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Preparing and Testing a Magnetic Antimicrobial Silver Nanocomposite for Water Disinfection To Gain Experience at the Nanochemistry–Microbiology Interface

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ABSTRACT: We describe a 2 h introductory laboratory procedure that prepares a novel magnetic antimicrobial activated carbon nanocomposite in which nanoscale sized magnetite and silver particles are incorporated (MACAg). The MACAg nanocomposite has achieved the synergistic properties derived from its components and demonstrated its applicability as an effective and recoverable antimicrobial agent for water disinfection. The principle is successfully illustrated by a significant reduction in the number of microbes in an *Escherichia coli* (*E. coli*) solution of 2×10^6 colony forming units following its treatment with MACAg for 10 min. The exercise allows the college students to (1) be introduced to an exciting class of advanced materials, known as nanocomposites, at an early stage, (2) gain working experiences at nanochemistry–microbiology interface, and (3) see the use and experience the fun of chemistry. The experiment uses readily available materials, can be run in a general or introductory chemistry laboratory environment, and is well received and enjoyed by the students. The experiment is also suitable for advanced high school students.

INTRODUCTION

Numerous cutting-edge “nano”-based laboratory activities have appeared in this Journal over the past 25 years.^{1–14} Classic examples include the preparations, properties, and applications of ferrofluids, gold nanoparticles, silver nanoparticles, nickel nanowires, liquid crystals, quantum dots, and polyaniline nanofibers.^{5–11} These attractive, attention-grabbing, and easily adaptable laboratory exercises serve as a successful strategy to raise undergraduates’ enthusiasm toward science, technology, engineering, and mathematics (STEM) and to enhance their academic and occupational advancement opportunities.^{1–3} While the

number of published “nano” activities rapidly increases, laboratory experiments illustrating the concept, synthesis, and applications of nanocomposites, especially at the introductory level, remain limited.^{3,4} A nanocomposite is a multiphase solid material where one of the phases has one, two,

or three dimensions of less than 100 nm. Nanocomposites represent an exciting class of advanced materials due to their synergistic and/or hybrid properties derived from their components. An early exposure to this important class of materials may peak students’ interest in science.³

Silver has been known for its antimicrobial properties all around the world for centuries. It has long been used for treating infections, burns, and chronic wounds. Today's emerging nanotechnology only leads to even greater use of silver, in the form of silver nanoparticles (AgNPs), in everyday products. Examples range from burn creams, to food packaging, to odorless clothing and socks. Recently, intensive studies have

been focused on the possibility of using silver nanoparticles for water disinfection.^{15,16} Microbial contaminated drinking water poses a serious health concern, especially in developing countries.¹⁷ In the case of our students, who are studying for maritime careers, treating ballast water to remove invasive microorganisms, minimizing the dispersal of aquatic nuisance species via shipping, is of increasing importance.^{18,19} Current water disinfection methods include chemical disinfection, size exclusion, and UV radiation. However, chemical methods may produce carcinogenic side products, and size exclusion and UV radiation may be costly and time-consuming.²⁰⁻²² AgNPs may therefore provide an attractive alternative due to their (1) strong antimicrobial activity toward many different bacteria, fungi, algae, and viruses;²³⁻²⁶ (2) relatively low toxicity to humans (AgNPs concentrations of 2-4 ppm, effectively inhibit bacterial growth but are not toxic for human healthy cells);²⁷ and (3) relative inertness in water. However, two challenges may limit AgNPs' use in water disinfection: (1) their aggregation in water, and (2) their removal from treated water. Nanoparticles are known to have a strong tendency to form aggregates in water due to van der Waals forces and high surface energy. The antimicrobial activities of silver nanoparticles are a result of nanometer size, with the smaller particles having higher activities on the basis of equivalent silver mass contents.^{25,27} The aggregation of silver nanoparticles may reduce or diminish the particles' ability to disinfect. Recently, it has been reported that a high concentration of AgNPs (≥ 26 ppm) harms mammalian cells.²⁵ For this health concern, removing silver nanoparticles from water after disinfection is necessary. The removed AgNPs may also be reused for water disinfection, reducing the material cost and avoiding any adverse environmental effects which would be involved in their disposal.²³

As a sequel to our early publication,³ we describe here a 2 h introductory laboratory procedure that prepares a AgNP-based magnetic nanocomposite using activated carbon (AC), magnetite nanoparticles (MNPs), and AgNPs. AC is renowned for being an effective absorbent for the removal of a wide variety of organic matter, including microbes,^{3,28-30} due to its extended surface area, complex porous structure, high adsorption capacity, and high degree of surface reactivity. In the previous publication,³ we have successfully demonstrated that magnetic nanocomposite, prepared by embedding MNPs into AC (will be labeled as MAC), can serve as a fast, effective, low-cost, and recyclable organic water pollutant adsorbent. The current lab proposes the idea of incorporating AgNPs into the MAC matrix, referred to as MACAg, through chemical reduction of silver from its salt

solution to create additional biocidal properties. The approach aims to achieve and demonstrate the synergistic and/or hybrid properties of the nanocomposite derived from its components: (1) the superior antimicrobial properties of AgNPs for water disinfection; (2) the magnetic properties of MNPs for easy and fast removal of AgNPs from treated water and for making antimicrobial agent recoverable; (3) the superior adsorption ability of AC for removing various organic contaminants, including microbes; and (4) the immobilization of well-dispersed nanoparticles on the AC and/or the MAC surfaces that prevents the aggregation of magnetite and silver nanoparticles in water, helping retain the superior properties of these nanoparticles.

Figure 1 shows the antimicrobial properties of relevant components of the MACAg nanocomposite: while AgNPs (20- 40 nm, Aesar) have demonstrated a remarkable ability at stopping the growth of *Escherichia coli* (*E. coli*), a well-studied

Figure 1. Samples containing 0.25 mmol of AgNPs (B), AC (C), and MNPs (D) atoms were placed on a nutrient agar plate covered with 100 μ L of *E. coli* solution of 2×10^8 colony forming units per milliliter (control is in space A). Results after 2 days' incubation at 30 $^{\circ}$ C are shown in the photo: a large zone of inhibition was observed for AgNPs while the zones of inhibition of other components, MNPs and AC, were little or none. This work was done by the authors.

prokaryotic indicator organism, in a nutrient agar plate, MNPs and AC have shown little or none. The concerted properties of MACAg components will allow MACAg's potential application as an effective and recoverable antimicrobial agent for water disinfection and overcome the challenges the AgNPs alone may face.

The activity also addresses basic concepts of the nanoscale, electron configuration, lattice structure, oxidation-reduction reactions, as well as stoichiometry calculations. Other than nanotechnology, environmental, and material science courses, the activity supports typical curricula of college general chemistry, introductory chemistry, and advanced placement chemistry courses. Supporting Information includes handouts for students and notes for instructors.

EXPERIMENTAL OVERVIEW

Synthesis of Magnetic Silver (MACAg) Nanocomposite

Students prepared the magnetic nanocomposite matrix by embedding magnetite nanoparticles onto the AC microparticles to form the MAC nanocomposite using a simple one-step room temperature process published in this Journal.³ Various procedures for making silver nanoparticles are available in this Journal.^{7,12-14} We adapted the procedure reported by Solomon et al.⁷ The method produces a stable yellow colloidal silver with particles' sizes of 10–12 nm. To integrate silver nanoparticles onto the MAC matrix, students first coated the MAC matrix with borohydride ions by mixing MAC vigorously with 30 mL of 2.0 mM of chilled sodium borohydride (NaBH_4) solution for 5 min in a 150 mL beaker in an ice bath. With stirring, the students subsequently dripped a 2 mL volume of 1.0 mM silver nitrate into the chilled MAC matrix and NaBH_4 mixture. The flask was then placed on a magnet to allow the resultant MACAg nanocomposite to settle, and the clear liquid without any tint of yellow was decanted with the magnet in contact with the bottom of the flask. Finally, students washed MACAg with deionized water.

Antimicrobial Activity of MACAg Nanocomposite

After preparing MACAg, students transferred a 20 mL portion of an *E. coli* solution with a concentration of 1×10^5 colony forming units (cfu) per millimeter into the 150 mL beaker containing MACAg. They treated water by stirring or swirling the content of the beaker for a total of 10 min. The “disinfected” water was sampled after allowing it to magnetically separate from MACAg at the end of 5 and 10 min treatments. Students obtained a Lysogeny broth (LB) agar plate, marked the plate into six sections, and labeled the sections as 0-1, 0-2, 5-1, 5-2, 101, and 10-2, for *E. coli* solution “disinfected” for 0 min-control trial 1, 0 min-control trial 2, 5 min-trial 1, 5 min-trial 2, 10 min-trial 1, and 10 min-trial 2, respectively. With a sterilized inoculation loop, students inoculated 4 μL of each of the water samples, control or “disinfected”, onto the corresponding agar section. After this, students sealed the plates and stored them in the dark in the laboratory drawers at around 25 °C. The colony growth was ready to be observed 2–3 days later.

Silver Nanoparticles in Disinfected Water

Students transferred a 5 mL portion of the “disinfected” water in a test tube and compared its color to that of a 5 mL portion of a yellow colloidal silver in a test tube provided. The colloidal silver contains 7 ppm AgNPs. The amount of AgNPs in the “disinfected” water was estimated on the basis of a visual inspection of the intensity of the yellow color in the solution.

HAZARDS

Gloves and goggles must be worn at all times. Aqueous ammonia, FeCl_2 , FeCl_3 , NaBH_4 , and AgNO_3 are corrosive. FeCl_2 is also a mutagen. Handle all of these materials with

care and wash immediately with water in the case of skin contact. The magnets strongly attract each other. Avoid placing two magnets in close proximity to avoid pinching. Once the nutrient agar plates have been exposed or inoculated, they should be sealed and not be reopened by students.

RESULTS AND DISCUSSION

Characterization of MACAg Nanocomposite

MAC nanocomposite was prepared by thoroughly mixing AC with iron(II) and iron(III) ions and titrated with excess ammonia solution. A major advantage of MAC is its excellent absorption capacity and its ability to be isolated magnetically.³ To fully coat the MAC surface with sodium borohydride, a reducing agent for silver, MAC was vigorously mixed with a chilled sodium borohydride solution. With stirring, a silver nitrate solution was subsequently dripped into the mixture. The procedure proposes the idea that the sodium borohydride coated onto the surface of the MAC is the site for silver reduction. The site allows the silver nanoparticles, when formed, to be intimately integrated and well-dispersed onto the surface of the MAC matrix. If all the silver were incorporated into the MACAg product, there would be a maximum of 2.0×10^{-6} mol or 0.22 mg of silver, representing 1.9×10^{-4} by mole fraction or 0.038% by mass of the MACAg nanocomposite. The relatively minute amount of silver in MACAg is below the 0.1–1% mass detection limit by X-ray photoelectron spectroscopy, a commonly employed method for elemental analysis. The results of the following tests, however, provided evidence that the silver introduced to the reaction mixture was largely, if not completely, incorporated into the MACAg product: (1) The solution after making MACAg was clear, absent of yellow color, indicating a visually undetectable amount of silver nanoparticles as shown in Figure 2a, test tube on the right. The yellow color of the test tube on the left represents 7 ppm of silver nanoparticles in water, formed when the same procedure was employed without the presence of MAC: (2) When a drop of saturated potassium

Figure 2. (a) Left test tube: A colloidal solution containing 7 ppm AgNPs. Right test tube: Solution after making MACAg, absent of yellow color, indicating a visually undetectable amount of AgNPs.

(b) Left test tube: One drop of saturated KOH added to a sample of the solution containing 2.0 mL of 1.0 mM AgNO_3 in 30 mL of deionized water; a brown precipitate of AgOH or Ag_2O forms. Right test tube: One drop of saturated KOH added to a sample of the clear solution after making MACAg; the solution remains clear. This work was done by the authors.

hydroxide solution was added to a sample of the clear solution after making MACAg, the solution remained clear, as shown in Figure 2b, test tube on the right; however, as illustrated in Figure 2b, test tube on the left, when the drop was added to a sample of clear deionized water solution containing the same amount of silver ions introduced to the reaction mixture, a brown precipitate of silver hydroxide or oxide was formed. The absence of brown precipitate formation of the former suggests that a large amount of silver ions, if not all of them, left the solution after forming MACAg. Figure 3a,b shows the scanning electron microscopy (SEM) images of MNPs and AgNPs, respectively, prepared using the same procedures as used in preparing MACAg for these components, with the exception of having the nanoparticles stabilized with proper surfactants.^{5,7} The images show that the MNPs are of sizes ranging from 10 to 20 nm, and AgNPs around 10–12 nm. The plasmon absorbance of the yellow colloidal silver (see Figure 1a) produced a peak at 399 nm with PWHM (peak width at half max) of 50–70 nm in the UV-vis spectrum. This peak corresponds to AgNP sizes of 10–12 nm,⁷ supporting the SEM result. Figure 3c is an SEM image of MACAg, showing the agglomerates of the MNPs and AgNPs of sizes less than 20 nm embedded onto and covering the AC surface.

Antimicrobial Activity of MACAg Nanocomposite

In order to demonstrate the MACAg nanocomposite's antimicrobial activity, the water disinfection tests were carried out on inoculated *E. coli* solution containing 10^4 – 10^7 cfu/mL in which MACAg had been applied to 20 mL of such solutions for selected periods of time at room temperature. Water disinfection was made much easier due to the magnetic properties of MACAg. The reduction in the number of *E. coli* microbes following interaction with MACAg was investigated by incubating the *E. coli* solution treated with MACAg on LB nutrient agar plates for 2–3 days at 25 °C. For the MACAg containing 0.10 g of AC, 0.46 g of MPNs, and 2.2×10^{-4} g of AgNPs, the antimicrobial threshold within a 30 min treatment was found to be 10^6 – 10^7 cfu. The maximum silver concentration introduced in treating the solutions was 0.01 mg/mL. The effectiveness of MACAg against *E. coli* in a solution of 2×10^5 cfu/mL is shown in Figure 4. The results in Figure 4a demonstrated an increased microbial reduction effect with

Figure 3. Scanning electron microscopy (SEM) images of (a) MNPs, (b) AgNPs, and (c) MACAg. The SEM observation was carried out on a JEOL 6500F with a thermally assisted field emission gun. A small sample was coated on an Al stub for each. The imaging employed 10 keV accelerating voltage for the electron beam with a beam current of 50 pA, and the image was captured with a secondary electron detector. The scale bar = 100 nm. This work was done by the authors.

Figure 4. (a) Effectiveness of MACAg against *E. coli* in a solution containing 4×10^6 cfu: A = control, B = 10 min. MACAg treatment, C = 20 min. MACAg treatment, D = 30 min. MACAg treatment (absent of *E. coli*). (b) The absence of viable microbes on MACAg after treating *E. coli* solution (B); in 20 mL wash (C); and on MACAg after wash (D) (space A shows the colonies formed from a sample of the *E. coli* solution before the MACAg treatment). This work was done by the authors.

increasing treatment time. A significant reduction in the number of microbial colonies was observed after 20 min treatment with MACAg. The colony numbers were reduced to zero after 30 min treatment. For the general chemistry student laboratory, no sterilization was applied to the labware or the surface in the lab, except the agar and the inoculation loops. Considering the laboratory conditions and the 2 h laboratory period limit, we set the successful measure of MACAg as an effective antimicrobial agent by its ability to effectively “reduce” rather than “eliminate” microbes in a solution containing 10^5 – 10^6 cfu of *E. coli* after 10 min application of MACAg. Figure 4b shows the incubated samples of MACAg magnetically separated from the treated solution after 30 min treatment, 20 mL of deionized water used to wash this MACAg, and the MACAg after the wash, respectively. No viable microbes were found in these samples, indicating the microbes, absent from the treated water, were not only removed from water but also killed.

The antimicrobial effect of MACAg could perhaps be largely explained by the proposed mechanisms explaining the antimicrobial effect of Ag.^{31,32} AgNPs coming in contact with the microbes are thought to be oxidized into the silver ions which disrupt permeability and respiration functions of the cell, and penetrate inside the microorganisms. This leads to cell death or cellular inactivation. AgNPs act as a silver ion reservoir and provide a relatively low but sufficient concentration of silver antimicrobial species and can thus remain active for a long period of time. This in fact makes AgNPs very attractive in water disinfection applications, since higher than necessary levels of silver application not only are wasteful but also may pose health and environmental concerns. Additionally, one nanoparticle immobilized to a bacterium is able to release several tens of thousands of silver ions in this vicinity, producing a local high concentration of antimicrobial ions. The AgNPs’ antimicrobial activity is very likely enhanced in MACAg since the AC particles may adsorb microbes.³⁰ This allows more microbes to be effectively brought to AgNPs and to be subsequently killed by silver ions. This idea was supported by our results: we found that MAC without AgNPs was able to remove *E. coli*; however, unlike MACAg as shown in Figure 4b, the *E. coli* attached to MAC was found to remain viable after treatment and wash. The MACAg may therefore achieve a synergistic effect that none of its components alone could achieve.

Silver Nanoparticles in Disinfected Water

The incorporation of magnetite nanoparticles in MACAg nanocomposite enabled the fast and effective removal of silver nanoparticles from “disinfected” water. A visual comparison of the clear and colorless “disinfected” water and the yellow colloidal solution containing less than 7 ppm of silver nanoparticles, shown in the left test tube of Figure 2a, indicates visually nondetectable amount of silver nanoparticles being left in the “disinfected” water. This demonstrates the general stability of the MACAg nanocomposite and the silver nanoparticles incorporated into the MACAg nanocomposite remained undetached after use.

The procedure developed here would then be considered “green” since it would not leak AgNPs to a level harming mammalian cells. Although students will not investigate the reusability of their MACAg for water disinfection due to limited lab time, its possibility and its impact on material cost and conservation should be discussed. In our study, MACAg was found to be able to remove all microbes from *E. coli* solutions containing 10^4 cfu after 10 min treatment for three repeated cycles. MACAg was rinsed three times with deionized water before each reuse.

Antimicrobial Results and Feedback from Students

The experiment was implemented in the Chemistry for Marine Engineers course and carried out by 25 midshipmen majoring in marine engineering in 10 groups. These students were sophomores who took a one-term General Chemistry course during their plebe year, had one-term sea-year experiences, and will sail for two additional terms immediately after the course. The lab offered students’ first experience working with agar plates and inoculating onto agar in a Petri dish. Students treated 20 mL of an *E. coli* solution containing 1×10^5 cfu/mL with their MACAg for 5 and 10 min, respectively. Figure 5 shows the

Figure 5. Typical results of the students 2 days after storing the agar plates in the dark at 25 °C. The student plates have produced expected results showing clear and consistent reduction in the colony counts for the treated water after 10 min treatment (samples 10-1 and 10-2), relative to the control (samples 0-1 and 0-2). (Results shown were by Midshipmen Nicholas Anders, Jordan Brown, Kevin Dinh, Hannah Gizzi, and John Olsen. Used with permission.)

typical results of the students 2 days after storing the agar plates in the dark at 25 °C. Accurate colony count was difficult due to indistinguishable colony overlap. This was especially true for the control samples where the colony densities were high. Nevertheless, 80% of the student plates produced the expected results that showed clear and consistent reduction in the number of colonies for the treated water after 10 min of treatment (samples 10-1 and 10-2), relative to the control (samples 0-1 and 0-2), as shown in Figure 5. The colony count ranged from 0 to 2 with an estimated average of 0.6 ± 0.5 for 4 μ L samples of *E. coli* solution after 10 min of treatment. The number of surviving *E. coli* species after 10 min treatment was reduced from 2×10^6 cfu to less than 6000 cfu, corresponding to an antimicrobial efficiency of more than 99%. The 20% failure of the student plates was largely due to contamination or

mishandling of the plates. The procedure was also utilized in the 2014–15 academic year where 70 groups of 140 sophomores and juniors taking the course treated the Long Island Sound water using their MACAg. Similar antimicrobial results were achieved by these students.

The experiment allowed students to (1) be exposed to water chemistry and microbiology, (2) be introduced to the modern field of nanoscience and technology, and (3) appreciate the roles that new and advanced materials play in keeping our water and environment safe and clean. Ninety students ($N = 90$) were given a postlab survey to fill out, which yielded very positive feedback. Of these students, 100% agreed that the lab exercise helped them learn new things, see the use of chemistry, experience the fun of science, and improve their interest in and attitude toward science. These sentiments were further illustrated by students' comments. Sample comments include the following:

This is an interesting lab. It allowed the hybrid properties of the nanocomposite by disinfecting water and allowing the silver to be removed.

The magnetic silver nanocomposite is very effective at disinfecting water and we enjoyed participating in this experiment.

It was very interesting that the colonies grew so quickly. I learned that nanocomposites are adaptable and recoverable structures that can provide a superior method of accomplishing tasks such as water disinfection. The ability to combine together the best attributes of different components is fascinating!

I had no idea silver could be used as disinfectant, and no idea more than 26 ppm AgNPs in water can be harmful to mammalian cells.

Lab was very fun and interesting—it showed how chemistry is applicable to real world issues—it would do it again and would recommend it for future classes!

This method of disinfecting water was proven effective by the lab we did as it reduced or eliminated bacteria growth in nearly every agar plate that was observed!

I learned that activated carbon has a large surface area which contributes to its absorption property. It is amazing our product can be easily made and effectively purify water. We found the usefulness of nanocomposites and the roles each component played and all components played together to be pretty interesting. We liked this lab! Thank you.

CONCLUSION

We have developed a 2 h experiment that introduces college students to the concepts, synthesis, and applications of an exciting class of materials, known as nanocomposites, at an early stage. The procedure prepares a AgNP-based magnetic nanocomposite, MACAg, using activated carbon, magnetite nanoparticles, and silver nanoparticles. The MACAg nanocomposite, containing a maximum of 1.9×10^{-4} by mole fraction and 0.038% by mass of silver, has achieved the synergistic properties derived from its components and

demonstrated its applicability as an effective and recoverable antimicrobial agent for water disinfection. The principle is successfully illustrated by a significant reduction in the number of microbes in an *E. coli* solution containing 2×10^6 cfu following its treatment with MACAg for 10 min. The maximum silver introduced via MACAg is 0.01 mg/mL which is removed magnetically after use. *E. coli* in water is cultivated using LB nutrient agar. The experience is well-received and enjoyed by the students who find it interesting and motivating. The experiment allows students to gain experiences working at the nanochemistry–microbiology interface, be exposed to the modern field of nanoscience and technology, and appreciate the roles that cutting-edge nanomaterials play in protecting our health and environment. The experiment is also suitable for advanced high school students.

The authors declare the views expressed in this article are the authors' own and not those of the U.S. Merchant Marine Academy, the Maritime Administration, the Department of Transportation or the United States government.

The authors declare no competing financial interest.

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