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Zinc oxide nanoleaves: A scalable disperser-assisted sonochemical approach for synthesis and an antibacterial application

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ABSTRACT

Current study reports a new and highly scalable method for the synthesis of novel structure Zinc oxide nanoleaves (ZnO-NLs) using disperser-assisted sonochemical approach. The synthesis was carried out in different batches from 50 mL to 1 L to ensure the scalability of the method which produced almost similar results. The use of high speed (9000 rpm) mechanical dispersion while bath sonication (200 W, 33 kHz) yield 4.4 g of ZnO-NLs powder in 1 L batch reaction within 2 h (> 96% yield). The ZnO-NLs shows an excellent thermal stability even at a higher temperature (900 °C) and high surface area. The high antibacterial activity of ZnO-NLs against diseases causing Gram-positive bacteria *Staphylococcus aureus* shows a reduction in CFU, morphological changes like eight times reduction in cell size, cell burst, and cellular leakage at 200 μg/mL concentration. This study provides an efficient, cost-effective and an environmental friendly approach for the synthesis of ZnO-NLs at industrial scale as well as new technique to increase the efficiency of the existing sonochemical method. We envisage that this method can be applied to various fields where ZnO is significantly consumed like rubber manufacturing, ceramic industry and medicine.

1. Introduction

Zinc oxide (ZnO) forms various nanostructures and is a well-known, versatile material because of its excellent biochemical activities, physical properties, and extensive applications [1]. Its structural and morphological diversity has attracted researchers in the past few years [1]. But, certain challenging factors like its large-scale production, purity, and production yield limits its applications [2–4]. However, currently, very few reports are available related to increase the production efficiency of any novel ZnO nanostructure. ZnO exists in three different lattice structures, wurtzite, rock salt and zinc blende, among all, wurtzite is the most common and thermodynamically stable structure, in which zinc and oxygen atoms are arranged in a tetrahedral manner [2]. The highly ionic nature of Zn-O bond makes it polar and thus it is widely used as the piezoelectric material [1]. The direct band gap energy 3.4 eV and crystal defects make it transparent in the visible region and active in UV region that makes it applicable in sunscreens lotions as UV protective-agents [2]. The silver nanoparticles (Ag NPs) and ZnO NPs are the most common bactericidal agents used nowadays but among these two, ZnO is preferred because of its chemical stability, easier synthetic procedures, cost-effectiveness, lesser toxicity, etc [3]. The antimicrobial property of ZnO strongly depends on its physical and chemical properties like size, shape, morphology, crystal size, surface area, porosity, crystal defects, etc [2,3].

There are numerous reports on the morphological modulation of the ZnO structures using various synthetic methodologies. Some common nanostructures are flowers, rods, cups, needles, belt, tube, sphere, walls, etc [4,5]. The two most common routes for the fabrication of ZnO are carried out in gas and solution phase. The later is comparatively easy and there are various methods for the synthesis of ZnO nanostructures in the solution phase like precipitation, hydrothermal, solvothermal, sol-gel, micro-emulsion, combustion, electrochemical, laser ablation, and sonochemical [6]. Lately, the sonochemical method has gained more attention due to large-scale production and morphological variation capabilities [7–9]. The advantage of ultrasound technology is that it is environmental friendly route as in many cases it not require any extra hazardous chemical to initiate or accelerate the reaction [10–15]. Sound wave is transmitted through a medium in a cyclic succession of compression and rarefaction (stretching) wave. The frequency of sound between 18 Hz and 18 kHz is related to human hearing, 20 to 100 kHz is ultrasound which can be used for sonochemistry, 100 kHz to 1 MHz is a high-frequency ultrasound and from 1 to 500 MHz is diagnostic ultrasound. The ultrasound frequency range 1 to 10 MHz is used for navigation, communication, echo location and diagnostic purposes [10,11]. Due to a cyclic succession of compression and rarefaction wave in the liquid, the molecules undergo

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mechanical vibration. When the pressure due to rarefaction exceeds the tensile strength of the liquid, it creates voids known as cavitation bubbles, and after a repeated cycle of these waves, the bubbles become unstable and collapse in micro-second during the compression phase of the cycle, the whole phenomena is known as cavitation [11,15–20]. These rapid collapsing bubbles create very high temperature (5000 K) and pressure (500 atm) inside the cavitation zone while moderate change in the bulk liquid. Increase in the temperature and pressure in the cavitation zone provides sufficient energy to initiate a chemical reaction. Ultrasonic irradiation parameters which can affect the cavitation phenomena are the frequency of sound, the intensity of sound, type of solvent, the presence of gas, external pressure and temperature [11,21–22]. According to the hot-spot theory of sonochemical effect, these hot collapsing microbubbles behave as microreactors which produce different types of reactive species like $OH^{\cdot}$, $H^{\cdot}$, hydroperoxyl radicals ($OOH^{\cdot}$) and $O^{\cdot}$ due to sonolysis of water and oxygen molecule. These free radicals react with each other to yield H_2O_2 and H_2. These chemical species are responsible for the ultrasound-based sonochemical reactions [11,22–24]. For the synthesis of ZnO nanoparticle through sonochemical method, $OH^{\cdot}$ may facilitate the synthesis of Zinc hydroxide, an intermediate product ($Zn(OH)_2$) as well as morphological variations [10–14]. During the sonochemical method of large-scale ZnO production, it is essential that reaction mixture is properly mixed to ensure the homogenous distribution of sonochemical energy to the reactants to give better production yield. In addition, there are chances of agglomeration of the reactants during the sonochemical synthetic process of ZnO nanostructures which could affect the reaction progress and ultimately the production yield. To address this issue and ensure the scalability and better yield, we have assisted sonochemical method with a disperser to achieve cost-effective large-scale production of ZnO nanostructure. Homogenizer disperser works on rotor-stator principle, the liquid medium that has to be dispersed axially drawn from the head part and radially forced with very high speed from the slots between rotor and stator [25,26]. Due to high speed and minimum gap between rotor and stator, a strong shear force is developed which results in better dispersion in the solution [25,26]. In this method, disperser not only created a homogenous condition during reaction progress but also prevents the formation of large shape ZnO nanostructures like a flower, yielding high surface area Zinc oxide nanoleaves (ZnO-NLs). Also, our method does not employ any capping agent or toxic surfactant like cetyltrimethylammonium bromide (CTAB), etc. for directional growth because its remnants in the final product may result in false positive antimicrobial activity of ZnO due to the microbicidal property of CTAB itself [4,27–30]. *S. aureus* is a Gram-positive bacteria commonly found in the respiratory tract, nose and on human skin [2–4]. Some common human infection and life threatening diseases caused by *S. aureus* are wound infection, surgical infection, respiratory tract infection, skin infection, sepsis, meningitis, osteomyelitis, pneumonia etc [2–4]. To avoid heavy doses of antibiotics due to the resistance develop in the bacteria, ZnO nanoparticles are promising to cure topical *S. aureus* infections [2–4]. We synthesized ZnO-NLs in three different batch reactions (50 mL, 500 mL and 1 L) to check the scalability of the method. We achieved ZnO-NLs as a novel structure with more than 96% production yield in every batch. In this study, we used Zinc acetate dihydrate as zinc precursor and sodium hydroxide as the base. No additives, surfactants, capping agents, etc. were used in this method. Hence, we are reporting a novel method for the large-scale production of ZnO-NLs through disperser-assisted sonochemical approach with excellent physical and chemical properties of the material (high thermal stability, high surface area, hydrophilic, pure, high antibacterial activity etc.), as well as a new technique to increase the efficiency and scalability of the existing sonochemical method. At industrial scale this method can produce more than 100 g of excellent quality ZnO nanostructure (ZnO-NLs) in 2 h only. The method would pave a way toward large-scale, cost effective production of novel structures ZnO-NLs for rubber and ceramic industries as well as for medical applications.

2. Materials and methods

2.1. Materials

Zinc acetate dihydrate [$Zn(CH_3COO)_2{\cdot}2H_2O$] was purchased from Sigma-Aldrich, and Sodium Hydroxide (NaOH) was purchased from Merck Pvt. Life Science (India). Tryptone Soya Yeast Extract Agar (TSYEA-M1214 F) and Tryptone Soya Yeast Extract Broth (TSYEB-M1263) both were purchased from HIMEDIA (India). An active culture of Gram-positive bacteria *S. aureus* [MCC 2043(T)] was purchased from National Centre for Cell Science, Pune, Maharashtra. Disperser (IKA-T25 Digital Ultra Turrax, IKA India Pvt. Limited, 3725022) and Bath Sonicator (LA-10L, Limplus, 33 ± 3 kHz, 200 watts) were used for the ZnO-NLs synthesis. All the glassware was properly cleaned with detergent and autoclaved before the use. All the chemicals were used without any further purification.

2.2. Disperser-assisted sonochemical synthesis of ZnO-NLs at large-scale and their characterizations

For the synthesis of ZnO-NLs, the dispersing element of the disperser was dipped in the beaker containing 55 mM $Zn(CH_3COO)_2{\cdot}2H_2O$. After proper mixing with a disperser, NaOH solution of 0.1 M concentration was added drop-wise to the $Zn(CH_3COO)_2{\cdot}2H_2O$ solution under bath sonication till its pH reaches up to 12 units, (experimental setup as shown in Fig. 1). The reactions were carried out in three different batches (50 mL, 500 mL, and 1 L) retaining the similar setup and the same concentration of reactants except dispersing element (Table 1). Dispersing element of large volume capacity (10–1500 mL working range) was used for the larger volumes (500 mL and 1 L). Due to sonication, the temperature of solution slowly increases up to 60 °C at the end of the reaction. After 2 h, the solution was allowed to cool down at ambient temperature. The resulting solution was centrifuged in four cycles at 5000 rpm, and after each cycle, the pellet was washed with pure MQ water (pH 6.7). Finally, the synthesized ZnO-NLs were dried in hot air oven at 60 °C for 12 h. The weight of synthesized ZnO-NLs powder from all three batches was measured and stored at ambient temperature for further characterization.

2.2.1. UV–Visible absorbance spectroscopy

A UV–Visible absorbance spectrum of ZnO-NLs solution from all three batches (50 mL, 500 mL, and 1 L) was recorded by Lambda UV–Visible spectrophotometer (Perkin-Elmer life and Analytical Science, USA) in the range 330 to 600 nm, using quartz cuvette with MQ water as a reference.

2.2.2. Fluorescence spectroscopy

The fluorescence spectrum of ZnO-NLs solution was recorded by Fluorescence spectrophotometer (F-2500 FL Spectrophotometer) and Confocal Laser Scanning Microscope (CLSM) (Zeiss LSM, Axio Observer Z1 Inverted Microscope). ZnO-NLs from all the three batches were dispersed in MQ water at a concentration of 100 µg/mL and bath sonicated for 10 min. Its fluorescence spectrum was recorded by using quartz cuvette, setting the excitation wavelength at 360 nm and emission range 388 to 707 nm. Due to broad emission spectra of ZnO-NLs, it was further characterized by CLSM. Briefly 20 µL of ZnO-NLs samples at the same concentration was drop cast on a glass slide and covered with glass cover slip. The samples on a glass slide were checked for its fluorescence imaging in the wavelength-scanning mode of CLSM. Excitation was set at 360 nm, and emission spectrum was recorded in the range 468 to 691 nm, using Plan Apochromatic 63X/1.4 oil objective lens.

2.2.3. Electron microscopy

The size and shape characterization of ZnO-NLs was done by using Field Emission Gun-Transmission Electron Microscope (FEF-TEM