Bactericidal, structural and morphological properties of ZnO$_2$ nanoparticles synthesized under UV or ultrasound irradiation

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Abstract
Nanoparticles of ZnO$_2$ were synthesized by a sol–gel method using Zn(CH$_3$COO)$_2$ and H$_2$O$_2$ in an aqueous solution exposed to either ultraviolet (UV) or ultrasound irradiation. X-ray diffraction and scanning electron microscopy showed that the nanostructures consisted of spherical blackberry-like clusters. Nanoparticles fabricated by using UV irradiation had smaller sizes and narrower size distributions than nanoparticles prepared by using ultrasound. Bacillus subtilis (B. subtilis), Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus) were used as test microorganisms, and the antibacterial activity of the ZnO$_2$ nanoparticles was studied by use of the well diffusion agar bacteriological test. ZnO$_2$ nanoparticles synthetized using UV had the best antibacterial properties. The inhibition zone was largest for B. subtilis but was present also for S. aureus and E. coli.

Keywords: zinc peroxide nanoparticles, UV irradiation synthesis, ultrasound irradiation synthesis
Classification numbers: 2.05, 4.02, 5.00

1. Introduction

Hospital-acquired infection, also known as nosocomial infection, can be developed in patients during hospital visits and among hospital staff due to their working environment. The biological agent for this type of infection can be a bacterium, virus, fungus or even a parasite. Avoiding the propagation of such infections is of great importance owing to their prevalence in some countries; for instance nosocomial infections rank fourth among causes of death in the United States, only behind heart disease, cancer and stroke [1]. Hospital-acquired infections are significantly reduced by employing guidelines for preventing healthcare-associated infections, but, in addition to such measures, bactericidal nanoparticles applied to textiles could help control the biological threat agents’ propagation [2].

This paper shows that zinc peroxide (ZnO$_2$) nanoparticles have bactericidal properties and may serve as an alternative to ZnO nanoparticles, whose antibacterial capacity was recently discovered [3]. We note that nanoparticles of ZnO$_2$ have attracted some attention in earlier work as a consequence of their many possible industrial applications, which include rubber manufacturing [4, 5], cosmetics and pharmaceutical products [6] and therapeutic applications [7, 8].

Materials in the nanometer-size regime often exhibit properties distinct from those of their bulk counterparts. Thus, the bactericidal effectiveness of metal nanoparticles has been suggested to be due to both their size and their high surface-to-volume ratio [9]. These characteristics may allow the nanosized materials to interact closely with bacterial membranes so that the bactericidal effect would go beyond the one caused solely by the release of metal ions [10].

Several methods have been reported for the preparation of ZnO$_2$ nanoparticles. Some of these are based on the mixture of hydrogen peroxide with compounds such as ZnO [11, 12], ZnSO$_4$ [13], ZnCl$_2$ [14], Zn$_5$(CO$_3$)$_2$(OH)$_6$ [15],
Zn(CH$_3$COO)$_2$ [16] and Zn(NO$_3$)$_2$ [17]. The utilization of ultrasound [18] or ultraviolet (UV) irradiation [19] provides powerful routes for the synthesis of nanostructured materials.

The present work describes the preparation of ZnO$_2$ nanoparticles via a sol–gel technique involving ultrasound with regard to *Bacillus subtilis* (B. subtilis), *Escherichia coli* (E. coli) and *Staphylococcus aureus* (S. aureus).

2. Experimental

All chemicals were of analytical grade and bought from Merck. One gram of zinc acetate dehydrate, Zn(CH$_3$COO)$_2$·2H$_2$O, was dissolved under vigorous stirring in a mixture of 50 ml distilled water and 5 ml of 30% H$_2$O$_2$. The resulting solution was then irradiated with a 300 W Ultra-Vitalux lamp (Osram), positioned 10 cm from the solution, for 30 min at ambient temperature or, alternatively, treated in an ultrasonic bath (Branson Model MT 1510) at 42 kHz and 75 W for 30 min at 60 °C. Both procedures resulted in the formation of a white zinc peroxide nanosol, presumably via the chemical reaction [16]

\[
\text{Zn(CH}_3\text{COO)}_2 + 2\text{H}_2\text{O}(\text{aq}) + \text{H}_2\text{O}_2 \xrightarrow{\text{UV/Ultrasound}} \text{ZnO}_2 \downarrow + 2\text{(CH}_3\text{COOH)}(\text{aq}) + 2\text{H}_2\text{O}. \tag{1}
\]

The UV irradiation synthesis was performed in a dark chamber in order to avoid light-induced effects. The nanosols were precipitated by centrifugation (Eppendorf Centrifuge 5810R). The precipitate was then washed using distilled water until a pH of 8 was reached. Finally the resultant white solid was dried at 80 °C for 12 h.

The structure and domain size of the nanoparticles were determined by x-ray diffraction using a Rigaku Miniflex II Desktop diffractometer operating with CuK$_\alpha$ radiation (0.15045 mm wavelength) at 30 kV and 20 mA with a scan speed of 3° min$^{-1}$. The x-ray diffraction data were subjected to a general convolution process (Topas-Academic) allowing, in principle, any combination of appropriate functions to be employed for modeling the whole powder profile. These functions can represent the aberrations of the diffractometer as well as various contributions from the specimens; the technique is known as a ‘fundamental parameters approach’ [20]. It was used together with the Rietveld refinement method [21, 22] and gave the crystallite domain size of the ZnO$_2$ nanoparticles. The morphology of the nanoparticles was investigated by scanning electron microscopy (SEM) using a JEOL JSM-6300 instrument operated at an acceleration voltage of 5 kV.

The bactericidal properties of the ZnO$_2$ nanoparticles were evaluated with the well diffusion agar method [23] and employed *B. subtilis* ATCC 27853, *E. coli* ATCC 25922 and *S. aureus* ATCC 25923. These strains were grown aerobically in nutrient broth for 24 h at 37 °C before being used as target organisms. The density of bacterial isolates was adjusted to an optimal value of 0.5 McFarland standards. ZnO$_2$ nanoparticles were added to the wells, and the inhibition ring was observed after 24 h incubation.

3. Results and discussion

Zinc peroxide has a cubic structure with the space group *Pm-3* (205) and a lattice parameter of 4.871 Å [24]. Zn and O ions are located at (0, 0, 0) and (0.412, 0.412, 0.412), respectively. Figure 1 shows that all diffraction peaks can be indexed as belonging to the zinc peroxide phase according to the JCPDS card number 13-311. Hence, the prepared powder samples were of high purity.

Domain sizes were determined from the broadening of the x-ray diffraction lines by assuming a Voigt function [25]. The average domain sizes for samples of ZnO$_2$ nanoparticles synthesized using UV or ultrasound irradiation were found to be approximately 6 and 10 nm, respectively.

The morphology of the ZnO$_2$ nanoparticles was studied by SEM, and figure 2(a) shows a typical micrograph for a sample prepared under UV irradiation. It indicates the presence of blackberry-like spherical clusters with a narrow size distribution and a particle size of 98±11 nm; these clusters have aggregates of small domains with sizes of ~10 nm. Figure 2(c) is a corresponding SEM image of ZnO$_2$ nanoparticles synthesized under ultrasonic irradiation. Blackberry-like spherical clusters appear again and display a...
Figure 2. SEM images of ZnO_2 nanoparticles synthesized under (a) and (b) UV, and (c) and (d) ultrasound irradiation for 30 min. Scale bars indicate magnifications.

broader size distribution than for the sample in figure 2(a); now the particle size is 134 ± 32 nm and the aggregates include domains that are ~10 nm in size. The domain sizes are consistent with the crystallite sizes determined by x-ray diffraction.

The bactericidal activity of the ZnO_2 nanoparticles was tested by the well diffusion agar method [23]. Figure 3 shows results for ZnO_2 nanoparticles prepared under UV irradiation. Inhibition zones are clearly seen and indicate the antibacterial effect of the nanoparticles. The diameters of these zones were 7.2, 2.9 and 3.7 mm for B. subtilis, E. coli and S. aureus, respectively.

Figure 4 displays corresponding results for ZnO_2 nanoparticles fabricated by ultrasound irradiation. Now the diameters of the inhibition zone were 3.8, 0.8 and 2.6 mm for B. subtilis, E. coli and S. aureus, respectively.
Figure 4. Antibacterial activity of ZnO nanoparticles, obtained under ultrasound irradiation, on (a) *B. subtilis*, (b) *E. coli* and (c) *S. aureus* as determined by the well diffusion agar technique. The diameter of the dishes was 10 cm.

The differences of the inhibition zones are noteworthy, and it is evident that the technique for fabricating the ZnO nanoparticles is important for the antibacterial properties. We note that the sample with the smallest crystallite size and the narrowest size distribution, prepared under UV irradiation, gives the most pronounced inhibition zones, which is the expected result [10].

4. Conclusion

ZnO nanoparticles with average particle sizes of 98 and 134 nm and crystallite sizes of about 6 and 10 nm were prepared through UV or ultrasound irradiation of a zinc precursor solution, respectively. Thus, ZnO nanoparticles synthesized using UV have the smallest crystallite size and narrowest size distribution, and they also display best antibacterial properties. The inhibition zones, observed using the well diffusion agar method, were of diminishing size for *B. subtilis*, *S. aureus* and *E. coli*, respectively.

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