It is clear that scale up cost becomes problematic as the cost of grinding media can rival or dominate the cost of the entire milling operation.

3.1.3.2.Media Separation

The media must be retained in the grinding chamber during grinding and during product discharge. Separation of the media at the outlet of the grinding chamber is achieved using two primary methods, rotating gap separators or screens. Rotating gap separator systems are useful because their dynamic nature helps prevent clogging and they can be produced from low contaminant materials (e.g. ceramics). When smaller media are used, however, the tolerances in manufacturing and wear during usage become problematic and make rotating gaps ineffective separators. Additionally, as grinders are scaled up in size, the surface area available for use as a gap separator does not scale proportionally and the increased flow rates result in hydraulic packing of media near the outlet, which can result in inefficient grinding and damage to the mill[20]. Static screens are seldom used because of their tendency to clog and become damaged and also suffer the same area-scale-up limitations as rotating gaps.

An improved method of media separation is through the use of cartridge screens. These are dynamic screening elements that have been implemented by using a cartridge screen that fit within slotted agitator. As the agitator turns, centrifugal forces throw grinding media back and reduce media contact with the screen. This prevents hydraulic packing and wear. Further, the cartridge design also increases the available surface area for separation, making scale-up easier[21].

Figure 3.3 – Left, Rotating Gap Separator & Right, Cartridge Screen Separator



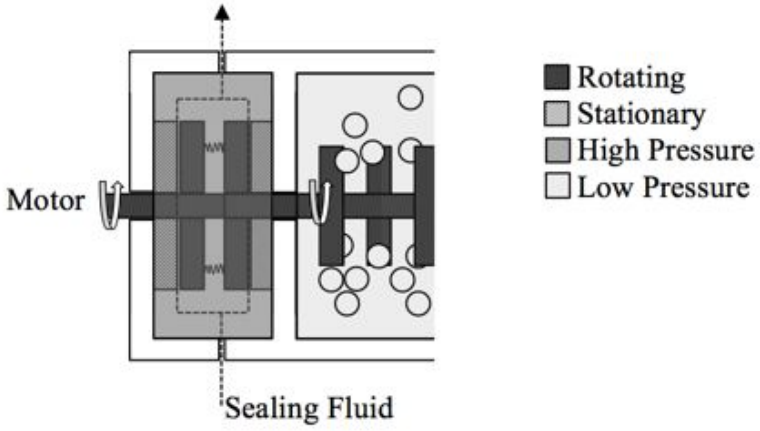
As media size decreases and as flow rate through the mill increases, the media are more easily swept towards the separator and care must be taken to avoid hydraulic packing and damage through the screen. Even if hydraulic packing is entirely prevented, the screen will still slowly be damaged through normal wear and tear. Typically, any separator should allow particles only less than half the size of the media to pass through to allow for minor tolerance and wear issues. Screens are typically stainless steel of wire mesh or laser cut construction.

3.1.3.3.Mechanical Sealing of the Agitator Shaft

Under-recognized and very important components of modern wet media mills are the sealing systems that contain the media and product fluids. Mechanical seals are used to separate the grinding chamber from the motor that turns the agitator. These mechanical seals are leakage control devices and require some leakage in order to maintain lubrication during operation. Leakage will always occur to some degree; thus it is important to think of these seals as dynamic components and not as static enclosures.

In its simplest form, a mechanical seal consists of one rotating seal face attached to the axle and one stationary seal face attached to a stationary casing. These seal faces are pressed together to minimize fluid leakage as the axle turns. A small amount of fluid, however, must leak through to lubricate the seal faces and prevent seizing as the rotating face spins at the mill speed against the stationary face. A sealing fluid can be applied to prevent the product from contaminating and damaging the seal faces. This sealing fluid is applied at a higher pressure than the grinding chamber such that the sealing fluid is pushed into the product. This prevents cross contamination between products and protects the seal faces from being damaged by product particles.

Figure 3.4 – Schematic of Double Mechanical Seal



Thus the product is always contaminated with sealing fluid and care must be taken to ensure this fluid is compatible with the product slurry; and the internal sealing fluid handling systems, although not in direct contact with the product slurry, must be well cleaned. Similarly, positive pressure of the sealing fluid is usually maintained using a compressed air source, thus the air must assuredly clean and oil free to prevent contamination.

In wet media mills, both seal faces are constructed of ceramic (e.g. silicon carbide) to prevent wear from stray debris that may enter the seal since a flat seal surface is critical to minimize fluid leakage into the product. Despite their construction, the seals may require periodic resurfacing as wear occurs. The degree of leakage will increase as the mill is used due to wear of the seal faces.

3.1.3.4.Mill Operation Modes and Flow Rate Effects

Mill operation is fairy simple and in operation is similar to other attritors or tumbling ball mills, the ingredients are loaded, the correct settings are entered, and the mill is engaged. Several choices exist in operating settings, however.

A wet media mill can be operated in batch, recirculation, or continuous modes. In batch mode, the mill is charged and the material is not removed until completion. Recirculation is itself a batch process but the material continuously passed through the mill to an intermediate batch tank. In this fashion, large batch sizes can be prepared without being limited by the size of the grinding chamber. Continuous mode has the advantage of the highest throughput, but least control over processing since several passes are typically required for all particles to experience media-particle contact, let alone desired amount of comminution.

In recirculation mode, high flow rates are preferred in order to approach plug flow and increase uniformity of the grind; however, high flow rates also increase the likelihood of hydraulic packing of the grinding media and associated wear and clogging issues. Continuous mode is limited in flow rate according to the desired residence time; faster flow-through will result in less residence time and less comminution occurring.

3.1.3.5.Mill Speed / Rate of Energy Input / Tip Speed

The speed of the agitator in RPM, or mill speed, determines the rate of energy input to the mill. The actual energy supplied can be determined by first operating the mill without a load at various speeds to determine the frictional/bearing losses, which can be subtracted from the energy input during actual operation to estimate the energy input. The tip speed of the agitator can be directly determined based on the mill speed and agitator geometry. The rate of energy input as well as tip speed determines the rate of comminution.

Generally, higher energy inputs produce higher rates of comminution. In some cases, however, the high tips speeds concurrent with high-energy inputs and resulting the high impact collisions between grinding media and product result in aggregation and compaction of product rather than comminution and dispersion. The use of smaller media allows for high-energy inputs and fewer high impact collisions compared to larger media. It is important to note that tip speeds are system dependent, and equivalent energy input rates in different mills may yield different products. The improved performance using low tip speeds or small grinding media has been termed “mild dispersion”.

High mill speeds result in rapid heat generation. This heat may be useful in reducing slurry viscosity or may detrimentally degrade the product (especially organic pigments and pharmaceuticals). Although the grinding chamber is usually jacketed and cooled to maintain a certain temperature, scale-up is again an issue as the surface area for cooling does not scale proportionally with volume.

Various systems exist which incorporate internal sparging of liquid nitrogen or grinding in liquid nitrogen directly to overcome thermal issues. These systems are especially suited for grinding materials that are non-friable at room temperature (e.g. cryogenic grinding of plastics). Thus temperature may be controlled by other methods besides the jacketing systems; however, for routine scale-up considerations, the chiller capacity and available surface area should be considered as limiting parameters.

3.1.3.6.Milling Time / Total Energy Input

The total amount of energy applied is the integral of energy input rate over the time of grinding. Energy inputs are typically normalized with the mass of material processed. Equivalent total energy inputs, however, do not result in equivalent products, but are dependent of the rate of energy input, as described above and other aspects of the grind. For instance, 4 hours of 1000 kJ/ton/hour grinding is not equivalent to 2 hours of 2000 kJ/ton/hour grinding. Nor is 4 hours of 1000 kJ/ton/hour with 1 μ media equivalent to 4 hours of 1000 kJ/ton/hour with 1 μ in a different mill or different media type. Thus, care must be taken in directly comparing energy inputs across different systems since many other factors play important roles.

Besides total energy input, the chemical stability of the feed may dictate that time in the mill should minimized. Additionally, other mill components, such as pumps, seals, and gaskets also have a finite lifespan. Thus it is further beneficial to achieve short grinding times. The longer time spent in the mill, the greater the degree of contamination due to the leakage of mechanical seal fluid that will inevitably occur.

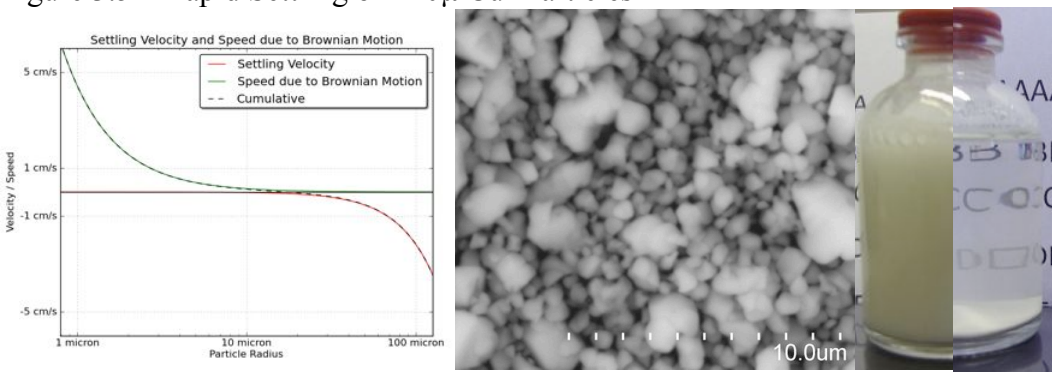
3.1.4. Small Particle Stabilization

As material is comminuted smaller and smaller, stability considerations become increasingly important. Under a given set of grinding conditions, an ultimate particle size will eventually be reached at some grinding limit. This ultimate particle size, or grinding limit, depends on the material being ground, grinding conditions, and stability of the resultant materials. The grinding limit is the point of dynamic equilibrium between aggregation and deaggregation where the mill is incapable supplying energy to fracture small particles any further.

A lack of primary particle stabilization may result in of energy being wasted on continuous disaggregation rather than comminution. Thus, in order for comminution to be achieved in the range of 10s to 100s of nm, the particles must be stabilized throughout grinding. A very brief description of particle stabilization is given here.

Small particles in a fluid are constantly moving due to Brownian motion and one might expect that particles having velocities due to Brownian motion greater than their settling velocities will remain stable in suspension. Using CuI, one finds that particles less than several microns in radius will have random speeds due to Brownian motion of at least six times than their settling velocities. Based on this, one might falsely predict that these particles should produce a stable suspension. The failure of this viewpoint is readily evidenced by the rapid instability of micron sized CuI particles, which readily settle in water as shown in Figure 3.5.

Figure 3.5 – Rapid Settling of <10μ CuI Particles



As these particles move, they experience collisions with one another. During these collisions, however, not all particle bounce apart, but many stick together and form agglomerates, which have settling velocities greater than Brownian motion and settle out of suspension. Accordingly, very small particles will form large agglomerates and fall out of suspension. Thus, these particles must be stabilized from such interaction. Stabilization can be achieved through electrostatic and/or steric stabilization techniques.

Electrostatic stabilization is accomplished by application of a charge on the surface of the particles such that repulsion between particles prevents particle agglomeration. Electrostatic stabilization, however, is highly dependent on the solution properties and is often ineffective at high concentrations or in organic solvents. At high concentrations, the electric double later, which acts to effect repulsion between the particles may be sufficiently compressed to allow Van der Waals forces to dominate and for aggregation to occur.

Steric stabilization is accomplished by the attachment of a molecule or a polymer to the particle surface such that approaching particles are prevented from colliding with one another. As particle approach each other, entropic repulsion of the attached polymers occurs preventing aggregation. Alternatively, the particles may be directly prevented

from aggregating by their containment in a high viscosity medium such that particle approach is physically prevented. In a liquid this may also be accomplished by using a high concentration of vesicles such that the particles are immobilized in locally high viscosity regions between the vesicles but globally the fluid remains low viscosity (e.g. vesicle stabilized TiO2 pigment[22]). Although seemingly similar, this technique is fundamentally different from steric stabilization in that if the particles happen to approach each other they will aggregate.

Any and all of these techniques can be combined. For instance, electrosteric stabilization refers to the combination of both electrostatic and steric techniques. Steric stabilization is focused on as a preferred method of stabilizing the particles such that large molecular or polymeric agents could be tailored to improve the compatibility of the produced particles with difference matrices.

3.1.5. Brief Description of Grinding Parameters

In the foregoing sections, practical descriptions of the relevant parameters of several aspects of a wet media mill have been given. This section provides a relevant framework for the treatment of these parameters. This framework has been largely adapted from the work of Kwade and Schwedes[23]. Most of the following description is applicable to grinding in general, but some aspects are only applicable to the particular operations carried out in this work (true comminution by horizontal media milling of brittle material).

3.1.5.1.Production Capacity and Optimum Values

Besides the possibility of producing improved CuI particle formulations with fewer regulatory hindrances, comminution processing was attractive because of its high production capacity and scalability. The production capacity can be defined as the mass of product capable of being produced in a given amount of time or with a given amount of energy.

If product quality is defined as a characteristic of the particle size (e.g. D_{50}, specific surface area), then the general integrated grinding law of presented in 3.1.2.2 can be applied,

Equation 3.4 – Integrated grinding rate law

$$E_m = \frac{C}{n-1}\left(\frac{1}{X_P^{n-1}} - \frac{1}{X_F^{n-1}}\right)$$

E_m = specific energy requirement (J/kg)
X_P = product size (m)
X_F = feed size (m)
C = constant
n = constant

In the case of the production of very small particles, however, where the feed size is much larger than the product size ($X_P << X_F$), the general solution to this equation can be reduced to a simple power function,

Equation 3.5 – Integrated grinding rate law with very large feed size

$$X_P = a(E_m)^b = c(t_{grind})^d$$

X_P = product size (m)
E_m = specific energy requirement (J/kg)
t_{grind} = time of grinding (min)
a-d = constants

This approach allows the determination of the specific energy required to reach some final average particle size, but it does it does not allow for the consideration of the relative impact of differently sized or density grinding media, filling ratio of grinding media in the grinding chamber, and many other wet-media-mill specific parameters. These parameters will of course affect how effectively milling can occur, thus it is useful to have a sense of the impact of these parameters.

3.1.5.2.Optimum Deagglomeration and Comminution

In order to describe the impact of different grinding conditions (mill speed, grinding media, etc.), several additional parameters are defined[24].

SN_{total} = Total stress, the total number of grinding media stress events
SN = Stress number, the number of stress events involving feed materials
$\overline{SE}$ = Stress energy, the energy supplied by each stress event

These parameters can be used to calculate the specific energy, E_m, required to achieve comminution, which can be given by,

Equation 3.6 – Specific energy relative to stress number

$$E_m = \frac{SN \cdot \overline{SE}}{m_p}$$

E_m = specific energy requirement (J/kg)
SN = stress number, the number of stress events involving feed materials
$\overline{SE}$ = average stress energy, average stress energy per stress event (J)
m_p = product mass

Stress Number (SN)

The total stress number (SN_{total}) describes the total number of stress events that occur throughout the grind from the perspective of the grinding media. Thus SN_{total} equals the product of the stress event frequency (SF) and grinding time (t_{grind}).

A more relevant value, however, is the total number of stress events from the perspective of the feed material. Physically, media-media contacts represent wasted energy and should not be counted as meaningful stress events. Thus, the normal stress number (SN) is given by the product of the probability of a feed particle being stressed and the ratio of feed particles to grinding media contacts.

Equation 3.7 – Stress number

$$SN = \frac{N_c \cdot P_S}{N_p}$$

N_c = number of grinding media contacts
P_s = probability a feed particle is sufficiently stressed by media contact
N_p = number of feed particles in grinding chamber

The number of media contacts, N_c, is assumed to be proportional to the mill speed and number of grinding media according to,

Equation 3.8 – Number of media contacts

$$N_C \propto n_{rev} \cdot t_{grind} \cdot N_{GM}$$

n_{rev} = agitator revolutions
t_{grind} = grinding time
N_{GM} = number of grinding media

The number of active feed particles, N_P, is proportional to the ratio of the total volume of feed particles ($V_{p,total}$) to the average feed particle volume(V_p).

Equation 3.9 – Active feed particles

$$N_p \propto \frac{V_{p,total}}{V_p}$$

N_P = Number of active feed particles
$V_{P,total}$ = Total volume of feed particles
V_P = Average feed particle volume

Furthermore, the number of active feed particles can be related to the grinding chamber, grinding media, and media fill according to,

Equation 3.10 – Active feed particles with respect to grinding media and chamber fill

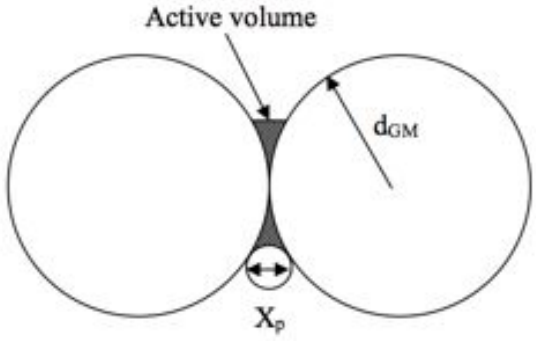
$$N_p \propto V_{GC} \frac{(1 - \varphi_{GM} \cdot (1 - \varepsilon)) \cdot c_v}{V_p}$$

N_P = Number of active feed particles
V_{GC} = volume of grinding chamber
φ_{GM} = grinding media fill ratio
ε = bulk porosity of grinding media (bulk media at rest)
c_v = solids volume concentration
V_P = average feed particle volume

The probability of a feed particle being sufficiently stressed by media contact (Ps) depends on whether deagglomeration or comminution is occurring. For deagglomeration, P_s depends of the surface area of the grinding media available for shearing to occur, and $P_s \propto d_{GM}^2$. For comminution, P_s is proportional to the active volume between grinding media, and $P_s \propto d_{GM}$.

The active volume is taken as the volume between grinding media, where the media-media distance is less than the feed size as shown in Figure 3.6.

Figure 3.6 – Active Volume Between Grinding Media



This difference between P_s for deagglomeration and comminution results in different equations for SN for each process. For comminution there is a greater dependence on the grinding media diameter than for deagglomeration processes.

Equation 3.11 – Stress number for deagglomeration

$$\mathrm{SN} \propto \frac{\varphi_{GM} \cdot (1-\varepsilon)}{(1-\varphi_{GM} \cdot (1-\varepsilon)) \cdot c_v} \cdot \frac{n_{rev} \cdot t_{grind}}{d_{GM}}$$

Equation 3.12 – Stress number for comminution

$$\mathrm{SN} \propto \frac{\varphi_{GM} \cdot (1-\varepsilon)}{(1-\varphi_{GM} \cdot (1-\varepsilon)) \cdot c_v} \cdot \frac{n_{rev} \cdot t_{grind}}{d_{GM}^2}$$

SN = stress number, the number of stress events involving feed materials
φ_{GM} = grinding media fill ratio
ε = bulk porosity of grinding media (bulk media at rest)
n_{rev} = agitator revolutions
t_{grind} = grinding time
d_{GM} = diameter of grinding media
c_v = solids volume concentration

If the solids concentration (c_v) and filling ratio (φ_{GM}) are kept constant, then the stress number becomes a function of only grinding time, tip speed, and media size. Thus, comparisons between different grinding media are easily facilitated by use of the reduced stress number (SN_r).

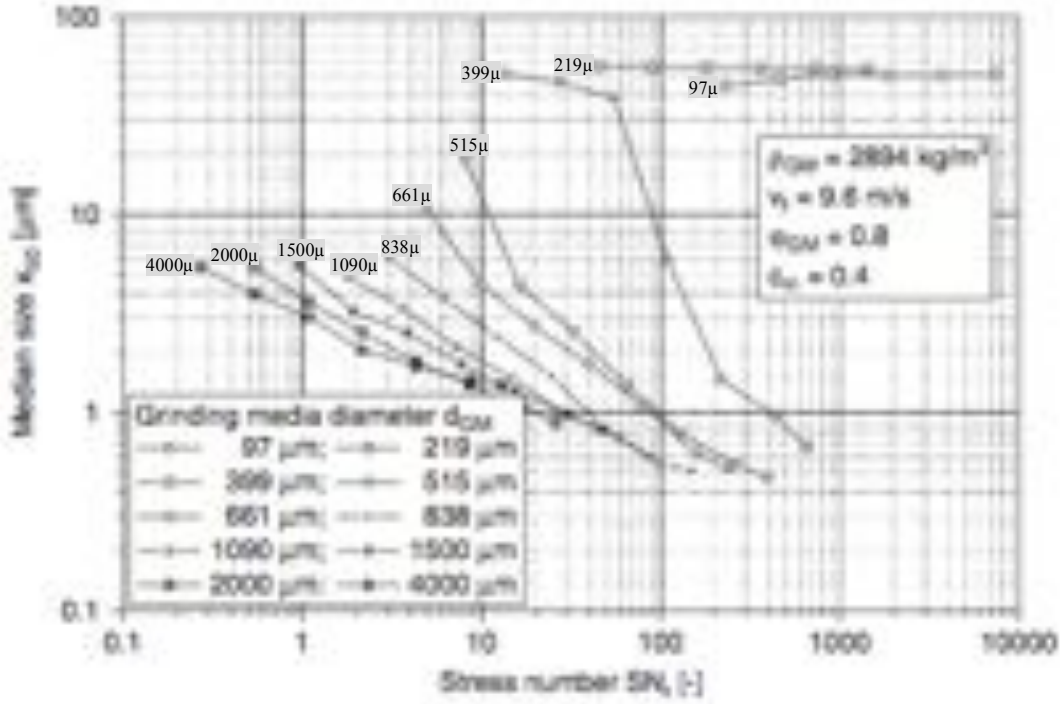
Equation 3.13 – Reduced stress number for comminution

$$\mathrm{SN} \propto \mathrm{SN}_R \propto n_{rev} \cdot t_{grind} \cdot \left(\frac{X_P}{d_{GM}}\right)^2$$

SN = stress number, the number of stress events involving feed materials
SN_R = reduced SN, useful if solid concentration & media fill are constant
n_{rev} = agitator revolutions
X_P = product size (m)
d_{GM} = size of grinding media

A comparison of different media sizes at equivalent SN_r for the comminution of a crystalline material was presented by Kwade (Figure 3.7), which demonstrates the expected result; that a higher SN results in greater comminution.

Figure 3.7 – Reduced Stress Number (SN_r) vs. Product Size [Kwade et al.]



From Figure 3.7, however, it is also evident that smaller grinding medias (97µ and 219µ) do not result in comminution even after much larger SN_r. At these small media sizes, the feed material does not receive sufficient stress energy (SE) to initiate fracture. In the intermediate case of d_{GM}=399µ, it is evident that a large number of repeated stress events were needed before meaningful fracture occurred, whereas in the case of larger media the initial stress energy was sufficient to immediately fracture the feed material.

Stress Energy (SE)

The stress event energy, SE, describes the energy exchanged from grinding media to the feed particle per stress event. Physically, SE is a distribution and cannot be described by a single value, but in practice the average SE, $\overline{SE}$, is useful. Typically $\overline{SE}$ is taken as proportional to the momentum of the grinding media, which is assumed to have the same speed as the agitator tip speed (v_t).

Equation 3.14 – Stress Energy of Grinding Media

$$SE_{GM} = d_{GM}^3 \cdot \rho_{GM} \cdot v_t^2$$

SE_{GM} = SE from point of view of grinding media
d_{GM} = diameter of grinding media
ρ_{GM} = grinding media density
v_t = tip speed / grinding media speed (assumed equal)

Any energy used to deform the grinding media is not used for comminution, so the energy transferred from the grinding media to the product should also account for the modulus of each material. By approximating the energy transfer between grinding media and feed as an undampened spring system and using a Hertzian model of collision, Becker determined the maximum stress energy transfer between two media and a feed particle[25]. This results in loss factor due to elastic deformation of the grinding media being applied to $SE_{GM.}$ For relatively soft materials, however, the energy loss due to deformation of the grinding media can be neglected and SE reverts to the original approximation of SE_{GM}.

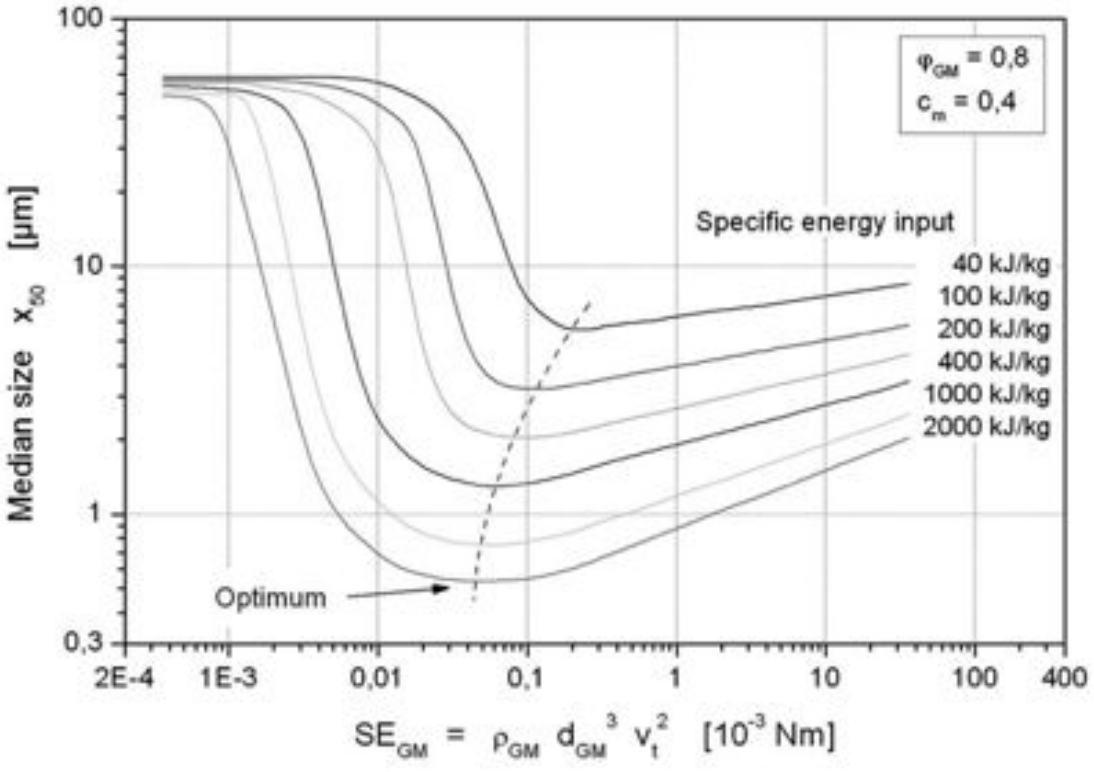
Equation 3.15 – Stress Energy of Product (SE_P)

$$SE_P = d_{GM}^3 \cdot \rho_{GM} \cdot v_t^2 \left(1 + \frac{Y_P}{Y_{GM}}\right)$$

SE_P = SE from point of view of feed material
d_{GM} = diameter of grinding media
ρ_{GM} = grinding media density
v_t = tip speed / grinding media speed (assumed equal)
Y_P = modulus of elasticity of feed
Y_{GM} = modulus of elasticity of grinding media

The graphical description of SE with respect to product size and specific energy input yields a characteristic curve shape. As described by Kwade, the optimum grinding condition is just beyond the step down. From Figure 3.8, it is apparent as the product size decreases, the optimum SE should also decrease (small SE to fracture small particles).

Figure 3.8 – SE_{GM} vs. Product Size vs. E_m



Using these formulae for SN and SE, the expected specific energy requirement (E_m) can be calculated by Equation 3.16 for comminution of a soft brittle material (e.g. CuI).

Equation 3.16 – Specific energy for comminution of soft brittle materials

$$E_m = \frac{SN \cdot \overline{SE}}{m_p} = \frac{\left[\frac{\varphi_{GM} \cdot (1-\varepsilon)}{(1-\varphi_{GM} \cdot (1-\varepsilon)) \cdot c_v} \cdot \frac{n \cdot t}{d_{GM}^2}\right] \cdot \left[\nu_E (d_{GM}^3 \cdot \rho_{GM} \cdot v_t^2)\right]}{m_p}$$

Many additional nuances must be taken in to account especially when dealing with grinding of hard and tough materials. Furthermore, scale-up becomes challenging as the distribution of SE at constant tip-speed can change per the geometry of the mill. The inclined reader is referenced work of Kwade and Schwedes for additional coverage.

3.2. Experimental Set Up

3.2.1. Wet Media Milling

3.2.1.1. Wet Media Mill

A Netzsch Minicer (NETZSCH Premier Technologies, LLC) wet media mill was customized for these experiments. The interior of the mill and agitator were constructed of zirconium oxide with a tandem dual-action mechanical seal adjoining the chamber to the agitator motor. An infinitely variable agitator drive was operated between working speeds of 1000 to 4200 RPM. The grinding chamber volume was approximately 160 mL. The exterior of the grinding chamber consisted of jacketed stainless steel for water-cooling.

Feed material was loaded in to a 400 mL batch tank and pumped through the grinding chamber. The batch tank was jacketed and water-cooled and of stainless steel construction. The batch tank agitator was controlled between 100 and 2000 rpm. A peristaltic pump was used to circulate material through an agitated batch tank using PharMed BPT peristaltic tubing. In the preparation of solvent based slurries a PTFE diaphragm pump was used when the solvent would otherwise degrade the peristaltic tubing. All wetted parts were constructed of 316 stainless steel, fluoropolymer (PTFE and VDF), zirconia, and peristaltic tubing .

Dynamic cartridge media screen separators of stainless steel with slot widths of 0.03, 0.06, or 0.15 mm were used. Typically the slot size was less than or equal to about half the diameter of the media used.

3.2.1.2.Grinding Media

Several grades of yttria stabilized zirconia (YZT) and lanthium glass ceramic (ZGC) grinding media were used throughout. The nominal media diameters used were, 0.1 mm YZT, 0.3 mm YZT, 1 mm YZT, as well as 0.05 mm ZGC, and 0.1 mm ZCG. The YZT media was acquired from Toshoh (Japan) or Inframat Advanced Materials (USA) and the ZGC grinding media were acquired from 3M Advanced Materials (USA). All media were spherical. Nominal sizes were confirmed within a ±15% range by microscopy before use. Media was supplied as preconditioned and was additionally conditioned before use by processing with 1% sodium lauryl sulfate for 24 hours at 4000 RPM before use.

3.2.1.3.Operation

Unless otherwise reported the mill was charged with 140 mL of grinding media. The mill was operated in recirculation mode with 5° C cooling water applied at 80 psi through both the batch tank jacket and the grinding chamber jacket. The following values were monitored during milling operations: agitator speed, pump speed, product pressure, product temperature, power consumption of the main drive in kW, total energy input in kWh.

For most experiments, the mill was set to shut off if pressure exceeded 1.9 barr or if temperature exceeded 40°C. The sealing pressure was set to approximately 4.7 bar. The mill was cleaned between runs using a mixture of ammonia, citric acid, and PVP mixture to dissolve and scavenge any residual CuI, followed by multiple passes of water. After every 10-20 runs the mill was disassembled and fully cleaned.

3.2.2. Particle Size Analysis

3.2.2.1. Light Scattering Techniques

Dynamic Light Scattering – Static Light Scattering Combined System (DLS-SLS)

A combined dynamic light scattering[a] (DLS) and static light scattering (SLS) system was used to monitor particle size (DLS) and particle mass (SLS) throughout processing. A practical discussion of this instrumentation is included briefly here to provide a rationale for the measurement parameters used.

A DyanaPro NanoStar (Wyatt, USA) equipped with a 661 nm laser was used and a scattering detector at 90° was used to monitor the mass fraction of particles of a given size at various processing times. Light scattering instrument control software, Dynamics, was used to interpret the received data using the regularization analysis methodology.

DLS measures the scattering intensity fluctuations of an incoming light source interacting with dispersed particles as they fluctuate in the solution due to Brownian motion. Since small particles fluctuate faster than large particles due to Brownian motion, these fluctuations can be analyzed to determine the distribution of particles that fits the observed fluctuations.

The intensity of scattered light increases very rapidly with particle size, however, which creates difficultly in interpreting the relative quantities of particles in polydisperse samples (e.g. sample with multiple peaks). In short, the average size presented in a DLS measurement corresponds to the intensity weighted average size of the particles. For particle size measurements of monodisperse samples this is acceptable. Example

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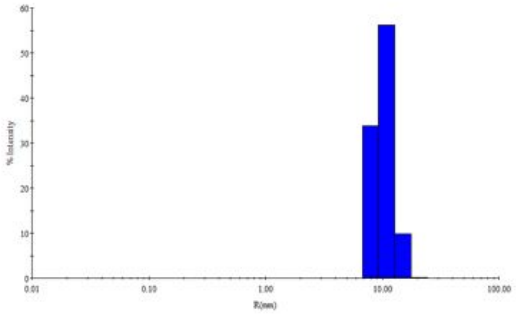
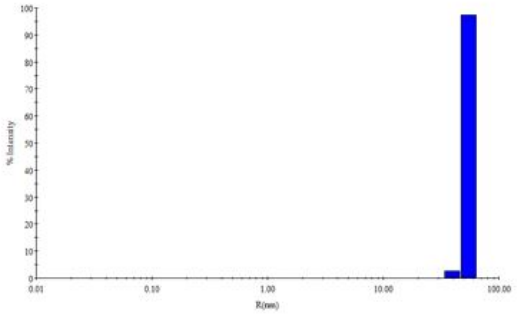
[a] DLS is also known as Photon Correlation Spectroscopy (PCS) or Quasi-Elastic Light Scattering (QELS)

measurements for R=10 nm and R=50 nm monodisperse polystyrene spheres are shown in Figure 3.9.

Figure 3.9 – Sample DLS/SLS measurement for Monodispersed Particles

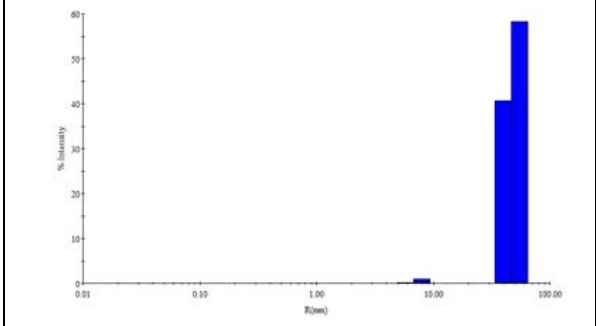
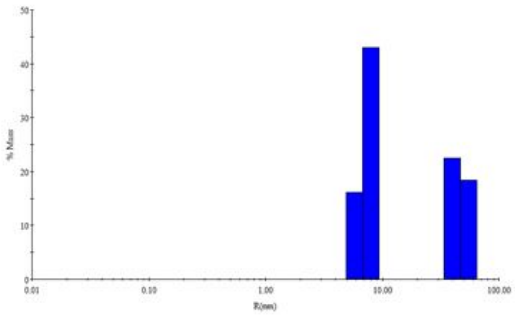
R=50 nm Nanosphere Standard			R=10 nm Nanosphere Standard		
					
R (nm)	%Pd	%Int	R (nm)	%Pd	%Int
54.3	5.9	100.0	9.3	18.8	100.0

It was preferred, however, to have a mass averaged particle size rather than an intensity-averaged size. This was especially the case when a polydisperse sample is measured. Utilization of SLS in combination with DLS measurements allows for the relative mass of each peak to be calculated, which provides improved interpretation of polydisperse samples and yields the relative mass of polydisperse sample peaks, which is more relevant in analyzing comminution processes since we are concerned with the mass of material that is or isn't properly comminuted.

In static light scattering, the measured amount of light scattered can be used to calculate molecular weight and radius of gyration of the scattering particles. A right angle light scattering system (RALS) as used, here, however, is only capable of measuring the molecular weight of isotropic scattering particles. Particles become more isotropic as they decrease in size, and thus particle weight measurements are more valid for smaller (more isotropic) particles. Complete isotropicity was assumed for all samples.

Figure 3.10 demonstrates the ambiguity in DLS measurements of a dispersion containing equal parts by mass of r=10 nm and r=50 nm particles. Figure 3.10 (b) demonstrates the improved representation of the same sample reported using the mass averaged particle size distributions compared to intensity averaged particle size distributions in Figure 3.10 (a).

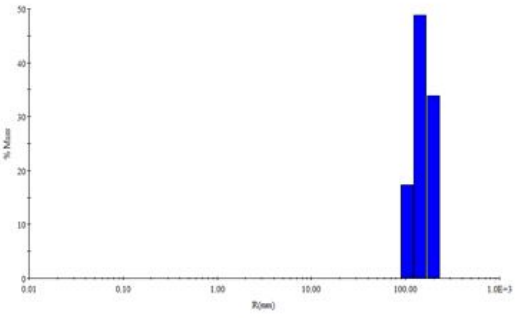
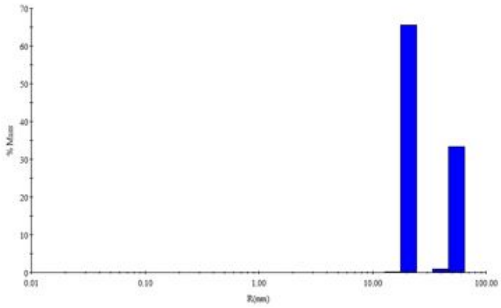
Figure 3.10 – Sample DLS/SLS measurement for Polydispersed Particles

(a) Intensity Representation			(b) Mass Representation		
					
R (nm)	%Pd	%Int	R (nm)	%Pd	%Mass
7.7	9.5	1.1	7.4	13.3	59.2
48.8	15.2	98.9	46.7	16.1	40.8

As a result of noise in the data, an infinite number of distributions can fit the data. Figure 3.10, above, could have been presented as a monodisperse sample. DLS is ideally capable of resolving peaks separated by at least 2x in size, however, the regularization of experimental data results in unresolved peaks being ignored as noise. Within the Dynamics software, however, the peak resolution can be increased by manually increasing the peak resolution. Thus, all measurements were performed with the peak resolution set to the maximum of six ticks higher than “optimal” in order to distinguish any peaks as milled samples were expected to be highly polydisperse.

Figure 3.11 demonstrates the measurement of a particle preparation containing 75wt% r=10 nm particles and 25wt% r=50 nm particles. The peaks are not resolved unless the resolution is increased. As can be seen, when the peaks are resolved there is a dramatic decrease in polydispersity for each peak.

Figure 3.11 – DLS Measurement with "Optimal" or "Maximum" Peak Resolution

(a) "Optimal" Peak Resolution 75wt% r=10nm, 25wt% r=50nm				(b) "Maximum" Peak Resolution 75wt% r=10nm, 25wt% r=50nm			
							
R (nm)	%Pd	%Int	%Mass	R (nm)	%Pd	%Int	%Mass
156.1	21.8	100.0	100.0	21.0	1.4	17.9	65.8
				54.6	4.5	82.1	34.2

Zeta Potential

An SZ-100 DLS system (Horiba) was employed as a secondary measure of particle sizes. This system was coupled with a Doppler electrophoresis system such that the electrophoretic mobility of the particles could be measured by tracking the movement of particles under the application of an electric field. This method interprets the frequency shift of the scattered light with respect to the incoming light beam the particle velocity can be measured. The measured particle velocity and particle size data can then be used to determine the zeta potential. These measurements were performed during a visit to a Horiba laboratory facility (CA, USA).

Laser Diffraction Particle Size Analysis

An LA-960 laser diffraction particle size analyzer (Horiba) system was employed as an additional measure of particle sizes. This system uses the diffraction pattern resultant from light-particle interactions to calculate particle size. One advantage of this type of system is that it provides a volume weighted particle size measurement, which may be more practical than intensity weighted measurements. Furthermore, since this system does not require particles to be suspended during measurements, unstable and large particle up to macro-dimensions can be analyzed. These measurements were performed during a visit to a Horiba laboratory facility (CA, USA).

3.2.2.2.Electron Microscopy

Analyses were conducted using a Hitachi S-4800 Type II Ultra-High Resolution Field Emission Scanning Electron Microscope at the University Spectroscopy and Imaging Facilities at the University of Arizona. Samples were imaged in scanning electron microscopy (SEM) and scanning transmission electron microscopy (STEM) modes.

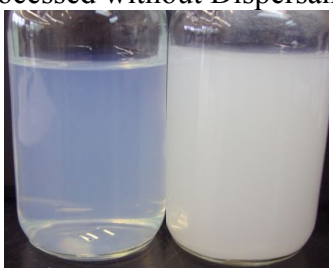
3.3. Preliminary Grinding Experiments

Several preliminary experiments were conducted in order to explore candidate CuI based formulations and to determine if useful material could be produced through wet media milling. Candidate formulations were evaluated based on their AM performance, dispersion stability, particle size stability after drying and redispersing, and compatibility with various material matrices.

It was found that the operating pressure of the mill was a useful indicator of the stability of trial formulations. Unstable formulations tended to increase the operating pressure due to build up of solids on the interior of the cartridge screen, leading to eventual blinding of the screen. Often, unstable formulations increased the pressure sufficiently to cause the mill to engage the pressure safety shut down controller. This provided a rapid qualitative method of determining the stability of trial formulations during processing.

Grinding raw CuI alone typically resulted in blinding of the separator screen and automatic pressure shutdown of the grind. Very small batches of CuI, however, did not substantially blind the screen and were capable of forming colorless and clear dispersions of small particle sizes. These dispersions, however, became turbid and unstable within hours after cessation of grinding. Thus it was evident, as expected, that grinding aids and stabilizers would be required. Several common polymers and surfactants were initially explored.

Figure 3.12 – Settling of CuI Processed without Dispersant



Immediately after processing (left) and after aging for 6 hours (right)

3.3.1. CuI with PVP

Based on the known ability of PVP to bind to metals and iodine/iodide (as was exploited in Chapter 2), PVP was explored as a protective agent. This yielded disappointing results. Grinding of CuI with large amounts of PVP (e.g. similar to CuI/PVP particles of Chapter 2) resulted in dispersions that appeared similar to those produced by the wet-chemical-acetonitrile-precipitation methods of Chapter 2. However, unlike the wet-chemical-acetonitrile-precipitation produced particles, these dispersions were unstable and particle growth was apparent after several days as was evidenced by DLS particle size measurements, increased turbidity, and sediment formation. Further, ground mixtures of CuI with lower proportions of PVP resulted in immediately unstable dispersions.

The lack of stability in the CuI-PVP system after grinding can be interpreted conceptually by considering the differences between the grinding process and the wet-chemical-acetonitrile-precipitation process. In the acetonitrile process, CuI is dissolved and precipitated to form crystallites with sizes controlled by capping with PVP; and since the particles are built-up they would be expected to adopt their lowest energy configuration. In the case of grinding, however, the particles are formed from high-energy comminution events due to impingement of grinding media, and hence some of

this energy might remain in CuI particles in the form of activated states, or localized regions of disorder.

This concept of activated states is exploited in many industries to achieve, for instance, improved mineral leaching, improved pharmacokinetic bioavailability, and increased clinker reactivity in cement processing[26]. In the case of stabilization of CuI, however, after cessation of grinding, the decay of these states seems to destabilize the protected particles and PVP. Although speculative, this perspective agrees well with the observation of stable dispersions, which after time decay to form unstable dispersions. In any case, the lack of utility of PVP to stabilize the CuI particles processed using grinding methods demanded that other materials be considered.

In the wet-chemical-acetonitrile-precipitation process, the particles are protected from water until they are formed, whereas in the case of grinding, they are continuously exposed to water during production. During grinding, the fractured CuI surfaces may become hydrated. As evidence of the role of water in this system, it was noticed that grinding in certain solvents (e.g. isopropyl alcohol) instead of water provided some improved stability to these ground PVP-CuI dispersions, but they were still relatively unstable compared to the wet-chemical process. The addition of up to 10% acetonitrile to the grind did not have any noticeable impact on the produced particles.

Figure 3.13 – Difference between processing routes of CuI/PVP formulation

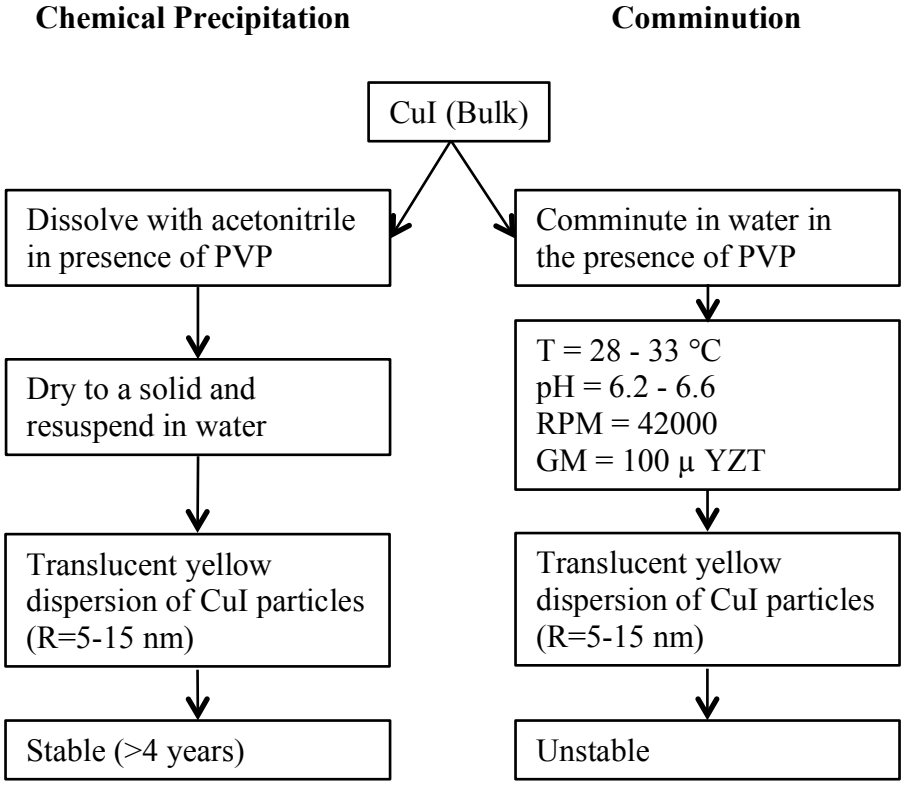
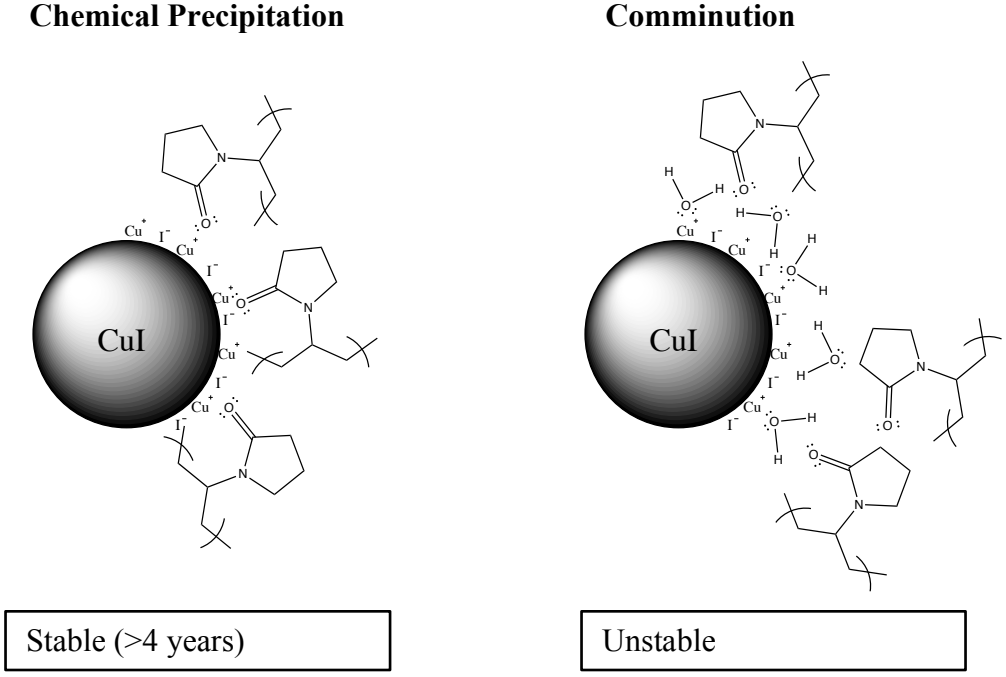


Figure 3.14 – Proposed physicochemical differences between CuI/PVP particles



On this basis it was decided to investigate other materials that might provide protection to the CuI particles if activated hydrated or oxidized surface layers formed during grinding. Several materials were explored, especially charged surfactant molecules. It was found that several cationic surfactants readily appeared to react with copper iodide and formed a second phase of yellow or brown material, which was presumed to be an iodide complex. Attention was thus given to anionic surfactants. In particular several sulfate- and sulfonate- based materials were found to form size stable particles during the grinding process.

3.3.2. CuI with Sodium Lauryl Sulfate

3.3.2.1. CuI/SLS Additive

Among an initial screening of several sulfate and sulfonate surfactants for their ability to stabilize CuI particles during grinding, sodium lauryl sulfate (SLS) was found to be effective. Processed mixtures of CuI with SLS were found to produce opaque colorless dispersions having a grinding limit of around 100 nm. These dispersions were capable of being dried and redispersed without a measurable increase in the particle size, and thus appeared to be suitable as a useful additive material.

A preliminary CuI-SLS dispersion was prepared by milling 25.7 g of CuI with 1.4 g of SLS in 200 mL DI-water for 24 hours at 4200 rpm with 100μ YZT grinding media. This resulted in particles that were no smaller than r=50 nm. It was not possible to comminute the particles below this size even after long grinding times (>24 hours) as measured by DLS. DLS measurements were performed by diluting the prepared dispersions with a 1wt% SLS solution until appropriate scattering intensity for DLS

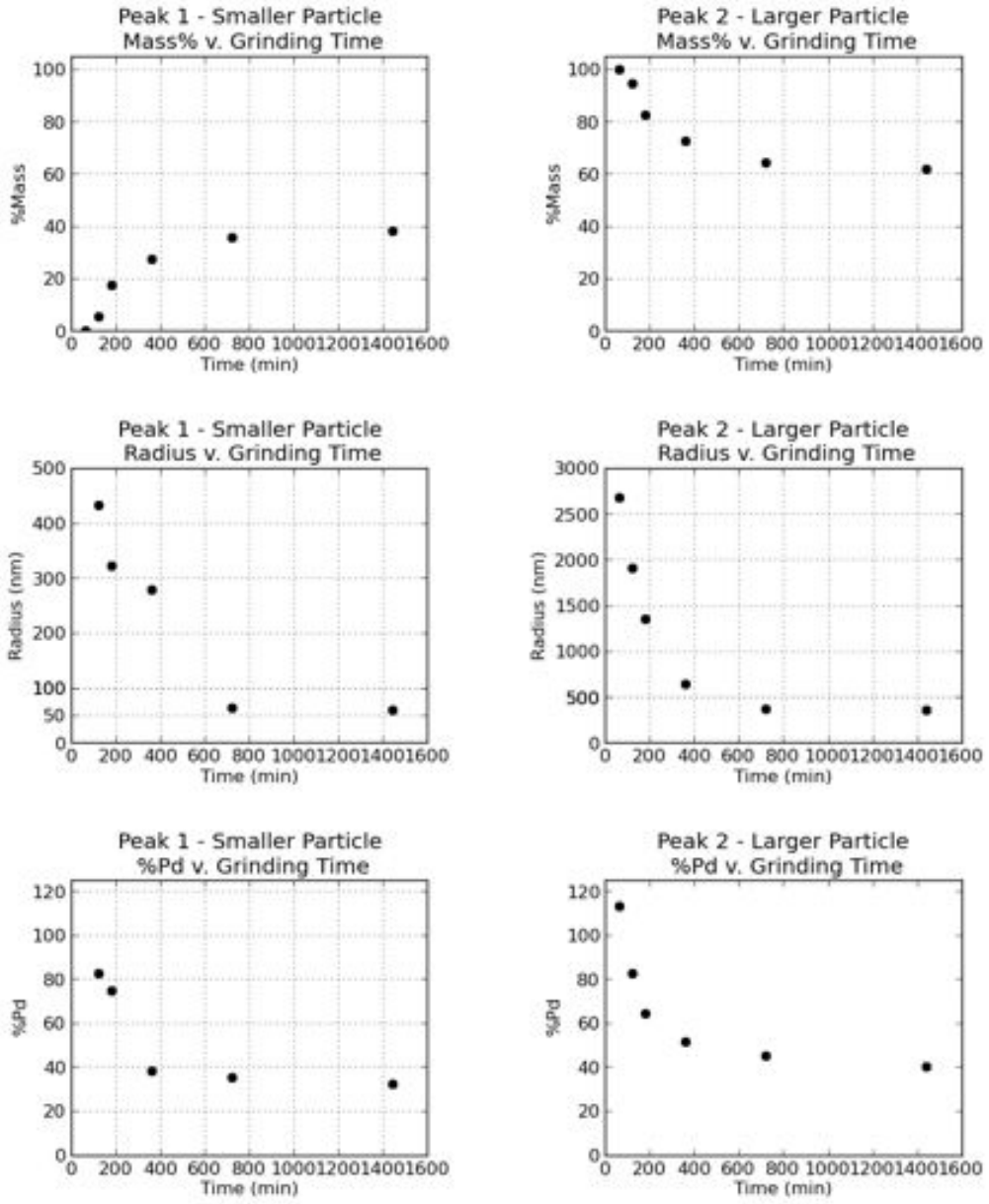
measurements was achieved. Dilution with DI-water resulted in increased instability as indicated by larger particle sizes observed by DLS and increased settling. This indicated that SLS was not stabilizing the CuI particles by strongly adsorbing on to the surface, but through weak physical adsorption.

Figure 3.15 shows the measured particle sizes at several times throughout grinding for four CuI/SLS batches. After around 600 minutes no change in particle size distributions were noticed up to 1440 minutes (24 hours). During grinding, typically two peaks were resolved in DLS measurements at each time point for these particles, corresponding to a small and large particle at each time point.

Figure 3.17 shows an SEM image the particles formed in this process after 24 hours of grinding. The agreement of the DLS and SEM measurements provided confidence in the use of the DLS system to monitor particle size during grinding.

The larger peak likely resulted from a lack of comminution of these larger fragments during grinding. This is likely the result of a lower stress event energy due to the use of small grinding media. Additionally, this may be attributed to the formation of lamellar packing of SLS, which would likely lead to an increased local viscosity and dampening of the grinding media. The presence of large tough particles in the raw CuI feed can be ruled out by reference to the comminuted CuI/PVP particles, which, although unstable were monodisperse in the 5-15 nm range immediately after grinding.

Figure 3.15 – Bimodal Distribution for grinding CuI/SLS



This figure is a consolidation of several individual DLS measurements. Each point corresponds to a measured value (mean radius, %Mass, %Pd) of each peak at each time point; for example each point corresponds to a measurement similar shown below,

Figure 3.16 – Example DLS measurement to explain Figure 3.15.

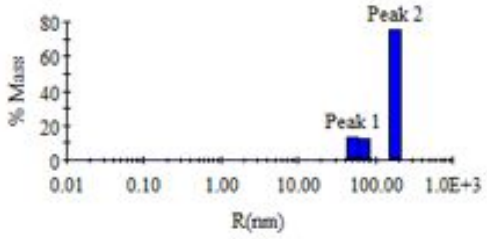
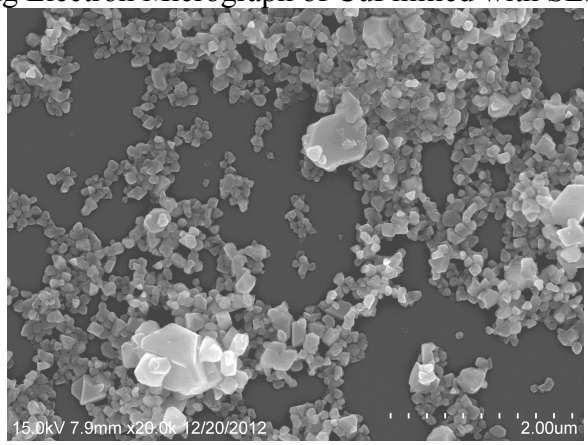


Figure 3.17 – Scanning Electron Micrograph of CuI milled with SLS for 24 hours



Several potential explanations on the stabilizing role of SLS exist. Likely, SLS was weakly adsorbed on to the CuI surface; and thus high levels were required in order to maintain sufficient CuI/SLS interaction as experienced during diluting the material for particle size analysis. Furthermore, it is likely that high concentrations of SLS also physically blocked the CuI particles from approaching one another through the formation of lamellar structures, as has been observed in the stabilization of titania based pigments[27]. In the dry state, SLS evidently was effective in maintaining effective separation of the CuI particles as was demonstrated by the ability of these particles to disperse to similar sizes as measured from the produced grinding slurry.

The usefulness of some CuI/SLS formulations was evaluated for AM efficacy as described in section Chapter 2 through cooperation with Jason Torrey (Gerba Lab,

University of Arizona). SLS concentration was increased to allow for redispersal of the material without addition of SLS to the solvent. A 40 g batch of 85% CuI 15% SLS (CuI/SLS-85/15) was prepared by grinding for 900 minutes followed by drying under reduced pressure at 60 °C. The CuI/SLS-85/15 particles were tested as dried and redispersed material and were found to be very effective against both *P. aeruginosa* and *S. aureus* (Table 3.1 and Table 3.2, respectively). This AM efficacy indicated that the particles were equivalently effective as the chemically prepared nanoparticles and that the AM efficacy was only a result of the CuI. In AM testing, the CuI/SLS particles were diluted in PBS immediately prior to AM testing.

Table 3.1 – Efficacy of CuI/SLS particles against *P. aeruginosa*

Time	Control	SLS Control (31 ppm SLS)	CuI/SLS (59.07 ppm Cu 31 ppm SLS)
15 min	-0.25 ± 0.00	-0.44 ± 0.05	3.96 ± 0.43
1 hr	-0.03 ± 0.12	-0.15 ± 0.01	> 4.56 ± 0.00
3 hr	-0.22 ± 0.24	-0.35 ± 0.14	> 4.56 ± 0.00
6 hr	-0.10 ± 0.02	-0.11 ± 0.02	> 4.56 ± 0.00

Original titer = 1.82E6 cfu/mL

Table 3.2 – Efficacy of CuI/SLS particles against *S. aureus*

Time	Control Coating	SLS Control (31 ppm SLS)	CuI/SLS (59.07 ppm Cu 31 ppm SLS)
15 min	-0.05 ± 0.09	-0.03 ± 0.00	4.01 ± 0.34
1 hr	-0.07 ± 0.07	-0.02 ± 0.04	> 4.25 ± 0.00
3 hr	-0.08 ± 0.03	0.01 ± 0.02	> 4.25 ± 0.00
6 hr	-0.06 ± 0.00	-0.02 ± 0.08	> 4.25 ± 0.00

Original titer = 8.95E5 cfu/mL

3.3.2.2.Polyurethane Coatings with CuI/SLS

The good AM efficacy of the CuI/SLS-85/15 material prompted the incorporation of this powder into several coating matrices. A CuI/SLS containing polyurethane based coating was prepared using a commercial polyurethane formulation. This polyurethane

formulation consisted of an aqueous dispersion of aliphatic polycarbonate diols and polyisocyanates with the addition of a trifunctional aziridine crosslinking agent.

Esacote PU 71 (Lamberti, Italy), polyurethane resin, was combined with 3% PZ-28 (PolyAziridne NJ, USA), crosslinker, and CuI/SLS material was added as a 25wt% dispersion in 7% N-ethyl-pyrrolidone as a cosolvent for the coating resin to achieve 1% Cu in the dried coatings.

This coating mixture was stirred and applied by hand on to stainless steel substrates. These coated substrates were then dried overnight under ambient conditions and cured for 2 hours at 70 °C in a convection oven. The finished coatings appeared slightly gray in color. Standard coatings were also prepared similarly without the addition of the CuI/SLS dispersion.

These coatings were evaluated for AM efficacy according to JIS Z 2801 through collaboration with Jason Torrey (Gerba Lab, University of Arizona) as described in Chapter 2. In addition to the prepared coatings, a commercial silver based AM powder coating manufactured by DuPont (PFW-669-S8A Sky white color) was also included as a comparative sample. In these coatings the silver content was not disclosed, but was based on an EPA registered silver additive. The results of this evaluation showed that the CuI based polyurethane coatings were not only very effective, but also much more effective than the commercial silver-based coatings.

Table 3.3 – Efficacy of CuI or Ag containing coatings against *S. aureus*

Time	Polyurethane Control	1% Cu Polyurethane	Commercial Silver Powder Coating
	Log_{10} Reduction	Log_{10} Reduction	Log_{10} Reduction
6 hr	-0.33 ± 0.11	4.56 ± 0.51	2.73 ± 0.75
24 hr	0.31 ± 0.32	> 4.79 ± 0.00	> 4.87 ± 0.00

Original Titer =5.25E+05 cfu/mL

It was subsequently determined that this polyurethane coating resin was incompatible with CuI after long-term storage. This was attributed to be largely due to the presence of the azidrine crosslinking agent used. Coating mixtures without this additive were more stable and from the literature several reports exist describing the ability of azidrines to form stable cupric complexes[28]. This accelerated the oxidation of copper in the resin, which was evidenced by color change during storage of the prepared CuI coatings resins. Crosslinker-free resin also resulted in notably slower but eventually similar incompatibility issues, and in this case was attributed to the aminic solvents used to prepare the resin dispersion.

In other coating matrices that were free of aminic solvents and other strongly copper-complexing species, it was found that much lower levels of CuI could be used to achieve similar efficacy as had been achieved in these polyurethane coatings. For this reason, the CuI/SLS material was evaluated for usefulness in several other matrices. A major drawback, however, was the inability to disperse this material in many non-aqueous coating solvents (e.g. methyl-ethyl-ketone, methyl-isobutyl-ketone, toluene). As such, a solvent-free epoxy-polyester powder coating system replaced the polyurethane system for further AM coating development. Additional CuI formulations were developed for solvent-based systems as presented later in this chapter and in Chapter 4.

3.3.2.3.Epoxy-Polyester Powder Coatings with CuI/SLS

A CuI containing polyester-epoxy based powder coating was prepared in partnership with an industrial powder-coating manufacturer. In making the powder coatings, CuI/SLS-85/15 powder was dry mixed with a carboxylated polyester resin along with leveling agents, crosslinking agent (triglycidylisocyanurate), and degassing agents. This mixture was then melt-blended and extruded to form a ribbon, which was then crushed to a powder to form the final powder for coating. This powder was fed into a Corona gun and applied to aluminum substrates, which were then cured at approximately 200 °C for 10 minutes. The final coating thickness was approximately 60±10 microns as measured by surface profilometry.

These powder coatings were prepared with the addition of CuI/SLS at 0.25, 1.0, and 3.0 wt% Cu in the finished coatings. The AM properties of these coatings were evaluated along with a commercial silver based AM powder coating manufactured by DuPont (PFW-669-S8A Sky white color) at 6 hr and 24 hr exposure in a modified JIS z2801 test through cooperation with Jason Torrey (Gerba Lab, University of Arizona). Again, these coatings demonstrated superior AM efficacy compared to commercial, silver-based, antimicrobial powder coatings.

Table 3.4 – Efficacy of CuI/SLS containing Powder Coating Against *P. aeruginosa*

Time	Standard	0.25 wt% Cu	1.0 wt% Cu	3.0 wt% Cu	Silver
	Log_{10} Reduction				
6 hr	-0.19±0.06	>5.63±0.00	>5.63±0.00	>5.63±0.00	1.79±0.36
24 hr	-0.59±0.08	>5.43±0.52	>5.83±0.35	>6.03±0.00	5.73±0.52

Original Titre = 1.37E06 CFU/mL

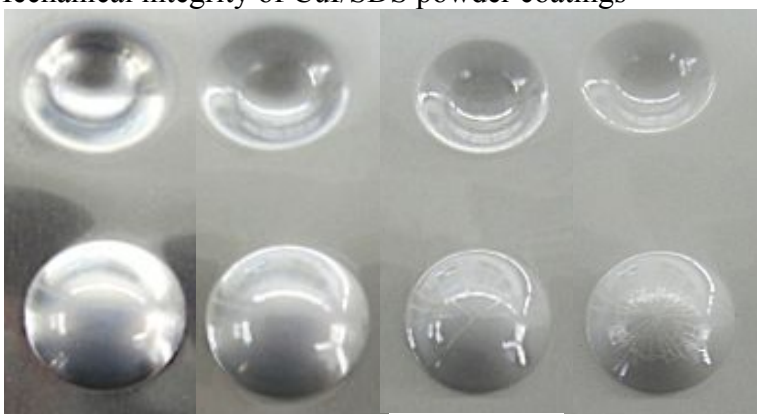
Table 3.5 – Efficacy of CuI/SLS containing Powder Coatings Against *S. aureus*

Time	Standard	0.25 wt% Cu	1.0 wt% Cu	3.0 wt% Cu	Silver
	Log_{10} Reduction				
6 hr	0.16±0.05	>4.77±1.65	>5.72±0.00	>5.72±0.00	3.29±1.45
24 hr	0.57±0.07	>5.31±0.00	>5.31±0.00	>5.31±0.00	4.65±0.61

Original Titre = 3.78E06 CFU/mL

A rapid test to assess the mechanical integrity of these coatings was performed by subjecting each coating to 160 lb force impacts and examining the coating for cracking or peeling. Impact craters were created on the front-side and reverse-side of the powder coatings. The control coating and 0.25% Cu coatings passed the test in both modes, but the 1% and 3% Cu coatings exhibited cracking in the reverse impact mode. The 3% Cu coating exhibited significantly more cracking, which indicated that the addition of the CuI/SLS additive was responsible for the degraded mechanical performance.

Figure 3.18 – Mechanical integrity of CuI/SDS powder coatings



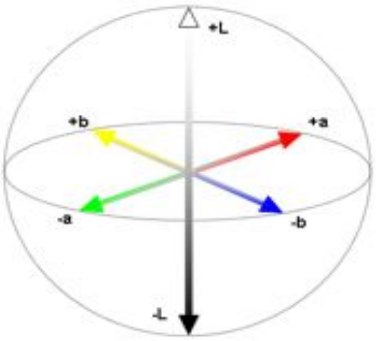
0% Cu, 0.25% Cu,1% Cu, 3% Cu

Additionally, with increasing levels of addition of the CuI/SLS additive the coatings became opaque and developed a gray color. Color coordinates were measured for each coating using a Hunter Labs colorimeter. It was found that the addition of the

CuI/SDS additive resulted in an increase in yellow and green coloration as indicated by the increase of the –a* (green) and +b* (yellow) ordinates in the *L*a*b** colorspace.

Table 3.6 – Colorimetric Measurements of CuI Powder Coatings

Powder Coating	L*	a*	b*
Standard	74.72	-0.25	2.69
3% Cu	77.92	-1.96	6.51
1% Cu	73.35	-1.84	6.60
0.25% Cu	73.56	-1.00	6.70
0.10% Cu	74.91	-0.76	3.57



Additional powder coatings were prepared using CuI/SLS material at 0.10 wt% Cu in the finished coating. This resulted in a dramatically improved appearance. It was indistinguishable from the standard coating by eye and only slightly more yellow than the standard by colorimetric measurements.

The AM efficacy of this reduced copper coating was tested along with the 0.25% Cu coating and the commercial material using the JIS z2801 protocol. At shorter times, 1 and 2 hours, the 0.10% Cu coating was equivalently effective compared to the 0.25% Cu coatings for *P. aeruginosa* but notably less effective against *S. aureus*. Both, however, were substantially more effective than silver based commercial powder coating samples in all cases.

Table 3.7 – Efficacy of Powder Coatings against *P. aeruginosa*

Time	Standard	0.10% Cu	0.25% Cu	Silver
	Log_{10} Reduction			
1 hour	-0.19 ± 0.04	3.87 ± 0.20	4.45 ± 0.38	0.49 ± 0.40
2 hour	0.19 ± 0.17	4.54 ± 0.15	4.74 ± 0.24	1.23 ± 0.56

Original Titer = 7.50E+05 cfu/mL

Table 3.8 – Efficacy of Powder Coatings against *S. aureus*

Time	Standard	0.10% Cu	0.25% Cu	Silver
	Log_{10} Reduction			
1 hour	-0.17 ± 0.02	0.89 ± 0.13	2.18 ± 0.29	0.26 ± 0.23
2 hour	-0.72 ± 0.09	1.29 ± 0.06	3.44 ± 0.56	0.26 ± 0.03

Original Titer = 1.81E+05 cfu/mL

Additional AM evaluations were conducted after exposing these coatings to several cleanings using common commercial cleaning agents. The AM performance of these coatings was typically unchanged or slightly decreased. Cleaning the coatings with concentrated ammonia or 50x cleanings with Windex ammonia based glass cleaner, however, severely degraded the performance of the coatings due to leaching of the CuI. In the cases where the AM efficacy was reduced, application of mild abrasion resulted in the regeneration of original AM properties as new CuI/SLS material was exposed.

Figure 3.19 – Antimicrobial CuI Powder Coatings with Cleaning and Abrasion

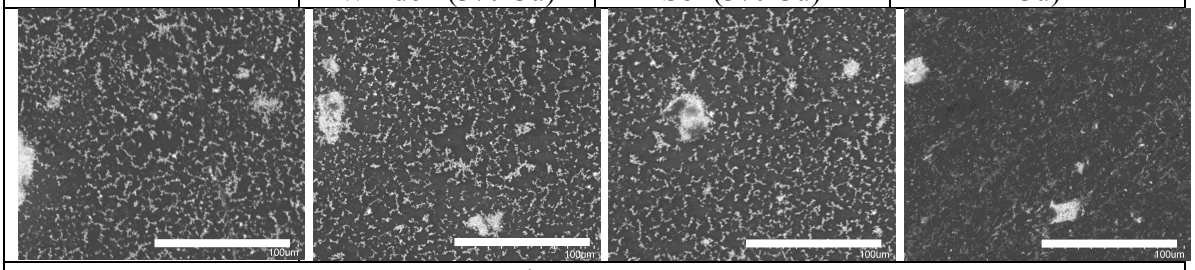
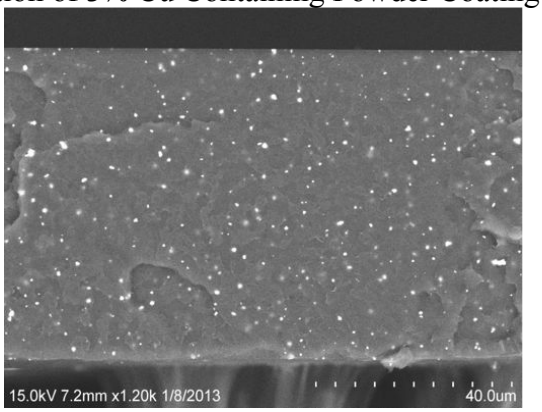
As is (3% Cu)	Cleaned with Windex (3% Cu)	Cleaned with Pine Sol (3% Cu)	Mild abrasion (3% Cu)
			
Scale Bar = 100 μ			

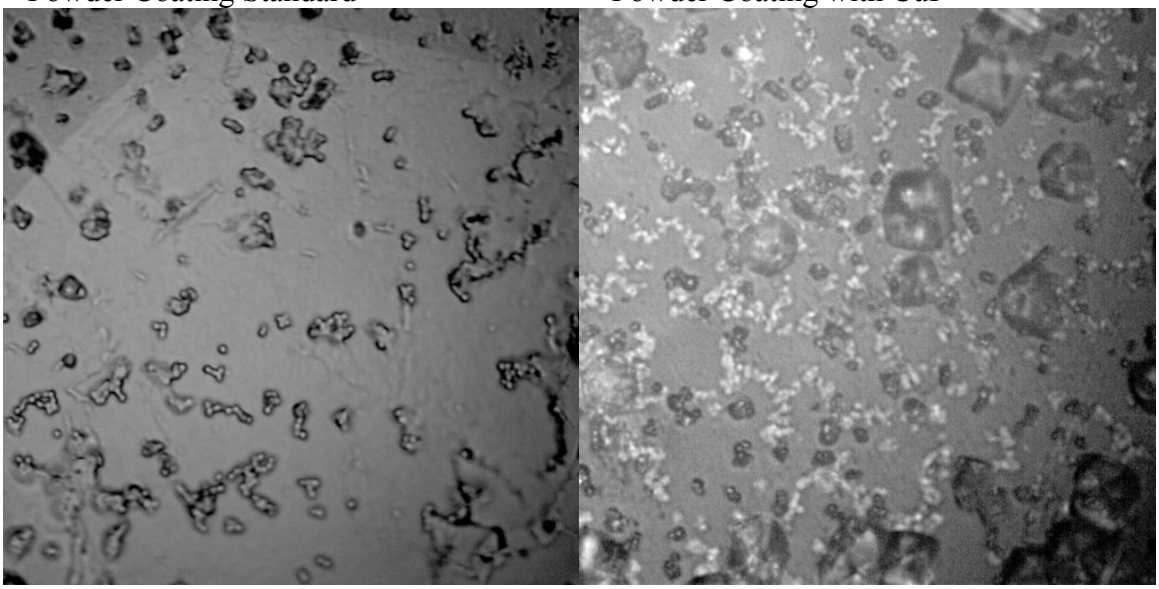
Figure 3.20 – Cross Section of 3% Cu Containing Powder Coating



Although these coatings were highly effective at the copper levels used, the CuI/SLS particles were noticeably poorly dispersed in the final coating. Examination of the coatings revealed that CuI/SLS in the final coating consisted of many large agglomerations rather than well dispersed particles. Under microscopic examination, it was readily apparent that there were many areas on the surface where bacteria would not be in direct or very close contact to apparent CuI. In these areas it was suspected that bacteria might have increased survival times.

Figure 3.21 – *S. aureus* on Powder Coatings

Powder Coating Standard | Powder Coating with CuI

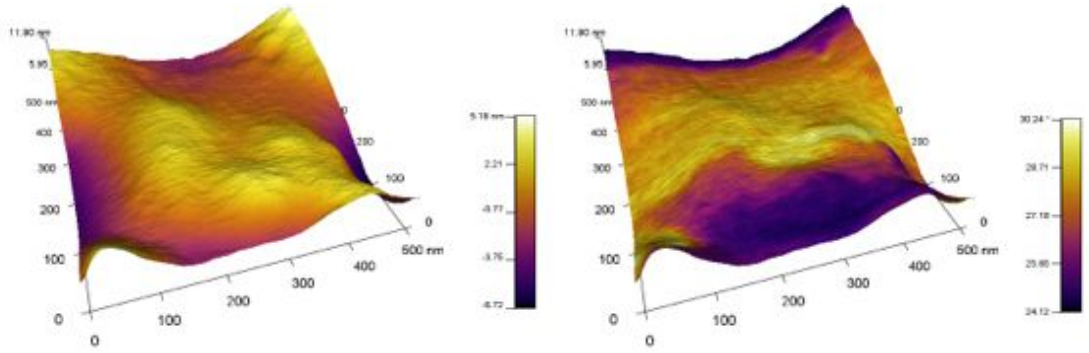


These observations were further confirmed by atomic force microscopy (AFM), which demonstrated large areas where CuI particles were evidenced by phase imaging. Large areas lacking CuI particles separated these areas. The CuI particles did not correspond well with surface topography, that is, the particles tended to present below the surface of the material. This finding confirmed the belief that CuI did not need to be directly exposed on the surface to exert an AM effect. As such, focus could be spent on

developing well-dispersed CuI particles rather than attempting to bring CuI preferentially to the surface.

Figure 3.22 – AFM Analysis of CuI Powder Coatings

Height Image with topo. map Phase Image (superimposed on topo. map)



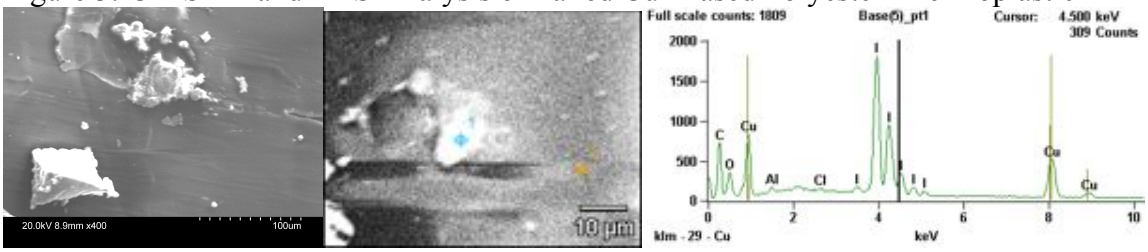
3.3.2.4.Polyester Thermoplastic with CuI/SLS

Based on the favorable performance of the CuI/SLS based powder coatings, polyester thermoplastics were prepared. CuI based polyester thermoplastics were prepared in partnership with an industrial plastics processing company. A similar CuI/SLS additive was used, but polyethylene glycol (PEG, MW=8000) was wet blended with the resultant CuI/SLS additive to reduce the friability of the powder during handling and to reduce concerns of dust formation during handling. This CuI/SLS/PEG material was first dry mixed with cryogenically ground polyester powder and then melt blended to form various masterbatch concentrates. These master batches were then melt blended with additional raw polyester and extruded to form plaques for AM testing.

The antimicrobial efficacy of these plaques was tested similarly to the powder coatings; however, they were found to be significantly less effective. Examination of the

plaques and masterbatch pellets revealed that the added CuI particles generally consisted of large 10-100µ agglomerates.

Figure 3.23 – SEM and EDS Analysis of Failed CuI Based Polyester Thermoplastic



These results prompted motivation for the development of improved CuI based materials, which could attain higher efficacies at lower use levels by reducing the size and improving the dispersion of the particles. To this end a series of experiments were conducted to find more useful dispersing agents and grinding aids such that smaller particles could be better dispersed in different matrices.

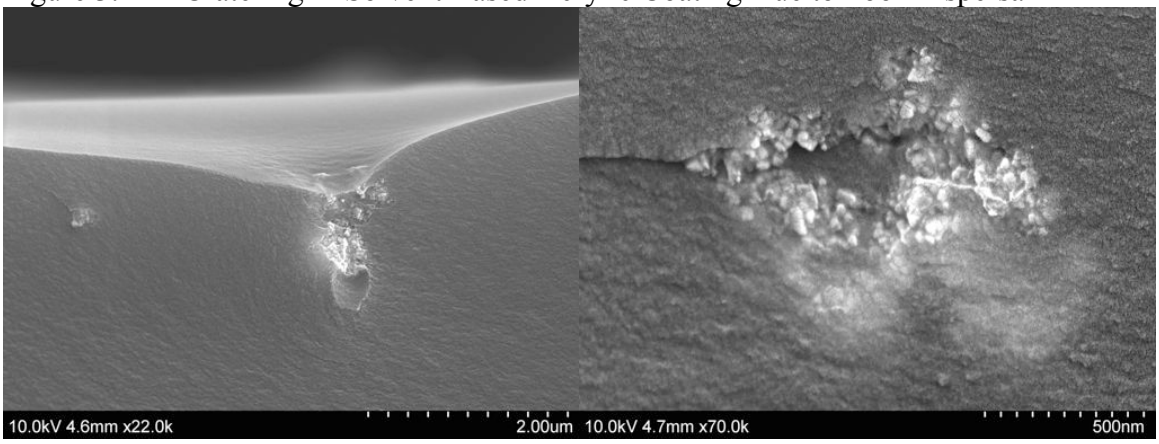
3.3.3. CuI with Other Commercial Surfactants and Dispersants

A large number of candidate formulations were tried by screening various additives, but few produced satisfactory results. Several of these were useful in producing stabilized dispersions, but none of the commercial dispersing agents evaluated produced useful material that could be dispersed in the matrices of interest. Furthermore, the proprietary nature of these dispersants precluded rational design of systems for compatibility with other matrices beyond the manufacturer's recommendations.

Several commercial dispersants were found to be effective in producing stable dispersions of small CuI particles from the mill. These dispersions consisted of smaller particles of CuI, but redispersion from the dry state tended to be poor.

For instance, combinations of CuI with a commercial fluorinated surfactant/dispersant were found to form stable nanoparticle of CuI. Again, however, the particles were not readily redispersed from their dry state. When these particles were added to coating systems, the undispersed particles appeared to cause various issues in the coating. For instance, in solvent based acrylic systems, poor dispersion of these particles resulted in regions of high surfactant concentration near the particle aggregates due and concomitant low surface tension. At these regions cratering occurred as shown in Figure 3.24.

Figure 3.24 – Cratering in Solvent Based Acrylic Coating Due to Poor Dispersal



In other cases, commercially available dispersing agents often had deleterious effects on the AM efficacy of the CuI particles themselves. For example, wet media milling of CuI with a commercial dispersant indicated for use in polyester resin resulted in stable small particles, but when incorporated into powder coatings these had far inferior antimicrobial properties compared to the CuI/SLS formulations (Table 3.9). Again, the lack of structural information on these materials precluded the development of improved systems based on these materials, which otherwise tended to show potential.

Thus the focus was largely maintained on systems of controllable and understood chemistry (e.g. nonproprietary chemicals).

Table 3.9 – Efficacy of several CuI powder coatings against *S. aureus*

Time	Standard Coating	CuI with Commercial Dispersant 0.05% Cu	CuI with SLS 0.05% Cu
	Log_{10} Reduction	Log_{10} Reduction	Log_{10} Reduction
24 hour	-0.13 ± 0.08	1.84 ± 1.02	4.50 ± 0.37

Original titer = 1.60E+07 cfu / mL

3.3.4. CuI with PVP and NaI

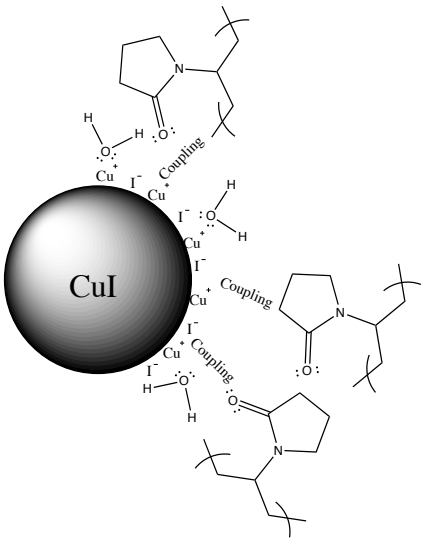
Initial grinding experiments of CuI with PVP resulted in very small particles, as evidenced by the formation of translucent yellow dispersions and measured particle sizes down to 5-10 nm. These dispersions, however, unlike their wet-chemical counterparts, were poorly stable and would form brown sediment after several days of storage. Despite this, it was decided that if this formulation could be stabilized it would provide improved results compared with the CuI/SLS system because (a) a small particle size is achievable and (b) the use of long chain polymers is more easily modified (e.g., using copolymers) for improved compatibility in other matrices (Chapter 4) as compared to small molecules.

Returning again to the differences between the chemically precipitated and wet-media milled CuI/PVP particles, it was proposed that the addition of a intermediate between the hydrated CuI particle and PVP molecule could be used to improve the stability of the CuI/PVP formulations.

It was hypothesized that the addition of a higher solubility, M^+ or X^- salt would act as an intermediate between the CuI particle and the PVP polymer. It was immediately found that the addition of NaI along with PVP but resulted in stable CuI small particle dispersions. These mixtures resulted in very stable dispersions that could be fully dried

and redispersed to similar particle size. Furthermore it was found that the produced particle sizes could be readily controlled through variation in the processing conditions.

Figure 3.25 – Proposed intermediate between CuI and PVP to improve stability



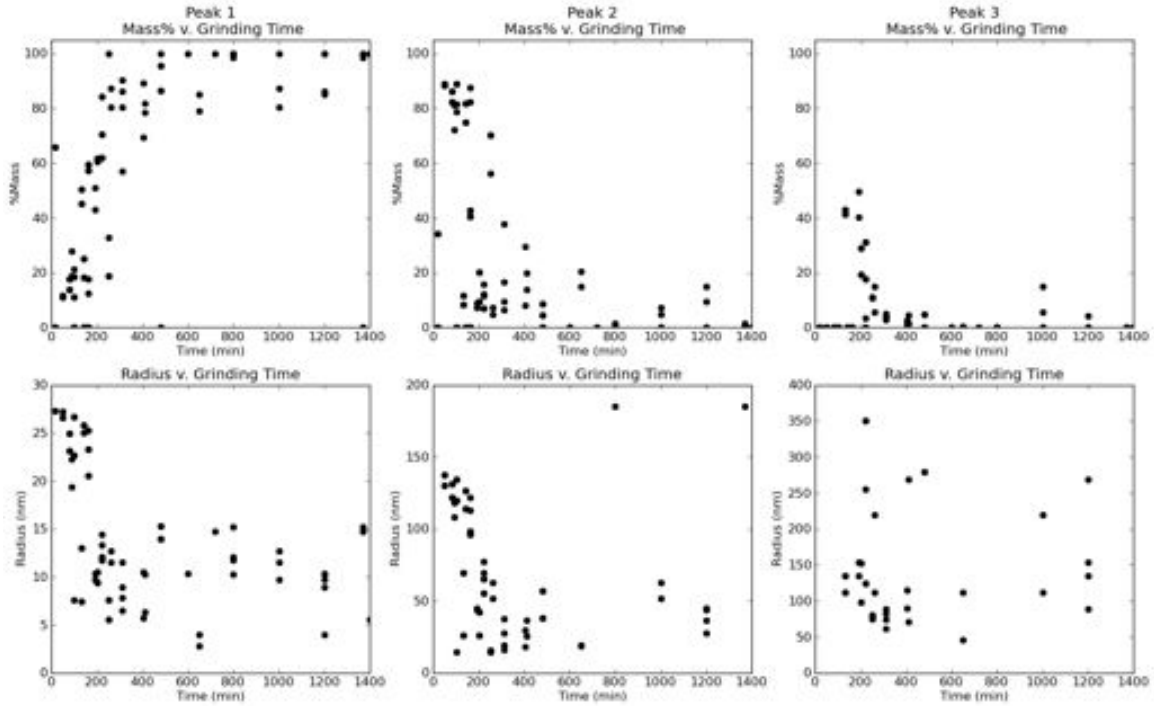
A preliminary formulation containing 50%CuI, 2% NaI, and 48% PVP was selected and a 10 g batch of this formulation was processed in 200 mL DI-water for 24 hours at 4200 rpm with 100μ YZT grinding media. This formulation was prepared several times and monitored throughout grinding by DLS.

These particle size measurements using DLS are shown in Figure 3.26, which were confirmed through scanning transmission electron microscopy (STEM) as shown in Figure 3.27. The morphology of the particles was as CuI particles embedded within a PVP polymer matrix wherein the size of the CuI particle tended to correspond with the particle sizes measured through DLS measurements.

Unlike the CuI/SLS system, this preparation resulted in rapid comminution of the primary particles, despite the use of similar grinding conditions. Typically three peaks were resolved. The distribution of these peaks suggested the rapid fracture of large CuI

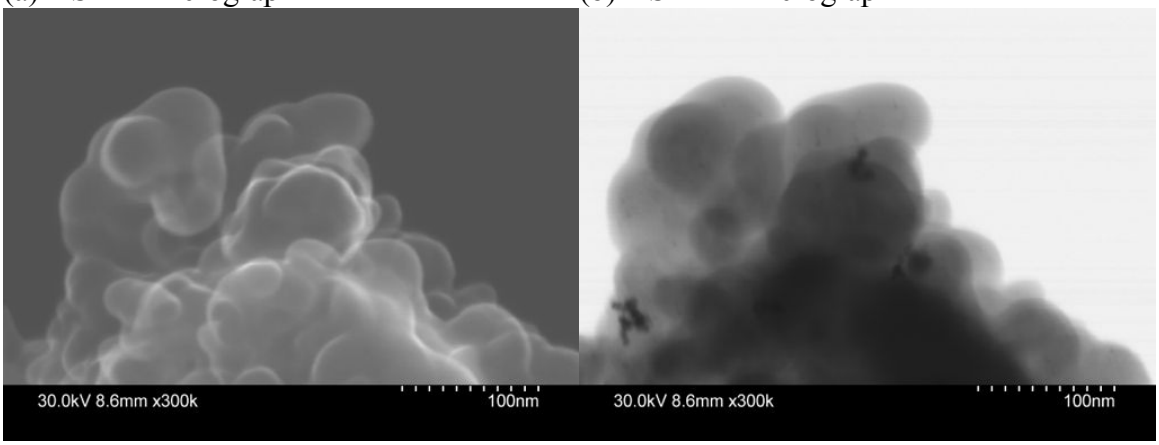
particles to form moderately size particles, followed by the slow generation of smaller particles. The rapidly formed moderate-sized particles were coincident with the ultimate particle sizes produced using the CuI/SLS system, which suggested that real comminution of the crystallites had not been actually occurring in the CuI/SLS system and that they were simply being dispersed.

Figure 3.26 – Particle Size Distributions for Grinding of CuI-PVP-NaI



Several batches of CuI-PVP-NaI material were prepared and size was monitored throughout grinding by DLS.

Figure 3.27 – SEM/STEM of 50%CuI, 2% NaI, and 48% PVP (600 minutes)
(a) – SEM Micrograph (b) – STEM Micrograph



It was further determined that both the PVP and NaI levels could be substantially reduced. A 90% CuI, 9%, PVP, 1% NaI formulation was selected for further development of optimal grinding procedures (CuI/PVP/NaI-90/9/1). Lower PVP/NaI formulations were capable of being comminuted to similar particle sizes and forming stable dispersions; however, these dispersions were incapable of being fully dried and redispersed to similar particles sizes without application of high shear using specialized equipment. Particles of CuI/PVP/NaI-90/9/1 were capable of being stored as liquid slurries or being dried and redispersed to similar particle sizes without application of high shear mixing in any form, and this formulation was ideal for the simple addition of the CuI additive.

Several additional formulations were prepared similarly utilizing the idea of a intermediate in order to investigate whether other salts would be effective. These were prepared as 10g of 90% CuI + 9% PVP + 1% intermediate, and milled for 24 hours in 200 mL water at 4200RPM with 100µ YZT grinding media. Other iodide salts (LiI, KI) provided some improved stabilization of the CuI/PVP particles. It was similarly found that the addition of Cu(I)Acetate, also provided improved stability to the CuI/PVP system

during grinding. The addition of Ag^+ as AgNitrate resulted in exceptionally stable suspensions of CuI/PVP particles.

In support of the view of these added salts as intermediates between CuI and PVP rather than simple electrostatic stabilization, it was observed that these added salts did not form stable particles in the absence of PVP.

The antimicrobial efficacy for these alternate formulations was generally comparable to that of the CuI/PVP/NaI formulation, and typically slightly better than the CuI/SLS formulation. It was found, however, that the CuI/PVP/NaI-90/9/1 particles were notably more effective against certain mycobacterium species. In particular, it was found that *Mycobacterium smegmatis* (a model organism for *Mycobacterium tuberculosis*) was more rapidly killed by CuI/PVP/NaI than by CuI/SLS at equivalent copper concentrations.

Table 3.10 – CuI Particulate Formulations against *P. aeruginosa*

Time	PBS Control	CuI/ SLS	CuI/PVP/NaI 90/9/1	CuI/PVP/$AgNO_3$ 90/9/1	CuI/PVP/CuOAc 90/9/1
	--	60 ppm Cu			
	Log10 Reduction				
15 min	0.34 ± 0.10	4.12 ± 0.04	>5.00 ± 0.00	4.99 ± 0.00	> 4.65 ± 0.49

Original titer = 4.94E+06 cfu / mL

M. smegmatis ATCC #14468, was tested as a fresh bacterial culture and after aging the organism for 24 and 48 hours to determine if metabolically slowed bacteria would also be readily killed and/or show similar different sensitivity to the CuI particles under different metabolic conditions. The results presented in Table 3.11 through Table 3.13 demonstrate that in all cases tested the CuI/PVP/NaI-90/9/1 particles were more effective than the CuI/SLS particles.

Table 3.11 – CuI/SLS or CuI/PVP/NaI against *M. smegmatis*, Fresh Culture

Time	PBS control	CuI/SLS 60 ppm Cu	CuI/PVP/NaI 60 ppm Cu
	Log_{10} Reduction	Log_{10} Reduction	Log_{10} Reduction
15 min	0.20 ± 0.08	0.81 ± 0.05	> 4.71 ± 0.00
1 hour	0.17 ± 0.08	> 4.71 ± 0.00	> 4.71 ± 0.00
24 hour	0.92 ± 0.09	> 4.71 ± 0.00	> 4.71 ± 0.00

Original titer =2.53E+06 cfu / mL

Table 3.12 – CuI/SLS or CuI/PVP/NaI against *M. smegmatis*, 24 Hour Culture

Time	PBS control	CuI/SLS 60 ppm Cu	CuI/PVP/NaI 60 ppm Cu
	Log_{10} Reduction	Log_{10} Reduction	Log_{10} Reduction
15 min	-0.01 ± 0.21	0.78 ± 0.01	3.50 ± 0.08
1 hour	0.04 ± 0.22	> 4.77 ± 0.00	> 4.77 ± 0.00
24 hour	0.88 ± 0.03	> 4.77 ± 0.00	> 4.32 ± 0.64

Original titer =2.93E+06 cfu / mL

Table 3.13 – CuI/SLS or CuI/PVP/NaI against *M. smegmatis*, 48 Hour Culture

Time	PBS control	CuI/SLS 60 ppm Cu	CuI/PVP/NaI 60 ppm Cu
	Log_{10} Reduction	Log_{10} Reduction	Log_{10} Reduction
15 min	0.03 ± 0.02	0.00 ± TNTC*	2.61 ± 0.01
1 hour	-0.14 ± 0.13	2.46 ± 0.54	4.40 ± 0.36
24 hour	0.63 ± 0.27	> 5.35 ± 0.00	> 5.35 ± 0.00

Original titer = 1.12E+07 cfu / mL

*Too numerous to count

In addition to these favorable antimicrobial results, it was found that other polymers could be used to produce particles with tailored properties in a similar manner using NaI as an intermediate. For instance, water insoluble graft copolymers were used to produce particles by milling in solvents that were not dispersible in water but would easily disperse in non-polar solvents (e.g. toluene). This reduced water-swelling problems of previous formulations in coatings and provided the ability to created tailored CuI

particle formulations for different materials. These modified formulations are discussed in chapter 4.

Depending upon the properties of the polymer, the dispersion behavior of the particles could be tailored to various materials matrices. Thus, the CuI-NaI-PVP system was decided upon as a useful formulation for further development as an AM additive. Since this formulation was prepared in water, it was decidedly easier to handle and was used as a model system for the development of other copolymer treated particles.

3.4. Production of PVP-NaI Stabilized CuI Small Particles

An experimental study was conducted to explore the impact of various processing conditions of the 90% CuI, 9%, PVP, 1% NaI formulation on the produced material. The goal was to investigate parameters relevant to producing these particles in an optimized processing system. Several processing variables were initially considered including: media size, media fill, media type (YZT or ZCG), grinding time, mill speed, pump rate, batch tank agitation rate, temperature, cooling (cooling applied to mill or batch tank, or both), slurry volume, and slurry solids concentration.

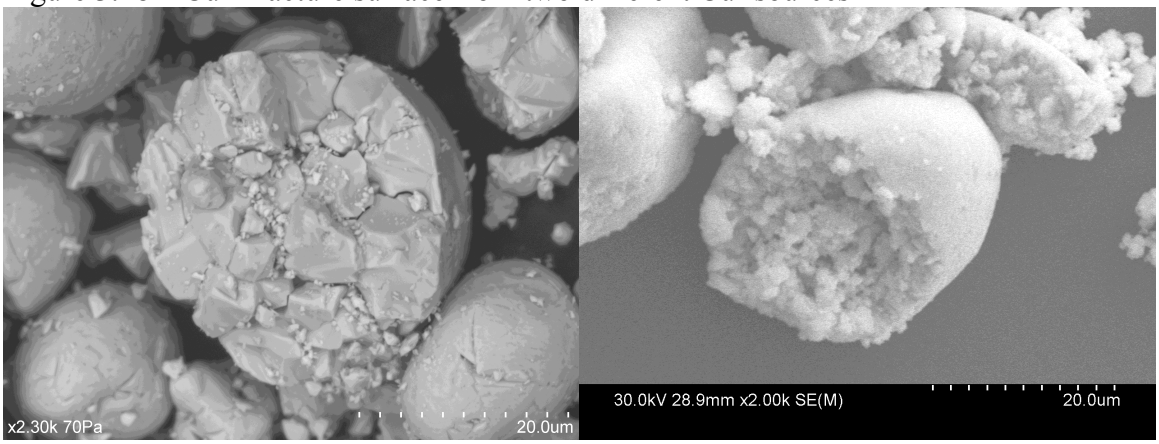
Preliminary exploration of grinding parameters suggested that changing the pump rate or batch tank agitation rates did not alter the produced particles, as long as caking was prevented and that feed particles could be carried entirely through the system (i.e. as long as minimum effective pump rates and agitation rates to prevent settling were observed such that the feed material did not form a hard pack cake within the mill). No difference was observed between the ZGC and YTZ media of the same size, but different

sizes resulted in obvious changes to the produced material. Mill speed as well as grinding time also affected the product quality, as expected.

Surprisingly, neither changes in temperature nor increasing slurry concentration up to around 40 wt% solids impacted the quality of the produced particles. The slurry viscosity did appreciably increase with concentration. However, it was observed that these slurries were highly thixotropic and thinned dramatically under high shear conditions. As such, the solids concentration did not seem to make a meaningful impact on the suspension viscosity due to the high shear conditions during grinding. Addition of chemicals which increased the viscosity (e.g. deacelyated chitosan or carboxymethylcellulose) resulted in immediate overpressurizing of the mill due to blinding of the separator screen.

The use of raw CuI of different starting feed sizes, from ~1μ to ~10μ in diameter, did not seem to impact the produced material. Similarly, the use of CuI from different manufacturers did not impact the product quality. This was likely due to the brittle nature of larger CuI particles as evidenced by microscopic examination of CuI as received from several different manufacturers, which indicated that large CuI particles were highly friable (Figure 3.28). Thus, larger feed material was likely rapidly shattered within the first few passes through the mill and was indistinguishable from smaller feed material after short grinding times.

Figure 3.28 – CuI Fracture surface from two different CuI sources



Media fill was generally maintained at a constant level recommended for the mill. Although deviation of this fill level did result in changes in the grinding rates of produced material, it was decided to maintain the optimum value suggested by the manufacturer of 140 mL.

Thus, mill speed, grinding time, and media sizes were varied in this study while the remaining parameters were typically maintained at fixed values per the above discussed rationale. Several batches of 90% CuI with 9% PVP and 1% NaI were processed through the mill according to various grinding conditions. Typically 100 g batches of 90% CuI, 9% PVP, and 1% NaI were milled in 200 mL DI-water and 100 µL samples were removed at various times throughout the grinding operation. The particle sizes of these prepared CuI samples were monitored throughout grinding.

It was quickly discovered that very CuI small particles could only be produced using the smaller, 100µ, grinding media at a high energy input. Greater total energy input using 100 µ media resulted in more translucent dispersions, and smaller particles were measured by DLS for these dispersions. Interestingly, however, in some cases similar particle sizes were measured by DLS for different grinding conditions, which had very

different dispersion stability. To address these conflicting data, several samples were analyzed by SEM/STEM to inspect the morphology of the solid dispersions and contained CuI particles.

3.4.1. Solid Dispersion Morphology

The morphology of several grinding runs was examined using scanning electron microscopy (SEM) and scanning transmission electron microscopy (STEM). 10g batches of 90% CuI, 9% PVP, and 1% NaI formulation were processed at 4000RPM for 6 hours, at 2000 RPM using 300 μ media for 6 hours, or at 1000RPM with 100 μ media for 24 hours. The product of each preparation was dried and redispersed to 2000 ppm Cu and dropped on to a lacey carbon grid for analysis and allowed to dry at ambient conditions. Some observations of these dispersions are shown in Table 3.14.

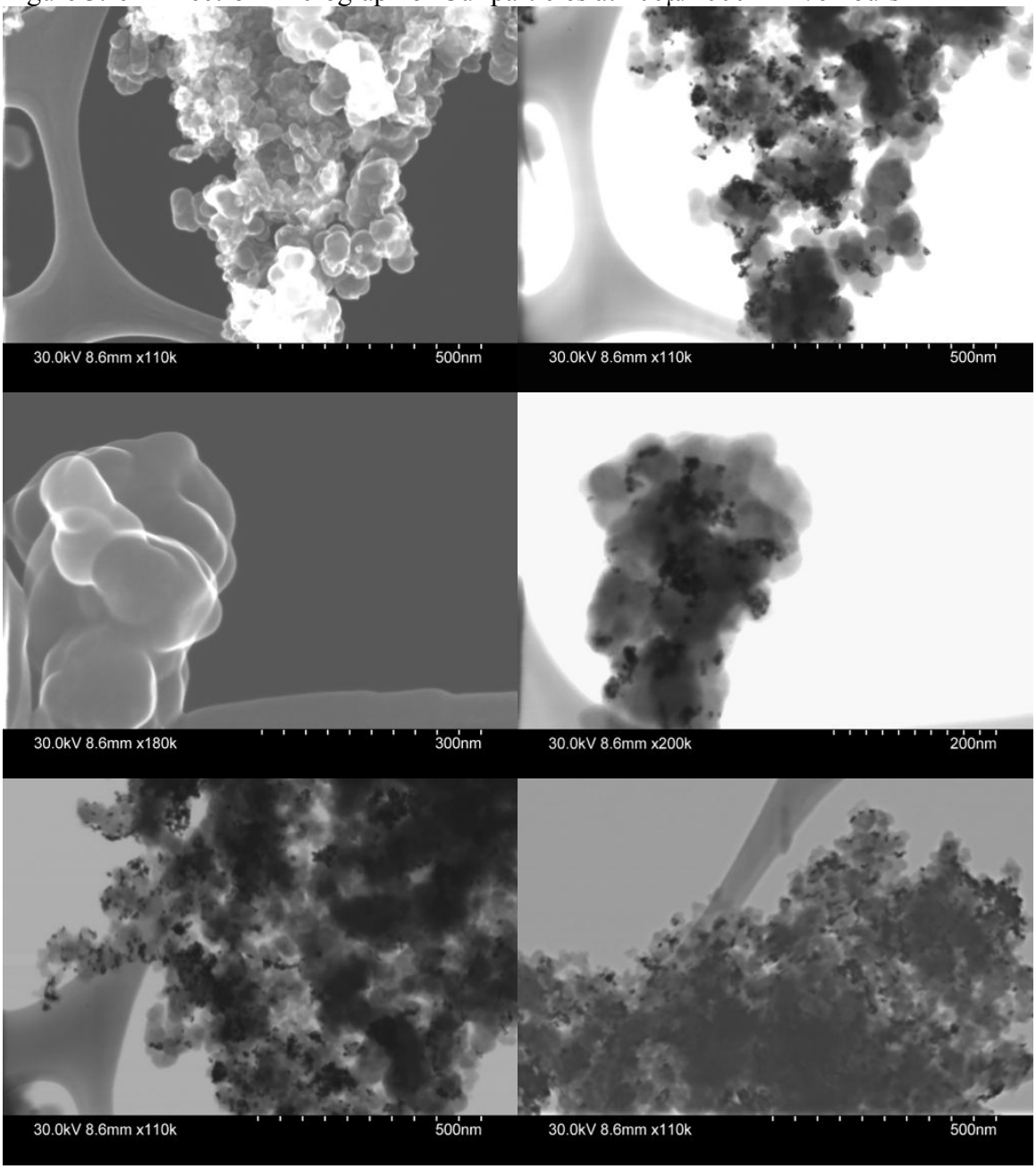
Table 3.14 – Appearance of several CuI materials after various processing conditions

Grinding Conditions	Appearance	Stability	Particle Size (DLS)*
100μ/4000RPM/6Hours	Translucent	Good	10-30 nm
300μ/4000RPM/6Hours	Opaque	Good	100-200 nm
100μ/1000RPM/24Hours	Opaque	Poor	200-1000 nm

*Particle size is reported for the peak with the dominant mass fraction

For the 90% CuI, 9% PVP, and 1% NaI processed with 100 μ media at 4000 RPM for 6 hours, the product appeared similar to the 50% CuI particles described in 3.3.4 and consisted of a dispersion of CuI particles in a PVP polymer matrix. The SEM image indicated that the dried material consisted of large pieces and the STEM indicated that CuI particles were dispersed throughout these pieces. The elemental identity of the observed particles was confirmed using energy-dispersive X-ray spectroscopy (EDS) to be CuI.

Figure 3.29 – Electron Micrograph of CuI particles at 100µ/4000RPM/6 hours



The particle sizes measured by DLS were similar to the particles sizes observed for the primary particles in STEM imaging. This material was capable of being fully dried and redispersed without increase in particle size as measured by DLS. This material appeared as a translucent suspension.

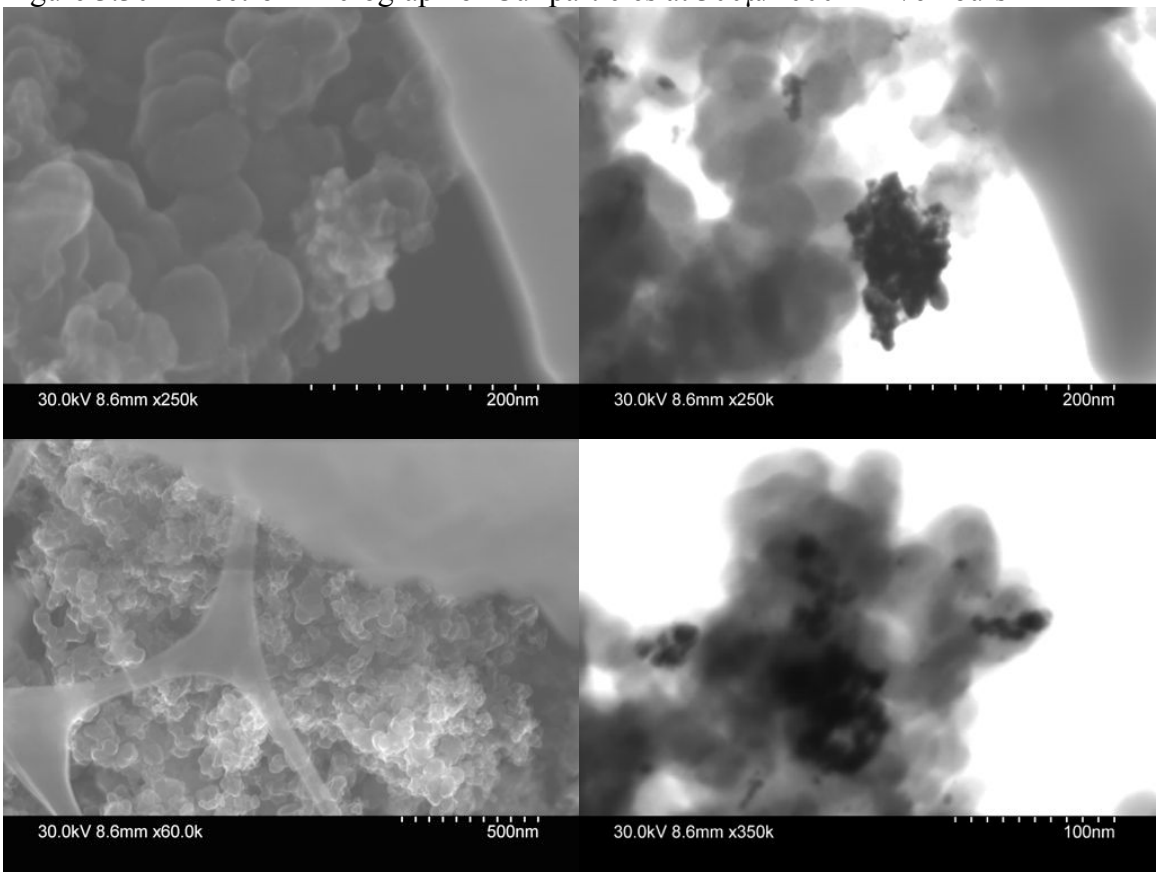
For the 90% CuI, 9% PVP, 1% NaI formulation processed with 300 μ media at 4000 RPM for 6 hours the produced material was notably different from that produced by the 100μ/4000RPM route. Surprisingly, primary CuI particles appeared to be the same size, but a significant number of large CuI aggregates were observed.

These aggregates consisted of smaller primary particles that appeared similar in size to those produced by the 100μ/4000RPM route. The identity of these aggregates and particles was again confirmed to be CuI by EDX.

The sizes of the aggregates, but not the primary particles, corresponded well with the DLS measurements of this sample. Despite the aggregated state of the CuI particles, these dispersions were quite stable and this material was capable of being fully dried and redispersed without change in particle size as measured by DLS, which indicated that aggregation was a function of mill conditions during processing.

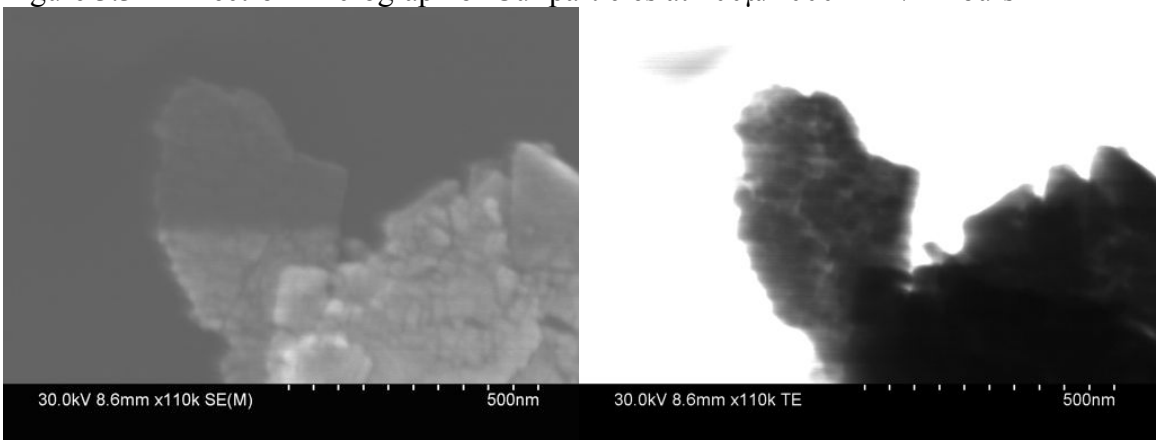
High shear mixing of the material using a high-speed disperser did not affect particle size as measured by DLS. This material appeared significantly more opaque as compared to the 100μ, 4000 RPM, 6 hours processed material.

Figure 3.30 – Electron Micrograph of CuI particles at 300µ/2000RPM/6 hours



For the 90% CuI, 9% PVP, 1% NaI formulation processed with 100 µ media at 1000 RPM for 24 hours, the produced material was significantly different from that produced by the 100µ/4000RPM route and the 300µ/4000RPM route. In this case, primary CuI particles were not easily identifiable or separable as they appeared in the former cases. The product appeared to consist fragmented but non-dispersed CuI, with composite sizes similar to the fragments observed for the shattered CuI bulk particles and CuI/SLS particles. This material was unstable and settling occurred within days for the produced slurry and very rapidly for the dried and redispersed material.

Figure 3.31 – Electron Micrograph of CuI particles at 100µ/1000RPM/24hours



These results are indicative of three scenarios. The 100µ/4000RPM process supplied sufficient energy to fracture and disintegrate the CuI and the impact events were sufficiently low impact that the fragments were dispersed rather than aggregated. The 100µ/1000RPM process supplied sufficient energy to shatter the bulk CuI particles but not enough to comminute the resultant fragments. The 300µ/4000RPM process supplied enough energy to comminute CuI, however, an excess of high-energy impacts resulted in compaction and aggregation of the CuI fragments rather than dispersal of the material. These results are consistent with the energy considerations graphically presented in Figure 3.7.

This demonstrates that CuI may be dispersed without being comminuted, but also that it may be comminuted without being well dispersed. The product depends both on the rate of energy input and total energy input. The rate of energy input can be described macroscopically as the rate of energy supplied to the mill and more appropriately as the energy supplied during media-product collision events.

Although, it is useful to distinguish between deagglomeration and comminution with respect to production capacity, it is apparent from these results that two processes

cannot be separated when very small particles are produced using wet media milling. In particular, the term “mild dispersion” has recently been popularized in the pigment processing literature for the redispersal of finished pigment particles using wet media milling. During wet media milling for the redispersal of finished pigment, it is generally recommended to apply low stress energy (i.e. small grinding media/low tip speed) to avoid destruction of specialized surface chemistries (e.g. silanes). From the present results, it seems that this teaching is equally important during the initial comminution of particulate matter.

To this end, several literature accounts on the ultimate grinding limits for various materials after very long times should be reconsidered with these concepts in mind. That is, the ultimate particle size achievable through grinding processes has likely not been achieved in studies where larger media are used. Or in cases where only hydrodynamic radii are measured (e.g. dynamic light scattering, laser diffraction particle size analysis), the prepared particles may in fact consist of aggregates.

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Chapter 4. CuI Small Particles with Modified Surface Chemistry

The previous chapters described the selection of surface modified copper iodide small particles as an antimicrobial additive and their production using wet media milling. The preference for use of PVP with NaI to form CuI particles of controllable size and with good redispersion characteristics was demonstrated. These particles have heretofore not been useful in non-aqueous matrices such as organic based solvent coatings or thermoplastics.

This chapter demonstrates the development of CuI small particles prepared by wet media milling that have been modified to be compatible with various non-aqueous systems. These formulations are mostly accomplished through the use of modified polymeric capping agents. The compatibility of these modified CuI formulations in various matrices is assessed with regard to their AM performance.

4.1. CuI Small Particles Using Vinyl-Acetate-PVP

The CuI-PVP-NaI particles described in Chapter 3 using wet media milling contained significantly less stabilizing agents than the particles described in Chapter 2 using wet chemical synthesis methods. These milled particles, however, still contained a relatively large amount of water-soluble polymer, PVP, which precluded their utility in certain applications such as in thermoplastics or with non-polar solvents where they could not be easily dispersed. Furthermore, large additions of the CuI-PVP-NaI particles to coating formulations resulted in negative effects on the coatings.

It was found that vinyl-acetate-PVP (e.g., BASF VA64) block copolymers were useful in their ability to form dryable and redispersible CuI small particles. They were

additionally capable of being more permanently incorporated into coating preparations. Coating formulations prepared using CuI small particles with VA64 instead of PVP resulted in reduced swelling and improved dispersibility in some solvents (e.g. MIBK) at higher CuI loading. It was then found that other copolymers were also useful and could be tailored to be compatible with different matrices.

4.2. Functionalized CuI Small Particles with Other Copolymers

This section summarizes the motivation for the use of small particles of CuI modified with other copolymers as an AM additive. Attention is given towards the comparative AM efficacy of particles prepared using differently copolymers in different matrices.

4.2.1. Introduction

The PVP/NaI stabilized CuI particles described in Chapter 3 demonstrated very high efficacy in the dispersed state, but the water soluble PVP limited the utility of the material in coatings due to swelling and disintegration at high CuI loading. Furthermore, the use of these particles was further limited by their inability to disperse in various matrices and solvent systems. In the previous section, the use of vinvylpyrrolidone-vinylacetate co-polymers with CuI was found to be a useful solution for creating improved CuI small particles. It was recognized, however, that the modification of the VA64 polymer was not practical and that alternative solutions were needed.

In particular, it was preferred the particles not be dispersible in water, such that they could be incorporated into various materials at a higher loading without rapid leaching of the particles upon wetting. Further, the protective polymer must be capable of

being tuned to the matrix in which it is to be added. Certain copolymers were found to be useful for accomplishing this task.

Nanoparticles of cuprous iodide were prepared using these other copolymers as a surface modifier with sodium iodide. The dispersibility of the nanoparticles in different solvents was controlled by appropriate selection of said copolymer. In order to demonstrate the importance of compatibility between the CuI particles and matrices to which they would be added, several combinations of different particles and matrices were prepared and evaluated for AM efficacy.

4.2.2. Materials and Methods

Preparation of CuI Particles

Copper iodide nanoparticles were prepared as described in Chapter 3 by wet media milling. Briefly, 4.95 g CuI, 4.95 g of copolymer, and 0.1 g NaI, and 200 mL of solvent were processed in a ceramic lined Netzsch MiniCer wet media mill using 100 micron yttria stabilized zirconia grinding media at a mill speed of 4000 RPM for 6 hours. The solvent used for each run was varied according to the copolymer used. The polymer and solvent combinations used to prepare the different copper iodide nanoparticles are described in Table 4.1.

Table 4.1 – Modified CuI Particles, Formulations

CuI ID	Copolymer	Solvent
1	A	Ethanol
2	B	Isopropanol
3	C	Toluene

Size Measurement of CuI Nanoparticles

The milled slurries were subsequently fully dried under reduced pressure and a portion of the dry powder was redispersed in the same solvents for particle size measurements.

The particle sizes of the produced particles were measured using laser diffraction (LA-950, measured at Horiba Facility, CA) and/or dynamic light scattering (DynaPro NanoStar, Wyatt) techniques in the same solvents that they were milled . Laser diffraction and/or dynamic light scattering indicated similar hydrodynamic radii for all samples as shown in Table Y.

Table 4.2 – Modified CuI Particles, Particle Sizes

CuI ID	Solvent	Hydrodynamic Particle Diameter
1	Ethanol	51 nm (LD; D_{50}), 62 nm (LD; D_{50}), 75 nm (LD; D_{50}), 45 (DLS)
2	Isopropanol	49 nm (LD; D_{10}), 60 nm (LD; D_{50}), 69 nm (LD; D_{90}), 49 (DLS)
3	Toluene	43 (LD; D_{10}), 54 (LD; D_{50}), 66 (LD; D_{90})

LD = Laser diffraction; DLS = Dynamic Light Scattering (dominant peak size)

Preparation of AM Coatings

Three different matrices were used to compare the effect of nanoparticle dispersibility on the antimicrobial effects for three of the particle formulations. The CuI-20VP-80C30, CuI-50VP-50C16, and CuI-90VP-10C4 particle formulations were each dispersed separately in each of three coating systems as described in Table C. Each copper iodide nanoparticle formulation was added to each coating matrix to achieve 0.1% copper in the dry solids.

The CuI particles were added directly to each coating formulation as described in Table 4.3. The particles were added to the ethyl cellulose and acrylic coating

formulations at room temperature and to the paraffin wax at 85°C (molten). All coating mixtures were mixed at 500 rpm for 60 minutes with a magnetic stirrer.

Table 4.3 – Modified CuI Particles, CuI Particle Containing Coatings

Matrix ID	Solvent	Coating Formulation
Ethylcellulose	Ethanol	10% Ethyl cellulose (Dow), 90% Ethanol
Acrylic	MIBK	60% MIBK, 20% pentaerythritol acrylate, 9% ethanol, 1% Irgacure 2959 (BASF), 0.1% fluorosurfacant 4959 (3M)
Polyolefin	None	Paraffin Wax (Tm , 65°C) [model system for polyolefin]

*MIBK = Methyl isobutyl ketone

The coating formulations were spin coated on 2"x2" pieces of aluminum. The paraffin wax samples were melted and spin coated on preheated pieces of roughened aluminum and allowed to solidify. The liquid coating formulations were spin coated and dried overnight at 85°C. Coating B was subsequently UV cured. Standard coatings without added CuI were also prepared for each coating matrix.

Testing of Antimicrobial Coatings

Antimicrobial evaluation of the coatings was conducted using a modified version of the Japanese Industrial Standard (JIS) method Z 2801 (Japanese Standards Association 2000). *P. aeruginosa* (ATCC #9027) was cultured on Trypic Soy Agar. Test cultures were grown for 16 hours (overnight) in Trypic Soy Broth at 37°C. The culture was subsequently centrifuged, washed, and redispersed to approximately 0.5 McFarland in sterile saline (0.9% NaCl) before antimicrobial testing.

Briefly, four 1/2" diameter rounds were punched from each coated aluminum piece. Each CS-2 and CS-3 coating was sterilized using 75% ethanol. The CS-1 coatings were sterilized by UV light and dry heat. Two coatings were inoculated with 10 microliters of an overnight culture of *P. aeruginosa*. Each round was contained in wells

of a 24 well plate. The inoculated samples/plates were then stored in a 100% humidity chamber at 37°C for 24 hours.

After 24 hours the samples were neutralized with 1 mL of DE broth. Each neutralized sample was further diluted to 10^{-5} in saline and plated in duplicate on tryptic soy agar. Samples were enumerated after 24 hours.

4.2.3. Results

Table 4.4 – Efficacy of CuI Particles in Matrices against *P. aeruginosa*

Matrix ID	CuI Particle ID	Log Reduction
Ethocellulose	1	-0.51 ± 0.52
	2	0.68 ± 0.02
	3	>5.40 ± 0.00
Acrylic	1	0.99 ± 0.32
	2	>5.40 ± 0.00
	3	1.22 ± 0.14
Polyolefin	1	>5.40 ± 0.00
	2	1.10 ± 0.03
	3	-0.18 ± 0.18

4.2.4. Discussion

These results demonstrate the dramatic dependence of proper dispersion compatibility of AM additives in the matrices to which they are added. These data demonstrate the strong dependence on having a properly formulated additive per each matrix. Similar results, although not presented here, have also been achieved for other common antimicrobial additives.

Based on these data it appears that small amounts of copper iodide can be used as an AM agent when supplied as a surface modified small particle that is compatible with a given matrix. The result of using small amounts of small particles is that matrices can be

prepared with AM properties that are less colored or transparent. These applications are discussed in the next chapter.

Chapter 5. Applications of Surface Modified CuI Small Particles

The development of CuI small particles as an antimicrobial additive was described in the previous chapters. This chapter is directed to the use of these CuI small particles in various applications. Focus is mostly placed on antimicrobial applications. Several non-antimicrobial uses of these materials are also presented.

5.1. Antimicrobial Coatings

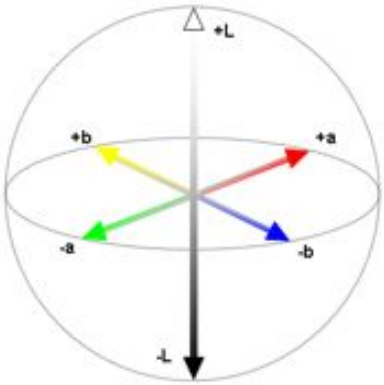
In the foregoing chapters, many antimicrobial coatings were presented in order to explain some of the motivations behind certain aspects of surface modified CuI small particles. This section provides a summary of some of the antimicrobial surfaces prepared using these particles.

UV Cured Acrylic Coatings

UV cured PETA acrylic coatings were prepared with and without the addition of vinyl-acetate vinylpyrrolidone modified CuI particles and tested for antimicrobial efficacy in partnership with Jason Torrey according to a modified JIS z2801 test using a 24 hour exposure time against *P. aeruginosa* and *S. aureus*. These CuI containing coatings were indistinguishable from standard acrylic coatings according colorimetric and reflective haze values (not shown) using an UltraScan XE Colorimeter by Hunter Labs.

Table 5.1 – Colorimetric Readings of CuI and Standard Acrylic Coatings

Acrylic Coatings	L*	a*	b*
0.5% Cu	82.64	-0.04	2.46
0.25% Cu	83.03	-0.05	2.43
0.10% Cu	84.01	-0.12	1.93
0.05% Cu	82.05	0.02	2.72
Standard Coating	82.03	-0.06	2.25



CIE 1976 (L*, a*, b*) Color space

Table 5.2 – Efficacy of CuI Acrylic Coatings against *P. aeruginosa*

Time	Standard	0.05% Cu
	Log_{10} Reduction	Log_{10} Reduction
24 hour	-0.68 ± 0.07	> 4.70 ± 0.00

Original Titer = 1.27E+06 cfu/mL

Table 5.3 – Efficacy of CuI Acrylic Coatings against *S. aureus*

Time	Standard	0.05% Cu
	Log_{10} Reduction	Log_{10} Reduction
24 hour	0.35 ± 0.21	> 4.40 ± 0.00

Original Titer = 1.25E+06 cfu/mL

Common Latex Paint

A commercial latex paint was acquired from a major paint company and modified with and without copper iodide (as CuI/SLS, see Chapter 3) and tested for antimicrobial efficacy in partnership with Jason Torrey according to a modified JIS z2801 test using a 6 hour exposure time against *P. aeruginosa*. The results shown in Table 5.4 demonstrate that the addition of CuI results in a dramatically improved antimicrobial efficacy.

Table 5.4 – Commercial latex paint with and without CuI against *P. aeruginosa*

Time	Sherwin Williams Harmony Paint	Sherwin Williams Harmony AM paint + 0.05% Cu
	Log_{10} Reduction	Log_{10} Reduction
6 hour	1.05 ± 0.15	> 6.06 ± 0.00

Original Titer = 5.77E+06 cfu/mL

5.2. Antimicrobial Wound Coverings

As with coatings and textiles, silver is a common antimicrobial material used in the preparation of antimicrobial wound dressings for the prevention of infection. Several infection control wound dressings and wound creams were prepared are compared to currently available medical silver and other wound dressings and creams.

5.2.1. Copper Iodide Based Wound Dressing Against Established Biofilms

An antimicrobial wound dressing for the elimination of developed biofilms were developed in cooperation with a major wound-dressing manufacturer. The requirement was for the elimination of established biofilms using a topical dressing. Wound dressings were prepared using hydroentangeled-polyester-cellulose textile substrates. The substrates were soaked in a dispersion of CuI-NaI-PVP particles and then dried to achieve a loading of 65 mg Cu / 100 cm2. The dressings were prepared with the addition of ascorbic acid and sodium ascorbate as an antimicrobial intensifier through supplying additional reducing power to convert cupric to cuprous ions. Additional control samples (without CuI, with only the hydroentangeled cellulose, and with nothing) and commercial silver dressings were also evaluated comparatively using a biofilm elimination assay.

Biofilm elimination testing was carried out against 24-hour-old biofilms through a partnership with Jason Torrey. The following procedure was used to determine the antimicrobial efficacy of wound dressings containing functionalized particles. Wound dressings were provided as sterile 10 mm diameter disks. An overnight culture of *P. aeruginosa* (ATCC #9027) was prepared as previously described but was not centrifuged or washed. Polycarbonate membrane films (25 mm diameter, 0.2 μm pore size, Whatman) were sterilized by autoclaving and placed in the center of TSA plates. The overnight culture was diluted 1:10,000 in PBS and 0.01 ml was inoculated as a single spot in the center of each membrane filter. These plates were incubated at 37°C for 24 hours to allow for the formation of bacterial biofilms. After growth, each membrane filter was transferred to a fresh TSA plate. Wound dressing samples were moistened with 0.05 - 0.2 ml DI water and applied gently to the biofilm spot. The biofilms with wound dressings were incubated at 37°C for 18 hours. Each wound dressing and membrane filter were transferred to 10 ml of D/E neutralizing broth, vortexed for 1 min and sonicated for 1 minute (described previously) to release bacteria from the surfaces. The neutralized samples were diluted/enumerated as described previously. Bacterial reductions were calculated and compared with the control dressings containing no antimicrobial agent and biofilm control samples without applied wound dressings.

Table 5.5 – Antimicrobial Wound Dressings Against 24 Hour Biofilm

Replicates after 18 hours	CuI 65 mg Cu / 100 cm2	Aquacel Ag ~10 mg Ag / 100 cm2	Acticoat ~100 mg Ag / 100 cm2
	Log_{10} Reduction (n=10)	Log_{10} Reduction (n=10)	Log_{10} Reduction (n=10)
Average	7.63 ± 0.00	0.64 ± 0.37	3.12 ± 3.18**

** These wound dressing demonstrated very high variability

Table 5.6 – Control Wound Dressings Against 24 Hour Biofilm

Replicates after 18 hours	Ascorbic/Ascorbate with NaI/PVP Control	Polyester-hydroentangeled cellulose substrate control	Biofilm Growth Control
	Log_{10} Reduction (n=3)	Log_{10} Reduction (n=3)	Log_{10} Reduction (n=3)
Average	2.51 ± 4.44**	-0.65 ± 0.04	-0.42 ± 0.03

** These wound dressing demonstrated very high variability

The Ascorbic/Ascorbate and CuI-NaI-PVP mixture on polyester-hydroentangeled cellulose substrate at 65 mg Cu / 100 cm2 wound dressings were supplied as a sample to a third party that similarly found very good efficacy, but dermal studies using porcine epidermis in-vitro revealed a staining issue associated with the ascorbic acid additive. In the absence of ascorbic acid it was found that the CuI based wound dressings provided inconsistent results unless the CuI level was increased. Alternative non-staining, reducing, organic acids were found to be effective replacements for ascorbic acid.

5.3. CuI Based Wound Cream

The common topical antimicrobial ointments, e.g., bacitracin (e.g. Neosporin) are mainly effective against gram-positive bacteria and have clinical issues relating to

antimicrobial resistance. Copper iodide containing ointments are believed to have potential as effective topical antimicrobial ointments.

Several CuI based wound creams ranging from were prepared and compared to commercially available ointments using a disk diffusion type assay. Similar zones of inhibition were achieved against *S. aureus* with commercial bacitracin ointment (0.9% bacitracin) and CuI containing cream. A significantly greater zone of inhibition for CuI containing cream compared to commercial bacitracin ointment (0.9% bacitracin) was achieved against *P. aeruginosa*.

5.4. Antimicrobial Surface Cleaners with Residual Disinfecting Films

Most common surface disinfectants dissipate by evaporation or otherwise become deactivated shortly after their application. Several products exist to effect both an immediate cleaning as well as leave a residual amount of AM agent to prevent further contamination. These are mostly silver based (commonly silver di-citrate, SDC). CuI is believed to be a more economical replacement for SDC based cleaning agents. A cleaning formulation was prepared using CuI and film-forming agents to achieve both an instantaneous surface disinfection and leave a residual antimicrobial layer to treat subsequent recontamination events.

The cleaning formulation was prepared containing 60 ppm Cu as CuI/VA64/NaI (see 4.1) dispersed in a solution of acetic acid, isopropanol, and chitosan biopolymer. The acetic acid-isopropanol provided sufficient antimicrobial activity to provide immediate (≤2 minute exposure) sterilization of the surface. Chitosan is polysaccharide derived from the biopolymer chitin. It is soluble in low pH solutions. Upon evaporation the acetic acid

from the cleaned surface, the chitosan formed a CuI containing, water-insoluble, antimicrobial thin film. This thin film was tested according JIZ z2801 after up to 1 month of storage and >3 log reductions were achieved for both *S. aureus* and *P. aeruginosa*.

Chapter 6. Summary and Outlook

6.1. Summary

Environmental microorganisms result in large costs to many various areas. They are implicated as the causative agents in a large portion of the occurrences of healthcare associated infections (HAI) and antimicrobial resistant infections (AMR), which result in increased costs and human suffering. Furthermore, microbiologically induced degradation of materials and biofouling of various systems results in a tremendous amount of damage to many different materials in many different sectors.

Current antimicrobial strategies have either not been effective in eliminating these microbes effectively through active strategies (e.g. enhanced cleaning). Self-disinfecting antimicrobial materials are a promising solution to this problem. Copper metal and it's alloys were the first generation of materials used as a self-disinfecting surfaces and they have been shown to reduce the rate of HAI's by 50% in some clinical settings. Copper metal, however, is expensive and impractical for many applications. High concentration (16 w/w%) copper oxide loaded polymers have recently received attention as an alternative self-disinfecting antimicrobial surface; however, this material is limited to a narrow variety of uses and cosmetic limitations.

The goal of this work was to develop an additive for common materials systems with minimal change in the processing conditions and a minimal change in the finished materials. This goal was achieved through a series of developments (Chapters 2-5).

In Chapter 2, small particles cuprous iodide was decided upon as a preferred antimicrobial agent. First, bulk CuI was shown to be more effective than various other materials as an AM agent. Next, a synthetic route was employed to produce small particles of CuI, which were shown to be more effective than bulk CuI material. These small particles, however, were of minimal usefulness because of the large amount of protecting capping-agent required to maintain them in a dispersed state and the inconvenient method of manufacture.

In Chapter 3, a convenient method of producing small particles of CuI in an efficient and scalable manner was developed. This allowed the CuI formulation to be improved upon and for a stable system with dramatically improved properties to be developed. These small particle CuI formulations were much more useful, however, still limited to only a few systems (e.g. polar solvents).

In Chapter 4, a series of CuI small particle formulations were prepared using a series of modified capping agent to control the dispersibility of the finished materials in a variety of different materials ranging from hydrophilic to hydrophobic. Additionally, a series of materials were produced using some of these CuI formulations in order to demonstrate the importance of compatibility between the additive and the matrix. In the absence of proper dispersion compatibility, significantly more antimicrobial agent was required to achieve equivalent antimicrobial efficacy. It is important to note, that these materials could not be produced using traditional wet-chemical precipitation methods because of a lack of solvent compatibility with the capping agent and bulk CuI.

In Chapter 5, a variety of applications utilizing the materials described in Chapters 3 and 4 were provided. These applications include various coatings, liquid additives to cosmetics, and preliminary medical applications of these materials.

6.2. Outlook

Self-disinfecting antimicrobial materials are a relatively new class of materials, which make claims to prevent disease through the elimination of environmental microorganisms. Only a few technologies exist, which are capable of meeting the requirements necessary to make self-disinfecting surface claims; and of these, only copper metal alloys have received significant attention and demonstrated clinical efficacy.

Surface-modified copper iodide small-particles can be used to achieve antimicrobial efficacy in any number of materials/systems with potential applications ranging from materials-preservation to self-disinfecting surfaces. A next step in the development of copper iodide and other self-disinfecting technologies is to further explore the areas where these materials may make a beneficial impact on public health and on which surfaces they might be most useful.

In conclusion, I recommend further work be directed towards the evaluation of self-disinfecting materials in a controlled settings in order to determine the responsible applications where disease prevention might provide both cost and human health savings through their use.

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