Materials Chemistry and Physics 179 (2016) 182–188

Contents lists available at ScienceDirect

Materials Chemistry and Physics

journal homepage: www.elsevier.com/locate/matchemphys

Antibacterial property of $CuCrO_2$ nanopowders prepared by a self-combustion glycine nitrate process

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HIGHLIGHTS

- $CuCrO_2$ nanopowders was prepared by low-temperature glycine nitrate process.
- 1250–1500 ppm of $CuCrO_2$ nanopowders were found to depress the growth of *Escherichia coli*.
- Bulk powders by the solid state reaction exhibited no antibacterial property.
- The antibacterial property of nanopowders was attributed to rapid Cu ion releases.
- Heavy adhesion of nanopowders to bacteria also resulted in antibacterial property.

ARTICLE INFO

Article history:
Received 23 November 2015
Received in revised form
23 April 2016
Accepted 9 May 2016
Available online 14 May 2016

Keywords:
Antibacterial
Delafossite
Nanopowder
Ceramic powder
Escherichia coli

ABSTRACT

Porous $CuCrO_2$ nanopowders were prepared via glycine nitrate process (GNP) at 175 °C in ambient air. The X-ray diffraction patterns showed a pure delafossite phase of $CuCrO_2$ with numerous broad peaks, indicating a crystallite size of approximately 20 nm. The surface area of the $CuCrO_2$ nanopowders was larger than 50 m^2/g, nearly 100 times greater than that of bulk powders (0.47 m^2/g). Pathogenic Gram-negative bacteria *Escherichia coli* (*E. coli*) were chosen as the antibacterial evaluation indicators for both the nanopowders and bulk powders. The results showed that 1750 ppm nanopowders inhibited the growth of *E. coli*. As a control, the bulk powders showed a normal growth profile. The antibacterial property of the $CuCrO_2$ nanopowders can be attributed to the extremely large surface area, which induces rapid release of Cu ions and strong adhesion of nanopowders to bacteria.

1. Introduction

Serious public health concerns on antibiotic and drug-resistant bacteria have necessitated the need to develop new antibacterial materials. Inorganic materials are considered to be superior to organic materials owing to their characteristics such as chemical stability, user safety, thermal resistance, and persistence of antibacterial effects. Therefore, antibacterial ceramics having silver, iron, and zinc as the functional components to suppress bacterial growth have attracted significant attention [1]. Owing to the rapid development of nanotechnology, numerous studies have been conducted on the antibacterial properties of synthesized nanoparticles [2], natural and modified nanomaterials [3], and nanocomposites, such as ZnO/CdS [4], TiO_2 [5], MgO [6], Cr_2O_3 [7], and montmorillonite clay [8].

Nanoparticles are known to exhibit antibacterial properties owing to the formation of reactive oxygen species (ROS), interaction with bacteria, abrupt release of metal ions, and the alkaline effect of a thin water layer on nanoparticles [9]. The bactericidal effect of Cu can be attributed to the release of ions from the carriers and their attachment to the negatively charged bacterial cell wall, thus inducing cell death [10–12]. Cai et al. reported that Cu-modified ZrP powders cause variations in ion concentrations and leakage of DNA, RNA, and protein of Gram-negative *Escherichia coli* (*E. coli*), thus presenting great potential for application as an antibacterial powder for microbial control [13]. Innovative 304 Cu-bearing stainless steels manufactured via a solution treatment to precipitate the saturated Cu-rich phase from the steel matrix [14] or

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http://dx.doi.org/10.1016/j.matchemphys.2016.05.026
0254-0584/

by surface plasma alloying with a mixture of pure Cu, expanded austenite phase, and a few Fe_3O_4 phases [15] exhibit excellent antibacterial activity against microbes and are suitable for water pipes, hospitals, food industries, and kitchen appliances.

Delafossite $CuCrO_2$ is a p-type semiconductor as CuO but with a wider band gap of 3.1 eV, corresponding to the 400 nm wavelength in the ultraviolet range, and has several applications such as transparent diodes, solar cells and photocatalysts. Further, Cu–Cr–O films prepared by reactive magnetron sputtering exhibit a high efficiency in killing *E. coli* bacteria in daylight or darkness, which depends significantly on the structure and content of Cu (≥15 at.%) in the Cr–Cu–O film [16,17]. The purpose of the present study is to evaluate the antibacterial properties and elution behavior of $CuCrO_2$ nanopowders against *E. coli* using the minimum inhibitory concentration (MIC) protocol [8]. In this study, the glycine nitrate process (GNP) was employed to prepare the $CuCrO_2$ nanopowders because it is cost effective and fast and does not require high pressure, energy, temperature, or highly toxic chemicals [18]. Further, the crystal structure, particle morphology, and surface chemistry of the prepared $CuCrO_2$ nanopowders were analyzed to elucidate the antibacterial mechanism.

2. Experimental

2.1. Materials and synthesis

In this study, $CuCrO_2$ nanopowders were synthesized via GNP by dissolving 24.16 g of $Cu(NO_3)_2{\cdot}3H_2O$ (99%, Showa), 40.01 g of $Cr(NO_3)_3{\cdot}9H_2O$ (99%, Acros), and 5.63 g of glycine ($C_2H_5NO_2$, 98%, TCI) in minimal amount of distilled water in a beaker and aged at 100 °C for 24 h to obtain a glassy material. The beaker was then covered with a fine-mesh sieve and heated to 175 °C for spontaneous ignition. Bulk powders of $CuCrO_2$ were also prepared for comparison using the solid state reaction (SSR) method by mixing stoichiometric amounts of Cu_2O (95%, Showa) and Cr_2O_3 (99%, Showa) powders in ethanol using zirconia balls as the milling media in a plastic jar for 24 h. The resulting slurries were dried at 70 °C in an oven and then fired in a crucible at 1200 °C for 6 h in air [18].

2.2. Antibacterial tests

To evaluate the antibacterial activity of both the nanopowders and bulk powders of $CuCrO_2$, 0.4 ml of *E. coli* (ATCC 8739, KWIK-STIK, ~10^5 CFU/ml) was inoculated in 30 ml of nutrient broth (NB; Conda) with different concentrations of $CuCrO_2$ powders (100 ppm–5000 ppm) in an orbital shaking baker (110 rpm/35 °C) for different time periods (0–24 h) in dark. The powders were first irradiated under a UV lamp for at least 15 min prior to the experiment. Subsequently, 1 ml or 200 μl of the aforementioned NB was diluted tenfold using phosphate buffer solution and transferred to solid nutrient agar (NA; Conda) in two replicates with a volume of 1 ml or 500 μl for viable colony counting after incubation at 35 °C for 24 h. The supernatant and solid powders from the used NB were collected separately by centrifuging for repeated antibacterial tests and composition analysis using an inductively coupled plasma mass spectrometer (ICP-MS; Thermo Element XR) or an X-ray photoelectron spectrometer (XPS; PHI 5000 VersaProbe) using Al Kα radiation. A spectrometer charge neutralizing system was used to compensate for sample charging during the XPS measurement, and the binding energy scale was referenced to C 1s at 284.8 eV. Precursor materials of the above metal nitrates were also dissolved in the NB in specific concentrations for antibacterial tests against *E. coli*.

2.3. Characterizations

An X-ray diffractometer (XRD; Bruker D8A25) and a scanning electron microscope (SEM; JEOL JSM-6360, Hitachi SU3500) equipped with an X-ray energy dispersive spectrometer (EDS; Bruker QUANTAX 400) were used to characterize the crystal structure, morphology, and composition of both the nanopowders and the bulk powders, respectively. The crystalline size (L) of the nanopowders was estimated using the Scherrer equation as follows:

$$L = \frac{K\lambda}{\beta \cos\theta}$$

where K is the Scherrer constant (0.9) and λ is the wavelength of XRD radiation (0.15406 nm). Further, β and θ are the full width at half maximum (FWHM) and the angle of diffraction peaks, respectively. The interplanar spacing (d) of the $CuCrO_2$ crystallites was calculated from the XRD patterns by using Bragg's law as follows:

$$2d \sin\theta = n\lambda$$

where n is set to be 1. A transmission electron microscope (TEM; JEOL JEM1400) was used for analyzing the microstructure of the $CuCrO_2$ nanopowders.

3. Results and discussion

3.1. Antibacterial tests

For the antibacterial tests against *E. coli*, $CuCrO_2$ nanopowders were dispersed in the NB to realize different concentrations ranging from 100 ppm to 5000 ppm. Fig. 1a shows that as the concentration of $CuCrO_2$ nanopowders increases from 1000 ppm to 1250 ppm (Sample C, 1250 ppm), the growth of *E. coli* was inhibited and maintained in the order of 10^5–10^6 CFU/ml from an initial concentration of approximately 10^3 CFU/ml after incubation at 35 °C for 24 h in 30 ml of the NB. Moreover, as the concentration was further increased to 1500 ppm (Sample D), the growth of *E. coli* was fully depressed because no colony was found (<1 CFU/ml) after 18 h. Therefore, the MIC and minimum bactericidal concentration (MBC) of $CuCrO_2$ nanopowders are 1250 ppm and 1500 ppm, respectively.

The 1750 ppm sample (Sample E) shown in the left-hand side column of Fig. 1b exhibited good antibacterial activity against *E. coli* (<1 CFU/ml). Furthermore, to investigate the mechanism and lifespan of the antibacterial property of $CuCrO_2$, the solution and solid powders of Sample E (1750 ppm), which were used in the above-mentioned antibacterial test, were separated by centrifugation and treated with a regular sterile process in an autoclave (121 °C, 1.05 kg/cm^2) to obtain other samples of solution (Sample E1) and solid powders (Sample E2). The powders (Sample E2) were refilled with a fresh NB to maintain a solid concentration of 1750 ppm, and both samples E1 and E2 were inoculated with fresh *E. coli* (~10^3 CFU/ml). However, the used powder (Sample E2) no longer exhibited any antibacterial property as opposed to the fresh nanopowders in Sample E (<1 CFU/ml), thus indicating the same growth rate of control samples (samples A and B, 10^9–10^{10} CFU/ml). In contrast, the used solutions referred to as supernatants of Sample E1 and Sample E1-2, sequentially derived from Sample E1, could depress the growth of *E. coli* to less than <10 CFU/ml. Another sample (Sample E′) with the same concentration (1750 ppm) as Sample E exhibited antibacterial reproducibility against *E. coli*, as shown in Fig. 1b. Sample E″, which was Sample E′ directly