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Investigation of Antibacterial Activity by Silver Nanoparticles Prepared by Microwave-Assisted Green Syntheses with Soluble Starch, Dextrose, and Arabinose

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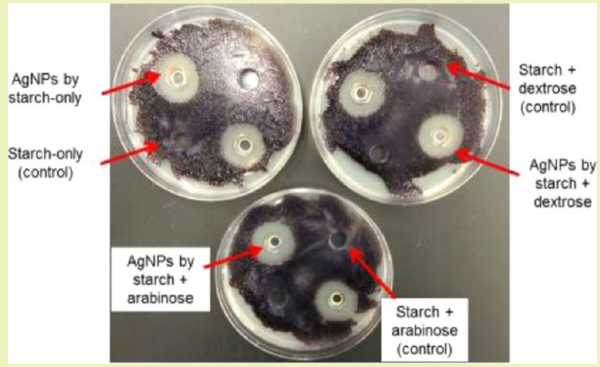
S *Supporting Information*

ABSTRACT: The objective of this study was to assess the antibacterial activity and inhibition of biofilm formation of silver nanoparticles (AgNPs) against *Escherichia coli* (MG1655), *Bacillus subtilis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Janthinobacterium lividum*. The AgNPs utilized in this study were prepared through one-pot microwave-assisted syntheses guided by principles of green chemistry. The AgNPs were synthesized in three different schemes by reducing Ag^+ ions (from $AgNO_3$) with reducing agents dextrose, arabinose, and soluble starch. Formation of AgNPs occurred in less than 15 min, and nanoparticles had diameters of 30 nm or less. Successful synthesis of AgNPs was confirmed using multiple orthogonal approaches, including UV−visible spectroscopy, fluorescence emission spectroscopy, powder X-ray diffraction, and transmission electron microscopy, while size analysis was gathered from transmission electron microscopy images and dynamic light scattering. All AgNPs prepared in this study exhibited antibacterial effects on a variety of organisms as determined by a well diffusion assay with no antibacterial effects observed in the control wells.



KEYWORDS: *Silver nanoparticles, Starch, Dextrose, Arabinose, Microwave synthesis, Green chemistry, Antibacterial activity*

INTRODUCTION

Engineered nanoparticles and nanomaterials are now being manufactured in increasing quantities for a broad range of applications, including uses in medicine, cosmetics, clothing, engineering, electronics, renewable energy, environmental remediation, and fertilizer and pesticide applications,[1−3] given their unique mechanical, catalytic, optical, and electrical conductivity properties.[4] The proliferation of nanotechnology has prompted discussions over the safety of these materials to ecological and human health[1] as the production, use, and disposal of nanomaterials will lead to their appearance in various environmental compartments and organisms.[2] In particular, silver nanoparticles (AgNPs) are widely used in numerous consumer products, including textiles, personal care products, food storage containers, laundry additives, household appliances, paints, and food supplements.[5,6] Though the use of AgNPs is quite high in commercial products owing to its antimicrobial activity, the effect of AgNPs versus silver ions on microbial communities[7,8] is of significant research interest given the broad range of environmental conditions and effects of ligands on AgNP dissolution rate and toxicity.[9] Interest in bacterial toxicity and mode of action by AgNPs is of interest as well given the rising rates of developed resistance of pathogenic organisms against antibiotics.[10]

The use of greener techniques for the synthesis of AgNPs has been recently reviewed.[11] Presented here are several environmentally friendly syntheses of AgNPs that result in nanoparticles stable in aqueous solutions. The AgNP synthesis methods described here utilize nontoxic environmentally benign reagents in minimal time (less than 15 min) including the use of soluble starch, arabinose, and dextrose. The preparation of AgNPs using solely soluble starch was first presented by Vigneshwaran et al.,[12] and the use of dextrose as a reducing agent in conjunction with the starch cap to AgNPs was first presented by Raveendran et al.[13] The use of arabinose as a reducing agent by itself has never before been proposed but was one of the many sugars present in a green synthesis proposed by Vinod et al.[14] The first objective of the work presented here was to use microwave technology and soluble starch as a capping agent with individual reducing agents

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dextrose and arabinose to create AgNPs. The advantages of microwave heating over traditional heating methods make it an ideal choice for research applications. The importance of this technique for NP synthesis has been reviewed,[11] and successful syntheses using amino acids,[15,16] certain sugars,[17−19] starch,[20] and orange peel[21] have been demonstrated. Soluble starch (used as the capping agent in all syntheses presented here) is the amylose component of starch, which acts like a linear polymer and forms films and complexes with ligands.[22] It requires high temperatures and pressures in order to expand the molecules,[23,24] thereby enhancing creation of AgNPs that can easily become embedded within (and hence capped by) the starch molecules. It is also reported that high temperatures accelerate the reductive properties of aldehydes.[25] It has been proposed that the aldehyde terminus of soluble starch may serve to reduce the silver nitrate,[12] while both dextrose and arabinose contain aldehyde groups in higher concentrations that serve to reduce Ag^+ to its elemental state.

Three AgNP synthesis protocols were developed using (1) soluble starch (hereafter referred to as starch-only, serving as both capping and reducing agent), (2) starch + dextrose, and (3) starch + arabinose. Though the use of starch[20] and sugars[17,18] with microwave technology has been previously individually reported, the work here is important for examining all three synthetic protocols simultaneously. The AgNPs that were produced with these three methods by microwave heating were characterized using multiple orthogonal approaches: UV−visible spectroscopy (UV−vis), fluorescence spectroscopy, powder X-ray diffraction (XRD), transmission electron microscopy (TEM), and differential light scattering (DLS). After characterization of these AgNPs, the second objective of the described work was to determine the antibacterial activities against multiple organisms: *Escherichia coli* (MG1655), *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Janthinobacterium lividum*. Past studies that have examined the antibacterial activities of AgNPs created with these green syntheses have also been investigated against various types of Gram positive and Gram negative organisms. AgNPs created using the extract of orange (*Citrus sinensis*),[26] *Eucalyptus chapmaniana*,[27] or *Artocarpus heterophyllus*[28] have been tested against such organisms as *E. coli*,[26,27,29] *P. aeruginosa*,[26−28] *S. aureus*,[26−28] *K. pneumoniae*,[27] *Proteus vulgaris*,[27,28] *B. cereus*,[28] *B. subtilis*,[28] and yeast (*Candida albicans*).[27] Toxicity against the human acute promyelocytic leukemia (HL-60) cell line has also been investigated.[27]

Thus, this work is novel for demonstrating comparable formation of AgNPs by microwave technology using three different synthetic protocols: (1) starch-only, (2) starch + dextrose, and (3) starch + arabinose. These AgNPs were characterized by multiple orthogonal approaches before being tested for their antibacterial activity, which to our knowledge has not been completed for AgNPs produced using green synthetic protocols employing starch and sugars. This work also demonstrated that these AgNPs have comparable bacterial toxicity to previous reports.

MATERIALS AND METHODS

Chemicals and Reagents. Silver nitrate (ultrapure grade; part no. 41936-1000) and soluble starch (ACS reagent grade, part no. 424495000) were from Acros Organics (through Thermo Fisher Scientific, Fairlawn, NJ). D-(-)-arabinose (≥98% purity; part no. A-3131) was from Sigma-Aldrich (St. Louis, MO). Anhydrous dextrose (*d*-glucose; USP grade, part no. D19−212) was from Fisher Scientific (Fairlawn, NJ) as were all other chemicals unless specified otherwise. Only HPLC-grade water (18 MΩ) from Barnstead E-pure system (Thermo Scientific, Asheville, NC) was used.

Synthesis of AgNPs. Aqueous starch solutions were freshly prepared volumetrically for all syntheses and used within two weeks of creation. For 3% (w/w) solutions, 3 g of soluble starch was first added to approximately 80 mL of 18 MΩ double deionized (DDI) H_2O to create 1% (w/w) solutions, and 1 g was added to about 80 mL of DDI H_2O. These solutions were then heated in a commercial food-grade microwave for approximately 1 min or until the solution became translucent with intermittent stirring. The solutions were then allowed to come to room temperature (25 °C) and raised to 100 mL in a volumetric flask. The laboratory-grade microwave synthesizer Discover SP (CEM Corporation, Matthews, NC) was used in all syntheses. Syntheses were performed in CEM-supplied single-use 10 mL reaction vessels and septa designed for high temperature/pressure reactions in the microwave. Stir bars used were of appropriate small size, supplied along with the CEM microwave.

Synthesis of AgNPs in Soluble Starch-Only. Seven milliliters of fresh 3% (w/w) starch solution were added to a 10 mL microwave reaction vessel along with approximately 0.020 g of $AgNO_3$ (1.17×10^{-4} mol) with a stir bar. The vial was capped and syntheses performed in the Discover SP microwave at 150 °C for 15 min at 50 psi pressure. The contents of the reaction vessel were amber in color at the conclusion of the reaction, indicating formation of AgNPs.

Synthesis of AgNPs with Dextrose Reducing Agent + Starch. Approximately 0.024 g of dextrose (1.33×10^{-4} mol) was added in slight molar excess based on previous studies[30] to a reaction vessel with 0.020 g of $AgNO_3$ (1.17×10^{-4} mol), along with 6 mL of DDI H_2O, 1 mL of freshly prepared 1% (w/w) starch solution (creating a 0.14% solution by weight), and a stir bar. The percent concentration of starch was reduced (when compared to the starch-only synthesis) to ensure that dextrose was the reducing agent. The solution was then reacted in the microwave at 150 °C for 10 min under 50 psi of pressure. The reaction vessel contents were deep yellow, indicating formation of AgNPs.

Synthesis of AgNPs with Arabinose Reducing Agent + Starch. Approximately 0.020 g of arabinose (1.33×10^{-4} mol) was added in slight molar excess to reaction vessel with 0.020 g of $AgNO_3$ (1.17×10^{-4} mol), along with 6 mL of DDI H_2O, 1 mL of freshly prepared 1% (w/w) starch solution (creating a 0.14% solution by weight), and a stir bar as was just described in the synthesis of AgNPs with dextrose as a reducing agent. Again, the percent concentration of starch in solution was less than in the starch-only synthesis to ensure that arabinose was the reducing agent. The solution was prestirred for 30 s and reacted in the microwave at 150 °C for 8 min under 50 psi of pressure. The vessel contained a deep yellow solution, again indicating formation of AgNPs.

AgNP Characterization. *UV−Visible Spectroscopy.* UV−vis spectra were collected on an Agilent 8453 system (Santa Clara, CA). This method is detailed elsewhere.[21]

Fluorescence Emission. All emission spectra were collected using a F-3010 fluorescence spectrophotometer (Hitachi, Pleasanton, CA). Details regarding this method may be found elsewhere.[21]

Dynamic Light Scattering Analysis. All sizing DLS experiments were carried out on a ZetaPALS Zeta Potential Analyzer (Brookhaven Instruments Corp., Holtsville, NY) using ZetaPlus Particle Sizing Software Version 4.20, with a backscatter angle of 90° and a laser wavelength of 657.0 nm. Details regarding this method may be found elsewhere.[21]

Powder X-ray Diffraction. X-ray diffraction was performed on a Scintag X-2 advanced diffraction system (Cupertino, CA) equipped with Cu Kα radiation (λ = 1.54 Å). Owing to the interference of the high percentage of starch in solution in the starch-only synthesis, this method required centrifugation at 13,300 rpm for 3 or more hours to obtain a concentrated sample for XRD. This aided in the removal of precipitated starch, allowing for the separation of AgNPs for characterization. Further details may be found in another study.[21]

Transmission Electron Microscopy. Low- resolution transmission electron micrographs were obtained on a JEOL TEM 1400 (Peabody,

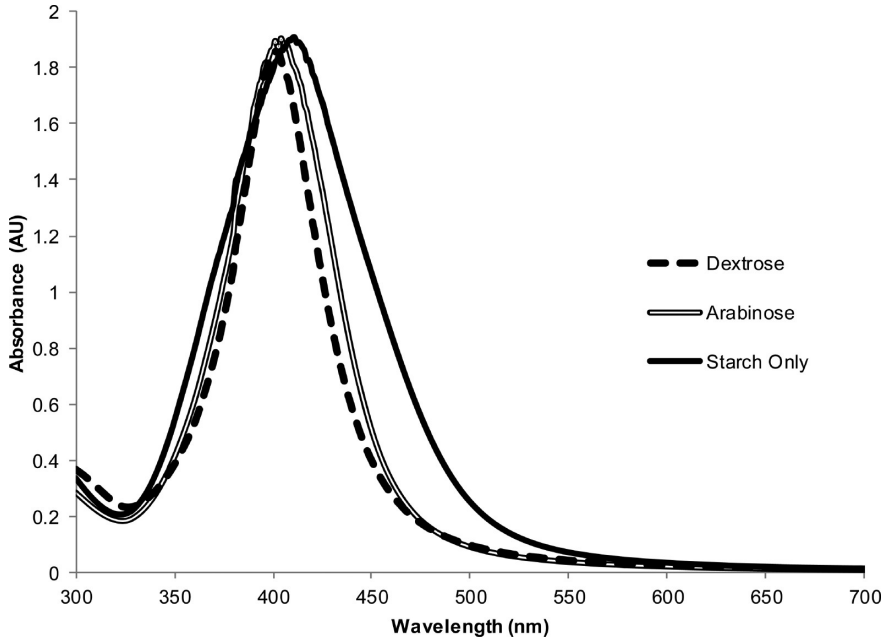

Figure 1. UV−visible absorbance spectrum of arabinose + starch, dextrose + starch, and starch-only syntheses. Relative λ_{max} occurs at 401 nm for dextrose + starch synthesis (dashed line), 404 nm for arabinose + starch synthesis (double line), and 411 nm for starch-only synthesis (solid line).

MA) at a working voltage of 100 kV. This procedure is detailed within another study.[21]

Antibacterial Assay. Gram positive and Gram negative bacteria were selected for study of the bactericidal effect of the silver nanoparticles on organisms with different cell wall structures. Laboratory strains of Gram negative bacteria include *Escherichia coli*, (MG1655), *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, as well as *Janthinobacterium lividum* (collected from a stream in Buena Vista, CO). Gram positive strains *Staphylococcus aureus* and *Bacillus subtilis* were also used.

In preparation for the well-diffusion assay, bacterial cells were grown in LB broth. Measuring turbidity at 600 nm, cell culture concentration was adjusted such that 100 μL containing 10^7 cells were spread in a lawn on LB agar plates. Eight millimeter diameter wells were formed by pressing the wide end of a 1000 μL pipet tip into the agar and removing the agar plug. All wells were filled with either 50 μL of the capping/reducing solution or 50 μL of the nanoparticle suspension (106, 10.6, or 1.06 ng of nanoparticles). The plates were incubated overnight at 37 °C or for *J. lividum* at room temperature for 3 days. The diameter of the zone of inhibition at each well was measured in millimeters.

RESULTS AND DISCUSSION

AgNP Characterization. The three syntheses presented all ran rapidly and produced AgNPs stable in suspension over the course of two months. This stability was due to the common capping agent, starch, which acted as a linear polymer in solution.[12] It is well-known that AgNPs are synthesized with great success and stability in other polymer-stabilizing solutions such as poly(vinyl alcohol),[31] poly(methyl methacrylate),[32] and poly(vinylpyrrolidone).[33] Polyhydroxylated macromolecules such as starch formed molecular-level capsules as a result of the inter- and intra-molecular hydrogen bonding that serve as templates for nanoparticle growth,[12,13] which were then well-stabilized by the high number of hydroxyl groups.[13]

After allowing reaction solutions to cool to room temperature (25 °C), solutions of AgNPs were subjected to optical studies. All three AgNP syntheses described showed the characteristic broad absorbance due to the surface plasmon resonance of the AgNPs[34,35] at ∼410 nm (Figure 1). Batch-to-batch variability in the λ_{max} for the three syntheses are presented in Table S1 of the Supporting Information. Plasmon resonance, an intrinsic property of nanoparticles in general, is due to the coupling between the conduction band electrons on the AgNP surface with incident electromagnetic radiation.[36−39] In the case of AgNPs, this absorbance typically occurs between 380 to 420 nm depending on the size (and the shape) of particles being analyzed. The symmetry of the plasmon resonance as shown in Figure 1 indicates a low level of agglomeration of AgNPs in solution,[12] which agrees with the microscopy results below. Note that in the dextrose and arabinose syntheses, a "starch blank" of the reaction containing an equal amount of starch was run to verify the increased activity of the chosen reducing agent over soluble starch alone; this was confirmed via a drastic increase in λ_{max} absorbance (not shown), indicating that a vastly higher concentration of AgNPs was synthesized.

The synthesized AgNPs were also characterized via fluorescence emission spectroscopy, which all exhibit photoluminescence (Figure 2). The AgNP samples synthesized here showed the strongest emission around λ_{em} = 500 nm when excited by light at λ_{ex} = 330 nm, values that were optimized experimentally. These emission peaks were not observed in the reaction blanks (Figure S1, Supporting Information), indicating that they were a result of the synthesized AgNPs and not inherent to the sugars or starch used. Photoluminescence of AgNPs reported in literature ranged anywhere from 465[40−42] to 550 nm[12,30] and tends to vary drastically as a function of both the size of the AgNPs studied as well as their capping agents. It has been previously observed that a red-shift in the photoluminescence can be caused by bulky capping agents.[12,40] Starch, which acts as a polymer and is therefore extremely bulky, was most likely the cause of the slightly longer λ_{em} than the "characteristic" photoluminescence peak at 465 nm.[12] Excitation at λ_{ex} = 380 nm (as was used by some groups) also produced emission at around λ_{em} = 500 nm but at far less

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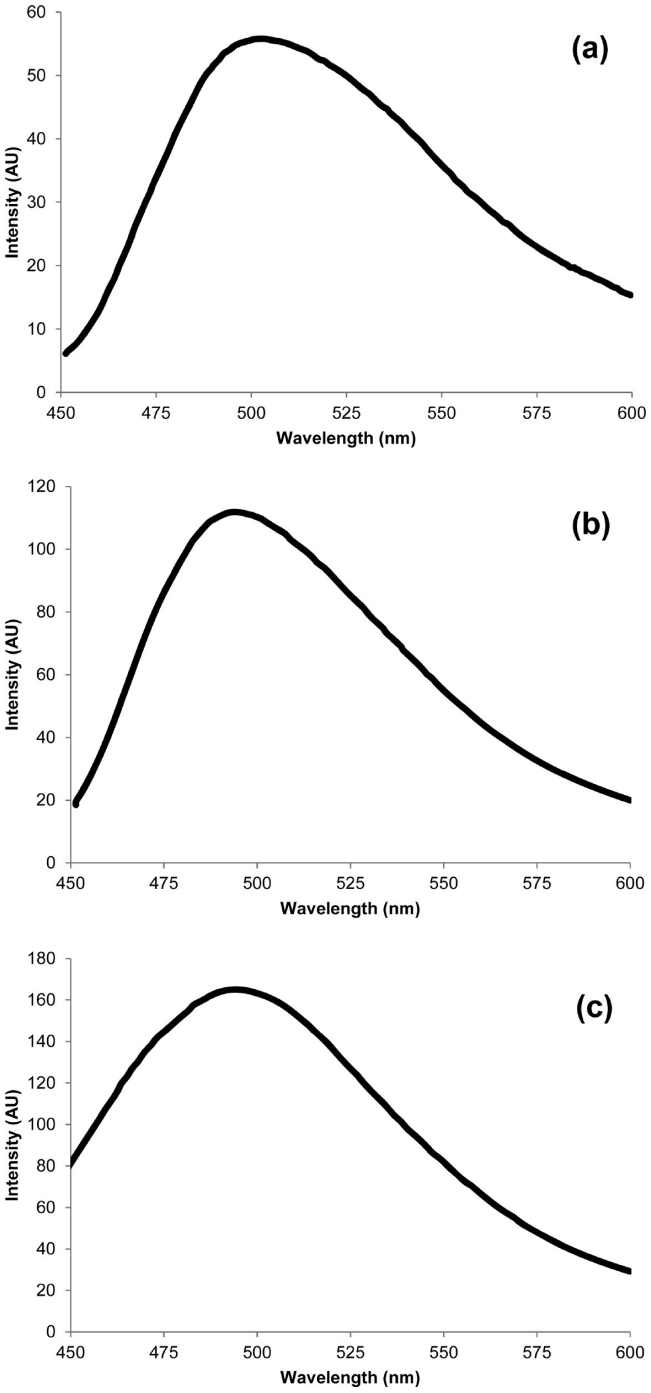

Figure 2. Emission spectroscopy of all AgNP syntheses when excited at λ_{ex} = 330 nm: (a) arabinose + starch synthesis with emission maximum at λ_{em} = 504 nm, (b) dextrose + starch synthesis with emission maximum at λ_{em} = 493 nm, and (c) starch-only synthesis with emission maximum at λ_{em} = 495 nm.

intensity than λ_{ex} = 330 nm; this unchanging emissions peak was consistent with other studies on AgNP fluorescence.[42]

In order to establish the presence of crystalline silver in the synthesized AgNPs and not silver oxide, dried AgNP samples (which were compositions of a minimum of four separate syntheses) were analyzed via XRD (Figure 3). From these obtained XRD patterns, it was clearly seen that the silver present in the synthesized AgNPs was face-centered cubic (FCC) crystalline, with peaks at 2θ = 38.1°, 44.3°, 64.4°, 77.4°,

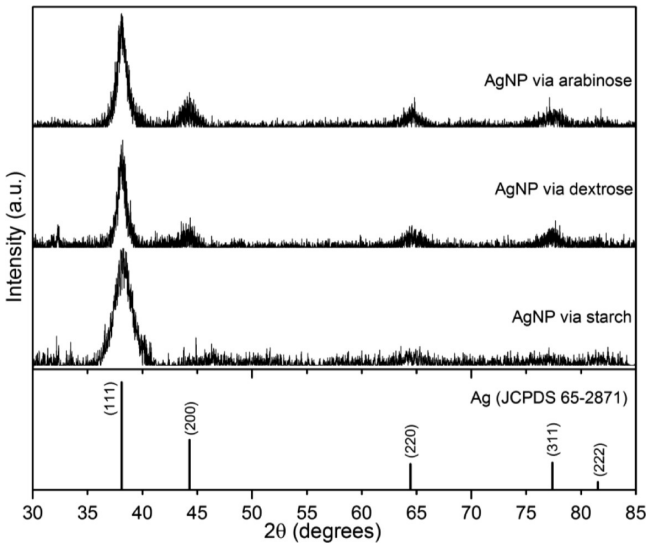

Figure 3. Powder XRD pattern of AgNPs synthesized via arabinose + starch (top panel), dextrose + starch (middle panel), and starch-only (middle panel). Peaks occurred at 2θ = 38.1°, 44.3°, 64.4°, 77.4°, and 81.5° corresponding to the (111), (200), (220), (311), and (222) planes (bottom panel), respectively.

and 81.5° corresponding to the (111), (200), (220), (311), and (222) planes, respectively, except in the case of the starch-only synthesis, where only one peak was obtained corresponding to the (111) plane. The peaks displayed in the pattern all corresponded to the NIST bulk silver reference (JCPDS 65-2871) with the exception of some small peaks at low 2θ values (about 34°) in the arabinose synthesis. These small peaks were a result of the crystalline arabinose present in the dried samples (PDF 00-038-1802). The peaks were also broader than those of bulk silver, an indication of the small size of the AgNPs. The lattice constant "*a*" was calculated from the AgNP samples using the instrument software with multiple peaks to be 4.08610 Å, a value in perfect agreement with the value reported in the literature for FCC silver (a = 4.086 Å, Joint Committee on Powder Diffraction Standards file no. 04-0783). It was therefore concluded that the nanoparticles synthesized were of crystalline silver without metal oxides present.

Size Analysis of AgNPs. The formation of roughly spherical AgNPs was confirmed via TEM, as shown in Figure 4, with some agglomeration present. Individual particles were, however, present, so sizing analysis was completed by both TEM and DLS to account for agglomeration, a solution phenomenon. The starch-only AgNPs exhibited the least amount of agglomeration, presumably due to the increased concentration of capping agent in the solution when compared to the arabinose + starch or dextrose + starch syntheses. The size of particles synthesized, as determined by TEM, when using each synthesis method varies slightly as shown in Figure 5; however, 99.4% of all AgNPs (where n = 595 particles counted for each synthesis mode) created by these three methods were under 30 nm making them ideal for fundamental studies because nanoparticles in general begin to exhibit nonbulk properties at diameters of less than 20−30 nm.[43]

The size of the AgNP composite samples (where reaction products from four separate syntheses were pooled) was also determined using DLS and compared with TEM data (Figure 6). By DLS, the weighted average of the AgNP diameters by the arabinose + starch method was 23.0 ± 1.3 nm (mean ±

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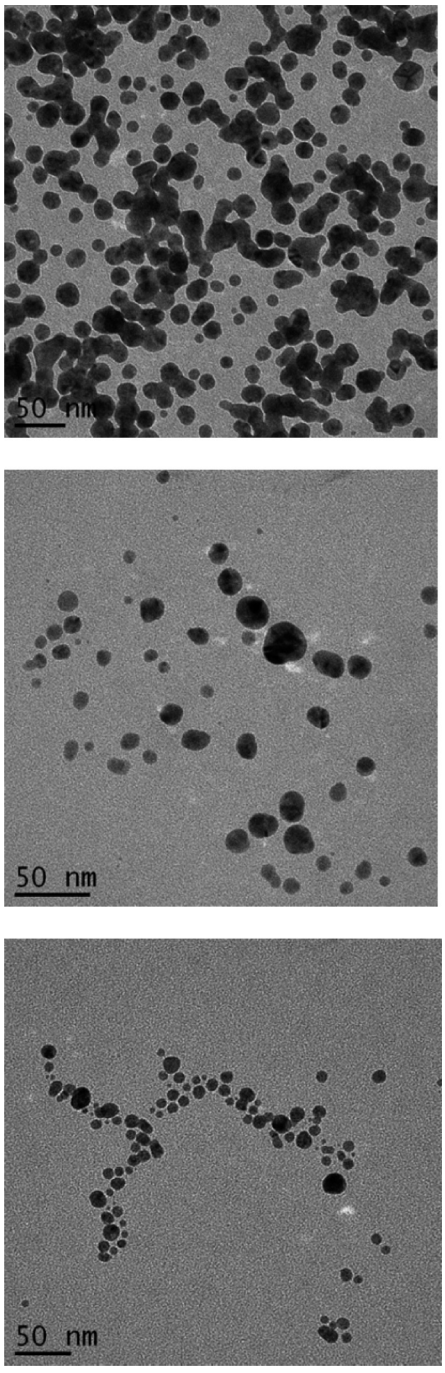

Figure 4. TEM images of synthesized AgNPs using arabinose + starch (top image), dextrose + starch (middle image), and starch-only (bottom image).

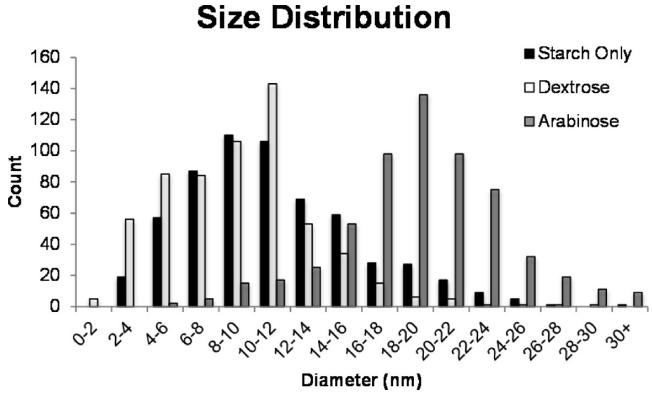

Figure 5. Size distribution of synthesized AgNPs (as composites from four separate syntheses) as gathered from TEM images. A total of 595 particles were counted for each synthesis.

standard deviation; n = 316) for a relative standard deviation (RSD, %) of 5.7% versus 20.2 ± 4.5 nm by TEM, RSD = 22% (Figure 6a). The sizing analysis by DLS and TEM for the dextrose + starch method was not in agreement. By DLS, the mean diameter ± standard deviation was 28.9 ± 2.8 nm (RSD = 9.7%) versus TEM data, where the mean AgNP size was 10.2 ± 4.1 nm, RSD = 40% (Figure 6b). A previous report that utilized starch + β-(d)-glucose for the formation of AgNPs demonstrated a mean diameter of 5.3 ± 2.6 nm (RSD = 49%) for n = 624 particles following a 20 h synthesis.[13] In a more recent study, AgNPs synthesized with starch and d-glucose produced AgNPs ranging in size from 3.7 to 4.8 nm at pH 10 following a 3 h synthesis.[19] The final synthetic procedure examined in the present study, the starch-only synthesis, produced AgNPs with mean size of 15.4 ± 1.8 nm. TEM data for the starch-only synthesis indicated that AgNPs were 12.1 ± 4.8 nm, RSD = 40% (Figure 6c). Use of the microwave for the creation of AgNPs by starch-only synthesis improved the size distribution from a previous report, where an autoclave had been used to create the AgNPs; in that report, the mean particle size was 22.85 ± 12.94 nm (RSD = 57%).[12]

The successful synthesis of starch-capped AgNPs using the environmentally friendly reagents dextrose, arabinose, and soluble starch utilizing microwave-assisted synthesis has thus been presented. Creation of AgNPs was confirmed by UV−vis spectroscopy, fluorescence spectroscopy, and XRD, while morphology and size were determined via DLS and TEM. Each of the methods presented produced AgNPs with diameters nearly exclusively under 30 nm and with better size control than previous reports.[12,13] Batch-to-batch variability for the various synthetic protocols is presented in Table S1 of the Supporting Information. As compared to methods using traditional heat,[12−14] these microwave-assisted syntheses provided drastically increased experimental efficiency, either producing a far more robust reaction and/or completing in a fraction of the time. In the case of dextrose, the reaction took under 1% of the time needed for the original reaction[13] to run to completion, while in the case of starch, an intense color (amber yellow) was obtained (as opposed to clear-yellow[12]) in a comparable time indicating creation of a much higher concentration of AgNPs. The synthesis utilizing arabinose as a reducing agent improved upon robustness and time of the original synthesis as well as energy savings, yielding a AgNP solution of darker yellow in 13% of the time taken by the original reaction.[14] It has thus been demonstrated that microwave-assisted synthesis of AgNPs using green chemistry serves as an excellent alternative to traditional heat methods and these presented data compare well to our previous report of AgNPs synthesized by orange peel extract using microwave irradiation.[21] In our previous work, putative compounds (aldehydes) responsible for reduction of Ag^+ to AgNPs in orange peel extract were identified by GC/MS.[21] Here, relatively pure reagents of soluble starch, arabinose, and dextrose in 18 MΩ DI water were utilized in the synthesis of AgNPs. Relatively recent work suggested that the synthesis of AgNPs when using starch and sugars at a high temperature (>70 °C) is important. Under high temperature, the starch is

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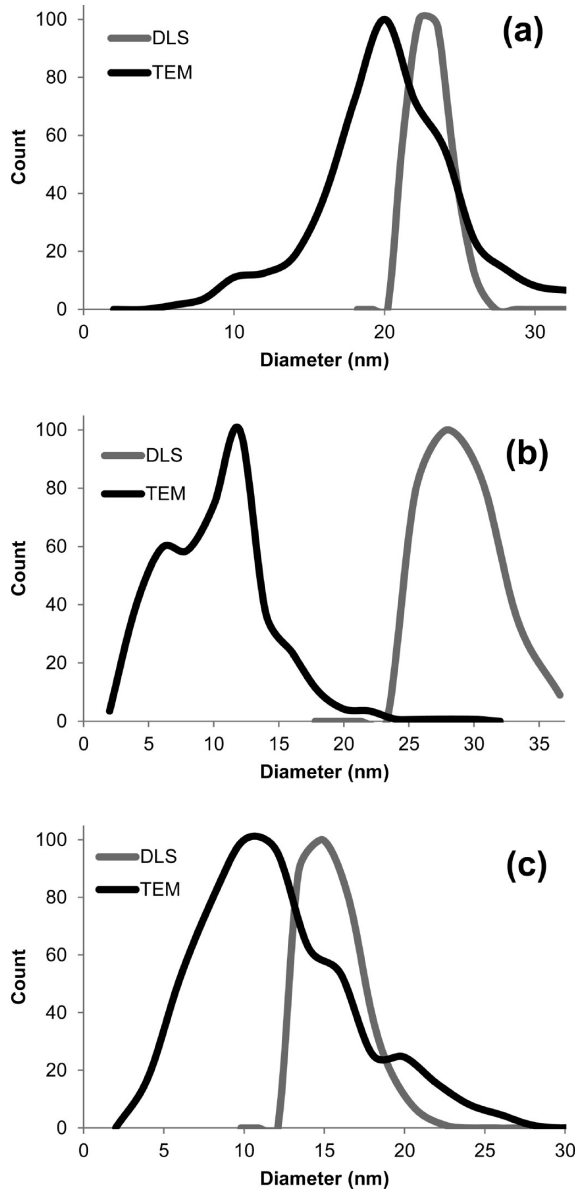

Figure 6. Comparison of DLS data (blue line) for size analysis of AgNPs versus TEM (red line) for (a) arabinose + starch syntheses, (b) dextrose + starch syntheses, and (c) starch-only syntheses. The sum of the weighted averages for size distribution indicated that for (a) arabinose + starch AgNPs were 23.0 ± 1.3 nm (DLS; mean ± standard deviation; n = 316) vs 20.2 ± 4.5 nm (TEM; n = 595), (b) dextrose + starch AgNPs were 28.9 ± 2.8 nm (DLS; n = 305) vs 10.2 ± 4.1 nm (TEM; n = 596), and (c) starch-only AgNPs were 15.4 ± 1.8 nm (DLS; n = 311) vs 12.1 ± 4.8 nm (TEM; n = 595).

hydrolyzed, and thus, more primary hydroxyl groups are available for oxidation to produce AgNPs, which may in turn produce aldehydes and carboxylate functional groups to stabilize these newly formed AgNPs.[19]

Upon completion of AgNP synthesis and characterization, several Gram negative and Gram positive bacterial strains were exposed to the AgNPs to examine effects on bacteria with different cell wall structures. Bacterial cells that stain Gram positive are characterized by having a thick mesh-like peptidoglycan layer surrounding the cell's plasma membrane, while Gram negative cells have a plasma membrane encased in a thin peptidoglycan mesh, which is in turn enveloped in an outer lipid bilayer. Chemicals, such as antibiotics, must first interact with or pass through the cell wall structure in order to function and often act differently on Gram positive as compared to Gram negative cells. For the first well diffusion assay, 50 μL of nanoparticle suspension (106 ng particles) were deposited in 8 mm-wide wells after bacteria cultures had been spread on the surface of the agar plate, such that overnight growth of the cells could be affected by diffusion of the nanoparticle suspension through the agar medium. A difference of 0.5−1.0 mm in the zone of inhibition affects a considerable number of cells as these bacteria range in size from 1−5 μm in length or diameter.

We determined that the capping-reducing solution, used as a control, had no effect on bacterial growth (Figure 7) for any of

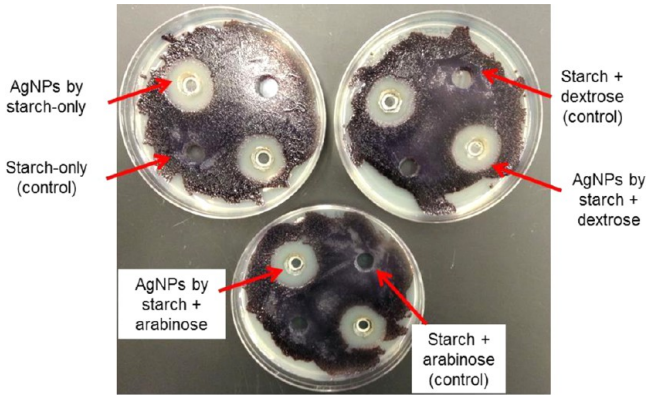

Figure 7. *Janthinobacterium lividum* exposed to capping/reducing agent only (as control) or AgNP suspensions. The wells showing no zone of inhibition of bacterial growth are control solutions (n = 2 per plate). AgNP suspensions (106 ng/50 μL) were added to the two remaining wells on each plate. In addition to the zone of inhibition, a ring of precipitate is visible at the bottom of each well.

the strains. Diameters of zones of inhibition for AgNP-bacterial interaction are listed in Table 1, including a comparison to published values. Nanoparticle suspensions applied at 106 ng/50 μL produced darkly colored sides on the wells and left a ring of pale precipitate on the bottom of the well (Figures 7 and 8, column 1). Diluting the nanoparticle suspension (with the respective capping/reducing control solution) by 10-fold or 100-fold gave color-free diffusion into the agar (Figure 8, columns 2 and 3) and typically resulted in slightly larger zones of inhibition than the more concentrated suspensions (Table 2). Though there are different effects among the bacterial strains exposed to nanoparticles, there was no distinction between strains strictly on cell wall structure (compare the Gram negative *E. coli* to the Gram negative *P. aeruginosa* and *P. aeurginosa* to the Gram positive *S. aureus*). Differences between these data and published reports[26−28] may be due to variation in AgNP concentration or to diffusion effects caused by agar medium composition. In one of those reports, AgNPs synthesized by citrus peel were tested for antibacterial effects.[26] Given some of the differences between our reported data and the previously reported antibacterial effects of green AgNPs, we included orange-peel AgNPs that we synthesized and characterized as well.[21] These AgNPs were effective against Gram positive and Gram negative bacteria, whereas a previous reported suggested improved antibacterial activity against Gram negative organisms (*E. coli* and *P. aeruginosa*). Among all strains tested, the widest zone of inhibition was seen for *J. lividum*, a

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Table 1. Diameter (mm) of Zone of Inhibition for AgNPs Synthesized with (1) Starch-Only, (2) Starch + Dextrose, or (3) Starch + Arabinose at Full Concentration (~2 μg/mL silver)[a]

AgNP type	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	*J. lividum*	*control*[b]
starch-only[c]	19	18	13	18	13.5	24	no effect
starch + dextrose	19	17	15	19	14	24	no effect
starch + arabinose	18	17	14	18	13	24	no effect
literature[26−28] for 10-mm wells	7.8−27	12	12.5−23	6−23	19−23	no report	−

[a]There was a significant amount of precipitate that formed for the full strength applications of the AgNP preparation. The wells were lined with a dark orange color, and the bottom of the well had a faint hazy ring in it. [b]Controls consisted of starch-only, starch + dextrose, or starch + arabinose without the addition of $AgNO_3$. All controls were carried through the microwave procedure. [c]AgNPs were dispensed into 8-mm wells compared to well sizes of 10 mm reported in the literature.

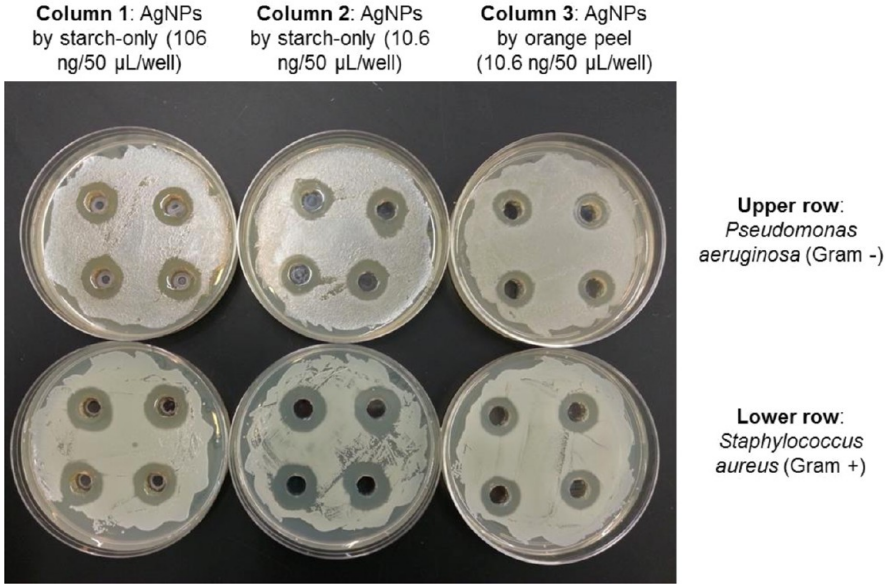

Figure 8. *Pseudomonas aeruginosa* (Gram negative) and *Staphylococcus aureus* (Gram positive) exposed to starch-only AgNPs (106 ng/50 μL), starch-only AgNPs (10.6 ng/50 μL), and orange peel AgNPs (10.6 ng/50 μL).

Table 2. Diameter (mm) of Zone of Inhibition for AgNPs Synthesized with (1) Starch-Only, (2) Starch + Dextrose, (3) Starch + Arabinose at Diluted AgNP Preparation Concentrations

organism[a]	AgNP type	zone of inhibition (mm) for 10.6 ng/50 μL (*n*)	zone of inhibition (mm) for 1.06 ng/50 μL (*n*)	AgNP TEM mean size (±diameter), nm
S. aureus	starch-only	19.5 (4)	18.5 (3)	12.1 (±4.8)
	starch + dextrose	19.5 (4)	14.5[c] (4)	10.2 (±4.1)
	starch + arabinose	20.5 (4)	15.5 (4)	20.2 (±4.5)
	orange peel[b]	16.5 (4)	− (0)	7.36 (±8.06)
P. aeruginosa	starch only	17.0 (4)	15.5[c] (4)	12.1 (±4.8)
	starch + dextrose	16.0 (4)	− (3)	10.2 (±4.1)
	starch + arabinose	16.5 (4)	14.5[c] (4)	20.2 (±4.5)
	orange peel	16.0 (4)	− (0)	7.36 (±8.06)

[a]*S. aureus* is a Gram positive organism, and *P. aeruginosa* is a Gram negative organism. [b]Included in this study were AgNPs synthesized using orange peel extract.[21] [c]Small colonies were observed growing in the zone of inhibition.

bacterium collected from a mountain stream, which only grows at room temperature and more slowly than the other bacteria. The slow growth may have been a factor in the greater effect of nanoparticle exposure.

The objective of this work was to examine the use of the microwave to create AgNPs with (1) starch-only, (2) starch + dextrose, and (3) starch + arabinose. Though the starch-only and dextrose syntheses had been previously reported[12,13,17,18,20] and the use of the microwave to create AgNPs has been well reviewed,[11] we utilized these well-characterized AgNPs to examine antibacterial effects in several Gram positive and Gram negative strains. Preliminary work demonstrated the following: (1) Use of the microwave allowed for improved control of AgNP size when using the starch-only or starch + dextrose syntheses when compared to previous reports.[12,13] (2) Energy savings was achieved. While we did not prepare AgNPs using previously published protocols that relied upon traditional heat, we can speculate on the energy savings. The microwave method we utilized had a maximum power consumption of 200 W over 15 min for each synthesis, so each synthesis required 0.05 kWh. In one seminal manuscript,[13] the synthesis of AgNPs by starch was carried out over 20 h and heated to 40 °C. Though the

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authors did not specify how they heated their reaction mixture in that previous work,[13] if they used a heating stirring mantle (such as one available through Fisher Scientific), 287.5 W are used at a fixed amperage (230 V × 1.25 A). Thus, 5.75 kWh are used for that particular reaction, using 115 times the amount of energy (in kWh) compared to our microwave-assisted synthesis. (3) Successful synthesis of starch + arabinose was demonstrated. (4) All synthesized AgNPs had antibacterial effects against Gram positive and Gram negative organisms at all AgNP suspensions tested per 50 μL deposited per well (106, 10.6, and 1.06 ng), whereas the controls (capping/reducing agent solutions) did not.

Given the interesting preliminary results for the antibacterial effects of AgNPs, future work will examine the effects of AgNPs versus antibiotics and the effect of AgNPs on chemical signaling compounds or metabolites (such as indole). For example, the normal human intestinal bacterium *E. coli* secretes indole into its environment, producing greater amounts of indole under stressful conditions.[44] Indole produced by norfloxacin-resistant *E. coli* has been shown to protect nonantibiotic resistant *E. coli* cells in broth culture, demonstrating an unexpected mechanism of bacterial survival in the presence of antibiotic concentrations previously shown to be lethal.[45] Bacterial evasion of antibiotics, and perhaps AgNPs, is a more complex phenomenon than simple mutation in the genome or on a plasmid. The information provided by these studies will expand our understanding of bacterial evolutionary response to the prevalence of environmental stressors such as AgNPs.

Safety Concerns. Solvents used (methanol, acetone, methylene chloride) should be used in a chemical fume hood and disposed of properly. Silver waste (from $AgNO_3$ and AgNP syntheses) should be disposed of properly. All work with bacterial organisms was handled with biosafety level 2 precautions.

■ ASSOCIATED CONTENT

Supporting Information

Data concerning batch-to-batch variability in UV−vis absorption data and fluorescence spectra (and spectra of blanks). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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