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Silver nanoparticles in combination with acetic acid and zinc oxide quantum dots for antibacterial activities improvement—A comparative study

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ABSTRACT

Due to their remarkable antibacterial/antivirus properties, silver nanoparticles (Ag NPs) and zinc oxide quantum dots (ZnO Qds) have been widely used in the antimicrobial field. The mechanism of action of Ag NPs on bacteria was recently studied and it has been proven that Ag NPs exerts their antibacterial activities mainly by the released Ag^+. In this work, Ag NPs and ZnO Qds were synthesized using polyol and hydrothermal method, respectively. It was demonstrated that Ag NPs can be oxidized easily in aqueous solution and the addition of acetic acid can increase the Ag^+ release which improves the antibacterial activity of Ag NPs. A comparative study between bactericidal effect of Ag NPs/acetic acid and Ag NPs/ZnO Qds on *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia* and *Staphylococcus aureus* was undertaken using agar diffusion method. The obtained colloids were characterized using UV–vis spectroscopy, Raman spectrometry, X-ray diffraction (XRD), transmission electron microscopy (TEM) and atomic force microscopy (AFM).

1. Introduction

Zinc oxide (ZnO) is a versatile and important semiconductor which found applications not only in the optoelectronic field [1,2] but also in the bio applications because of its environmentally friendly properties [3,4]. ZnO nanoparticles are recently investigated and seem to have significantly higher antibacterial effect without reaction with human cells [5]. Liu et al. [6] have addressed an important topic about the mechanism of action of ZnO NPs on bacteria. They proved that under light irradiation, ZnO can produce electron–hole (e^-, h^+) pairs with high energies. At the surface of ZnO, the created (e^-, h^+) pairs influence the redox reaction which generates hydroxyl and perhydroxil radicals (OH^-, HO_2^-) in addition to the superoxide anions (O_2^-). The microorganism cells were immediately destructed in the presence of these radicals. Another mechanism has been proposed by Sharma et al. [7]: the bactericidal effect of ZnO NPs is mainly explained by the rupture of the lipid bilayer of bacterium which results in leakage of cytoplasmic contents. Several studies have been, also, devoted to silver nanoparticles as a broad spectrum antimicrobial agent [8]. The toxicity of these nanoparticles may be explained by: (i) degradation of the cell membrane caused by the generation of reactive oxygen species [9–11], (ii) the release of silver ions from the crystalline core of silver nanoparticles may contribute to the toxicity causing a decrease in the pH of the cell cytoplasm [12,13].

In this work, Ag NPs and ZnO Qds were synthesized using polyol and hydrothermal method, respectively. The bactericidal effect of Ag NPs on *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia* and *Staphylococcus aureus* was examined, after ZnO Qds addition firstly, and acetic acid in a second step, using agar diffusion method.

2. Materials and methods

2.1. Ag NPs and ZnO QDs preparation

Ag NPs were synthesized using polyol method. As a precursor, silver nitrate ($AgNO_3$) was chosen for the synthesis of Ag NPs. Ethanol 96% was adopted as reducing agent [14] and polyvinylpyrrolidone (PVP) was used as surfactant. A flask

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containing a solution of 0.1 g of $AgNO_3$, 1 g of PVP and 20 ml of ethanol 96% was immersed in an oil bath. The solution was then heated under reflux at 160 °C with a constant stirring for 30 min. During the process, the color of the solution becomes reddish after it was transparent. After cooling, the obtained solution remains stable without any precipitation for many months. For ZnO Qds preparation, hydrothermal method was used. In this method, 20 mg of multi walled carbon nanotubes (MWCNTs) was attacked by nitric acid (HNO_3) as reported by [15]. This mixture was then dispersed in 20 ml of dimethylformamide (DMF) by ultra-sonication for 30 min. 6 ml aliquot of the MWCNTs-HNO_3/DMF solution was then mixed with 36 ml of DMF in which 0.39 g of zinc nitrate tetrahydrate ($Zn(NO_3)\cdot 4H_2O$) was dissolved, and the resulting mixture was vigorously stirred for 30 min at room temperature. The mixture was then heated to 105 °C at a heating rate of 2 °C/min, and maintained at this temperature for 3 h. The transparent upper part of the container is composed of suspended ZnO Qds (dispersed in DMF) and the dark bottom layer contained the isolated MWCNTs-HNO_3.

2.2. Characterization method

For XRD characterization, ZnO Qds were deposited on glass substrates using spin coating (100 t/min) followed by drying at 160 °C to eliminate DMF (boiling point: 153 °C). These films were then annealed at 400 °C for 1 h and Ag NPs were deposited on titanium substrates using spin coating (100 t/min) followed by drying at 430 °C to decompose PVP (decomposition temperature: 420 °C). The XRD analysis was conducted on a D8 Siemens Advance diffractometer with Cu$K\alpha$ radiation ($\lambda = 0.15418$ nm). The 2θ range was from 10° to 100°. The formation of Ag NPs and ZnO Qds, also, was further confirmed using UV–vis Jasco V-670 spectrometer. Raman spectra were carried out, using Bruker SENTERRA R200L spectrometer, to control the reactions which occurred after acetic acid addition in Ag NPs colloid. Furthermore, the surface enhanced Raman spectroscopy of ZnO Qds on Ag NPs films with different thicknesses was studied. The Raman spectra were recorded using the 532 nm line of an Argon ion laser. AFM images of ZnO Qds were obtained using Horiba Jobin Yvon (AIST-NT Smart SPM/AFM) in non-contact mode. To examine the size and the morphology of the Ag NPs, transmission electron microscopy (TEM; Tecnai TF20, BFTEM at 200 kV) was used.

2.3. Antibacterial tests

The agar diffusion method (Kirby-Bauer) is a relatively quick and easy to execute as semi-quantitative test to determine antibacterial activity of diffusible antimicrobial agents on treated textile material [16,17]. Bacterial inactivation tests were carried out using *P. aeruginosa* (ATCC: 27853), *E. coli* (ATCC: 25922), *K. pneumonia* (ATCC: 70603) and *Staphylococcus aureus* (ATCC: 25923) as test organisms. A spectrophotometer (JENWAY 7305) was used to ensure an identical bacterial concentration in each test by measuring the optical density (10^8 cells/cm^3). One colony of each bacteria was taken out in a petri dish and grown in nutrient broth medium (Müller-Hinton agar: beef infusion 300.0 ml, caseinhydrolysate 17.5 g, starch 1.5 g, agar: 17.0 g, pH adjusted to neutral at 25 °C). 10 mm diameter discs (3 M perti film) immersed in ethanol 96% and acetic acid were used as control fabrics. As test fabrics, discs immersed in Ag NPs (100 μg/cm^3) with different concentration of added acetic acid and ZnO Qds were used. These discs were gently pressed onto the surface of the petri dishes and were incubated in darkness at 37 °C for 18–24 h (INB 200-Memmert incubator). The antibacterial activity of fabrics was demonstrated by the diameter of the zone of inhibition in comparison to the control fabric.

3. Theory

Based on the principle that Ag^+ is the definitive toxicant agent responsible for killing bacteria [18], we have added acetic acid (CH_3COOH) to the Ag NPs colloid which would increase H^+ concentration according to Eq. (3) and give rise to the increase of Ag^+ content according to Eq. (2).

Eq. (1) shows that Ag NPs can be oxidized in aqueous solution [18] and rapidly ionized in the presence of H^+ to give Ag^+ according to Eq. (2).

$$4Ag + O_2 \rightarrow 2Ag_2O \quad (1)$$

$$2Ag_2O + 4H^+ \rightarrow 4Ag^+ + 2H_2O \quad (2)$$

$$CH_3COOH \rightarrow CH_3COO^- + H^+ \quad (3)$$

This phenomenon is well confirmed subsequently in the Raman spectroscopy analysis section.

4. Results and discussion

4.1. XRD analysis

XRD spectra of the synthesized ZnO Qds (dried at 160 °C and annealed at 400 °C) recorded in the 2θ range 10–75° are presented in Fig. 1a. The diffraction pattern of the annealed ZnO Qds films exhibits seven peaks at $2\theta = 31.86, 34.59, 36.36, 47.78, 56.60, 62.98, 68.28$ degree which were assigned to the (1 0 0), (0 0 2), (1 0 1), (1 0 2), (1 1 0), (1 0 3) and (1 1 2) (JCPDS No. 36-1451) planes of hexagonal zinc oxide. No characteristic peaks of any impurities were detected; suggesting that high quality of ZnO was obtained. However, the related ZnO peak intensities appear lower in the dried samples (Fig. 1a) which can be due to the presence of the organic matter that appears as amorphous phase in the range 10–30°. Fig. 1b shows the diffraction pattern of Ag NPs deposited on titanium substrates using spin coating and drying at 430 °C. The peaks

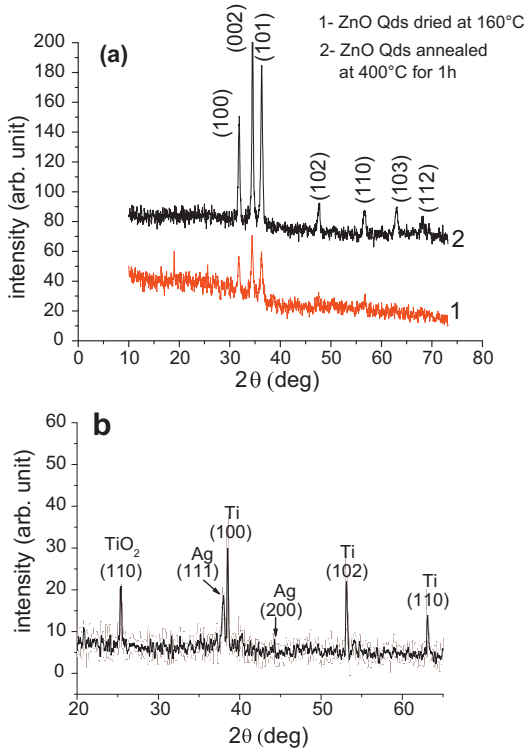

Fig. 1. XRD patterns of (a) dried and annealed ZnO Qds, (b) Ag NPs deposited on Ti.substrate and heat treated at 430 °C.

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