Antibacterial Properties of an In Situ Generated and Simultaneously Deposited Nanocrystalline ZnO on Fabrics

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ABSTRACT Zinc oxide (ZnO) nanoparticles were synthesized and deposited on the surface of cotton fabrics using ultrasound irradiation. Optimization of the process resulted in a homogeneous distribution of ZnO nanocrystals, 30 nm in size, on the fabric surface. The mechanism of the ultrasound-assisted coating was proposed. The antibacterial activities of the ZnO−fabric composite were tested against *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram positive) cultures. A significant bactericidal effect, even in a 0.75% coated fabric (wt %), was demonstrated.

KEYWORDS: ZnO nanoparticles • fabrics • ultrasound irradiation • antibacterial activity

INTRODUCTION

Nanotechnology is an emerging interdisciplinary field that has become very popular in many areas during the past decade, including materials science, mechanics, electronics, optics, medicine, plastics, and the textile industry (1). The production of nanostructured fibers is one area where nanotechnology is already having a huge impact within the textile industry (2). The impact of nanotechnology in the textile industry has made it possible to produce a new generation of antimicrobial textiles by innovative finishes of the fabric surface. Nanosized particles have a larger surface area per unit mass and hence higher efficiency than bulk materials. Nanoparticles are used in textile finishing to alter surface properties and impart textile function. These "smart" textiles can be widely used for wound healing and medical applications in hospitals and other places where bacteria present a hazard (3).

Antibacterial agents can be broadly classified into two types, organic and inorganic. Organic antibacterial materials are often less stable particularly at high temperatures and/or pressures compared to inorganic antibacterial agents (4). Indeed, inorganic materials such as metal and metal oxides have attracted a lot of attention because of their ability to withstand harsh processing conditions. One of these inorganic materials is zinc oxide (ZnO). ZnO belongs to a group of metal oxides that are characterized by the following properties: photocatalytic ability, electrical conductivity, UV absorption, photooxidizing capacity versus chemical and biological species, antimicrobial, and self-sterilization (2). Moreover, ZnO is generally regarded as a safe material for human beings and animals, and it has been used extensively in the formulation of personal care products (5, 6).

There are only a few methods in the literature that describe the coating of fabrics with ZnO nanoparticles, for example, the "pad-dry-cure" method (7), radiation, and thermal treatments (8). These techniques have some disadvantages: first, they are rather complicated and involve several stages; second, a stabilizer agent is used in order to get smaller ZnO nanoparticles, resulting in the presence of impurities in the final products.

In the present work, we have developed a new simple method for preparing cotton bandages with antibacterial properties by immobilizing ZnO nanoparticles on the fabric surface via ultrasound irradiation. Sonochemical irradiation has been proven as an effective technique for the synthesis of nanophased materials (9), as well as for the deposition and insertion of nanoparticles on/into the mesoporous ceramic and polymer supports, fabrics, and glasses (10−12). This process involves in situ generation of ZnO nanoparticles under ultrasound irradiation and their subsequent deposition on fabrics in a one-step reaction. This coating is a "green" chemistry approach because it does not involve any toxic materials. In addition, it is simple and inexpensive. Moreover, this research shows that even with a low coating concentration of ZnO in the composite (less than 1%), excellent antibacterial activity is achieved. The minimum concentration reported in the literature until now for efficient killing of bacteria is 2 wt % ZnO (7).

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Received for review September 24, 2008 and accepted October 2, 2008

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DOI: 10.1021/am8000743

EXPERIMENTAL SECTION

All of the chemical reagents, of chemical grade, were purchased from Aldrich and used without further purification. ZnO sonication was conducted in the presence of a cotton bandage, 13 treads/cm^2 density. Reaction parameters were varied to obtain the best conditions for the coating of ZnO nanoparticles on the bandages. The optimal results representing a typical experiment were as follows: 1 piece of bandage 10 × 10 cm (0.7 g) was added to an 1 mM $Zn(Ac)_2 \cdot H_2O$ solution of ethanol/water (10:1) in a 100 mL sonication flask. The pH was adjusted to 8−9 with the addition of $NH_3{}^xH_2O$. The reaction mixture was irradiated for 30 min with a high-intensity ultrasonic horn (Ti horn, 20 kHz, 750 W at 70% efficiency) under a flow of argon. The sonication flask was placed in a cooling bath, maintaining a constant temperature of 30 °C during the reaction. At the end of the procedure, the color of the fabric did not change. The product was first washed thoroughly with water to remove traces of ammonia and then with ethanol and dried under vacuum.

Antimicrobial Testing. The antimicrobial activity of the coated bandages was tested against the Gram-negative bacterial strain *Escherichia coli* and the Gram-positive strain *Staphylococcus aureus*. Overnight cultures of the two strains were grown in an NB medium at 37 °C with aeration and transferred the next day into a fresh medium at an initial optical density (OD) of 0.1 at 600 nm. When the culture reached an OD of 0.3 at 600 nm, the cells were harvested by centrifugation and washed twice with a 0.9% solution of NaCl at pH 6.5. The cells were diluted to a final concentration of OD = 0.65 (600 nm) with a 0.9% solution of NaCl. A total of 1 cm^2 of the coated fabric and the uncoated fabric, which served as a reference, respectively, was poured into a vial containing 4.5 mL of a 0.9% NaCl solution. A total of 500 μL of the washed and diluted cells was pipetted into the vial. The initial bacterial concentration in the vial was approximately 10^7 CFU/mL. To ensure that any decrease in the bacterial number was likely to be due to exposure to coated bandage treatment, a 0.9% solution of NaCl without any fabric was included in the experiment as an additional control. The bacterial suspensions were incubated for up to 2 h at 37 °C and 170 rpm. Samples of 100 μL each were taken at the beginning and after 30 min, 1 h, and 2 h. Different dilutions were then transferred onto nutrient agar plates. The plates were allowed to grow overnight at 37 °C and counted for viable bacteria. The viable bacteria were monitored by counting the number of colony-forming units from the appropriate dilution on nutrient agar plates.

Characterization. The ZnO content in the bandage was determined by volumetric titration with ethylenediaminetetraacetic acid after treatment of the sample in 0.5 mol of HNO_3 and controlled by inductively coupled plasma (ICP) analysis on the device ULTIMA JY2501. The X-ray diffraction (XRD) patterns of the product were measured with a Bruker D8 diffractometer (Karlsruhe, Germany) with Cu Kα radiation. The particle morphology and size were studied with a high-resolution scanning electron microscope (HRSEM), JEOL-JSN 7000F. The determination of ZnO nanoparticles in the solution was conducted by dynamic light scattering (DLS) measurements employing a Coulter particle analyzer (Malvern Zetasizer). The mechanical tests were performed on a mechanical testing machine, ZWICK 1445 (Zwick Gmbh & Co., Ulm, Germany). Fourier transform infrared spectroscopy (FTIR) spectra were recorded using a Nicolet model 860 FTIR spectrometer.

RESULTS AND DISCUSSION

Optimization of Reaction Conditions. The first aim of this research was to reach a minimal effective concentration of the deposited ZnO nanoparticles on the fabrics, which will still demonstrate antibacterial activity. The antibacterial activity of ZnO depends on the particle size: decreasing the particle size leads to an increase in the antibacterial activity (13). Therefore, the second goal was to obtain a homogeneous coating of small nanopartices with a narrow size distribution.

Table 1. Optimization of the ZnO Coating Process[a]

expt	concn of the precursor (mM)	reaction time (h)	content of ZnO (wt %)	particle size (nm)
1	20	2	10.3	400
2	20	1	5.6	300
3	2	1	2.1	200
4	1	1	0.95	100
5	1	0.5	0.75	30

[a] Precursor $Zn(Ac)_2 \cdot 2H_2O$; solvent, 90 mL of ethanol + 9 mL of H_2O.

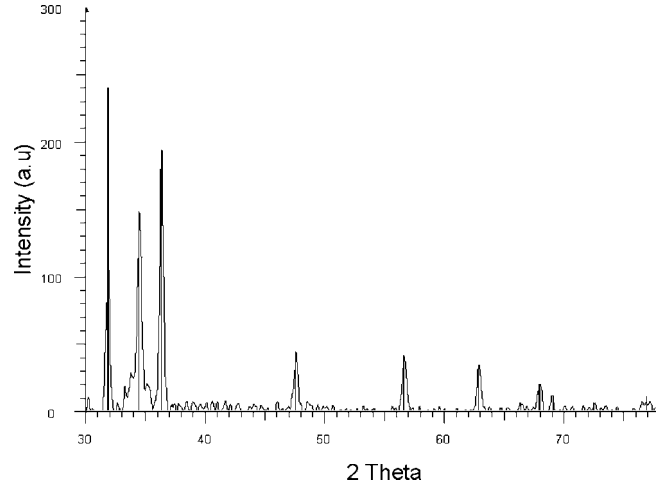

FIGURE 1. XRD diffraction pattern of the cotton bandage coated with ZnO nanoparticles.

Our previous studies indicated that the yield of the product and particle size is strongly dependent on the rate of particle collision and on the concentration of the reagents during sonochemical synthesis (8). That is why experimental parameters such as the time and concentration of the precursor were selected as important factors for the optimization of the sonochemical reaction. The different compositions used in the reaction are presented in Table 1. All of the products have been characterized by XRD and HRSEM, and their antibacterial activity has been tested.

The results indicate that the amount of coated ZnO and the particle size depend on the concentration of $Zn(Ac)_2 \cdot 2H_2O$ in the solution and on the reaction time. Table 1, which presents the results of the HRSEM, demonstrates that a decrease in the reaction time from 2 to 1 h leads to a reduction in the particle size from 400 to 300 nm (samples 1 and 2). Lowering the initial concentrations of $Zn(Ac)_2 \cdot 2H_2O$ leads to a decrease of the ZnO content in the nanocomposite and to smaller particle size (samples 2 and 3). By reducing the initial concentration and reaction time, we have obtained the optimal conditions for the particle size, content of ZnO, and antibacterial activity, corresponding to sample 5. Thus, this sample was chosen for further investigation.

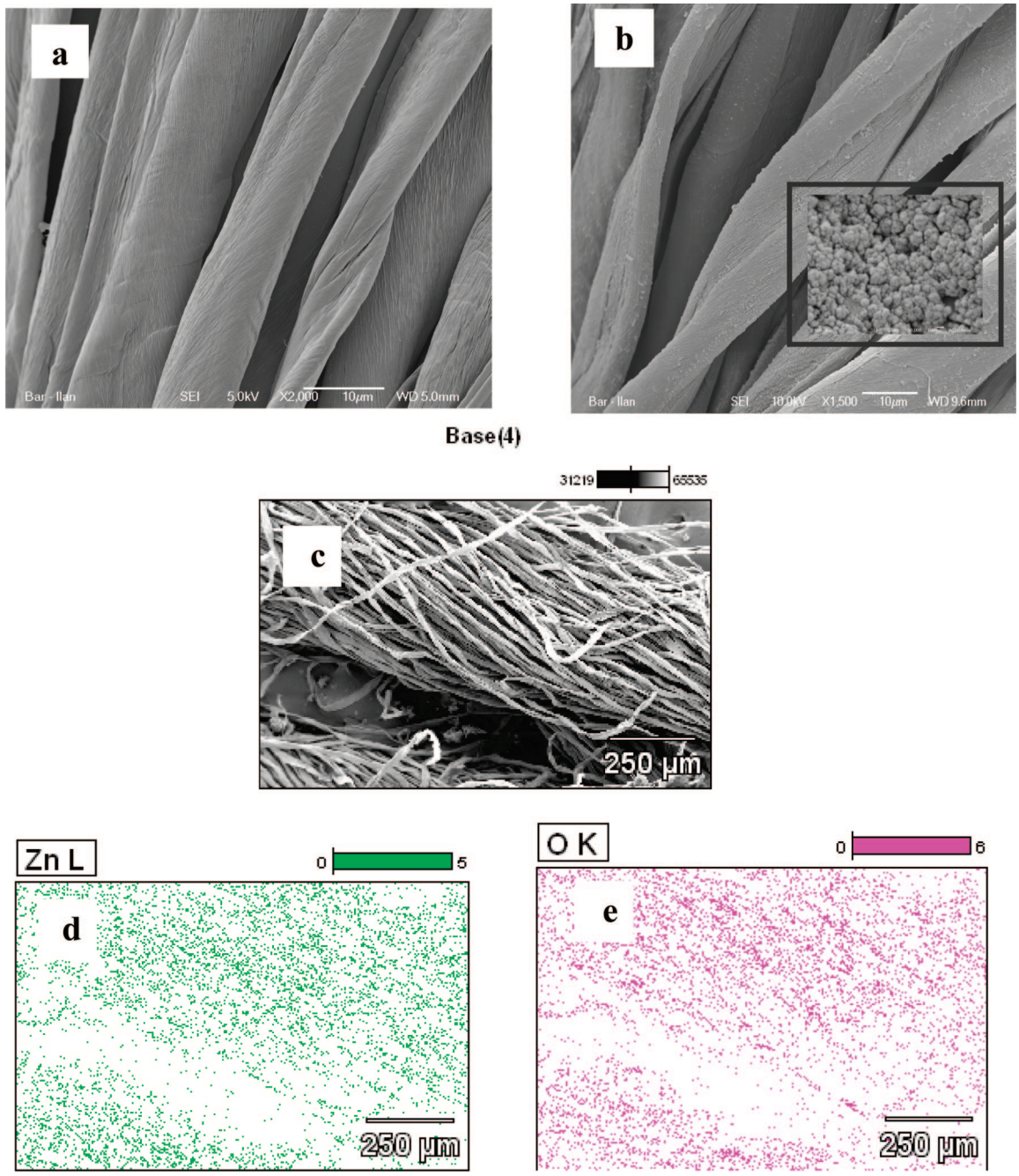

FIGURE 2. HRSEM images of (a) pristine bandage fibers (magnification ×2000), (b) a bandage coated with ZnO nanoparticles [magnification ×1500; the inset shows a magnified image (×50 000) of the nanoparticles coated the fibers], (c) selected image for X-ray dot mapping, (d) X-ray dot mapping for zinc, (e) X-ray dot mapping for oxygen.

Structure and Morphology of the ZnO−Fabric Nanocomposite. The XRD patterns (Figure 1) of sonochemically deposited ZnO nanoparticles on a cotton bandage demonstrate that ZnO is crystalline in nature, and the diffraction peaks match very well the hexagonal phase of ZnO (PDF: 89-7102). The peaks at $2\theta = 31.77$, 34.42, 36.25, 47.54, 56.6, 62.85, and 67.95° are assigned to the (100), (002), (101), (102), (110), (103), and (112) reflection lines of hexagonal ZnO particles, respectively. No peaks characteristic of any impurities were detected. The particle size estimated by the Debye−Scherrer equation is 30 nm.

The morphology of the fiber surface area before and after the deposition of ZnO nanoparticles studied by HRSEM is presented in Figure 2. Figure 2a demonstrates the smooth texture of the pristine cotton bandage. Fibers after sonication are homogeneously coated with nanoparticles (Figure 2b). The inset image in Figure 2b has been taken at higher magnification in order to obtain the particle size distribution. The distribution of the particles is quite narrow, and primary particles are in a very low nanometric range (~30 nm) that matches well with the XRD results. The selected-area HRSEM image studied with the elemental dot-mapping technique is shown in Figure 2c. The contents of zinc and oxygen in the mapped area are presented in parts d and e of Figure 2, respectively. These images verify a homogeneous coating of the fibers with ZnO nanoparticles.

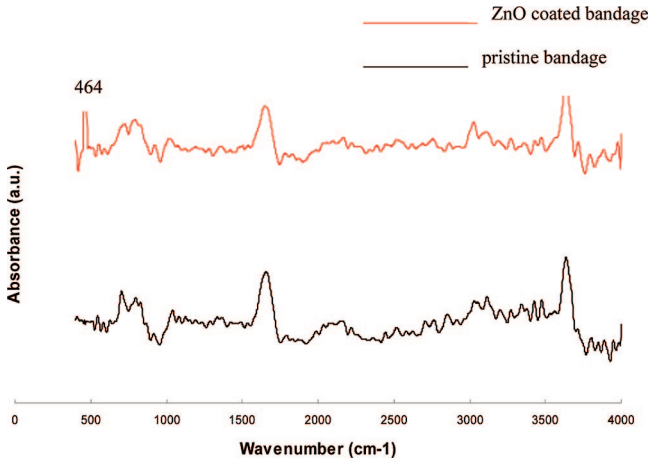

FIGURE 3. FTIR spectra of (black line) the pristine bandage and (red line) the ZnO-coated bandage.

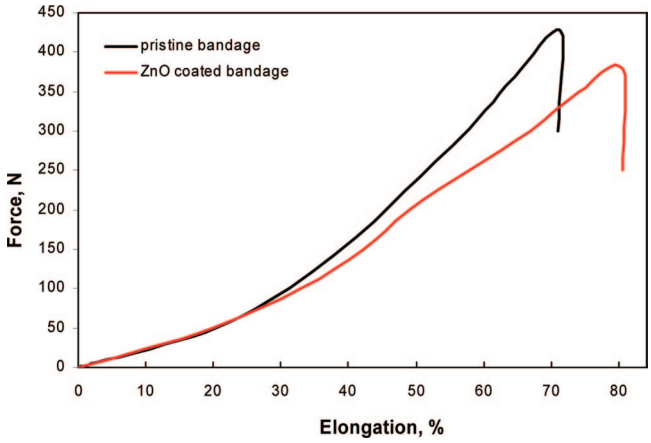

FIGURE 4. Mechanical properties of the cotton bandage before and after the deposition of ZnO nanoparticles.

Coating Mechanism. The coating process involves the in situ generation of ZnO nanoparticles and their subsequent deposition on fabrics in a one-step reaction via ultrasound irradiation. ZnO nanoparticles are formed during irradiation according to the following reactions:

$$Zn^{2+}(aq) + 4NH_3 \cdot H_2O(aq) \rightarrow [Zn(NH_3)_4]^{2+}(aq) + 4H_2O \quad (1)$$

$$[Zn(NH_3)_4]^{2+}(aq) + 2OH^-(aq) + 3H_2O \rightarrow ZnO(s) + 4NH_3 \cdot H_2O(aq) \quad (2)$$

Ammonia works as a catalyst of the hydrolysis process, and formation of ZnO takes place through the ammonium complex $Zn(NH_3)_4^{2+}$. ZnO nanoparticles produced by this reaction are thrown at the surface of the bandages by the sonochemical microjets resulting from the collapse of the sonochemical bubble. Our assumption is that the coating of the bandages with ZnO is, in fact, a physical adsorption of the nanoparticles on the substrate. We have already studied the sonochemical deposition of nanoparticles on different types of substrates such as ceramics (11), polymers (14), and wool fibers (15).

The sonochemical irradiation of a liquid causes two primary effects, namely, cavitation (bubble formation, growth, and collapse) and heating. When the microscopic cavitation bubbles collapse near the surface of the solid substrate, they generate powerful shock waves and microjets that cause effective stirring/mixing of the adjusted layer of the liquid. The aftereffects of the cavitation are several hundred times greater in heterogeneous systems than in homogeneous systems (16). In our case, the ultrasonic waves promote the fast migration of the newly formed ZnO nanoparticles to the fabric's surface. This fact might cause a local melting of the fibers at the contact sites, which may be the reason why the particles strongly adhere to the fabric.

To further support the coating mechanism, the FTIR spectra of the pristine cotton bandage and the ZnO-coated bandage have been recorded (Figure 3). Both spectra show the characteristic bands of cellulose. The recorded spectrum of the ZnO-coated bandage revealed an additional sharp single band at 464 cm^{-1}, which is attributed to a Zn−O vibrational band (17). Ling and co-workers (18) claim that the coating of ZnO nanoparticles on a paper surface by ultrasound might take place via hydrogen bonding. From our experience, coating assisted by ultrasound irradiation is a physical phenomenon that occurs regardless of the surface properties, especially when there are chemical interacting groups on the surface (19).

Mechanical Properties. The tensile mechanical properties of a cotton-impregnated fabric were studied on a universal testing machine, ZWICK 1445. Four-folded fabric samples with a gauge length of 60 mm and a width of 40 mm were placed in special grips. The tensile force for the ZnO-coated sample was observed to be ~11% less than that of the pristine bandage (Figure 4). The observed changes in the mechanical behavior of the yarn are in a range that is acceptable for standard cotton fabrics. According to this result, we conclude that the sonochemical treatment of the bandage did not cause any significant damage to the structure of the yarns.

Leaching of ZnO Nanoparticles. One of the factors influencing the commercial exploitation of the antibacterial bandages is the release of nanoparticles into the surrounding environment. Although the main hazard arises from the release of ZnO nanoparticles into water upon laundering, we have first examined the release of Zn^{2+} ions, realizing that these ions are environmentally friendly ions. To examine the leaching of Zn^{2+} ions, we have conducted some control experiments. Namely, we treated the bandages with an aqueous solution of NaCl. We placed a piece (0.08 g) of the coated bandage (sample 5a) into 50 mL of a 0.9% NaCl solution overnight at room temperature (25 °C), while sample 5b was soaked in the solution and heated to 37 °C. After this procedure, the content of zinc in the solution was determined by ICP. The concentrations of Zn^{2+} ions in the washing solution were 1.2 and 2.4 ppm for samples 5a and 5b, respectively. Thus, 8 and 16% of the Zn^{2+} ions were removed by the NaCl solution. The results indicate that the release of Zn ions depends on the temperature of the surrounding medium. While leaching of the Zn^{2+} ions is governed by the K_{sp} of ZnO and Zn^{2+} is considered as an environmentally safe ion, a much more important and

Table 2. Antibacterial Activity Test of the Coated Bandages against *E. coli* and *S. aureus*[a]

E. coli						
	duration of treatment					
	1 h			2 h		
sample	CFU, mL^{-1}	N/N_0	% reduction in viability	CFU, mL^{-1}	N/N_0	% reduction in viability
clean fabric	1.02×10^7	0.98	2.4	1.34×10^7	1.28	−28.23
no fabric	1.17×10^7	1.14	−28.57	1.23×10^7	1.35	−35.16
0.75% ZnO (sample 5)	1.71×10^4	1.58×10^{-3}	99.84	0	$\sim 0.9 \times 10^{-8}$	100
S. aureus						
	duration of treatment					
	1 h			3 h		
sample	CFU, mL^{-1}	N/N_0	% reduction in viability	CFU, mL^{-1}	N/N_0	% reduction in viability
clean fabric	0.7×10^7	0.71	20.46	0.99×10^7	1.125	−12.5
no fabric	0.98×10^7	1.1	−10.11	0.67×10^7	0.75	24.72
0.75% ZnO (sample 5)	3.9×10^6	3.36×10^{-1}	66.4	7.6×10^3	6.55×10^{-4}	99.93

[a] The viable bacteria were monitored by counting the number of colony-forming units (CFU); N/N_0 = survival fraction.

serious issue is the leaching of ZnO nanoparticles. In light of a recent paper (20) that found that silver nanoparticles of 10−500 nm in diameter leach from sock textile, we have conducted special experiments in an attempt to find the amount of leached ZnO nanoparticles. The methods we used for the leaching examination were DLS and transmission electron microscopy (TEM). The DLS and TEM studies did not reveal the presence of any nanoparticles in the leaching solution. That means that the sonochemically deposited ZnO nanoparticles are strongly anchored to the textile substrate.

Bactericidal Tests. The antibacterial activity of cotton fabrics coated with 0.75% ZnO (sample 5) was determined using the Gram-positive bacterium *S. aureus* and the Gram-negative bacterium *E. coli*. As shown in Table 2, treatment for 1 h with the coated cotton leads to the complete inhibition of *E. coli* growth. Regarding *S. aureus*, 100% reduction in viability was reached after 3 h, while after 1 h of treatment, a reduction of 60% could be seen.

One of the factors influencing the antibacterial activity of the developed coating is the release of the active ingredient into the surrounding medium. Zinc is an essential micronutrient for prokaryotic organisms. However, at superphysiological levels, zinc inhibits the growth of many bacteria (21). The leaching experiment indicates that only 16% (2.4 ppm) of the Zn^{2+} ions are removed by a NaCl solution after incubation of a piece of bandage (0.08 g) overnight at 37 °C, which was determined by ICP. This corresponds to a concentration of Zn ions of 36.7 μM. Compared to the minimum inhibitory concentration reported in the literature of 4−8 mM (22), the amount of zinc released from fabrics measured in this study is much lower (36.7 μM).

Mechanism of the Antibacterial Activity of ZnO Nanoparticles. We assume that the Zn ions have a minor influence on the antibacterial activity. The major component responsible for the bactericidal effect is ZnO nanoparticles. Although ZnO nanoparticles were not found in the solution, they can generate some species of oxyradicals in the solution (23). These active oxygen species are detected in electron spin resonance (ESR) studies conducted with and without the bacteria present in the ESR tube. In light of these results, the difference in the antibacterial action of ZnO-coated bandages upon two strains of bacteria can be explained by the difference in their sensitivity toward oxyradicals. In this respect, it has been found recently that *S. aureus* contains a large amount of cartenoid pigment, which promotes higher resistance to oxidative stress (24). A detailed study of the mechanism of the activity of ZnO nanoparticles will soon be submitted for publication.

CONCLUSION

ZnO nanoparticles are uniformly deposited onto the surface of a cotton bandage by the sonochemical method. The process is a simple, efficient, one-step synthesis. The physical and chemical analyses have shown that nanocrystalline ZnO, 30 nm in size, is finely dispersed onto the cotton surface without significant damage to the structure of the yarns. The mechanism of ZnO formation and adhesion to the fibers was discussed. It is based on the local melting of the substrate due to the high rate and temperature of ZnO nanoparticles thrown at the solid surface by sonochemical microjets. The performance of fabrics coated with 0.75 wt % ZnO nanoparticles as an antibacterial agent was investigated and their excellent bactericidal effect demonstrated.

Acknowledgment. This research was carried out as part of the activities of the LIDWINE consortium. Contract No. NMP2-CT-2006-026741 LIDWINE is an IP project of the 6th EC program.

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AM8000743