

Utilization of Fluorescent Microspheres as a Surrogate for *Cryptosporidium* Removal in
Conventional Drinking Water Treatment

by

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ABSTRACT

The purpose of this study was to determine the applicability of fluorescent microspheres as a surrogate to measure the removal of *Cryptosporidium* oocysts through the coagulation, flocculation, sedimentation, and filtration steps of conventional water treatment. In order to maintain accuracy and applicability, a local water treatment facility was chosen as the system to model. The city of Chandler Arizona utilizes conventional treatment methodologies to remove pathogens from municipal drinking water and thus the water, coagulant, polymer, and doses concentrations were sourced directly from the plant. Jar testing was performed on four combinations of coagulant, polymer, and fluorescent microsphere to determine if the log removal was similar to that of *Cryptosporidium* oocysts.

Complications with the material properties of the microspheres arose during testing that ultimately yielded unfavorable but conclusive results. Log removal of microspheres did not increase with added coagulant in the predicted manner, though the beads were seen aggregating, the low density of the particles made the sedimentation step inefficient. This result can be explained by the low density of the microspheres as well as the potential presence of residual coagulant present in the system. Given the unfavorable properties of the beads, they do not appear to be a suitable candidate for the surrogacy of *Cryptosporidium* oocysts in conventional drinking water treatment. The beads in their current state are not an adequate surrogate; however, future testing has been outlined to modify the experiment in such a way that the microspheres should behave like oocysts in terms of physical transportation.

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1.0 INTRODUCTION

1.1. Background

Drinking water treatment is perhaps the most important aspect of a community's development. Without clean drinking water, a population cannot thrive and develop into the modern and sustainable societies that the world so desperately needs. A common method of removing contaminants from drinking water is the conventional drinking water treatment process that includes coagulation, flocculation, sedimentation, and filtration. This process removes contaminants via formation of large clumps (flocs) that then settle out of the water in the sedimentation phase. The efficiency of this process was historically measured by the decrease in the water's turbidity; however, in recent years, the actual log removal of pathogens has been applied to coagulation, flocculation, and sedimentation steps of the water treatment process.

In researching pathogen removal through conventional water treatment, it was discovered that there is a gap in knowledge regarding the use of fluorescent microspheres as a surrogate to measure the removal of *Cryptosporidium* oocysts. *Cryptosporidium* is an enteric parasite with a low infectious dose of a single microbe and thus its removal is highly monitored.

The concept of a surrogate in water treatment is a substitute for a targeted pathogen that is likely easier to detect, less harmful if consumed, and more resilient to treatment variables like adverse environmental conditions, presence of microbes, treatment residuals, etc. Surrogates can be used to determine the performance of treatment processes without directly handling potentially harmful contaminants.

The measure of microbial log removal for drinking water treatment is done by simply measuring influent and effluent concentrations; however, when measuring microbes that are sensitive to a multitude of potential factors it is best to instead measure a surrogate that is not as sensitive and thus yield a more conservative result. The utilization of fluorescent microbeads was suggested due to several physical characteristics that would make them an optimal choice in water treatment. The first and possibly most important characteristic is that the microspheres are inanimate. Non-living surrogates can provide a more conservative representation of physical removal because they cannot be broken down by microbes or residual chemicals present in water. Additionally, microspheres are much easier to detect than their living counterpart because they do not require culturing, thus, eliminating a source of error in the detection process. Given that there is little existing literature on the topic of microspheres as surrogates for *Cryptosporidium* oocysts in coagulation, flocculation, sedimentation, and filtration the results were unpredictable but nonetheless useful.

1.2. Objective

The goal of this study is to determine the viability of a potential surrogate to measure the removal of *Cryptosporidium* oocysts during the conventional treatment processes.

- Evaluation of removal efficiencies for coagulation, flocculation, sedimentation, and filtration.
- Determine the impact of coagulant dose variations on removal efficiency.
- Evaluate the use of fluorescent microspheres as a surrogate for *Cryptosporidium* oocyst removal.

- Compare settling velocities of known and potential surrogates to that of the targeted pathogen to determine viability of surrogacy.

2.0 LITERATURE REVIEW

2.1. Conventional Drinking Water Treatment

2.1.1. History

Water purification through coagulation processes is no new technology. Aluminum sulfate (Alum) was used as a coagulant in water treatment as early as the 17th century; however, it is known that Alum was used by the illiterate poorer class along the great Chinese Rivers and thus it is believed that Alum was used regularly prior to this documentation (Pearls 2015). Many centuries before the addition of chemical coagulants, settling basins were utilized by cultures such as the ancient Minoans to decrease turbidity and provide the community with cleaner drinking water (Mays 2012). This utilization of gravity as a treatment methodology was prevalent for generations before the invention of regulated conventional treatment processes.

2.2. Treatment Processes (Coagulation, Flocculation, Sedimentation, and Filtration)

2.2.1. Overview

Conventional water treatment (coagulation, flocculation, sedimentation, and filtration) is used to remove drinking water contaminants present as particles, organics, and chemicals. Typically the contaminants targeted by coagulation and flocculation are those that are stable in a water source and thus not likely to settle out on their own. The usage of a coagulant (potentially with a polymer) destabilizes contaminants and thus makes them vulnerable to gravitational forces. The addition of coagulants is not, however, enough to instigate the immediate removal of contaminants. The process of flocculation imparts energy on the water and coagulant solution to increase particle

collisions and thus produce an aggregated particle (floc) that will have a much faster settling velocity (Edzwald 1993).

Filtration occurs after the sedimentation step and is used to remove particles that remain suspended after coagulation and flocculation. Many different filtration methodologies are in use worldwide; however, the process relevant to this study is media filtration with anthracite coal, sand, and coarse media. Media filtration has demonstrated high removal efficiencies of oocysts; however, removal efficiency is highly dependent on filter age, water quality, and presence of coagulants (Gitis 2008).

2.2.2. Traditional Uses of Coagulation, Flocculation, and Sedimentation

A common contaminant targeted by water treatment plants is Natural Organic Matter (NOM). NOM causes unpleasant odor, color, and taste in water, making it undesirable to consumers. The two varieties of NOM present in water are hydrophobic and hydrophilic which have higher and lower molar masses respectively. Hydrophobic and high molar mass compounds are easily removed through coagulation and flocculation as they are easily destabilized and settled out. Hydrophilic and low molar mass compounds are difficult to remove and require the usage of advanced coagulation and other more costly methods for their efficient removal (Matilainen et al. 2010).

2.2.3. Non-Traditional Uses of Coagulation, Flocculation, and Sedimentation

Traditionally, coagulation and flocculation were used for the removal of particles such as microbes, metals, soil particulates, and anything present in water as a solid (Edzwald 1993). Recent research has demonstrated that conventional water treatment can also be used to remove pesticides found in surface water. According to one estimate, approximately 95% of surface waters in the United States are contaminated with

pesticides such as DDT (dichlorodiphenyltrichloroethane) (Ballard and Mackay 2005). Research has shown that DDT is easily removed with traditional coagulation and flocculation methodologies (Jordan 2015); however, other anthropogenic organic compounds are not so easily destabilized (Ballard and Mackay 2005). Ballard cites experimentation in which humic acid was added to contaminated source water in hopes of sorption of organic materials to humic materials, thus creating hydrophobic materials that are destabilized and able to settle (Rebhun et al. 1998).

2.3. Jar Testing

Jar testing is the small scale replication of coagulation, flocculation, and sedimentation that is used daily at drinking water treatment plants to assess the day's water quality and determine the dose of coagulants needed to meet the treatment requirements. Traditionally jar testing was developed to determine optimum coagulant doses but has been proven to be useful in predicting other water quality related factors (Hudson and Wagner). Jar testing can be used to determine the quality of water after the sedimentation step, the necessary filtration equipment, and the lifespan of filtration infrastructure.

Similar to many other testing procedures, the steps required for jar testing are outlined by the American Society for Testing and Materials (ASTM). The standard used in the replication of jar testing is ASTM D2035 Standard Practice for Coagulation-Flocculation Jar Test of Water (ASTM 2011). This testing procedure outlines the water volumes, mixing speeds, mixing times, and settling times necessary for proper replication of conventional water treatment methodologies. For specific volumes and times, refer to the methodology section of this report (Ebeling et al. 2003).

2.4. Microbial Removal Surrogates for Coagulation Processes

The use of surrogates in the treatment process is a common practice. Traditionally surrogates are selected to be more conservative than the targeted pathogen. Surrogates can be living organisms like *Escherichia coli* (*E. coli*) or aerobic spore formers such as *Bacillus*, or non-living surrogates like chemical tracers or microspheres. *E. coli* is an ideal living indicator for many applications because of its low cost detection, its live span in water (4-12 weeks), and its sensitivity to oxidants (Edberg et al. 2000). *E.coli* is also a highly desirable biological indicator because of its presence in the mammalian digestive system. Enteric pathogens are of great concern for drinking water treatment and thus having a simple organism that can be easily observed provides a great advantage for the engineers designing treatment processes.

Typically the usage of biological surrogates is best applied to treatment methodologies in which the target pathogen is to be inactivated rather than physically removed. In the inactivation process of microbes like *Cryptosporidium*, it is wise to select a surrogate with similar features to ensure that the results are as similar as possible to the actual inactivation of the target pathogen. One example of a biological surrogate for *Cryptosporidium* is bacterial aerobic spores. Spores are an ideal surrogate for pathogens such as *Giardia* and *Cryptosporidium* due to their enteric presence, their ease of detection, and their resistance to chemical treatment (Facile et al. 2000).

2.5. Microspheres

In recent years, the utilization of fluorescent microspheres has become a popular option for experiments in which a surrogate is needed to assess the efficacy of treatment methodologies. Fluorescent microspheres are optimum candidates because unlike

chemical tracers and microbial surrogates, microspheres will not react, decay, or multiply during a treatment process. Fluorescent microspheres are available in a wide range of sizes which enhances their applicability in terms of modeling microbes or other colloids. Microspheres have been studied as surrogates for *Cryptosporidium* oocysts in filtration experiments (Dai and Hozalski 2003). A recent experiment performed by Gottinger et al. 2013 cites the usage of 4.5 micron microspheres as surrogates for *Cryptosporidium* oocysts in slow sand biofiltration beds.

2.5.1. Microsphere Dose Concentration

The utilization of microspheres as surrogates for similarly sized microbes requires an estimation to be made by researchers regarding the dose concentration at the head of the experiment. Different sources yielded different dose concentrations; however, the applications for each were different. Given that there is little information regarding the use of microspheres as surrogates from *Cryptosporidium* in conventional water treatment, experiments were selected in which *Cryptosporidium* oocyst were and were not the desired target. In the coagulation paper by Gottinger et al. 2013, the dose concentration was on the order of 10^6 beads/liter, whereas the article by Hogan et al. 2013 dosed their hydrologic removal experiment with only 10^3 beads/liter. Given the large variation in selected dose concentrations, it seems apparent that the numeric value for the dose is irrelevant as long as the bead removal is quantifiable and within reason.

2.5.2. Microsphere Composition

Fluorescbright[®] Microspheres are composed of polystyrene latex with a carboxylate coating. Each vile is distributed as 2.5% suspended solids in water and has a density of 1005 kg/m^3 (Fluoresbrite [®] 2013).

2.5.3. Microsphere Fluorescence

Fluorescent microspheres are available in a range of colors. Colors are chosen at the discretion of the researcher to aid in detection. For example, yellow and Nile red microspheres were reportedly used in *Cryptosporidium* detection experimentation (Gottinger et al. 2013). Another experiment in which microspheres were used as a surrogate for *Cryptosporidium parvum* oocysts cited the use of Dragon Green and Glacial Blue spheres as these researcher believed these colors would provide the clearest detection (Hogan et al. 2013).

2.5.4. Microsphere Surface Chemistry

Biotin-and-Glycoprotein coated carboxylate polystyrene beads have been used to counter the effects of buoyancy neutral traditional carboxylate-coated beads. *Cryptosporidium* oocysts produce Glycoprotein on the cell surface, and thus coating microspheres enables a higher level of surrogacy. As stated by Stevenson et al., the utilization of Biotin and Glycoprotein coatings have only proved beneficial in filtration models of *Cryptosporidium* oocysts (Stevenson et al. 2015).

2.6. *Cryptosporidium*

One of the most widely recognized water borne pathogens is the parasite *Cryptosporidium*. Since its discovery in 1907 by Ernest Tyzzer, environmental engineers have been working to establish methods of its identification, removal, and inactivation. In recent years, *Cryptosporidium* infection has become more prevalent in developed countries and is currently the leading cause of gastrointestinal parasitic infection (Sunnotel et al. 2006). Thus, verifying the utilization of fluorescent microspheres as a surrogate for *Cryptosporidium* oocyst removal is crucial.

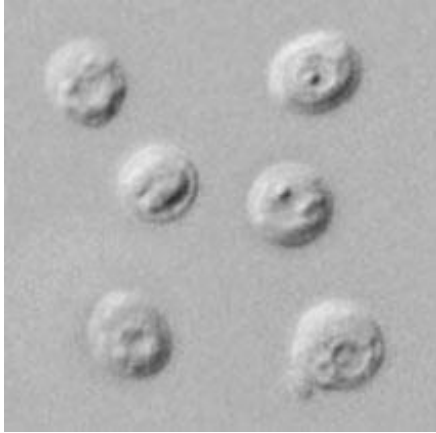


Figure 1:

***Cryptosporidium* Oocysts**

(Ref: Centers for Disease Control, Atlanta, Georgia)

2.6.1. Species

Currently there are 26 recognized species of *Cryptosporidium*. The species, host, and presence in humans has been summarized in Table 1 by Ryan et al. 2014 in their work entitled *Cryptosporidium species in humans and animals: Current understanding and research needs*.

Table 1:

Cryptosporidium Species and Target Hosts

Species Name	Author (s)	Type Host	Major Host	Reports in Humans
<i>C. muris</i>	Tyzzer (1907, 1910)	Tame mice	Rodents	Numerous reports
<i>C. wrairi</i>	Vetterling et al. (1971)	<i>Cavia porcellus</i> (Guinea pig)	Guinea Pigs	None reported
<i>C. felis</i>	Iseki (1979)	<i>Felis catis</i> (Cat)	Snakes and Lizards	Many reports
<i>C. serpentis</i>	Levine (1980)	<i>Elaphe guttata</i> , <i>E. Subocularis</i> , <i>Sanizinia madagascarensis</i> (Snakes)	Birds and Humans	None reported
<i>C. meleagridis</i>	Slavin (1955)	<i>Meleagris gallopavo</i> (Turkey)	Ruminants	Commonly reported in humans
<i>C. parvum</i>	Re: Upton and Current (1985) & Tyzzer (1912)	<i>Bos taurus</i> (Cattle)	Birds and Humans	Commonly reported in humans
<i>C. baileyi</i>	Current et al. (1986)	<i>Gallus gallus</i> (Chicken)	Birds	None reported

<i>C. varanii</i>	Pavilasek et al. (1995)	<i>Varanus prasinus</i> (Emerald Monitor)	Lizards	None reported
<i>C. andersoni</i>	Lindsay et al. (2000)	<i>Bos taurus</i> (Cattle)	Cattle	Reported
<i>C. canis</i>	Fayer et al. (2001)	<i>Canis familiaris</i> (Dog)	Dogs	Many reports
<i>C. molnari</i>	Alvarez-Pellitero and Sitja-Bobadilla (2002)	<i>Sparus aurata</i> and <i>Dicentrarchus labrax</i> (Fish)	Fish	None reported
<i>C. hominus</i>	Morgan-Ryan et al. (2002)	<i>Homo sapiens</i> (Humans)	Humans	Most common species in humans
<i>C. galli</i>	Re: Ryan et al. (2003) & Pavilasek et al. (1995)	<i>Spermestidae, Frangillidae, Gallus, gallus, Tetrao urogallus, Pinicola enucleator</i> (Birds)	Birds	None reported
<i>C. suis</i>	Ryan et al. (2004)	<i>Sus scrofa</i> (Pig)	Pigs	Reported
<i>C. bovis</i>	Fayer et al. (2005)	<i>Bos taurus</i> (Cattle)	Cattle	Reported
<i>C. fayeri</i>	Ryan et al. (2008)	<i>Macropus rufus</i> (Kangaroo)	Marsupials	Numerous reports
<i>C. fragile</i>	Jirku et al. (2008)	<i>Duttaphrynus melanostictus</i> (Toad)	Toads	None reported

<i>C. macropodum</i>	Power and Ryan (2008)	<i>Macropus giganteus</i> (Kangaroo)	Marsupials	None reported
<i>C. ryanae</i>	Fayer et al. (2008)	<i>Bos taurus</i> (Cattle)	Cattle	None reported
<i>C. xiaoi</i>	Fayer et al. (2010)	<i>Ovis aries</i> (Sheep)	Sheep and Goats	Numerous reports
<i>C. ubiquitum</i>	Fayer et al. (2010)	<i>Bos taurus</i> (Cattle)	Ruminants, Rodents, Primates	Commonly reported in humans
	Re: Robinson et al.			
<i>C. cuniculus</i>	(2010), Inman and Takeuchi (1979)	<i>Oryctolagus cuniculus</i> (European rabbit)	Rabbits	Reported
	Re: Ren et al.			
<i>C. tyzzeri</i>	(2012), Tyzzer (1912)	<i>Mus musculus</i> (Mouse)	Rodents	Reported
<i>C. viatorum</i>	Elwin et al. (2012)	<i>Homo sapiens</i> (Humans)	Humans	Reported
<i>C. scrofarum</i>	Kvac et al. (2013)	<i>Sus scrofa</i> (Pig)	Pigs	Reported
<i>C. erinacei</i>	Kvac et al. (2014)	<i>Erinaceus europaeus</i> (European hedgehog)	Hedgehogs and Horses	Reported

Ref: (Ryan et al. 2014)

2.6.2. Oocyst Characteristics

Outside the body of the host, *Cryptosporidium* is found as oocysts. Oocysts are approximately 3-6 µm in diameter and are spherical in shape. When shed from an infected host, contaminated feces can contain up to 10^7 oocysts/gram (Ongerthl and Stibbs 1987). An appropriate summary of *Cryptosporidium* is provided by Fayer et al. 2000.

“The genus *Cryptosporidium* is classified as a eukaryote in the phylum Apicomplexa. All species of *Cryptosporidium* are obligate, intracellular, protozoan parasites that undergo endogenous development culminating in the production of an encysted stage discharged in the feces of the host” (Walker et al. 2001).

2.6.3. Oocyst Occurrence in Water

The necessity for modeling the removal of *Cryptosporidium* was addressed in a study performed by Ryu and Abbaszadegan 2008, which quantified the presence of *Cryptosporidium* oocysts in Arizona surface waters over the course of four years. Of the samples tested, 10% were positive for *Cryptosporidium* oocysts (Ryu and Abbaszadegan 2008). Given that the infectious dose of *Cryptosporidium* is a single oocyst, the presence of oocysts in 10% of the surface water in the Phoenix Metro area indicates that drinking water treatment facilities are at a high risk of encountering oocysts in their treatment processes.

2.6.4. Oocyst Decay Rate

Cryptosporidium oocysts are shed in the fecal matter of an infected host and thus introduced to the surrounding environment. Oocysts that are free in the environment can potentially be introduced to any number of host related consumables (drinking water, crops, accidental ingestion of surface water, etc.). As previously stated, *Cryptosporidium* is an intracellular organism and thus cannot reproduce outside of its host; this means, that the oocysts in the environment must either infect a host or perish. Modeling the decay of oocysts in the environment helps assess the risk of treating contaminated drinking water. Using a first order decay function, Walker, et al. modeled the decay of *Cryptosporidium* oocysts when exposed to several harsh environmental conditions including freeze/thaw cycles, long durations of freezing cold, and long durations of heat. The results of those experiments are seen Table 2 (Walker et al. 2001).

Table 2:
Oocyst Decay Rates

Temperature (°C)	Fitted Model	Model Significance	Parameter Significance
4 and 30	$K = 0.003 \cdot \text{bars} - 0.0004 \cdot \text{Temp}$	$P < 0.001$	Temp, $P < 0.001$; Bars, $P = 0.003$
-14	$k = 0.013 \cdot \text{bars}$	$P = 0.001$	Bars, $P = 0.001$
Freeze-thaw cycling (-14 to 10)	$k = 0.055 \cdot \text{bars}$	$P < 0.001$	Bars, $P < 0.001$

2.6.5. *Cryptosporidium* Infection (Cryptosporidiosis)

As stated above, *Cryptosporidium* is an obligate, intracellular parasite, meaning that it cannot replicate outside of its host. Once *Cryptosporidium* has entered the digestive tract of its host it can begin replicating and causing illness. *Cryptosporidium* infects its vertebrate host by targeting the microvillus border of the gastrointestinal epithelium, leading to severe diarrhea. The prevalence of Cryptosporidiosis outbreaks and the most affected age groups vary throughout the world depending on societal cleanliness and age development. In under-developed countries, children under the age of five typically experience cryptosporidiosis; however, developed countries typically experience outbreaks in adults. Adult outbreaks in developed countries are usually due to food or water contamination; whereas, child exposure in underdeveloped countries occurs due to person to person transmission (i.e. poor sanitation) (Xiao et al. 2004).

2.6.6. Oocyst Removal in Conventional Treatment

According to the United States Environmental Protection Agency (U.S. EPA), the average removal of *Cryptosporidium* through conventional water treatment with Alum as the primary coagulant is between 2 and 3 Log. The variation in removal efficiency is due to plant performance and is highly dependent on influent water quality (Nieminski 1997). According to the EPA Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) the maximum credit allotted to the conventional water treatment for the removal of *Cryptosporidium* oocysts is 2.5 Log. This value assumed a high influent dose of oocyst as well as the addition of water softening which is something that is not present at the drinking water facility in Chandler, Arizona (Nieminski 1997). Multiple coagulants are available and widely used in treatment plants throughout the world; however they do

not all perform to the same efficiency. Experiments highlighted in Water Treatment and Pathogen Control noted that the removal efficiency of iron-based coagulants was typically higher than that of alum or polyaluminum chloride; however, the change in efficiency was negligible when compared to the efficiency impact of influent water quality (Bartram 2004).

The removal of oocysts occurs at multiple steps in the drinking water treatment process. Oocysts, are settled, filtered, broken down, and inactivated to achieve the highest removal possible to ensure that the public is not in harm's way. The effectiveness of removal in all stages has been linked directly to the efficiency of the coagulation phase. The same article suggests that the most efficiency removal of oocysts during conventional treatment is to replace the sedimentation step with Dissolved Air Flotation (DAF) to remove low density particles by bringing them to the surface rather than forcing them to settle (Betancourt 2004).

3.0 MATERIALS AND METHODS

3.1. Coagulant and Polymer Dosage

In order to accurately determine the removal of fluorescent microspheres from water using coagulation, flocculation, sedimentation, and filtration it was decided that various combinations of coagulant and polymer doses would need to be tested to ensure a broad enough range of data. The conventional water treatment model being assessed was designed to mimic that of Chandler, Arizona's drinking water treatment facility; thus, the coagulant dose range was determined based on typical plant averages. Per discussions with Dr. Anupa Jain, it was decided that coagulant would be added in 10, 25, and 40 mg/L doses while maintaining a polymer concentration of 2.7 mg/L, as well as maintain a control which contained no polymer and no coagulant (Jain, Anupa, Personal Communication. 30 Aug. 2015).

The raw water, coagulant, and polymers selected for this study were sourced directly from the Chandler Drinking Water Treatment Plant. The raw water from the plant had a measured turbidity of 26.86 NTU and a pH of 6.49. The provided coagulant and polymer were aluminum sulfate and Magnafloc[®] LT-7996 (2-Propen-1-aminium, N, N-dimethyl-N-2-propenyl-, chloride, homopolymer) respectively. The calculated volumetric doses are shown in Table 4 (BASF Safety Data Sheet).

3.1.1. Coagulant and Polymer Stock Concentration Determination

The coagulant and polymer stock solutions as well as all relevant information regarding the material properties were provided graciously by the city of Chandler. The Aluminum sulfate and polymer solutions were of unknown concentrations; however, the weight percentage of each chemical in solution was provided. As stated by the city of

Chandler officials, the weight percentages of aluminum sulfate and polymer in their respective solutions were 48% and 20% (Jain, Anupa, Personal Communication. 30 Aug. 2015). Using known volumes of 10 mL, the samples were weighed and the exact concentration of coagulant (in mg/L) was calculated, see Table 3 and calculation below.

$$\text{Concentration} = \frac{\text{Measure Weight}}{\text{Known Volume}} * \text{Weight \%}$$

Table 3:

Coagulant and Polymer Concentration

Material	Measured Weight (g)	Volume (mL)	Conc. Total (g/mL)	Weight (%)	Conc. (mg/L)
Alum	13.05	10	1.305	0.48	626400
Polymer	9.64	10	0.964	0.2	192800

3.1.2. Coagulation and Polymer Dosage Concentration Determination

Having calculated the concentration of each material, the next step was to calculate the volume of coagulant needed to achieve the desired dose for each jar. The values represented in Table 4 were calculated as follows:

$$\text{Stock Concentration} \left[\frac{\text{mg}}{\text{L}} \right] * \text{Dose Volume [L]}$$

$$= \text{Dose Concentration} \left[\frac{\text{mg}}{\text{L}} \right] * \text{Jar Volume [L]}$$

$$\text{Dose Volume [L]} = \frac{\text{Dose Concentration} \left[\frac{\text{mg}}{\text{L}} \right] * \text{Jar Volume [L]}}{\text{Stock Concentration} \left[\frac{\text{mg}}{\text{L}} \right]}$$

Table 4:

Coagulant and Polymer Dose Concentrations for Jar Testing

Material	Target Concentration in Jar (mg/L)	Volume of Stock to Achieve Target (uL)
Alum	10	15.964
Alum	25	39.911
Alum	40	63.857
Polymer	2.7	14.004

3.2. Bead Selection and Stock Solution Preparation

Since the average size of a *Cryptosporidium* oocyst is in the range of 3-6 μm , all experimentation was performed with a 3 micron sphere, thus providing a more conservative set of results. The Fuoresbright[®] beads were selected due to their availability and variety of sizes. For ease of detection, fluorescent green colored beads were selected. The 2 mL solution of beads arrived with a concentration of $\sim 10^9$ beads, thus a dilution was necessary. Our stock solution for experimentation was produced by diluting 20 μL of bead solution in 10 mL of deionized water. This solution was vortexed and counted using 2 μL drops under 20X magnification. This process was performed in triplicate and averaged to yield a result of 2.56×10^6 beads per mL.

3.3. Jar Testing

Jar testing was performed in accordance with ASTM standard D2035 using the jar tester shown in Figure 4; specifically, 1L samples were subjected to 1 minute of rapid mixing at 120 rpm, then transitioned to slow mixing at 30 rpm for 15 minutes, and finally settling for 15 minutes. Each jar was dosed with 10^6 beads (0.39 mL of stock solution) in order to ensure visibility of beads in the effluent.



Figure 4:
Jar Testing Device

3.4. Effluent Analysis

In order to determine the efficiency of a jar test, the influent and effluent concentrations of the water are measured to find the log removal of the targeted pathogen. Various methods exist for determining effluent concentration, including:

- Measuring the settled sludge and calculating the volume in order to determine the total number of pathogens removed
- Measuring the turbidity of the water before and after to determine overall improved clarity and quality (this is more typical for present organic matter), or

- Measuring the effluent concentration and a value of the effluent to determine the total number of pathogens still present in the water after treatment.

All of the above methodologies were considered when determining the best way to assess the removal of microspheres during the coagulation, flocculation, and sedimentation process. After executing several trial runs, it was determined that the volume of sludge produced for this particular water was negligible, and thus the quantification of how much sludge was produced would introduce an unnecessary source of error into the analysis step of this study. Next, the measure of turbidity was determined to not be an accurate enough measure given that the infectious dose of *Cryptosporidium* is but one organism, thus turbidity could not reflect the removal of all constituents on a microbial level. Thus, measuring effluent concentration was chosen as it would allow a direct measure of pathogens present and could be assumed to represent the entire volume of the jar given that the sludge layer was negligible.

3.4.1. Effluent Sampling

Each jar was dosed with its respective coagulant dose and tested under the ASTM D2035 standard. After completing each step in the approved process, 40 mL of water was sample one inch from the surface of the water and in the middle of the jar. This sampling location was used to avoid any aggregation of microspheres near the surface of the water as well as any spheres that may have adhered to the walls of the jar.

3.4.2. Sample Preparation

After several trial runs, it became apparent that the concentration of the effluent was too dilute to be directly measurable, so a centrifugation step was implemented. Each

collected sample was centrifuged at 3,000 rpm for 15 minutes and suspended in 0.4 mL of deionized water in order to obtain a 100X concentration.

3.4.3. Sample Counting

After sampling and concentrating the effluent from each jar, slides from each jar were prepared in triplicate in order to provide an accurate count of beads present in the effluent. Each slide was comprised of 2 μL samples that were counted under ultraviolet light. Table 5 in section 4.1 outlines effluent bead concentrations.

3.5. Media Filtration

After performing jar testing with the aforementioned microspheres, a column built to the specifications of those in use at the city of Chandler, Arizona plant was set to operate at a flow rate of 0.2 gal/min and was dosed with 10^7 beads and monitored for breakthrough. 50 mL of water was sampled hourly and centrifuged and re-suspended to achieve 100X concentration; however, after 20 hours of monitoring, no beads were observed leaving the filter. Samples of anthracite coal were taken from the top 5 inches of the filter and observed under the microscope which revealed as many as 15 microspheres adhered to each grain. As seen in Figures 5-6, many beads are present on a single grain of carbon; however, to properly see them, the focus must be set for each depth, thus, one cannot observe all beads present on the granule at once.

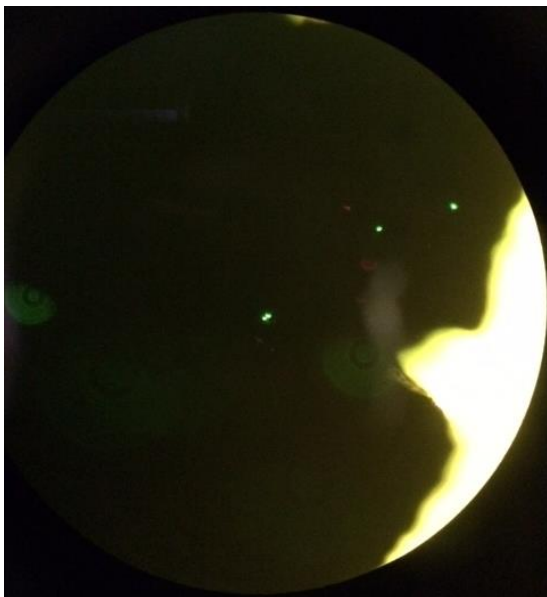


Figure 5:
Anthracite Coal Granule Depth
of View 1

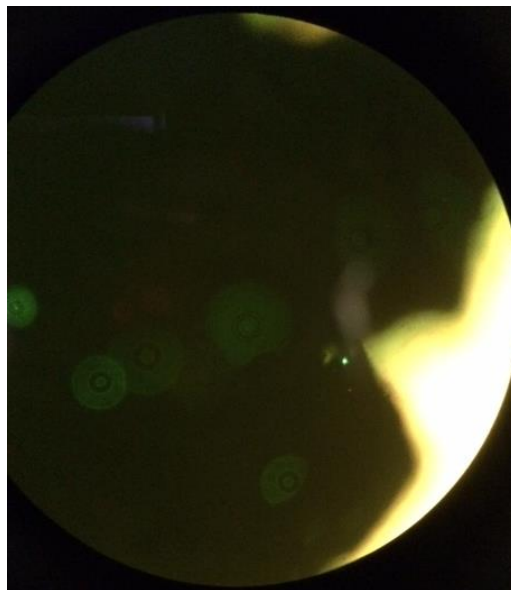


Figure 6:
Anthracite Coal Granule Depth
of View 2

4.0 RESULTS AND DISCUSSION

4.1. Effluent Count Results

The jar testing procedure was performed three times for each concentration to provide triplicate data. The effluent water of each jar was sampled, centrifuged, placed on slides, and counted. Three repeat measurements were made for each jar and the results were averaged to most accurately represent effluent concentrations. The results are summarized in Table 5.

Table 5:

Bead Removal Results

Dose	Control	10 mg/L	25 mg/L	40 mg/L
(Beads/mL)				
Trial 1	95.0	50.0	411.7	91.7
Trial 2	113.3	125.0	806.7	311.7
Trial 3	86.7	106.7	261.7	210.0
Avg.	98.3	93.9	493.3	204.4
Log Removal	2.3	2.4	0.7	1.6

As seen in the table above, the desired removal of the beads was not achieved. It appears as though an increase in coagulant concentration can drive down removal efficiency. The apparent decrease in removal efficiency can be attributed to several factors, including but not limited to the surface composition of the beads and the density of the beads.

4.1.1. Bead Removal Complications

Unlike many pathogens that flow through the conventional water treatment process, the fluorescent microspheres have a carboxylate coating. This coating appears to react in such a way with the added polymer that the beads aggregate with one another rather than other particles present in the untreated water. This aggregation of smaller particles should, in theory, lead to a large enough floc that they would settle out and be removed; however, this assumption is only valid for particles with a high enough density. The density of the beads was provided from the manufacturer as 1.05 g/mL which is only 5% higher than that of water. Typical *Cryptosporidium* oocysts have a density of approximately 1075 kg/m³ (Komisar 2005). Additionally, the average density of *Bacillus* spores (a current surrogate for oocysts) is approximately 1180 kg/m³ (Carrera et al. 2008). In order to properly compare the affect density has on the settling of a particle, Stokes Law was applied to determine the settling velocity of both the beads and average oocysts.

In addition to unnecessary bead aggregation, the low turbidity of the raw water generated a nearly negligible sludge layer. Turbidity is the driving force of conventional treatment, in that removal efficiency is directly proportional to turbidity. That being said, for microspheres to be a viable surrogate they must perform ideally under all influent water conditions regardless of turbidity variations.

Stokes Law for settling velocity (Crittenden 2005).

$$v_s = \frac{g(\rho_P - \rho_W)D_P^2}{18\mu}$$

Table 6:

Variable Definitions

v_s	Settling Velocity
g	Gravitational Constant
ρ_P	Density of Particle
ρ_W	Density of Water
D_P	Diameter of Particle
μ	Dynamic Viscosity of Water

- Settling velocity of fluorescent microspheres:

$$v_s = \frac{\left(9.81 \frac{m}{s^2}\right) * \left(1050 \frac{kg}{m^3} - 1000 \frac{kg}{m^3}\right) * (3 * 10^{-6}m)^2}{18 * \left(1.002 * 10^{-3} N * \frac{s}{m^2}\right)}$$

$$v_s = 2.448 * 10^{-7} \frac{m}{s}$$

- Settling velocity of *Cryptosporidium* oocysts (assuming 3 µm diameter):

$$v_s = \frac{\left(9.81 \frac{m}{s^2}\right) * \left(1075 \frac{kg}{m^3} - 1000 \frac{kg}{m^3}\right) * (3 * 10^{-6}m)^2}{18 * \left(1.002 * 10^{-3} N * \frac{s}{m^2}\right)}$$

$$v_s = 3.671 * 10^{-7} \frac{m}{s}$$

- Settling velocity of *Bacillus* spores (assuming 1 µm average diameter) (Carrera et al. 2007):

$$v_s = \frac{\left(9.81 \frac{m}{s^2}\right) * \left(1180 \frac{kg}{m^3} - 1000 \frac{kg}{m^3}\right) * (1 * 10^{-6} m)^2}{18 * \left(1.002 * 10^{-3} N * \frac{s}{m^2}\right)}$$

$$v_s = 9.790 * 10^{-8} \frac{m}{s}$$

- Settling velocity comparison of oocysts and microspheres using absolute relative difference:

$$\left| \frac{v_{Scryptosporidium} - v_{Smicrospheres}}{v_{Smicrospheres}} \right| * 100\%$$

$$\left| \frac{\left(3.67 * 10^{-7} \frac{m}{s}\right) - \left(2.488 * \frac{10^{-7} m}{s}\right)}{\left(2.448 * 10^{-7} \frac{m}{s}\right)} \right| * 100\% = 47.56\%$$

- Settling velocity comparison of oocysts and *Bacillus* using absolute relative difference:

$$\left| \frac{v_{Scryptosporidium} - v_{SBacillus}}{v_{SBacillus}} \right| * 100\%$$

$$\left| \frac{\left(3.67 * 10^{-7} \frac{m}{s}\right) - \left(9.790 * \frac{10^{-8} m}{s}\right)}{\left(9.790 * 10^{-8} \frac{m}{s}\right)} \right| * 100\% = 274.90\%$$

4.1.2. Conclusions

Conventional treatment processes have been proven to remove *Cryptosporidium* oocysts with relatively high efficiency; however, the same cannot be said for the aforementioned fluorescent microspheres. The relationship between coagulant and polymer dose and bead removal efficiency is not predictable and does not increase proportionally as expected. Beads were observed forming larger flocs (Figure 7); however, due to their low density this was not enough to initiate settling. As seen in the above calculations, the settling velocity of the beads is approximately 50% slower than that of oocysts and thus the high concentrations of beads that remained in the jar tester effluent is not an accurate representation of oocyst behavior. Additionally, when subjected to media filtration, the beads do not migrate well through the system. After 20 hours of continuous flow, no beads were seen breaking through in the effluent. This would seem to be a favorable result; however, the filter was dosed with 10^7 beads and no detectable concentration was able to migrate through the system which demonstrates unreasonably high removal efficiency. This result can be explained by the low density of the microspheres as well as the potential presence of residual coagulant present in the system. Given the unfavorable properties of the beads, they do not appear to be a suitable candidate for the surrogacy of *Cryptosporidium* oocysts in conventional drinking water treatment.

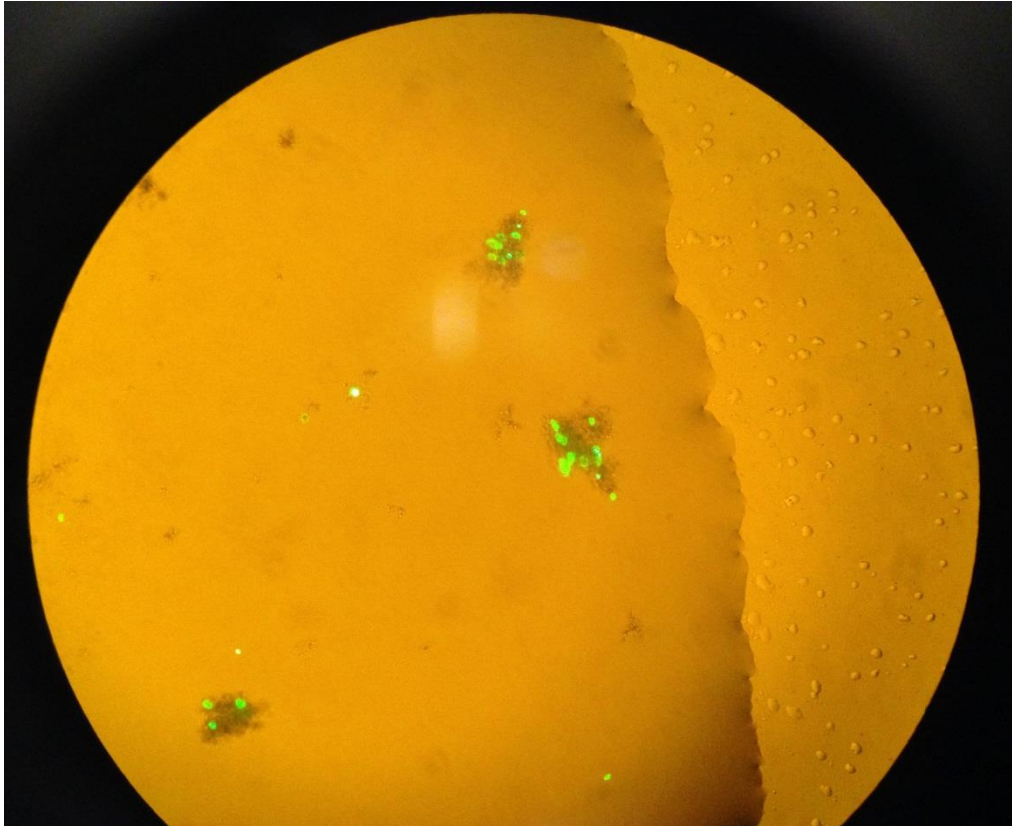


Figure 7:
Unsettled Bead Floc

5.0 SUMMARY

5.1. Significance of Results

Given the importance of *Cryptosporidium* removal in conventional treatment processes, the efficiency seen with fluorescent microspheres needed to match or exceed required minimum removal values to be considered an optimal surrogate. Without similar removal efficiency, the microspheres are not a suitable surrogate for *Cryptosporidium* oocysts in the conventional drinking water treatment process at the city of Chandler Drinking Water Treatment Plant.

5.2. Future Work

It is my belief that the addition of the polymer instigated the aggregation of the microspheres with one another, and without this chemical, the removal efficiency would have increased as coagulant dose increased; however, this task was to model the potentially surrogacy specifically for the Chandler plant and thus the polymer was not removed. The issue regarding the density of the beads could be resolved by contacting bead manufacturers and special ordering beads of higher density. From my research, the average density of *Cryptosporidium* oocysts is approximately 7.5% greater than that of water; thus, the density of the beads should be tailored to match.

Additionally, studies performed by Stevenson et al. 2015 indicate that a chemical bath can be applied to beads to negate the chemical coating and make the beads less likely to aggregate when exposed to the polymer. This could be applied to mitigate the unnecessary aggregation of microspheres in further tests to evaluate their potential surrogacy for *Cryptosporidium* oocysts.

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