

An Innovative Antibacterial Water Filter to Improve Public Health Outcomes

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Abstract Poor access to clean drinking water is responsible for millions of water-borne disease deaths each year and is a critical issue worldwide. Because most household water filters are not antibacterial, impure drinking water disproportionately impacts communities with poor public water treatment. Chitosan is an abundant natural biopolymer that has remarkable antibiotic properties due to its molecular structure. An antibacterial water filter was developed by adsorbing two molecular weights of chitosan onto activated carbon (AC) to synthesize novel compounds which effectively deliver chitosan for water treatment. An in vitro evaluation of the filter's antibacterial properties was performed by measuring Colony Forming Units (CFUs) in the water sample. Additionally, a Kirby-Bauer Disk Diffusion Susceptibility Test was used to distinguish between the efficacies of high molecular weight chitosan (HMWC) and low molecular weight chitosan (LMWC). Based on inhibition zone measurements, it was determined that HMWC is most effective at eliminating bacteria from contaminated water. Water purified using the HMWC filter had bacterial growth of 0 colonies/mL, whereas the control sample contained 7600 colonies/mL, indicating a 100% statistical reduction. The chitosan-AC compound filter developed in this experiment has several practical applications and can be used to enhance the performance of traditional household water filters used worldwide. This innovative, low-cost filter may also be used during emergencies and natural disasters as well as in the military. Furthermore, since chitosan is derived from nature and is biodegradable unlike synthetic antibiotics, its use does not contribute to environmental pollution or antimicrobial resistance.

Keywords: Low Molecular Weight Chitosan, High Molecular Weight Chitosan, Colony Forming Units, Zone of inhibition

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1. Introduction

Water, the most basic necessity, sustains life on earth. However, many developing regions of the world still lack access to clean drinking water. In fact, 3.5 million people die from water-borne diseases annually, which is the single largest cause of death in the world today [1].

Currently, ceramic water filters are used in many developing regions because their porous structures can trap particulate matter. Although this solution is low-cost, environmentally friendly, and easily accessible, ceramic, along with most commercial-grade household water filters, lack antibacterial properties. Additionally, although the use of synthetic antibiotics for water treatment has been proposed, such a solution would worsen already pressing concerns of antimicrobial resistance, as many strains of water-borne bacteria have already become resistant to synthetic antibiotics.

The challenge of designing an effective antibacterial water filter that is both environmentally friendly and globally accessible remains unresolved. Along with the

two challenges mentioned, there is also the added challenge of designing a filter that is also relatively inexpensive. This paper proposes the design of an innovative water filter using chitosan, a natural antibiotic agent, in order to treat water cost-effectively without contributing to antimicrobial resistance.

Chitosan, a byproduct of chitin, is widely available in natural sources such as shrimp, crabs, crawfish, and insects. As the world's second largest naturally occurring biopolymer, chitosan is an abundant and biodegradable resource [2]. Chitosan's hydroxyl (-OH) and amino (NH₂) functional groups are thought to play an important role in its antibacterial properties [3]. Chitosan binds to negatively charged bacterial cell walls, disrupting cellular operations and molecular exchanges across the cell membrane. It then attaches to bacterial DNA and inhibits DNA repair, accelerating cellular stress, which eventually leads to bacterial cell death.

2. Rationale

Chitosan compounds have been used in prior research

for particle filtration and purification [4,5]. Although its potential for antibacterial water treatment has already been somewhat understood, chitosan's practical applications to purify contaminated water in the real-world have not yet been achieved due to complications with creating chitosan compounds that can be delivered efficiently [6,7]. Activated carbon has an extremely high surface area to volume ratio. By adsorbing two different molecular weights of chitosan onto activated carbon (AC) novel compounds were synthesized which can effectively deliver chitosan to treat water.

Therefore, the goals of this research are: (1). to establish in vitro the most appropriate molecular weight and concentration of chitosan to use in an antibacterial water filter and (2). to develop chitosan-based compounds for designing an antibacterial water filter capable of removing bacteria from natural water sources.

It can be hypothesized that chitosan-based compounds with optimized delivery and concentration will eliminate waterborne bacteria due to chitosan's natural antibacterial properties. These compounds, when applied to a water filter, will enable practical applications that effectively reduce water-borne diseases.

The independent variables are the molecular weights and concentrations of the chitosan solutions. The dependent variable is the number of colony forming units (CFUs) observed when the treated water is cultured. The control group of this study is a water filter without the chitosan-AC compound.

3. Methods

Two sterile 5 mL containers were filled with pond water from Shakerag Park in Johns Creek, GA. The containers were sealed and placed in a sterile bag to avoid contamination during transport. They were then stored at room temperature until inoculation. Pond water has an abundance of bacteria and offers a excellent way to test the filter performance [9].

Both the 90% deacetylated and 86.4% deacetylated chitosan solutions, which were diluted with acetic acid, used in this study were obtained from Tidal Vision. The 4% LMWC solution was used as the starting concentration. First, 5 mL of the 4% LMWC solution was added to a sterile container. This solution was then successively diluted to make the 2%, 1%, and 0.5% LMWC solutions. A similar method was used to create 2% and 1% HMWC solutions.

3.1. Kirby-Bauer Disk Diffusion Susceptibility Test

The Kirby-Bauer Disk Diffusion Susceptibility Test was used in this study as a reliable method for measuring the resistance of bacteria against chitosan [8]. After the agar plate was thoroughly inoculated, it was split into five sections using a permanent marker. Each section was used to observe a different concentration of chitosan. A filter paper disk was then soaked in each concentration of chitosan solution. Once the disks were fully soaked, they were carefully placed onto the agar plate in their respective sections. Afterwards, the agar plates were

incubated at 37°C for 24 hours. After the agar has properly diffused and the bacteria has grown, the efficacy of chitosan was defined by the diameter of the empty ring, or zone of inhibition, that surrounds the disk. The zone of inhibition is proportional to the overall effectiveness of the antibiotic. These steps were repeated four times for a total of five trials. This procedure was also used in the HMWC experiments.

3.2. Antimicrobial Water Filter Design

3.2.1. Chitosan-Carbon Compound

A water filter that can effectively deliver chitosan to untreated water is currently unknown of. However, due to its structure and properties, activated carbon (AC) has the potential to solve this issue. AC has a porous structure due to its microtubules, drastically increasing surface area to 32,000 ft²/g. AC is also capable of adsorption. Due to these benefits, a novel compound made by adsorbing chitosan onto AC is being proposed. The high surface area of AC allows for increased contact between chitosan and the bacteria. The AC used in this study is granulated charcoal derived from coconuts.

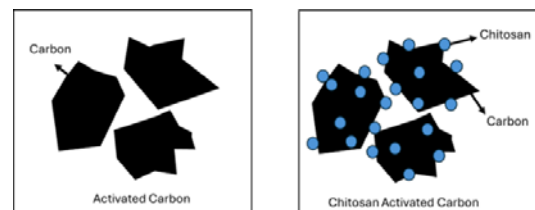


Figure 1. Chitosan activated carbon compound

A mixture of chitosan and AC was agitated at 80°C to deposit chitosan molecules onto AC, allowing the AC to adsorb the chitosan. This mixture was then added to a magnetic stirrer to ensure that the chitosan was uniformly distributed onto the AC. The mixture was filtered and dried to extract the chitosan-AC compound.

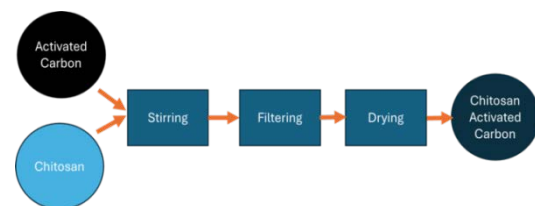


Figure 1. Method for synthesizing chitosan infused activated carbon

Two types of the compound are synthesized: one with LMWC and the other with HMWC.

3.2.2. Water Filter with Chitosan Compound

Ceramic water filters are widely used in many developing regions of the world because they are easily manufactured, causing them to be inexpensive and widely accessible. However, these filters do not possess antibacterial properties, so enhancing their performance will have a wide impact and extensive applications. This goal can be achieved using the novel chitosan-AC compounds that were developed. Running water through a ceramic filter without chitosan serves as a baseline of the water quality in many parts of the world. Adding chitosan-AC compounds

as a layer to this filter provides an accurate measurement of the benefit that is desired to be achieved.

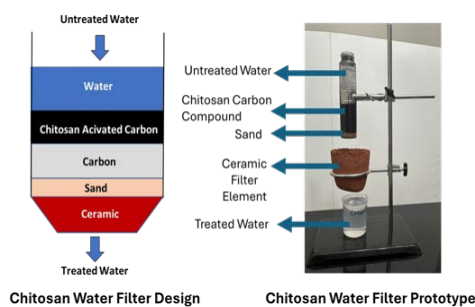


Figure 2. Water filter prototype design using chitosan activated carbon compounds

3.3. Performance Evaluation of the Filter

The efficiency of the proposed filter was evaluated by measuring the number of bacteria/mL in the treated water. The control group of this study is a water filter without the chitosan-AC compound. To measure the bacteria/mL, filtered water was cultured and the bacterial colonies were counted. Each colony is thought to have grown from a single bacterium, and by counting the number of colonies, the total amount of bacteria can be estimated. However, as the number of bacteria is expected to be too large to count, the serial dilution method is used.

In the serial dilution method 1 mL of the filtered water was diluted by 9 mL of distilled water. 1 mL of this solution was then diluted once again with 9 mL of distilled water. This process was repeated 3-4 times as needed. This allows the bacterial colonies to be counted using the viable cell count method.

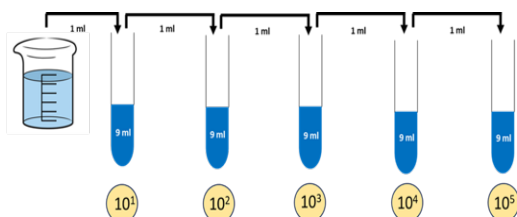


Figure 3. CFU counting with viable cell count method and serial dilution

After the sample was diluted to the desired factor, 0.1 mL was swabbed onto an agar plate and incubated at 32°C for 24 hours. The number of Colony Forming Units (CFUs) were then counted. By counting the number of CFUs/mL in the diluted sample, the number of CFUs/mL in the original sample can be estimated using this formula:

$$\frac{\text{CFUs}}{\text{mL}} \text{ in water sample} = \frac{\text{CFUs on plate}}{\text{Volume in mL cultured}} \times 10^N \quad (1)$$

The procedure is repeated for the scenarios as described below:

1. The CFUs/mL in untreated water were measured.
2. The CFUs/mL were measured in water passed through an AC and ceramic filter. This filter did not contain the chitosan-AC compound.
3. The CFUs/mL were measured in water passed through a ceramic filter containing a chitosan-AC compound derived from LMWC.

4. The CFUs/mL were measured in water passed through a ceramic filter containing a chitosan-AC compound derived from HMWC.
5. trials were performed for each type of filter. Each trial was cultured in a separate agar plate.

4. Discussion

The experiment contained thirty different experimental groups, five for four different concentrations of LMWC, and five for two different concentrations of HMWC. Trials with 4%, 2%, 1%, 0.5%, and 0% LMWC had an average zone of inhibition of 36.2 mm, 27 mm, 17.4 mm, 14.6 mm, and 0 mm respectively. In the case of HMWC, trial with 2%, 1% and 0% concentrations had average inhibition zones of 35.2 mm, 29 mm and 0 mm respectively.

Table 1. Diameter of the Zone of Inhibition with Different Concentrations of LMWC Solution

Diameter of Inhibition Zone (mm)	4% LMWC	2% LMWC	1% LMWC	0.5% LMWC
Trial 1	34	27	11	14
Trial 2	37	27	16	16
Trial 3	36	25	18	15
Trial 4	42	28	27	15
Trial 5	32	28	15	13
Average	36.32	27	17.4	14.6

Table 2. Diameter of the Zone of Inhibition with Different Concentrations of HMWC Solution

Diameter of Inhibition Zone (mm)	2% HMWC	1% HMWC
Trial 1	38	32
Trial 2	33	27
Trial 3	30	22
Trial 4	34	25
Trial 5	41	36
Average	35.2	29

It should also be considered that Trials 1-5, which were done using HMWC, displayed a larger zone of inhibition than the experiments conducted with LMWC. For example, the diameter of the zone of inhibition for 2% LMWC and 2% HMWC had a mean difference of 8.2 mm. 4% LMWC, compared to the other concentrations of LMWC tested, provides the largest zone of inhibition. However, for a given concentration, HMWC displays a larger zone of inhibition compared to LMWC.

4% LMWC exhibits a zone of inhibition of 36.32 mm. This is close to the zone of inhibition of 2% heavy weight chitosan at 35.2 mm. This shows that LMWC, at twice the concentration of HMWC, has the same antibacterial potency. Similar observation can be seen from the zones of inhibition of 2% LMWC and 1% LMWC.

Untreated pond water had the highest number of CFUs/mL. From the 5 trials that were performed, Trial 4 showed the highest number of CFUs/mL, while Trial 3 had the lowest. The mean CFUs/mL from untreated pond water was 7600. The standard deviation was 2073.6. When the water was treated with AC only, the CFU/mL was lower than the untreated water. Trial 2 recorded the

highest number of CFUs/mL, while Trial 5 had the lowest. The mean CFUs/mL was 1620 with a mean absolute deviation of 432.4.

Table 3. Comparison of CFUs/mL of different chitosan-AC compounds

CFUs/mL	Untreated Water	Activated Carbon	LMW Chitosan Carbon Compound	HMW Chitosan Carbon Compound
Trial 1	6000	1500	70	0
Trial 2	8000	2200	0	0
Trial 3	5000	1900	40	0
Trial 4	10000	1400	30	0
Trial 5	9000	1100	0	0
Average	7600	1620	28	0

As shown by the CFUs/mL, water treated with the LMWC-AC compound almost completely eliminated the bacteria. In Trials 3 and 5 no colonies could be detected. Trial 2 had the highest number of CFUs/mL of 60. The mean CFUs/mL for water treated with the LMWC-AC compound is 16. The standard deviation is 29.4. The HMWC-AC compound was the most effective and completely eliminated bacteria from pond water. There was no noticeable growth of bacteria in the 5 trials of water with the HMWC-AC compound.

In order to evaluate the performance of the chitosan compounds developed in this paper and quantify the improvement filters the % efficacy of the filter is defined as below:

$$\% \text{ Efficacy} = \frac{|\text{Final CFU} - \text{Initial CFU}| * 100}{\text{Initial CFU}} \quad (2)$$

% Efficacy represents how much of the bacteria from the original sample was successfully removed by the compound. The % efficacy of the different filters is shown in Table 4.

Table 4. Efficacy of water filter with different compounds

Compound	% Efficacy
Activated Carbon	78.68%
LMWC Activated Carbon Compound	99.63%
HMWC Activated Carbon Compound	100%

From the table, it can be seen that AC filter eliminated approximately 78.68% of bacteria from the original sample on average. This is good improvement but LMWC-AC compound filters eliminate approximately 99.63% of bacteria from untreated water which is a significant improvement. HMWC-AC compound completely eliminated all bacteria.

The results from this research strongly suggests that chitosan-activated carbon compounds can be used to create an antibacterial water filter. In regions where people often consume unsafe water, chitosan-activated carbon filters can address the non-potable water associated public health challenges, enhancing the quality of life for hundreds of millions of people. This filter may serve as a

solution for obtaining clean drinking water when an existing infrastructure is compromised. Similarly the filter may provide access to safe drinking water from natural sources during emergencies and times of war when other options are scarce. As chitosan is a natural substance, its manufacture and application do not contribute to environmental pollution. It is biodegradable and non-toxic, aiding in the fight against antimicrobial resistance and environmental degradation.

5. Conclusions

Innovative compounds of chitosan and activated carbon have been created by incorporating chitosan into the porous structure of activated carbon. As seen from the reduced CFU/mL counts, the chitosan-AC compound filter is an effective natural antibacterial filter to treat water from contaminated sources such as ponds and lakes. In accessing the antibacterial efficiency, the size of the observed inhibition zone was proportional to the concentration of the chitosan applied. Furthermore, the molecular weight of the chitosan was found to have a noticeable impact on the diameter of the zone of inhibition. HMWC has stronger antibiotic properties than LMWC. LMWC at twice the concentration had the similar antibacterial efficacy as HMWC. Antibacterial water filters developed using LMWC and HMWC carbon compounds have shown high efficacy rates. The research has shown that chitosan-activated carbon compound is a multifunctional material that offers an efficient delivery system due to the porosity of activated carbon, expanding the applications of chitosan.

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