

The potential of gallic acid and ascorbic acid as green reducing agent in ZnO nanoparticle synthesis

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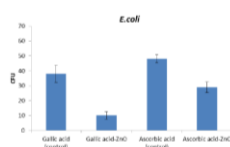
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GRAPICAL ABSTRACT



ABSTRACT

In this study, method for the synthesis of zinc oxide (ZnO) nanoparticles for biomedical applications ideally involve the use of nontoxic, less hazardous reducing and capping agent as well as the selection of environmentally benign solvents. In this study, we had investigated the potential of gallic acid and ascorbic acid as both reducing and capping agent in a green approach using microwave heating method. Two parameters including microwave power (400W and 800W) and time of heating (4 and 8 min) were investigated. UV-Vis absorption spectrum showed a typical spectrum for ZnO nanoparticles around 300nm wavelength. This microwave heating method with green reducing and capping agent successfully been advocated as a possible environmentally friendly alternative to chemical methods in synthesizing ZnO nanoparticles. ZnO nanoparticles synthesis from gallic acid and ascorbic acid were both found to exhibit antibacterial activity against a Gram-positive bacterium *Bacillus subtilis* and a Gram-negative bacterium *Escherichia coli*.

Keywords: Zinc oxide nanoparticle, gallic acid, ascorbic acid, microwave heating, phytochemical

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1. INTRODUCTION

Metal oxides nanoparticle has increased interest in many areas of technology [1]. Among metal oxides nanoparticles, Zinc oxide (ZnO) has received much attention in the recent past due to its unique physical, chemical, effective biological properties and wide applications in various fields, including medicine [2]. Because of increased outbreaks, infections of pathogenic strains and bacterial antibiotic resistance, ZnO nanoparticle is currently being investigated as novel antibacterial agent against bacterial strain [3]. Recently, Zinc oxide nanoparticles exhibit excellent biocidal and biostatic action against Gram-positive and Gram –negative bacteria including *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus* [4].

Currently, chemically green synthesis of metal nanoparticles is an interesting issue of nanoscience especially for the used in biomedical application. Chemically green synthesise of nanoparticles not only help to control the chemical toxicity in the environment but also responsible in producing small size, shape controlled nanoparticle with increasing ability and performance especially in extensive antimicrobial activity that is important in medical application. Green synthesis also can eliminate biological risk in pharmaceutical and biomedical application to ensure it safe and not provoke unexpected side effect. Chemically green synthesis involves the use of non-toxic, less hazardous reducing and capping agent, as well as the selection of environmentally benign solvents [5]. In our study, we are focusing the green synthesis of ZnO nanoparticle using gallic acid and ascorbic acid as reducing and capping agent.

Gallic acid (3,4,5- trihydroxyl-benzoic acid) which is a polyphenol naturally found in various plants (gallnut, tea leaves, oak bark) and fruits (bananas, strawberries, grapes) [7], has a good potential and highly relevant biomolecule as green reducing agent that have additional value in wide range of health benefits such as antibacterial [8], antiviral [9], anti-inflammatory [10] anticancer and antioxidant application [11]. Gallic acid is well-known phytochemical that has been used to synthesis silver and gold nanoparticles. This molecule has been used as both reducing and stabilizing agents in nanoparticles synthesis because it contain more than two hydroxyl groups that can reduce metal ions [12, 13, 14].

Ascorbic acid has been widely used as a reducing agent in the synthesis process of nanomaterials such as metal oxide and metal nanoparticle [15, 16, 17, 18]. Ascorbic acid can form complexes with various metal ions such as Fe³⁺, Cu²⁺, Co³⁺, and Zn²⁺ in solution. Ascorbic acid acts as an inhibitor of the hydrolysis of Zn²⁺ and lead to a reduction in the concentration of the growth units of ZnO crystal [17,19].Therefore, ascorbic acid can be used as modifier material for modified the structural and morphology properties of ZnO nanoparticles [20].

The achievement of green synthesis of nanoparticles depend not only on the selection of environmentally benign chemicals on capping agent, reducing agents and solvents , but also on the methodological used. In this study we were using the microwave heating methods. As an environmentally benign technology with wide applications, microwave synthesis has the advantages of homogeneous volumetric heating, and high reaction rate compared with other physical and chemical methods. Microwave heating methods can

address the problem of heating inhomogeneity, while providing a scalable platform for industrial application [21].

2. MATERIALS AND METHODS

2.1 Materials

Zinc sulphate heptahydrate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) of analytical grade was purchased and used without further chemical treatment and purification. L-Ascorbic Acid and gallic acid were functioning as reducing agents, were purchased from Sigma chemicals and used freshly after dissolving in de-ionized water. De-ionized water was obtained from Laboratory of Faculty Chemical and Natural Resources Engineering, University Malaysia Pahang.

2.2 Methodology

Synthesis of ZnO nanoparticles

5% of ascorbic acid and 5% of gallic acid were respectively dissolved in deionized water and then mixed with 50mL of 60-mmol/L (0.06M) $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$. The color of solution will change following the reduction process of zinc ions. The products were then subjected to microwave irradiation for 4 minutes. The microwave power was set to 800W. After 4 minutes, the mixture was sat to cool at room temperature [22, 23].

Antibacterial assays

A single colony of *E.coli* and *B. subtilis* was grown overnight in Luria Broth (LB) at 37° C until late log phase. 100µL of bacteria was transferred into 50 mL of LB, and 2mL of that solution was placed in separate sterile tubes. 50 µL of gallic acid-ZnO nanoparticles, ascorbic acid-ZnO nanoparticles, ascorbic acid monomer and gallic acid monomer were added. All tubes were then incubated at 37° C with shaking for approximately 24 h. After that, 5 µL of the solution was spread onto the surface of LB agar plates and incubated at 37° C for 24 h and tested. The number of viable cells in the sample was determined by choosing the appropriate dilution of the sample onto LB agar plates and counting colonies that appeared on the plates. The number of viable cells was obtained by averaging the numbers in three replicate plates [6].

2.3 Characterization

UV-vis spectrophotometer

The band gap of ZnO nanoparticles was analysed by a UV-vis spectrophotometer (U-1800 spectrophotometer Hitachi). The samples were measured for its maximum absorption spectra in the range of 200 to 700 nm, with a slit width of 2 nm and a

scanning rate of 100 nm/min. All the samples were measured to get absorption and normalized the spectra to compare peak shift.

3. RESULTS AND DISCUSSION

Gallic acid and ascorbic acid in 5% of concentration were used respectively as green reducing agent as well as capping agent for ZnO nanoparticle synthesis. The microwave heating method accelerated the processes and the reaction was completed in a very short period of time (4-8 minutes). The white cloudy colloidal suspension indicated the synthesis of ZnO nanoparticle. The reaction did happen in the absence of microwave heating, but it takes longer time almost 24 hours and more at room temperature. The formation of ZnO nanoparticle was confirmed by UV-vis spectrophotometry.

UV-vis analysis

The optical absorption spectra of ZnO dispersed in water were recorded using UV-vis spectrophotometer. UV spectra were measured at room temperature in a plastic cuvette with the path length of 1 cm. UV-Vis spectroscopy is the most widely used technique for the structural characterization of nanoparticle. It is generally recognized that UV -Vis spectra could be used to examine the size and shape controlled nanoparticle in aqueous suspension. The absorption edge systematically shifts to the lower wavelength or higher energy with the decreasing size of nanoparticle [33].

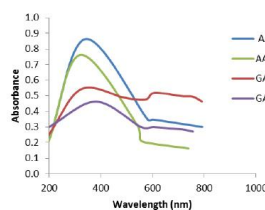


Fig. 1 UV-vis absorbance spectra of ZnO nanoparticles synthesized at different microwave heating power. AA; ascorbic acid, GA; gallic acid

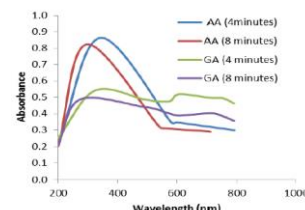


Fig. 2 UV-vis absorbance spectra of ZnO nanoparticles synthesized at 800W in different time of microwave heating. AA; ascorbic acid, GA; gallic acid.

Fig. 1 shows the UV Vis spectra of the synthesized ZnO nanoparticle using gallic acid and ascorbic acid as reducing agent respectively at microwave power 400W and 800W. An absorption peak was observed in range between 200 nm to 700 nm. Ascorbic acid and gallic acid show a similar trend respectively. A broad absorption peak was observed in each spectrum at 300nm-380 nm which is characteristic band for pure ZnO. The UV-Vis absorption spectroscopy of ZnO nanoparticles using ascorbic acid as reducing agent shows an exciton absorption peak at about 341 nm at 400W and 321nm at 800 W, and using gallic acid as reducing agent shows an exciton absorption peak at about 323 nm at 400W and 381nm at 800 W.

Fig. 2 shows the UV-vis spectra of the synthesized ZnO nanoparticle using gallic acid and ascorbic acid under microwave time of heating for 4 minutes and 8 minutes. An absorption peak was observed in range between 200 nm to 700 nm. The UV-Vis absorption spectroscopy of ZnO nanoparticles using ascorbic acid as reducing agent shows an exciton absorption peak at about 341 nm for 4 minutes of microwave heating and 321nm for 8 minutes of microwave heating, and using gallic acid acid as reducing agent shows an exciton absorption peak at about 323 nm for 4 minutes of icrowave heating and 273 nm for 8 minutes of microwave heating. The absorption is caused due to the excitation of the electrons from the valence band to the conduction band when they are irradiated with UV light, which causes the absorption of UV radiation. It also due to the light scatter which the particle size of a colloidal solution within the wavelength of UV light results in light scattering, which also causes the absorption.

The result indicated that the blueshift is observed with decrease in particle size. The dependence of the absorption band gap on the size of ZnO nanoparticles is shown in Figure 3. The average particles size of ZnO nanoparticles has been estimated by using the following hyperbolic band model (25):

$$R = \sqrt{\frac{2\pi^2 \times h^2 \times E_{gb}}{m^* \times (E_{gn}^2 - E_{gb}^2)}} \quad (1)$$

where R is the radius, m* is the effective mass of the specimen (m*= 29.15×10⁻³¹ kg for ZnO), E_{gb} is bulk band gap, h is Planck's constant (6.6261×10⁻³⁴ J s) and E_{gn} is the band gap at strong absorption edge. E_{gn} can be calculated by the formula (26):

$$E_{gn} = \frac{hc}{\lambda_{gn}} \quad (2)$$

where c is the velocity of light (3×10⁸ m s⁻¹) and λ_{gn} is the strong absorption edge obtained from the absorption spectra. The strong absorption edge for both samples is found to be 298 nm. The average particle size for gallic acid-ZnO nanoparticle and ascorbic acid-ZnO nanoparticle in optimum condition (800W for 8 minutes microwave heating) is calculated and found to be 6.03 nm and 11.16 nm respectively.

Antibacterial studies

Antibacterial study was carried out on 2 isolated strains namely, a Gram-positive bacteria *Bacillus subtilis* and Gram-negative bacteria *Escherichia coli*. The ZnO nanoparticle synthesized from using gallic acid and ascorbic acid were tested on the growth of both of these strains. The relative number of colony forming units (CFU) was counted as shown in Fig 3 and Fig 4. From the graphs, we observed that the growth of bacteria was significantly inhibited in the presence of gallic acid-ZnO nanoparticle and ascorbic acid-ZnO nanoparticle. However, Gallic acid-ZnO nanoparticle shows

better antibacterial effect compare to ascorbic acid-ZnO nanoparticle for both strains.

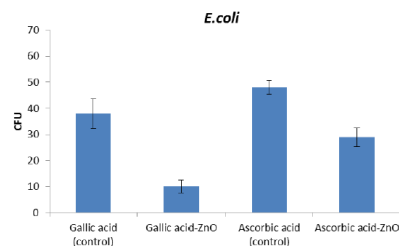


Fig. 3 Inhibition of *E. coli* growth in the presence ZnO nanoparticle synthesis using gallic acid and ascorbic acid.

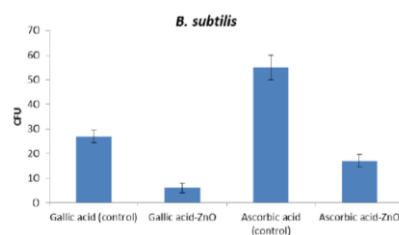


Fig. 4 Inhibition of *B. subtilis* growth in the presence ZnO nanoparticle synthesis using gallic acid and ascorbic acid.

In addition, we also investigated the effects of control (5% of gallic acid and 5% of ascorbic acid). Gallic acid controls showed less antibacterial effect compared to that gallic acid-ZnO nanoparticle. Plant polyphenols such as gallic acid has been shown to possess antibacterial activity (29). While ZnO nanoparticle exhibit attractive antibacterial properties in many reserch study. Therefore, it is expected the combination of these gallic acid and ZnO will enhanced antibacterial activity.

Ascorbic acid also showed antibacterial acitivity in both *E. coli* and *B. subtilis*. There is finding suggested the application of ascorbic acid, in combination with lactic acid, may have potential as preservative to inhibit the growth of *E. coli* O157:H7 in food (30). Ascorbic acid also reported has antibacterial effect on *Pseudomonas aeruginosa* (31).

From the Fig. 3 and Fig. 4, ZnO nanoparticle synthesized from both gallic acid and ascorbic acid as reducing agent show better inhibitory effect of growth on *B. subtilis* compared to *E. coli*. These result not only caused by the influence of cell wall of Gram-positive bacteria, but also influenced by the character of *B. subtilis* is an obligate aerobe and hence is expected to experience high level of oxidativestress which may lead to cellular disruption. *E. coli*, although being a facultative anaerobe and gram negative bacteria, is highly affected by the nanoparticle (27).

Generally, Gram-positive bacteria are more sensitive to ZnO than Gram-negative bacteria. This is might be caused by the difference in the nature and organization of the cell wall of bacteria. Gram-positive bacteria have relatively a thick cell wall containing large amounts pf peptidoglycan, as well as compenents like lipoteichoic acids (LTA). In contrast, Gram-

negative bacteria have relatively complex structure and thin cell wall. The outer membrane is composed of high concentration of lipids, polysaccharides and protein. Outside of the central membrane is an open area called the periplasmic region, followed by a thin layer or peptidoglycan which is surrounded by an additional membrane (27).

There are various factors that influenced the antibacterial effect of ZnO nanoparticles. Various mechanism like release of toxic ions, production of reactive oxygen species from the nanoparticles, mechanical stress of stimuli caused by the surface, small size and shape of the particles, damage to cell membrane by adhesion to the surface, penetration through the cell wall and subsequently cellular internalization (28) have been reported to induce the antibacterial activity and toxicity in ZnO.

4. CONCLUSION

Chemically green synthesis of ZnO nanoparticles is much safer, environment friendly and important to eliminate the biological risk to ensure it safe and not provoke unexpected side effect. In this study we demonstrated the green synthesis of ZnO nanoparticle using gallic acid and ascorbic acid with act as an effective reducing and capping agent. The synthesized ZnO nanoparticle were characterized by UV-vis absorption spectroscopy. From the microwave heating method, the average particle size for gallic acid-ZnO nanoparticle and ascorbic acid-ZnO nanoparticle in optimum condition (800W for 8 minutes microwave heating) are 6.03 nm and 11.16 nm respectively. The synthesized gallic acid-ZnO nanoparticle and ascorbic acid-ZnO nanoparticle have shown antibacterial activity on , *Bacillus subtilis* and *Escherichia coli*.

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