Investigating the Synergistic Effect of Free Chlorine and Silver Ions in Natural Waters for Antibacterial Activity, and Developing a Material for Low-Dose Chlorine Release in Water for Pathogen Inactivation

---

A

Dissertation

Presented to

the faculty of the School of Engineering and Applied Science

University of Virginia

---

in partial fulfillment

of the requirements for the degree

Doctor of Philosophy

by

Ana Elizabeth Estrella You

April 2023

APPROVAL SHEET

This

Dissertation

is submitted in partial fulfillment of the requirements
for the degree of

Doctor of Philosophy

Author: Ana Elizabeth Estrella You

Advisor: James A. Smith, PhD

Advisor:

Committee Member: Rachel A. Letteri, PhD

Committee Member: Lisa Colosi Peterson, PhD

Committee Member: Andrés F. Clarens, PhD

Committee Member: Valeria Ochoa-Herrera, PhD

Committee Member:

Committee Member:

Accepted for the School of Engineering and Applied Science:

Jennifer L. West, School of Engineering and Applied Science

April 2023

Abstract

Point-of-use (POU) water treatment technologies that contain free chlorine (e.g., chlorine tablets, bleach) release high quantities of chlorine (up to 4,000 μg/L) to disinfect water. These high concentrations can create an unpleasant taste and odor in the treated water and may lead to discontinuation of the disinfection treatment. Other POU technologies that use silver provide effective inactivation of bacterial pathogens and do not change the taste and odor of the water, but they do not perform as well as chlorine against viruses. Multiple studies in well water or solutions inoculated with bacteria or viruses have evaluated the use of combinations of metals (i.e., silver and copper) and chlorine in doses that meet the World Health Organization guidelines for drinking water and have demonstrated the presence of a synergistic pathogen inactivation effect with these combinations. This dissertation describes the evaluation of the silver-chlorine synergistic effect in natural waters and the development and assessment of a material that releases low doses of chlorine in water for pathogens' inactivation.

We first investigated the synergistic inactivation of bacteria in natural waters (from a pond with 4.82 NTU and upstream with 11.9 NTU in Virginia) using low doses of silver (10 μg/L) and free chlorine (100 μg/L). There was a significant synergistic effect at 3-hr contact time and chlorine effectiveness was significantly reduced by higher turbidity, whereas silver effectiveness was not. In addition, bacteria inactivation by the MadiDrop+, MD (a commercial silver-ceramic tablet that releases silver ions for POU water disinfection), and low doses of free chlorine (50-200 μg/L) in water from a stream in South Africa was tested. The MD alone at 8-hr contact time obtained ~1-$\log_{10}$ reduction for *E. coli* and ~2-$\log_{10}$ reduction for total coliform bacteria. However, some of the MD-free chlorine combinations achieved a similar bacteria reduction with a substantial reduction of contact time (between 6- and 7-hr less). Overall, these results show that the silver-chlorine synergistic effect demonstrated in previous studies with solutions inoculated with pathogens is also present in the more realistic scenario with natural waters which contain more complex matrixes.

We also developed gels that release low doses of chlorine in water. We tested the gels against *E. coli* bacteria and MS2 bacteriophage virus in deionized water that contains salts to simulate groundwater. In addition, we evaluated the gels together with the MD and a copper releasing material or copper screen. Results show that after 8-hr of treatment the gels are effective for bacteria inactivation, and in combination with the MD, the inactivation was close to 2-$\log_{10}$ reduction (*E. coli* reduction for gels-MD combination, gels alone, and MD alone: 1.86-, 1.10-, and 0.69-$\log_{10}$ reduction, respectively). But when the gels were combined with the copper screen there was essentially no increase in the reduction of bacteria compared to when the gels were used alone. On the other hand, after 8- or 24-hr of treatment, the gels were not effective for MS2 inactivation. However, contrary to *E. coli* inactivation, combining the gels with the copper screen did increase the reduction of MS2 compared to the screen alone (8-hr treatment: 0.4-$\log_{10}$ reduction for the gels-screen combination, and 0.2-$\log_{10}$ reduction for the copper screen; 24-hr treatment: 3.3-$\log_{10}$ reduction for the gels-screen combination, and 2.1-$\log_{10}$ reduction for the copper screen), and when the gels were combined with the MD there was no increase in virus reduction compared to when the MD was used alone. Moreover, the greatest reduction of MS2

(0.9- and 4.4-$\log_{10}$ reduction for 8- and 24-hr treatment, respectively) occurred when combining all three materials: copper screen, MD, and gels.

These results from laboratory settings are encouraging and contribute towards future development of the gels to become an alternative to current commercial chlorine based POU technologies and improvement for silver based POU technologies. Future work should focus on long term usage of the gels, including stability and rechargeability evaluations, and field studies considering natural water sources, social acceptability and affordability. The ultimate goal would be to produce a certified metal-chlorine-releasing POU technology for drinking water disinfection.

Acknowledgements

This work was possible because of the support of many people. I would like to acknowledge Qinmo Luo and Israt Duti for their significant contributions to this research. I am also grateful to members of the Smith and Letteri groups for their mentorship, helpful feedback and support, especially Wuhuan Zhang, Jamie Harris, Sydney Turner, Courtney Hill, Tahmina Ahmed, Gail Hayes, and Zixian Cui.

Part of our study of the silver-chlorine synergistic effect was made possible through the support of people at the University of Venda in Thohoyandou, South Africa, especially, Joshua Edokpayi and Amidou Samie. Working in Thohoyandou has been the highlight of my research experience. Kathy Nguyen also contributed to this part of the study. I would also like to thank Derek Henderson for his support in Charlottesville as our laboratory manager.

I am thankful to my advisor Jim Smith for his flexibility, guidance and support. I would also like to thank my committee members Rachel Letteri, Lisa Colosi Peterson, Andres Clarens, and Valeria Ochoa-Herrera for helpful feedback and support.

I am grateful to my family for always supporting me and cultivating a sense of discipline, responsibility, curiosity, sincerity, and simplicity. I am thankful to my paternal grandmother and my mother's family for the support they provided to our family. I am grateful to my parents, Edison and Young Ran, and my brother, Pablo, for providing me with support and inspiration, and to Vladimir for his unconditional love and support.

Table of contents

List of figures

List of tables

Publications and Presentations

This dissertation has resulted in the following publications:

1. In preparation: Estrella-You, A., Luo, Q., Duti, I. J., Letteri, R. A., Conti, C. & Smith, J. A. "*N*-chloramine crosslinked polyethylene glycol methacrylate networks for inactivation of *E. coli* in water."
2. In preparation: Harris, J. D.; Estrella-You, A. & Smith, J. A. "Development and Evaluation of a Novel Copper- and Silver-Based Household Water Treatment."
3. Estrella-You, A. and J. A. Smith. "Synergistic bacterial inactivation by silver ions and free chlorine in natural waters." *Journal of Environmental Engineering*. 148.11 (2022): 04022072 https://doi.org/10.1061/(ASCE)EE.1943-7870.0002053
4. Estrella-You, A., Harris, J. D., Singh, R. & Smith, J. A. (2022). Inactivation of Waterborne Pathogens by Copper and Silver Ions, Free Chlorine, and *N*-chloramines in Point-of-Use Technology: A Review. In P. LeBlanc (Ed.), *Water Purification: Processes, Applications and Health Effects* (pp. 1-88). New York: Nova Science Publishers, Inc.

This dissertation has resulted in the following presentations:

"Rechargeable gels designed to release chlorine for inactivation of pathogens in water." (2023).

1. 26th Triennial Borchardt Conference on Advancements in Water & Wastewater. *Poster* presentation. University of Michigan, Ann Arbor, MI.
2. UVa Engineering Research Symposium (UVERS). Charlottesville, VA.

"Synergistic bacterial inactivation by silver ions and free chlorine in natural waters." (2021).

3. UNC Water and Health Conference. Virtual *poster*.
4. WaterJAM Fresh Ideas Young Professional *Poster* Contest. Virginia Beach, VA.
5. UVa Global Water Initiative Graduate Water Symposium. Virtual.

6. "Investigating the synergistic effects of chlorine and silver on waterborne pathogen disinfection." (2019). UVa Global Water Initiative Graduate Water Symposium. Charlottesville, VA.

Chapter 1

Introduction

1.1 Background and motivation

The World Health Organization (WHO) estimates that at least 2.2 billion people in the world (approximately 30% of the world population) lack clean drinking water at home (World Health Organization 2019). From these people, 490 million use surface water sources (e.g., lakes, ponds, rivers, streams) contaminated with fecal material or water from unprotected wells and springs (World Health Organization 2022). The contaminated water can transmit bacterial (e.g., *E. coli*, *Vibrio cholerae*, *Shigella*), viral (e.g., norovirus, poliovirus, rotavirus, adenovirus, hepatitis A and E) and protozoan (e.g., *Giardia*, *Cryptosporidium*) species that can cause severe diarrheal diseases (e.g., schistosomiasis, cholera, dysentery, typhoid, polio, and hepatitis) and lead to death and other health problems. Each year diarrheal diseases associated with unsafe drinking water, sanitation, and hand hygiene claim the lives of around 829,000 people, from which more than one third are children aged under 5 years old (World Health Organization 2022). Diarrheal diseases can also lead to decreased food intake and nutrient absorption, reduced resistance to infection, and impaired physical growth and cognitive development (Lantagne et al. 2010). However, with appropriate water management and disinfection, and sanitation services these health risks and deaths can be prevented.

Highly effective water disinfection can be achieved through different means such as chlorination, ozonation, UV radiation, etc. (Hassen et al. 2000). Yet in many areas of the world, there is a lack of infrastructure to support these kinds of disinfection processes nor a distribution network with safe water because of insufficient funds (Jain et al. 2010; Patil et al. 2015). Therefore, one possible solution to this problem is to treat contaminated water in the households before consumption. Such point-of-use (POU) water treatment along with safe water storage options have the potential to significantly improve the collected source water quality and reduce the risk of diarrheal diseases and death, especially in children (Jackson and Smith 2018; Singh et al. 2019). Common POU technologies that have been developed, tested,

and disseminated include porous ceramic tablets and filters infused with silver (such as the MadiDrop+ tablets), chlorine tablets, and bleach or liquid chlorine (Jain et al. 2010).

Chlorine tablets and bleach release free chlorine to effectively disinfect water or inactivate bacteria and viruses (Ercumen et al. 2015). However, when chlorinating waters that in addition to pathogens contain high amounts of organic compounds, free chlorine will readily react with the organics which leads to not having enough chlorine for disinfection. For this reason, it is recommended to apply high quantities of free chlorine (up to 4,000 μg/L) in natural waters to have enough for microorganisms' inactivation. As a reference, the WHO guideline value that represents the concentration of free chlorine that does not result in any significant risk to the health of people over a lifetime of consumption is 5,000 μg/L. Nevertheless, some people can taste or smell free chlorine in water at concentrations as low as 300 μg/L (World Health Organization 2017). These unpleasant changes in aesthetics may lead to rejection by users or discontinuation of the disinfection treatment (Firth et al. 2010; Patil et al. 2015).

Another POU technology, the MadiDrop+ tablet, releases silver ions into the water at a controlled and sustained rate for waterborne pathogens' inactivation. To date, multiple studies have quantified the performance of these tablets in both the field and laboratory (Ehdaie et al. 2014, 2017, 2020; Hill et al. 2020; Jackson et al. 2019; Kahler et al. 2016; Singh et al. 2019) and their results indicate that the MadiDrop+ performs well against coliform bacteria, including *E. coli* (can achieve a 4-$\log_{10}$ reduction in 8 hours of contact time), but only provides about a 1-$\log_{10}$ reduction in protozoan (*Cryptosporidium* and *Giardia sp.*) and viral pathogens (adenovirus). Compared to free chlorine, silver requires higher doses and longer contact times to achieve the same level of water disinfection [the secondary drinking water standard for silver is 100 μg/L (US EPA 2020)]. However, silver has an advantage over free chlorine because it does not change the taste and odor of the treated water (Jackson and Smith 2018), which does not lead to rejection by users and discontinuation of the disinfection treatment.

The synergistic effects of different combinations of silver and/or copper ions, and free chlorine on waterborne pathogens' inactivation (including viruses, bacteria, and protozoa) have been demonstrated in multiple prior laboratory studies (Abad et al. 1994; Biurrun et al. 1999; Chen et al. 2008; Cromeans et al. 2010; Liu et al. 1994; Lucier et al. 2017; Straub et al. 1995; Yahya

et al. 1990). Therefore, this dissertation focused on developing a material that could minimize chlorine POU technologies' challenges and at the same time improve pathogens' inactivation performance of the MadiDrop+. To our knowledge, no commercial POU technology uses a combination of silver and chlorine for water disinfection.

1.2 Dissertation goals

The overall goals of this research were to evaluate the silver-chlorine antibacterial synergistic effect in natural waters and to develop a POU water treatment technology that combines silver and chlorine and takes advantage of their synergistic effect. Chapter 2 presents a comprehensive literature review about POU technologies that use silver or chlorine for water disinfection. This review includes the technologies' laboratory and field studies' findings, and silver and chlorine drinking water standards, pathogen inactivation mechanisms, and findings about their synergistic effect in laboratory settings. Chapter 3 evaluates silver and chlorine synergistic effect in natural waters (sampled in South Africa and the US). Chapter 4 focuses on the development of a rechargeable material that releases chlorine in water and describes the selection of optimal formulation and chlorine charging or loading time. And Chapter 5 presents the antimicrobial efficacy assessment of the developed material alone and of the material combined with the MadiDrop+ and/or with a copper releasing material.

1.3 References

Abad, F. X., R. M. Pinto, J. M. Diez, and A. Bosch. 1994. "Disinfection of Human Enteric Viruses in Water by Copper and Silver in Combination with Low Levels of Chlorine." *Appl Environ Microbiol*, 2377–2383. https://doi.org/10.1128/aem.60.7.2377-2383.1994

Biurrun, A., L. Caballero, C. Pelaz, E. León, and A. Gago. 1999. "Treatment of a Legionella pneumophila-Colonized Water Distribution System Using Copper-Silver Ionization and Continuous Chlorination." *Infect Control Hosp Epidemiol*, 20 (6): 426–428. https://doi.org/10.1086/501645

Chen, Y. S., Y. E. Lin, Y. C. Liu, W. K. Huang, H. Y. Shih, S. R. Wann, S. S. Lee, H. C. Tsai, C. H. Li, H. L. Chao, C. M. Ke, H. H. Lu, and C. L. Chang. 2008. "Efficacy of point-of-entry copper-silver ionisation system in eradicating Legionella pneumophila in a tropical tertiary care hospital: implications for hospitals contaminated with Legionella in both hot and cold water." *Journal of Hospital Infection*, 68 (2): 152–158. W.B. Saunders. https://doi.org/10.1016/j.jhin.2007.10.020

Cromeans, T. L., A. M. Kahler, and V. R. Hill. 2010. "Inactivation of adenoviruses, enteroviruses, and murine norovirus in water by free chlorine and monochloramine." *Appl Environ Microbiol*, 76 (4): 1028–1033. American Society for Microbiology. https://doi.org/10.1128/AEM.01342-09

Ehdaie, B., C. Krause, and J. A. Smith. 2014. "Porous ceramic tablet embedded with silver nanopatches for low-cost point-of-use water purification." *Environ Sci Technol*, 48 (23): 13901–13908. American Chemical Society. https://doi.org/10.1021/es503534c

Ehdaie, B., C. T. Rento, V. Son, S. S. Turner, A. Samie, R. A. Dillingham, and J. A. Smith. 2017. "Evaluation of a Silver-Embedded Ceramic Tablet as a Primary and Secondary Point-of-Use Water Purification Technology in Limpopo Province, S. Africa." *PLoS One*, 12 (1). https://doi.org/10.1371/journal.pone.0169502

Ehdaie, B., Y.-H. Su, N. S. Swami, and J. A. Smith. 2020. "Protozoa and Virus Disinfection by Silver- and Copper-Embedded Ceramic Tablets for Water Purification." *Journal of Environmental Engineering*, 146 (4): 04020015. American Society of Civil Engineers (ASCE). https://doi.org/10.1061/(asce)ee.1943-7870.0001664

Ercumen, A., A. Mohd. Naser, L. Unicomb, B. F. Arnold, J. M. Colford Jr., and S. P. Luby. 2015. "Effects of Source- versus Household Contamination of Tubewell Water on Child Diarrhea in Rural Bangladesh: A Randomized Controlled Trial." *PLoS One*, (M. Ali, ed.), 10 (3): e0121907. Public Library of Science. https://doi.org/10.1371/journal.pone.0121907

Firth, J., V. Balraj, J. Muliyil, S. Roy, L. M. Rani, R. Chandresekhar, and G. Kang. 2010. "Point-of-Use Interventions to Decrease Contamination of Drinking Water: A Randomized, Controlled Pilot Study on Efficacy, Effectiveness, and Acceptability of Closed Containers, Moringa oleifera, and In-home Chlorination in Rural South India." *Am J Trop Med Hyg*, 82 (5): 759–765. The American Society of Tropical Medicine and Hygiene. https://doi.org/10.4269/AJTMH.2010.09-0206

Hassen, A., A. Heyouni, H. Shayeb, M. Cherif, and A. Boudabous. 2000. "Inactivation of indicator bacteria in wastewater by chlorine - A kinetics study." *Bioresour Technol*, 72 (1): 85–93. Elsevier Science Ltd. https://doi.org/10.1016/S0960-8524(99)00086-3

Hill, C. L., K. McCain, M. E. Nyathi, J. N. Edokpayi, D. M. Kahler, D. J. Operario, D. D. J. Taylor, N. C. Wright, J. A. Smith, R. L. Guerrant, A. Samie, R. A. Dillingham, P. O. Bessong, and E. T. Rogawski McQuade. 2020. "Impact of low-cost point-of-use water treatment technologies on enteric infections and growth among children in limpopo, South Africa." *American Journal of Tropical Medicine and Hygiene*, 103 (4): 1405–1415. American Society of Tropical Medicine and Hygiene. https://doi.org/10.4269/ajtmh.20-0228

Jackson, K. N., D. M. Kahler, I. Kucharska, D. Rekosh, M.-L. Hammarskjold, and J. A. Smith. 2019. "Inactivation of MS2 Bacteriophage and Adenovirus with Silver and Copper in Solution and Embedded in Ceramic Water Filters." *Journal of Environmental Engineering*, 146 (3): 04019130. American Society of Civil Engineers. https://doi.org/10.1061/(ASCE)EE.1943-7870.0001634

Jackson, K. N., and J. A. Smith. 2018. "A New Method for the Deposition of Metallic Silver on Porous Ceramic Water Filters." *J Nanotechnol*, 2018. https://doi.org/10.1155/2018/2573015

Jain, S., O. K. Sahanoon, E. Blanton, A. Schmitz, K. A. Wannemuehler, R. M. Hoekstra, and R. E. Quick. 2010. "Sodium dichloroisocyanurate tablets for routine treatment of household drinking water in periurban Ghana: A randomized controlled trial." *American Journal of Tropical Medicine and Hygiene*, 82 (1): 16–22. The American Society of Tropical Medicine and Hygiene. https://doi.org/10.4269/ajtmh.2010.08-0584

Kahler, D., N. Koermer, A. Reichl, A. Samie, and J. Smith. 2016. "Performance and Acceptance of Novel Silver-Impregnated Ceramic Cubes for Drinking Water Treatment in Two Field Sites:

Limpopo Province, South Africa and Dodoma Region, Tanzania." *Water (Basel)*, 8 (3): 95. MDPI AG. https://doi.org/10.3390/w8030095

Lantagne, D. S., F. Cardinali, and B. C. Blount. 2010. "Disinfection by-product formation and mitigation strategies in point-of-use chlorination with sodium dichloroisocyanurate in Tanzania." *American Journal of Tropical Medicine and Hygiene*, 83 (1): 135–143. The American Society of Tropical Medicine and Hygiene. https://doi.org/10.4269/ajtmh.2010.09-0431

Liu, Z., J. E. Stout, L. Tedesco, M. Boldin, C. Hwang, W. F. Diven, and V. L. Yu. 1994. "Controlled Evaluation of Copper-Silver Ionization in Eradicating Legionella pneumophila from a Hospital Water Distribution System." *J Infect Dis*, 169 (4): 919–922.

Lucier, K. J., S. E. Dickson-Anderson, and C. J. Schuster-Wallace. 2017. "Effectiveness of silver and copper infused ceramic drinking water filters in reducing microbiological contaminants." *Journal of Water Supply: Research and Technology - AQUA*, 66 (7): 528–536. IWA Publishing. https://doi.org/10.2166/aqua.2017.028

Patil, R. A., D. Ahmad, S. B. Kausley, P. L. Balkunde, and C. P. Malhotra. 2015. "A compact point-of-use water purification cartridge for household use in developing countries." *J Water Health*, 13 (1): 91–102. IWA Publishing. https://doi.org/10.2166/wh.2014.111

Singh, R., C. Rento, V. Son, S. Turner, and J. A. Smith. 2019. "Optimization of Silver Ion Release from Silver-Ceramic Porous Media for Household Level Water Purification." *Water (Basel)*, 11 (4): 816. MDPI AG. https://doi.org/10.3390/w11040816

Straub, T. M., C. P. Gerba, X. Zhou, R. Price, and M. T. Yahya. 1995. "Synergistic inactivation of Escherichia coli and MS-2 coliphage by chloramine and cupric chloride." *Water Res*, 29 (3): 811–818. Pergamon. https://doi.org/10.1016/0043-1354(94)00213-Q

US EPA, O. 2020. "Drinking Water Regulations and Contaminants." Accessed January 4, 2021. https://www.epa.gov/sdwa/drinking-water-regulations-and-contaminants

World Health Organization. 2017. *Guidelines for drinking-water quality, 4th edition, incorporating the 1st addendum*. Geneva: WHO. . https://www.who.int/publications/i/item/9789241549950

World Health Organization. 2019. "1 in 3 people globally do not have access to safe drinking water – UNICEF, WHO." *News release*. Accessed March 8, 2023. https://www.who.int/news/item/18-06-2019-1-in-3-people-globally-do-not-have-access-to-safe-drinking-water-unicef-who

World Health Organization. 2022. "Drinking-water." Accessed March 8, 2023. https://www.who.int/news-room/fact-sheets/detail/drinking-water

Yahya, M. T., L. K. Landeen, M. C. Messina, S. M. Kutz, R. Schulze, and C. P. Gerba. 1990. "Disinfection of bacteria in water systems by using electrolytically generated copper:Silver and reduced levels of free chlorine." *Can J Microbiol*, 36 (2): 109–116. https://doi.org/10.1139/m90-020

Chapter 2

Literature review

The work presented in this chapter resulted in a published book chapter.

Estrella-You, A., Harris, J., Singh, R., & Smith, J. A. (2022). Inactivation of Waterborne Pathogens by Copper and Silver Ions, Free Chlorine, and *N*-chloramines in Point-of-Use Technology: A Review. In P. LeBlanc (Ed.), *Water Purification: Processes, Applications and Health Effects* (pp. 1-88). New York: Nova Science Publishers, Inc.

2.1 Introduction

This review, which mainly considers selected field studies and publications of the past 20 years, is primarily focused on presenting an overview of silver and chlorine-based compounds (free chlorine, monochloramine, and *N*-chloramines) when used as water disinfectants. More specifically, the outcomes of when these chemicals are used in point-of-use (POU) drinking water treatment technologies are discussed. Then, research findings on mechanisms of inactivation of pathogens, and toxicity of the chemical disinfectants are discussed. Finally, information about synergistic effects of different combinations of these disinfectants are introduced.

The importance of this review lies in addressing the knowledge gap and the potential to improve the technologies for POU water treatment by considering the synergism among chemicals to avoid or minimize the formation of disinfection byproducts and chemical disinfectants residuals toxicity while maintaining them affordable, accessible, and user friendly.

2.2 Water chemical disinfectants

2.2.1 Chlorine-based disinfectants

2.2.1.1 Free chlorine

Free chlorine is the most widely used chemical for water disinfection (Pereira et al. 2008). It was first used for this purpose in the early 1900s and since then has contributed to substantial

reductions in waterborne diseases (Lantagne et al. 2010). It has been proven that free chlorine can be very effective for deactivation of bacteria such as *Escherichia coli*, *Legionella*, *Salmonella* Typhi, and *Shigella*, and it can be reasonably effective for the inactivation of viruses such as adenoviruses, norovirus, and rotaviruses. Additionally, if free chlorine is used with the aid of turbidity reduction (i.e., keeping <5 nephelometric turbidity units or NTU with processes like filtration, coagulation and/or flocculation) or in higher concentrations and during longer contact times it can more effectively inactivate cysts and oocysts of protozoan species such as *Giardia* and *Cryptosporidium* (which are more resistant pathogens) (Ercumen et al. 2015; Pereira et al. 2008; World Health Organization 2017). However, high concentrations of free chlorine in water can produce harmful disinfection byproducts (see section 2.4.1 for more toxicity details).

Commonly used products that are sources of free chlorine used for POU water disinfection are commercial household bleach (diluted solution of sodium hypochlorite, NaOCl), sodium dichloroisocyanurate (NaDCC) tablets, and calcium hypochlorite, $Ca(OCl)_2$, tablets. These products are convenient, relatively safe to handle, inexpensive and easy to dose (World Health Organization 2017). Furthermore, they react with water to form free chlorine or the sum of two chlorine species: hypochlorous acid (HOCl) and hypochlorite ion (OCl^-) at pH above 2. Both species are in equilibrium which depends on the pH and temperature (Yahya et al. 1992). Even though both species are effective in deactivating waterborne pathogens, HOCl is more reactive and more effective than OCl^-. Therefore, to obtain a highly effective disinfection with free chlorine, the pH should ideally be between 7 and 7.5 because within this range free chlorine is less likely to be corrosive and the protonated species, HOCl, is predominant (Sharma et al. 2017; World Health Organization 2017).

Sodium hypochlorite and calcium hypochlorite solutions decompose slowly, decreasing the concentration of free chlorine due to chlorate, perchlorate, and chlorite ion formation. Even so, solid calcium hypochlorite decomposition is much slower (World Health Organization 2017). Bleach, which generally has a shelf life of six months to one year, can be 2.4 times cheaper than NaDCC tablets but the latter have several advantages such as: shelf life for 5 years, resistance to sunlight degradation, single use packaging, low weight in distribution, and easy dissemination and handling (Ercumen et al. 2015; Jain et al. 2010; Lantagne et al. 2010).

Free chlorine residual is the free chlorine available to protect the already treated water against recontamination (World Health Organization 2017). But the use of free chlorine for drinking water disinfection and the presence of its residual can alter the water taste and odor, which is often not appealing to consumers (Patil et al. 2015). Some people can taste or smell free chlorine in treated water at concentrations as low as 300 μg/L. Nevertheless, the WHO recommends maintaining a free chlorine residual concentration between 200 and 500 μg/L to avoid recontamination. Besides, the WHO recommends for POU drinking water disinfection a free chlorine dose of 2,000 μg/L for clear water (<10 NTU) and 4,000 μg/L for turbid water (≥10 NTU), with both values below the health-based standard of 5,000 μg/L (World Health Organization 2017).

Several studies have been carried out to evaluate the efficacy of free chlorine when used for disinfection of drinking water (usually a dose of 2,000 μg/L free chlorine is tested). Generally, in field studies chlorination is employed as a POU intervention. Its efficacy with respect to *E. coli* inactivation and its impact related to waterborne diseases are usually assessed. Also, variables such as source water conditions (e.g., turbidity), season variability (i.e., rainy, or dry), type of water storage container, handling of treated water, etc. are key in field studies. Whereas laboratory studies include a broader range of pathogens such as viruses and protozoa and are more focused on determining the inactivation kinetics by changing parameters like pH and temperature. In the next sections, examples of these kinds of studies are described.

2.2.1.1.1 Water chlorination in field studies

Levy et al. (2014) compared *E. coli* deactivation with free chlorine under controlled conditions (laboratory settings) versus chlorination under household or real-world use practices (observational study); see Table 2.1 for study conditions information. They also compared two disinfectant products: a commercial product used with a free chlorine dosage of 1,875 μg/L and a local product applied with a dose approximately twice as high. The researchers first found that 38% households that used low turbidity (<10 NTU) source water had <1 MPN/100 mL *E. coli* after treatment as compared to 17% households that used high turbidity (≥10 NTU) source water ($p = 0.007$). Hence, turbidity ≥10 NTU in source water hindered chlorination effectiveness.

Table 2.1: Summary of conditions in recent field studies that evaluate chlorination efficacy

	Jain et al. (2010)	Barzilay et al. (2011)	Levy et al. (2014)	Ercumen et al. (2015)
Study site	Periurban population in Tamale, Ghana	Lagos, Nigeria	7 rural villages in Esmeraldas Province, Ecuador	87 rural villages in Bangladesh
Study period	12.5 weeks between August and November 2006 (rainy season)	23 weeks from, February to August 2005 (from dry to rainy season)	4 weeks in June and July 2010	1 year (dry season: Oct 2011-May 2012; rainy season: June-Nov 2012)
Participants	240 households (3,240 individuals) with at least one child <5 years old	187 HIV infected women	138 households	1,800 households with a child aged 6–18 months old
Disinfectant	Intervention group: NaDCC tablets (AQUATABS) Control group: placebo tablets	WaterGuard: a 150 mL bottle of 1.25% sodium hypochlorite solution with a 4 mL screw cap used to measure and dispense the disinfectant	Arm 1: none Arm 2: locally available chlorine (diluted NaOCl in plastic container) Arm 3: commercial bleach (provided by research team, Ajax brand); on average the concentration was 2x as high as the local product	33 mg NaDCC tablets (AQUATABS)
Dosage	• 1 tablet per 20 L (general) • 2 tablets per 20 L (for turbid water) ○ Contact times and corresponding free chlorine concentration not reported	Not reported	Arm 1: none Arm 2 (high dose): chose by household members or local health promoter, no instruction from researchers; ~2x as high as arm 3 dose Arm 3 (low dose): 1,875 μg/L • Contact time: 24±3 hours	Arm 1: 1 tablet per 10 L (equivalent to 2,000 μg/L free chlorine) Arms 2 and 3: none • Contact time: 24 hours
Source water	Tap (samples yielded no *E. coli*), surface water (median *E. coli*: 178 MPN/100 mL), well, rain, borehole, water tanker	Direct household connection, public standpipe, borehole, well, spring, rain, vendor provided, bottled, tanker truck	Rain (*E. coli* $\log_{10}$ geometric mean: 0.67), tap (0.59), well (2.13), river (2.83), stream (2.84)	Groundwater with low concentrations of iron: • 41% samples were positive for *E. coli* • 14%: >10 CFU/100 mL • 3%: >100 CFU/100 mL
Storage containers	20-liter plastic vessel with a plastic lid and metal spigot (provided by researchers for both intervention and control groups)	25-liter jerry can with a narrow mouth, spigot, and a comfortable handle (provided by researchers)	Small mouth (≤8 cm) or large mouth (>8 cm) containers	Arms 1 and 2: narrow mouth (10.5 cm diameter) vessel with a tightly fitting lid and tap provided by researchers Arm 3: kolshis (lidless aluminum vessels with a narrow mouth) or jugs
Materials provided for disinfection improvement	• A guinea worm cloth (commonly used in the community) for filtering turbid water • Alum was provided on request	None	None	Brush and detergent to clean the provided safe storage container

Secondly, the WHO guideline of <1 MPN/100 mL *E. coli* for drinking water was met by 51% households using local chlorine and 39% households using the commercial product (p = 0.291). At the same time, 73% laboratory samples with local product and 52% laboratory samples with commercial bleach (p = 0.059) met the WHO *E. coli* guideline. In consequence, water chlorination effectiveness under controlled conditions (laboratory settings) was significantly better than chlorination under household conditions (p = 0.0012 when comparing local chlorine results, and p = 0.0008 when comparing commercial product effects). Finally, household samples with free chlorine residual between 200 and 2,000 μg/L were 35.0% in the local chlorine group and 53.3% in the commercial chlorine group, and laboratory samples that met this residual range were 44.7% in the local chlorine group and 42.9% in the commercial chlorine group.

In an earlier study, Jain et al. (2010) conducted an intervention study (with ideal use practices) to assess the health impact of NaDCC tablets and storage containers daily use for drinking water treatment (see Table 2.1 for study conditions information). Enrolled households were randomly assigned to one of the following groups: control (with placebo tablets) and intervention (with NaDCC tablets). Also, each household was provided with a 20-liter plastic vessel. At the end of the study, 92% stored water samples in the intervention group and 46% in the control group (p = 0.002) met the WHO guideline of <1 MPN/100 mL *E. coli* for drinking water. Overall, water quality significantly improved in both groups. And safe water storage containers may have served as a water quality intervention for the control group because diarrhea rates decreased over the study period in this group. However, these rates were much lower than anticipated resulting in diarrhea reductions that were not significantly different between the two studied groups.

A similar intervention study was performed by Ercumen et al. (2015) (see Table 2.1 for study conditions information). The objective of this study was to evaluate the impact of safe storage with and without chlorination on the quality of household water and diarrhea among children under 2 years old. Each enrolled household was randomized in one of three arms: 1, chlorination with safe storage; 2, only safe storage; and 3, no intervention. The results of this study show that during the dry season, the WHO guideline of <1 CFU/100 mL *E. coli* for

drinking water was met in 83% arm 1 samples, 41% arm 2 samples, and 16% arm 3 samples. Nevertheless, during the rainy season these percentages decreased (65% arm 1 samples, 18% arm 2 samples, and 6% arm 3 samples). In addition, a higher percentage of source water samples were positive for *E. coli* during the rainy season compared to the dry season across all 3 arms. Furthermore, 70% arm 1 samples met the WHO guideline of free chlorine residual of 200–5,000 μg/L. Another finding of this study was that safe storage, alone or combined with chlorination, significantly improved the quality of stored water. And, compared to the control arm (no intervention), 7-day diarrhea episodes in children 8 to 32 months old was reduced by 36% in arm 1 and 31% in arm 2; in other words, there was no added benefit from water chlorination on diarrhea reduction.

Another intervention study was conducted by Barzilay et al. (2011) to examine the impact of POU water chlorination along with safe water storage on diarrhea prevention among human immunodeficiency virus (HIV) infected women (see Table 2.1 for study conditions information). After the intervention, diarrhea rates in HIV infected women were reduced by 36%. More specifically, for participants who were confirmed to have free chlorine residual in stored water during ≥85% of home visits, the diarrhea rate reduction was 46% when comparing post-intervention with baseline ($p = 0.04$). And, for participants confirmed to have free chlorine residual in stored water during <85% visits, there was no statistically significant difference in diarrhea rates ($p = 0.47$). Even though these rates were low because all diarrhea episodes were reported by 36.9% of participants, regular POU water chlorination combined with the use of safe water storage containers reduced the risk of diarrhea in HIV infected women.

Even though this is not a comprehensive review of water chlorination field studies, next we present general observations. First, the results of the studies indicate that turbidity in source water can significantly affect chlorination effectiveness. According to the WHO, this can happen because suspended particles that contribute to high levels of turbidity can protect pathogens from the effects of disinfection, stimulate their growth and give rise to a significant chlorine demand (World Health Organization 2017). For this reason, it is important to consider the inclusion of aids or processes (such as cloths, alum, filtration, sedimentation, etc.) to reduce turbidity before disinfection in order to improve the efficacy of chlorination. Also, the weather

conditions can impact the outcome of water chlorination. In other words, a rainy season can mean an increase in the concentration of pathogens in source water. Yet, if the dry season dosage of free chlorine is applied to the more contaminated rainy season water, there is the potential to have a less effective treatment.

Secondly, in the reviewed studies, the percentage of treated water samples that met the WHO guideline of <1 MPN/100 mL *E. coli* varied widely. For instance, in the Levy et al. (2014) study, 39% to 51% samples met this guideline while treated with NaOCl solutions. But, in Jain et al. (2010) and Ercumen et al. (2015) studies where NaDCC tablets were used, 65% to 92% samples met the guideline. Different source water microbial quality could explain these differences in WHO guideline compliance, since the more contaminated the source water, the more free chlorine and contact time would be required to achieve a complete pathogen reduction. Additionally, the type of applied free chlorine product (i.e., diluted NaOCl solutions or NaDCC tablets) and its dosage can affect the disinfection outcome. Free chlorine tablets are easy to use and have a long shelf life, so it is more probable that a consistent dose can be achieved when the user employs it. However, liquid products decompose slowly, decreasing the concentration of free chlorine, which can make dosage less uniform and in consequence affect the chlorination effectiveness.

Third, with respect to water chlorination effects on waterborne diseases reduction, diarrhea as a symptom of these diseases is usually analyzed. Both Ercumen et al. (2015) and Barzilay et al. (2011) studies reported a 36% decrease in diarrhea rates after intervention with POU water chlorination accompanied with safe water storage. Ercumen et al. (2015) analyzed these rates among children under 2 years old, and Barzilay et al. (2011) among HIV infected women.

Finally, the use of new storage containers alone (i.e., without water chlorination) may serve as a water quality intervention. Jain et al. (2010) reported that 46% samples tested negative for *E. coli* over the study period in the group that was provided with safe storage vessels and placebo tablets. Similarly, in the Ercumen et al. (2015) study, the safe storage group results show that 41% dry season household stored samples and 18% rainy season household stored samples had <1 MPN/100 mL *E. coli*. Furthermore, in both studies, the use of storage containers alone resulted in a decrease of diarrhea rates comparable to when chlorination along with safe storage was

used. This outcome could be explained by the need of a routine cleaning procedure of the storage containers that were being used in the households before the intervention studies.

2.2.1.1.2 Water chlorination in laboratory studies

A laboratory study by Pereira et al. (2008) assessed the efficacy of free chlorine for the inactivation of *Cryptosporidium parvum* oocysts in water from the public supply of a state in Southern Brazil. The water characteristics were pH 7.5 and temperature of 20°C, and the seeded concentration of the protozoa species was 2×10^4 oocysts/mL. The results of the study show that a dose of 2,000 μg/L free chlorine had a low deactivation efficacy of 49.04% after 120 min. Other researchers found a similar efficacy of 58.42% even though they used a doubled dose (4,000 μg/L free chlorine at 20°C) and even after 4 days of treatment (the inoculum dose was prepared to be 2.5×10^4 oocysts/mouse, where mice were infected with *Cryptosporidium* oocysts previously exposed to free chlorine) (Soliman et al. 2018). It is known that *C. parvum* is very resistant to free chlorine so higher concentrations of the disinfectant or longer contact times could be used to achieve higher inactivation efficacies. However, this can be impractical because the retention time in a conventional water treatment plant is not generally greater than 120 min and it is not recommended to use high concentrations of free chlorine because of the formation of byproducts. As a result, more efficient disinfecting chemicals such as chlorine dioxide and ozone could be considered as alternatives for complete *C. parvum* deactivation.

Venczel et al. (2004) evaluated the inactivation of bacterial (*E. coli*, *Vibrio cholerae*, *Clostridium perfringens* spores), viral (MS2 coliphage as a surrogate for human pathogenic viruses) and protozoan (*Cryptosporidium parvum* oocysts) species with free chlorine at different pH values and temperatures, and with or without the presence of humic acid. The results from deactivation of *E. coli*, *V. cholerae*, MS2 and *C. perfringens* spores with 2,000 μg/L free chlorine in water without humic acid are presented in Table 2.2. According to these results, *E. coli*, *V. cholerae*, and MS2 were inactivated to 4-$\log_{10}$ reduction by 30 minutes under all conditions tested. But the spores were deactivated by no more than 2-$\log_{10}$ reductions in >60 minutes. Table 2.2 results show that the deactivation time generally decreased with the 20°C increase in temperature (except for the spores where the time increased slightly) which means inactivation

becomes faster as temperature increases. And the inactivation time varied differently for each pathogen as pH changed between 6 and 10.

Table 2.2: Mean time required for deactivation of pathogens with 2,000 μg/L free chlorine in water without humic acid. Adapted from Venczel et al. (2004) with permission from the copyright holders, IWA Publishing.

Pathogen	pH 6		pH 8		pH 10		log_{10} reduction
	5°C	25°C	5°C	25°C	5°C	25°C	
	Mean time for inactivation (min)						
E. coli	24	0	10	5	14	2.3	4
Vibrio cholerae	3	1.3	4.3	4	7.3	1.9	4
MS2 coliphage	11	5	14	18	9.5	7	4
Clostridium perfringens spores	77	83	155	168	104	110	2

Note: Venczel et al. (2004) mention that only 2-log_{10} reductions are shown for *C. perfringens* because first order linear regression estimations of 4-log_{10} inactivation times were not appropriate for downward extrapolation. In other words, the inactivation kinetics became progressively retardant with the slope of the inactivation curve approaching a value of zero.

Results from Venczel et al. (2004) for deactivation of *E. coli*, MS2 and *C. perfringens* spores with 2,000 μg/L free chlorine in water with 4 mg/L humic acid are shown in Table 2.3. Analyzing the effects of the presence of organic matter, the inactivation time for the spores was shorter in the water with humic acid (113 min) than in water without it (168 min). On the contrary, *E. coli* deactivation time increased from 5 to 14 minutes when there was humic acid, but for MS2 inactivation the time increased much more from 18 to 103 minutes. Finally, *C. parvum* oocysts inactivation tests were only performed at 7 and 25°C, and with a free chlorine dose of 5,000 μg/L. These pathogens were not inactivated, since the log_{10} reduction results were: 0 at 30 min and 0.12 at 90 min.

Table 2.3: Mean time required for deactivation of *E. coli*, MS2 and *C. perfringens* spores with 2,000 µg/L free chlorine in water at pH 8 and 25°C, and with 4 mg/L humic acid. Adapted from Venczel et al. (2004) with permission from the copyright holders, IWA Publishing.

Pathogen	Mean time for inactivation (min)	$\log_{10}$ reduction
E. coli	14	4
MS2 coliphage	103	4
Clostridium perfringens spores	113	2

2.2.1.2 N-chloramines

Biocidal polymers have been developed as antimicrobial agents or coatings to effectively inhibit the growth of microorganisms in drinking water or surfaces. *N*-chloramines are chlorine based biocidal polymers that have a great potential of applications such as paints, healthcare products, air purification, food processing, water disinfection, odor control in water treatment facilities and recirculating baths, and coatings on plastics, textiles, or metals (Bastarrachea et al. 2014; Hui and Debiemme-Chouvy 2013; Liang et al. 2005). Some characteristics of *N*-chloramines are long term stability in aqueous solution and in dry storage, proven effective against a broad spectrum of microorganisms (e.g., *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Shigella boydii*, *Candida tropicalis*, MS2 virus), weakly toxic, relatively cheap and, compared to sodium hypochlorite (bleach), they are less corrosive (Dong et al. 2017; Hui and Debiemme-Chouvy 2013).

Structurally, *N*-chloramines contain one or more nitrogen-chlorine covalent bonds which provide powerful antimicrobial properties due to the strong oxidative state +1 of the chlorine atom (Figure 2.1). *N*-chloramines can be organic or inorganic compounds, and are also classified as cyclic, acyclic or a combination of the two as well. To synthesize *N*-chloramines there are two main steps: precursor preparation and chlorination. The most common precursor preparations include polymerization of monomers bearing N-H groups and grafting or coating monomers onto polymer backbones or substrates such as resins, membranes, fibers, etc. (for example see Figure 2.2). Then, the chlorination step can be accomplished by exposing the precursor with a

Cl^+ donor compound such as sodium hypochlorite (Dong et al. 2017; Hui and Debiemme-Chouvy 2013).

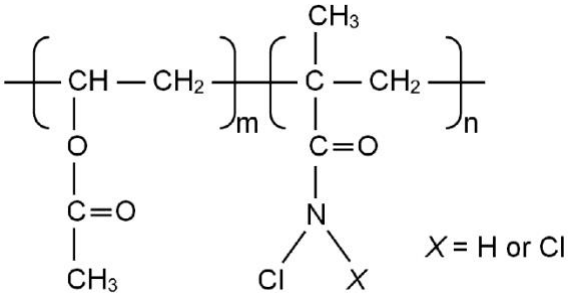

Figure 2.1: Acyclic *N*-chloramine copolymer poly(vinyl acetate-co-methacrylamide). Reprinted with permission from Hui & Debiemme-Chouvy (2013). Copyright 2013 American Chemical Society.

Insoluble *N*-chloramines used for POU water disinfection have antimicrobial properties by release (the polymers have an inhibitory zone around them) or by contact (direct contact with the waterborne pathogen) (Dong et al. 2017); see more information about the inactivation mechanisms in section 2.3. These polymers can be substantial reservoirs of chlorine which translates to very high effective chlorine concentrations. Consequently, very short contact times are sufficient for effective deactivation of pathogens (Chen et al. 2003).

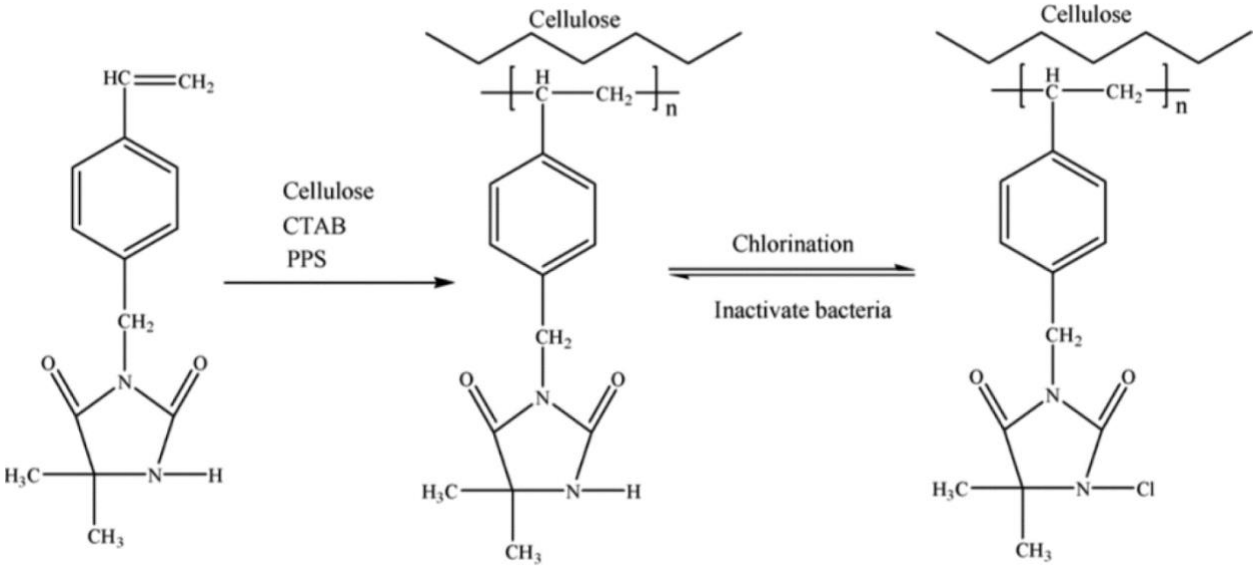

Figure 2.2: Admicellar polymerization of 3-(4′-vinylbenzyl)- 5,5-dimethylhydantoin (VBDMH) on cellulose and its conversion to an *N*-chloramine biocidal cellulose. Reprinted with permission from Hui & Debiemme-Chouvy (2013). Copyright 2013 American Chemical Society.

A unique property of *N*-chloramines is that they are rechargeable or regenerable. In other words, after using *N*-chloramines for inactivation of pathogens, its chlorine supply will get exhausted. But interestingly, chlorine can be easily and repeatedly replenished on the polymers by simply exposing them with a Cl^+ donor compound such as bleach or NaDCC tablets (Figure 2.2) (Bastarrachea et al. 2014; Chen et al. 2003; Hui and Debiemme-Chouvy 2013).

Compared to the more than 100 years of use of free chlorine for water disinfection, the implementation of insoluble *N*-chloramines as biocidal agents for drinking water is relatively new. To our knowledge there are no *N*-chloramines field studies related to POU water treatment applications. *N*-chloramines are more commonly used in membranes and textiles or as coatings and paints for surfaces to inhibit the growth of pathogens. Next, we present laboratory studies where *N*-chloramines have been tested for drinking water disinfection.

2.2.1.2.1 Water disinfection with N-chloramines in laboratory studies

A laboratory study was conducted by Chen et al. (2003) to evaluate the efficacy of inactivation of pathogens, chlorine stability, and rechargeability of *N*-chloramine beads in a water filtration application (see Table 2.4 for detailed experimental settings). The filtration system consisted of glass columns containing insoluble porous beads made of poly[1,3-dichloro-5-methyl-5-(4'-vinyl phenyl)hydantoin] or Poly1-Cl. The first main finding of this study was that *S. aureus* and *E. coli* were completely inactivated (~7 $\log_{10}$ removal) in very brief contact times (≤1.1 s). It was also demonstrated that the bacteria were inactivated and not only removed by filtration. Secondly, for poliovirus complete deactivation, longer contact times (107 s for 1.4 $\log_{10}$ removal, and 120 s for 4–5 $\log_{10}$ removal) were needed compared to the times for MS2 full inactivation (3.2–91 s for 5 $\log_{10}$ removal). The reason why the required contact times are so short when using Poly1-Cl beads for inactivation of pathogens could be related to the very high effective concentration of Cl (in the order of g/mL, more details in Table 2.4), in contrast to the concentrations (mg/L or μg/L) that can be used safely with water soluble disinfectants (e.g., silver ions, free chlorine, monochloramine), which can require several minutes for deactivation. Third, with respect to chlorine stability, about 5.4% of the initial chlorine loading (17.16%) was lost over a 90-day period under dry, vacuum conditions (final Cl load: 16.23%).

Table 2.4: Settings in recent laboratory studies that evaluate *N*-chloramines biocidal efficacy

	Chen et al. (2003)	Liang et al. (2005)	McLennan et al. (2009)	Coulliette et al. (2013)
Disinfectant	Glass columns containing insoluble porous beads made of poly[1,3-dichloro-5-methyl-5-(4′-vinyl phenyl)hydantoin] or Poly1-Cl	Cartridge filter containing sand particles coated with an adhered film composed of chlorinated 3-triethoxysilylpropyl-5,5-dimethylhydantoin polymer	AquaSure water purifier device: • consists of an upper reservoir for influent water that flows by gravity and later passes through a disinfectant cartridge within a lower storage tank from which the water is dispensed through a spigot • the contact disinfectant cartridge contains HaloPure media or *N*-chloramine beads • the device usually includes cloth and activated charcoal prefilters, but these were removed to directly evaluate the *N*-chloramine inactivation performance (the prefilter normally removes large particulates, preventing the device from clogging and indirectly increasing the life of the charged beads)	
Dose	• Chlorine loading of 14-18 wt % in the Poly1-Cl beads • **Cl available for inactivation tests:** ○ Bacteria: 0.55-0.70 g Cl in beads packed into a column with an empty bed volume of 3.3 mL ○ MS2: 5.32 g Cl in beads packed into a pipet with an empty bed volume of ~6.0 mL ○ Poliovirus: 2.52-5.22 g Cl in beads packed similarly to the MS2 tests	Polymer bound sand contained about 0.28 wt % of Cl • packed into a glass column with empty bed volume of 6.42 mL	Not reported	Not reported
Tested water	Chlorine-demand-free water at pH of 6.5-7.5	Distilled water buffered to pH 7.0	Well water contaminated with raw sewage (1:10 dilution); turbidity of 132 ± 12 NTU	• Well water seeded without raw sewage • Well water seeded with 10% raw sewage
Pathogens analyzed	• *Staphylococcus aureus* • *Escherichia coli* O157:H7 • MS2 virus • Poliovirus	• *Staphylococcus aureus* • *Escherichia coli*	• Total coliforms • Heterotrophic bacteria • *E. coli* • *Enterococcus* • *Clostridium* • Coliphage	• *Salmonella* serovar Typhimurium • *Vibrio cholerae*
Other analysis/ measurements	Free chlorine residual: • <100 μg/L were detected in the water treated with the columns containing the Poly1-Cl beads	Free chlorine residual: • in the effluent was about 200 μg/L	• First flow (when water first began to exit the spigot on the lower reservoir): ○ 6 to 12 min • Free chlorine residual: ○ did not produce a measurable residual	• First flow: ○ Without raw sewage: 11 min ○ With raw sewage: 7 min • Mean free chlorine residual: ○ Without raw sewage: 0.0 μg/L ○ With raw sewage: 150 μg/L

Also, it is indicated that the Cl content can decrease more with time if moist conditions are present. Finally, around 12% less of the initially loaded Cl was regenerated after 100 recharge cycles under alkaline conditions (final Cl load: 15.3%). Yet, it has previously been shown that a chlorine loading of only 10.5% in the beads still works very well for inactivation of pathogens.

Further, Liang et al. (2005) tested the bacterial deactivation efficacy of sand coated with chlorinated 3-triethoxysilylpropyl-5,5-dimethylhydantoin polymer in a packed column (see Table 2.4 for detailed experimental settings). The study results show that complete inactivation was observed within 1 min of contact for *S. aureus* (6.5 log removal) and in the interval of 1–5 min for *E. coli* (7.4 log removal). The Cl stability of the coated sand was assessed by filling a cartridge with the sand and continuously flowing water at 5 mL/min. It was found that the stability is high because after over 1 week, the Cl loading in the sand declined from 0.274 to 0.251 wt %. And, after that time, the sand was recharged and yielded a Cl loading of 0.271 wt % which means that the loss of bounded polymer was very small.

Coulliette et al. (2013) and McLennan et al. (2009) examined the efficacy of other type of *N*-chloramine beads from a water purifier device (AquaSure brand, also a filtration application) for the inactivation of pathogens in well water with and without raw sewage (see Table 2.4 for detailed experimental settings). McLennan et al. (2009) only used water contaminated with sewage and obtained a complete bacterial removal. The geometric mean $\log_{10}$ reductions after 30 min contact time were: 4.5 for total coliforms, 4.2 for heterotrophic bacteria, 3.7 for *E. coli*, 3.0 for *Enterococcus*, and 1.5 for *Clostridium*. Nevertheless, ideal coliphage virus reduction was not achieved since the geometric mean $\log_{10}$ removal was 1.1 (the seeded geometric mean $\log_{10}$ concentration was 2.0). On the other hand, Coulliette et al. (2013) found that *S*. Typhimurium was more resistant to inactivation with *N*-chloramine beads compared to *V. cholerae*. A similar *S*. Typhimurium mean $\log_{10}$ reduction was reached with and without sewage (6.06 and 5.44, respectively). Whereas a higher *V. cholerae* mean $\log_{10}$ reduction was attained with sewage than without sewage (7.78 and 6.07, respectively).

Chen et al. (2003) and Liang et al. (2005) studies had very similar experimental settings, therefore it is possible to compare their results. There is a clear difference in Cl loading between the studies and consequently a difference in the required contact time for the complete

inactivation of the same waterborne bacteria. Liang et al. (2005) tested sand particles containing 0.28 wt % of Cl and obtained 6.5 log removal for *S. aureus* within 1 min of contact and 7.4 log removal for *E. coli* in the interval of 1–5 min. But Chen et al. (2003) used beads containing 14-18 wt % of Cl and reported ~7 $\log_{10}$ removal of *S. aureus* and *E. coli* in very brief contact times (≤1.1 s), which made these beads more efficacious than the sand because of the higher Cl loading. Additionally, these two studies covered rechargeability evaluations. The results show that the oxidative chlorine that is lost from the beads or sand particles after many recharge cycles (which simulates losing the Cl after biocidal activity), can be partially regenerated to amounts that do not affect the inactivation efficacy.

In general, the studies show that there is a free chlorine residual in the effluent water. Since the studies used filtration settings, Cl is leached out and forms the residual in the effluent. Coulliette et al. (2013) results show that the tested well water with raw sewage resulted in a higher free chlorine residual than when the water did not contain any sewage. This means that more frequent recharging will be necessary with water containing a large chlorine demand.

2.2.1.3 Monochloramine

The focus of this review is to discuss chemical disinfectants used in POU interventions. For this reason, the main chlorine-based disinfectants described are free chlorine and *N*-chloramines. A brief background information about monochloramine is also discussed here because this chemical is mentioned in other sections.

Monochloramine (NH_2Cl), dichloramine ($NHCl_2$) and trichloramine (NCl_3) are considered chlorination byproducts because they are formed when ammonia reduces free chlorine. However, only monochloramine is useful for water disinfection because the higher chloramines can produce undesirable changes in taste and odor in the treated water (World Health Organization 2017). In addition, at the pH and free chlorine concentrations used at water treatment plants, monochloramine is the predominant form of chloramine (Cromeans et al. 2010; Straub et al. 1995).

Monochloramine can be used in drinking water treatment plants as a secondary disinfectant to minimize the formation of trihalomethanes (THMs) and biofilm. It can also

maintain residual disinfection activity throughout water distribution systems with long residence times and elevated temperatures since monochloramine has a lower disinfection effectiveness, but higher stability compared to free chlorine (Cromeans et al. 2010; World Health Organization 2017). The typical monochloramine concentrations found in drinking water supplies range from 500 to 2,000 μg/L (the WHO guideline for monochloramine in drinking water is 3,000 μg/L) (World Health Organization 2017).

Dichloramine and trichloramine can be formed when production of monochloramine is not properly controlled. In other words, dichloramine and trichloramine are produced when there is an inappropriate free chlorine to ammonia weight ratio (i.e., >2:1 or free chlorine in excess) or when the pH is >7.5 (Cromeans et al. 2010; Straub et al. 1995). Taking this information into account, chloramination systems are designed with conditions to minimize the formation of higher chloramines to avoid changes in taste and odor that can be unappealing to some consumers (World Health Organization 2017).

2.2.2 Silver

Silver and silver-based compounds have been used as disinfectants for centuries. Their use dates back as early as the first century (Deshmukh et al. 2019). Many other metals (e.g., copper, zinc, iron, gold) are effective for disinfection, but silver has proven superior and most popularly researched for its biocidal efficacy (Lalley et al. 2014). Silver as an antimicrobial agent is popular in the form of metallic or metallic compounds. Silver is used as silver nitrate (Singh et al. 2019), silver nanoparticles (AgNPs) (Das et al. 2020), and silver wires (Hill and Smith 2019) for water disinfection. Recently, silver has become more popular in the nano form, which provides a larger surface area for contact with microorganisms resulting in better disinfection. Technologies based on these forms of silver provide POU water purification including ceramic filters (Kallman et al. 2011; Oyanedel-Craver and Smith 2008), bactericidal paper (Dankovich and Gray 2011), silver nanoparticle-coated rice (Lin et al. 2013), AgNPs-alginate composite beads, and polyurethane foams homogeneously coated with AgNPs (Jain and Pradeep 2005).

The U.S. EPA secondary drinking water standard and the WHO guideline for silver in drinking water is 100 μg/L. Silver disinfection efficacy depends on various parameters which

include temperature, pH, contact time, organic matter, and calcium and chloride concentration. Silver-based technologies using ceramics are of particular interest in scenarios with underserved communities. Among these, ceramic filters impregnated with AgNPs can have a better disinfection efficacy because they provide effective metallic disinfection in addition to physical filtration (Abebe et al. 2014; Kallman et al. 2011).

The reachability of these so-called 'household drinking water treatment systems' is vast among many people in the short-term, which certainly demands a degree of expertise and commitment by the users. The ceramic silver-impregnated pot filter (CSF) is one such household drinking water treatment system used worldwide. These pot filters have manufacturing factories in various countries including Honduras, Kenya, Cambodia, Ghana, South Africa, and Nicaragua (van Halem et al. 2009). Ceramic filter elements are commonly manufactured with local materials, and skills and labor to support the local economy. A blend of clay, sawdust, and water is mixed in some ratio and pressed into a specific pot shape using press molds. Once the filter element gets its shape, it is air-dried and then fired in a kiln so that the sawdust is combusted to leave porous material. Then, the filter can be impregnated with a mixture of colloidal silver, for assumed disinfection purposes, before distribution to the customers (Oyanedel-Craver and Smith 2008).

Silver can also be added at mixing steps due to underlying disadvantages with this conventional practice of application of silver after firing. The new method involves adding silver nitrate dissolved in water and combining with clay and sawdust uniformly (Jackson and Smith 2018).

Apart from ceramic silver technology, silver electrodes have potential as a means for electrolytic disinfection. Silver ion can be recovered, proving electrolytic silver disinfection an economical and environmentally friendly option (Justia Patents 1977). Electrolytic silver disinfection has a potential for application in point-of-use water treatment devices to provide resilient and sustainably treated water in a setting with underserved populations (Hill and Smith 2019).

2.2.2.1.1 Water Disinfection with Silver in Laboratory and Field Studies

Several studies have been carried out using silver-based technologies for the disinfection of drinking water. Silver in field studies is employed as a POU technology at household level.

van Halen et al. (2009) demonstrated the sustainability of a household drinking water treatment system based on five criteria: accessibility, water quality, water production, functionality, and environmental footprint (see in Table 2.5 for study conditions information). In this study, the authors used CSF to monitor *E. coli*, protozoan (oo)cysts, and MS2 phage to check water quality for microbial contaminants and effectiveness of CSF in the reduction of diarrheal cases. There was a significant removal of *Escherichia coli* and protozoan (oo)cysts (van Halem 2006; van Halem et al. 2007), which was supported by the reduction in diarrhea cases observed by CSF users. Therefore, CSF was found effective in improving water quality. Although CSF performed excellently for diarrhea inducing microbes, it was not effective for the removal of MS2 bacteriophage (log_{10} reductions below 1).

van Halen et al. (2009) also discussed the water accessibility criterion, which depends on two factors: availability and affordability. Availability is limited to the surrounding area and at a low price, which is directly related to affordability. The limiting factor was the reduced water production resulted from a decreased flow rate. The decrease in flow rate requires frequent scrubbing which results in a higher risk of recontamination and breakage. Based on this finding, the authors recommended an optimization study to increase the initial flow rate with no effect on removal efficiency. Besides, water production is directly related to affordability and functionality. The environmental footprint criterion was complex to measure, but the authors stated it is an eco-friendly system as compared to other household drinking water treatment systems.

A similar study by Brown and Sobsey (2010) evaluated the performance of two Cambodian porous ceramic water purifiers or filters (CWFs) against bacterial and viral pathogen (*E. coli* and MS2 bacteriophage) surrogates in the laboratory under replicated household use conditions using actual drinking water sources and harvested rainwater (see in Table 2.5 for study conditions information). This laboratory testing preceded a field-based intervention study.

Table 2.5: Summary of conditions in recent field and laboratory studies that evaluate silver efficacy as a water disinfectant

	van Halem et al. 2006, 2007, 2009	Brown & Sobsey 2010	Nawaz et al. 2012	Abebe et al. 2014	Guerrero-Latorre et al. 2019
Study site	Cambodia	Cambodia	Seoul, South Korea	Limpopo Province, South Africa	Rural Ecuador
Study period	12 weeks	Over 3 months	168 h	Over a year (June 2009 through August 2010)	6 Months
Participants	80 households	Laboratory based	Laboratory Roof Top Harvesting	74 participants	10 household in each community (Total=20)
Technology	Ceramic colloidal silver-impregnated pot filters	$AgNO_3$ solution painted on filters	$AgNO_3$ suspension solution	Ceramic water filters (CWFs) impregnated with silver nanoparticles	Black ceramic water filters (BCWF) with silver
Dosage	Not reported	Not reported	Concentrations of 10–100 μg/L of silver	Not reported	Colloidal silver 250 mL of a solution at 375 mg/L
Source water	Canal water	Rainwater and surface water	Rainwater	Home taps and community taps from a treated source	Surface water
Storage containers	Ceramic filters in a plastic container	Ceramic water purifier units (filter enclosed in plastic container)	Storage tanks	5-gallon plastic container-safe storage reservoir	Ceramic filter units in plastic container
Pathogens analyzed	*E. coli K12*, MS2 phage, *Clostridium* spores	*E. coli*, MS2 bacteriophage	*Pseudomonas aeruginosa, Escherichia coli*	Total coliform and *Cryptosporidium* sp.	*E. coli*, adenovirus, MS2 bacteriophage
Reduction in pathogen loads	Successful removal of coliforms from the canal water, high concentrations of *Clostridium* spores (2-5 log_{10}) and *E. coli* K12 (4-7 log_{10}). Even the MS2 bacteriophages (0.5-3.0 log_{10}) of 23-25 nm are partially retained by CSF	• For *E. coli*: mean log_{10} reduction was 2.9 log_{10} (95% CI 2.5–3.4) within the first 100 L of testing and 2.1 log_{10} (95% CI 2.0–2.2) thereafter ($p = 0.0001$). • For MS2: mean log_{10} reduction was 4.1 log_{10} (95% CI 3.5–4.8) within the first 100 L of testing and 1.2 log_{10} (95% CI 1.1–1.3) thereafter ($p = 0.0001$).	• Initial concentration of *P. aeruginosa* (350-440 CFU/100 mL) and *E. coli* (740-920 CFU/100 mL). • The inactivation was not completed at lower concentration of silver (10 and 20 μg/L) for either *P. aeruginosa* or *E. coli* except at 40 μg/L of silver for *E. coli* inactivation. • Complete inactivation was achieved at higher silver concentration (80-100 μg/L)	• Removal of total coliform bacteria (median 2 log_{10} removal) • A subset of six filters was tested for the removal of 106 cfu/100 mL and exhibited a 6-log removal of *E. coli*	• Microbiological efficiency showed reduction values of 5.36 logarithms for *E. coli* (standard error ±0.38) and 3.83 logarithms for MS2 bacteriophage (standard error ±1.47)

The main objective of this study was to evaluate the extent to which filters could be effective against bacteria and viruses at the household level for long-term use. The main objective of this study was to evaluate the extent to which filters could be effective against bacteria and viruses at the household level for long-term use. Two types of filters, one treated with silver nitrate to inhibit microbial growth, and one without $AgNO_3$, were tested to assess the impact of silver nitrate amendment on the microbial reduction efficiency of filters. CWFs under investigation significantly reduced surrogates for waterborne bacterial and viral pathogens, with a mean of approximately 99% (2 $\log_{10}$) reduction for *E. coli* bacteria and 90–99% (1–2 $\log_{10}$) reduction for viruses; reductions of *E. coli* and MS2 were not significantly different between filters tested or challenge waters; the CWF with no application of silver was observed to be comparable in microbiological effectiveness to the CWFAg (with silver amendment). Authors reported that locally produced ceramic filters may be a promising solution at the household level for safe drinking water treatment and safe storage.

Nawaz et al. (2012) reported silver disinfection of *Pseudomonas aeruginosa* and *E. coli* in rooftop harvested rainwater for potable purposes in Seoul, South Korea (see in Table 2.5 for study conditions information). Rainwater harvesting systems as storage tanks with $AgNO_3$ suspension solution (concentrations of 10–100 μg/L of silver) were used to test regrowth of *Pseudomonas aeruginosa* and *Escherichia coli*. However, re-growth at lower concentrations shows that the residual effect of silver did not last for a long time. No re-growth was seen in either *P. aeruginosa* or *E. coli* when inactivated with silver at higher concentrations.

A pilot study by Abebe et al. (2014) was conducted to determine whether a household-level CWF intervention can improve drinking water quality and decrease days of diarrhea in people living with HIV in rural South Africa (see in Table 2.5 for study conditions information). CWFs impregnated with silver were used. Microbial contaminants total coliform bacteria and *Cryptosporidium* spp. were measured. The authors concluded that the use of CWFs "markedly reduced days of diarrhea of HIV-positive individuals" since there was "an 80% reduction in diarrhea in the intervention group in comparison to the control group" (Abebe et al. 2014).

Another study by Guerrero-Latorre et al. (2019) tested a household water treatment technology in Ecuador to evaluate its performance after specific local production and its

potential implementation in a rural scenario (see in Table 2.5 for study conditions information). Black ceramic water filters (BCWF) were used to check efficacy for *E. coli*, adenovirus, and MS2 bacteriophage and physicochemical pollutants. Results in BCWF filter assays at laboratory level for 600 liters of usage have shown 5.36 logarithms of bacterial removal, 3.83 logarithms for viral removal, and significant reductions of physicochemical pollutants considering international standards. The obtained results suggest that viral indicators of fecal contamination complement important information on drinking water microbial risks. In a nutshell, BCWF produced for the first time in Ecuador showed great laboratory performance in reducing microbial and chemical parameters with natural and artificial waters. At the field level, a baseline study on water quality and hygiene practices results revealed poor drinking water quality in the selected communities. At 6 months, BCWF implementation at field level revealed promising results for microbial contaminants. However, maintenance should be reinforced correctly for better performance while using this technology at a household level.

For discussion on the findings of these field studies, ceramic filters with or without silver impregnation were effective for bacteria and viruses (*E. coli*, adenovirus, MS2 phage, *Pseudomonas aeruginosa*, total coliforms, and *Cryptosporidium* spp.). These treatment technologies were effective for surface water as well as harvested rainwater. Abebe et al. (2014) concluded that there was 80% reduction in diarrhea in the intervention group in comparison to the control group and the use of ceramic water filters reduced the days of diarrhea of HIV-positive individuals. The authors reported a median 2-$\log_{10}$ removal of total coliforms and total silver below the WHO drinking water guideline (100 μg/L). Guerrero-Latorre et al. (2019) showed reduction values of 5.36 logarithms for *E. coli* and 3.83 logarithms for MS2 bacteriophage with BCWF produced for the first time in Ecuador. Although, to access the sustainability of a household drinking water treatment system, some other factors play an important role including accessibility, water production, functionality, environmental sustainability (footprint), and ease-of-use. Some of these parameters were studied by van Halen et al. (2009). The authors reported that the criterion of the environmental footprint was more complex to assess, but the energy and chemical consumption is low compared to other household water treatment systems. The environmental impact depends on the energy source used by the manufacturers to fire the

kiln as the other manufacturing methods and natural sources needed for this treatment system are eco-friendly in nature. The users' effort to operate this system is similar to traditional household water storage. The flow rate of the pot filter is used as a quality check at the factories. If the flow rate is within the 1–3 L/h range, then it is approved to be sold. Rejected filter elements are crushed for recycling purposes. The percentage of discarded filters for an experienced facility should be able to achieve less than 15% waste.

If we compare antimicrobial nanotechnology with chlorination, chlorine-based disinfection technologies are very effective. But these technologies have several limitations, including their ineffectiveness against protozoan pathogens, poor social acceptance due to a change in the taste of the water, the possible formation of various disinfection byproducts (DBPs), and the emergence of microorganisms that are resistant to chlorine disinfection (Singh et al. 2019; World Health Organization 2013). Despite being the most widely used nanomaterial for disinfection, having applications in over 100 consumer products such as commercial home water purification systems, emerging concerns about the possible release of nanosilver in the environment is alarming. Also, the impact on human health and the ecosystem and the emergence of resistance is the current challenge for antimicrobial nanotechnology. Moreover, increasing bacterial resistance has an impact on public health which is addressed by a synergistic combination of antibiotics (Deshmukh et al. 2019).

2.3 Mechanisms of waterborne pathogens' inactivation

Despite decades of research efforts to elucidate how water chemical disinfectants deactivate pathogens, the understanding of the inactivation mechanisms is still unclear (Wigginton et al. 2012; Yahya et al. 1992), partly because the deactivation involves complex and diverse pathways even when a single microorganism is considered (Cho et al. 2010). However, the need of a unified understanding of these mechanisms is critical not only to identify rate limiting steps involved in the inactivation process, but also to provide an explanation on presence or absence of synergism in sequential or parallel application of chemical disinfectants. Consequently, this will also contribute to engineer more effective disinfection strategies as well as reduce chemical residuals toxicity (Cho et al. 2010; Page et al. 2009).

One example mechanism that has been linked to the inactivation of pathogens with free chlorine and silver ions is the modified site-specific Fenton mechanism. This mechanism starts when a chemical disinfectant binds to a biological target (bacteria or virus), and then cyclic redox reactions occur. More specifically, the disinfectant is first reduced by superoxide radicals or other reductants, and then re-oxidized by hydrogen peroxide yielding highly reactive hydroxyl radicals near the target site. As these hydroxyl radicals are formed, it is believed that they damage the targets located in the external structure of the pathogen and produce a multi-hit damage (Samuni et al. 1984; Yahya et al. 1992).

But the mechanisms of inactivation of pathogens strongly depend on the type of microorganisms as well as disinfectants (Cho et al. 2010). For simplicity and based on these distinctions, in the next subsections we present the suggested mechanisms found in the literature. However, since the chemical disinfectants considered in this review, when used singly in doses that are safe for drinking water, are practically ineffective for inactivating protozoan cysts (Corona-Vasquez et al. 2002), we first present briefly in the next paragraphs the available information related to the mechanisms of protozoan species inactivation with these chemicals.

Protozoan cysts present a high resistance to the disinfectants discussed in our work due to the protective bilayer cyst wall (Gyürék et al. 1997). For this reason, in places where POU technologies are commonly used, physical sieving is considered to be the main mechanism for removal of protozoan cysts. Similarly, in drinking water treatment plants, protozoan cysts are less sensitive than most of the bacteria and viruses to conventional treatment methods such as chlorination and chloramination (Omarova et al. 2018). The low cysts inactivation efficacy of these methods at commonly used doses and contact times, and potential increased byproducts formation (when seeking to increase deactivation efficacy by applying higher doses of the chemicals) had led to consideration of alternative disinfectants (Soliman et al. 2018). Among these alternative chemicals or technologies that result in effective inactivation of protozoan cysts are ozone, chlorine dioxide, UV irradiation, or combinations of ozone and free chlorine, ozone and monochloramine (Rennecker et al. 2000), and solar irradiation and free chlorine (with

potential for POU water treatment by employing free chlorine in conventional SODIS procedures) (Zhou et al. 2014).

Free chlorine treatment for protozoan species inactivation has been attributed to thinning, perforation, or complete removal of only the outer layer of the oocyst wall (the inner zone oocyst wall was reported unaffected). Other researchers examined protozoan cysts deactivation with a sequential combination of free chlorine and monochloramine and proposed a similar inactivation mechanism. First, pretreatment with free chlorine was suggested to affect the oocyst walls sufficiently to alter the permeability but not provide a measurable inactivation. This change in permeability subsequently would permit monochloramine to better diffuse through the oocyst wall and damage sporozoites (Gyürék et al. 1997). Further, with respect to giardial cysts inactivation by *N*-chloramines, it has been postulated that the polymers are able to penetrate the thick walls of the cysts and oxidize crucial enzymes on the walls (Kong et al. 1988).

Ehdaie et al. (2020) reported that a POU drinking water treatment technology embedded with metallic ions can deliver effective levels of silver and copper (<100 μg/L and < 10,000 μg/L, respectively) to inhibit sporozoite metabolic ability, and thus inactivate protozoa. Authors reported that fluorescence microscopy demonstrated two mechanisms for protozoa inactivation by metallic ions which includes first, structural damage of oocyst walls, and second, alteration of sporozoite structure. Cameron et al. (2016) demonstrated a clear AgNPs and Ag ions concentration dependence for oocyst destruction. Both forms of silver induced oocyst death at very high concentrations (5×10^8 μg/L), where AgNPs interact with the cell wall and are able to fully break the oocyst wall, while Ag ions enter the oocyst and destroy the sporozoites.

2.3.1 Bacterial species inactivation mechanisms

Cho et al. (2010) have proposed that regardless of the complexity involved, the mechanisms of bacterial inactivation can generally be classified into two groups: those which involve bacterial cell surface damage and those which do not. In the first case, cell wall physico-chemical changes or damages on its components, would be the first, and perhaps major, step for bacterial inactivation. Then, damage in intracellular constituents and their functions would follow. On

the contrary, for deactivation mechanisms that do not include cell surface damage, direct intracellular impairment would be the primary reason for inactivation.

Further, Cho et al. (2010) suggest that since the chemical disinfectants are oxidants, their oxidation potentials (Table 2.6) are well correlated with the inactivation mechanisms. In other words, the higher the oxidation potential of the chemical, the more reactive it is, and the more likely it will generate a pronounced bacterial cell membrane damage while oxidizing the various membrane components and undergoing a reactive and retarded diffusion through the cell's protective barrier. But the lower the oxidation potential of the disinfectant, the more likely it will mainly oxidize cell inner components without causing significant cell surface damage since it will have limited reactions with membrane components and the diffusive transport through the membrane will be less retarded. As a result, they concluded that the extent of reaction compared to diffusion determines the mechanism of bacterial cell death by the chemical disinfectants.

Table 2.6: Standard potential at 298.15 K (25 °C) and 101.325 kPa (1 atm) of chemicals used for water disinfection (Lide 2004; Rajasekharan et al. 2007)

Half reaction	Standard potential (volts)
$O_3 + 2\ H^+ + 2\ e \leftrightharpoons O_2 + H_2O$	2.08
$HOCl + H^+ + 2\ e \leftrightharpoons Cl^- + H_2O$	1.48
$ClO_2 + H^+ + e \leftrightharpoons HClO_2$	1.28
$OCl^- + H_2O + 2\ e \leftrightharpoons Cl^- + 2\ OH^-$	0.81
$Ag^+ + e \leftrightharpoons Ag$	0.80
$NH_2Cl + H_2O + 2\ e \leftrightharpoons Cl^- + OH^- + NH_3$	0.74
$Cu^{2+} + 2\ e \leftrightharpoons Cu$	0.34
$Cu^{2+} + e \leftrightharpoons Cu^+$	0.15

According to Table 2.6, the protonated form of free chlorine (HOCl) is more reactive than the unprotonated form (OCl^-), monochloramine and silver ions, since hypochlorous acid has the

highest oxidation potential in this group of chemicals (1.48 V). Monochloramine and silver ion have similar standard potentials (0.74 and 0.80 V, respectively), and copper ions have the lowest oxidation potential (0.34 V when it accepts two electrons and 0.15 V when it accepts one), which makes them the least reactive disinfectant among the ones that are the focus in this review.

2.3.1.1 Mechanisms of bacteria inactivation with chlorine-based disinfectants

The suggested mechanisms of bacterial inactivation with free chlorine mainly involve cell membrane damage. For example, Bajszár and Dekonenko (2010) propose that some of the more reactive free chlorine targets are in the membrane and seem to be associated with oxidative protein unfolding or degradation. They also suggest that the bactericidal effect of free chlorine involves the action of hydroxyl radicals generated by a Fenton type reaction. Rose et al. (2007) suggest similar mechanisms where free chlorine targets many parts of the bacterial cell structure and metabolism, such as oxidation of the membrane, DNA damage, and respiration inhibition.

Cho et al. (2010) conducted tests where *E. coli* was inactivated with disinfectants used in water treatment plants (ozone, chlorine dioxide, free chlorine, and UV light) to first determine the dominant inactivation mechanism i.e., whether cell membrane attack is more likely to occur compared to intracellular attack. Then, they evaluated the presence or absence of synergism when some of these disinfectants were applied sequentially. Bacterial cell membrane damage was associated with significant levels of protein release, oxidative degradation of lipids, and change in the membrane permeability. On the other hand, intracellular attack was related to limited levels of the previously mentioned observations. The results obtained in this study suggest that the stronger oxidants (e.g., ozone) effectively damage the cell membrane and that this could enhance the penetration of subsequently applied less stronger oxidants (e.g., free chlorine) confirming the presence of synergism. Consistently, when a less strong oxidant was first applied and then a stronger oxidant, a reduced synergistic effect was found since minimal membrane damage occurred during the first inactivation step.

Further, with respect to inactivation mechanisms with *N*-chloramines, studies suggest that these polymers can perform inactivation of bacteria (e.g., *E. coli* and *S. aureus*) in water solution

by release killing, or contact killing, or a combination of both mechanisms (Bastarrachea et al. 2014; Dong et al. 2017; Hui and Debiemme-Chouvy 2013). With the contact mechanism there is no dissociation of the halogen because of the direct transfer of Cl^+ from *N*-chloramines to bacterial cells. *N*-chloramines with stable N-Cl bonds (i.e., amine and amide-based *N*-chloramines) are more suitable for contact killing. Moreover, the release inactivation mechanism is linked with the appearance of inhibitory zones around the *N*-chloramines. This could be indicative of Cl^+ diffusing away from *N*-chloramines into solution, with subsequent bacteria inactivation (Dong et al. 2017).

It is likely that the Cl^+ transferred or released from *N*-chloramines penetrates the bacterial cells and since the halogen is a strong oxidant it has a strong tendency to disrupt the bacteria membrane and also react with bacterial receptors (e.g., sulfhydryl groups and thiol containing constituents) destroying the metabolic process in the cells (Chen et al. 2003; Dong et al. 2017). In addition, bacterial growth could be inhibited, particularly bacterial DNA, RNA, and protein synthesis (Dong et al. 2017). Other researchers have attributed *N*-chloramines cytotoxicity and genotoxicity to cellular oxidative stress involving the generation of reactive oxygen species (ROS), and radical induced damage to DNA (How et al. 2017; Natan et al. 2015). Finally, another held explanation is that protons of the amide functional groups of the protein sheet in the bacterial cell membrane are oxidized by the Cl^+ that has been transferred from the *N*-chloramine (Ahmed et al. 2011). This could alter the equilibrium between intra- and extracellular protons, and potentially have a harmful effect on numerous metabolic processes of the bacteria (Ahmed et al. 2011).

Similar to inactivation mechanisms with *N*-chloramines and free chlorine, Cromeans et al. (2010), Straub et al. (1995) and Rose et al. (2007) suggest that the mechanism of bacteria inactivation with monochloramine may be the result of oxidation of the thiol groups in amino acids and tryptophan in the cell membrane. This may successively lead to structural changes in the membrane (Straub et al. 1995).

2.3.1.2 Mechanisms of bacteria inactivation with silver

The mechanism of the antimicrobial action of silver ion is still not well understood, several hypotheses have been reported by various investigators (Chatterjee et al. 2015; Deshmukh et al. 2019; Gibbins and Warner 2005; Marimuthu et al. 2020; Pal et al. 2007). It is believed that the formation of highly reactive oxygen species (ROS), including OH^-, H_2O_2, O_2^{2-}, is responsible for antimicrobial activity of silver nanomaterials (Chatterjee et al. 2015; Guo et al. 2016). It is closely related to amino acids, such as cysteine, sodium thioglycolate, but other target sites are also a possibility. Silver has different modes of action for the inactivation of bacterial cells, which again differs for gram-positive and gram-negative bacteria (Dibrov et al. 2002; Nawaz et al. 2012; Rai et al. 2012). The biocidal mechanism of silver is also hypothesized to be associated with electrostatic repulsion or attraction between the negatively charged bacterial surface and positively charged silver ion (Dibrov et al. 2002).

The bacterial cell membranes contain sulfur/thiol group (-SH) in proteins and amino acids and their interaction with silver results in bacterial inactivation (Deshmukh et al. 2019). The findings of the study by Jung et al. (2008) suggest that the cellular components, like the H-bonding and thiol group of L-Cysteine residue of proteins and enzymes, play an essential role in its antimicrobial action by silver. The most widely used mechanism is that the silver can bind to functional groups of enzymes that cause the release of K ions, affecting bacterial plasma or cytoplasmic membranes (Figure 2.3 a). Additionally, silver ion inhibits bacterial growth, cell division, DNA replication, generation of intracellular reactive oxygen species, and damage the cell envelope and contents of bacteria, resulting in structural abnormalities. Although lethal action of silver ion on nucleic acid is unclear, their preferential interaction is with purine and pyrimidine bases in DNA, which stabilize the DNA helix and prevent replication of the DNA. Subsequent cell division is also believed to play a role in disinfection by silver. Ag^+ ions are believed to bind to the functional groups of proteins which causes protein denaturation. Silver hampers respiratory functions and other essential cellular functions which leads to bacterial death (Figure 2.3 b). Recent findings indicate that the effectiveness and broad-spectrum activity of silver lies in its ability to attack multiple targets sites and that makes it difficult for bacteria to develop resistance against silver (Chakraborty et al. 2017). Similarly, Lucier et al. (2017)

explained that silver promotes bacterial and viral oxidation, it affects bacterial cell viability, and it inhibits enzyme function.

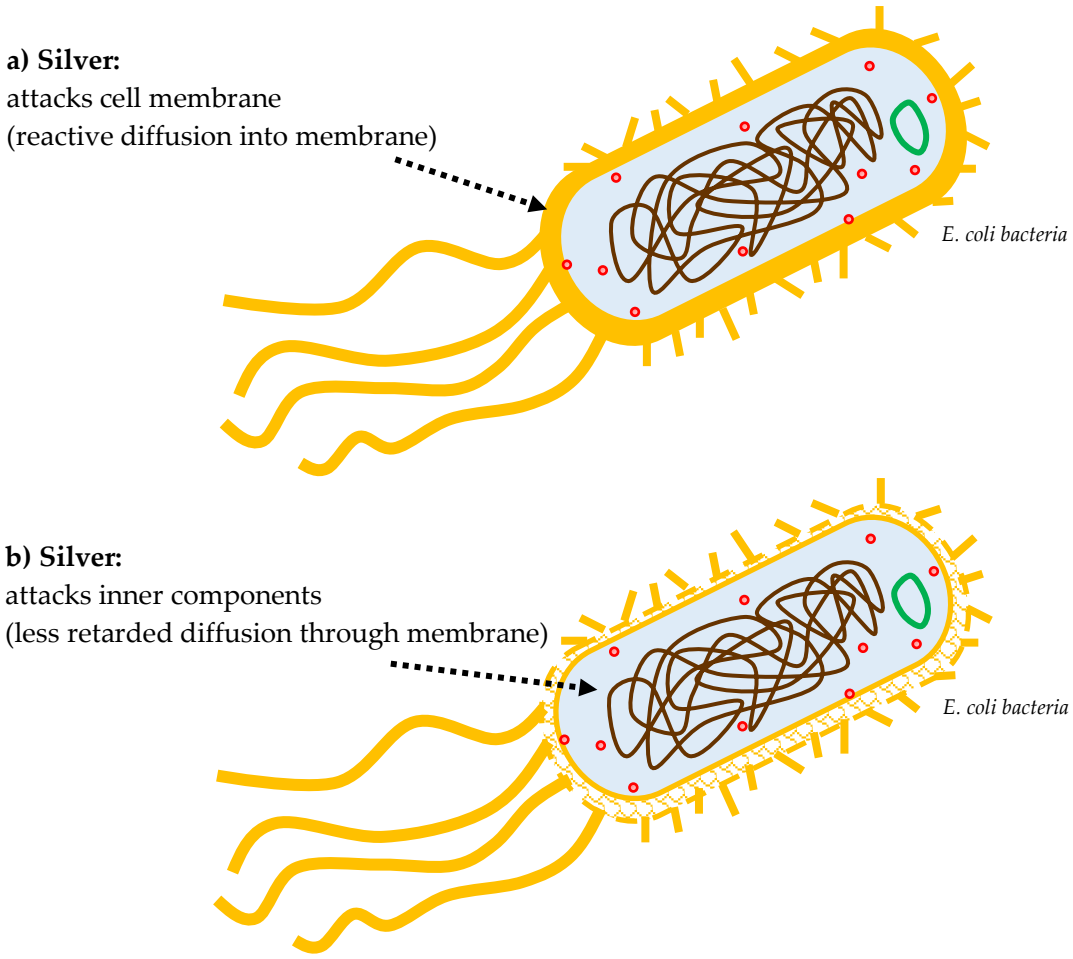

Figure 2.3: Summary diagram of the potential mechanisms/modes of action for silver nanomaterials' antibacterial activity. a) It may attack the membrane; b) After the membrane is compromised it may attack the inner cellular components.

2.3.2 Viral species inactivation mechanisms

Virus deactivation is considered successful when infection of a susceptible host cell is prevented. In particular, the following virus functions must be inhibited or impaired in order to deactivate it: binding to the host cell (protein mediated function), injection of the nucleic acid (i.e., RNA or DNA) genome into the host (protein mediated function), and replication in the host (genome mediated function) (Wigginton et al. 2012). With chemical disinfectants this may be accomplished by destroying host cell receptors on the virus or inactivating the nucleic acid within the viral capsid (Yahya et al. 1992).

2.3.2.1 Mechanisms of viral inactivation with chlorine-based disinfectants

Deactivation studies on viruses suggest that free chlorine attacks both proteins on the capsid and the RNA of the virus (Kahler et al. 2011; Yahya et al. 1992). However, for echovirus 1 inactivation only conformational changes in capsid structure have been reported, and for picornavirus only RNA degradation was associated with its deactivation with free chlorine (Cromeans et al. 2010). Furthermore, inactivation of adenovirus with free chlorine has been attributed to damage in the proteins necessary for genome injection (Wigginton et al. 2012). More specifically, it has been proposed that free chlorine readily reacts with the proteins forming the adenovirus capsid (which contain amine, thiol, and other functional groups). This could result in a reduction of the injection, gene transcription and genome replication functions of the virus within the host cell (Gall et al. 2016; Page et al. 2009), see Figure 2.4. Also, it has been suggested that deactivation of bacteriophage MS2 (a common surrogate for enteric viruses due to its similar shape and composition) with free chlorine is primarily caused by inhibition or impairment of genome injection and replication, which is linked to extensive destruction of genome and proteins (specifically coat and maturation or assembly proteins). Overall, it can be concluded that free chlorine is not a selective oxidant because it does not affect only specific proteins (Wigginton et al. 2012).

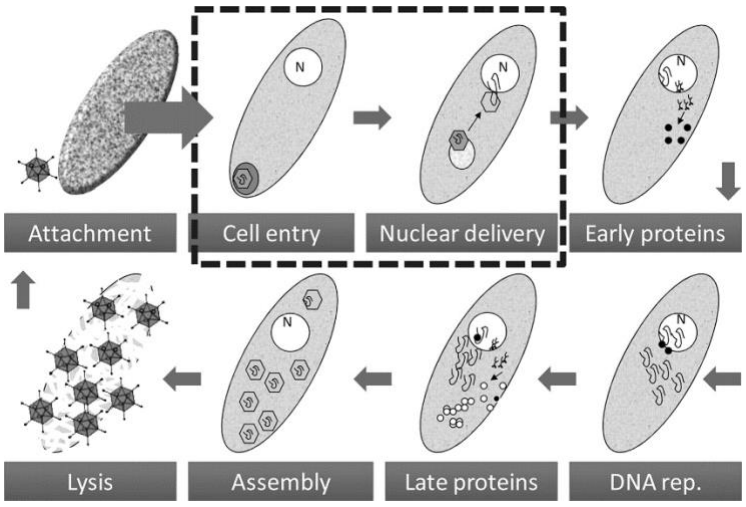

Figure 2.4: Summary of the adenovirus (Ad2) life cycle. The larger arrow shows the observed lack of binding inhibition by free chlorine. The dashed box contains the step(s) thought to be targeted by free chlorine. Reprinted with permission from Page et al. (2010). Copyright 2010 American Society for Microbiology.

In the same ways as the mechanism of adenovirus inactivation with free chlorine, the mechanism with monochloramine is also suggested to involve DNA damage without affecting the binding function (Kahler et al. 2011). In other words, after binding to the host cell, inhibition in replication cycle events, including early gene transcription, genome replication, and late gene transcription is believed to occur (Gall et al. 2016).

Finally, studies show that MS2 and poliovirus deactivation with *N*-chloramines have the same mechanism as for bacteria (Chen et al. 2003), see previous section 2.3.1.1 for more details.

2.3.2.2 Mechanisms of viruses inactivation with silver

Studies suggest that silver nanoparticles, AgNPs, main antiviral properties affect the initial stage of a virus cycle: attachment to the host cell (Lara et al. 2010). Two different mechanisms of respiratory syncytial virus (RSV) inactivation by AgNPs have been proposed: preventing virus attachment to the host cell by binding of the nanoparticles to surface proteins and interfering with virus assembly by blocking cellular factors with the nanoparticles (Morris et al. 2019). A different study with Peste des petits ruminants virus (PPRV) similarly suggests that AgNPs interacted with the virus surface and its core affecting its attachment to target cells, and that the nanoparticles also significantly inhibited the virus replication (Khandelwal et al. 2014).

2.4 Chemical disinfectants toxicity

2.4.1 Harmful water chlorination byproducts

The use of high concentrations of free chlorine in water containing natural organic matter (NOM), metal ions, metal oxides, nanoparticles, bromide (Br^-) and/or iodide (I^-) is associated with the generation of numerous chlorinated byproducts that are potentially hazardous to human health (Abad et al. 1994; Lantagne et al. 2010; Patil et al. 2015; Pereira et al. 2008; Rajasekharan et al. 2007; Sharma et al. 2017). These disinfection byproducts (DBPs) can be correlated to reproductive complications, cardiovascular defects, neural tube defects and even cancer (Chakraborty et al. 2017; Sharma et al. 2017). The main organic DBPs are trihalomethanes (THMs), haloacetic acids (HAAs), haloketones and haloacetonitriles. Inorganic DBPs include chlorite (ClO_2^-), chlorate (ClO_3^-), bromate (BrO_3^-), hypobromous acid (HOBr) and hypoiodous

acid (HOI) (Sharma et al. 2017; World Health Organization 2017). Overall, DBPs are a very complex mixture and presently more than 600 DBPs have been identified which may or may not have mutagenic activity (Lantagne et al. 2010; Sharma et al. 2017). For this reason, THMs and to a lesser extent HAAs are used as indicator chemicals of water chlorination byproducts (Lantagne et al. 2010; World Health Organization 2017).

When using monochloramine for water disinfection, DBPs can also form. Disinfection with monochloramine produces lower THM concentrations than free chlorine but produces other DBPs (e.g., cyanogen chloride). Also, monochloramine residuals in drinking water can oxidize lead present in pipes, solder, and brass and this could lead to elevated soluble Pb^{2+} levels while free chlorine residuals may form more insoluble lead (PbO_2) containing deposits (Rajasekharan et al. 2007; World Health Organization 2017).

Organic DBPs may be classified into four categories (Table 2.7). For several of these DBPs, the WHO has established guideline values in drinking water that are fully protective for cancer and non-cancer effects (Table 2.8). Additionally, the guideline values are set below the expected threshold for these effects (Lantagne et al. 2010). Similarly, the U.S. EPA has set maximum contaminant levels (MCLs) for DBPs in drinking water (Table 2.8) (Straub et al. 1995).

There are several factors that can influence the formation of DBPs. First, the nature and concentration of NOM (particularly the ones containing phenolic parts and amines) affects the formation of DBPs and compounds such as humic and fulvic acids, and other particulates can also shield the surfaces of pathogens and protect them from the action of free chlorine. Therefore, one way to reduce THMs and HAAs formation and to improve the inactivation effectiveness is to control these precursor compounds by incorporating or enhancing processes such as coagulation or filtration (Chakraborty et al. 2017; Sharma et al. 2017; World Health Organization 2017). Second, metal ions (e.g., Ca^{2+}, Mg^{2+}, Cu^{2+}, Pb^{2+}, Fe^{2+}, Fe^{3+}) can enhance the generation of DBPs and metal oxides (e.g., CuO, Fe_3O_4, α-FeOOH, δ-MnO_2, TiO_2, CeO_2) can catalyze DBPs formation. The ions can form complexes with NOM (e.g., carboxylic acids) and then undergo different reactions (e.g., hydrolysis, substitution, and decarboxylation) that can produce DBPs. Lastly, even though there is a disagreement in the literature about whether the

Table 2.7: Chlorinated organic byproducts classification (Straub et al. 1995)

	Categories			
	Volatile	Nonvolatile	Carbonaceous	Nitrogenous
Trihalomethanes (THMs): • chloroform or trichloromethane ($CHCl_3$) • bromoform ($CHBr_3$) • bromodichloromethane ($CHCl_2Br$) • dibromochloromethane ($CHClBr_2$)	✓		✓	
Haloketones	✓		✓	
Halophenols	✓		✓	
Haloacetonitriles (HANs)	✓			✓
Haloacetic acids (HAAs)		✓	✓	
Nitrosamines (NAs): • nitrosodimethylamine (NDMA) • nitrosomorpholine (NMor)	✓			✓

Table 2.8: Maximum contaminant levels (MCLs) and guideline values of disinfection byproducts in drinking water (US EPA 2020; World Health Organization 2017)

	WHO Guideline value (μg/L)	**U.S. EPA MCL (μg/L)**
Chloroform ($CHCl_3$)	300	Not specified
Bromoform ($CHBr_3$)	100	Not specified
Bromodichloromethane ($CHCl_2Br$)	60	Not specified
Dibromochloromethane ($CHClBr_2$)	100	Not specified
Total trihalomethanes (TTHMs)	Sum of the four actual values of the THMs divided by their guideline value should not exceed 1	80
Haloacetic acids (HAAs)	• Dichloroacetic acid: 50 • Monochloroacetic acid: 20 • Trichloroacetic acid: 200	60
Bromate (BrO_3^-)	10*	10
Chlorite ion (ClO_2^-)	700*	1,000
Chlorate ion (ClO_3^-)	700*	Not specified

*Provisional

presence of nanoparticles decreases or increases the concentration of DBPs, the nanoparticles are likely to impact the levels of DBPs during water chlorination (Straub et al. 1995).

2.4.2 Silver toxicity

The silver concentrations used in water disinfection (<100 μg/L) have no apparent detrimental effects on mammalian cells; however, when ingested in gram quantities, silver causes argyria (irreversible skin discoloration) (Nawaz et al. 2012). When silver nanoparticles, AgNPs, are released into the environment, they can be toxic in water bodies and soil because they can inhibit nitrification (Marimuthu et al. 2020), and can have adverse long-term effects on soil bacterial community structure by causing disadvantages for the organic carbon transformation and the chitin degradation (Grün and Emmerling 2018).

2.5 Synergistic inactivation of waterborne pathogens by silver and/or copper and chlorine combinations

Several studies have reported improved efficacy and synergistic effects when combinations of chemical disinfectants are used to inactivate waterborne pathogens (Patil et al. 2015). This synergism can produce higher levels of inactivation with shorter contact times and reduced levels of disinfectants, and consequently less disinfection byproducts and chemicals residuals (Straub et al. 1995). Generally, in studies where combinations of chlorine-based disinfectants and metal ions are examined for inactivation of pathogens, the chemicals are applied simultaneously. However, in water treatment plants where there is more than one disinfection process, these are set in sequence. With these settings, the synergistic effect in the deactivation of pathogens is identified as an enhancement of the secondary disinfection because of the application of the primary disinfectant (Cho et al. 2010).

Bacterial and viral inactivation with either silver and monochloramine require higher doses and longer contact times to achieve the same level of inactivation as free chlorine because of their relatively lower reactivity (Cromeans et al. 2010; Straub et al. 1995). Taking this into account, many studies have tested combinations of reduced levels of free chlorine and silver.

Abad et al. (1994) evaluated the efficacy of combining free chlorine and copper and silver ions for the inactivation of hepatitis A virus (HAV), human rotavirus (HRV), human adenovirus (ADV), poliovirus (PV) and bacteriophage B40-8 in seeded well and tap water. Copper and silver ions were electrolytically generated in doses of 700 μg/L and 70 μg/L, respectively. But different free chlorine doses were applied: 200, 500, and 1,000 μg/L. In general, the results of this study show that the addition of Cu and Ag ions may not provide a reliable alternative to using only reduced levels of free chlorine (200 and 500 μg/L) for the inactivation of human enteric viruses. The presence of virus aggregates in the treatments that included copper and silver ions could have influenced the resistance of viruses to inactivation. Besides, the aggregates were not observed on the free chlorine only treatments. Overall, HAV and HRV had the lowest inactivation in all conditions (<2.6-$\log_{10}$ reduction), and on the contrary, the highest deactivation achieved was with PV (from 3- to even more than 4-$\log_{10}$ reduction).

Biurrun et al. (1999) studied the same combination of chemicals (free chlorine and copper and silver ions) but for the eradication of *Legionella pneumophila* colonies in both the hot and cold-water circuits of a hospital. Similar free chlorine concentrations were used (800 μg/L at the cold-water outlets), but lower electrolytically generated copper ion concentrations were applied (20–250 μg/L in the hot water and 20–80 μg/L in the cold water). The electrolytically generated silver ion concentrations are not reported. The main finding of this study was that the high rate of colonization (62%) initially found could not be reduced by conventional systems, such as hyperchlorination, but it fell dramatically (17%) after 5 months of Cu–Ag electrodes installation, even though the achieved copper levels were under those recommended by other studies. And, although the *L. pneumophila* colonies were not completely eradicated during the study period, no new cases of nosocomial legionellosis were detected.

Patil et al. (2015) have demonstrated the synergistic inactivation of *E. coli* in seeded well water when using low concentrations of free chlorine (200–500 μg/L) and ionic silver (10–70 μg/L). For this, they used a gravity driven water treatment cartridge consisting of a rice husk ash and clay porous disc treated with nano silver, and trichloroisocyanuric acid tablets that are the source of free chlorine. This system achieved ≥6 $\log_{10}$ removal of *E. coli* within 30 minutes of contact time (seeded *E. coli* concentration of 10^{6}–10^{7} CFU/mL). However, when using only the

porous disc, a 6 $\log_{10}$ removal was not reached even after 3 hours of contact time (the highest was 5.28). And, with only the free chlorine treatment, close to 6 $\log_{10}$ removal was obtained after 2 hours of contact time. These results demonstrate the presence of a synergistic effect when combining free chlorine and silver ions. Besides, the WHO guidelines of free chlorine (200–5,000 μg/L) and silver (<100 μg/L) for drinking water were met in the treated water.

2.6 Conclusions

Water disinfection with free chlorine is effective for the inactivation of bacterial and viral pathogens. Free chlorine products are low-cost and simple to use. Nonetheless, depending on the quality of the source water that is treated, unappealing taste and odor, and harmful byproducts can be formed by the chlorination treatment. In addition, free chlorine in liquid form is unstable (it can start decomposing after 6 months of storage), which results in loss of efficacy over time. On the other hand, silver is popular as a microbial inactivating agent with low sensitivity for humans. While this metal requires higher doses and longer contact times to achieve the same level of disinfection as free chlorine, silver has an advantage over chlorine because this metal does not change the taste and odor of the treated water. Alternatively, technologies that include insoluble *N*-chloramines are promising as these polymers can provide very high effective Cl^+concentration. This creates the advantage that only short contact times (in the order of seconds or a few minutes) are required to effectively inactivate pathogens. These polymers also have the capacity to deactivate the pathogens by contact and not by release of chemicals without leaving residuals in the treated water. Furthermore, *N*-chloramines' rechargeability (by simply exposing them with bleach) and their high stability provides them with a long shelf life.

Little information is available on the use of combinations of free chlorine and silver in POU applications because research has been focused on the study of synergism of powerful oxidants used in water treatment plants (e.g., ozone, UV, chlorine dioxide, free chlorine). Published research shows that reduced levels of free chlorine (200 and 500 μg/L) combined with copper and silver ions did not show promise for the inactivation of human enteric viruses over the use of free chlorine alone. This was linked to the presence of virus aggregates in the treatments that

included the metals, which could have created resistance of the viruses to inactivation. However, a POU device that combined free chlorine and silver demonstrated the presence of a synergistic effect in the inactivation of *E. coli* with doses that met the WHO guidelines for drinking water. Therefore, future research should focus on combinations of these chemicals in POU water treatment that can produce synergistic effects enabling higher efficacy of microbial inactivation with shorter contact times and low disinfectant doses. Consequently, this would lower the formation of disinfection byproducts and the presence of chemical residuals in the treated water.

One of the challenges of designing more effective disinfection strategies with combinations of chemicals is the lack of a unified understanding of individual disinfectant inactivation mechanism. As discussed in section 2.3 (inactivation mechanisms), it is believed that some disinfectants are highly selective or react with specific components of the pathogens and cause inactivation. If this is the case, these types of chemicals would have an advantage over non- or less-selective oxidants because high selectivity is linked with less mass transfer limitations or diffusion-reaction interactions that are not hindered, so the disinfectants can penetrate the pathogens more rapidly and cause inactivation (Hosni et al. 2009). Other researchers suggest that the modified site-specific Fenton mechanism in the pathogen surface is responsible for their inactivation. The generation of reactive oxygen species with this mechanism is believed to strengthen the inactivation ability of the chemical disinfectants. Other several hypotheses presented on antimicrobial action of the disinfectants include their binding to other electron donor groups containing nitrogen, oxygen, and sulfur such as amines, hydroxyls, phosphates, and thiol (—SH) groups.

In the field, apart from challenges such as negative product perceptions by the users, handling and maintenance issues, lack of compliance, etc., what impacts the effectiveness of the deactivation of pathogens are the factors that influence the contact between the disinfectants and the microorganisms. Among these factors are temperature, pH, alkalinity, and hardness of the water to be treated, presence of inorganic and organic reduced compounds (e.g., iron, sulfide, ammonia, natural organic matter, etc.), and aggregation of pathogens (e.g., microorganisms adsorption onto particles, biofilms, turbidity, etc.). Therefore, future work

should focus on examining combinations of chemicals and their synergistic effects in field studies, i.e., using POU interventions, natural waters with different qualities, and active monitoring of variables affecting the inactivation efficacy. This implies the need of equipment, qualified staff, and resources in general, which could potentially be achieved with the collaboration of research institutes, or private and government sectors. Additionally, in the field, it is important to prioritize the compliance with WHO microbial guidelines (e.g., <1 CFU/100 mL *E. coli*) over highlighting the removal of the load of pathogens (i.e., $\log_{10}$ removal or reduction). Finally, the optimized doses of chemicals in combinations that produce the best efficacy should be determined through long term randomized trials.

2.7 References

Abad, F. X., R. M. Pinto, J. M. Diez, and A. Bosch. 1994. "Disinfection of Human Enteric Viruses in Water by Copper and Silver in Combination with Low Levels of Chlorine." *Appl Environ Microbiol*, 2377–2383. https://doi.org/10.1128/aem.60.7.2377-2383.1994

Abebe, L. S., J. A. Smith, S. Narkiewicz, V. Oyanedel-Craver, M. Conaway, A. Singo, S. Amidou, P. Mojapelo, J. Brant, and R. Dillingham. 2014. "Ceramic water filters impregnated with silver nanoparticles as a point-of-use water-treatment intervention for HIV-positive individuals in Limpopo Province, South Africa: A pilot study of technological performance and human health benefits." *J Water Health*, 12 (2): 288–300. IWA Publishing. https://doi.org/10.2166/wh.2013.185

Ahmed, A. E.-S. I., G. Cavalli, M. E. Bushell, J. N. Wardell, S. Pedley, K. Charles, and J. N. Hay. 2011. "New approach to produce water free of bacteria, viruses, and halogens in a recyclable system." *Appl Environ Microbiol*, 77 (3): 847–853. American Society for Microbiology. https://doi.org/10.1128/AEM.01645-10

Bajszár, G., and A. Dekonenko. 2010. "Stress-induced Hsp70 gene expression and inactivation of Cryptosporidium parvum oocysts by chlorine-based oxidants." *Appl Environ Microbiol*, 76 (6): 1732–1739. American Society for Microbiology. https://doi.org/10.1128/AEM.02353-09

Barzilay, E. J., T. S. Aghoghovbia, E. M. Blanton, A. A. Akinpelumi, M. E. Coldiron, O. Akinfolayan, O. A. Adeleye, A. LaTrielle, R. M. Hoekstra, U. Gilpin, and R. Quick. 2011. "Diarrhea prevention in people living with HIV: An evaluation of a point-of-use water quality intervention in Lagos, Nigeria." *AIDS Care*, 23 (3): 330–339. Taylor & Francis Group. https://doi.org/10.1080/09540121.2010.507749

Bastarrachea, L. J., L. A. McLandsborough, M. Peleg, and J. M. Goddard. 2014. "Antimicrobial N-halamine Modified Polyethylene: Characterization, Biocidal Efficacy, Regeneration, and Stability." *J Food Sci*, 79 (5): E887–E897. Blackwell Publishing Inc. https://doi.org/10.1111/1750-3841.12455

Biurrun, A., L. Caballero, C. Pelaz, E. León, and A. Gago. 1999. "Treatment of a Legionella pneumophila-Colonized Water Distribution System Using Copper-Silver Ionization and

Continuous Chlorination." *Infect Control Hosp Epidemiol*, 20 (6): 426–428. https://doi.org/10.1086/501645

Brown, J., and M. D. Sobsey. 2010. "Microbiological effectiveness of locally produced ceramic filters for drinking water treatment in Cambodia." *J Water Health*, 8 (1): 1–10. IWA Publishing. https://doi.org/10.2166/WH.2009.007

Cameron, P., B. K. Gaiser, B. Bhandari, P. M. Bartley, F. Katzer, and H. Bridle. 2016. "Silver nanoparticles decrease the viability of Cryptosporidium parvum oocysts." *Appl Environ Microbiol*, 82 (2): 431–437. American Society for Microbiology. https://doi.org/10.1128/AEM.02806-15

Chakraborty, D., V. Sharma, S. Agnihotri, S. Mukherji, and S. Mukherji. 2017. "Disinfection of water in a batch reactor using chloridized silver surfaces." *Journal of Water Process Engineering*, 16: 41–49. https://doi.org/10.1016/j.jwpe.2016.12.009

Chatterjee, T., B. K. Chatterjee, D. Majumdar, and P. Chakrabarti. 2015. "Antibacterial effect of silver nanoparticles and the modeling of bacterial growth kinetics using a modified Gompertz model." *Biochimica et Biophysica Acta (BBA) - General Subjects*, 1850 (2): 299–306. Elsevier. https://doi.org/10.1016/J.BBAGEN.2014.10.022

Chen, Y., S. D. Worley, J. Kim, C.-I. Wei, T.-Y. Chen, J. I. Santiago, J. F. Williams, and G. Sun. 2003. "Biocidal Poly(styrenehydantoin) Beads for Disinfection of Water." *Ind Eng Chem Res*, 42: 280–284. American Chemical Society. https://doi.org/10.1021/IE020266+

Cho, M., J. Kim, J. Y. Kim, J. Yoon, and J. H. Kim. 2010. "Mechanisms of Escherichia coli inactivation by several disinfectants." *Water Res*, 44 (11): 3410–3418. Elsevier Ltd. https://doi.org/10.1016/j.watres.2010.03.017

Corona-Vasquez, B., A. Samuelson, J. L. Rennecker, and B. J. Mariñas. 2002. "Inactivation of Cryptosporidium parvum oocysts with ozone and free chlorine." *Water Res*, 36 (16): 4053–4063. Pergamon. https://doi.org/10.1016/S0043-1354(02)00092-1

Coulliette, A. D., K. S. Enger, M. H. Weir, and J. B. Rose. 2013. "Risk reduction assessment of waterborne Salmonella and Vibrio by a chlorine contact disinfectant point-of-use device." *Int J Hyg Environ Health*, 216 (3): 355–361. https://doi.org/10.1016/j.ijheh.2012.08.007

Cromeans, T. L., A. M. Kahler, and V. R. Hill. 2010. "Inactivation of adenoviruses, enteroviruses, and murine norovirus in water by free chlorine and monochloramine." *Appl Environ Microbiol*, 76 (4): 1028–1033. American Society for Microbiology. https://doi.org/10.1128/AEM.01342-09

Dankovich, T. A., and D. G. Gray. 2011. "Bactericidal paper impregnated with silver nanoparticles for point-of-use water treatment." *Environ Sci Technol*, 45 (5): 1992–1998. American Chemical Society. https://doi.org/10.1021/es103302t

Das, C. G. A., V. G. Kumar, T. S. Dhas, V. Karthick, K. Govindaraju, J. M. Joselin, and J. Baalamurugan. 2020. "Antibacterial activity of silver nanoparticles (biosynthesis): A short review on recent advances." *Biocatal Agric Biotechnol*. Elsevier Ltd.

Deshmukh, S. P., S. M. Patil, S. B. Mullani, and S. D. Delekar. 2019. "Silver nanoparticles as an effective disinfectant: A review." *Materials Science and Engineering: C*, 97: 954–965. Elsevier. https://doi.org/10.1016/J.MSEC.2018.12.102

Dibrov, P., J. Dzioba, K. K. Gosink, and C. C. Häse. 2002. "Chemiosmotic mechanism of antimicrobial activity of Ag+ in Vibrio cholerae." *Antimicrob Agents Chemother*, 46 (8): 2668–2670. American Society for Microbiology . https://doi.org/10.1128/AAC.46.8.2668-2670.2002/ASSET/94954A7F-91EE-4757-8F2B-652259076882/ASSETS/GRAPHIC/AC0820108003.JPEG

Dong, A., Y. J. Wang, Y. Gao, T. Gao, and G. Gao. 2017. "Chemical Insights into Antibacterial N-Halamines." *Chem Rev*. American Chemical Society.

Ehdaie, B., Y.-H. Su, N. S. Swami, and J. A. Smith. 2020. "Protozoa and Virus Disinfection by Silver- and Copper-Embedded Ceramic Tablets for Water Purification." *Journal of Environmental Engineering*, 146 (4): 04020015. American Society of Civil Engineers (ASCE). https://doi.org/10.1061/(asce)ee.1943-7870.0001664

Ercumen, A., A. Mohd. Naser, L. Unicomb, B. F. Arnold, J. M. Colford Jr., and S. P. Luby. 2015. "Effects of Source- versus Household Contamination of Tubewell Water on Child Diarrhea in Rural Bangladesh: A Randomized Controlled Trial." *PLoS One*, (M. Ali, ed.), 10 (3): e0121907. Public Library of Science. https://doi.org/10.1371/journal.pone.0121907

Gall, A. M., J. L. Shisler, and B. J. Mariñas. 2016. "Inactivation Kinetics and Replication Cycle Inhibition of Adenovirus by Monochloramine." *Environ Sci Technol Lett*, 3 (4): 185–189. American Chemical Society. https://doi.org/10.1021/acs.estlett.6b00079

Gibbins, B., and L. Warner. 2005. "The Role of Antimicrobial Silver Nanotechnology." *Medical Device and Diagnostic Industry Magazine*. Accessed March 5, 2023. https://www.mddionline.com/rd/role-antimicrobial-silver-nanotechnology

Grün, A. L., and C. Emmerling. 2018. "Long-term effects of environmentally relevant concentrations of silver nanoparticles on major soil bacterial phyla of a loamy soil." *Environ Sci Eur*, 30 (1): 1–13. Springer Verlag. https://doi.org/10.1186/S12302-018-0160-2/TABLES/4

Guerrero-Latorre, L., P. Balseca-Enriquez, C. Moyota-Tello, R. Bravo-Camino, S. Davila-Chavez, E. Bonifaz-Arcos, B. Romero-Carpio, and M. Chico-Terán. 2019. "Performance of black ceramic water filters and their implementation in rural Ecuador." *Journal of Water, Sanitation and Hygiene for Development*, 9 (4): 694–702. IWA Publishing. https://doi.org/10.2166/washdev.2019.185

Guo, H., J. Zhang, M. Boudreau, J. Meng, J. jie Yin, J. Liu, and H. Xu. 2016. "Intravenous administration of silver nanoparticles causes organ toxicity through intracellular ros-related loss of interendothelial junction." *Part Fibre Toxicol*, 13 (1): 1–13. BioMed Central Ltd. https://doi.org/10.1186/S12989-016-0133-9/FIGURES/8

Gyürék, L. L., G. R. Finch, and M. Belosevic. 1997. " Modeling Chlorine Inactivation Requirements of Cryptosporidium parvum Oocysts ." *Journal of Environmental Engineering*, 123 (9): 865–875. American Society of Civil Engineers (ASCE). https://doi.org/10.1061/(asce)0733-9372(1997)123:9(865)

van Halem, D. 2006. "Ceramic silver impregnated pot filters for household drinking water treatment in developing countries." Delft University of Technology.

van Halem, D., S. G. J. Heijman, A. I. A. Soppe, J. C. van Dijk, and G. L. Amy. 2007. "Ceramic silver-impregnated pot filters for household drinking water treatment in developing countries: material characterization and performance study." *Water Supply*, 7 (5–6): 9–17. IWA Publishing. https://doi.org/10.2166/WS.2007.142

van Halem, D., H. van der Laan, S. G. J. Heijman, J. C. van Dijk, and G. L. Amy. 2009. "Assessing the sustainability of the silver-impregnated ceramic pot filter for low-cost household drinking water treatment." *Physics and Chemistry of the Earth, Parts A/B/C*, 34 (1–2): 36–42. Pergamon. https://doi.org/10.1016/J.PCE.2008.01.005

Hill, C. L., and J. A. Smith. 2019. "Electrolytic Release of Metal Cations in Water." *Encyclopedia of Water*, 1–10. Wiley.

Hosni, A. A., W. T. Shane, J. G. Szabo, and P. L. Bishop. 2009. "The disinfection efficacy of chlorine and chlorine dioxide as disinfectants of Bacillus globigii, a surrogate for Bacillus anthracis , in

water networks: A comparative studyA paper submitted to the Journal of Environmental Engineering and Science." *Canadian Journal of Civil Engineering*, 36 (4): 732–737. https://doi.org/10.1139/L09-001

How, Z. T., I. Kristiana, F. Busetti, K. L. Linge, and C. A. Joll. 2017. "Organic chloramines in chlorine-based disinfected water systems: A critical review." *Journal of Environmental Sciences*, 58: 2–18. https://doi.org/10.1016/j.jes.2017.05.025

Hui, F., and C. Debiemme-Chouvy. 2013. "Antimicrobial N-halamine polymers and coatings: A review of their synthesis, characterization, and applications." *Biomacromolecules*. American Chemical Society.

Jackson, K. N., and J. A. Smith. 2018. "A New Method for the Deposition of Metallic Silver on Porous Ceramic Water Filters." *J Nanotechnol*, 2018. https://doi.org/10.1155/2018/2573015

Jain, P., and T. Pradeep. 2005. "Potential of silver nanoparticle-coated polyurethane foam as an antibacterial water filter." *Biotechnol Bioeng*, 90 (1): 59–63. John Wiley & Sons, Ltd. https://doi.org/10.1002/bit.20368

Jain, S., O. K. Sahanoon, E. Blanton, A. Schmitz, K. A. Wannemuehler, R. M. Hoekstra, and R. E. Quick. 2010. "Sodium dichloroisocyanurate tablets for routine treatment of household drinking water in periurban Ghana: A randomized controlled trial." *American Journal of Tropical Medicine and Hygiene*, 82 (1): 16–22. The American Society of Tropical Medicine and Hygiene. https://doi.org/10.4269/ajtmh.2010.08-0584

Jung, W. K., H. C. Koo, K. W. Kim, S. Shin, S. H. Kim, and Y. H. Park. 2008. "Antibacterial activity and mechanism of action of the silver ion in Staphylococcus aureus and Escherichia coli." *Appl Environ Microbiol*, 74 (7): 2171–2178. American Society for Microbiology. https://doi.org/10.1128/AEM.02001-07

Justia Patents. 1977. "US Patent for Method of purifying water Patent (Patent # 4,098,660 issued July 4, 1978) ." Accessed February 20, 2023. https://patents.justia.com/patent/4098660

Kahler, A. M., T. L. Cromeans, J. M. Roberts, and V. R. Hill. 2011. "Source water quality effects on monochloramine inactivation of adenovirus, coxsackievirus, echovirus, and murine norovirus." *Water Res*, 45 (4): 1745–1751. Elsevier Ltd. https://doi.org/10.1016/j.watres.2010.11.026

Kallman, E. N., V. A. Oyanedel-Craver, and J. A. Smith. 2011. "Ceramic Filters Impregnated with Silver Nanoparticles for Point-of-Use Water Treatment in Rural Guatemala." *Journal of Environmental Engineering*, 137 (6): 407–415. American Society of Civil Engineers. https://doi.org/10.1061/(ASCE)EE.1943-7870.0000330

Khandelwal, N., G. Kaur, K. K. Chaubey, P. Singh, S. Sharma, A. Tiwari, S. V. Singh, and N. Kumar. 2014. "Silver nanoparticles impair Peste des petits ruminants virus replication." *Virus Res*, 190: 1–7. Elsevier. https://doi.org/10.1016/j.virusres.2014.06.011

Kong, L. I., L. J. Swango, B. L. Blagburn, C. M. Hendrix, D. E. Williams, and S. D. Worley. 1988. "Inactivation of Giardia lamblia and Giardia canis cysts by combined and free chlorine." *Appl Environ Microbiol*, 54 (10).

Lalley, J., D. D. Dionysiou, R. S. Varma, S. Shankara, D. J. Yang, and M. N. Nadagouda. 2014. "Silver-based antibacterial surfaces for drinking water disinfection — an overview." *Curr Opin Chem Eng*, 3: 25–29. Elsevier. https://doi.org/10.1016/J.COCHE.2013.09.004

Lantagne, D. S., F. Cardinali, and B. C. Blount. 2010. "Disinfection by-product formation and mitigation strategies in point-of-use chlorination with sodium dichloroisocyanurate in Tanzania." *American Journal of Tropical Medicine and Hygiene*, 83 (1): 135–143. The American Society of Tropical Medicine and Hygiene. https://doi.org/10.4269/ajtmh.2010.09-0431

Lara, H. H., N. V. Ayala-Nuñez, L. Ixtepan-Turrent, and C. Rodriguez-Padilla. 2010. "Mode of antiviral action of silver nanoparticles against HIV-1." *J Nanobiotechnology*, 8 (1): 1–10. BioMed Central. https://doi.org/10.1186/1477-3155-8-1

Levy, K., L. Anderson, K. A. Robb, W. Cevallos, G. Trueba, and J. N. S. Eisenberg. 2014. "Household effectiveness vs. laboratory efficacy of point-of-use chlorination." *Water Res*, 54: 69–77. https://doi.org/10.1016/j.watres.2014.01.037

Liang, J., R. Wu, T. S. Huang, and S. D. Worley. 2005. "Polymerization of a hydantoinylsiloxane on particles of silicon dioxide to produce a biocidal sand." *J Appl Polym Sci*, 97 (3): 1161–1166. John Wiley & Sons, Ltd. https://doi.org/10.1002/app.21814

Lide, D. 2004. *CRC handbook of chemistry and physics*.

Lin, S., R. Huang, Y. Cheng, J. Liu, B. L. T. Lau, and M. R. Wiesner. 2013. "Silver nanoparticle-alginate composite beads for point-of-use drinking water disinfection." *Water Res*, 47 (12): 3959–3965. Elsevier Ltd. https://doi.org/10.1016/j.watres.2012.09.005

Lucier, K. J., S. E. Dickson-Anderson, and C. J. Schuster-Wallace. 2017. "Effectiveness of silver and copper infused ceramic drinking water filters in reducing microbiological contaminants." *Journal of Water Supply: Research and Technology - AQUA*, 66 (7): 528–536. IWA Publishing. https://doi.org/10.2166/aqua.2017.028

Marimuthu, S., A. J. Antonisamy, S. Malayandi, K. Rajendran, P. C. Tsai, A. Pugazhendhi, and V. K. Ponnusamy. 2020. "Silver nanoparticles in dye effluent treatment: A review on synthesis, treatment methods, mechanisms, photocatalytic degradation, toxic effects and mitigation of toxicity." *J Photochem Photobiol B*, 205: 111823. Elsevier. https://doi.org/10.1016/J.JPHOTOBIOL.2020.111823

McLennan, S. D., L. A. Peterson, and J. B. Rose. 2009. "Comparison of point-of-use technologies for emergency disinfection of sewage-contaminated drinking water." *Appl Environ Microbiol*, 75 (22): 7283–7286. American Society for Microbiology. https://doi.org/10.1128/AEM.00968-09

Morris, D., M. Ansar, J. Speshock, T. Ivanciuc, Y. Qu, A. Casola, and R. Garofalo. 2019. "Antiviral and Immunomodulatory Activity of Silver Nanoparticles in Experimental RSV Infection." *Viruses*, 11 (8): 732. MDPI AG. https://doi.org/10.3390/v11080732

Natan, M., O. Gutman, R. Lavi, S. Margel, and E. Banin. 2015. "Killing mechanism of stable N-halamine cross-linked polymethacrylamide nanoparticles that selectively target bacteria." *ACS Nano*, 9 (2): 1175–1188. American Chemical Society. https://doi.org/10.1021/nn507168x

Nawaz, M., M. Y. Han, T. il Kim, U. Manzoor, and M. T. Amin. 2012. "Silver disinfection of Pseudomonas aeruginosa and E. coli in rooftop harvested rainwater for potable purposes." *Science of the Total Environment*, 431: 20–25. Elsevier. https://doi.org/10.1016/j.scitotenv.2012.05.022

Omarova, A., K. Tussupova, R. Berndtsson, M. Kalishev, and K. Sharapatova. 2018. "Protozoan Parasites in Drinking Water: A System Approach for Improved Water, Sanitation and Hygiene in Developing Countries." *Int J Environ Res Public Health*, 15 (3): 495. MDPI AG. https://doi.org/10.3390/ijerph15030495

Oyanedel-Craver, V. A., and J. A. Smith. 2008. "Sustainable colloidal-silver-impregnated ceramic filter for point-of-use water treatment." *Environ Sci Technol*, 42 (3): 927–933. American Chemical Society. https://doi.org/10.1021/es071268u

Page, M. A., J. L. Shisler, and B. J. Mariñas. 2009. "Kinetics of adenovirus type 2 inactivation with free chlorine." *Water Res*, 43 (11): 2916–2926. Elsevier Ltd. https://doi.org/10.1016/j.watres.2009.03.047

Page, M. A., J. L. Shisler, and B. J. Mariñas. 2010. "Mechanistic Aspects of Adenovirus Serotype 2 Inactivation with Free Chlorine." *Appl Environ Microbiol*, 76 (9): 2946–2954. American Society for Microbiology. https://doi.org/10.1128/AEM.02267-09

Pal, S., Y. K. Tak, and J. M. Song. 2007. "Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium Escherichia coli." *Appl Environ Microbiol*, 73 (6): 1712–1720. https://doi.org/10.1128/AEM.02218-06/FORMAT/EPUB

Patil, R. A., D. Ahmad, S. B. Kausley, P. L. Balkunde, and C. P. Malhotra. 2015. "A compact point-of-use water purification cartridge for household use in developing countries." *J Water Health*, 13 (1): 91–102. IWA Publishing. https://doi.org/10.2166/wh.2014.111

Pereira, J. T., A. O. Costa, M. B. De Oliveira Silva, W. Schuchard, S. C. Osaki, E. A. De Castro, R. C. Paulino, and V. T. Soccol. 2008. "Comparing the efficacy of chlorine, chlorine dioxide, and ozone in the inactivation of cryptosporidium parvum in water from parana state, Southern Brazil." *Appl Biochem Biotechnol*, 151 (2–3): 464–473. Springer. https://doi.org/10.1007/s12010-008-8214-3

Rai, M. K., S. D. Deshmukh, A. P. Ingle, and A. K. Gade. 2012. "Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria." *J Appl Microbiol*, 112 (5): 841–852. Oxford Academic. https://doi.org/10.1111/J.1365-2672.2012.05253.X

Rajasekharan, V. V., B. N. Clark, S. Boonsalee, and J. A. Switzer. 2007. "Electrochemistry of free chlorine and monochloramine and its relevance to the presence of Pb in drinking water." *Environ Sci Technol*, 41 (12): 4252–4257. American Chemical Society . https://doi.org/10.1021/es062922t

Rennecker, J. L., A. M. Driedger, S. A. Rubin, and B. J. Mariñas. 2000. "Synergy in sequential inactivation of Cryptosporidium parvum with ozone/free chlorine and ozone/monochloramine." *Water Res*, 34 (17): 4121–4130. Pergamon. https://doi.org/10.1016/S0043-1354(00)00188-3

Rose, L. J., E. W. Rice, L. Hodges, A. Peterson, and M. J. Arduino. 2007. "Monochloramine inactivation of bacterial select agents." *Appl Environ Microbiol*, 73 (10): 3437–3439. American Society for Microbiology. https://doi.org/10.1128/AEM.00051-07

Samuni, A., M. Chevion, and G. Czapski. 1984. "Roles of copper and O2 in the radiation-induced inactivation of T7 bacteriophage." *Radiat Res*, 99 (3): 562–572. https://doi.org/10.2307/3576330

Sharma, V. K., X. Yang, L. Cizmas, T. J. McDonald, R. Luque, C. M. Sayes, B. Yuan, and D. D. Dionysiou. 2017. "Impact of metal ions, metal oxides, and nanoparticles on the formation of disinfection byproducts during chlorination." *Chemical Engineering Journal*. Elsevier B.V.

Singh, R., C. Rento, V. Son, S. Turner, and J. A. Smith. 2019. "Optimization of Silver Ion Release from Silver-Ceramic Porous Media for Household Level Water Purification." *Water (Basel)*, 11 (4): 816. MDPI AG. https://doi.org/10.3390/w11040816

Soliman, A., A. El-Adawy, A. A. Abd El-Aal, M. A. Elmallawany, R. K. Nahnoush, A. R. Abd Eiaghni, M. S. Negm, and A. Mohsen. 2018. "Usefulness of sunlight and artificial UV radiation versus chlorine for the inactivation of cryptosporidium oocysts: An in vivo animal study." *Open Access Maced J Med Sci*, 6 (6): 975–981. Open Access Macedonian Journal of Medical Sciences. https://doi.org/10.3889/oamjms.2018.180

Straub, T. M., C. P. Gerba, X. Zhou, R. Price, and M. T. Yahya. 1995. "Synergistic inactivation of Escherichia coli and MS-2 coliphage by chloramine and cupric chloride." *Water Res*, 29 (3): 811–818. Pergamon. https://doi.org/10.1016/0043-1354(94)00213-Q

US EPA, O. 2020. "Drinking Water Regulations and Contaminants." Accessed January 4, 2021. https://www.epa.gov/sdwa/drinking-water-regulations-and-contaminants

Venczel, L. V., C. A. Likirdopulos, C. E. Robinson, and M. D. Sobsey. 2004. "Inactivation of enteric microbes in water by electro-chemical oxidant from brine (NaCl) and free chlorine." *Water Science and Technology*, 50 (1): 141–146. IWA Publishing. https://doi.org/10.2166/wst.2004.0042

Wigginton, K. R., B. M. Pecson, T. Sigstam, F. Bosshard, and T. Kohn. 2012. "Virus inactivation mechanisms: Impact of disinfectants on virus function and structural integrity." *Environ Sci Technol*, 46 (21): 12069–12078. American Chemical Society. https://doi.org/10.1021/es3029473

World Health Organization. 2013. "Pocket book of hospital care for children: Second edition." Accessed February 20, 2023. https://www.who.int/publications/i/item/978-92-4-154837-3

World Health Organization. 2017. *Guidelines for drinking-water quality, 4th edition, incorporating the 1st addendum*. Geneva: WHO.

Yahya, M. T., T. M. Straub, and C. P. Gerba. 1992. "Inactivation of coliphage MS-2 and poliovirus by copper, silver, and chlorine." *Can J Microbiol*, 38 (5): 430–435. NRC Research Press Ottawa, Canada . https://doi.org/10.1139/m92-072

Zhou, P., G. D. Di Giovanni, J. S. Meschke, and M. C. Dodd. 2014. "Enhanced Inactivation of Cryptosporidium parvum Oocysts during Solar Photolysis of Free Available Chlorine." *Environ Sci Technol Lett*, 1 (11): 453–458. American Chemical Society. https://doi.org/10.1021/ez500270u

Chapter 3

Synergistic bacterial inactivation by silver ions and free chlorine in natural waters

The work presented in this chapter resulted in a published journal article.

Estrella-You, A. and J. A. Smith. "Synergistic bacterial inactivation by silver ions and free chlorine in natural waters." *Journal of Environmental Engineering*. 148.11 (2022): 04022072 https://doi.org/10.1061/(ASCE)EE.1943-7870.0002053

3.1 Abstract

Using high amounts of chlorine to disinfect contaminated natural waters for drinking purposes can produce an unpleasant taste and odor and contribute to the formation of toxic byproducts. These challenges can be addressed through the combined use of lower amounts of chlorine and silver. Several studies in well water or solutions inoculated with bacteria or viruses have demonstrated that this combination produces a synergistic effect in the inactivation of pathogens. This study investigated the synergistic inactivation of bacteria in natural waters (from a pond with 4.82 NTU and upstream with 11.9 NTU in Virginia) using low doses of silver (added as silver nitrate) and free chlorine (from Aquatabs). There was a significant synergistic effect at 3-hr contact time and the $\log_{10}$ reductions of *E. coli* and total coliform bacteria (TCB) were ≥1.44 and ≥2.73, respectively, with the lower-turbidity water, and 0.87 and 1.29, respectively, with the higher-turbidity water. Chlorine effectiveness was significantly reduced by higher turbidity, whereas silver effectiveness was not. Thus, for waters with higher turbidity, silver alone or a combination of low doses of silver and chlorine may produce a higher bacteria inactivation than chlorine alone. In addition, bacteria inactivation by the MadiDrop+, MD (a commercial silver-ceramic tablet that releases silver ions for point-of-use water disinfection), with low doses of free chlorine in water from a stream in South Africa, was tested. The MD alone at 8-hr contact time obtained ~1-$\log_{10}$ reduction for *E. coli* and ~2-$\log_{10}$ reduction for TCB. However, some of the MD-free chlorine combinations achieved a similar bacteria reduction

with a substantial reduction of contact time (between 6- and 7-hr less). Overall, these results show that the silver-chlorine synergistic effect demonstrated in previous studies with solutions inoculated with pathogens is also present in the more realistic scenario with natural waters which contain more complex matrixes.

3.2 Introduction

The World Health Organization (WHO) estimates that 490 million people around the world use contaminated water (including surface waters from lakes, ponds, rivers and streams, and water from unprotected wells and springs) for drinking purposes. This untreated water can transmit bacterial, viral, and protozoan species to people and cause severe diarrheal diseases that can lead to death and other health problems, especially in children under the age of 5 years old (World Health Organization 2022). The absence of safe drinking water can be linked to a lack of a centralized infrastructure to support effective water disinfection and/or a defective water distribution network (Jain et al. 2010; Patil et al. 2015). Treatment of contaminated water in households immediately before consumption using point-of-use (POU) technologies (Clasen and Edmondson 2006) can offer a remedy for the previously mentioned challenges.

Currently, some POU technologies use silver (e.g., porous ceramic tablets or filters where the metal is embedded) or free chlorine (e.g., chlorine tablets, and bleach or liquid chlorine) to inactivate waterborne pathogens (Ercumen et al. 2015; Kallman et al. 2011). Silver is more expensive than chlorine and may require higher doses and longer contact times to be as effective as chlorine against pathogens (Kannan et al. 2021). However, silver has an advantage over chlorine because it does not change the taste and odor of the treated water (Jackson and Smith 2018), which can prevent rejection by users or discontinuation of the disinfection treatment (Firth et al. 2010). Moreover, high doses of chlorine (to account for variable chlorine demands of natural waters) can form toxic disinfection byproducts (DBPs), and commonly used products that are sources of free chlorine have a short shelf life (6 months to 1 year) (Lantagne et al. 2010).

A MadiDrop+ (MD) tablet (Silivhere Technologies, Inc., Charlottesville, VA, USA) is a POU technology that uses silver. When placed in a household container that stores water, the MD can

release silver ions into the water at a controlled and sustained rate for waterborne pathogens' inactivation (Ehdaie et al. 2014). This occurs because the metallic silver embedded in the ceramic tablet is oxidized to silver ions in the presence of water, and these ions gradually diffuse out of the tablet into the stored water. Treatment of 10- to 20-L of water with the MD requires 8-hr contact time, in which an average of 30 μg/L Ag^+ is released from the tablet. To date, multiple studies have quantified the performance of these tablets in both the field and laboratory (Ehdaie et al. 2014, 2017, 2020; Hill et al. 2020; Jackson et al. 2019; Kahler et al. 2016; Singh et al. 2019) and their results indicate that the tablets perform well against coliform bacteria, including *E. coli* (can achieve a 4-$\log_{10}$ reduction in 8-hr contact time), but only provide about a 1-$\log_{10}$ reduction in protozoan (*Cryptosporidium* and *Giardia sp.*) and viral pathogens (adenovirus).

Several studies have evaluated the combined use of metals and chlorine in well water or solutions inoculated with bacteria or viruses (i.e., under controlled conditions) for waterborne pathogens' inactivation (including viruses, bacteria, and protozoa) (Abad et al. 1994; Biurrun et al. 1999; Chen et al. 2008; Cromeans et al. 2010; Liu et al. 1994; Lucier et al. 2017; Straub et al. 1995; Yahya et al. 1990). These studies have demonstrated that there is a synergistic effect in the inactivation of pathogens. But there is a data gap in testing this effect in natural waters that are considered contaminated or non-potable. By using natural waters, a more realistic scenario can be tested. In this scenario, the physicochemical quality of the waters, the presence of inorganic and organic reduced compounds (which contribute to significant chlorine demand and/or silver ions complexation), the aggregation of pathogens, or other conditions play important roles in the pathogens' inactivation kinetics and potentially affect the synergistic effect (Barbeau et al. 2005; Russel et al. 2004). Therefore, the objectives of this study were to (1) evaluate if a synergistic effect is produced when combining low doses of silver ion and free chlorine in the inactivation of *E. coli* and total coliform bacteria (TCB) in natural waters; (2) test the influence of turbidity and dissolved organic carbon (DOC) in silver ion and free chlorine effectiveness; and (3) determine if there is an improvement in the performance of the MD when it is combined with low doses of free chlorine.

3.3 Materials and methods

3.3.1 Water collection

Samples of stream water were collected from a stream located near the University of Venda (Univen) in the town of Thohoyandou, Limpopo province, South Africa. This province is in the northeast corner of South Africa, next to Zimbabwe and Mozambique, and it is the second poorest and most rural province of the country (90% of its population lives in rural areas) (Mellor et al. 2013). Samples of pond water and upstream were collected from a pond located at the University of Virginia (UVa) in Charlottesville, VA, USA. All samples were transported in plastic buckets between collection and testing sites (laboratories at Univen and at UVa, respectively).

3.3.2 Charlottesville water quality analysis

Prior to disinfection, Charlottesville sample waters were analyzed for pH (Orion Versa Star Pro benchtop pH meter; Thermo Scientific, Waltham, MA, USA), turbidity (2100Q portable turbidimeter; Hach, Loveland, CO, USA), nitrate (Vacu-vials Kit K-6913; CHEMetrics, Inc. Midland, VA, USA) and phosphate (TNTplus 843; Hach) using a spectrophotometer (DR6000; Hach), dissolved organic carbon and total dissolved nitrogen (DOC and TDN; Shimadzu TOC-L with a coupled TNM-L analyzer, Shimadzu Scientific Instruments, Columbia, MD, USA), and *E. coli* and total coliform bacteria (Colilert Defined-Substrate Technology System; IDEXX, Westbrook, ME, USA). Before DOC and TDN analysis, samples were passed through a 0.45 μm pore PTFE filter and acidified to 2% hydrochloric acid (34-37% TraceMetal™ Grade; Fisher Chemical, Pittsburgh, PA, USA).

3.3.3 Preparation of disinfectants

Free chlorine ($HOCl/OCl^-$) and silver ion were the disinfectants considered in this study. At the Univen laboratory, before every water disinfection test, a stock solution of free chlorine was generated when dissolving an Aquatab (Medentech, Wexford, Ireland) in 1 L of reverse osmosis water, producing approximately 40,000 μg/L free chlorine as Cl_2. A stock solution of 300,000 μg/L silver ion was made by dissolving silver nitrate (ACS grade; Artcraft Chemicals, Altamont, NY, USA) in reverse osmosis water. Similarly, at the UVa laboratory, the free chlorine stock

solution was prepared dissolving an Aquatab (Medentech) in 100 mL of reverse osmosis water, producing approximately 400,000 μg/L free chlorine as Cl_2. And a 20,000 μg/L silver ion stock solution was made by dissolving silver nitrate (ACS grade; Artcraft Chemicals) in reverse osmosis water. At the start of each bacteria inactivation test (t = 0), the appropriate aliquots of silver ion and/or free chlorine stock solutions were added to achieve the desired chemical concentration in the water samples (see Experimental design, below).

3.3.4 Bacteria inactivation tests (Experimental design)

E. coli and total coliform bacteria (TCB) inactivation tests were performed in the natural water samples using silver and chlorine. The $\log_{10}$ reduction of bacteria over time was measured for each chemical treatment.

At the Univen laboratory, two tests were performed. First, appropriate aliquots of silver ion (Ag^+) and/or free chlorine (as Cl_2) stock solutions were added to three different 10-L water samples to obtain the following concentrations at t = 0: (i) combination of 15 μg/L Ag^+ and 100 μg/L Cl_2 (applied simultaneously); (ii) 15 μg/L Ag^+; and (iii) 100 μg/L Cl_2. Water samples were taken before chemicals addition, and at 1- and 3-hr contact time to analyze for *E. coli* and TCB. Second, the MadiDrop+ tablet (MD; Silivhere Technologies, Inc.) and appropriate aliquots of free chlorine stock were added to four different 10-L water samples to obtain the following concentrations at t = 0: (i) MD; (ii) MD + 50 μg/L Cl_2; (iii) MD + 100 μg/L Cl_2; and (iv) MD + 200 μg/L Cl_2 (for the combinations, the tablet and free chlorine aliquot were added simultaneously). Similarly, water samples were taken before MD and chemical addition, and at 0.5-, 1-, 2-, 4-, and 8-hr contact time to analyze for *E. coli* and TCB, and total residual silver. In both tests, when sampling after t = 0, the antibacterial activity of silver and/or chlorine was quenched by the addition of 2.64 mL of 60 g/L sodium thiosulfate solution (sodium thiosulfate anhydrous; Fisher Chemical) to each 100 mL of sample as indicated in Ehdaie et al. (2014). Additionally, in both tests each treatment had a replicate, and a no treatment or control was also included. Finally, tests were performed under ambient conditions.

At the UVa laboratory, four tests were performed. First, appropriate aliquots of silver ion and/or free chlorine stock solutions were added to three different 10-L pond water samples to obtain the following concentrations at t = 0: (i) combination of 10 μg/L Ag^+ and 100 μg/L Cl_2

(applied simultaneously); (ii) 10 μg/L Ag^+; and (iii) 100 μg/L Cl_2. Second, the first test was replicated but with upstream water samples. Third, appropriate aliquots of silver ion or free chlorine stock solutions were added to four different 10-L pond water samples to obtain the following concentrations at t = 0: (i) 5 μg/L Ag^+; (ii) 70 μg/L Ag^+; (iii) 50 μg/L Cl_2; and (iv) 500 μg/L Cl_2. Fourth, appropriate aliquots of silver ion or free chlorine stock solutions were added to four different 10-L upstream water samples to obtain the following concentrations at t = 0: (i) 40 μg/L Ag^+; (ii) 70 μg/L Ag^+; (iii) 250 μg/L Cl_2; and (iv) 500 μg/L Cl_2. In test four (upstream water), compared to test three (pond water), a 40 μg/L Ag^+ dose instead of 5 μg/L Ag^+, and a 250 μg/L Cl_2 dose instead of 50 μg/L Cl_2 were tested. These changes were done considering that the higher turbidity in the upstream water could inhibit the bacteria inactivation when using the lower disinfectant doses (5 μg/L Ag^+ and 50 μg/L Cl_2). For all tests, water samples were taken before chemical addition, and at the contact times specified on Table 3.1 to analyze for *E. coli* and TCB. Sodium thiosulfate was added to samples to stop the antibacterial activity of silver and/or chlorine as indicated above. Also, in all tests each treatment had a replicate, and a no-treatment or control was included. Lastly, tests were performed under ambient conditions. The log_{10} reduction results of these tests were used to evaluate the synergistic effect according to the data analysis procedure explained later.

Table 3.1: Sampling times for disinfection tests using Charlottesville water

	Pond		**Upstream from the pond**	
Disinfectant dose	**Sampling times (h)**			
	Test 1	**Test 3**	**Test 2**	**Test 4**
5 μg/L Ag^+	-	0.5, 1, 1.5, 2, 2.5, 4	-	-
10 μg/L Ag^+	0.5, 1, 2, 3, 4	-	0.5, 1, 2, 3, 4	-
40 μg/L Ag^+	-	-	-	0.5, 1, 2, 3, 4
70 μg/L Ag^+	-	0.5, 1, 1.5, 2, 2.5, 4	-	0.5, 1, 2, 3, 4
50 μg/L Cl_2	-	0.5, 1, 1.5, 2, 2.5, 4	-	-
100 μg/L Cl_2	0.5, 1, 2, 3, 4	-	0.5, 1, 2, 3, 4	-
250 μg/L Cl_2	-	-	-	0.5, 1, 2, 3, 4
500 μg/L Cl_2	-	0.5, 1, 1.5, 2, 2.5, 4	-	0.5, 1, 2, 3, 4
10 μg/L Ag^+ and 100 μg/L Cl_2	1, 3	-	1, 3	-

Note: "-" indicates that the concentration of the chemical disinfectant was not assessed in the test.

3.3.5 Quantification of *E. coli* and total coliform bacteria (TCB)

At the Univen laboratory, viable *E. coli* and TCB were quantified in samples (before starting the disinfection tests and at the different contact times indicated in the Experimental design) via membrane filtration as described in the US Environmental Protection Agency (EPA) m-ColiBlue24 Test or the Hach Company method 10029. Briefly, sterile, individually packaged, 0.45 μm pore filter paper disks (MilliporeSigma, Burlington, MA, USA) were placed on the surface of the manifold cup holders using forceps and an aseptic technique. At the same time, the sample cups of the manifold were introduced in a hot water bath (set to 100°C) for a minimum of 1 minute (the cup holders were not sterilized but for each filtration round, blanks were included, and these did not show any colonies). Next, the cups were placed on top of the filters and waited a couple of minutes for them to cool down. Then, 100 mL of water sample (full-strength) or diluted sample (10^{-2} dilution) were passed per filter. This dilution provides a range of <1 to 20,000 CFU/100 mL for both *E. coli* and TCB. Blank tests were run with reverse osmosis water. After filtration, the filters were transferred by aseptic technique to sterile petri dishes, each containing an absorbent pad (MilliporeSigma). A sterile, 2 mL ampule of selective growth media solution (m-ColiBlue24; MilliporeSigma) was added to each petri dish. The petri dishes were incubated at 35°C for 24 hours. After incubation, *E. coli* and TCB colonies were counted.

At the UVa laboratory, viable *E. coli* and TCB were quantified in samples (before starting the disinfection tests and at the different contact times indicated in the Experimental design) as described in the Standard Method 9223 for the Examination of Water and Wastewater (American Public Health Association et al. 2017). Briefly, Colilert media (IDEXX) was added to 100 mL of water sample (full-strength) or diluted sample. Next, the solution was mixed thoroughly. Then, the solution was poured into an IDEXX Quanti-Tray/2000 (IDEXX) which provides counts up to 2,419 MPN/100 mL in a sample without dilution. The trays were sealed and then incubated at 35°C for 24 hours. After incubation, viable *E. coli* and TCB were determined using the MPN table provided by IDEXX and a UV lamp for *E. coli*.

3.3.6 Free chlorine and silver analysis

The concentration of free chlorine in samples (as mg/L Cl_2) was determined using the *N*,*N*-diethyl-*p*-phenylenediamine (DPD) colorimetric method with a reagent set (Test 'N Tube Vials; Hach) and a spectrophotometer (at Univen: Orion AquaMate 7000, Thermo Fisher Scientific; and at UVa: DR6000, Hach) with a measuring wavelength of 530 nm. The concentration of total silver in samples acidified to 2% nitric acid (70% TraceMetal™ Grade; Fisher Chemical) was analyzed using atomic absorption. This analysis has been described elsewhere (Singh et al. 2019). Briefly, a graphite furnace (HGA 900, PerkinElmer, Waltham, MA, USA) atomic absorption spectrometer (AAnalyst 200, PekinElmer), which includes a silver cathode lamp, was used with the US EPA method 7010.

3.3.7 Bacteria inactivation data analysis

Following a previously reported data analysis method by Straub et al. (1995), synergism was assessed using the mathematical model developed by Berenbaum (1989) and modified for disinfection kinetics by Kouame and Hass (1991). In this model, the effect produced by the combination of chemical disinfectants can be determined with the following formula:

$$\sum_{i=1}^{n} \frac{d_i}{D_i} = \begin{cases} 1 & \text{no interaction} \\ < 1 & \text{synergism} \\ > 1 & \text{antagonism} \end{cases} \quad (1)$$

where n = total number of disinfectants, i = individual disinfectant, d_i = concentration of the individual disinfectant in the combination, and D_i = concentration of the disinfectant that individually would produce the same effect (i.e., bacteria $\log_{10}$ reduction) as that of the combination. When the formula result is equal to 1 there is no interaction among the chemicals. However, when the solution is less than 1, there is a synergistic interaction, which means that the overall effect of the combination is greater than the sum of the individual effects. Conversely, when the solution is greater than 1, there is an antagonistic interaction (Straub et al. 1995).

3.4 Results

3.4.1 Charlottesville water quality

Physicochemical and bacterial analysis results of the Charlottesville sample waters are shown in Table 3.2. These waters were chosen for the disinfection tests because they naturally contained *E. coli* and total coliform bacteria, and dissolved organic carbon (DOC), and they had different turbidity levels. The upstream water compared with the pond water had more than double turbidity units, and higher nitrate, DOC, and bacteria concentrations.

Table 3.2: Charlottesville sample waters' chemical and bacterial analysis

	Pond	**Upstream from the pond**
pH	6.4	6.5
Turbidity (NTU)	4.82	11.9
Nitrate (mg/L NO_3-N)	0.28	0.43
Total Dissolved Nitrogen (mg/L TDN)	0.63	0.60
Phosphate (mg/L PO_4-P)	<0.05	<0.05
Dissolved Organic Carbon (mg/L DOC)	1.80	2.49
***E. coli* (log_{10})**	1.44	3.19
Total coliform bacteria (log_{10})	2.73	3.81

Note: All values are means (n=2).

3.4.2 Synergistic inactivation of *E. coli* and total coliform bacteria (TCB) in natural waters

Figure 3.1 and Table 3.3 results suggest that the combined use of silver ions and free chlorine for bacteria inactivation in the sampled natural waters may have a synergistic effect. This effect was best demonstrated in the stream water disinfection (see Figure 3.1). After 3-hr contact time, *E. coli* inactivation by silver plus chlorine resulted in a 2.42-log_{10} reduction, whereas treatment of the same water by silver or chlorine separately yielded 0.42- and 0.31-log_{10} reduction, respectively. Similarly, TCB inactivation by silver plus chlorine resulted in a 2.32-log_{10} reduction, whereas treatment by silver or chlorine separately yielded 0.62- and 0.19-log_{10} reduction, respectively.

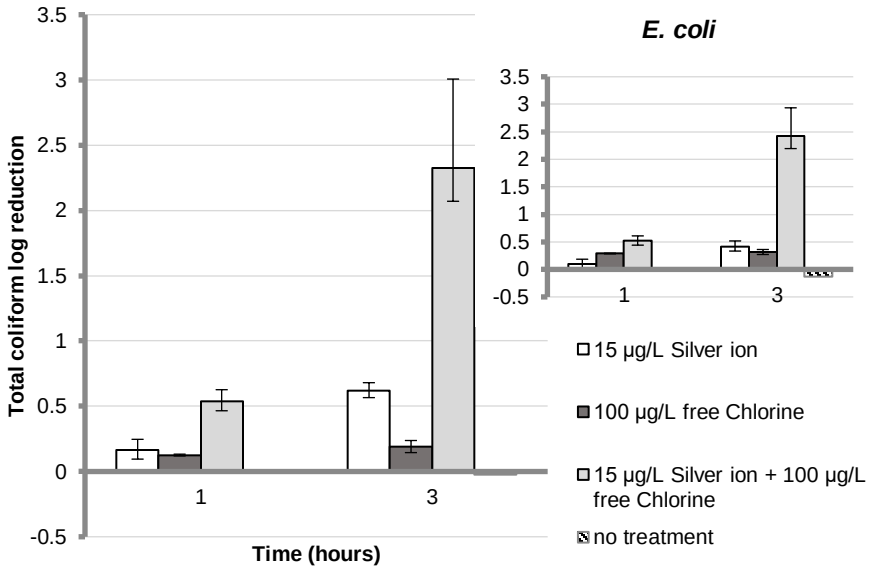

Figure 3.1: Inactivation of *E. coli* and total coliform bacteria by silver ion and free chlorine in stream water collected in Thohoyandou, Limpopo province, South Africa for 1- and 3-hr contact time. Error bars indicate range (n = 2).

Table 3.3: Bacteria inactivation in natural waters after 3-hr contact time with silver ion and/or free chlorine

	Pond (4.82 NTU)		**Upstream from the pond** (11.9 NTU)	
Disinfectant dose (d_i)	**$\log_{10}$ reduction**			
	E. coli	**Total coliform**	***E. coli***	**Total coliform**
10 µg/L Ag^+ and 100 µg/L Cl_2	≥1.44	≥2.73	0.87	1.29
10 µg/L Ag^+	0.69	1.03	0.55	1.04
100 µg/L Cl_2	1.18	0.44	-0.03	0.17
Limit of sensitivity[a]:	1.44	2.73	3.19	3.81

[a]The limit of sensitivity is the maximum reduction that could be observed in each test. It depends on the initial bacteria concentration.

The results from the UVa trials with different turbidity water (see Table 3.3) suggest that an increase in turbidity does not significantly change the bacterial inactivation by silver. However, and increase in turbidity does decrease the bacterial inactivation by chlorine. In other words, TCB were more sensitive to 10 or 15 µg/L silver ion used alone compared to 100 µg/L free chlorine as Cl_2 alone (see Figure 3.1 and Table 3.3). Bacteria inactivation by silver plus chlorine in pond water reached the limit of sensitivity for the tests (this limit was calculated based on the

bacteria concentration at t = 0, and it represents the maximum $\log_{10}$ reduction that can be achieved). Therefore, if a higher bacteria concentration (i.e., *E. coli*: $>10^{1.44}$ MPN/100 mL; or TCB: $>10^{2.73}$ MPN/100 mL) was present before starting the pond water disinfection tests, a higher $\log_{10}$ reduction may have been achieved.

Synergism between silver and chlorine in the inactivation of *E. coli* and TCB in samples taken from pond and upstream waters was assessed with the mathematical model, equation (1). Values for d_i are given in Table 3.3. D_i values were determined from regression equations that represent bacteria $\log_{10}$ reductions from each disinfectant. With the pond water disinfection results, the *E. coli* inactivation regression equations for 50, 100, and 500 µg/L free chlorine as Cl_2 (see Figure 3.2) and 5, 10, and 70 µg/L Ag^+ (see Figure 3.3) were calculated. These equations were determined by plotting the $\log_{10}$ reduction versus contact time using data from duplicate disinfection tests at each concentration. Next, *E. coli* $\log_{10}$ reductions for each disinfectant concentration at 1 and 3 hours were calculated with the regression equations (e.g., at 3-hr the calculated $\log_{10}$ reduction for 10 µg/L Ag^+ is $0.13 \times 3 + 0.28 = 0.67$). Then, plots of the calculated $\log_{10}$ reduction versus disinfectant concentration at each contact time were generated (see Figure 3.4 for free chlorine and Figure 3.5 for silver ion). Finally, calculation of the D_i values was done by graphical extrapolation from the $\log_{10}$ reduction versus concentration plots (see Figure 3.4 and Figure 3.5) as detailed next.

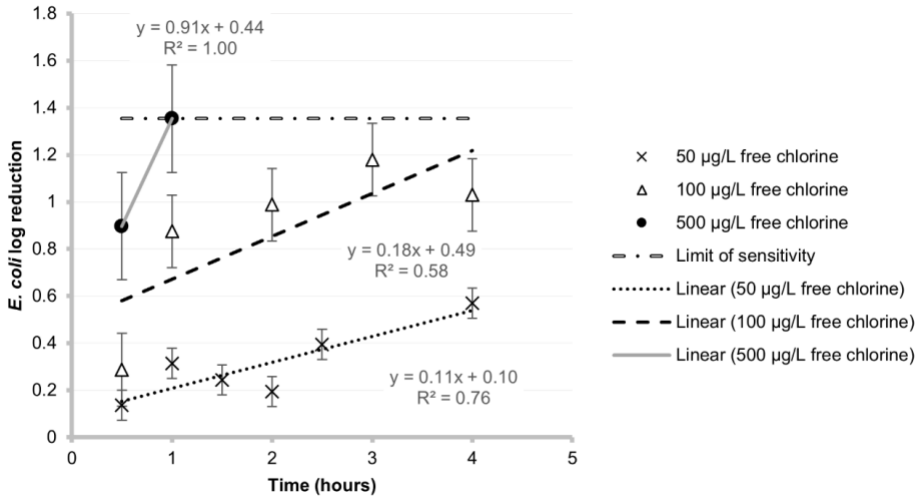

Figure 3.2: Regression analysis of *E. coli* inactivation by free chlorine in pond water collected in Charlottesville, VA, USA. Solid, dashed, and dotted lines represent the linear function fittings of the plotted data. Error bars indicate standard error (n = 2).

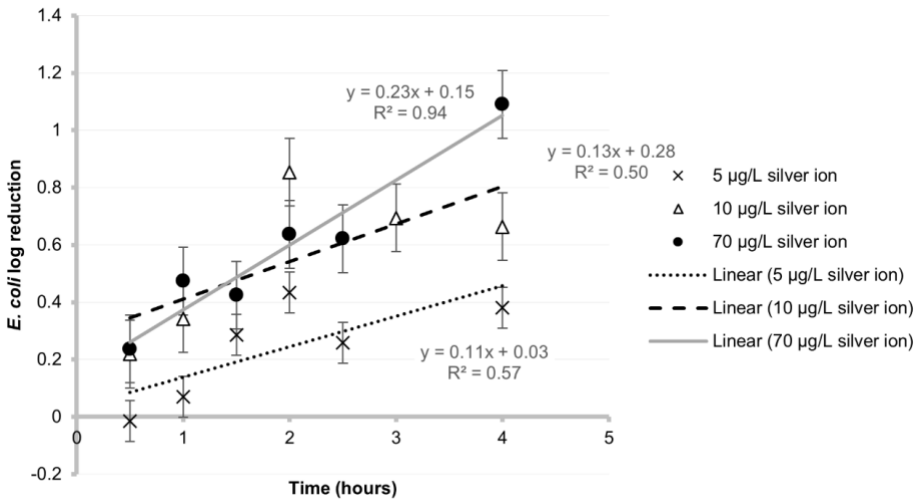

Figure 3.3: Regression analysis of *E. coli* inactivation by silver ion in pond water collected in Charlottesville, VA, USA. Solid, dashed, and dotted lines represent the linear function fittings of the plotted data. Error bars indicate standard error (n = 2).

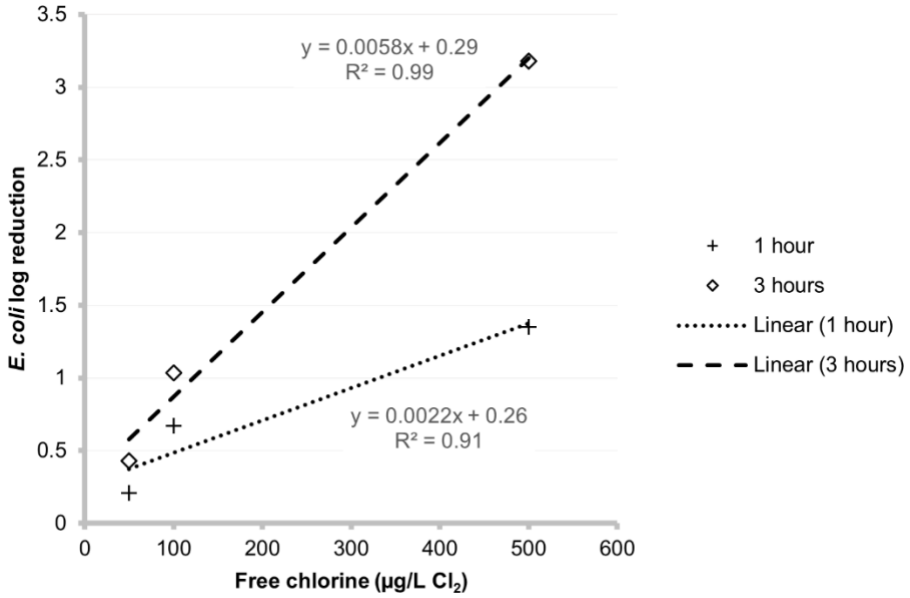

Figure 3.4: Calculated *E. coli* $\log_{10}$ reduction by free chlorine at 1- and 3-hr contact time in pond water collected in Charlottesville, VA, USA. Dashed and dotted lines represent the linear function fittings of the plotted data.

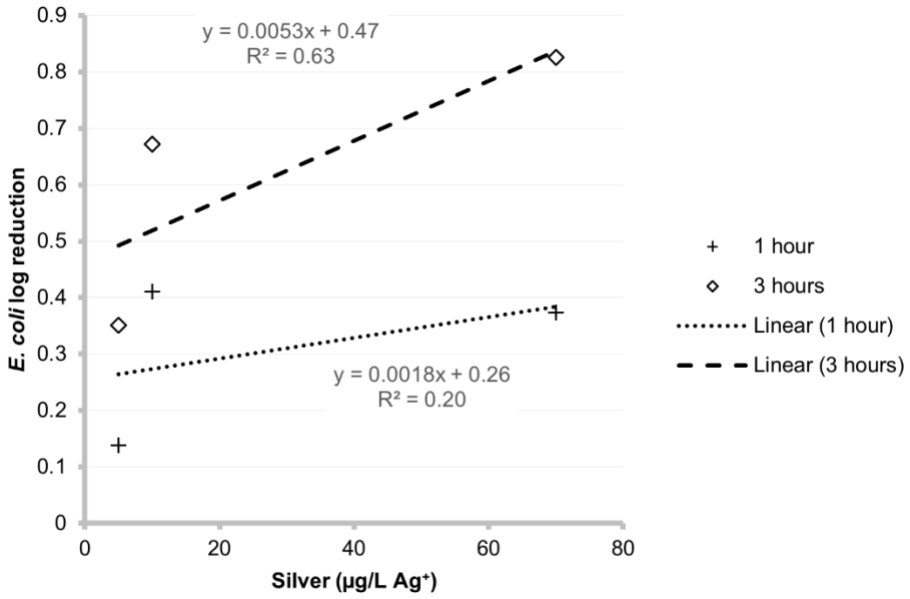

Figure 3.5: Calculated *E. coli* $\log_{10}$ reduction by silver ion at 1- and 3-hr contact time in pond water collected in Charlottesville, VA, USA. Dashed and dotted lines represent the linear function fittings of the plotted data.

In Figure 3.2, the linear regression analysis for 500 µg/L Cl_2 was performed using two data points. In this case, all the bacteria were inactivated by the second time point (1-hr) because of the high chlorine concentration. Additionally, the natural water samples used in this study represent a complex water matrix. Because of this, the bacteria inactivation in those waters can deviate from the linear model used to assess synergism and result in low R^2 values (Figure 3.5). Overall, trends where higher disinfectant concentration and greater contact time produce higher $\log_{10}$ reduction are present in the results.

The combined system with 100 µg/L Cl_2 + 10 µg/L Ag^+ in pond water produced a *E. coli* $\log_{10}$ reduction of 1.44 after 3-hr contact time (see Table 3.3). By extension of the lines for the 3-hr time in Figure 3.4 and Figure 3.5, these intersect the $\log_{10}$ reduction of 1.44 at the concentrations of approximately 200 µg/L free chlorine as Cl_2 and 184 µg/L Ag^+, respectively. These are the D_i values or the predicted concentration of the chemicals when used individually to achieve the same 1.44-$\log_{10}$ reduction after 3-hr contact time. Therefore, the corresponding $\Sigma(d_i/D_i)$ is 100/200 + 10/184 = 0.56. Since this sum is less than 1, according to equation (1) the combined use of the disinfectants produced a synergistic effect.

Similar calculations and plots were made for TCB inactivation in pond water and *E. coli* and TCB inactivation in upstream water. A summary table with the values of D_i and sum of ratios for each case is presented in Table 3.4. Even though, as mentioned previously, the bacteria inactivation by 10 μg/L silver plus 100 μg/L Cl_2 in pond water reached the limit of sensitivity for the tests, all sums in Table 3.4 are less than 1. Based on equation (1), this means that silver plus chlorine produced a synergistic effect in the inactivation of *E. coli* and TCB in pond and upstream waters.

Table 3.4: Evaluation of the synergistic effect in bacteria inactivation in natural waters after 3-hr contact time with 10 μg/L silver ion (d_{Ag}) and 100 μg/L free chlorine (d_{Cl})

	Pond (4.82 NTU)		**Upstream from the pond** (11.9 NTU)	
	E. coli	**Total coliform**	***E. coli***	**Total coliform**
Target $\log_{10}$ reduction[a]	1.44	2.73	0.87	1.29
D_{Ag}: Ag^+ predicted to achieve target reduction[b] (μg/L)	184	207	58	86
D_{Cl}: Cl_2 predicted to achieve target reduction[b] (μg/L)	200	248	164	185
$\sum_{i=1}^{2} \frac{d_i}{D_i}$	0.56	0.45	0.78	0.66

[a] Values from Table 3.3.

[b] Determined using regressions.

3.4.3 MadiDrop+ performance improvement

Figure 3.6 and Figure 3.7 results suggest that the performance of the MadiDrop+ (MD) tablet improves with the addition of low doses of free chlorine in stream water disinfection. When following the MD recommended contact time of 8-hr to treat 10-L water, the *E. coli* inactivation by MD plus 50, 100, and 200 μg/L Cl_2 resulted in 1.76-, 2.30-, and 2.12-$\log_{10}$ reduction, respectively. Whereas treatment of the same water with the MD alone yielded a 1.02-$\log_{10}$ reduction. A similar *E. coli* reduction (1.12-$\log_{10}$) was achieved at 1-hr contact time when using the combination MD plus 100 μg/L Cl_2.

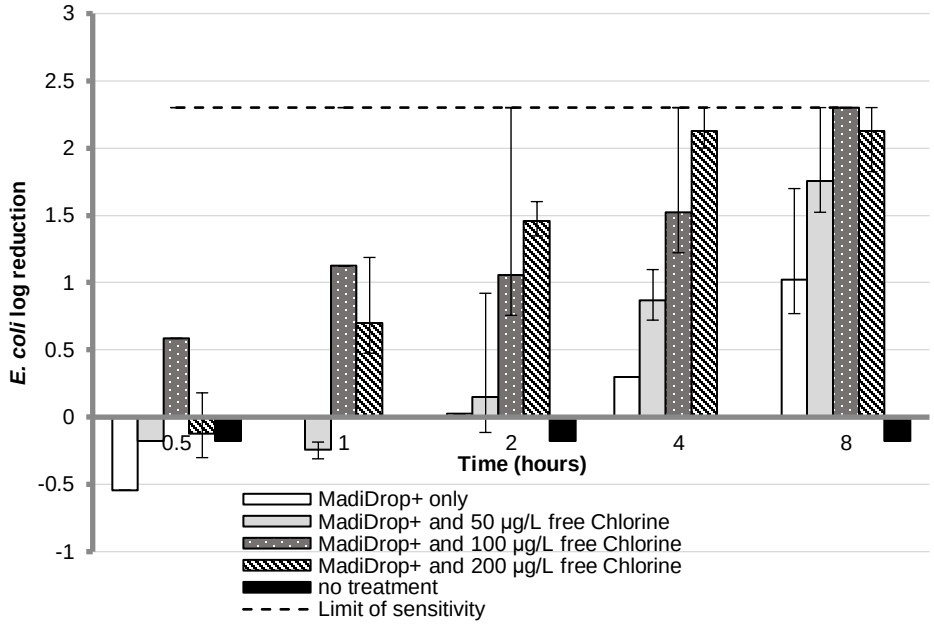

Figure 3.6: Inactivation of *E. coli* by MadiDrop+ tablets and free chlorine in stream water collected in Thohoyandou, Limpopo province, South Africa. Error bars indicate range (n = 2).

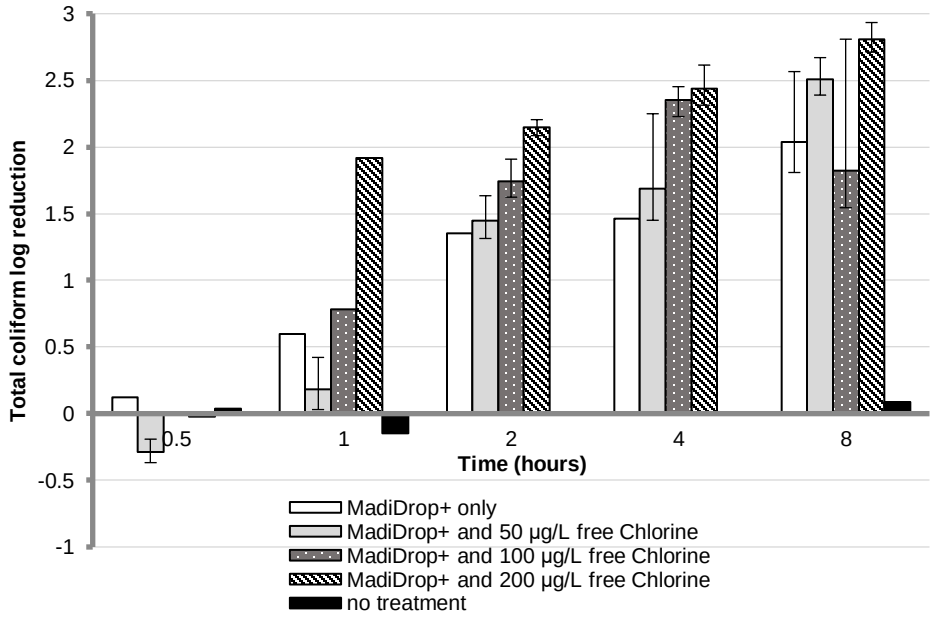

Figure 3.7: Inactivation of total coliform bacteria by MadiDrop+ tablets and free chlorine in stream water collected in Thohoyandou, Limpopo province, South Africa. Error bars indicate range (n = 2).

Overall, in Figure 3.6, MD plus 100 or 200 μg/L Cl_2 produced similar bacteria reductions as contact time increased. Moreover, these combinations always resulted in greater bacteria inactivation compared to MD alone and MD plus 50 μg/L Cl_2. Figure 3.8 shows the total residual silver (diffused out from the MD tablets to the sample water and what is left after bacteria inactivation) at different contact times. All results are below the silver secondary drinking water standard of 100 μg/L (US EPA 2020). In addition, most silver results are similar at each contact time except for the ones corresponding to the combination MD plus 200 μg/L Cl_2 which are lower.

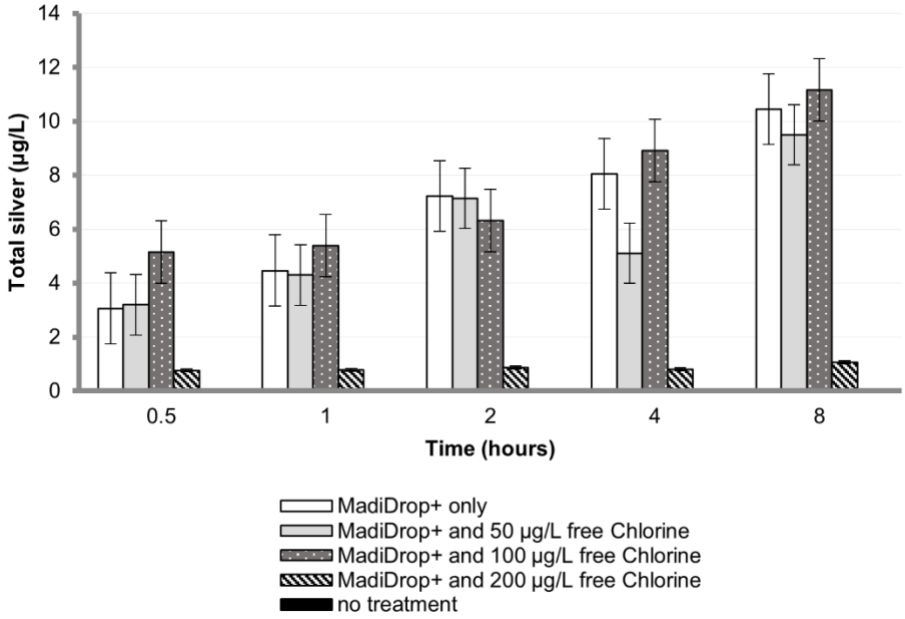

Figure 3.8: Total residual silver in treated stream water collected in Thohoyandou, Limpopo province, South Africa. Error bars indicate standard error (n = 2). "No treatment" results were all 0 μg/L.

3.5 Discussion

Silver ions and free chlorine worked together synergistically in the inactivation of *E. coli* and total coliform bacteria. This synergism was true for different natural waters (pond in Virginia and stream in South Africa) with low doses of silver ion (10-15 μg/L) and free chlorine (100 μg/L as Cl_2) and even with different water chemistries (mainly lower vs. higher turbidity and organic matter). Although free chlorine disinfection effectiveness was negatively impacted by turbidity,

silver ion effectiveness did not appear to be affected. Additionally, the performance of the MadiDrop+ was improved significantly by the addition of low doses of free chlorine (50, 100, and 200 μg/L as Cl_2).

3.5.1 Synergism and bacteria reduction values

Patil et al. (2015) reported the synergistic inactivation of *E. coli* (seeded in well water with 0.31 NTU) by low doses of silver ion (50 μg/L) and free chlorine (200 μg/L). Silver plus chlorine, silver alone, and chlorine alone achieved 6.20-, 0-, and 4.44-log_{10} reduction of *E. coli*, respectively, after 30-min contact time. A different study by Straub et al. (1995) reported that monochloramine (1,000 μg/L) and cupric chloride (400 μg/L) in combination to inactivate *E. coli* in filtered well water (0.08 NTU) also produced a synergistic effect. The achieved *E. coli* log_{10} reductions by the combination of the chemicals after 30- and 60-min contact time were 1.80 and 4.90, respectively. Even though Straub et al. (1995) used different chemicals, their findings can be compared to our study in the sense that a chlorine compound and a metal compound produced the synergistic *E. coli* inactivation. In our study, the calculated sum of ratios (see Table 3.4) demonstrated that the inactivation of bacteria in natural waters (pond and upstream waters) by the combination of low doses of silver ion and free chlorine was synergistic. However, in contrast to Patil et al. (2015) and Straub et al. (1995), the *E. coli* log_{10} reduction obtained in our study was lower (≥1.44 in pond water and 0.87 in upstream water) at a longer contact time of 3-hr. These differences in *E. coli* reduction may have occurred for several reasons: lower doses of chemicals used (i.e., 10 μg/L Ag^+ and 100 μg/L Cl_2); lower limit of sensitivity for the tests or lower initial bacteria concentration; higher turbidity that may have protected bacteria from the effects of silver and chlorine; and, even though no data of DOC was reported in the other studies, as they used well water, the chlorine demand may not have been significant to interfere with bacteria inactivation.

Synergism was more pronounced (ratios moved away from 1; see Table 3.4) in the disinfection of less turbid water samples that also had a lower DOC concentration. Nevertheless, in the tests where these waters were used, the limit of sensitivity was reached at the 3-hr contact time. Because this may also mean that the limit of sensitivity was reached before this sampling

time, the following discussion was made only with the upstream or more turbid water disinfection results where the limit of sensitivity was not reached. In Table 3.4, the synergistic effect (due to the combination of silver ions and free chlorine) allows to reduce the concentration of the chemicals according to the mathematical model. For example, in the inactivation of *E. coli*, silver can be reduced approximately six-fold (from 58 to 10 μg/L Ag^+) and free chlorine approximately twofold (from 164 to 100 μg/L Cl_2), and by combining those low doses the target reduction of 0.87 was achieved.

Further evaluations with longer contact times are required to determine when the World Health Organization (WHO) drinking water guideline for *E. coli* of <1 MPN/100 mL (World Health Organization 2017) can be achieved. This way, new predicted silver and chlorine concentrations can be calculated, and then the reduced amount of the chemicals for different target reductions can be obtained. Moreover, by considering longer contact times, it can be evaluated if the synergistic effect changes as time increases. For instance, a possible strengthening of the synergistic interaction between free chlorine and monochloramine in the inactivation of *E. coli* (in 0.00263 M potassium phosphate buffer solution) as mean residence time increased was reported in another study (Kouame and Haas 1991).

3.5.2 MadiDrop+ combined with free chlorine

Overall, in our study, when the MadiDrop+ (MD) was combined with low doses of free chlorine, a substantial reduction of contact time was observed when achieving a bacteria reduction that is similar to that achieved by the MD alone at 8-hr. In other words, with the addition of 100 μg/L Cl_2, the *E. coli* $\log_{10}$ reduction was 1.1 at 1-hr, compared to 1.0 by the MD alone at 8-hr (see Figure 3.6). Moreover, with the addition of 200 μg/L Cl_2, a 2.2-total coliform bacteria (TCB) $\log_{10}$ reduction was achieved at 2-hr compared to the 2.0-$\log_{10}$ reduction obtained by the MD alone at 8-hr (see Figure 3.7).

Figure 3.6 shows that, at 30-min contact time, the *E. coli* $\log_{10}$ reduction in stream water by the different combinations of the MD tablet and chlorine was ≤0.59, and with the MD alone there was no reduction. Moreover, the average total residual silver in the treated water across all treatments at 30-min was 3 μg/L (see Figure 3.8). For comparison, Patil et al. (2015) tested the

inactivation of *E. coli* (seeded in well water) by a sequential treatment system that consisted of water first flowing through a rice husk ash and clay porous disc treated with nanosilver (releasing 10-70 μg/L Ag^+), and then entering another section containing free chlorine (200-500 μg/L Cl_2). In that study, the achieved log_{10} reduction after 30-min contact time was significantly higher than our results: ≥5.9 (limit of sensitivity of the assays) with the combined system, and 0.31-0.9 with silver alone. Additionally, the average residual silver was 20 μg/L (but it is not specified at what contact time these residuals were found). Analyzing longer contact times, in the other study, after 3-hr, the average *E. coli* log_{10} reduction with the silver treatment in well water was 4.23. In our study, at 4-hr with the MD alone in stream water, the *E. coli* log_{10} reduction was 0.3 (see Figure 3.6) and the total residual silver in the treated water was 8.1 μg/L (see Figure 3.8). The differences in *E. coli* reduction compared to our study may be explained by the difference in the water matrices, the higher chemical doses, and the additional physical barrier provided by the water passing through the disc in the other study. The flow of water may aid the release of silver from the disc, resulting in higher silver concentrations available for bacteria inactivation and higher residual silver in the treated water. In contrast, when the MD is used for water disinfection, the water does not flow through it because the tablet stays at the bottom of the storage container and silver is released to the water over time.

Further, at 8-hr contact time (recommended contact time to treat 10-L of water with the MD) with the MD alone, the *E. coli* log_{10} reduction was 1.0, and the TCB log_{10} reduction was 2.0 (see Figure 3.6 and Figure 3.7, respectively). However, in the MD plus chlorine treatments, the *E. coli* log_{10} reduction was between 1.8 and 2.3, and the TCB log_{10} reduction was between 1.8 and 2.8 (see Figure 3.6 and Figure 3.7, respectively). At this contact time, the average total residual silver in the treated water across all treatments was 8 μg/L (see Figure 3.8). The bacteria reductions from the MD plus chlorine treatments are similar to results from field studies where the MD alone was tested: 2.7-log_{10} reduction of *E. coli* at 8-hr in surface water (Singh et al. 2019), and after overnight treatment approximately 3-log_{10} reduction of TCB in different water sources (Hill et al. 2020; Kahler et al. 2016).

3.5.3 Impact of water quality in the disinfection

Due to the lower reactivity of silver ion and other chemicals, such as monochloramine, compared to free chlorine [standard potentials: 0.80 V for Ag^+, 0.74 V for NH_2Cl, 1.48 V for HOCl, and 0.81 V for OCl^- (Lide 2004)], it has been suggested that lower concentrations of free chlorine are required to achieve the same bacteria reduction levels as silver and monochloramine (Straub et al. 1995). First, it is necessary to consider the water matrix where the disinfection is performed. In other words, if the water is not highly contaminated (e.g., turbidity <1 NTU or no organic material present), the chlorine demand will be negligible. In consequence, chlorine may be mostly consumed in the inactivation of pathogens allowing to achieve higher bacteria $\log_{10}$ reductions such as >4. In contrast, in more contaminated waters, such as those used in our study (with turbidities of 4.82 and 11.9 NTU, and DOC of 1.80 and 2.49 mg/L, respectively), it is predicted that *E. coli* inactivation with free chlorine will require higher doses to achieve the same level of inactivation as silver ion, according to the mathematical model (see predicted concentrations in Table 3.4). The difference in reactivity between silver ion and free chlorine can lead to silver being a more selective oxidant and chlorine producing non-discriminative oxidations (Wigginton et al. 2012). This may be linked to high chlorine demands interfering with bacteria inactivation by low doses of chlorine; hence higher concentrations of chlorine will be needed. For this reason, the World Health Organization (2017) recommends keeping the turbidity of the collected water <1 NTU using filtration or other aids to support effective chlorination. However, where this is not practical, the WHO recommends trying to keep the turbidity <5 NTU and applying higher free chlorine doses or longer contact times. As a comparison, the WHO recommends applying 2,000 μg/L Cl_2 to water with <10 NTU. This concentration is 20 times the dose of 100 μg/L Cl_2 that we used in our study. Despite the effects of turbidity and DOC in chlorine performance, it is important to highlight the presence of the synergistic effect, as explained previously, when using low doses of silver ion and free chlorine in the disinfection of water with turbidity of 11.9 NTU.

Bacteria inactivation by 10 μg/L silver ion was not significantly affected by an increase in turbidity and DOC (from 4.82 NTU and 1.80 mg/L to 11.9 NTU and 2.49 mg/L; see Table 3.3). In other words, the *E. coli* $\log_{10}$ reduction was 0.69 in the water with lower turbidity and DOC, and

0.55 in the water with higher turbidity and DOC; when the dose of 100 μg/L Cl_2 was tested, *E. coli* log_{10} reductions were 1.18 and -0.03, respectively. For comparison, Sicairos-Ruelas et al. (2019) reported that the reduction of *E. coli* in demand-free 0.01 M phosphate buffer by 100 μg/L silver was not affected by the presence of organic matter at concentrations that completely inhibited the performance of 200 μg/L chlorine (i.e., 3 and 10 mg/L TOC from humic acids). After 3-hr contact time, the average *E. coli* log_{10} reduction when using chlorine was ≥5.70 (limit of sensitivity of the assay) with 0 mg/L TOC, 0.15 with 3 mg/L TOC, and 0.17 with 10 mg/L TOC. On the other hand, when silver was used, the average *E. coli* log_{10} reduction was 5.55 for all the tested TOC concentrations. Although we simultaneously tested an increase in turbidity and DOC in natural waters, and the silver, chlorine, and DOC concentrations in our study are lower than those evaluated by Sicairos-Ruelas et al. (2019), we can suggest that the increase in turbidity and DOC were enough to completely inhibit the chlorine disinfection effectiveness but not enough to affect silver effectiveness. However, it is important to recognize that the bioavailability of silver can be reduced by chelation or complexation with organic matter, sulfide, chloride, and phosphate (Sicairos-Ruelas et al. 2019).

3.6 Conclusions

There was a synergistic bacteria inactivation across multiple natural waters (pond and upstream waters) when using low doses of silver ion and free chlorine. The bacteria inactivation effectiveness by 10 μg/L silver ion was not affected by the presence of turbidity and organic matter at levels that inhibited the effectiveness of 100 μg/L free chlorine. Also, by combining two point-of-use technologies (MadiDrop+ tablet and free chlorine generated when dissolving an Aquatab) there was substantial reduction of contact time to achieve a specific bacteria reduction in a sample of stream water when compared with cases when one of the technologies was used alone. These results provide knowledge about the synergist effect in a realistic scenario with natural waters that contain inorganic and organic compounds, and other conditions that play important roles in the bacteria inactivation process.

3.7 References

Abad, F. X., R. M. Pinto, J. M. Diez, and A. Bosch. 1994. "Disinfection of Human Enteric Viruses in Water by Copper and Silver in Combination with Low Levels of Chlorine." *Applied and Environmental Microbiology*, 2377–2383. https://doi.org/10.1128/aem.60.7.2377-2383.1994

American Public Health Association, American Water Works Association, and Water Environment Federation. 2017. *Standard methods for the examination of water and wastewater*. 23rd ed. Washington, DC: American Public Health Association.

Barbeau, B., R. Desjardins, C. Mysore, and M. Prévost. 2005. "Impacts of water quality on chlorine and chlorine dioxide efficacy in natural waters." *Water Research*, 39 (10): 2024–2033. Elsevier Ltd. https://doi.org/10.1016/j.watres.2005.03.025

Berenbaum, M. C. 1989. "What is Synergy?" *Pharmacological Reviews*, 41 (2): 93–141.

Biurrun, A., L. Caballero, C. Pelaz, E. León, and A. Gago. 1999. "Treatment of a Legionella pneumophila-Colonized Water Distribution System Using Copper-Silver Ionization and Continuous Chlorination." *Infection Control & Hospital Epidemiology*, 20 (6): 426–428. https://doi.org/10.1086/501645

Chen, Y. S., Y. E. Lin, Y. C. Liu, W. K. Huang, H. Y. Shih, S. R. Wann, S. S. Lee, H. C. Tsai, C. H. Li, H. L. Chao, C. M. Ke, H. H. Lu, and C. L. Chang. 2008. "Efficacy of point-of-entry copper-silver ionisation system in eradicating Legionella pneumophila in a tropical tertiary care hospital: implications for hospitals contaminated with Legionella in both hot and cold water." *Journal of Hospital Infection*, 68 (2): 152–158. W.B. Saunders. https://doi.org/10.1016/j.jhin.2007.10.020

Clasen, T., and P. Edmondson. 2006. "Sodium dichloroisocyanurate (NaDCC) tablets as an alternative to sodium hypochlorite for the routine treatment of drinking water at the household level." *International Journal of Hygiene and Environmental Health*, 209 (2): 173–181. Elsevier GmbH. https://doi.org/10.1016/j.ijheh.2005.11.004

Cromeans, T. L., A. M. Kahler, and V. R. Hill. 2010. "Inactivation of adenoviruses, enteroviruses, and murine norovirus in water by free chlorine and monochloramine." *Applied and Environmental Microbiology*, 76 (4): 1028–1033. American Society for Microbiology. https://doi.org/10.1128/AEM.01342-09

Ehdaie, B., C. Krause, and J. A. Smith. 2014. "Porous ceramic tablet embedded with silver nanopatches for low-cost point-of-use water purification." *Environmental Science and Technology*, 48 (23): 13901–13908. American Chemical Society. https://doi.org/10.1021/es503534c

Ehdaie, B., C. T. Rento, V. Son, S. S. Turner, A. Samie, R. A. Dillingham, and J. A. Smith. 2017. "Evaluation of a Silver-Embedded Ceramic Tablet as a Primary and Secondary Point-of-Use Water Purification Technology in Limpopo Province, S. Africa." *PLoS One*, 12 (1). https://doi.org/10.1371/journal.pone.0169502

Ehdaie, B., Y.-H. Su, N. S. Swami, and J. A. Smith. 2020. "Protozoa and Virus Disinfection by Silver- and Copper-Embedded Ceramic Tablets for Water Purification." *Journal of Environmental Engineering*, 146 (4): 04020015. American Society of Civil Engineers (ASCE). https://doi.org/10.1061/(asce)ee.1943-7870.0001664

Ercumen, A., A. Mohd. Naser, L. Unicomb, B. F. Arnold, J. M. Colford Jr., and S. P. Luby. 2015. "Effects of Source- versus Household Contamination of Tubewell Water on Child Diarrhea in Rural Bangladesh: A Randomized Controlled Trial." *PLOS ONE*, (M. Ali, ed.), 10 (3): e0121907. Public Library of Science. https://doi.org/10.1371/journal.pone.0121907

Firth, J., V. Balraj, J. Muliyil, S. Roy, L. M. Rani, R. Chandresekhar, and G. Kang. 2010. "Point-of-Use Interventions to Decrease Contamination of Drinking Water: A Randomized, Controlled Pilot Study on Efficacy, Effectiveness, and Acceptability of Closed Containers, Moringa oleifera, and In-home Chlorination in Rural South India." *The American Journal of Tropical Medicine and Hygiene*, 82 (5): 759–765. The American Society of Tropical Medicine and Hygiene. https://doi.org/10.4269/AJTMH.2010.09-0206

Hill, C. L., K. McCain, M. E. Nyathi, J. N. Edokpayi, D. M. Kahler, D. J. Operario, D. D. J. Taylor, N. C. Wright, J. A. Smith, R. L. Guerrant, A. Samie, R. A. Dillingham, P. O. Bessong, and E. T. Rogawski McQuade. 2020. "Impact of low-cost point-of-use water treatment technologies on enteric infections and growth among children in limpopo, South Africa." *American Journal of Tropical Medicine and Hygiene*, 103 (4): 1405–1415. American Society of Tropical Medicine and Hygiene. https://doi.org/10.4269/ajtmh.20-0228

Jackson, K. N., D. M. Kahler, I. Kucharska, D. Rekosh, M.-L. Hammarskjold, and J. A. Smith. 2019. "Inactivation of MS2 Bacteriophage and Adenovirus with Silver and Copper in Solution and Embedded in Ceramic Water Filters." *Journal of Environmental Engineering*, 146 (3): 04019130. American Society of Civil Engineers. https://doi.org/10.1061/(ASCE)EE.1943-7870.0001634

Jackson, K. N., and J. A. Smith. 2018. "A New Method for the Deposition of Metallic Silver on Porous Ceramic Water Filters." *Journal of Nanotechnology*, 2018. https://doi.org/10.1155/2018/2573015

Jain, S., O. K. Sahanoon, E. Blanton, A. Schmitz, K. A. Wannemuehler, R. M. Hoekstra, and R. E. Quick. 2010. "Sodium dichloroisocyanurate tablets for routine treatment of household drinking water in periurban Ghana: A randomized controlled trial." *American Journal of Tropical Medicine and Hygiene*, 82 (1): 16–22. The American Society of Tropical Medicine and Hygiene. https://doi.org/10.4269/ajtmh.2010.08-0584

Kahler, D., N. Koermer, A. Reichl, A. Samie, and J. Smith. 2016. "Performance and Acceptance of Novel Silver-Impregnated Ceramic Cubes for Drinking Water Treatment in Two Field Sites: Limpopo Province, South Africa and Dodoma Region, Tanzania." *Water (Basel)*, 8 (3): 95. MDPI AG. https://doi.org/10.3390/w8030095

Kallman, E. N., V. A. Oyanedel-Craver, and J. A. Smith. 2011. "Ceramic Filters Impregnated with Silver Nanoparticles for Point-of-Use Water Treatment in Rural Guatemala." *Journal of Environmental Engineering*, 137 (6): 407–415. American Society of Civil Engineers. https://doi.org/10.1061/(ASCE)EE.1943-7870.0000330

Kannan, U., P. C. Sabumon, and S. M. Maliyekkal. 2021. "Development of an eco-friendly and reusable point-of-use disinfection system." *Process Safety and Environmental Protection*, 148: 104–113. Institution of Chemical Engineers. https://doi.org/10.1016/j.psep.2020.09.055

Kouame, Y., and C. N. Haas. 1991. "Inactivation of E. coli by combined action of free chlorine and monochloramine." *Water Research*, 25 (9): 1027–1032. Pergamon. https://doi.org/10.1016/0043-1354(91)90195-V

Lantagne, D. S., F. Cardinali, and B. C. Blount. 2010. "Disinfection by-product formation and mitigation strategies in point-of-use chlorination with sodium dichloroisocyanurate in Tanzania." *American Journal of Tropical Medicine and Hygiene*, 83 (1): 135–143. The American Society of Tropical Medicine and Hygiene. https://doi.org/10.4269/ajtmh.2010.09-0431

Lide, D. 2004. *CRC handbook of chemistry and physics*.

Liu, Z., J. E. Stout, L. Tedesco, M. Boldin, C. Hwang, W. F. Diven, and V. L. Yu. 1994. "Controlled Evaluation of Copper-Silver Ionization in Eradicating Legionella pneumophila from a Hospital

Water Distribution System." *The Journal of Infectious Diseases*, 169 (4): 919–922. https://doi.org/10.1093/infdis/169.4.919

Lucier, K. J., S. E. Dickson-Anderson, and C. J. Schuster-Wallace. 2017. "Effectiveness of silver and copper infused ceramic drinking water filters in reducing microbiological contaminants." *Journal of Water Supply: Research and Technology - AQUA*, 66 (7): 528–536. IWA Publishing. https://doi.org/10.2166/aqua.2017.028

Mellor, J. E., J. A. Smith, A. Samie, and R. A. Dillingham. 2013. "Coliform Sources and Mechanisms for Regrowth in Household Drinking Water in Limpopo, South Africa." *Journal of Environmental Engineering*, 139 (9): 1152–1161. American Society of Civil Engineers (ASCE). https://doi.org/10.1061/(asce)ee.1943-7870.0000722

Patil, R. A., D. Ahmad, S. B. Kausley, P. L. Balkunde, and C. P. Malhotra. 2015. "A compact point-of-use water purification cartridge for household use in developing countries." *Journal of Water and Health*, 13 (1): 91–102. IWA Publishing. https://doi.org/10.2166/wh.2014.111

Russel, M., H. B. Lowman, and T. Clackson. 2004. "Introduction to phage biology and phage display." In T. Clackson and H. B. Lowman (Ed.), *Phage Display: A practical approach*, 1–26. Oxford University Press.

Sicairos-Ruelas, E. E., C. P. Gerba, and K. R. Bright. 2019. "Efficacy of copper and silver as residual disinfectants in drinking water." *https://doi.org/10.1080/10934529.2018.1535160*, 54 (2): 146–155. Taylor & Francis. https://doi.org/10.1080/10934529.2018.1535160

Singh, R., C. Rento, V. Son, S. Turner, and J. A. Smith. 2019. "Optimization of Silver Ion Release from Silver-Ceramic Porous Media for Household Level Water Purification." *Water (Basel)*, 11 (4): 816. MDPI AG. https://doi.org/10.3390/w11040816

Straub, T. M., C. P. Gerba, X. Zhou, R. Price, and M. T. Yahya. 1995. "Synergistic inactivation of Escherichia coli and MS-2 coliphage by chloramine and cupric chloride." *Water Research*, 29 (3): 811–818. Pergamon. https://doi.org/10.1016/0043-1354(94)00213-Q

US EPA, O. 2020. "Drinking Water Regulations and Contaminants." Accessed January 4, 2021. https://www.epa.gov/sdwa/drinking-water-regulations-and-contaminants

World Health Organization. 2017. *Guidelines for drinking-water quality, 4th edition, incorporating the 1st addendum*. Geneva: WHO. https://www.who.int/publications/i/item/9789241549950

World Health Organization. 2022. "Drinking-water." Accessed March 9, 2023. https://www.who.int/news-room/fact-sheets/detail/drinking-water

Wigginton, K. R., B. M. Pecson, T. Sigstam, F. Bosshard, and T. Kohn. 2012. "Virus inactivation mechanisms: Impact of disinfectants on virus function and structural integrity." *Environmental Science and Technology*, 46 (21): 12069–12078. American Chemical Society. https://doi.org/10.1021/es3029473

Yahya, M. T., L. K. Landeen, M. C. Messina, S. M. Kutz, R. Schulze, and C. P. Gerba. 1990. "Disinfection of bacteria in water systems by using electrolytically generated copper:Silver and reduced levels of free chlorine." *Canadian Journal of Microbiology*, 36 (2): 109–116. https://doi.org/10.1139/m90-020

Chapter 4

Development of a chlorine-releasing material

4.1 Motivation and objectives

The combination of hypochlorous acid (HOCl) and hypochlorite ions (OCl^-) makes up free chlorine, with their proportions varying depending on the water pH. Both forms possess antimicrobial properties, but HOCl is a more potent microbial disinfectant than OCl^- (Sharma et al. 2017; Yahya et al. 1992). Antimicrobial capacity in both forms arises from oxidative chlorine or Cl^+, which acts as a strong oxidizer due to its high affinity for electrons. This property allows free chlorine to react easily with pathogens' external membrane components, leading to their damage and eventual death (Ahmed et al. 2011; Chen et al. 2017; Si et al. 2017; Timofeeva and Kleshcheva 2010). Consequently, free chlorine effectively eliminates bacteria and viruses in contaminated water (Jain et al. 2010).

According to Jain et al. (2010), chlorine tablets and bleach can release high amounts of free chlorine into water, which effectively kill bacteria and viruses. However, if the water being disinfected contains high levels of organic compounds in addition to pathogens, the free chlorine will react with the organics (this constitutes the chlorine demand) leading to a reduction in the amount of available chlorine for disinfection purposes. To address this issue, it is recommended to use higher quantities of free chlorine (up to 4,000 μg/L) when treating natural waters to ensure sufficient inactivation of microorganisms. The WHO has set a guideline value of 5,000 μg/L for free chlorine concentration in drinking water, which poses no significant health risks over a lifetime of consumption. But some individuals may detect the taste or odor of free chlorine in water at concentrations as low as 300 μg/L (World Health Organization 2017). These sensory changes can lead to user dissatisfaction and may even result in discontinuation of the disinfection treatment (Firth et al. 2010; Patil et al. 2015).

To mitigate these challenges, an alternative approach could be to disinfect water (containing low levels of organics or that has undergone pretreatment to reduce the organic content) with low chlorine concentrations (<300 μg/L) combined with low doses of silver (<100

μg/L). The secondary drinking water standard for silver is 100 μg/L (US EPA 2020). Several studies have investigated the combined use of low levels of metals (10-70 μg/L silver ions and 20-700 μg/L copper ions) and chlorine (50-1,000 μg/L) for water disinfection. The results of these studies suggest that the combinations provide higher reduction in pathogens than either metal or chlorine alone (Abad et al. 1994; Biurrun et al. 1999; Chen et al. 2008; Cromeans et al. 2010; Estrella-You and Smith 2022; Liu et al. 1994; Lucier et al. 2017; Straub et al. 1995; Yahya et al. 1990).

The main objective of our research was to develop a material that could release chlorine in water at levels effective for pathogen inactivation but below 300 μg/L to prevent any unpleasant taste in the disinfected water. In this chapter, we outline the development of this material, and the following chapter, we provide details on its potential for pathogen inactivation.

4.2 Background information

Chlorine-releasing materials include biocidal polymers that contain *N*-chloramines with one or more nitrogen-chlorine, N-Cl, covalent bonds (Dong et al. 2017; Hui and Debiemme-Chouvy 2013), which release oxidative chlorine or Cl^+. Regular usage of these materials for pathogen inactivation exhausts their chlorine content. However, their Cl^+ supply can be conveniently and repeatedly restored by exposing the materials to a Cl^+ donor compound such as bleach or a concentrated free chlorine solution (Bastarrachea et al. 2014; Chen et al. 2003; Hui and Debiemme-Chouvy 2013), as shown in Figure 4.1. This recharging property prolongs the shelf life of these chlorine-releasing materials.

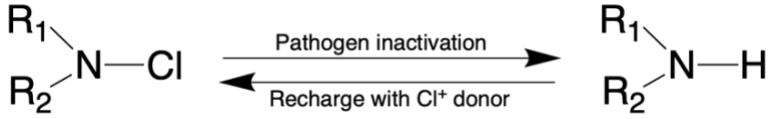

Figure 4.1: *N*-chloramines rechargeability property

To eliminate pathogens from water, *N*-chloramine containing materials are primarily utilized as filtration media (Coulliette et al. 2013; McLennan et al. 2009). These materials directly come in contact with microorganisms in the contaminated water as it flows through the filter,

resulting in their inactivation (Bastarrachea et al. 2014; Dong et al. 2017; Hui and Debiemme-Chouvy 2013). Alternatively, another mechanism of pathogen inactivation by *N*-chloramines can occur, which does not require direct contact. In this process, the Cl^+ dissociates from *N*-chloramines in water, forming free chlorine (Tsao et al. 1991) (see Figure 4.2). Moreover, studies in filtration settings have found that low concentrations of free chlorine (<200 μg/L) are present in the effluent water (Chen et al. 2003; Coulliette et al. 2013; Liang et al. 2005; McLennan et al. 2009).

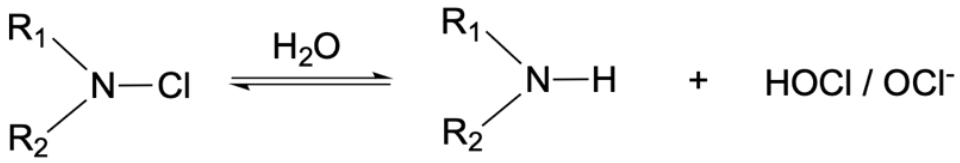

Figure 4.2: Cl+ dissociation from *N*-chloramines and free chlorine formation (Qian and Sun 2003; Williams et al. 1988)

Our first step towards developing a chlorine-releasing material was to select the optimal monomer. This monomer would have one or more amine groups with N-H bonds, where the H^+ could be substituted by Cl^+ upon exposure to a free chlorine solution to form *N*-chloramines. The second step involved determining the precursor, which is the material that would contain the amine monomer but would not yet have chlorine. We provide further details on these steps in the following subsections.

4.2.1 Monomer selection

According to Hui and Debiemme-Chouvy (2013), the stability of *N*-chloramines' N-Cl bond relies on the existence of an α-hydrogen adjacent to this bond, i.e., the hydrogen bound to the carbon adjacent to the nitrogen atom. When an α-hydrogen is present, dehydrochlorination or an elimination reaction with the α-hydrogen and the chlorine in the N-Cl bond occurs, resulting in the production of hydrochloric acid or HCl (see Figure 4.3). Conversely, in cyclic *N*-chloramines, the absence of an α-hydrogen prevents dehydrochlorination (Dong et al. 2017).

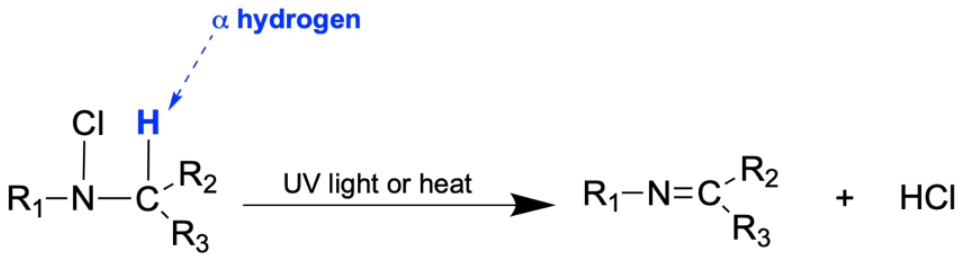

Figure 4.3: Dehydrochlorination in *N*-chloramines (Hui & Debiemme-Chouvy, 2013)

Among the most common cyclic *N*-chloramines are the ones that contain a hydantoin group, see Figure 4.4 (a). Several studies on this type of *N*-chloramines (Chen and Sun 2006; Demir et al. 2017; Liang et al. 2005; Panangala et al. 1997; Sun and Sun 2002; Zhao et al. 2014) have demonstrated their high antimicrobial efficacy in applications ranging from water treatment to coating in different materials. These studies have also examined their stability and have shown promising results including a shelf life of up to 6 months without being recharged.

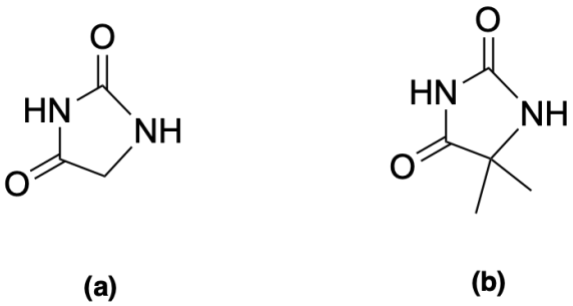

Figure 4.4: (a) Hydantoin. (b) 5,5-dimethylhydantoin or DMH.

Initially, our approach for developing a chlorine-releasing material involved exploring the possibility of modifying the surface of the MadiDrop+ to create a new product capable of simultaneously releasing silver and chlorine, leveraging their synergistic effect in pathogen inactivation. To pursue this strategy, we reviewed studies where cyclic *N*-chloramines had been added to ceramic like materials. We found that the 5,5-dimethylhydantoin or DMH group, which had been integrated into materials such as sand particles, polymers, and textiles (Chen and Sun 2006; Liang et al. 2005; Sun and Sun 2001, 2002; Zhao et al. 2014), was a suitable monomer for our purposes, see Figure 4.4 (b). Thus, we opted to utilize monomers that contained DMH.

4.2.2 Precursor selection

4.2.2.1 MadiDrop+ surface modification

Our initial strategy to achieving our goal of developing a chlorine-releasing material involved assessing the integration of monomers containing 5,5-dimethylhydantoin or DMH into the surface of the ceramic MadiDrop+ tablets. We followed a previously reported procedure by Liang et al. (2005) and outlined the fabrication process illustrated in Figure 4.5. First, we synthesized triethoxysilane-modified DMH monomers, compound 1 in Figure 4.5, and then attempted to coat the ceramic tablet with the monomers to obtain compound 2.

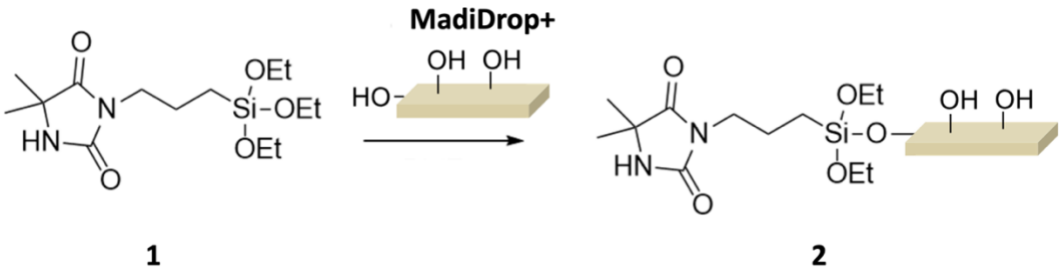

Figure 4.5: Proposed fabrication of silver ceramic tablets functionalized with *N*-chloramines

To characterize the coating of the ceramic, we intended to utilize Fourier Transform Infrared (FTIR) Spectroscopy to observe the appearance of vibrations of C-H and C=O bonds. According to Tran et al. (2013), FTIR spectra of silica or SiO_2 (a key component of ceramics) exhibit OH groups, as shown in Figure 4.6. However, the FTIR spectra of the MadiDrop+ (see Figure 4.7) showed that the tablet did not possess the OH groups necessary for bonding with the modified DMH. We suspect that these OH groups are lost during sintering, which occurs when the tablet is fired under high temperatures to reduce its porosity.

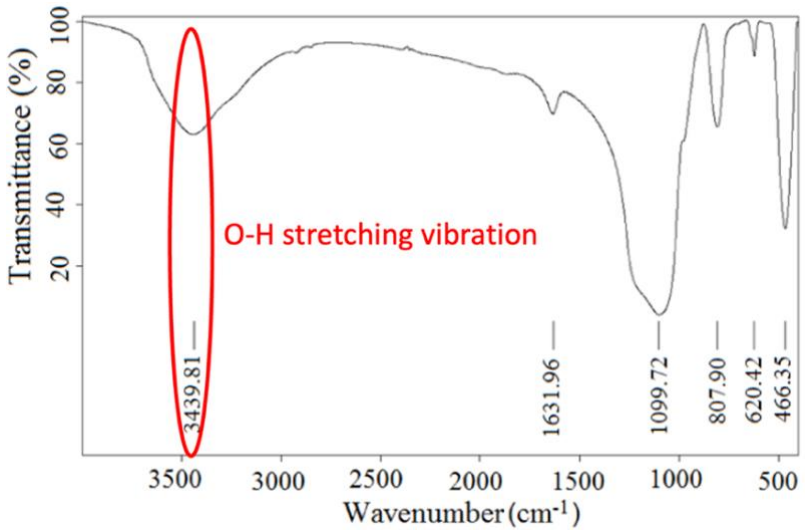

Figure 4.6: Fourier transform infrared (FTIR) spectrum of amorphous silica (SiO_2). Figure modified from (Tran et al. 2013)

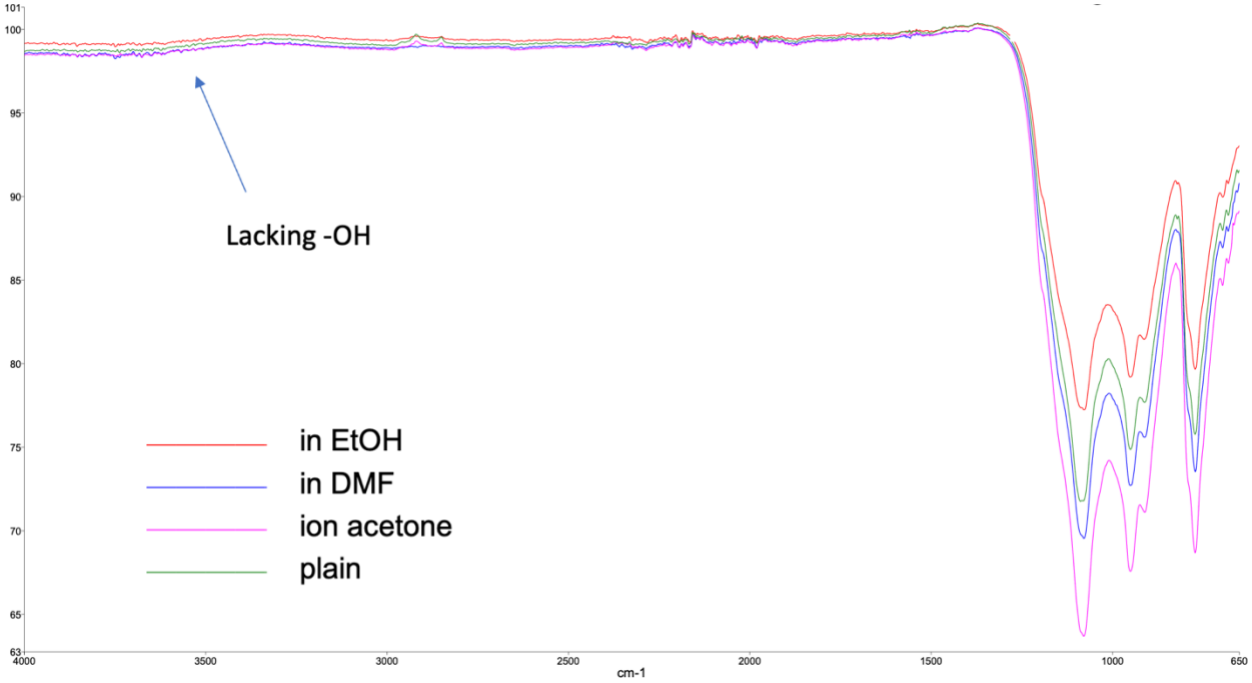

Figure 4.7: Fourier transform infrared (FTIR) spectra of the MadiDrop+

Upon finding this, we explored the option of introducing OH groups to the ceramic by conducting the coating process under basic conditions. However, the inability to confirm the presence of modified DMH monomers within the resulting MadiDrop+ directed us away from this first strategy. Consequently, we pursued the development of an alternative precursor, which is described in the following section.

4.2.2.2 Polymer gel

Our second strategy was to prepare polymer hydrogel pellets that would swell in water. These gels would be made up of crosslinked and porous polymer networks with DMH containing monomers. The porosity of the gels would facilitate contact between the water and monomers, thereby promoting the transport or diffusion of free chlorine into and out of the network to and from the bulk solution.

Sun and Sun (2001) developed biocidal polymers with DMH containing monomers known as 3-(4'-vinylbenzyl)-5,5-dimethylhydantoin or VBDMH. These monomers, instead of having a triethoxysilane group (compound 1 in Figure 4.5), have a vinylbenzyl group in addition to the DMH group (see Figure 4.8). To prepare the polymer gels, we crosslinked the VBDMH monomers with a hydrophilic compound to increase contact between water and N-Cl bonds in the chlorinated gels. To achieve this, we used poly(ethylene glycol) methacrylate or PEGMA as the hydrophilic chemical and 1,6-hexanediol diacrylate or HDDA as the crosslinker (see Figure 4.9).

The final step in fabricating the chlorine-releasing polymer gels is the precursor chlorination. The precursor or polymer gel contains amine or N-H groups with H^+ that can be substituted with Cl^+ after exposure to a free chlorine solution or a Cl^+ donor solution (e.g., aqueous sodium hypochlorite). Once the gels are chlorinated, they are ready for water disinfection (see Figure 4.10).

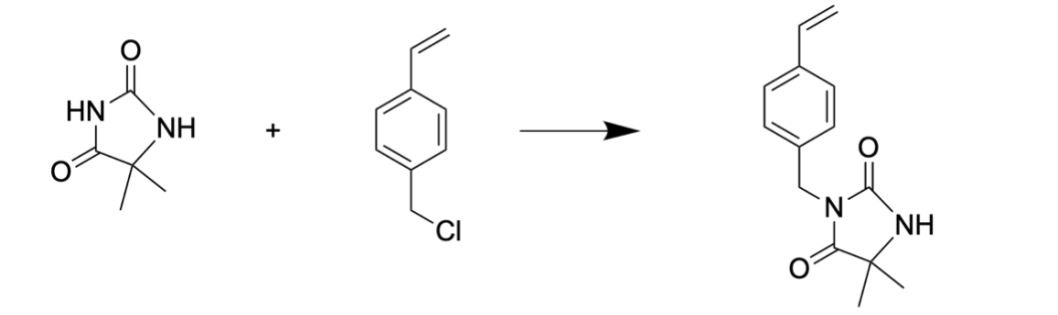

Figure 4.8: Synthesis of 3-(4'-vinylbenzyl)-5,5-dimethylhydantion or VBDMH monomer (a simplified reaction is shown here)

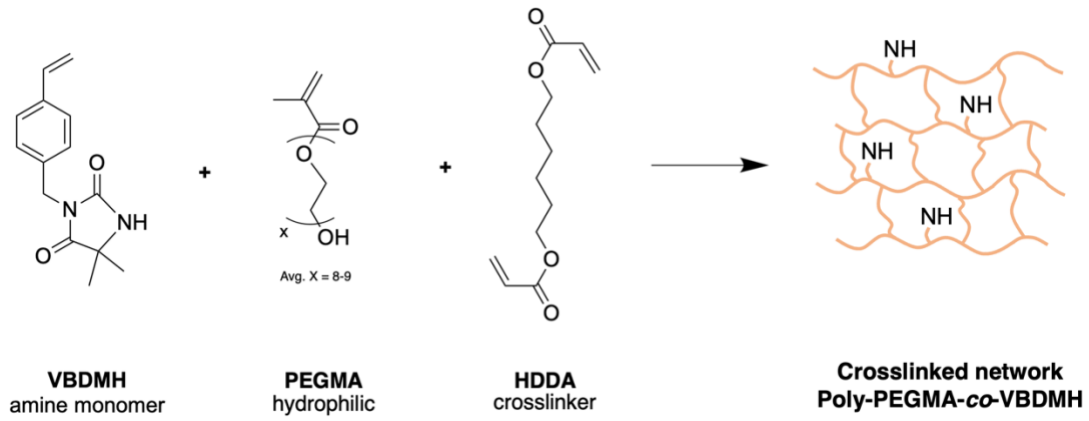

Figure 4.9: Synthesis of amine containing porous networks or polymer gels
(a simplified reaction is shown here)

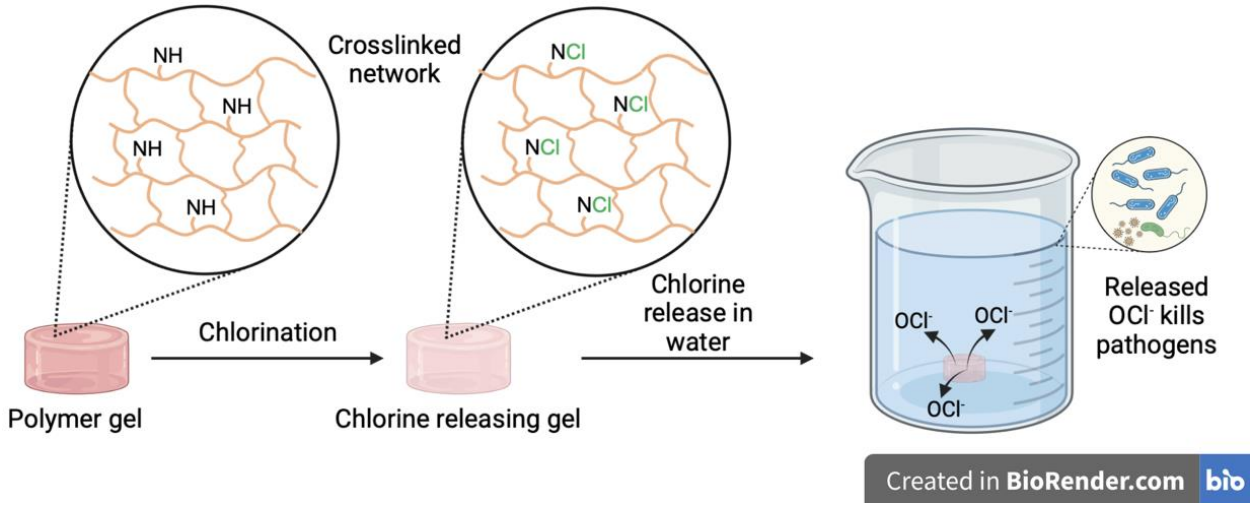

Figure 4.10: Preparation of chlorine-releasing porous networks or chlorinated polymer gels

4.3 Methods

4.3.1 Synthesis of 3-(4′-vinylbenzyl)-5,5-dimethylhydantion or VBDMH monomers

Based on prior work conducted by Sun and Sun (2001), VBDMH was synthesized from 4-vinylbenzyl chloride (VBC) and 5,5-dimethylhydantoin (DMH) in the presence of potassium hydroxide (KOH). First, a mixture of 12.8 g (0.1 mol) DMH (Acros Organics, Pittsburgh, PA), 5.6 g (0.1 mol) KOH (VWR, Radnor, PA), and 80 mL ethyl alcohol (Sigma-Aldrich, St. Louis, MO) was stirred at 60°C until the solution became clear. Then, the mixture was combined with a

solution of 14 mL (0.1 mol) VBC (Sigma-Aldrich) in 50 mL methanol (Sigma-Aldrich) and stirred at 65°C overnight. After cooled, unreacted DMH, VBC, and the potassium chloride produced in the reaction were removed and VBDMH was recrystallized (adding 200 mL methanol first and then slowly 300 mL water), vacuum filtered, and vacuum dried. In the recrystallization step, methanol was added first to the cooled reaction mixture to dissolve the VBDMH monomer. Then, water was added to selectively precipitate or crystallize the monomer out of solution while keeping the VBC and other impurities dissolved.

The purities of the crude and purified monomer were compared from high performance liquid chromatography (HPLC) traces. The structure of the VBDMH monomers were confirmed using ^{1}H nuclear magnetic resonance (NMR) spectroscopy (^{1}H NMR, 500 MHz Varian INOVA-500 NMR spectrometer with deuterated DMSO). HPLC and NMR analyses were performed by Israt Jahan Duti.

4.3.2 Synthesis of amine containing crosslinked networks or polymer gels

Hydrophilic crosslinked polymer networks or polymer gel pellets were synthesized through UV light photo-crosslinking of hydrophilic poly(ethylene glycol) methacrylate (PEGMA), purified VBDMH monomers, and 1,6-hexanediol diacrylate (HDDA) crosslinker in *N*,*N*-dimethylformamide (DMF), using 2-hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone photo-initiator. Gels with the following VBDMH monomer masses were prepared: 0.2, 0.5, 1, and 3 g. For this, a mixture of 3 g (1 mol eq.) VBDMH, 0.28 mL (0.1 mol eq.) HDDA (Sigma-Aldrich), 2.8 mL (0.5 mol eq.) PEGMA (Sigma-Aldrich), and the photo-initiator (Sigma-Aldrich) in the amount of 1% of the total mass was dissolved in 10 mL DMF (Thermo Fisher Scientific, Waltham, MA) in a 25 mL glass vial (proportional amounts of the reagents were mixed to prepare the gels with less VBDMH). The vial was introduced in a crosslinker (Analytik Jena UVP Crosslinker, CL-3000L) and exposed to 365 nm UV light for 4-hr.

After removing the gels from the vials, they were washed by soaking them in five consecutive mixtures of methanol and deionized water for a few hours to prevent any unreacted chemicals from being released in the water where the bacteria inactivation tests were later performed. 150 mL of each of the following washes per gel theoretically containing 1 g

VBDMH were used: (i) and (ii) with 100% methanol; (iii) and (iv) with 50% methanol; and (v) with 33% methanol. The supernatants of the washes were analyzed by high performance liquid chromatography (HPLC) to confirm removal of unreacted material. HPLC was performed by Israt Jahan Duti.

4.3.2.1 Styrene controls

To provide evidence that any disinfection efficacy does not come from just free chlorine that gets trapped in the gels during chlorination and later comes out of the gels, styrene control gels were synthesized. To synthesize these gels, chlorine binding VBDMH monomers were replaced by styrene (Sigma-Aldrich) which has a similar chemical structure with VBDMH but does not contain amines. Same mol eq. were used but less DMF (about half from what was used for the VBDMH gels) was added to account for the difference in molecular weight between VBDMH and styrene. The styrene gels were washed and chlorinated the same way as the VBDMH gels.

4.3.3 Loading gels with chlorine

Exposing the VBDMH monomers in the polymer gels to a sodium hypochlorite (NaOCl) solution, a Cl^+ donor, should convert the amines to chloramines. A 0.25% or 2,500 mg/L Cl_2 solution was prepared from a 12% stock NaOCl solution (Sigma-Aldrich) and reverse osmosis water. To adjust the pH of the diluted NaOCl solution to ~7, a 5% acetic acid (Sigma-Aldrich) solution was used (Miner, 2006). This pH adjustment shifts the free chlorine equilibrium toward HOCl, which is the more reactive species.

The oxidative chlorine (Cl^+ or Cl_2) concentration in the diluted NaOCl solution was measured through a standard iodometric/thiosulfate titration (Zhao et al. 2014). Between 2-5 mL of sample was added to 50 mL of 0.04 N sulfuric acid (Fisher Chemical, Pittsburgh, PA) solution. After addition of 0.20 g of potassium iodide (Fisher Chemical) and stirring, the solution turned yellow-brownish due to the oxidation of iodide to iodine by Cl^+ in acidic medium. Titration was started by adding aliquots of 0.01 N of sodium thiosulfate (Fisher Chemical) solution until the yellow color began to fade. Then, 0.40 mL of 0.50 % of starch (Thermo Fisher Scientific) water solution, as an indicator, was added (starch formed a dark blue complex with iodine), and the

titration was continued adding sodium thiosulfate solution until the blue color disappeared at the end point. Chlorine in the sample was calculated using the following equations:

$$Cl^{+}\left(\text{in } \frac{\text{mg}}{\text{L}}\right) = \left(N \times V_{\text{T}} \times \frac{35.45}{2 \times V}\right) \times 10^{6} \quad (1)$$

$$Cl_2\left(\text{in } \frac{\text{mg}}{\text{L}}\right) = 2 \times \left[Cl^{+}\left(\text{in } \frac{\text{mg}}{\text{L}}\right)\right] \quad (2)$$

where N and V_T are the normality (eqv/L) and the total volume (L) of the sodium thiosulfate consumed in the titration, respectively, and V is the sample volume (mL). The detection limit of this analysis (354.5 μg/L Cl_2) was determined considering the sensitivity of the burette used to add the thiosulfate solution (volume increments of 0.1 mL) and the sample volume added (we chose a high volume, 100 mL, to determine this limit).

Three gels (each theoretically containing 1 g VBDMH) were exposed to 400 mL of the pH ~7 0.25% Cl_2 for 48-hr to load them with chlorine. Then, the gels were soaked in 750 mL deionized water for 30 min to remove any unbound chlorine. Finally, the gels were kept in a closed glass container to avoid changes in their mechanical integrity or breakage after they get dry under ambient conditions. To quantify the Cl^+ loaded into the gels the following method was developed.

4.3.3.1 Quantification of loaded chlorine in the gels

To estimate the Cl^+ loaded into the polymer gels we developed a method considering the natural decrease of Cl^+ concentration in the sodium hypochlorite solution because we used extended chlorination times (>12 hours). Hypochlorite solutions over time decompose and their concentration of free chlorine (and in consequence Cl^+) decreases due to chlorate, perchlorate, and chlorite ion formation (World Health Organization 2017). Therefore, we estimated that the amount of Cl^+ in the gels was the difference between 'remaining Cl^+ in the solution with *no* gel' and 'remaining Cl^+ in the solution with the gel' (see Figure 4.11).

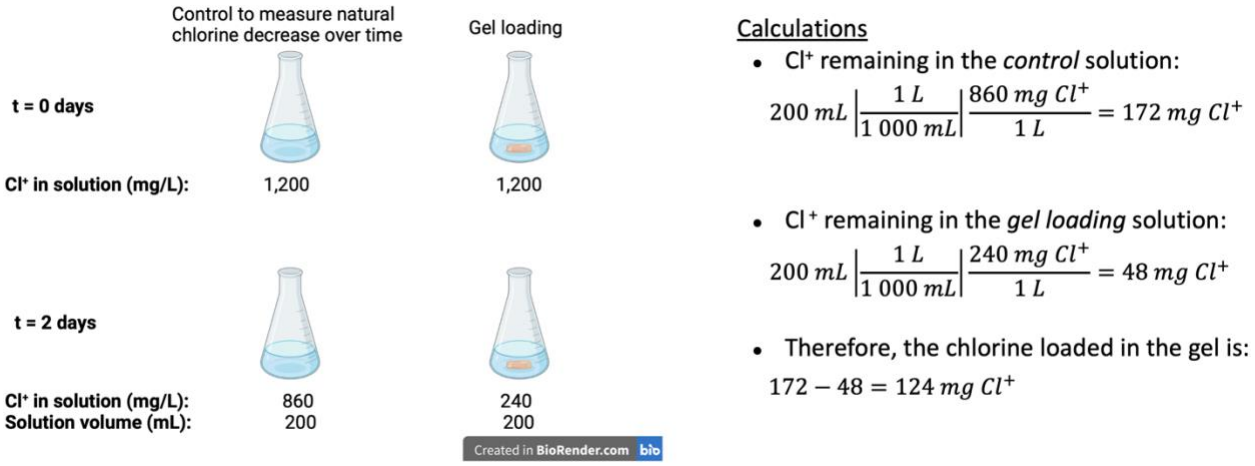

Figure 4.11: Schematic and calculations example of the method developed to examine loading of Cl^+ into polymer gels

4.3.4 Chlorine releasing test

Once we had the chlorinated gels ready, we evaluated the release of chlorine from a gel submerged in synthetic groundwater (SGW), which is a solution that contains salts to simulate groundwater. The preparation of this solution has been described elsewhere (Singh et al. 2019). Briefly, 9 L deionized water, 0.60 g $MgSO_4$ (Alfa Aesar, Tewksbury, MA), 0.96 g $NaHCO_3$ (Fisher Chemical), and 0.04 g KCl (Fisher Chemical) were combined and mixed. In a separate container, 0.47 g $CaSO_4$ (Alfa Aesar) was mixed with 1 L deionized water until the calcium sulfate was completely dissolved. Finally, the two solutions were combined and mixed again.

In the chlorine release test, a gel, theoretically containing 0.5 g VBDMH monomer or 72.5 mg Cl^+, was added to 15 mL SGW. A water sample was taken at 8-hr contact time to analyze for free chlorine. The concentration of free chlorine (as μg/L Cl_2) in the sample was determined using the colorimetric method Hach 10241 (range 40 – 4,500 μg/L Cl_2) with a reagent set (Freechlor F Reagent and Monochlor F Reagent; Hach, Loveland, CO) and a spectrophotometer (DR6000, Hach) with a measuring wavelength of 655 nm.

4.4 Results and discussion

4.4.1 Synthesized VBDMH monomers

Figure 4.12 displays the VBDMH monomer, which was obtained after recrystallization and vacuum filtration. We achieved an average yield of 64% for the monomer. The final purity of the monomer was 89% which was determined by high performance liquid chromatography (HPLC). In Figure 4.13, the crude and purified monomer chromatograms are shown. The crude monomer chromatogram shows the presence of impurities. On the other hand, the purified monomer chromatogram contains only the VBDMH peak without any impurities, indicating the removal of essentially all the VBC while still recovering about 89% of monomer.

Figure 4.12: Synthesized 3-(4′-vinylbenzyl)-5,5-dimethylhydantoin or VBDMH monomers

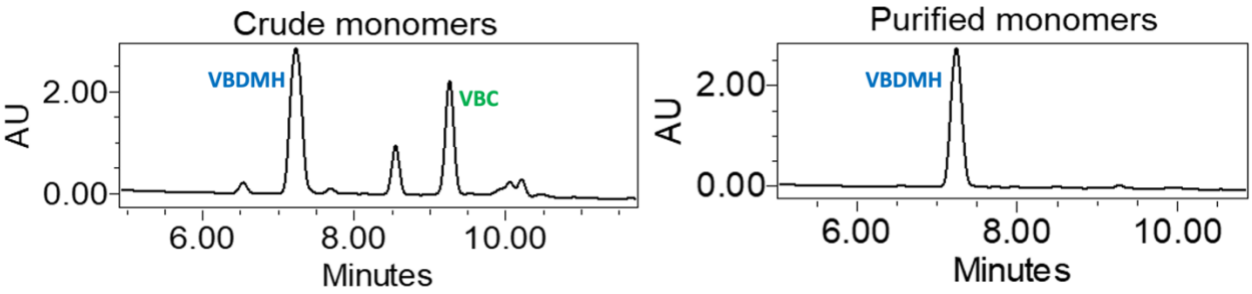

Figure 4.13: High performance liquid chromatography (HPLC) traces of the crude and purified VBDMH monomers

The VBDMH monomer structure was confirmed by ^{1}H NMR. The spectra (see Figure 4.14) show good agreement between the reagents (DMH and VBC) and the produced VBDMH monomer, which means that the reaction was occurring successfully. Chemical shift changes occurred for protons *e* (peak at ~7.5 ppm) and *f* (peak at ~4.75 ppm) in the benzyl ring region, indicative of conformational changes due to the loss of Cl from VBC. In the VBDMH spectrum, the disappearance of the signal corresponding to proton *a* (peak at ~10.5 ppm) from DMH and appearance of protons *g* (peak at ~10.5 ppm) and *h* (peak at ~1.25 ppm) demonstrates successful VBC modification with DMH. We also observed that the VBC peaks decreased in intensity as the VBDMH monomer formed.

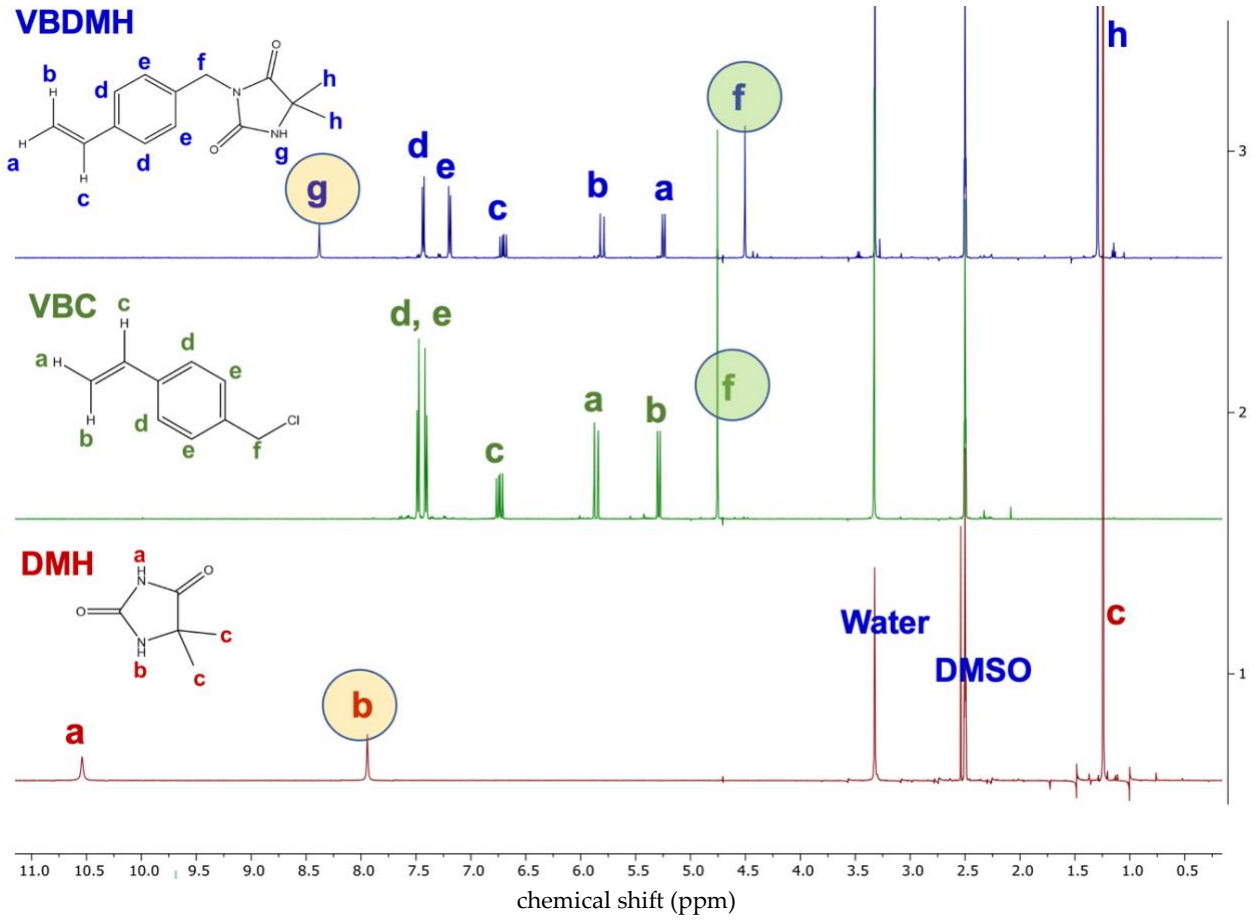

Figure 4.14: ^{1}H-NMR spectra of VBDMH, VBC, and DMH

4.4.2 Synthesized polymer gels

To tune water uptake and therefore loading and release of chlorine, molar ratios of VBDMH:PEGMA (amine monomer : hydrophilic group) were varied. We tested the following molar ratios 1:0, 1:0.5, 1:1, and 1:5 (see Table 4.1). Due to the increase in the amount of PEGMA, the crosslinker HDDA was proportionally increased for the last two formulations and the solvent DMF was also increased for the last formulation.

Table 4.1: Polymer gel formulations where the molar ratio VBDMH:PEGMA was varied

	Molar ratio			
VBDMH:PEGMA (mol eq.)	1:0	1:0.5	1:1	1:5
VBDMH:HDDA (mol eq.)	1:0.1	1:0.1	1:0.2	1:0.5
	Reagent amount			
VBDMH (g)	0.5			
PEGMA (mL)	0	0.47	0.93	4.64
HDDA (μL)	46	46	92	229
Photo-initiator, 1% total mass (mg)	5.5	10.6	16.2	58.5
DMF (mL)	1.67	1.67	1.67	3.33

Upon preparing the different formulations, we observed that increasing the amount of PEGMA generally reduced the stability of the gel. Consequently, the mechanical integrity of the gels limited us to a composition range of around 1:0.5 (see Figure 4.15). Hence, we selected the formulation consisting of 1:0.5 VBDMH:PEGMA, 1:0.1 VBDMH:HDDA, 1% total mass for the photo-initiator, and 1.67 mL DMF per 0.5 g VBDMH to prepare the precursors or polymer gels. Figure 4.16 shows crosslinked gels ready to be washed (to remove unreacted compounds) and then chlorinated.

Figure 4.15: Polymer gels with varying VBDMH:PEGMA molar ratios (see formulations in Table 4.1)

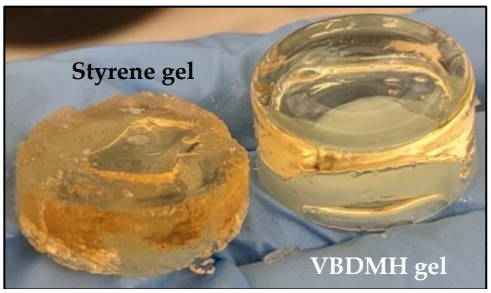

Figure 4.16: Gels with 1:0.5 VBDMH:PEGMA or styrene:PEGMA molar ratio after crosslinking. The diameter of each gel is ~2 cm, like the diameter of a nickel coin. Gel on the right has 1 g or 4 mmol VBDMH. Gel on the left has 0.43 g or 4 mmol styrene.

Removal of unreacted material and solvent from the synthesized polymer gels was analyzed by HPLC (see Figure 4.17). As we showed the VBDMH monomer was pure (see Figure 4.13), the washing procedure of the gels using five methanol/water mixtures successfully eliminated unreacted compounds. Figure 4.17 shows that the initial supernatant washes contain solvent (DMF) and unreacted VBDMH and PEGMA, but the last wash (purple line) shows removal of these reagents. The peaks in the HPLC chromatographs were identified by running the reagents in water or methanol (VBDMH is insoluble in water).

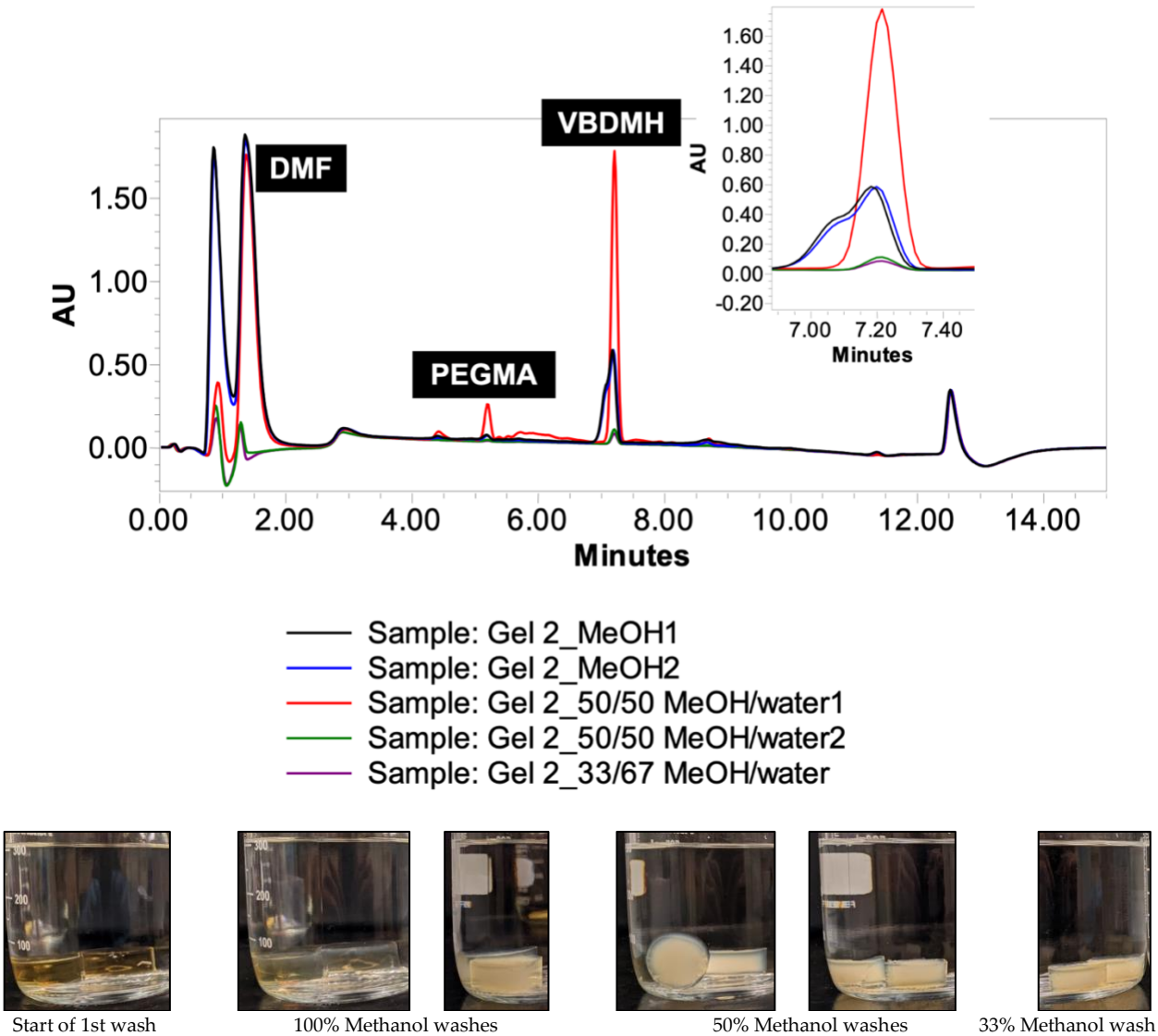

Figure 4.17: High performance liquid chromatography (HPLC) traces of the supernatants from the gel washes

4.4.3 Chlorine loading tests

We tested different loading or chlorination times (12, 24, and 48 hours) to determine how the Cl^+ content correlates with loading time. Upon chlorination, the gels changed in appearance, becoming stiffer and darker (see Figure 4.18). The Cl^+ content (mg of Cl^+) in the polymer gels increased with increasing chlorination time (see Figure 4.19), but after 48 hours of loading, the average Cl^+ content was ~65% of the theoretical. This result is reasonable because the theoretical Cl^+ content assumes 100% incorporation of VBDMH monomer into the gel, but we showed

previously that unreacted VBDMH was removed during the gel washes (see Figure 4.17). Based on these findings, we chose to proceed with 48-hr chlorination of synthesized gels to increase their Cl^+ content without requiring several days of loading.

Before chlorination 12-hr chlorination

Figure 4.18: Change in appearance of the gels after chlorination

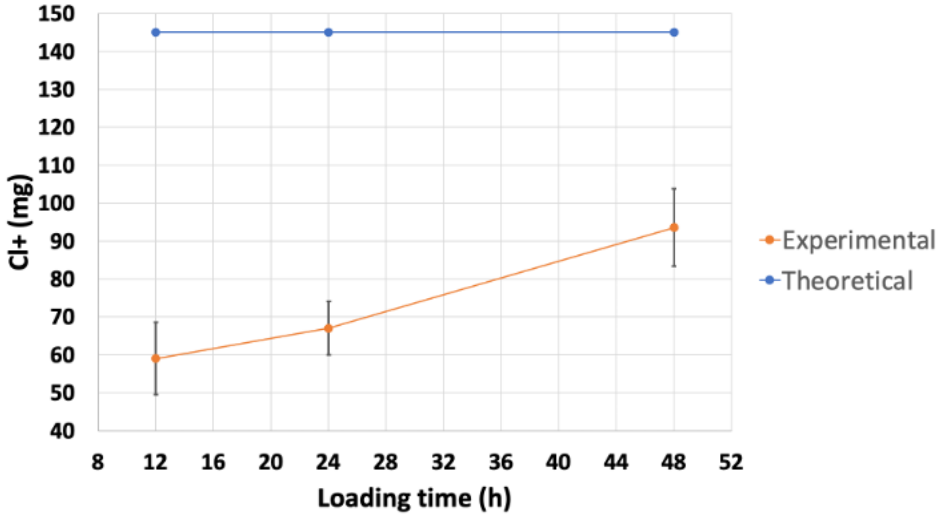

Figure 4.19: Calculated Cl^+ content in gels (theoretically containing 1 g VBDMH) with respect to loading time. NaOCl solution at t=0: 300 mL of 2,464 mg Cl_2 or 1,232 mg Cl^+ for each 1 g VBDMH monomer gel. Error bars indicate standard error (n=2).

4.4.4 Chlorine release test

The release of chlorine from a chlorinated gel in SGW was evaluated. We found that the concentration of the released chlorine was below the detection limit (40 µg/L Cl_2) of our spectroscopic method Hach 10241. However, we confirmed the presence of Cl^+ in the polymer gel by staining it with iodide (Zhao et al. 2014). Figure 4.20 shows that the chlorinated gel

stained in the presence of iodide in acidic medium (iodide reacts with chlorine forming yellow-brownish iodine), while the non-chlorinated gel did not stain.

As stated earlier in this chapter, Cl^+ dissociates from *N*-chloramines in water and forms free chlorine (Tsao et al. 1991). Although we could not detect the released chlorine from the gels, we hypothesize that chlorine in concentrations below 40 μg/L is released in water, and it would be available for pathogen inactivation. Chapter 5 outlines the series of experiments we conducted to examine this hypothesis.

Figure 4.20: Iodide staining test for a chlorinated gel (left) and a non-chlorinated gel (right)

4.5 Conclusions

We successfully synthesized chlorine containing polymer gels through UV crosslinking. We tested different gel formulations and selected the most mechanically stable gel formulation. Before loading chlorine into the gels, thorough washes were conducted to remove unreacted chemicals and avoid their release in water. The presence of chlorine in the gel was confirmed by iodide staining. While we were not able to measure the amount of chlorine released by the polymer gels in water, we hypothesize that these chlorine concentrations are sufficient for pathogen inactivation.

4.6 References

Abad, F. X., R. M. Pinto, J. M. Diez, and A. Bosch. 1994. "Disinfection of Human Enteric Viruses in Water by Copper and Silver in Combination with Low Levels of Chlorine." *Appl Environ Microbiol*, 2377–2383. https://doi.org/10.1128/aem.60.7.2377-2383.1994

Ahmed, A. E.-S. I., G. Cavalli, M. E. Bushell, J. N. Wardell, S. Pedley, K. Charles, and J. N. Hay. 2011. "New approach to produce water free of bacteria, viruses, and halogens in a recyclable system." *Appl Environ Microbiol*, 77 (3): 847–853. American Society for Microbiology. https://doi.org/10.1128/AEM.01645-10

Bastarrachea, L. J., L. A. McLandsborough, M. Peleg, and J. M. Goddard. 2014. "Antimicrobial N-halamine Modified Polyethylene: Characterization, Biocidal Efficacy, Regeneration, and Stability." *J Food Sci*, 79 (5): E887–E897. Blackwell Publishing Inc. https://doi.org/10.1111/1750-3841.12455

Biurrun, A., L. Caballero, C. Pelaz, E. León, and A. Gago. 1999. "Treatment of a Legionella pneumophila-Colonized Water Distribution System Using Copper-Silver Ionization and Continuous Chlorination." *Infect Control Hosp Epidemiol*, 20 (6): 426–428. https://doi.org/10.1086/501645

Chen, A., H. Peng, I. Blakey, and A. K. Whittaker. 2017. "Biocidal Polymers: A Mechanistic Overview." *Polymer Reviews*. Taylor and Francis Inc.

Chen, Y. S., Y. E. Lin, Y. C. Liu, W. K. Huang, H. Y. Shih, S. R. Wann, S. S. Lee, H. C. Tsai, C. H. Li, H. L. Chao, C. M. Ke, H. H. Lu, and C. L. Chang. 2008. "Efficacy of point-of-entry copper-silver ionisation system in eradicating Legionella pneumophila in a tropical tertiary care hospital: implications for hospitals contaminated with Legionella in both hot and cold water." *Journal of Hospital Infection*, 68 (2): 152–158. W.B. Saunders. https://doi.org/10.1016/j.jhin.2007.10.020

Chen, Y., S. D. Worley, J. Kim, C.-I. Wei, T.-Y. Chen, J. I. Santiago, J. F. Williams, and G. Sun. 2003. "Biocidal Poly(styrenehydantoin) Beads for Disinfection of Water." *Ind Eng Chem Res*, 42: 280–284. American Chemical Society. https://doi.org/10.1021/IE020266+

Chen, Z., and Y. Sun. 2006. "N-Halamine-Based Antimicrobial Additives for Polymers: Preparation, Characterization, and Antimicrobial Activity." *Ind Eng Chem Res*, 45 (8): 2634–2640. American Chemical Society . https://doi.org/10.1021/IE060088A

Coulliette, A. D., K. S. Enger, M. H. Weir, and J. B. Rose. 2013. "Risk reduction assessment of waterborne Salmonella and Vibrio by a chlorine contact disinfectant point-of-use device." *Int J Hyg Environ Health*, 216 (3): 355–361. https://doi.org/10.1016/j.ijheh.2012.08.007

Cromeans, T. L., A. M. Kahler, and V. R. Hill. 2010. "Inactivation of adenoviruses, enteroviruses, and murine norovirus in water by free chlorine and monochloramine." *Appl Environ Microbiol*, 76 (4): 1028–1033. American Society for Microbiology. https://doi.org/10.1128/AEM.01342-09

Demir, B., R. M. Broughton, M. Qiao, T. S. Huang, and S. D. Worley. 2017. "N-Halamine Biocidal Materials with Superior Antimicrobial Efficacies for Wound Dressings." *Molecules 2017, Vol. 22, Page 1582*, 22 (10): 1582. Multidisciplinary Digital Publishing Institute. https://doi.org/10.3390/MOLECULES22101582

Dong, A., Y. J. Wang, Y. Gao, T. Gao, and G. Gao. 2017. "Chemical Insights into Antibacterial N-Halamines." *Chem Rev*. American Chemical Society.

Estrella-You, A., and J. A. Smith. 2022. "Synergistic Bacterial Inactivation by Silver Ions and Free Chlorine in Natural Waters." *Journal of Environmental Engineering*, 148 (11): 04022072. American Society of Civil Engineers. https://doi.org/10.1061/(ASCE)EE.1943-7870.0002053

Firth, J., V. Balraj, J. Muliyil, S. Roy, L. M. Rani, R. Chandresekhar, and G. Kang. 2010. "Point-of-Use Interventions to Decrease Contamination of Drinking Water: A Randomized, Controlled Pilot Study on Efficacy, Effectiveness, and Acceptability of Closed Containers, Moringa oleifera, and In-home Chlorination in Rural South India." *Am J Trop Med Hyg*, 82 (5): 759–765. The American Society of Tropical Medicine and Hygiene. https://doi.org/10.4269/AJTMH.2010.09-0206

Hui, F., and C. Debiemme-Chouvy. 2013. "Antimicrobial N-halamine polymers and coatings: A review of their synthesis, characterization, and applications." *Biomacromolecules*. American Chemical Society.

Jain, S., O. K. Sahanoon, E. Blanton, A. Schmitz, K. A. Wannemuehler, R. M. Hoekstra, and R. E. Quick. 2010. "Sodium dichloroisocyanurate tablets for routine treatment of household drinking water in periurban Ghana: A randomized controlled trial." *American Journal of Tropical Medicine and Hygiene*, 82 (1): 16–22. The American Society of Tropical Medicine and Hygiene. https://doi.org/10.4269/ajtmh.2010.08-0584

Liang, J., R. Wu, T. S. Huang, and S. D. Worley. 2005. "Polymerization of a hydantoinylsiloxane on particles of silicon dioxide to produce a biocidal sand." *J Appl Polym Sci*, 97 (3): 1161–1166. John Wiley & Sons, Ltd. https://doi.org/10.1002/app.21814

Liu, Z., J. E. Stout, L. Tedesco, M. Boldin, C. Hwang, W. F. Diven, and V. L. Yu. 1994. "Controlled Evaluation of Copper-Silver Ionization in Eradicating Legionella pneumophila from a Hospital Water Distribution System." *J Infect Dis*, 169 (4): 919–922.

Lucier, K. J., S. E. Dickson-Anderson, and C. J. Schuster-Wallace. 2017. "Effectiveness of silver and copper infused ceramic drinking water filters in reducing microbiological contaminants." *Journal of Water Supply: Research and Technology - AQUA*, 66 (7): 528–536. IWA Publishing. https://doi.org/10.2166/aqua.2017.028

McLennan, S. D., L. A. Peterson, and J. B. Rose. 2009. "Comparison of point-of-use technologies for emergency disinfection of sewage-contaminated drinking water." *Appl Environ Microbiol*, 75 (22): 7283–7286. American Society for Microbiology. https://doi.org/10.1128/AEM.00968-09

Miner, N. 2006. "Effective use of bleach as an antiseptic and disinfectant." *Microbe Magazine*, 1 (6): 257. American Society for Microbiology. https://doi.org/10.1128/microbe.1.257.1

Panangala, V. S., L. Liu, G. Sun, S. D. Worley, and A. Mitra. 1997. "Inactivation of rotavirus by new polymeric water disinfectants." *J Virol Methods*, 66 (2): 263–268. https://doi.org/10.1016/S0166-0934(97)00062-1

Patil, R. A., D. Ahmad, S. B. Kausley, P. L. Balkunde, and C. P. Malhotra. 2015. "A compact point-of-use water purification cartridge for household use in developing countries." *J Water Health*, 13 (1): 91–102. IWA Publishing. https://doi.org/10.2166/wh.2014.111

Qian, L., and G. Sun. 2003. "Durable and regenerable antimicrobial textiles: Synthesis and applications of 3-methylol-2,2,5,5-tetramethyl-imidazolidin-4-one (MTMIO)." *J Appl Polym Sci*, 89 (9): 2418–2425. John Wiley & Sons, Ltd. https://doi.org/10.1002/APP.12405

Sharma, V. K., X. Yang, L. Cizmas, T. J. McDonald, R. Luque, C. M. Sayes, B. Yuan, and D. D. Dionysiou. 2017. "Impact of metal ions, metal oxides, and nanoparticles on the formation of disinfection byproducts during chlorination." *Chemical Engineering Journal*. Elsevier B.V.

Si, Y., J. Li, C. Zhao, Y. Deng, Y. Ma, D. Wang, and G. Sun. 2017. "Biocidal and Rechargeable N-Halamine Nanofibrous Membranes for Highly Efficient Water Disinfection." *ACS Biomater Sci Eng*, 3 (5): 854–862. American Chemical Society. https://doi.org/10.1021/acsbiomaterials.7b00111

Singh, R., J. N. Edokpayi, J. O. Odiyo, and J. A. Smith. 2019. "E. coli Inactivation by Metals and Effects of Changes in Water Chemistry." *Journal of Environmental Engineering*, 145 (2): 04018136.

American Society of Civil Engineers (ASCE). https://doi.org/10.1061/(ASCE)EE.1943-7870.0001486

Straub, T. M., C. P. Gerba, X. Zhou, R. Price, and M. T. Yahya. 1995. "Synergistic inactivation of Escherichia coli and MS-2 coliphage by chloramine and cupric chloride." *Water Res*, 29 (3): 811–818. Pergamon. https://doi.org/10.1016/0043-1354(94)00213-Q

Sun, Y., and G. Sun. 2001. "Durable and refreshable polymericN-halamine biocides containing 3-(4?-vinylbenzyl)-5,5-dimethylhydantoin." *J Polym Sci A Polym Chem*, 39 (19): 3348–3355. John Wiley & Sons, Ltd. https://doi.org/10.1002/pola.1317

Sun, Y., and G. Sun. 2002. "Synthesis, Characterization, and Antibacterial Activities of Novel N-Halamine Polymer Beads Prepared by Suspension Copolymerization." *Macromolecules*, 35 (23): 8909–8912. American Chemical Society . https://doi.org/10.1021/MA020691E

Timofeeva, L., and N. Kleshcheva. 2010. "Antimicrobial polymers: mechanism of action, factors of activity, and applications." *Applied Microbiology and Biotechnology 2010 89:3*, 89 (3): 475–492. Springer. https://doi.org/10.1007/S00253-010-2920-9

Tran, T. N., T. V. A. Pham, M. L. P. Le, T. P. T. Nguyen, and V. M. Tran. 2013. "Synthesis of amorphous silica and sulfonic acid functionalized silica used as reinforced phase for polymer electrolyte membrane." *Advances in Natural Sciences: Nanoscience and Nanotechnology*, 4 (4): 045007. IOP Publishing. https://doi.org/10.1088/2043-6262/4/4/045007

Tsao, T. -C, D. E. Williams, C. G. Worley, and S. D. Worley. 1991. "Novel N-Halamine Disinfectant Compounds." *Biotechnol Prog*, 7 (1): 60–66. American Chemical Society (ACS). https://doi.org/10.1021/BP00007A010

US EPA, O. 2020. "Drinking Water Regulations and Contaminants." Accessed January 4, 2021. https://www.epa.gov/sdwa/drinking-water-regulations-and-contaminants

Williams, D. E., E. D. Elder, and S. D. Worley. 1988. "Is free halogen necessary for disinfection?" *Appl Environ Microbiol*, 54 (10): 2583–2585. American Society for Microbiology. https://doi.org/10.1128/AEM.54.10.2583-2585.1988

World Health Organization. 2017. *Guidelines for drinking-water quality, 4th edition, incorporating the 1st addendum*. Geneva: WHO. https://www.who.int/publications/i/item/9789241549950

Yahya, M. T., L. K. Landeen, M. C. Messina, S. M. Kutz, R. Schulze, and C. P. Gerba. 1990. "Disinfection of bacteria in water systems by using electrolytically generated copper:Silver and reduced levels of free chlorine." *Can J Microbiol*, 36 (2): 109–116. https://doi.org/10.1139/m90-020

Yahya, M. T., T. M. Straub, and C. P. Gerba. 1992. "Inactivation of coliphage MS-2 and poliovirus by copper, silver, and chlorine." *Can J Microbiol*, 38 (5): 430–435. NRC Research Press Ottawa, Canada . https://doi.org/10.1139/m92-072

Zhao, L., X. Yan, Z. Jie, H. Yang, S. Yang, and J. Liang. 2014. "Regenerable antimicrobial N-halamine/silica hybrid nanoparticles." *Journal of Nanoparticle Research*, 16 (7): 1–12. Kluwer Academic Publishers. https://doi.org/10.1007/s11051-014-2454-7

Chapter 5

Bacteria and virus inactivation efficacy of the chlorinated polymer gels in water

5.1 Introduction

Chapter 4 provides a detailed account of the development of a rechargeable polymer gel or chlorine-releasing material. The chapter outlines the testing of various gel formulations and the selection of the best one that maintained its mechanical integrity. The optimal formulation was used to synthesize gels, which were then washed thoroughly to eliminate any unreacted material before being loaded with chlorine.

The concentration of released chlorine from the chlorinated gels in water was below the detection limit (40 μg/L Cl_2) of our spectroscopic method (see section 4.4.4). Thus, the first objective of this chapter was to assess the efficacy of the gels in inactivating target bacteria in water by evaluating if the concentration of chlorine released from the gels is sufficient to inactivate *E. coli* bacteria. Subsequently, our second objective was to examine whether the chlorination time and Cl^+ content of the gels are correlated with the efficacy of bacteria inactivation.

During each water disinfection test, the Cl^+ content in the polymer gels decreases. However, after Cl^+ depletion, these gels can be recharged or reloaded by exposing them again to a solution that contains a Cl^+ donor (Bastarrachea et al. 2014; Chen et al. 2003; Hui and Debiemme-Chouvy 2013; Si et al. 2017). Therefore, our third objective was to evaluate the rechargeability capacity of a gel that had been used in several *E. coli* inactivation experiments.

Previous research has demonstrated that using low levels of metals (≤70 μg/L silver ions and ≤700 μg/L copper ions) and chlorine (≤1,000 μg/L) in water disinfection yield higher pathogen reduction than using either metal or chlorine alone (Abad et al. 1994; Biurrun et al. 1999; Chen et al. 2008; Cromeans et al. 2010; Estrella-You and Smith 2022; Liu et al. 1994; Lucier et al. 2017; Straub et al. 1995; Yahya et al. 1990). Given this context, our fourth objective was to test the efficacy of inactivating *E. coli* bacteria and MS2 bacteriophage virus using combinations

of polymer gels with materials that release silver or copper in water. The silver source that we used was the MadiDrop+, a commercial silver-ceramic tablet for point-of-use water disinfection. For our copper source, we employed a copper screen tested by Harris (2023), which consistently releases 190-330 μg/L copper in 10-L of water over 24-h.

5.2 Methods

5.2.1 Preparation of free chlorine solution

A 0.01% or 100 mg/L Cl_2 solution was prepared from a 12% stock NaOCl solution (Sigma-Aldrich, St. Louis, MO) and reverse osmosis water. The oxidative chlorine (Cl_2) concentration in the diluted NaOCl solution was measured through a standard iodometric/thiosulfate titration as detailed in Chapter 4, section 4.3.3.

5.2.2 Preparation of *E. coli* suspension

A ~10^{12} MPN/100 mL *E. coli* suspension was prepared from a frozen nonpathogenic wild strain stock in Luria-Bertani or LB broth. Following a previously reported method by Singh et al. (2019), the LB broth was prepared by adding 0.5 g yeast extract (Sigma-Aldrich), 0.5 g NaCl (Fisher Chemical, Pittsburgh, PA), and 0.25 g tryptone (Sigma-Aldrich) to 50 mL deionized water. This mixture was sterilized for 21 min at 121°C (autoclave 3545E-B/L, Tuttnauer Brinkmann, Hauppauge, NY). After the broth reached room temperature, 50 μL of thawed *E. coli* stock (QC *E. coli* kit, IDEXX, Westbrook, ME) were added and the culture was incubated (B4 incubator, ELCONAP, Newark, NJ) for ~12-h at 37°C while mixing at 200 rpm in an orbital shaker (VWR Scientific, Radnor, PA). After incubation, the culture was centrifuged for 20 min at 2,500 rpm (Sorvall Legend XTR centrifuge, Thermo Fisher Scientific, Waltham, MA). The *E. coli* pellet from the bottom of the centrifuge tube was re-suspended in 50 mL 10 mM phosphate buffer solution composed of 6.43 mM or 1.12 g/L K_2HPO_4 (Fisher Chemical) and 3.53 mM or 0.48 g/L KH_2PO_4 (Fisher Chemical). The suspension was stored at 4°C to maintain the viability of *E. coli* in solution while preventing growth prior to any inactivation experiment. This suspension was viable for up to 5 days. The concentration of *E. coli* in suspension was

determined using the IDEXX Colilert Defined-Substrate Technology System as described in Chapter 3, section 3.3.5.

5.2.3 Bacteria inactivation tests (Experimental design)

5.2.3.1 Inactivation of E. coli with low doses of free chlorine

This test was performed in synthetic groundwater (SGW) inoculated with an aliquot of the *E. coli* suspension to obtain ~10^5 MPN/100 mL. SGW was prepared as described in Chapter 4, section 4.3.4. Appropriate aliquots of the 100 mg/L Cl_2 solution were added to four different glass containers with 120 mL SGW to obtain four treatments with the following free chlorine concentrations at t = 0: 5, 10, 15, and 20 μg/L Cl_2 (after chlorine addition, each glass container was sealed using Parafilm M (HACH, Loveland, CO). Water samples were taken before chlorine addition, and at 8-h contact time to analyze for *E. coli* and quantify $\log_{10}$ reduction of the bacteria by each treatment. At the 8-h sampling, the antibacterial activity of chlorine was quenched by the addition of 2.64 mL of 60 g/L sodium thiosulfate solution (Fisher Chemical) to each 100 mL water sample as indicated in Ehdaie et al. (2014).

Each treatment had 3 replicates, and a no treatment or "control" was also included to determine the bacteria natural reduction during the contact time. Tests were conducted under ambient conditions. The concentration of *E. coli* in the SGW samples was determined using the IDEXX Colilert Defined-Substrate Technology System as described in Chapter 3, section 3.3.5.

5.2.3.2 Inactivation of E. coli with the chlorinated polymer gels

We performed the following tests to evaluate the efficacy of bacteria inactivation by the chlorinated gels: (i) compared VBDMH gel effects vs. styrene gel effects; (ii) evaluated the effect of gel chlorination time; and (iii) assessed the effect of Cl^+ content in the gels. These tests were conducted the same way as the one with low doses of free chlorine (section 5.2.3.1) but instead of adding aliquots of free chlorine solution, VBDMH or styrene gels were introduced in the 120 mL SGW inoculated with *E. coli*.

Test (i) compared 8-h treatment for *E. coli* inactivation by 3 styrene gels (introduced together in SGW; each gel theoretically containing 4 mmol styrene) against 3 VBDMH gels

(added together to SGW; each gel theoretically containing 1 g or 4 mmol VBDMH or 145 mg Cl^+). Test (ii) compared *E. coli* inactivation by gels theoretically containing 1 g VBDMH and chlorinated for 12, 24, or 48 hours (each gel was added to a separate glass container with SGW) but shorter contact times (between 4 and 6 hours) were evaluated. Test (iii) compared 8-h treatment for *E. coli* inactivation by gels theoretically containing 0.2, 0.5, and 3 g VBDMH (each gel was added to a separate glass container with SGW and used several times, changing the water and container each time). Additionally, in test (iii) the amount of chlorine in the gels varied from 29 to 435 mg Cl^+ because the amount of VBDMH that can bind Cl^+ was varied from 0.2 to 3 g.

5.2.3.3 Chlorinated polymer gels combined with the MadiDrop+ or the copper screen

We performed two tests to evaluate the efficacy of *E. coli* inactivation by the chlorinated gels combined with the MadiDrop+ (MD) or the copper screen in SGW with a *E. coli* concentration of ~10^5 MPN/100 mL. The MD is designed to be used in a water volume of 10-L. These tests were done using 5-L, so half of the MD was tested (see Figure 5.1).

Figure 5.1: *E. coli* inactivation tests combining the chlorinated polymer gels with the MadiDrop+ or the copper screen, 8-h treatment in 5-L synthetic groundwater

In the first test, at t = 0, half MD and/or 2 gels (each theoretically containing 1 g VBDMH) were added to three different buckets with SGW to obtain the following treatments: (i) 2 gels; (ii) half MD; and (iii) half MD + 2 gels (added simultaneously). Similarly, in the second test, at t = 0, 5 g of copper screen and/or 2 gels were added to three different buckets with SGW to obtain the following treatments: (i) 2 gels; (ii) screen; and (iii) screen + 2 gels (added simultaneously). After

adding the materials to the buckets, these were covered with their lids. *E. coli* quantification and water sampling were done the same way as in the free chlorine test (section 5.2.3.1) but before taking the samples, the water was gently stirred to ensure water quality homogeneity. Each treatment had a replicate, and a no treatment or "control" was also included. Tests were conducted under ambient conditions.

5.2.4 Virus inactivation tests

The culture and quantification methods, and the experimental design for these tests were established by Harris (2023). Briefly, bacteriophage MS2 virus (15597-B1, ATCC, Manassas, VA) was cultured and quantified by adapting methods from the manufacturer and double layer plaque assay (Cormier and Janes 2014). Two virus inactivation tests were performed, one testing the polymer gels with the MD and/or copper screen, and the other testing the MD with the screen. In the first test, the following treatments were evaluated in 5-L SGW containing MS2 bacteriophage: (i) 2 gels (each theoretically containing 1 g VBDMH); (ii) 5 g screen + 2 gels; (iii) half MD + 2 gels; and (iv) 5 g screen + half MD + 2 gels. The second test assessed the following treatments in 10-L SGW: (i) MD; (ii) 10 g screen; and (iii) MD + 10 g screen. Both tests included a no treatment or "control". Water samples were taken at 8-h and 24-h contact time to analyze for MS2 and quantify $\log_{10}$ reduction of the virus by each treatment. These virus inactivation tests were performed by Jamie D. Harris.

5.2.5 Rechargeability test

This test was conducted with a gel that theoretically contained 3 g VBDMH. This gel was previously used in 14 different *E. coli* inactivation tests. After the last test, the gel was not used for ~2 months and then it was recharged the same way as it was originally loaded with chlorine (see Chapter 4, section 4.3.3). Once recharged, the polymer gel was tested again for *E. coli* inactivation efficacy several times (changing the water and container each time) as detailed in section 5.2.3.2, i.e., 8-h treatment in 120 mL SGW with ~10^5 MPN/100 mL *E. coli*.

5.3 Results and discussion

5.3.1 Bacteria inactivation efficacy tests

5.3.1.1 E. coli inactivation tests with low doses of free chlorine

Initially, we tested the efficacy of low doses of free chlorine (5-20 µg/L Cl_2) to determine if concentrations below the detection limit of our spectroscopic method (40 µg/L) were sufficient to inactivate *E. coli* bacteria in SGW. Using these chlorine concentrations, we observed *E. coli* inactivation ranging from 0.43- to 1.69-log_{10} reduction with a contact time of 8 hours (see Figure 5.2).

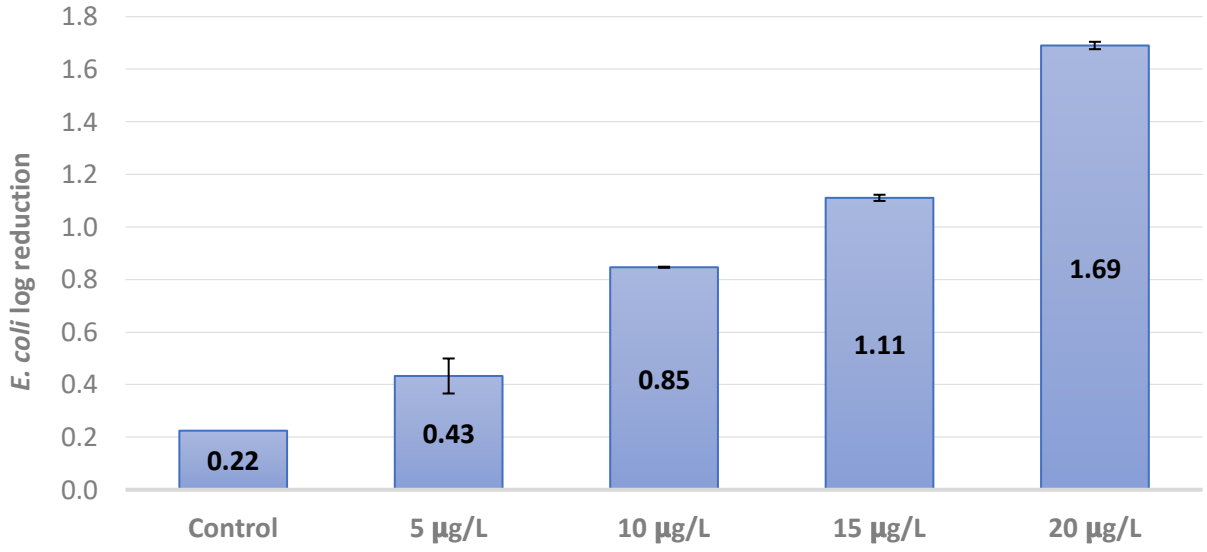

Figure 5.2: *E. coli* inactivation by low doses of free chlorine, 8-h treatment in synthetic groundwater. Initial bacteria concentration in the water was ~60,000 MPN/100 mL or 4.79 in log_{10} scale. "Control": bacteria natural reduction. Error bars indicate standard deviation (n=3, except for "Control" where n=1).

Based on these findings, our next step was to examine the hypothesis that the introduction of chlorinated polymer gels in SGW containing *E. coli* leads to the bacteria reduction because the gels release chlorine in levels below 40 µg/L. Furthermore, by evaluating this hypothesis, we could indirectly confirm that the gels were effectively releasing chlorine in water. However, to verify that no other chemical besides chlorine was causing bacteria reduction, we would need to include a styrene control gel in this test.

5.3.1.2 *E. coli inactivation tests with chlorinated polymer gels*

While *N*-chloramine polymers are known to inactivate bacteria mostly upon contact, we reasoned that without filtering through the gel, most bacteria would not contact the gel. Therefore, any antibacterial activity should stem from released chlorine at concentrations below our spectroscopic method detection limit (40 μg/L Cl_2). Figure 5.3 shows that the chlorinated polymer gels were effective for *E. coli* inactivation (achieving complete bacteria reduction), whereas the styrene control gels were not. This provides evidence that the efficacy of bacteria inactivation does not come from chlorine in solution that gets trapped in the polymer gels during loading and later comes out of the gels.

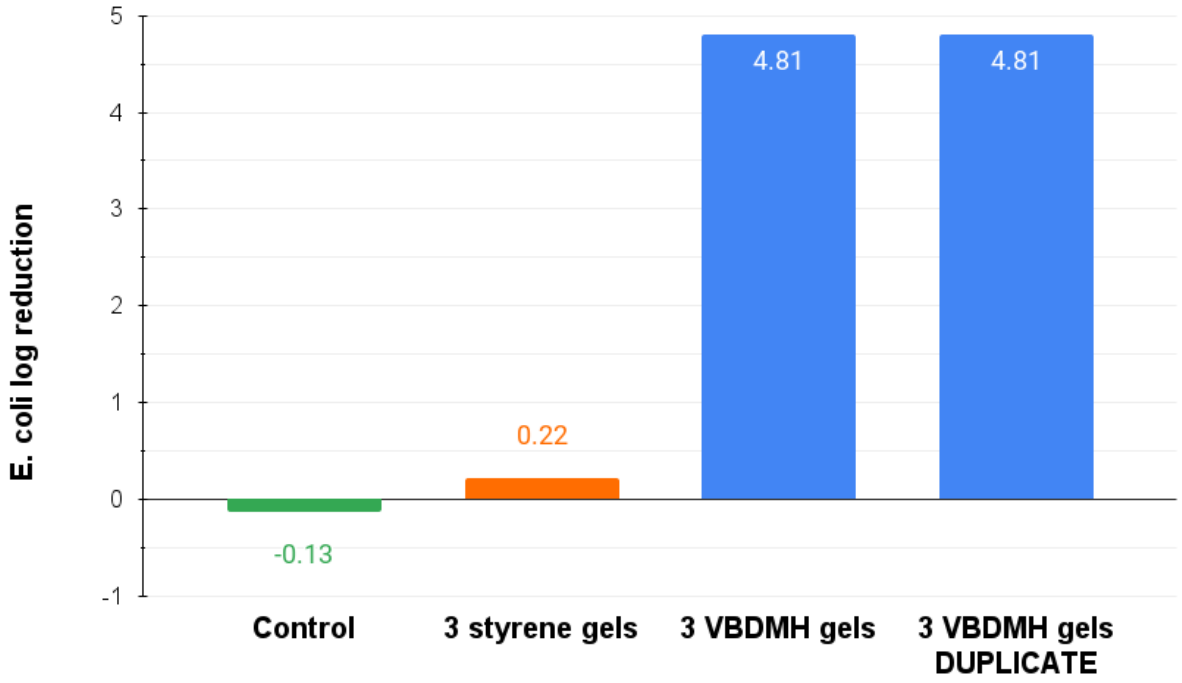

Figure 5.3: *E. coli* inactivation by chlorinated VBDMH and styrene gels, 8-h treatment in 120 mL synthetic groundwater. Initial bacteria concentration in the water was ~65,000 MPN/100 mL or 4.81 in $\log_{10}$ scale. "Control": bacteria natural reduction. Each VBDMH gel contained 1 g of this monomer (or 4 mmol). Each styrene gel contained the 4 mmol styrene instead of VBDMH. All the gels were chlorinated for 48 hours.

Furthermore, these findings corroborate previous studies that reported the release of chlorine from *N*-chloramines. In part, the bacteria inactivation results could be attributed to a slow dissociation of chlorine from the amines.

5.3.1.3 *Effect of gel chlorination time in bacteria inactivation efficacy*

In SGW, we tested the *E. coli* inactivation efficacy of polymer gels that had been chlorinated for different times (i.e., 12, 24, and 48 hours). The results in Figure 5.4, demonstrate that the $\log_{10}$ reduction of bacteria increases with the gels' chlorination time. While the bacteria reduction was similar for gels chlorinated for 12- and 24-h, those chlorinated for 48-h produced a greater reduction in bacteria after 6 hours of contact time.

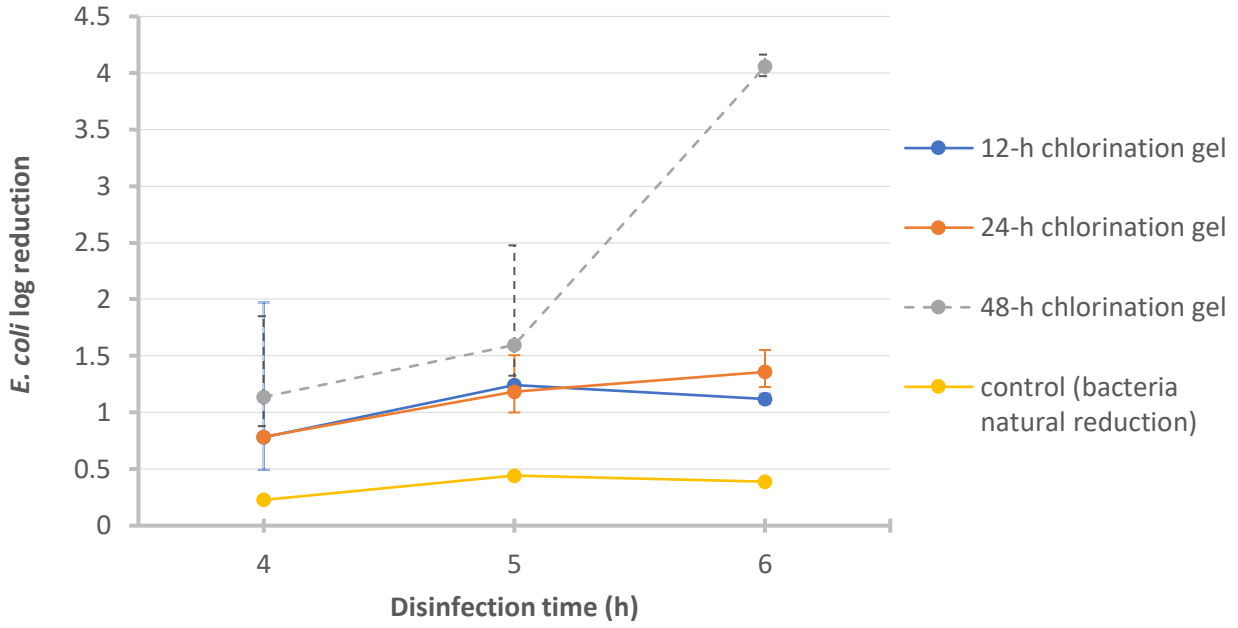

Figure 5.4: *E. coli* reduction with respect to 1 g VBDMH gel's chlorine loading time in 120 mL synthetic groundwater. Initial bacteria concentration in the water was ~30,000 MPN/100 mL or 4.46 in $\log_{10}$ scale. Error bars indicate range (n=2 except for "control" where n=1).

5.3.1.4 *Effect of gel Cl^+ content in bacteria inactivation efficacy*

We conducted tests to determine the efficacy of *E. coli* inactivation using gels containing various theoretical amounts of VBDMH (ranging from 0.2 to 3 g VBDMH, or 29 and 435 mg Cl^+). While these Cl^+ amounts were high, release of chlorine was suspected to occur at only very low levels because we could not detect it. The first data points in Figure 5.5 show that after 8 hours in 120 mL SGW inoculated with *E. coli*, a gel containing 0.5 g VBDMH produced a $\log_{10}$ reduction of 1.74, while a gel containing 3 g of VBDMH led to a $\log_{10}$ reduction of 4.14. Thus, Figure 5.5

results demonstrate that the $\log_{10}$ reduction of *E. coli* increases with the amount of VBDMH present in the gels.

Figure 5.5 also shows that repeated use of the gels leads to a decline in the $\log_{10}$ reduction of bacteria. This can be attributed to the expected decrease in the amount of Cl^+ in the polymer gels each time they are submerged in water. However, the gels can be recharged with a sodium hypochlorite solution once the Cl^+ has been depleted, using the same method of chlorination described in section 5.3.3.

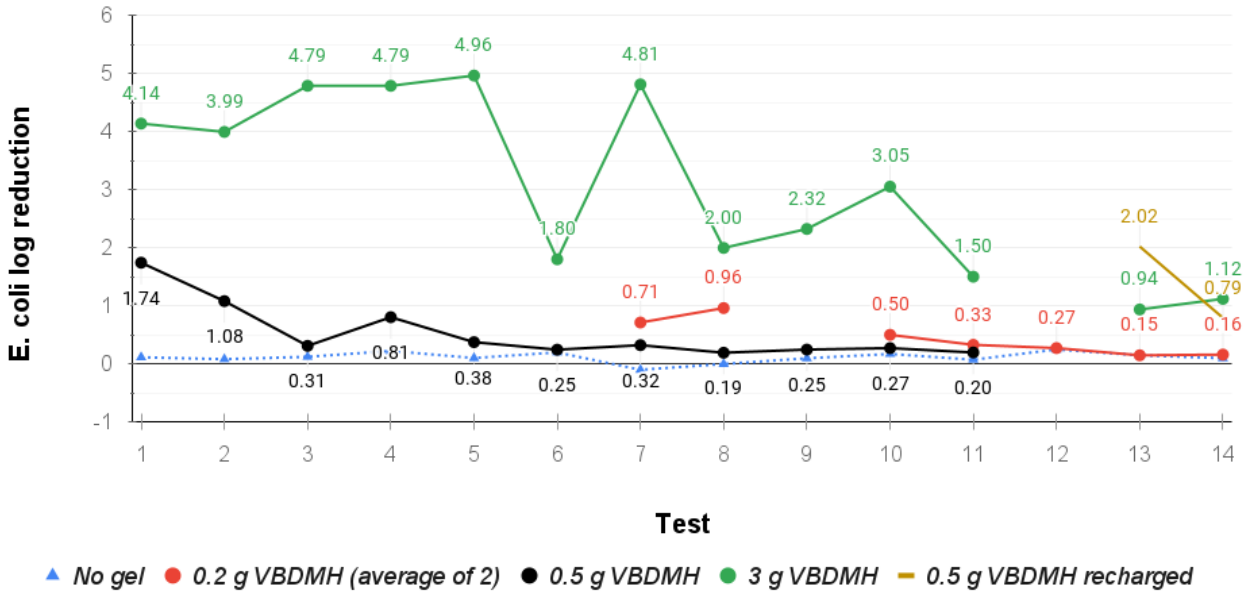

Figure 5.5: *E. coli* inactivation tests using chlorinated polymer gels with different theoretical amounts of VBDMH monomer, 8-h treatment in 120 mL synthetic groundwater. Gels with 0.2 g VBDMH were used for the first time for disinfection in test 7. Test 9: 0.2 g VBDMH gels average result not shown because unable to have the accurate bacteria count (result was <100 MPN/100 mL; so, should have diluted the water sample by a lower factor). Test 12: 3 g VBDMH gel result not shown because unable to have the accurate bacteria count (result was >2,419.6 MPN/100 mL; so, should have diluted the water sample by a higher factor). The 0.5 g VBDMH gel was recharged after test 11 and then used for disinfection in tests 13 and 14. All the gels were chlorinated for 48 hours. Each data point involves a water change.

5.3.2 Polymer gels combined with the MadiDrop+ and/or copper screen

As mentioned in section 5.1, prior studies have shown that combining silver, copper, and/or chlorine can enhance pathogen reduction in water disinfection compared to the use of these chemicals individually (Abad et al. 1994; Biurrun et al. 1999; Chen et al. 2008; Cromeans et al.

2010; Estrella-You and Smith 2022; Liu et al. 1994; Lucier et al. 2017; Straub et al. 1995; Yahya et al. 1990). To investigate this further, we combined the polymer gels with either the MadiDrop+ (MD) or the copper screen.

5.3.2.1 Bacteria inactivation efficacy tests

Figure 5.6 shows that after an 8-hr treatment, 2 polymer gels (yellow bar) produced a greater $\log_{10}$ reduction than half MD (blue bar). Combining the gels with half MD (green bar) led to a higher reduction than when either material was used independently, approaching a 2-$\log_{10}$ reduction. It is possible that the $\log_{10}$ reduction of the MD-gel combination could be further increased by increasing the number of gels used or by extending the contact time. Additionally, Figure 5.6 shows that the copper screen was not effective for *E. coli* inactivation within an 8-hr contact time. Furthermore, when the screen was combined with the polymer gels (orange bar), there was essentially no improvement in the reduction of bacteria compared to the gels alone (yellow bar).

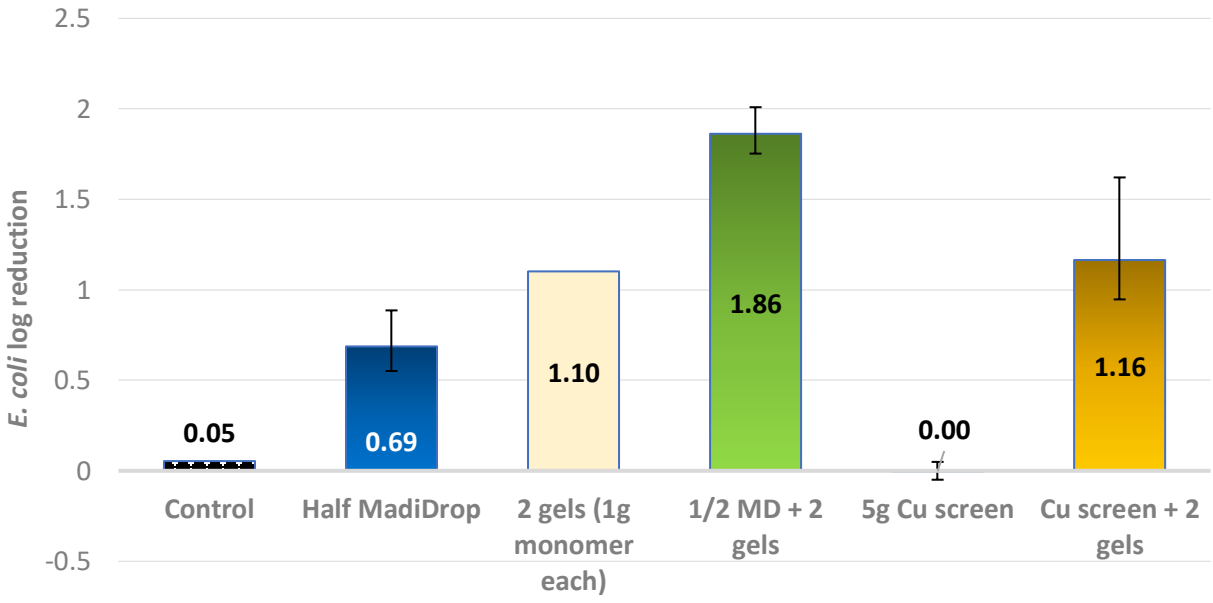

Figure 5.6: Testing *E. coli* inactivation efficacy of the chlorinated gels combined with the MadiDrop+ or copper screen, 8-h treatment in 5-L synthetic groundwater. The theoretical Cl^+ content of a gel that contains 1 g VBDMH monomer is 145 mg. "Control": bacteria natural reduction. n=2 except for: "2 gels" (yellow bar) where n=4, and "Control" where n=1. Error bars represent the range. For "2 gels" the range was 0.67 - 4.81, not shown in the figure. All the gels were chlorinated for 48 hours.

5.3.2.2 *Virus inactivation efficacy tests*

Figure 5.7 shows that when 2 polymer gels (each theoretically containing 1 g VBDMH) were tested for MS2 bacteriophage inactivation in 5-L SGW for 8 or 24 hours, they were not effective. The combination of the polymers with half MD (green bar) did not improve virus reduction compared to half MD alone (blue bar). However, the addition of 5 g copper screen to the polymers (orange bar) increased the reduction of MS2 compared to the copper screen alone (red bar), which is different from the results observed for *E. coli* inactivation. Notably, the greatest reduction in MS2 was achieved when all three materials were combined (gray bar): copper screen, MD, and polymer gels.

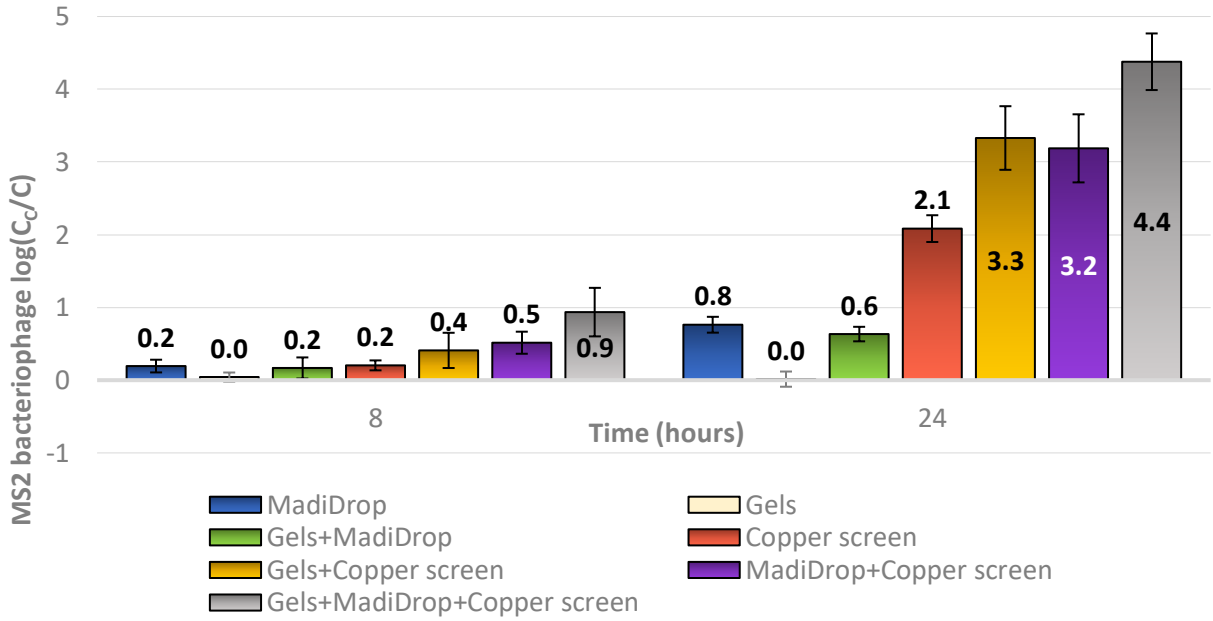

Figure 5.7: Inactivation of MS2 bacteriophage with disinfectant (chlorine, copper, or silver) releasing materials individually and in combination. The $\log_{10}$ (C_C/C) was calculated dividing the concentration of the control (C_C) at 8 and 24 hours by the concentration of the sample (C) at the same time point. Error bars represent standard error of triplicate data except for any treatments that include a polymer, which were collected in duplicate.

5.3.3 Polymer gel rechargeability test

We evaluated the rechargeability of a gel previously synthesized with a theoretical content of 3 g VBDMH monomer (or 435 mg Cl^+). As shown in Figure 5.5, this gel underwent testing for *E. coli* inactivation 14 times earlier. The *E. coli* inactivation results of the reloaded gel are presented

in Figure 5.8, with each data point involving a water change. The gel's performance is comparable to that of Figure 5.5, with most $\log_{10}$ reductions consistently above 4 for the first 7 uses. But there is a noticeable trend with the recharged gel where the $\log_{10}$ reduction decreases and then increases again. After each disinfection test, the gel was not stored in a closed container, exposing it to laboratory ambient conditions. The humidity in the air may have altered the chlorine content in the gel, contributing to the significant variability in bacteria reduction.

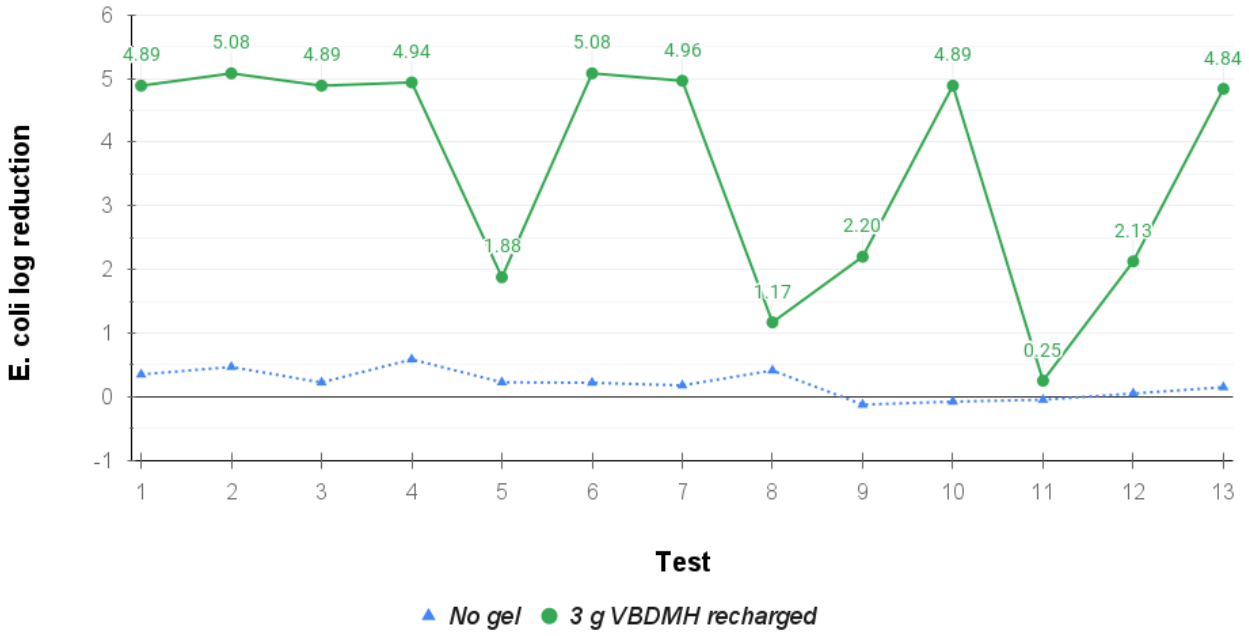

Figure 5.8: *E. coli* inactivation tests using a recharged gel that theoretically contains 3 g of VBDMH monomer, 8-h treatment in 120 mL synthetic groundwater. Each data point involves a water change.

5.4 Conclusions

The polymer gel formulation that we selected has the ability to inactivate bacteria in synthetic groundwater (SGW). When the gel is added to water, the *N*-chloramines present in the gel gradually release chlorine into the bulk solution (at levels below 40 μg/L during an 8-hr contact time), which leads to pathogen inactivation. Furthermore, the degree of bacteria reduction is linked to the polymer gels' loading or chlorinating time, as well as the quantity of *N*-chloramine or VBDMH monomer present in the gels.

We found that the use of polymer gels in conjunction with the MadiDrop+ (MD) and/or the copper screen results in greater reduction of *E. coli* bacteria and MS2 bacteriophage virus in SGW, compared to when the materials are used alone. Combining the gels with MD increased *E. coli* reduction compared to either material alone. Similarly, combining the gels with the screen increased MS2 virus reduction compared to the screen alone (the gels alone were not effective for the virus inactivation). Notably, the highest viral reduction was observed when all three materials were used together.

Preliminary results from a gel rechargeability test demonstrate that the gel can effectively inactivate bacteria similarly to when it was first charged. Our laboratory findings are promising and contribute towards the future development of the gels, ultimately aiming to create a chlorine based POU technology that can work in conjunction with the MD and/or copper screen for extended periods and produce potable water.

5.5 References

Abad, F. X., R. M. Pinto, J. M. Diez, and A. Bosch. 1994. "Disinfection of Human Enteric Viruses in Water by Copper and Silver in Combination with Low Levels of Chlorine." *Appl Environ Microbiol*, 2377–2383. https://doi.org/10.1128/aem.60.7.2377-2383.1994

Bastarrachea, L. J., L. A. McLandsborough, M. Peleg, and J. M. Goddard. 2014. "Antimicrobial N-halamine Modified Polyethylene: Characterization, Biocidal Efficacy, Regeneration, and Stability." *J Food Sci*, 79 (5): E887–E897. Blackwell Publishing Inc. https://doi.org/10.1111/1750-3841.12455

Biurrun, A., L. Caballero, C. Pelaz, E. León, and A. Gago. 1999. "Treatment of a Legionella pneumophila-Colonized Water Distribution System Using Copper-Silver Ionization and Continuous Chlorination." *Infect Control Hosp Epidemiol*, 20 (6): 426–428. https://doi.org/10.1086/501645

Chen, Y. S., Y. E. Lin, Y. C. Liu, W. K. Huang, H. Y. Shih, S. R. Wann, S. S. Lee, H. C. Tsai, C. H. Li, H. L. Chao, C. M. Ke, H. H. Lu, and C. L. Chang. 2008. "Efficacy of point-of-entry copper-silver ionisation system in eradicating Legionella pneumophila in a tropical tertiary care hospital: implications for hospitals contaminated with Legionella in both hot and cold water." *Journal of Hospital Infection*, 68 (2): 152–158. W.B. Saunders. https://doi.org/10.1016/j.jhin.2007.10.020

Chen, Y., S. D. Worley, J. Kim, C.-I. Wei, T.-Y. Chen, J. I. Santiago, J. F. Williams, and G. Sun. 2003. "Biocidal Poly(styrenehydantoin) Beads for Disinfection of Water." *Ind Eng Chem Res*, 42: 280–284. American Chemical Society. https://doi.org/10.1021/IE020266+

Cormier, J., and M. Janes. 2014. "A double layer plaque assay using spread plate technique for enumeration of bacteriophage MS2." *J Virol Methods*, 196: 86–92. Elsevier. https://doi.org/10.1016/J.JVIROMET.2013.10.034

Cromeans, T. L., A. M. Kahler, and V. R. Hill. 2010. "Inactivation of adenoviruses, enteroviruses, and murine norovirus in water by free chlorine and monochloramine." *Appl Environ Microbiol*, 76 (4): 1028–1033. American Society for Microbiology. https://doi.org/10.1128/AEM.01342-09

Ehdaie, B., C. Krause, and J. A. Smith. 2014. "Porous ceramic tablet embedded with silver nanopatches for low-cost point-of-use water purification." *Environ Sci Technol*, 48 (23): 13901–13908. American Chemical Society. https://doi.org/10.1021/es503534c

Estrella-You, A., and J. A. Smith. 2022. "Synergistic Bacterial Inactivation by Silver Ions and Free Chlorine in Natural Waters." *Journal of Environmental Engineering*, 148 (11): 04022072. American Society of Civil Engineers. https://doi.org/10.1061/(ASCE)EE.1943-7870.0002053

Harris, J. D. 2023. "Improving Silver-Ceramic-Based Point-of-Use Water Treatment with Novel Copper Addition and Commercial Water Filters." PhD. University of Virginia.

Hui, F., and C. Debiemme-Chouvy. 2013. "Antimicrobial N-halamine polymers and coatings: A review of their synthesis, characterization, and applications." *Biomacromolecules*. American Chemical Society.

Liu, Z., J. E. Stout, L. Tedesco, M. Boldin, C. Hwang, W. F. Diven, and V. L. Yu. 1994. "Controlled Evaluation of Copper-Silver Ionization in Eradicating Legionella pneumophila from a Hospital Water Distribution System." *J Infect Dis*, 169 (4): 919–922.

Lucier, K. J., S. E. Dickson-Anderson, and C. J. Schuster-Wallace. 2017. "Effectiveness of silver and copper infused ceramic drinking water filters in reducing microbiological contaminants." *Journal of Water Supply: Research and Technology - AQUA*, 66 (7): 528–536. IWA Publishing. https://doi.org/10.2166/aqua.2017.028

Si, Y., J. Li, C. Zhao, Y. Deng, Y. Ma, D. Wang, and G. Sun. 2017. "Biocidal and Rechargeable N-Halamine Nanofibrous Membranes for Highly Efficient Water Disinfection." *ACS Biomater Sci Eng*, 3 (5): 854–862. American Chemical Society. https://doi.org/10.1021/acsbiomaterials.7b00111

Singh, R., C. Rento, V. Son, S. Turner, and J. A. Smith. 2019. "Optimization of Silver Ion Release from Silver-Ceramic Porous Media for Household Level Water Purification." *Water (Basel)*, 11 (4): 816. MDPI AG. https://doi.org/10.3390/w11040816

Straub, T. M., C. P. Gerba, X. Zhou, R. Price, and M. T. Yahya. 1995. "Synergistic inactivation of Escherichia coli and MS-2 coliphage by chloramine and cupric chloride." *Water Res*, 29 (3): 811–818. Pergamon. https://doi.org/10.1016/0043-1354(94)00213-Q

Yahya, M. T., L. K. Landeen, M. C. Messina, S. M. Kutz, R. Schulze, and C. P. Gerba. 1990. "Disinfection of bacteria in water systems by using electrolytically generated copper:Silver and reduced levels of free chlorine." *Can J Microbiol*, 36 (2): 109–116. https://doi.org/10.1139/m90-020

Chapter 6

Conclusions and Future work

This dissertation examines the effectiveness of the silver-chlorine synergistic effect in bacteria inactivation across multiple natural waters sources. The results confirm that the synergist effect is present in real-world scenarios with natural waters that contain inorganic and organic compounds, and other factors that affect the bacteria inactivation process. This thesis also includes the development and evaluation of a chlorine-releasing material that can be used for pathogen inactivation in water. The chlorine-releasing material or rechargeable polymer gel shows *E. coli* bacteria inactivation capability in synthetic groundwater, and when used together with silver and/or copper releasing materials (MadiDrop+ and copper screen, respectively), it produces greater MS2 bacteriophage virus inactivation (the gels alone were not effective for the virus inactivation). These laboratory results are promising and suggest the potential for the gels to serve as an alternative to existing commercial chlorine-based POU technologies while also improving silver-based POU technologies.

In terms of limitations, this study has some, including the gels' chlorination time limited to a long period (48 hours), as well as the fact that only one concentration of sodium hypochlorite solution (0.25% Cl_2) was tested for chlorination. Future studies could explore the use of more concentrated hypochlorite solutions to determine if shorter chlorination times are feasible. Moreover, it is important to examine chlorinating the gels with diluted commercial bleach, which is a solution that primarily contains concentrated sodium hypochlorite (~4% Cl_2), as this is a readily available chlorine product that users may use to recharge the gels in household settings.

For *E. coli* bacteria inactivation, two gels, each containing 1 g VBDMH monomer theoretically, were able to achieve a 1.10-log_{10} reduction after an 8-hr contact time in 5-L synthetic groundwater. Using this as a baseline, future optimization studies could be conducted to determine the minimum number of gels required and the corresponding contact time to increase the bacteria reduction and approach 100% reduction. It would also be beneficial to

determine the number of gels required to release >40 ug/L Cl_2. Additionally, it could be explored whether there is a correlation between number of gel units and contact time, since shorter treatment times would be ideal.

The results of combining the gels with the MadiDrop+ and/or the copper screen indicate that there are no antagonistic or negative effects on *E. coli* and MS2 reduction. This suggests that increasing the number of gels could further enhance the reduction of the pathogens. However, as the number of gels increases, so does the amount of chlorine released, requiring an evaluation of the effect on the silver and copper release from the other materials due to the strong oxidation potential of chlorine. It is important to consider the World Health Organization (WHO) and EPA guidelines for silver, copper, and chlorine concentrations in drinking water to prevent any setting where the concentration of these chemicals exceed recommended levels.

Future work should also focus on both short-term and long-term application of the gels, including evaluations of stability and rechargeability. While there are initial findings regarding the use of the gels for a short period, additional research could provide answers to questions like: how many times can the gels be used before the chlorine becomes depleted? Considering that recharging the gels can prolong their shelf life, studies can be conducted to determine a suitable frequency for recharging the gels, such as how often would the gels need to be recharged during a 12-month span of daily water disinfection.

Could the gels behave differently in the field? Would pretreatment of the source water be necessary to reduce organics prior to using the gels? Are there any potential risks associated with this technology, and does it change the taste and odor of the treated water? To address these questions, field studies will be required once it can be confirmed that the water treated with the gels is safe for human consumption. These studies will allow to closely match environmental conditions and to consider household settings, social acceptability, and affordability. In addition, in these studies, prioritizing compliance with WHO microbial guidelines (e.g., <1 CFU/100 mL *E. coli*) over emphasizing the reduction of pathogen loads by the gels (i.e., $\log_{10}$ reduction) will be important. The results of these studies will also contribute to WHO certification and commercialization of the gels for POU drinking water disinfection.