Applied and Environmental Microbiology, May 2009, p. 2973–2976 Vol. 75, No. 9
0099-2240/09/$08.00+0 doi:10.1128/AEM.01658-08

Antimicrobial Properties of a Novel Silver-Silica Nanocomposite Material[▽]

Salome Egger,[1] Rainer P. Lehmann,[1] Murray J. Height,[2,3] Martin J. Loessner,[1] and Markus Schuppler[1]*

Institute of Food Science and Nutrition, ETH Zurich, Schmelzbergstrasse 7, 8092 Zurich, Switzerland[1]; HeiQ Materials, Zürcherstrasse 42, 5330 Bad Zurzach, Switzerland[2]; and Particle Technology Laboratory, ETH Zurich, Sonneggstrasse 3, 8092 Zurich, Switzerland[3]

Received 18 July 2008/Accepted 22 February 2009

Nanotechnology enables development and production of novel silver-based composite materials. We used in vitro tests to demonstrate the antimicrobial activity of a silver-silica nanocomposite compared to the activities of conventional materials, such as silver nitrate and silver zeolite. A silver-silica-containing polystyrene material was manufactured and shown to possess strong antimicrobial properties.

Many applications, including medicine and food production and storage, would benefit greatly from incorporation of safe and inexpensive long-lasting biocides into polymers, paints, or textiles (1, 5). The antimicrobial effect of silver additives is broadly used in various injection-molded plastic products, in textiles (13), and in coating-based applications, including air ducts, countertops, and food preparation areas (12). Some important advantages of silver-based antimicrobials are their excellent thermal stability and their health and environmental safety (19). However, like the use of all biocide products, the use of silver is strictly controlled by various national laws and control agencies. In the United States, the Environmental Protection Agency has regulated the use of silver as a biocide since 1954 (2) under the Federal Insecticide Fungicide and Rodenticide Act. In the European Union, a European biocide product directive (EU/BPD/98) imposes regulatory requirements on the use and claims associated with all biocide products (3).

In the past few years, there has been a tremendous push for development of inorganic nanoparticles with structures that exhibit novel physical, chemical, and biological properties (34). In particular, the potential benefits of nano-silver materials have been recognized by many industries due to the strong antimicrobial activity of silver against a broad spectrum of bacteria, viruses, and fungi and the low frequency of development of resistance (10, 30).

Generally, silver-based antimicrobial additives consist of silver ions integrated into inert matrices consisting of ceramic, glass, or zeolite. Other silver additives based on silver salts or metallic silver may be readily incorporated into thermoplastic polymers, such as polyethylene, polypropylene, polystyrene, or nylon (5). The bactericidal efficacy of silver-containing polymers is based on the release of silver ions (Ag^+) through interaction with a liquid watery phase (19). Although the antimicrobial effects of silver ions and salts have been intensively studied, the mechanism of the inhibitory action of silver on microbes is still not fully understood. It has been proposed that silver ions interact with disulfide or sulfhydryl groups of enzymes, causing structural changes that lead to disruption of metabolic processes followed by cell death (8, 11). The inhibitory action of silver nanoparticles is also based on the release of Ag^+ (20, 24). Exposure of microorganisms to silver nanoparticles was shown to result in strong antimicrobial activity (6, 9, 26, 33). In addition to the increased surface area and associated increased potential for the release of Ag^+, when dispersed in liquid suspensions, silver nanoparticles may accumulate in the bacterial cytoplasmic membrane, causing a significant increase in permeability and cell death (33), and penetrate bacterial cells (28). Recently, it has been suggested that the antimicrobial mechanism of silver nanoparticles may also be related to membrane damage due to free radicals that are derived from the surface of the nanoparticles (16). This bactericidal activity also appears to be dependent on the size and shape of the silver nanoparticles (25).

In this study, we evaluated the properties of a novel silver-silica nanocomposite material (HeiQ AGS-20; HeiQ Materials, Bad Zurzach, Switzerland) used as an antimicrobial additive and compared its efficacy to the efficacies of the conventional silver additives silver nitrate ($AgNO_3$; 63.5% Ag) and silver zeolite (38% Ag bound to type A zeolite; Sigma-Aldrich, Buchs, Switzerland). The novel silver-silica nanocomposite material was produced using an industrial flame spray pyrolysis process. This process involves combustion of a flammable solvent containing homogeneously dissolved compounds as the source of components for the synthesis of the material (14, 21, 22). A representative transmission electron micrograph of the silver-silica material is shown in Fig. 1. The nanocomposite consists of silver nanoparticles embedded in a matrix of amorphous silicon dioxide (SiO_2). The SiO_2 fine structure consists of aggregate matrix particles with an average diameter of approximately 1 μm (Fig. 1A). Silver metal particles are located on the surface of the silica and are also embedded within the matrix (Fig. 1B). High-magnification scanning transmission electron microscopy imaging of a localized region of a nanoparticle indicated that each silica particle contains many small silver metal particles with a typical diam-

* Corresponding author. Mailing address: Institute of Food Science and Nutrition, ETH Zurich, Schmelzbergstrasse 7, 8092 Zurich, Switzerland. Phone: (41) 44 632 7862. Fax: (41) 44 632 1266. E-mail: markus.schuppler@ilw.agrl.ethz.ch.

▽ Published ahead of print on 6 March 2009.

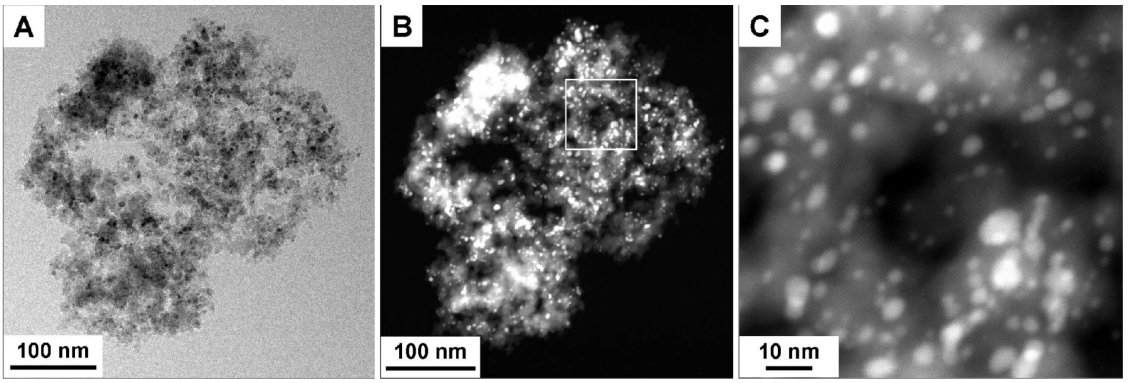

FIG. 1. (A) Transmission electron micrograph showing an amorphous silicon dioxide aggregate particle (gray structure) together with numerous supported silver metal particles (dark spots). (B) Scanning transmission electron micrograph of the structure shown in panel A, providing better contrast between the silica structure (gray) and the silver metal particles (bright spots). (C) Higher magnification of the region in panel B enclosed in a box. The silver metal particles are typically between 1 and 10 nm in diameter. Transmission electron microscope and scanning transmission electron microscope images were obtained using an FEI Tecnai F30 FEG microscope operated at 300 kV.

eter between 1 and 10 nm (Fig. 1C). The specific surface area of the nanocomposite powder, as measured by nitrogen adsorption (7), is typically about 250 m^2/g, a value which is consistent with the open structure of the silica aggregate shown in Fig. 1. It can be concluded that, upon contact with moisture, the pure silver particles act as a source that releases silver ions, which represent the active antimicrobial principle (27). Some key advantages of the novel nanocomposite are the dispersion of the discrete silver particles throughout the silica (which prevents agglomeration of the silver particles), the small diameter of the silver particles (which results in a large surface area and release of a large amount of Ag^+, which results in high antimicrobial efficiency), and the small size of the silver-silica composite (ca. 1 μm) (which allows the material to be uniformly dispersed and readily incorporated into a variety of substrates, including synthetic fibers, plastics, and other thin or delicate materials). The silica structure acts as a convenient carrier for incorporating the fine silver particles into plastics, textiles, and coatings. A further advantage is that the immobilization of silver nanoparticles within the silica structure limits the potential for release and disposal of the nanoparticles themselves. This property may be highly desirable because of the possible abilities of nanoparticles to cross biological membranes and other barriers (31).

The microorganisms and growth conditions used for antimicrobial testing are shown in Table 1. The MICs for all combinations of silver materials and microorganisms were determined by preparing twofold serial dilutions of the additives in an appropriate growth medium (Table 1). The tubes were then inoculated with 10^7 CFU/ml from overnight cultures of the bacteria or 10^6 CFU/ml for *Candida albicans* and incubated on a shaker (180 rpm) for 24 h. The MIC was defined as the lowest

TABLE 1. Comparison of the antimicrobial activities of silver nanocomposite powder, silver nitrate, and silver zeolite

Microorganism	Silver nanocomposite		Silver nitrate		Silver zeolite	
	MIC (μg/ml)[a]	MBC or MFC (μg/ml)[b]	MIC (μg/ml)[a]	MBC or MFC (μg/ml)[b]	MIC (μg/ml)[a]	MBC or MFC (μg/ml)[b]
Escherichia coli ATCC 2732[c]	62.5	125	7.8	15.6	3.9	15.6
Klebsiella pneumoniae ATCC 4352[c]	62.5	125	3.9	7.8	7.8	31.2
Pseudomonas fluorescens LME 2333[d]	62.5	250	7.8	7.8	15.6	31.2
Salmonella enterica serovar Enteritidis D1[c]	62.5	250	3.9	7.8	15.6	62.5
Salmonella enterica serovar Typhimurium DB 7155[c]	62.5	250	3.9	15.6	15.6	31.2
Enterococcus faecalis ATCC 19433[e]	62.5	250	3.9	7.8	7.8	7.8
Bacillus cereus ATCC 14579[e]	250	500	31.2	31.2	62.5	250
Listeria monocytogenes Scott A[f]	500	1,000	31.2	31.2	31.2	62.5
Staphylococcus aureus ATCC 29213[f]	250	1,000	15.6	15.6	15.6	125
Candida albicans ATCC 10259[g]	125	2,000	31.2	250	62.5	250
Aspergillus niger ATCC 9642[h]	2,000	ND[i]	15.6	ND	125	ND

[a] The MIC was determined at least in duplicate.
[b] The MBC or minimum fungicidal concentration (MFC) was determined at least in duplicate.
[c] Cultured in Luria-Bertani broth (10 g/liter peptone from casein, 5 g/liter yeast extract, 10 g/liter NaCl; Merck, Darmstadt, Germany) at 37°C.
[d] Cultured in Biotone tryptose broth (Biolife, Milan, Italy) at 30°C.
[e] Cultured in Biotone tryptose broth (Biolife, Milan, Italy) at 37°C.
[f] Cultured in half-strength brain heart infusion broth (Biolife, Milan, Italy) at 37°C.
[g] Cultured in malt extract broth (Merck, Darmstadt, Germany) at 37°C.
[h] Cultured on malt extract agar (Merck, Darmstadt, Germany) at 30°C. The MIC for *A. niger* was defined as the lowest concentration not associated with visible growth on malt extract agar after 72 h of incubation at 30°C.
[i] ND, not determined.

concentration of the silver additive at which no visual turbidity of the growth medium developed. The minimal bactericidal concentration (MBC) was determined by surface plating 0.2-ml aliquots from the nonturbid tubes, followed by incubation at 37°C for 24 h. The MBC was defined as the lowest concentration of silver additive resulting in less than 200 colonies per plate (corresponding to a killing rate of more than 4 logs). *Aspergillus niger* spores were harvested by floating the spores in densely grown lawns on malt extract agar plates in an extraction buffer (0.1% [vol/vol] Tween 20, 145 mM sodium chloride, 20 mM sodium phosphate; pH 7.4) and removing them. The MIC was determined by spreading approximately 200 spores on malt extract agar plates containing serial dilutions of the silver additives (Table 1) and was defined as the lowest concentration that prevented visible growth after 72 h of incubation at 30°C.

MICs and MBCs for the silver additives tested are shown in Table 1. All experiments were performed at least in duplicate. For bacteria, the MICs of the nanocomposite material ranged from 62.5 to 500 μg/ml, corresponding to 12.5 to 100 μg pure Ag/ml. The MICs of silver nitrate varied from 3.9 to 31.2 μg/ml (corresponding to 2.4 to 19.8 μg Ag/ml), and the MICs of silver zeolite ranged from 3.9 to 31.2 μg/ml (corresponding to 2 to 12 μg Ag/ml). The MBCs determined were in the ranges from 125 to 1,000 μg/ml for the nanocomposite powder, from 7.8 to 31.2 μg/ml for silver nitrate, and from 7.8 to 125 μg/ml for silver zeolite. Growth of *C. albicans* was inhibited by 125 μg/ml silver nanocomposite, and the minimal fungicidal concentration was 2 mg/ml. Development of visible colonies of *A. niger* on agar plates was also completely inhibited by 2 mg silver nanocomposite per ml agar.

In general, gram-positive bacteria appeared to be more tolerant to silver than gram-negative cells (Table 1), except for *Enterococcus faecalis*, for which the MICs and MBCs were similar to those for gram-negative bacteria. It has previously been reported that gram-positive bacteria are less susceptible to the antimicrobial activity of silver (15, 16, 29). It was speculated that this may be due to differences in the cell wall structure (15). The cell wall of gram-positive bacteria contains multiple layers of peptidoglycan compared to the cell wall of gram-negative bacteria. Peptidoglycan is a complex structure and often contains teichoic acids or lipoteichoic acids which have a strong negative charge, which may contribute to sequestration of free Ag^+ ions. Thus, gram-positive bacteria may allow less Ag^+ to reach the cytoplasmic membrane than gram-negative bacteria allow (15) and may therefore be less susceptible.

Susceptibility tests using different silver compounds in previous studies revealed that the MICs of silver particles for *Escherichia coli* ranged from 2 to 75 μg/ml (24, 32). However, because corresponding silver concentrations were not specified, it is not possible to compare these values to our results. For silver zeolite containing 1.9% (wt/wt) Ag, the previously reported MICs determined by using a similar protocol ranged from 256 to 2,048 μg/ml, corresponding to 4.8 to 38.4 μg/ml of Ag (15). Here we used silver zeolite containing 38% (wt/wt) Ag to determine the MIC for *E. coli*. The MIC determined (1.9 to 3.9 μg/ml) was much lower than the previously reported MICs of the silver zeolite containing 1.9% Ag. The nanocomposite material had an MIC of 62.5 μg/ml (12.5 μg Ag/ml) for *E. coli*. Not considering the relative Ag content, silver nitrate and silver zeolite (38% Ag) resulted in inhibition that was approximately 10 times more effective than the inhibition observed with the nanocomposite. This can be explained by the fact that in aqueous systems silver nitrate dissolves completely and the silver is completely available in its biologically active ionic form. The silver ions held in the zeolite structure are also relatively rapidly released into solution. In contrast, the silver nanoparticles embedded within the silica matrix release Ag^+ in a more gradual, controlled manner and at a much lower rate (18). Thus, although silver nitrate and silver zeolite are more effective in applications where high Ag^+ concentrations are required immediately, the effect is only short lived. In contrast, the nanocomposite powder allows slow and controlled release of Ag^+, resulting in long-term antimicrobial activity. This should be a clear advantage in any long-term antimicrobial applications (e.g., contact surfaces, fibers, plastics, medical devices, food-manufacturing equipment, cutting boards, etc.).

To examine the antimicrobial properties of a typical application product, silver-containing polystyrene plates were manufactured from commercially available polystyrene polymer (clear, unfilled) using a thermoplastic injection-molding process (17, 23). Test coupons that were 50 by 50 by 1.5 mm and contained the nanocomposite material (0.25% [wt/wt], corresponding to approximately 500 ppm Ag) were produced by dry blending the polystyrene polymer with the required amount of polymer concentrate containing the silver nanocomposite additive, which was followed by injection molding. The antimicrobial activity was determined by using the Japanese industrial standard test (JIS Z 2801:2000) (4). In brief, the test samples were placed in petri dishes and inoculated with 0.4 ml of a bacterial culture containing 10^5 to 10^6 CFU/ml. The inoculum was covered with a polyester film (X-131 transparent copier film; Folex Imaging), and the petri dishes were incubated at 37°C for 24 h in a humid chamber to prevent desiccation. After the incubation period 20 ml of extraction solution (0.1% [vol/vol] Tween 20, 145 mM sodium chloride, 20.5 mM sodium phosphate; pH 7.4) was added to the petri dishes and shaken for 2 min. Subsequently, serial dilutions of the extraction solution were spread on agar plates in triplicate and incubated at 37°C overnight. Colonies were counted visually, and the numbers of CFU per sample were determined. The activity value was calculated from the mean value for the individual samples by subtraction of the log value determined for the test sample from the log value determined for the control. The results for viable counts determined for the control and the silver nanocomposite-containing samples are shown in Table 2. The activity values determined by the JIS Z 2801:2000 method (4) were 4.4 for *E. coli* and 2.1 for *Staphylococcus aureus* ($P < 0.05$, Student's t test [$n = 3$]). The results demonstrate that the silver-silica nancomposite-containing polystyrene material has significant antibacterial activity against both *E. coli* and *S. aureus*.

In this study, a silver-silica nanocomposite material with a novel structure and composition was investigated to determine its antimicrobial properties. The material exhibited very good antimicrobial activity against a wide range of microorganisms. The inhibition of microbial growth due to surface contact with the silver-silica nanocomposite-containing polystyrene demonstrated that materials functionalized with the silver nanocom-

TABLE 2. Antimicrobial activity of silver nanocomposite-containing polystyrene plates[a]

Organism	No. of cells (CFU/sample)[b]			Activity
	Polystyrene control after inoculation	Polystyrene control after 24 h	Polystyrene with silver after 24 h	
Escherichia coli	$1.7 \times 10^5 \pm 4.9 \times 10^4$	$2.6 \times 10^6 \pm 6.5 \times 10^4$	<100	4.4
Staphylococcus aureus	$1.7 \times 10^5 \pm 7.9 \times 10^4$	$1.8 \times 10^5 \pm 6.9 \times 10^4$	$1.4 \times 10^3 \pm 9.0 \times 10^2$	2.1

[a] Activity was tested by using Japanese industrial standard JIS Z 2801:2000 (4).
[b] The values are means ± standard deviations for measurements obtained for three independent sample pieces. The values for the polystyrene control after 24 h and polystyrene with silver after 24 h were significantly different according to the Student *t* test ($P < 0.05$; $n = 3$).

posite have excellent antimicrobial properties. Further studies of the mode of action of the silver-silica nanocomposite material with gram-positive and gram-negative bacteria and also with yeasts and molds are required to fully evaluate its potential for use as an antimicrobial additive in various materials.

We are grateful to Joos Kiener for excellent technical assistance and to Elisabeth Müller Gubler from the Electron Microscopy Center of ETH Zürich for the electron microscopy images. M.J.H. thanks S. E. Pratsinis for his suggestions and ideas concerning synthesis of the material and the Particle Technology Laboratory, Department of Mechanical and Process Engineering at the Swiss Federal Institute of Technology (ETH Zurich), for supporting initial development of the material.

REFERENCES

1. **Adams, A. P., E. M. Santschi, and M. A. Mellencamp.** 1999. Antibacterial properties of a silver chloride-coated nylon wound dressing. Vet. Surg. **28:** 219–225.
2. **Anonymous.** 1993. EPA reregistration eligibility document for silver, case 4082. U.S. Environmental Protection Agency, Washington, DC. http://www.epa.gov/oppsrrd1/REDs/old_reds/silver-pdf.
3. **Anonymous.** 1998. Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market. Off. J. Eur. Communities L123/1. http://ec.europa.eu/environment/biocides/pdf/dir_98_8_biocides.pdf.
4. **Anonymous.** 2000. JIS Z 2801:2000. Antimicrobial products—test for antimicrobial activity and efficacy. Japanese Standards Association, Tokyo, Japan.
5. **Appendini, P., and J. H. Hotchkiss.** 2002. Review of antimicrobial food packaging. Innov. Food Sci. Emerg. Technol. **3:**113–126.
6. **Baker, C., A. Pradhan, L. Pakstis, D. J. Pochan, and S. Ismat Shah.** 2005. Synthesis and antimicrobial properties of silver nanoparticles. J. Nanosci. Nanotechnol. **5:**244–249.
7. **Brunauer, S., P. H. Emmett, and E. Teller.** 1938. Adsorption of gases in multimolecular layer. J. Am. Chem. Soc. **60:**309–319.
8. **Butkus, M. A., L. Edling, and M. P. Labare.** 2003. The efficacy of silver as a bactericidal agent: advantages, limitations and considerations for future use. J. Water Supply Res. Technol. AQUA **52:**407–416.
9. **Cho, K.-H., J.-E. Park, T. Osaka, and S.-G. Park.** 2005. The study of antimicrobial activity and preservative effects of nanosilver ingredient. Electrochim. Acta **51:**956–960.
10. **Damm, C., M. Neumann, and H. Münstedt.** 2006. Properties of nanosilver coatings on polymethyl methacrylate. Soft Mater. **3:**71–88.
11. **Feng, Q. L., J. Wu, G. Q. Chen, F. Z. Cui, T. N. Kim, and J. O. Kim.** 2000. A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. J. Biomed. Mater. Res. **52:**662–668.
12. **Galeano, B., E. Korff, and W. L. Nicholson.** 2003. Inactivation of vegetative cells, but not spores, of *Bacillus anthracis*, *B. cereus*, and *B. subtilis* on stainless steel surfaces coated with an antimicrobial silver- and zinc-containing zeolite formulation. Appl. Environ. Microbiol. **69:**4329–4331.
13. **Gao, Y., and R. Cranston.** 2008. Recent advances in antimicrobial treatment of textiles. Text. Res. J. **78:**60–72.
14. **Height, M. J., and S. E. Pratsinis.** February 2006. Antimicrobial and antifungal powders made by flame spray pyrolysis. European patent EP1846327.
15. **Kawahara, K., K. Tsuruda, M. Morishita, and M. Uchida.** 2000. Antibacterial effect of silver-zeolite on oral bacteria under anaerobic conditions. Dent. Mater. **16:**452–455.
16. **Kim, J. S. K., E. Kuk, K. N. Yu, J.-H. Kim, S. J. Park, H. J. Lee, S. H. Kim, Y. K. Park, Y. H. Park, C.-Y. Hwang, Y.-K. Kim, Y.-S. Lee, D. H. Jeong, and M.-H. Cho.** 2007. Antimicrobial effects of silver nanoparticles. Nanomed. Nanotechnol. Biol. Med. **3:**95–101.
17. **Ku, P. L.** 1988. Polystyrene and styrene copolymers. I. Their manufacture and application. Adv. Polym. Technol. **8:**177–196.
18. **Kumar, R., S. Howdle, and H. Münstedt.** 2005. Polyamide/silver antimicrobials: effect of filler types on the silver ion release. J. Biomed. Mater. Res. Part B **75:**311–319.
19. **Kumar, R., and H. Münstedt.** 2005. Polyamide/silver antimicrobials: effect of crystallinity on the silver ion release. Polym. Int. **54:**1180–1186.
20. **Lok, C.-N., C.-M. Ho, R. Chen, Q.-Y. He, W.-Y. Yu, H. Sun, P. K.-H. Tam, J.-F. Chiu, and C.-M. Che.** 2007. Silver nanoparticles: partial oxidation and antibacterial activities. J. Biol. Inorg. Chem. **12:**527–534.
21. **Mädler, L., H. K. Kammler, R. Mueller, and S. E. Pratsinis.** 2002. Controlled synthesis of nanostructured particles by flame spray pyrolysis. J. Aerosol Sci. **33:**369–389.
22. **Mädler, L., W. Stark, and S. E. Pratsinis.** 2003. Simultaneous deposition of gold nanoparticles during flame synthesis of titania and silica. J. Mater. Res. **18:**115–120.
23. **Martin, M. F., J. P. Viola, and J. R. Wuensch.** 2003. Preparation, properties and applications of high-impact polystyrene, p. 247–280. *In* J. Scheirs and D. B. Priddy (ed.), Modern styrenic polymers: polystyrenes and styrenic copolymers. John Wiley & Sons, New York, NY.
24. **Morones, J. R., J. L. Elechiguerra, A. Camacho, K. Holt, J. B. Kouri, J. T. Ramírez, and M. J. Yacaman.** 2005. The bactericidal effect of silver nanoparticles. Nanotechnology **16:**2346–2353.
25. **Pal, S., Y. K. Tak, and J. M. Song.** 2007. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium *Escherichia coli*. Appl. Environ. Microbiol. **73:**1712–1720.
26. **Panáček, A., L. Kvítek, R. Prucek, M. Kolár, R. Večeřová, N. Pizúrová, V. K. Sharma, T. Nevěčná, and R. Zbořil.** 2006. Silver colloid nanoparticles: synthesis, characterization, and their antimicrobial activity. J. Phys. Chem. B **110:**16248–16253.
27. **Radeshkumar, C., and H. Münstedt.** 2006. Antimicrobial polymers from polypropylene/silver composites—Ag^+ release measured by anode stripping voltammetry. React. Funct. Polym. **66:**780–788.
28. **Raffi, M., F. Hussain, T. M. Bhatti, J. I. Akhter, A. Hameed, and M. M. Hasan.** 2008. Antibacterial characterization of silver nanoparticles against *E. coli* ATCC-15224. J. Mater. Sci. Technol. **24:**192–196.
29. **Rhim, J.-W., S.-I. Hong, H.-M. Park, and P. K. W. Ng.** 2006. Preparation and characterization of chitosan-based nanocomposite films with antimicrobial activity. J. Agric. Food Chem. **54:**5814–5822.
30. **Russell, A. D., and W. B. Hugo.** 1994. Antimicrobial activity and action of silver. Prog. Med. Chem. **31:**351–370.
31. **Sanvicens, N., and M. P. Marco.** 2008. Multifunctional nanoparticles—properties and prospects for their use in human medicine. Trends Biotechnol. **26:**425–433.
32. **Sarkar, S., A. D. Jana, S. K. Samanta, and G. Mostafa.** 2007. Facile synthesis of silver nano particles with highly efficient anti-microbial property. Polyhedron **26:**4419–4426.
33. **Sondi, I., and B. Salopek-Sondi.** 2004. Silver nanoparticles as antimicrobial agent: a case study of *E. coli* as a model for gram-negative bacteria. J. Colloid Interface Sci. **275:**177–182.
34. **Weiss, J., P. Takhistov, and D. J. McClements.** 2006. Functional materials in food nanotechnology. J. Food Sci. **71:**R107–R116.