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N-Chloramine Functionalized Polymer Gels for Point-of-Use Water Disinfection

Ana Estrella-You [1], Israt Jahan Duti [2], Qinmo Luo [2], Jamie D. Harris [1], Rachel A. Letteri [2] and James A. Smith [1,*]

[1] Department of Civil and Environmental Engineering, University of Virginia, Charlottesville, VA 22903, USA; aee8xq@virginia.edu (A.E.-Y.); jw3ek@virginia.edu (J.D.H.)
[2] Department of Chemical Engineering, University of Virginia, Charlottesville, VA 22903, USA; id8nu@virginia.edu (I.J.D.); rl2qm@virginia.edu (R.A.L.)
* Correspondence: jas9e@virginia.edu

Abstract: Combinations of metal disinfectants (i.e., silver and copper) with chlorine in doses that meet the World Health Organization guidelines for drinking water operate synergistically to provide superior drinking water disinfection across a wide range of pathogens. Moreover, the combination of disinfectants allows for lower chlorine levels and a less objectionable taste and odor to the treated water (some people can taste or smell chlorine at concentrations as low as 300 μg/L). Towards chlorine-releasing materials for combination with silver- or copper-releasing materials in point-of-use water disinfection, *N*-chloramine containing polymer gels were developed and their potential for *E. coli* bacteria inactivation was assessed in deionized water that contained salts to simulate groundwater. Following the chlorination of gels containing chloramine precursors, these gels capably inactivated *E. coli*, achieving $\log_{10}$ reductions—depending on the gel mass—ranging from 1.1 to 4.5. While chlorine released from the gels was not spectroscopically detected, free chlorine solutions inactivated *E. coli* in a concentration-dependent way, with 5 and 20 μg/L Cl_2 yielding $\log_{10}$ reductions of 0.43 and 1.69, respectively, suggesting that low levels of chlorine, below both the limit of detection of spectroscopic assays (ca. 40 μg/L Cl_2) and levels known to create adverse taste and smell, are sufficient to inactivate bacteria. Unchlorinated gels or chlorinated control styrene gels (without chloramine precursor) did not inactivate bacteria, suggesting that disinfection did not come from the precursor or from chlorine trapped in the gels after chlorination. In addition, these gels were evaluated together with the MadiDrop (MD, a commercial silver-ceramic tablet) and a copper screen that release silver and copper disinfectants, respectively. Combinations of the gel and MD produced *E. coli* inactivation close to 2-$\log_{10}$ reduction, with the combination, gels alone, and MD alone achieving 1.86-, 1.10-, and 0.69-$\log_{10}$ reduction, respectively. When the gels were combined with the copper screen, however, neither an increase nor a decrease in bacterial reduction was observed compared to that achieved with the gels alone. The laboratory results in this study are promising and suggest the potential for chloramine-functionalized gels to serve as an alternative to existing commercial chlorine-based POU technologies and in combination with silver-based POU technologies.

Keywords: *N*-chloramines; free chlorine; silver; point-of-use water treatment; water disinfection; *E. coli* inactivation

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1. Introduction

The World Health Organization (WHO) estimates that approximately 490 million people around the world use untreated water sources contaminated with bacterial, viral, and protozoan pathogens for drinking purposes [1]. These microorganisms cause gastrointestinal infections that especially affect children, who, as a consequence, can experience cognitive impairment and stunted growth [2]. A solution to address these problems is to treat the water before consumption in households with point-of-use (POU) technologies [3].

One class of POU technologies includes those that release free chlorine-based disinfectants (e.g., chlorine tablets, and bleach). Free chlorine includes a combination of hypochlorous acid (HOCl) and hypochlorite ions (OCl^-), with their proportions varying with pH [4,5]. Antimicrobial capacity in both forms arises from the strong oxidative activity of chlorine in the Cl^+ form. This property allows free chlorine to react easily with external membrane components of pathogens, leading to their damage and eventual death [6–9]. Consequently, free chlorine effectively eliminates bacterial and viral pathogens in water [10].

Since chlorine is a strong oxidant, besides pathogen membranes, it can also react with other organic compounds present in water (this constitutes the chlorine demand), leaving less chlorine available for pathogen inactivation. For this reason, it is recommended to apply high quantities of free chlorine (up to 4000 µg/L) in natural waters to ensure sufficient availability for microorganism inactivation. As a reference, the WHO guideline value that represents the concentration of free chlorine that does not result in any significant risk to human health over a lifetime of consumption is 5000 µg/L. Nevertheless, some people can taste or smell free chlorine in water at concentrations as low as 300 µg/L [11]. The unpleasant changes in the aesthetics of the water may lead users to reject these disinfecting treatments [12,13]. Moreover, some chlorine-based POU technologies have a short shelf life (six months to one year) [14], and others are single-use, significantly elevating costs and user burden.

To mitigate these challenges, an alternative approach is to disinfect water with a combination of low chlorine concentrations (<300 µg/L) and low doses of silver (<100 µg/L). The secondary drinking water standard (non-enforceable guideline regulating contaminants that may cause cosmetic effects or aesthetic effects) for silver is 100 µg/L [15]. Several studies investigating the combined use of low levels of metals (10–70 µg/L silver ions and 20–700 µg/L copper ions) and chlorine (50–1000 µg/L) show that such combinations provide a higher reduction in pathogens than either a metal or chlorine alone [16–24].

While single-use chlorine disinfectants exist (e.g., Aquatabs®), as previously mentioned, the use of these technologies can result in user rejection or discontinuation of the disinfection treatment due to changes in the aesthetic of the treated water. Therefore, the main objective of this research is to develop a novel material that releases chlorine in water at levels effective for pathogen inactivation and below 300 µg/L to prevent any unpleasant taste in the disinfected water. Additionally, to date, no POU technology has a combination of metals and chlorine. For this reason, another objective is to test the efficacy of inactivating *E. coli* bacteria using combinations of the novel chlorine-releasing material with materials that release silver (e.g., the MadiDrop, MD, a commercial silver-ceramic tablet for POU water disinfection) or copper (e.g., a copper screen tested by [25], which consistently releases 190–330 µg/L copper in 10 L of water over 24 h) in water to leverage their synergistic effects in pathogen inactivation. In summary, the development of a novel material to sustain the release of low levels of chlorine is sought, and its disinfection performance alone and in combination with the MD or the copper screen is tested.

Chlorine-releasing materials include biocidal polymers that contain *N*-chloramines with one or more nitrogen–chlorine (N-Cl) covalent bonds [26,27], which release oxidative chlorine (Cl^+). Regular usage of these materials for pathogen inactivation exhausts their chlorine contents. However, their Cl^+ supplies can be conveniently and repeatedly restored by exposing the materials to a Cl^+ donor compound, such as bleach or a concentrated free chlorine solution [27–29]; see Figure 1. This recharging property prolongs the shelf lives of these chlorine-releasing materials.

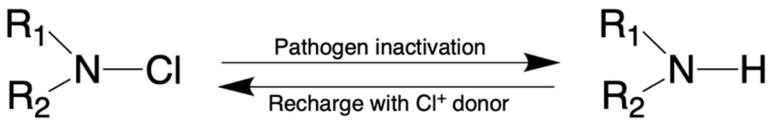

Figure 1. Rechargeability of *N*-chloramines.

To eliminate pathogens from water, *N*-chloramine containing materials are primarily utilized as filtration media [30,31]. These materials are thought to kill microorganisms in contaminated water upon contact as the water flows through a filter [26–28]. Alternatively, Cl^+ may dissociate from *N*-chloramines in water, thereby forming free chlorine [32]; see Figure 2. Moreover, studies in filtration settings have found that low concentrations of free chlorine (<200 µg/L) are present in the effluent water [29–31,33]. If such low concentrations of chlorine are effective against pathogens, then networks can be designed that release free chlorine over time; these networks can be recharged with household bleach for repeated use.

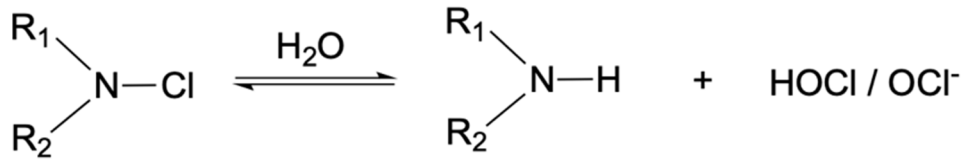

Figure 2. Cl^+ dissociation from *N*-chloramines and free chlorine formation [34,35].

Therefore, in this work, networks containing chloramine precursors were prepared and then chlorinated to generate chloramine, and their disinfection efficiencies alone and in combination with copper- and silver-releasing materials are tested. The networks include chloramine precursor units, crosslinkers, and hydrophilic monomers. Hydrophilic monomers are intended to promote the swelling of the network in water and enable the diffusion of water and chlorine. For the chloramine precursor units, a monomer reported by [36] for biocidal polymers, namely, 3-(4′-vinyl benzyl)-5,5-dimethyl hydantoin or VBDMH, is used. Several studies on *N*-chloramines that contain a hydantoin group [33,37–41], have demonstrated their high antimicrobial efficacies in applications ranging from water treatment to coatings. These studies have also examined their stabilities and have shown promising results, including a shelf life of up to six months without being recharged. For the crosslinker and hydrophilic monomer, hexanediol diacrylate or HDDA and poly(ethylene glycol) methacrylate or PEGMA, respectively, are selected. Since it has been observed that the networks capably disinfect water, the released chlorine is not detected, a range of control experiments are designed and conducted to demonstrate the necessity of both the chloramine precursor monomer and chlorination for effective disinfection, and the feasibility of disinfection at chlorine levels below the limit of detection of the spectroscopic assays is determined. Combining these chloramine-containing gels with silver-releasing MDs further improves disinfection, motivating the use of chlorine-releasing materials in combination with other disinfectant-releasing materials.

2. Materials and Methods

2.1. Synthesis of Hydantoin-Containing Crosslinked Networks or Polymer Gels

Hydrophilic crosslinked polymer networks or water-swellable polymer gel pellets were synthesized through the photocrosslinking of hydrophilic PEGMA, the hydantoin monomer VBDMH, and the HDDA crosslinker in *N*,*N*-dimethylformamide (DMF) using the 2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone photo-initiator (Figure 3).

A molar ratio of VBDMH:PEGMA:HDDA = 1:0.5:0.1 was selected to promote swelling in water and, therefore, the diffusion of chlorine without compromising mechanical integrity (Appendix B). Gels with the following VBDMH monomer masses were prepared: 0.5 g (used in the chlorine release test) and 1 g (used in the chlorine loading tests and in the *E. coli* inactivation tests). As an example, for the synthesis of gels with 1 g VBDMH, a mixture of VBDMH (1 g, 1 mol. eq, synthesized as described in Appendix A with a protocol adapted from [36]), HDDA (0.092 mL, 0.1 mol eq.; technical grade, 80%, Sigma-Aldrich, St. Louis, MO, USA), PEGMA (0.929 mL, 0.5 mol eq.; average Mn 500 contains 900 ppm monomethyl ether hydroquinone as inhibitor, Sigma-Aldrich), and photo-initiator (52.60 mg, 1% of the total formulation mass; 98%, Sigma-Aldrich) was dissolved in DMF (3.33 mL; ACS, ≥99.8%, Thermo Fisher Scientific, Waltham, MA, USA) in a 25 mL glass

vial. The vials were covered with watch glasses, placed in a crosslinker (Analytik Jena UVP Crosslinker, CL-3000L, Jena, Germany), and exposed to UV light (365 nm) for 4 h. A VBDMH-containing crosslinked gel is shown in Figure 4.

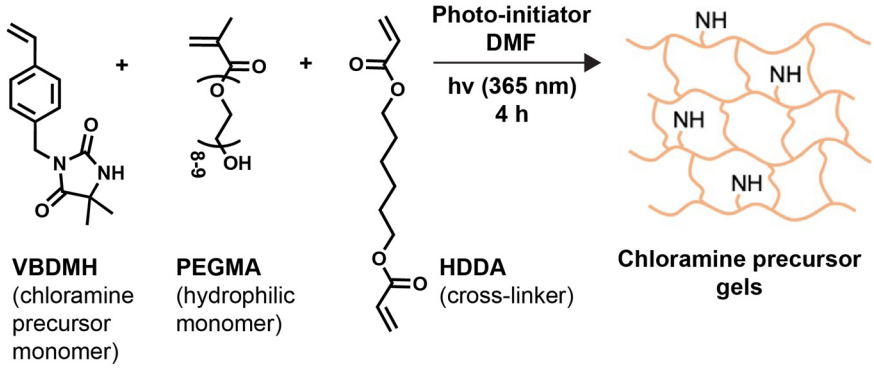

Figure 3. Synthesis of amine-containing porous networks or polymer gels.

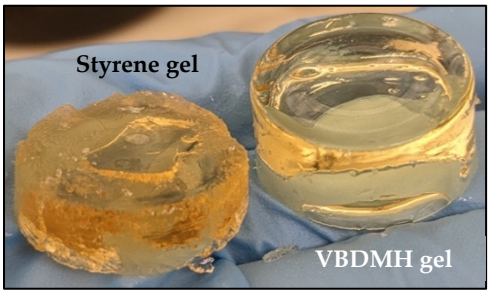

Figure 4. Photograph of gels prepared from 4 mmol VBDMH with 1:0.5 mol VBDMH: mol PEGMA (**right**) and 4 mmol styrene with 1:0.5 mol styrene: mol PEGMA (**left**) after crosslinking. The diameter of each gel is ~2 cm.

After removing the gels from the vials, unreacted monomer, solvent, and photo-initiator were removed by washing each gel with 150 mL of each of the following washes per gram of VBDMH used in the gelation reaction: (i) and (ii) with 100% methanol; (iii) and (iv) with 50% v/v methanol in water; and (v) with 33% v/v methanol in water. The supernatants of the washes were analyzed by high-performance liquid chromatography (HPLC) to confirm the removal of unreacted material (Appendix C).

2.2. Styrene Controls

To provide evidence that disinfection efficacy did not come from free chlorine alone trapped in the gels during chlorination and later came out of the gels, styrene control gels were prepared similarly as the 1 g VBDMH-containing gels from styrene (0.470 mL; ReagentPlus®, contains 4-tert-butylcatechol as stabilizer, ≥99%, Sigma-Aldrich), HDDA (0.092 mL), PEGMA (0.929 mL), photo-initiator (31.18 mg), and DMF (1.67 mL). The same mol eq. of styrene as VBDMH was used, but less DMF (about half of what was used for the VBDMH gels) was added to keep the DMF content at ca. 30% w/v, accounting for the difference in molecular weight between VBDMH and styrene. Figure 4 shows a photograph of the styrene gel after crosslinking and before the wash steps.

2.3. Loading Gels with Chlorine

The chloramine precursor polymer gel contained amine (N-H groups) that could be substituted with Cl^+ upon exposure to a free chlorine solution or a Cl^+ donor solution (e.g., aqueous sodium hypochlorite); see Figure 5.

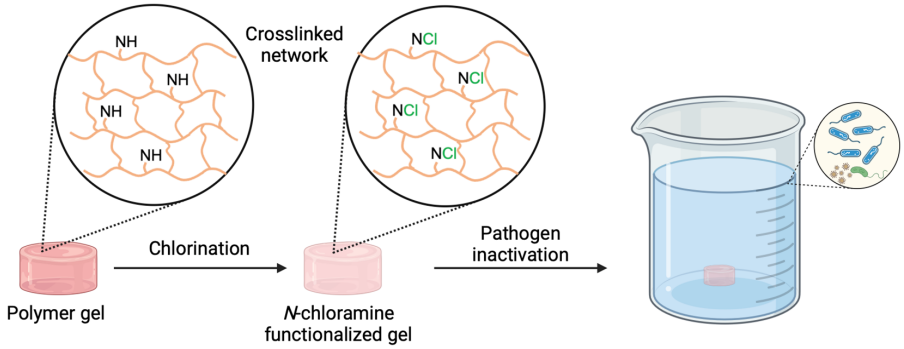

Figure 5. Preparation of *N*-chloramine functionalized porous networks or chlorinated polymer gels.

A 0.25% *w*/*v* or 2500 mg/L Cl_2 solution, with Cl_2 representing available chlorine, which is a measurement of the oxidizing strength of chlorine solutions expressed in terms of an equivalent quantity of chlorine gas [42], was prepared from a 12% stock NaOCl solution (reagent grade, Sigma-Aldrich) and reverse osmosis water. To adjust the pH of the dilute NaOCl solution to ~7, a 5% acetic acid solution (glacial, ACS, ≥99.7%, Sigma-Aldrich) was used [43]. This pH adjustment shifted the free chlorine equilibrium toward HOCl, the more reactive species.

Three gels (each prepared with 1 g VBDMH) were loaded with chlorine by immersion in aqueous NaOCl (400 mL, 0.25% *w*/*v* Cl_2, pH ~7) for 48 h. To remove unreacted chlorine, the gels were soaked in 750 mL deionized water for 30 min prior to storage in a closed glass container until use to avoid drying-induced changes in their mechanical integrity or breakage.

2.4. Chlorine Analysis in Solution

The oxidative chlorine (Cl^+ or Cl_2) concentration in dilute NaOCl solutions was measured through a standard iodometric/thiosulfate titration [41]. The sample solution (2–5 mL) was added to the sulfuric acid solution (50 mL, 0.04 N; ACS Plus, 96.2%, Fisher Chemical, Pittsburgh, PA, USA). After the addition of potassium iodide (0.20 g; >95%, Fisher Chemical) and stirring, the solution turned a yellowish-brown color due to the oxidation of iodide to iodine by Cl^+ in an acidic medium; there was a subsequent reaction with I^- to form more soluble, less volatile triiodide (I_3^-). The titration was then performed by adding aliquots of sodium thiosulfate solution (0.01 N; anhydrous, ≥98%, Fisher Chemical) until the yellow color began to fade. Then, aqueous starch solution (0.40 mL, 0.50%; soluble, ACS, Thermo Fisher Scientific) was added as an indicator since starch formed a dark blue complex with triiodide. The titration was continued by adding the sodium thiosulfate solution until the blue color disappeared at the endpoint. The 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 or mol/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 (a high volume, 100 mL, was chosen to determine this limit).

2.5. Quantification of Chlorine Loaded in the Gels

A method was developed to estimate the Cl^+ loaded into the polymer gels considering the natural decrease in Cl^+ concentration over time in the sodium hypochlorite solution because extended chlorination times (>12 h) were used. Hypochlorite solutions decomposed over time, and their concentrations of free chlorine (and, in consequence, Cl^+) decreased due

to chlorate, perchlorate, and chlorite ion formation [11]. Therefore, as shown in Figure A5, the amounts of Cl^+ in the gels were estimated as the difference between the 'remaining Cl^+ in the solution with *no* gel' and the 'remaining Cl^+ in the solution with the gel'. Based on the example of Figure A5, while in the absence of gels, the free chlorine solution prepared to theoretically contain 1200 mg/L Cl^+ was found to contain 860 mg/L Cl^+ after 2 days, and the supernatant of the same solution containing a VBDMH gel contained just 240 mg/L Cl^+. Considering the volume of the solution (200 mL) and taking the difference between the Cl^+ concentrations in solutions with and without gels allowed us to calculate a theoretical amount of chlorine loaded in the gels (in this case, 124 mg Cl^+).

2.6. Chlorine Release Measurements

After loading and washing the chlorinated gels, the release of chlorine from a gel submerged in synthetic groundwater (SGW) was evaluated, a solution that contained salts to simulate groundwater.

The preparation of SGW has been described elsewhere [44]. Briefly, deionized water (9 L), $MgSO_4$ (0.60 g; anhydrous, >99.5%, Alfa Aesar, Tewksbury, MA, USA), $NaHCO_3$ (0.96 g; powder, ACS, >99.7%, Fisher Chemical), and KCl (0.04 g; crystalline, ACS, >99%, Fisher Chemical) were combined and mixed. In a separate container, $CaSO_4$ (0.47 g; anhydrous, 99%, Alfa Aesar) was mixed with deionized water (1 L) until the calcium sulfate dissolved completely. Finally, the two solutions were combined and mixed again.

In the chlorine release test, a gel prepared with 0.5 g of VBDMH monomer and theoretically containing 72.5 mg Cl^+ (assuming all the monomer reacted into the gel) was added to SGW (15 mL). A water sample was taken at 8 h contact time to analyze free chlorine. The concentration of free chlorine (as μg/L Cl_2) in the sample was determined using the colorimetric method HACH 10241 (range 40–4500 μg/L Cl_2) with a reagent set (Freechlor F Reagent and Monochlor F Reagent; HACH, Loveland, CO, USA) and a spectrophotometer (DR6000, HACH) to measure absorbance at 655 nm.

2.7. Bacteria Inactivation Tests

2.7.1. Preparation of *E. coli* Suspension

A ~10^{12} most probable number (MPN)/100 mL *E. coli* suspension was prepared from a frozen nonpathogenic wild strain stock in Luria−Bertani (LB) broth. Following a previously reported method by [44], the LB broth was prepared by adding yeast extract (0.5 g; Sigma-Aldrich), NaCl (0.5 g; Fisher Chemical), and tryptone (0.25 g; Sigma-Aldrich) to deionized water (50 mL). This mixture was sterilized for 21 min at 121 °C (autoclave 3545E-B/L, Tuttnauer Brinkmann, Hauppauge, NY, USA). After the broth reached room temperature, thawed *E. coli* stock (50 μL; QC *E. coli* kit, IDEXX, Westbrook, ME, USA) was added, and the culture incubated (B4 incubator, ELCONAP, Newark, NJ, USA) for ~12 h at 37 °C while mixing at 200 rpm in an orbital shaker (VWR Scientific). After incubation, the culture was centrifuged for 20 min at 2500 rpm (Sorvall Legend XTR centrifuge, Thermo Fisher Scientific). The *E. coli* pellet was re-suspended in a 10 mM phosphate buffer solution (50 mL) composed of K_2HPO_4 (6.43 mM or 1.12 g/L; Fisher Chemical) and KH_2PO_4 (3.53 mM or 0.48 g/L; Fisher Chemical). The suspension was stored at 4 °C to maintain the viability of *E. coli* in solution while preventing growth prior to inactivation experiments. 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. Briefly, Colilert media (IDEXX) was added to a 100 mL 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 provided counts of up to 2419 MPN/100 mL in a sample without dilution. The trays were sealed and incubated at 35 °C for 24 h. After incubation, viable *E. coli* was determined using the MPN table provided by IDEXX and a UV lamp.

2.7.2. Preparation of Free Chlorine Solution

A Cl_2 solution (0.01% *w*/*v* or 100 mg/L) was prepared from a stock NaOCl solution (12% *w*/*v*, Sigma-Aldrich) 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 Section 2.4.

2.7.3. Inactivation of *E. coli* with Low Doses of Free Chlorine

To determine whether doses of free chlorine at concentrations below the limit of detection of the spectroscope HACH 10241 used to detect free chlorine (40 µg/L Cl_2) were sufficient to inactivate *E. coli* bacteria, inoculated water was treated with 5, 10, 15, and 20 µg/L free chlorine solution. 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 Section 2.5. 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 h: 5, 10, 15, and 20 µg/L Cl_2. After chlorine addition, each glass container was sealed using Parafilm M (HACH). Water samples were taken before chlorine addition and at 8 h of contact time to analyze for *E. coli* and quantify $\log_{10}$ reduction in the bacteria by each treatment. At the 8 h sampling time, the antibacterial activity of chlorine was quenched by the addition of sodium thiosulfate solution (2.64 mL, 60 g/L; Fisher Chemical) to each 100 mL water sample, as indicated in [45].

Each treatment involved n = 3 replicates, and a no-treatment sample ("control") was also included to determine the natural reduction in bacteria during the 8 h contact time. Tests were conducted under ambient conditions. The concentrations of *E. coli* in the SGW samples were determined using the IDEXX Colilert Defined-Substrate Technology System, as described in Section 2.7.1.

2.7.4. Inactivation of *E. coli* with the Chlorinated Polymer Gels

The following tests were performed to evaluate the efficacy of bacteria inactivation by the chlorinated gels: (i) compared disinfection provided by chlorinated gels containing the chloramine precursor VBDMH to those containing styrene; (ii) assessed the effect of gel mass (and therefore Cl^+ dose); and (iii) evaluated the effect of the gel chlorination time. Test (iii) was conducted the same way as those with low doses of free chlorine (Section 2.7.3), 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 of inactivation of *E. coli* by treatment in 250 mL SGW with a styrene gel (prepared with 4 mmol styrene) against a VBDMH gel (prepared with 1 g or 4 mmol VBDMH, corresponding to a theoretical chlorine amount = 145 mg Cl^+).

Test (ii) compared 8 h inactivation of *E. coli* by treatment in 250 mL SGW with gels prepared from 1, 2, and 3 g VBDMH (each was added to a separate glass container with 250 mL SGW). The theoretical amounts of chlorine in the gels varied from 145 to 435 mg Cl^+ as the amount of the chloramine precursor VBDMH varied from 1 to 3 g. In tests (i) and (ii), each treatment involved n = 3 replicates, each conducted with a gel prepared and chlorinated as described in Section 2.3, and a no-treatment sample ("control") was also included to determine the bacteria's natural reduction during the contact time.

Test (iii) compared *E. coli* inactivation by gels prepared from 1 g VBDMH and then chlorinated for either 12, 24, or 48 h (each gel was added to a separate glass container with SGW), but shorter contact times (between 4 and 6 h) were evaluated. Each treatment involved n = 2 replicates, and a no-treatment system ("control") was also included. All the tests were conducted under ambient conditions.

2.7.5. Chlorinated Polymer Gels Combined with the MadiDrop or the Copper Screen

Two tests were performed to evaluate *E. coli* inactivation by combining the chlorinated gels with the MadiDrop (MD, Silivhere Technologies, Inc., Charlottesville, VA) or the copper screen in SGW with an *E. coli* concentration of ~10^5 MPN/100 mL. The MD was designed to be used in a water volume of 10 L. Since these tests were performed in 5 L water, half of the MD tablet was tested. The length of an MD was 8 cm (3.1 in), so MD halves were obtained by vertically cutting the original tablet, resulting in 4-cm-long halves.

In the first test, at t = 0 h, half MD and/or two gels (each prepared with 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 h, 5 g of a copper screen and/or two 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 performed the same way as in the free chlorine test (Section 2.7.3). Before taking the samples, the water was gently stirred to ensure water quality homogeneity. In these tests, $n = 2$ was true for the replicates of the half MD and copper screen, $n = 4$ was for the two gels, and $n = 1$ was for the no-treatment control. Tests were conducted under ambient conditions.

2.8. AI Tool Use

In the writing and preparation of the original draft of this manuscript, the first author of this paper used ChatGPT to improve the language since her first language is not English. Specifically, what was asked to the chat was to re-write the paragraphs that the first author initially wrote. Then, in case there were significant improvements that were correct, they were used in the manuscript.

3. Results and Discussion

3.1. Quantification of Chlorine Loaded in the Gels

Upon exposure to a free chlorine solution, the gels changed in appearance, becoming stiffer and darker (more intense yellow). After chlorination, it was determined how the Cl^+ loaded into the gels correlated with loading time (12, 24, and 48 h). It was found that the Cl^+ content (mg of Cl^+) in the polymer gels increased with chlorination time (Figure 6), and after 48 h, the average Cl^+ content reached ~65% of the theoretical maximum. This result was reasonable because the theoretical maximum Cl^+ content assumed 100% incorporation of VBDMH monomer into the gel, but from monitoring VBDMH removal (Figure A4), it is known that the monomer did not incorporate fully into the gels. Based on these findings, it was chosen to proceed with 48 h of chlorination of synthesized gels to maximize Cl^+ content without requiring several days of loading. Future studies could explore the use of more concentrated hypochlorite solutions to determine if shorter chlorination times were feasible. Commercial bleach, a readily available chlorine product that users could employ to recharge the gels in household settings, was more concentrated (ca. ~4% Cl_2), so examining the loading of these gels under conditions of anticipated use would be an important future direction.

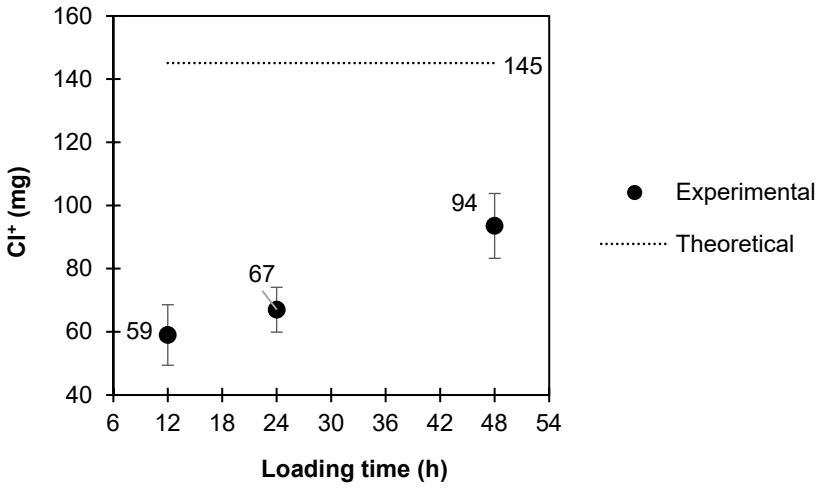

Figure 6. Calculated Cl^+ content in gels (theoretically containing 1 g VBDMH) with respect to loading time. NaOCl solution at t = 0 h: 300 mL of 0.25% w/v Cl_2 or 2464 mg Cl_2 equivalent to 1232 mg Cl^+ for each 1 g VBDMH monomer gel. Error bars indicate standard error ($n = 2$).

3.2. Chlorine Release Tests

The release of chlorine from a chlorinated gel in SGW was evaluated using the HACH 10241 spectroscope. Chlorine release above the detection limit of this assay (40 µg/L Cl_2) was not observed. However, the presence of Cl+ in the polymer gel was confirmed by staining it with iodide, similar to what was previously reported [41]. Figure 7 shows that the chlorinated gel turned red in the presence of iodide in an acidic medium (iodide reacted with chlorine, forming yellowish-brown iodine), while the non-chlorinated gel did not.

Figure 7. Iodide staining test for a chlorinated VBDMH gel (**left**) and a non-chlorinated VBDMH gel (**right**).

Although chlorine in the solution surrounding the gels was not detected, it was reasoned that if chlorine in concentrations below 40 µg/L was released in water, it would be available for pathogen inactivation in levels well below those known to cause adverse taste and smell. The next sections outlined the series of experiments conducted to examine this notion.

3.3. Bacteria Inactivation Efficacy Tests

3.3.1. *E. coli* Inactivation Tests with Low Doses of Free Chlorine

First, the efficacies of low doses of free chlorine (5–20 µg/L Cl_2) were tested to determine if concentrations below the detection limit of the spectroscopic method (40 µg/L) were sufficient to inactivate *E. coli* bacteria in SGW. Using these chlorine concentrations, *E. coli* inactivation ranging from 0.43- to 1.69-log_{10} reductions with a contact time of 8 h was observed (Figure 8).

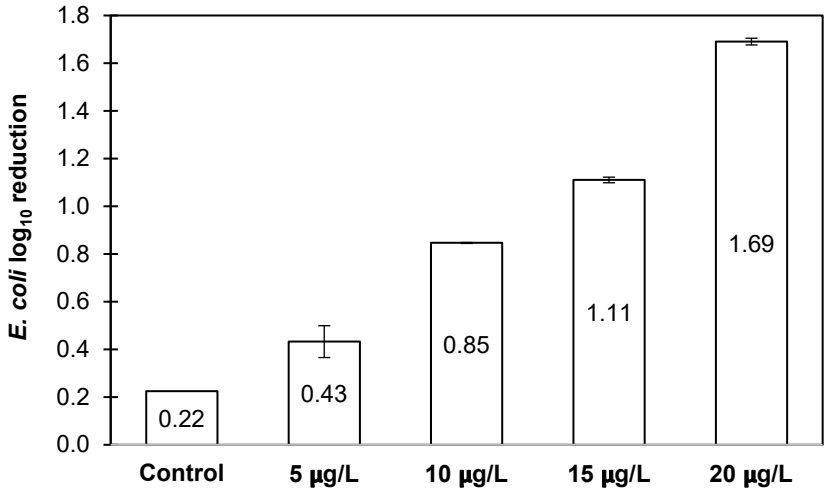

Figure 8. *E. coli* inactivation by low doses of free chlorine after 8 h treatment in 120 mL synthetic groundwater. Initial bacteria concentration in the water was ~60,000 MPN/100 mL or 4.79 in log_{10} scale. "Control": bacteria natural reduction. Error bars indicate standard deviation (n = 3, except for "control", where n = 1).

Encouraged by these findings, the next step was to examine the hypothesis that the introduction of chlorinated polymer gels in SGW containing *E. coli* led to bacteria reduction despite not detecting chlorine because the gels released chlorine at levels below 40 μg/L. Furthermore, by evaluating this hypothesis, it could indirectly be proven whether the gels were effectively releasing chlorine into the water.

3.3.2. *E. coli* Inactivation Tests with Chlorinated Polymer Gels

While *N*-chloramine polymers are known to inactivate bacteria primarily upon contact, it was 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 the spectroscopic method detection limit (40 μg/L Cl_2).

Figure 9 shows that the chlorinated polymer gels were effective for *E. coli* inactivation (some achieving complete bacteria reduction), whereas the chlorinated styrene control gels did not. This provided evidence that the efficacy of bacteria inactivation did not come from chlorine in a solution that was trapped in the polymer gels during loading and later came out of the gels. Additionally, the VBDMH gels that were not chlorinated were not effective for *E. coli* inactivation. This also suggested that bacteria reduction did not arise from the chloramine precursor gels. Through the process of elimination, these results suggested that the gels released sufficient chlorine for E. coli reduction but were insufficient for spectroscopic detection. Additionally, these findings corroborated previous studies that reported the release of chlorine from *N*-chloramines [29–31,33]. In other words, bacterial inactivation results could be attributed to a slow dissociation of chlorine from the amines.

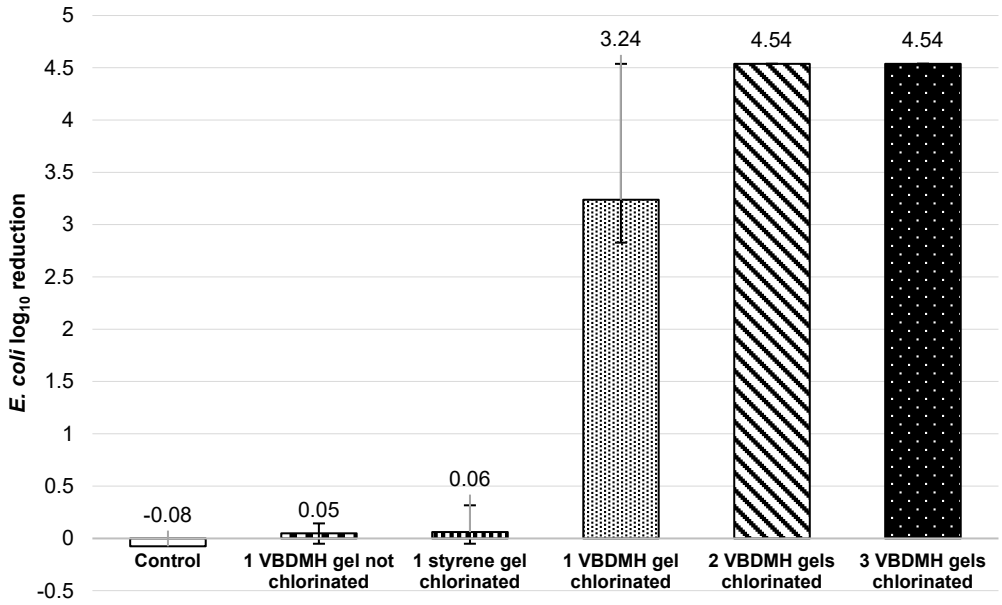

Figure 9. Tests (i) and (ii): *E. coli* inactivation by chlorinated VBDMH and styrene gels, 8 h treatment in 250 mL synthetic groundwater. Initial bacteria concentration in the water was ~35,000 MPN/100 mL or 4.54 in $\log_{10}$ scale. "Control": bacteria natural reduction. Each VBDMH gel contained 1 g of this monomer (or 4 mmol), while the styrene gels contained 4 mmol styrene instead of VBDMH. All the gels were chlorinated for 48 h. Error bars indicate the range (n = 3, except for "control", where n = 1). For tests with 2 and 3 VBDMH gels, error bars are not visible because complete inactivation was observed in all replicates.

It is important to clarify that the tests were performed in water that did not have chlorine demand. Therefore, all the chlorine was available for bacteria disinfection, and the results presented above could be viewed as confirmation that the gels had a bacteria inactivation capacity. However, the disinfection results could significantly change in waters containing high levels of organics or chlorine demand or in natural surface waters with

different compositions, and testing the gels' inactivation efficacies in these types of waters would be an important future direction.

3.3.3. Effect of Gel Cl^+ Content in Bacteria Inactivation Efficacy

Tests to determine the efficacy of *E. coli* inactivation using gels containing different theoretical amounts of VBDMH (ranging from 1 to 3 g VBDMH, or 145 to 435 mg Cl^+) were also conducted. While these Cl^+ amounts were high, the release of chlorine was suspected to occur at only very low levels because it could not be detected.

Figure 9 results demonstrate that the $\log_{10}$ reduction in *E. coli* increased with the amount of VBDMH present in the gels. After 8 h in 250 mL, SGW inoculated with *E. coli*, a gel containing 1 g VBDMH produced a $\log_{10}$ reduction of 3.24, while the 2 and 3 gels led to a $\log_{10}$ reduction of 4.54. It is important to clarify that when the 2 and 3 gels were used, the bacteria inactivation reached the limit of sensitivity for the test (this limit was calculated based on the bacteria concentration at t = 0 h, and it represented the maximum $\log_{10}$ reduction that could be achieved) or a 100% inactivation. Potentially, if a higher bacteria concentration (i.e., *E. coli*: $>10^{4.54}$ MPN/100 mL) was present before starting the inactivation test, a higher $\log_{10}$ reduction could have been achieved by the 2 and/or 3 gels.

3.3.4. Effect of Gel Chlorination Time on Bacteria Inactivation Efficacy

In SGW, the *E. coli* inactivation efficacy of polymer gels that had been chlorinated for different times (i.e., 12, 24, and 48 h) was tested. The results in Figure 10 demonstrate that the $\log_{10}$ reduction of bacteria increased with 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 h of contact time, which was consistent with the higher Cl^+ loading in gels loaded for 48 h (Figure 6).

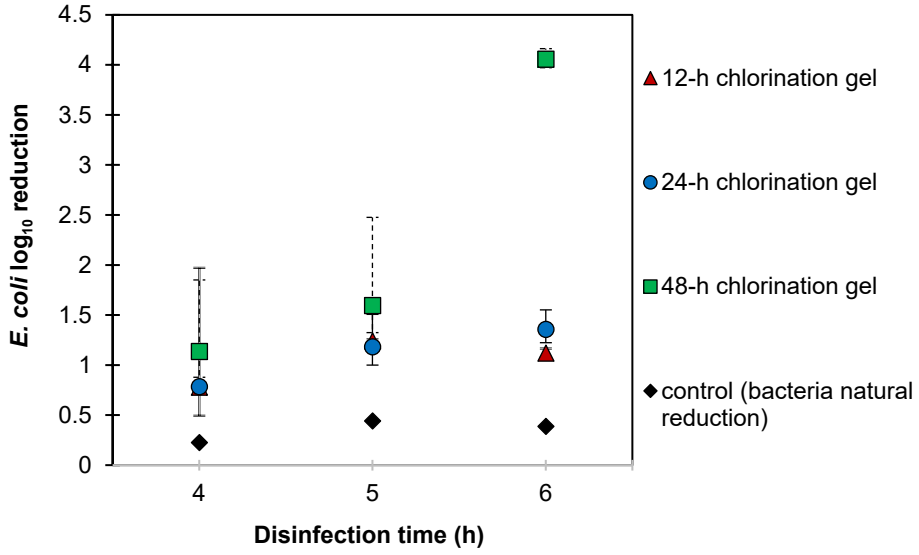

Figure 10. Test (iii): *E. coli* reduction over time with respect to 1 g VBDMH gels as a function of chlorine loading time in 120 mL synthetic groundwater. Initial bacteria concentration in the water was ~30,000 MPN/100 mL or 4.46 in $\log_{10}$ scale. Error bars indicate the range (n = 2, except for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

3.3.5. Polymer Gels Combined with the MadiDrop (MD) and/or Copper Screen

To determine the impacts of combining chloramine-functionalized gels with silver- or copper-releasing materials, *E. coli* inactivation by the chlorinated gels combined with the silver-releasing MD or a copper screen was evaluated in SGW (5 L) with an initial *E. coli* concentration of ~10^5 MPN/100 mL. For combinations of the gels with MD, the achieved inactivation, in terms of $\log_{10}$ reduction, was compared with (i) 2 gels, (ii) half MD, and (iii) half MD + 2 gels (added simultaneously). Similarly, for combinations of the

gels with a copper screen, the achieved inactivation was compared with (i) 2 gels, (ii) screen, and (iii) screen + 2 gels (added simultaneously). Rather than testing in 120 mL solutions, these combination experiments were conducted in 5 L solutions because MD tablets were designed for use in larger containers (10 L); it would be impractical and imprecise to cut the MD tablets into more than two pieces as done here for use with 5 L water.

Combining the gels with half MD led to a higher reduction than when either material was used independently, with the combination approaching a 2-$\log_{10}$ reduction (Figure 11). Potentially, 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 11 shows that the copper screen was not effective for *E. coli* inactivation within an 8 h contact time. Furthermore, when the screen was combined with the polymer gels, there was essentially no improvement or deterioration in the reduction of bacteria compared to the gels alone. Together, these results were promising and suggested the potential for the gels to serve as an alternative to existing commercial chlorine-based POU technologies while also improving silver-based POU technologies.

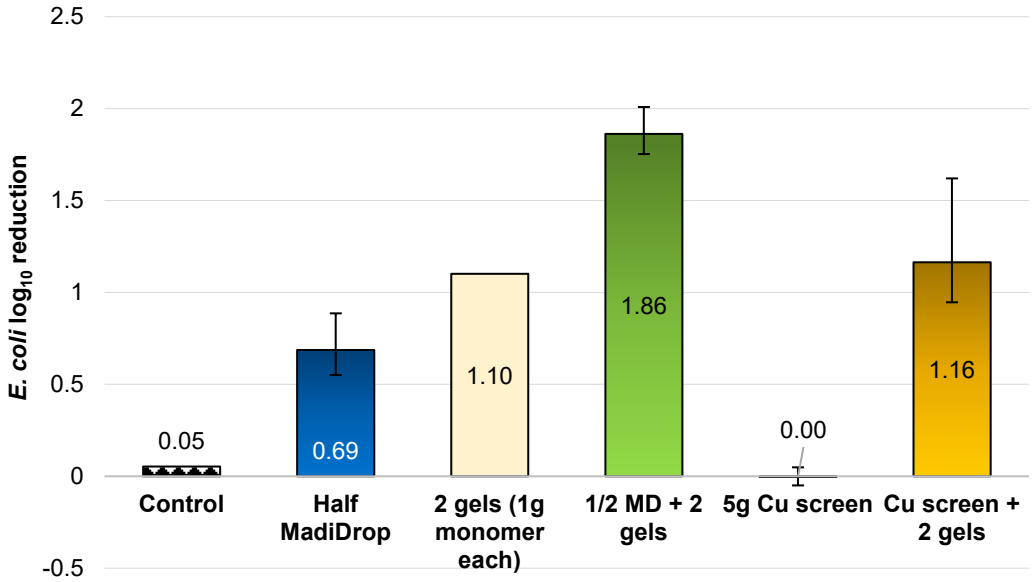

Figure 11. Testing *E. coli* inactivation efficacy of the VBDMH chlorinated gels combined with the MadiDrop (MD) or copper screen after 8 h treatment in 5 L synthetic groundwater. The MD is designed to be used in a water volume of 10 L. Since these tests were done using 5 L, half of the MD was tested. The theoretical Cl^+ content of a gel that contains 1 g VBDMH monomer is 145 mg. Initial bacteria concentration in the water was ~10^5 MPN/100 mL or ~5 in $\log_{10}$ scale. "Control": bacteria natural reduction. n = 2 except for: "2 gels", 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 h.

Figure 11 shows that for *E. coli* bacteria inactivation, two gels, each prepared with 1 g VBDMH monomer, achieved a 1.10-$\log_{10}$ reduction after an 8 h contact time in 5 L of 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 a 100% reduction. The lower $\log_{10}$ reductions observed here, as compared with those exceeding four shown in Figure 9, are attributed to the larger volume of inoculated solutions per gel in these combination experiments. Additionally, it could be explored whether there was a correlation between the number of gel units and contact time since shorter treatment times would be ideal.

The results of combining the gels with the MD and/or the copper screen indicated that there were no antagonistic or negative effects on *E. coli* reduction. This suggested

that increasing the number of gels could further enhance the reduction in the number of pathogens. However, as the number of gels increased, so did the amount of chlorine released, requiring an evaluation of the effect on the silver and copper released from the other materials due to the strong oxidation potential of chlorine. It was 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 concentrations of these chemicals exceeded the recommended levels.

3.4. Silver–Chlorine Interaction

As previously mentioned, since the water was not filtered through the gels, the mechanism of pathogen inactivation by the *N*-chloramines contained in the gels involved Cl^+ dissociation and free chlorine formation, which was available for water disinfection through diffusion into the bulk solution [32]. Therefore, it was important to emphasize that chloride (Cl^-) would not be released from the gels and that Cl^+ released from the gels would not form AgCl with the silver released from the MD.

However, the suggested inactivation mechanisms of bacteria with free chlorine included the oxidation of the cell membrane, DNA damage, and respiration inhibition where free chlorine targeted many parts of the cell structure and metabolism [46]. Based on these mechanisms, during bacteria inactivation, Cl^+, being a strong oxidant, could potentially reduce to Cl^-.

3.5. Gel Rechargeability Preliminary Results

Recharged gels were tested for *E. coli* inactivation, but there were not enough replicates. For this reason, those results were not included in this manuscript. The preliminary results showed that after the gels were recharged, they still showed bacteria inactivation capacities.

4. Conclusions

This work provides a detailed account of the development of a rechargeable *N*-chloramine polymer gel potentially capable of releasing chlorine at low doses in water. Different gel formulations were tested, and the most mechanically stable formulation was selected. The concentrations of potentially released chlorine from the chlorinated gels in water were below the detection limit (40 µg/L Cl_2) of the spectroscopic method. However, it was found that the concentration of chlorine released from the gels was sufficient to inactivate *E. coli* bacteria in synthetic groundwater (SGW). Furthermore, the results indicated that the degree of bacteria reduction was linked to the polymer gels' loading or chlorinating time, as well as the quantities of *N*-chloramine or VBDMH monomer present in the gels. Finally, it was found that the use of polymer gels in conjunction with the MadiDrop (a point-of-use, POU, technology that releases silver for water disinfection) resulted in a greater reduction in the amount of *E. coli* bacteria in SGW compared to when the materials were used alone.

These laboratory findings are promising and contribute to the future development of the gels, which could potentially become a chlorine-based POU water disinfection technology. This product could work in conjunction with silver-based technologies. Future work should focus on the long-term usage of the gels, including stability and rechargeability evaluation and field studies considering natural water sources, social acceptability, and affordability.

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Conflicts of Interest: J.A.S. is the Chief Technology Officer of Silivhere Technologies Inc., which produces and sells the MadiDrop technology used in this study. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

Appendix A. Synthesis of the Chloramine Precursor 3-(4′-Vinylbenzyl)-5,5-dimethylhydantion (VBDMH) Monomer

Based on prior work conducted by [36], VBDMH was synthesized from 4-vinyl benzyl chloride (VBC) and 5,5-dimethyl hydantoin (DMH) in the presence of potassium hydroxide (KOH). First, DMH (12.8 g, 0.1 mol; 97%, Acros Organics, Pittsburgh, PA) and KOH (5.6 g, 0.1 mol; VWR Scientific, Radnor, PA, USA) were added to ethyl alcohol (80 mL; Pure, 200 proof, Sigma-Aldrich, St. Louis, MO, USA) and stirred at 60 °C until the DMH dissolved. Then, VBC (14 mL, 0.1 mol; 90%, Sigma-Aldrich) was dissolved in methanol (50 mL; ACS, ≥99.8%, Sigma-Aldrich) and added to the DMH solution, which was stirred at 65 °C overnight. After cooling to room temperature, VBDMH was recrystallized by the addition of methanol (200 mL) to dissolve the VBDMH monomer, which was followed by the slow addition of water (300 mL) to crystallize VBDMH while keeping the other components dissolved. The crystals were collected by filtration and dried under vacuum conditions. The average yield was 64%.

The purities of the monomer before and after purification were determined using high-performance liquid chromatography (HPLC) traces; see Figure A1. The final purity of the monomer was 89%, which was determined by high-performance liquid chromatography (HPLC). In Figure A1, 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 the monomer.

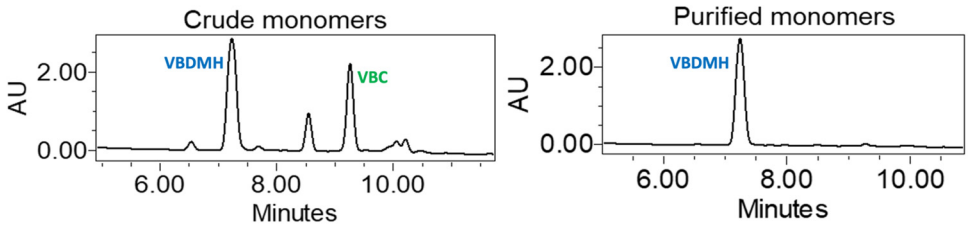

Figure A1. High-performance liquid chromatography (HPLC) traces of the crude and purified VBDMH monomers.

The structures 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); see Figure A2. Chemical shift changes occurred for protons *e* (peak at ~7.5 ppm) and *f* (peak at ~4.75 ppm) in the benzyl ring region, which was indicative of 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 the appearance of protons *g* (peak at ~8.5 ppm) and *h* (peak at ~1.25 ppm) demonstrated successful VBC modification with DMH.

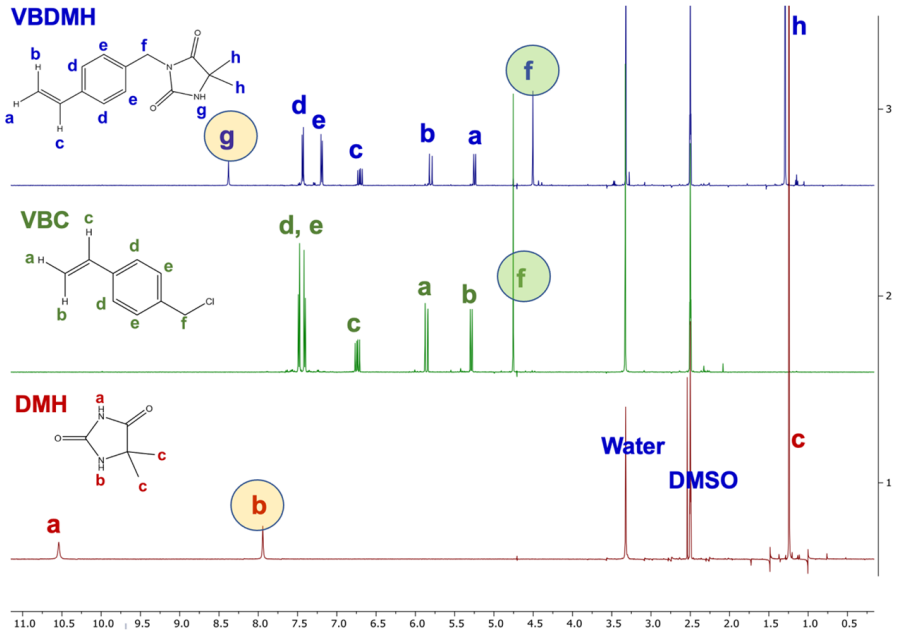

Figure A2. ^{1}H-NMR spectra of VBDMH, VBC, and DMH.

Appendix B. Gel Formulations

To tune water uptake and the loading and release of chlorine, molar ratios of VBDMH:PEGMA (amine monomer: hydrophilic group) were varied. The following molar ratios 1:0, 1:0.5, 1:1, and 1:5 (see Table A1) were tested. 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 A1. 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, it was observed that increasing the amount of PEGMA generally reduced the stability of the gel because their mechanical integrity changed or even broke into pieces. Consequently, the mechanical integrity of the gels limited us to a composition range of around 1:0.5 VBDMH:PEGMA (see Figure A3). Therefore, the formulation consisting of 1:0.5:0.1 mol VBDMH:PEGMA:HDDA, 1% of the total formulation mass (including the three other reagents and the solvent) for the photo-initiator, and DMF (30% *w*/*v*, or 1.67 mL DMF per 0.5 g VBDMH) was selected to prepare the precursors or polymer gels.

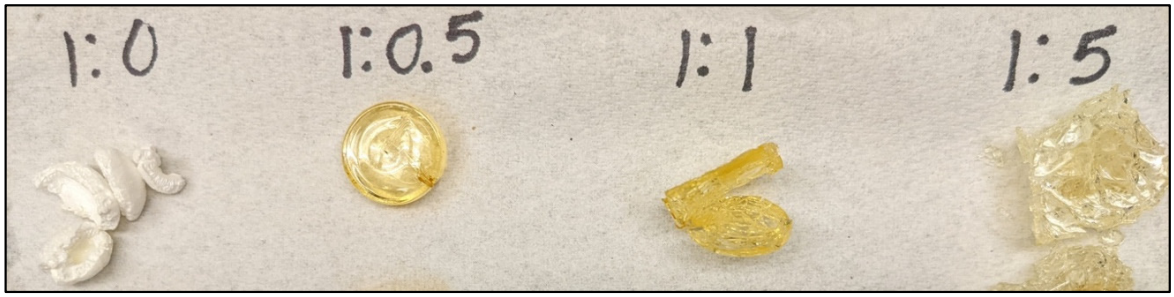

Figure A3. Polymer gels with varying VBDMH:PEGMA molar ratios (see the formulations in Table A1).

Appendix C. Gel Washes

Removal of unreacted material and solvent from the synthesized polymer gels was monitored by HPLC (see Figure A4). Following gelation, washing the gels using five methanol/water mixtures successfully eliminated unreacted monomers. Figure A4 shows that the initial supernatant washes contained solvent (DMF) and unreacted VBDMH and PEGMA, while the last wash (purple line) removed these reagents. The peaks in the HPLC chromatographs were identified by running each reagent in water or methanol (VBDMH was insoluble in water).

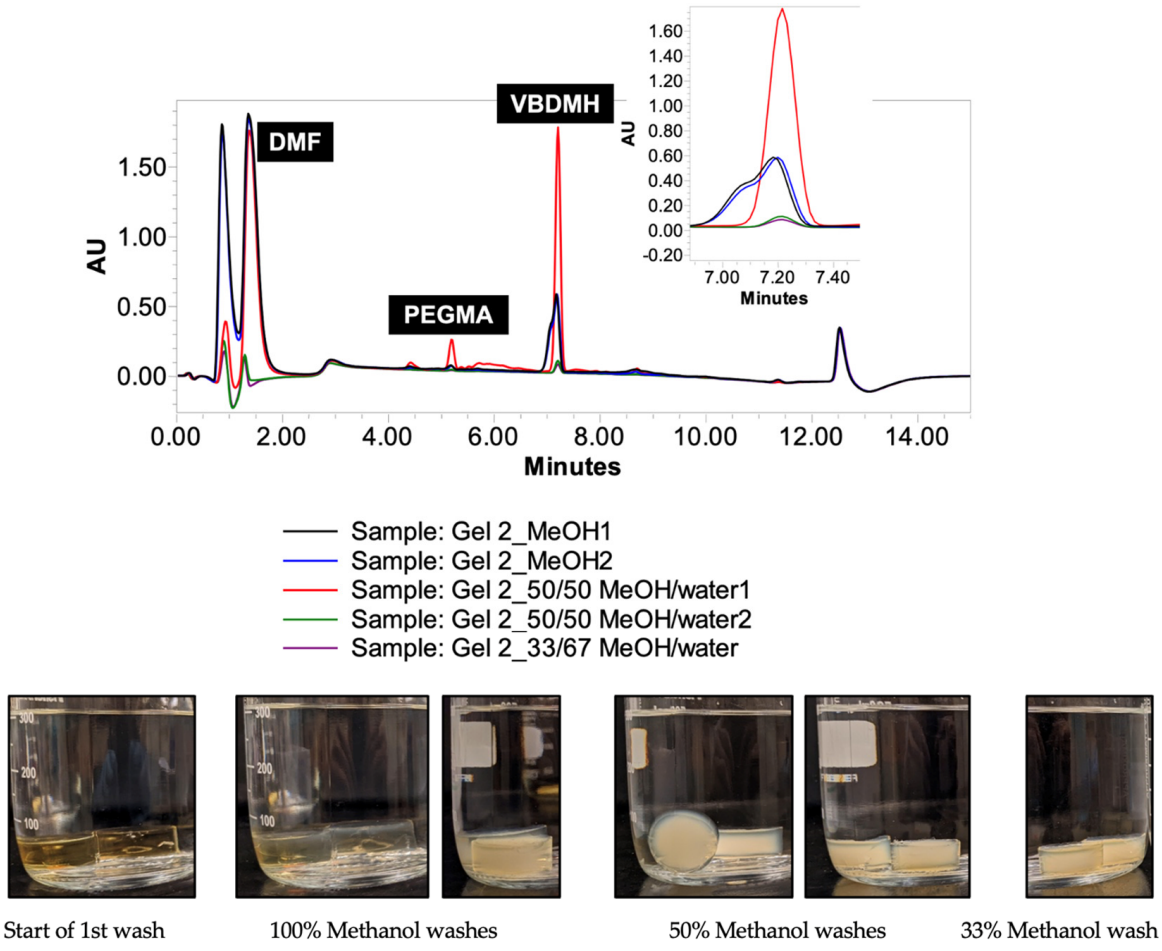

Figure A4. High-performance liquid chromatography (HPLC) traces of the supernatants from the gel washes: (i) 100% methanol, black trace; (ii) 100% methanol, blue trace; (iii) 50/50 methanol/water v/v, red trace; (iv) 50/50 methanol/water v/v, green trace; and (v) 33/67 methanol/water v/v, purple trace.

Appendix D. Quantification of Chlorine Loaded in the Gels

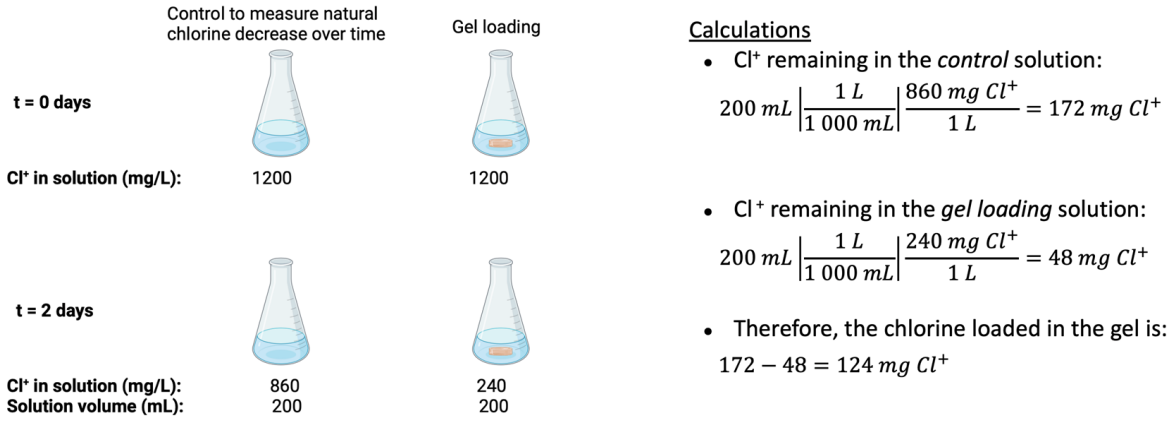

Figure A5. Schematic and calculations example of the method developed to examine loading of the Cl^+ into polymer gels.

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