In Situ Preparation of Silver Nanoparticles in Paper by Reduction with Alkaline Glucose Solutions

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ABSTRACT: Percolation of contaminated water through paper sheets containing silver nanoparticles is a promising way to provide emergency drinking water. The silver nanoparticles are deposited by the in situ reduction of silver nitrate on the cellulose fibers of an absorbent blotting paper sheet. Sodium borohydride has been used as the reductant but is toxic and expensive. Glucose is a benign alternative but is much less reactive. In this note, we demonstrate an improved way to produce silver nanoparticles in paper sheets by adding sodium hydroxide to the glucose reductant. The silver content of the sheets, measured by diffuse reflectance spectroscopy, was around 2–3 mg of silver per gram of dry paper. This was sufficient to reduce the concentration of a model Escherichia coli suspension after percolation through the sheet.

1. INTRODUCTION

Paper continues to generate interest as a renewable, cheap substrate for practical point-of-use methods to purify drinking water in an emergency. Two main approaches have been reported. The in situ reduction of aqueous solutions of metal salts (and especially silver salts) in the paper sheets leads on drying to metal nanoparticles strongly attached to the cellulose sheet matrix. When a suspension of bacteria passes through the sheet, a large reduction in the number of viable bacteria is observed, with minimal loss of silver from the sheet.3–5 A second approach that avoids the use of metal nanoparticles is based on the modification of the fiber surfaces in paper so that negatively charged bacteria6 or negatively charged bactericidal copolymer micelles6,7 are irreversibly adsorbed on positively charged paper surfaces. Both approaches exploit the availability of paper-based sheets with a wide range of porosities that can be used to control the flow rate and area available for interaction with bacteria.

In the first approach, the silver nanoparticles are attached by the in situ reduction of silver nitrate on the cellulose fibers of an absorbent blotting paper sheet. The aim is to achieve inactivation of bacteria during percolation through the sheet, rather than by removal of bacteria from the effluent by filtration.5 The sheets containing silver nanoparticles exhibit antibacterial properties with minimal silver loss, indicating the promise of the method. However, the use of sodium borohydride to reduce the silver salt in the sheet2,3 is problematic, due to toxicity.8 To avoid this, glucose was suggested as a benign reductant.9 No reduction was detected at room temperature, but drying the sheets containing excess glucose in conventional or microwave ovens resulted in the formation of silver nanoparticles in the paper sheet.9 Successful field testing of paper sheets containing silver and copper nanoparticles10 led to scale-up and commercial availability.11

Glucose or related reducing sugars are clearly more benign and more readily available than the original reductant, sodium borohydride, proposed for the preparation of AgNP paper sheets. However, the reducing sugars are less reactive than sodium borohydride and have been used in large excess or at elevated temperatures to reduce the silver salts in the paper sheet. This has not proved to be a problem to date, but it might be useful to find a simple additive that would enhance the reactivity of reducing sugars for formation of AgNP on paper sheets. On the basis of the report by Darroudi et al. that NaOH acts as an accelerator in the reduction of solution-dispersed silver nanoparticles,12 we here demonstrate an improved way to produce silver nanoparticles in paper sheets by adding sodium hydroxide to the glucose reductant.

2. RESULTS AND DISCUSSION

2.1. Reflectance Spectra of Dry AgNP Sheets. In a previous work with glucose as an in situ reductant for silver nitrate, the paper samples were heated and an excess of glucose was employed to enhance the formation of silver nanoparticles.
in the sheet.\textsuperscript{9} Here, we examine an alternative way to enhance the reductive effectiveness of glucose. In basic media, it is expected that formation of the acidic oxidation products would be favored, thus enhancing the silver-ion reduction. Darroudi et al. used NaOH as an accelerator in the preparation of gelatin-stabilized silver nanoparticles by reduction with glucose.\textsuperscript{12} The effectiveness of this approach to that of the in situ reduction in paper was tested by adding NaOH to the glucose reducing solution. The usual yellow-brown sheet coloration appeared at room temperature but at much lower glucose concentration than that required for glucose reduction without added base. Reflectance spectra for dried sheets dipped in 10 mM AgNO$_3$ solution with two different amounts of added NaOH are shown in Figure 1. For equimolar AgNO$_3$ and NaOH, the reflectance spectra show a broad peak at around 420 nm with reflectance of 20\% relative to a sheet without AgNP, in reasonable accord with the reflectance of sheets where the AgNO$_3$ was reduced with NaBH$_4$.\textsuperscript{2} Increasing the concentration of AgNO$_3$ with a corresponding increase in concentration of NaOH shows the expected increase in sheet coloration, as shown in Figure 2.

### 2.2. Silver Content of AgNP Sheets

Each 60 mm $\times$ 60 mm square of the AgNP paper absorbed about 4 mL of solution so that for a solution containing 10 mM AgNO$_3$, the maximum possible silver content in each dry sheet would be about 4.3 mg of silver per sheet or 8.6 mg of silver per gram of paper.

The actual content of silver in the dried sheets was measured by acid digestion followed by atomic absorption spectroscopy. The sheets made with glucose/NaOH as reductant contained somewhat less silver than those reported previously for sodium borohydride reduction\textsuperscript{2} and for heated sheets containing a large (>10-fold) molar excess of glucose,\textsuperscript{9} as illustrated in Table 1. While the reflectance spectra and silver content of the papers produced by NaOH are similar to those produced by other reductants, the size of the silver nanoparticles observed by transmission electron microscopy (TEM) for two of the samples was somewhat larger and more polydisperse than the dimensions usually observed (Table 1).

### 2.3. AgNP Eluent Properties

A suspension of Escherichia coli strain K12 was passed through the AgNP sheets, and the eluent was tested for viability by measuring the increase in optical density of the cultured suspension at a wavelength of 600 nm. Incomplete deactivation of the E. coli passing through the paper results in an increase in optical density over time. Results are shown in Figure 3. The sheet made by immersion

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**Table 1. Comparison of Silver Content of Paper Sheets Prepared by Different Methods**

| sample | precursors | AgNO$_3$ concn (mM) | reductant | silver content mg per gram of dry paper | silver nanoparticle diameter (nm) |
|--------|------------|---------------------|-----------|----------------------------------------|-----------------------------------|
| a      | 10         | excess NaBH$_4$      | 2.2 ± 0.7 | 4.9 ± 1.9                              |
|        | 25         | excess NaBH$_4$      | 6.0 ± 1.4 | 7.1 ± 3.5                              |
| b      | 10         | glucose, 1.0 M       | 2.4 ± 0.3 | 6.9 ± 4.8                              |
|        | 25         | glucose, 1.0 M       | 5.3 ± 0.6 | 7.5 ± 2.2                              |
| c      | 10         | glucose, 10 mM, NaOH, 10 mM | 1.7 | 15 ± 7                                  |
|        | 25         | glucose, 25 mM, NaOH, 25 mM | 2.9 | 26 ± 14                                |

\textsuperscript{a} From ref 2, Domtar Blotter paper substrate, 1 M sodium borohydride reductant. \textsuperscript{b} From ref 9, several paper substrates, heated in oven or microwave. \textsuperscript{c} This work, Whatman gel blotter GB003 paper substrate.
in the lowest silver salt content (5 mM AgNO₃, 5 mM glucose + 5 mM NaOH, giving a final silver content of 1.3 mg of Ag per gram of dry paper) displayed no inhibition of bacterial growth (not shown), being essentially identical to curve (i) for elution through untreated blotter paper. Increasing the silver content in the blotter paper to 1.7 mg of Ag per gram of dry paper resulted in some inhibition of bacterial growth in the eluent (curve (ii), Figure 3). For blotter paper containing 2.9 mg of silver per gram of paper (the highest silver content examined in this work), the bacterial growth rate in the eluent was about 1/3 that for the blotter paper without silver (curve (i)). While significant, this is far short of complete inhibition (curve (iv)). Note that bacterial growth in the first hour is inhibited to a much greater extent relative to the silver-free eluent (curve (iv) vs curve (i)) than at longer times. This suggests that elution through sheets with a threshold silver content of greater than ∼3 mg of Ag per gram of paper causes significant initial reduction of bacterial content but that sufficient viable bacteria remain in the eluent to increase over time. This regrowth may be enhanced by the presence of residual glucose in the eluent.

The bactericidal effectiveness of the AgNP papers depends not only on their nanoparticle content but also on factors such as the flow rate through the papers, the initial bacteria content, and the presence of interfering compounds in turbid influent water. It remains unclear whether the silver nanoparticles provide a local reservoir of silver ions in the sheet or direct contact between bacteria and silver nanoparticles is required. Negatively charged bacteria adsorb on positively charged surface-modified cellulose, and a similar electrostatic interaction may facilitate the initial interaction with AgNP/paper.

3. CONCLUSIONS

Adding NaOH to the glucose reducing solution enhances its effectiveness for the in situ reduction of silver nitrate in paper sheets. The effectiveness of paper containing silver nanoparticles for emergency water treatment in field tests suggests that further optimization of benign reductants for silver salts in a paper matrix would be worthwhile.

4. EXPERIMENTAL SECTION

4.1. Materials. We used a commercially available porous lightly bonded blotting paper (Whatman gel blotting paper grade GB003, ∼0.8 mm dry thickness as base paper sheet). AgNO₃ (s), NaOH 0.5 M, AgNO₃ 10 mM (aq), anhydrous D-glucose(s), nitric acid (70%), hydrogen peroxide (30%), and Lysogeny broth (LB) powder were all purchased from Sigma-Aldrich and used as received. E. coli strain K12 was kindly provided by Prof. N. Tufenkji, Department of Chemical Engineering, McGill University. Laboratory deionized (DI) water was used for solution preparation.

4.2. Preparation of Silver Nanoparticle Paper (AgNP Paper). The blotting paper, thickness 0.80 mm, was cut into 60 mm × 60 mm squares. Each square was allowed to soak in 30 mL of silver nitrate solution of a given concentration for 15 min. After the excess solution was allowed to drip from the sheet, the paper squares were then immersed in 30 mL of an alkaline glucose reducing solution for another 15 min, before drying under restraint in an air oven at 50 °C for 2 h.

The initial AgNO₃ solutions were 5, 10, and 25 mM. The glucose concentration in the reductant solutions (5, 10, and 25 mM) was the same as that of the silver solutions, but NaOH was added to the glucose solution to increase the effectiveness of the reduction. The molar ratio of AgNO₃ to Glu was always 1:1, but NaOH was added directly to the glucose solution in two different molar ratios; Glu/NaOH = 10:1 and 1:1.

The compositions of the solutions into which the paper was dipped are given in Table 1. For comparison, results are also listed in Table 1 for compositions employed in previous publications, in which 1 M sodium borohydride and 1 M glucose with heating were used to reduce the AgNO₃.

4.3. Bacterial Growth Measurements. The growth of bacteria in the eluent after passing through the AgNP paper was estimated from the increase in optical density at a wavelength of 600 nm (OD600), measured with a Cary 5000 UV-vis–NIR spectrophotometer from Agilent.

E. coli was seeded in 100 mL of autoclaved LB (20 g per litre) and incubated overnight by suspending the solution in a water bath at 37 °C with continuous shaking of the flask. OD600 was then measured to confirm that the colony had grown (the absorbance ranged between 0.73 and 1.12). The bacterial suspension was then centrifuged and the bacterial pellet redispersed in 100 mL of autoclaved DI water. OD600 was again measured before the bacterial suspension (aq) passed through a AgNP paper under gravity. An increase in absorbance is assumed to be due to growth of bacteria increasing the turbidity of the sample. After centrifugation and redispersion in water, the absorbance ranged between 0.51 and 1.03.

After passage through the AgNP paper, the supernatant was collected for analysis of the silver content by atomic absorption (see below). The bacterial pellet was separated by centrifugation, redispersed in 100 mL of LB, and incubated at 37 °C with continuous shaking. The OD600 was measured at hourly intervals to monitor bacterial growth.

4.4. Solid UV–vis Spectroscopy on Paper. Diffuse reflectance spectroscopy was employed to confirm the formation of AgNP in the paper. The percentage reflectance from the AgNP papers was measured for wavelengths between 300 and 800 nm with a Cary 5000 UV–vis–NIR spectrophotometer and diffuse reflectance accessory. Untreated Whatman blotting paper was used as a blank.

4.5. Transmission Electron Microscopy. The papers were imaged with transmission electron microscopy to measure the size of the silver nanoparticles. Some of the surface material of the paper samples was scraped off and deposited on copper grids. This material was imaged using a FEI Tecnai G2 Spirit Twin 120 kV Cryo-TEM and a Philips CM200 200 kV TEM coupled with an EDAX Genesis EDS Analysis System.

4.6. Silver Analysis by Atomic Absorption Spectroscopy. Nitric acid was added to the supernatant liquid from the bacterial growth experiment to bring the final concentration of nitric acid to 2 wt %. The AgNP papers were digested by adding 5 mL of DI water and 5 mL of nitric acid (70%) and heating at 40 °C for 30 min. Then, 5 mL of hydrogen peroxide (30%) was added and heating was continued for another 30 min at 40 °C. The suspension was diluted to 175 mL using DI water and filtered through a 0.22 μm Millipore filter prior to analysis with a PerkinElmer AAAnalyst 200 Flame Atomic Absorption Spectrometer using DI water as the blank.
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Notes
The authors declare no competing financial interest.

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