Green Synthesis of ZnO Nano Particles Using *Chlorella vulgaris* Extract as Additives

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Abstract. The application of nano zinc oxide in health-related fields calls for green and environmentally friendly synthetic methods. In this paper, *Chlorella vulgaris* extract was used to synthesize uniform nano sized zinc oxide particles. The results indicate that *Chlorella vulgaris* extract can effectively reduce the size of zinc oxide particles. The as synthesized ZnO particles using *Chlorella vulgaris* extract as additive are about 20-40nm.

1. Introduction

Nano sized ZnO particles have been integrated into various applications, including feed, cosmetics, chemical and health industries [1-2], which calls for a green and environmental eco-friendly approach to their synthesis [3]. High molecular weight substances can hinder grain growth [4-6]. Therefore, in the chemical methods for preparation of nano particles, soluble polymer compounds are often added as inhibitors. The plant extracts contain high molecular weight substances, such as proteins and polysaccharides, and thus can be used as grain growth inhibitors and stabilizers [7-9].

*Chlorella vulgaris* is a common health supplement and food ingredient due to its high protein content [10-11]. Its safety has been confirmed in literatures. In this paper, the *Chlorella vulgaris* extract is used as an additive to obtain nano zinc oxide particles.

2. Experimental procedure

2.1. Preparation of *Chlorella vulgaris* extract

The microalgae strain (*Chlorella vulgaris*) was obtained from National Key Laboratory of Food Science, Nanchang University. The *Chlorella vulgaris* were cultured in a 1000 ml Erlenmeyer flask containing TAP medium for a week with 24 h photoperiod at 28°C. The cultured microalgal solution was centrifuged (4000 r/min for 10 min), and the gained C. vulgaris cells were frozen in a refrigerator freezer at -18°C for 15 hours, and then thawed at room temperature. This freeze-thaw process was repeated three times to destroy the cell walls of *Chlorella vulgaris* 20 mL/(g algae) deionized water was added to the centrifuge tube for centrifugation (4000 r/min for 20 min), and the supernatant extract was collected to a test tube and stored at 4°C for the subsequent experiments.
2.2. Preparation of ZnO nanoparticles

*Chlorella vulgaris* extract (25 mL), zinc acetate (2.0 g) and deionized water (100 mL) were mixed in a 500 ml beaker, then adjusted the pH to 8 with 1 mol/L NaOH, set the constant temperature magnetic stirrer speed to 800 r/min. The reaction was conducted in a thermostatic magnetic stirrer set to 800 rpm and set to 80 °C for 1 h. The control experiment was carried out with ultrapure water. The product was cooled, washed repeatedly with suction and dried at 60 °C. The obtained product was subjected to heat treatment in a muffle furnace at a temperature of 500 °C and heated for 2 hours to obtain a powder for the next experiment.

2.3. Characterization

The crystal phase was studied with powder X-ray diffraction analysis (XRD, D/Max-2550 V, Rigaku, Japan; CuK, $\lambda = 0.15406$ nm) in an angle region from 10° to 80° with a scanning speed of 2°/min. The microstructure and morphology of products were examined with field emission scanning electron microscopy (FESEM, S-4800, Hitachi, Japan) and Transmission electron microscope (TEM, TECNAI G2 20 S-TWIN). Fourier transform infrared spectroscopy (IR, 960, Tianjin ruian technology Co., Ltd, China) was used to analyze the surface properties of as synthesized particles. The reaction process was analyzed using thermogravimetric analyzer (TGA, HTG-1, Beijing permanent, China).

![Figure 1. XRD patterns of as synthesized ZnO nanoparticles (C indicates the addition of *Chlorella vulgaris* extract, H indicates heat treatment).](image)

3. Results and discussion

3.1. XRD patterns

As shown in Figure 1, all the four samples have diffraction peaks at (100), (002), (101), (110) and (103), which matches ZnO on the standard card (JCPDS48-1066). However, since the diffraction peaks of zinc oxide and zinc hydroxide coincide at the above-mentioned peak positions, it was not confirmed from the diffraction pattern that the resulting products were pure zinc oxides or mixtures of the two. The heat treatment has the effect of purifying the crystal grains, and the diffraction peak of the zinc oxide is sharpened, and the impurity peaks disappeared after heat treatment. According to the calculation, the average sizes of ZnO, C-ZnO, ZnO-H and C-ZnO-H samples are 56 nm, 60nm, 40nm...
and 35nm, respectively. After heat treatment, the calculated grain size reduced, which implies that crystal transformation occurred during the heat treatment process, and that the samples without heat treatment were mixtures of zinc oxide and zinc hydroxide rather than pure zinc oxide.

3.2. IR spectrum
In the infrared spectrum of Figure 2, all four samples have absorption peaks at 3882 cm\(^{-1}\) and 450 cm\(^{-1}\). The 3882 cm\(^{-1}\) peak can be attributed to the stretching vibration of the -OH between molecules, while the 450 cm\(^{-1}\) is a characteristic absorption peak of Zn-O in ZnO. The two heat-treated samples, ZnO-H and C-ZnO-H have absorption peaks only at the above two positions, indicating that the heat treatment has the effect of purifying crystal grains, which is consistent with the XRD results. Protein and polysaccharide in the chlorella extract, coating on the surface of the zinc oxide particles, resulted in absorption peaks in the infrared spectrum at 1562 cm\(^{-1}\), 1506 cm\(^{-1}\), 1395 cm\(^{-1}\), 1353 cm\(^{-1}\) and 1048 cm\(^{-1}\). The amide I and amide II bands of the C=O vibration and N-H bending characteristic peaks in the protein corresponds to the absorption peaks at 1562 cm\(^{-1}\), 1506 cm\(^{-1}\), 1395 cm\(^{-1}\) and 1353 cm\(^{-1}\). The absorption peak at 1048 cm\(^{-1}\) may attribute to the vibration of the C-O-C of the polysaccharide in the extract. The 1509 cm\(^{-1}\) and 1392 cm\(^{-1}\) absorption peaks in the ZnO spectrum may be caused by the asymmetric C=O vibration present in residual zinc acetate.

Figure 2. IR spectrum of as synthesized ZnO nano particles.

Figure 3. TGA curves of as synthesized ZnO nano particles.
3.3. Thermal stability

In the TGA curves (as shown in Figure 3, sample ZnO-H and sample C-ZnO-H were stable, without obvious weight loss. The weight losses of sample ZnO and sample C-ZnO below 150°C were 2% and 3%, respectively, which can attribute to the evaporation of water. The weight losses at 150°C to 210°C were 12% and 11%, respectively, where Zn(OH)₂ was decomposed into ZnO and H₂O. Above 210°C, the weight losses of 4% and 6% were due to the decomposition of the remaining water and other substances. The weight loss of C-ZnO was higher than that of ZnO thanks to the organic substance in the *Chlorella vulgaris* extract.

![Figure 4. TEM images of as-synthesized nano ZnO particles.](image)

**Figure 4.** TEM images of as-synthesized nano ZnO particles. (a) C-ZnO, (b) ZnO, (c) C-ZnO-H, (d) ZnO-H, (e) C-ZnO, (f) C-ZnO-H.

![Figure 5. SEM images of as-synthesized nano ZnO particles.](image)

**Figure 5.** SEM images of as-synthesized nano ZnO particles. (a) C-Zn, (b) C-ZnO-H.

3.4. Microstructure analysis

As shown in Figure 4((a), (b), (c) and (d)), samples with addition of *Chlorella vulgaris* extract (sample C-ZnO and C-ZnO-H) have better dispersion and uniformity than sample ZnO and ZnO-H. The *Chlorella vulgaris* extract, containing abundant protein and polysaccharides, played a role in hindering
the growth of crystals. Since the particles appeared in a sheet shape, the calculation result according to XRD data cannot truly reflect the actual size of the particles. After heat treatment, the particles were transformed from plate-like into nearly spherical shape, which indicates that crystal transformation or rearrangement occurred during the heat treatment. A transition from polycrystalline to single crystalline was observed as shown in Figure 4 ((e) and (f)). The SEM results (as shown in Figure 5) also reflect the transformation of the crystal from a stacked sheet structure to a granular form after heat treatment.

3.5. Reaction mechanism
The reactions during synthesizing ZnO in this paper are as follows:

\[ \text{Zn(C}_2\text{H}_3\text{O}_2\text{)}_2 + 2\text{NaOH} \rightarrow \text{Zn(OH)}_2 \downarrow + 2\text{CH}_3\text{COONa} \]  
(1)

\[ \text{Zn(OH)}_2 + 2\text{OH}^- \rightarrow \text{Zn(OH)}_4^{2-} \]  
(2)

\[ \text{Zn(OH)}_4^{2-} \rightarrow \text{ZnO} + \text{H}_2\text{O} + 2\text{OH}^- \]  
(3)

Under hydrothermal conditions, Zn(OH)$_4^{2-}$ is the basic growth unit of ZnO crystals. The amino group of the protein in the Chlorella vulgaris extract formed a hydrogen bond with the (0001) crystal plane, inhibiting the migration of Zn(OH)$_4^{2-}$ growth unit to the ZnO (0001) crystal plane. The results are consistent with the decrease in the peak strength of the (100) and (002) diffraction peaks of XRD.

4. Conclusions
In this paper, nano zinc oxide particles are conveniently synthesized by hydrothermal method using Chlorella vulgaris extract as an additive. The Chlorella vulgaris extract acts to refine the zinc oxide grains. The heat treatment can remove the residue of the Chlorella vulgaris extract from the surface of the zinc oxide, and the heat treatment causes the original sheet-like precursor to be converted into a nearly spherical particle.

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