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Comparison of Bactericidal Properties of ZnO Nanoparticles Obtained By Two Synthesis Methods

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Abstract **-** The potential bactericidal properties of nanosized zinc oxide (ZnO) make this semiconductor suitable for medical and pharmaceutical applications. In this article we present two methods of synthesis of ZnO nanoparticles (Nps-ZnO): chemical precipitation and solution combustion synthesis. The synthesized nanopowders were characterized with Scanning Electron Microscopy-Energy Dispersive X-Ray Spectroscopy (SEM-EDS), X-ray Diffraction (XRD) and Transmission Electron Microscopy (TEM). The EDS analysis indicate a Zn:O atomic ratio of 0.81 and 1.36, for the chemical precipitation and solution combustion synthesis, respectively. The XRD results confirm that Nps-ZnO obtained by both methods show a hexagonal arrangement (Wurtzite). TEM images show nanoparticles with mean diameters between 15.5 ± 6.8 nm and 73.4 ± 6.0 nm. Finally, the Kirby−Bauer disk diffusion method for the determination of antibacterial activity shows that *Escherichia coli* was more sensitive to the antimicrobial action of Nps-ZnO obtained by chemical precipitation than the *Bacillus Subtilis* bacteria.

Keywords**:** Antibacterial activity, chemical precipitation, nanoparticles, solution combustion synthesis, zinc oxide.

1. Introduction

Zinc oxide (ZnO) has been extensively studied for its optical and semiconductor properties [1]. However, the study of the antibacterial properties of this oxide has attracted great interest in recent decades [2]. In fact, ZnO offers advantages over other antimicrobial agents, such as low toxicity, adequate chemical stability, and good selectivity, among others [3]. Among the bacteria used in the studies found in literature we can mention: *Bacillus subtilis* [4], *Escherichia coli* [2,4-6], *Pseudomonas aeruginosa* [4] and *Staphylococcus aureus* [3,4].

There are several routes to obtain ZnO oxide nanoparticles with different shapes and mean sizes. Among the reported synthesis methods, the solution combustion method [7,8] and precipitation [9,10] are presented as good alternatives to obtain ZnO nanoparticles. In the present work, the shape and mean size of the ZnO nanoparticles obtained by two synthesis methods are compared. Preliminary results of their antibacterial activity against gram-positive and gram-negative bacteria are also evaluated.

2. Experimental section

The reagents and analytical techniques used will be detailed below.

2.1. Chemicals

Glycine, $NH_2CH_2CO_2H$ (≥ 99%), Potassium nitrate, KNO_3 (≥ 98%); Sodium hydroxide, NaOH (≥ 99%) and Zinc nitrate, $Zn(NO_3)_2.6H_2O$ (≥ 98%) were purchased from Sigma-Aldrich (Darmstadt, Germany). All chemicals were of analytical purity and used as received without further purification. Milli-Q water (18 MΩ cm), obtained from a purification system (Millipore, Darmstadt, Germany), was used. A commercial sample of ZnO particles was provided by Sigma-Aldrich (Darmstadt, Germany).

2.2. Preparation of ZnO nanoparticles

For the synthesis of ZnO nanoparticles two methodologies were used. The chemical precipitation method based on previous work reported by Guzman et al. [11], and the solution combustion synthesis developed by Flores et al. [7] and Rostami et al. [12]. For the chemical precipitation method, solutions of $Zn(NO_3)_2$ (20 mM), NaOH (0,4 M) and KNO_3 (K^+/Zn^{+2}, 1:1) were used [11]. After the reaction at 60ºC and 800 rpm during more less 2 hours, the obtained colloids containing nanoparticles we centrifuged at 6500 rpm using a microcentrifuge (Eppendorf 5804). The nanoparticles were washed two times with deionized water to remove dissolved zinc ions. The product was finally dried at 60°C.

For solution combustion synthesis, 5 g of zinc nitrate as source of zinc and 0.3 g of glycine as fuel and oxidizer were dissolved in 20 ml of water to form a viscous solution under continuous magnetic stirring [7]. The mixture was taken to a closed furnace preheated to 500 °C so that, after approximately 2 min, combustion takes place. In a short time, the mixture was ignited with a flame, producing an evolution of large amounts of gases and leaving a porous product (nanocrystalline ZnO) inside the porcelain crucible [12]. This product was ground using an agate pestle to produce the final powder, without any further heat treatment.

2.3. Characterization techniques

- **Elemental Composition Analysis**

Chemical compositions of the samples were analysed by Scanning Electron Microscope (SEM) using a FEI Quanta 650 in the secondary electron mode at an accelerating voltage of 3-10 kV (FEI Europe BV; Eindhoven, The Netherlands). EDS analysis was performed with an Ametek EDAX TEAM system coupled to the SEM microscope

- **Structural Characterization**

X-ray diffraction analysis was carried out using a Bruker D8 Advance (Karlsruhe, Germany) X-ray diffractometer equipped with a copper anticathode (λ Cu Kα= 1.54056˚A). Data were obtained over the range 2θ = 25°–75° using a step size of 0.02° and counting time of 10 seconds per step. Reference intensity ratios were used to assign the phases observed in the X-ray pattern. Average crystallite size measurements were also carried out using the Scherrer equation, $D=k\lambda/ß\cos\theta$, where D is the crystallite size, k is a constant (=0.9 assuming that the particles are spherical), λ is the wavelength of the X-ray radiation, ß is the line width at half maximum intensity of the peak and θ is the angle of diffraction.

- **Morphology Analysis**

The size and morphology of the ZnO nanoparticles were evaluated by a LVEM5 microscope (Delong Instruments-Check Republic) in transmission electron microscopy (TEM) mode. Also, a Philips CM20-Ultra Twin TEM microscope operating at 200 kV (Philips; Eindhoven, The Netherlands) was used. A small amount of nanopowder was dispersed in ethanol (98%) by sonication. Then, 5 μL of this solution was deposited on a carbon-coated TEM copper grid. Histograms of size distribution were calculated from the TEM images by measuring the diameters of at least 50 particles using the ImageJ® software.

2.4. Antibacterial assays

The disc diffusion or Kirby-Bauer method was used to evaluate the antimicrobial susceptibility of zinc oxide nanoparticles against both gram-positive bacteria (*B. Subtilis*) and gram-negative (*E. Coli*) [13]. In each plate bacteria were seeded at a concentration of $1.5x10^8$ CFU and disks with different concentrations of nanoparticles ranging from

0.5-1.5 mg/mL were placed on top. After 24 h of incubation at 30 ºC (for *B. Subtilis*) and 37 ºC (for *E. Coli*) the halos formed, representing the inhibition zones, were measured (mean ± SD values) [14]. Discs containing antibiotics, *ampicillin* (for gram-positive bacterial controls) and *streptomycin* (for gram-negative bacterial controls) were prepared to make the necessary comparisons.

3. Results and discussion

Samples obtained by chemical precipitation and solution combustion synthesis were characterized using the techniques described above. Commercial zinc oxide nanoparticles were used as a reference. Elemental analysis of the samples was performed by energy dispersive X-ray spectroscopy (EDS). The results are shown in Figure 1. The EDS spectra of the three samples of ZnO nanoparticles exhibited well defined K and L emission peaks related with zinc and oxygen elements. Furthermore, the average Zn/O ratio are 0.81, 1.36 and 1.20 for samples obtained by precipitation method, solution combustion synthesis and commercially method, respectively. Those values are very close to 0.86, 1.32 and 1.18 reported by Manikandan et al. [15], Umar et al. [8] and Parra et al. [9], respectively.

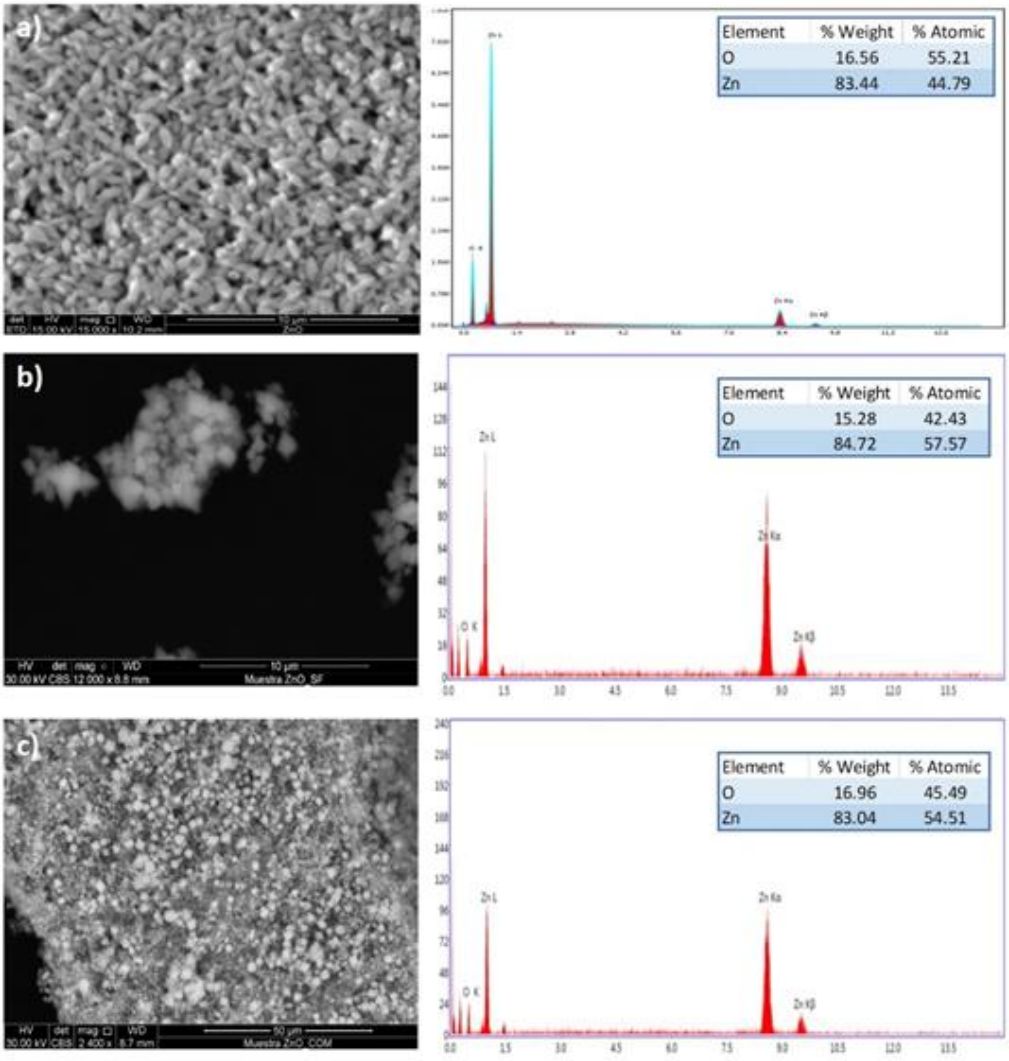

Fig. 1: EDS spectra of ZnO nanoparticles prepared by precipitation method a) solution combustion synthesis b) and commercial product c).

Figure 2 shows the XRD spectra of ZnO nanoparticles samples. The interplanar distances calculated for the family of planes (100), (002), (101), (102), (110), (103), (200), (112) and (201) of the XRD confirming the formation of hexagonal zinc oxide phase (JCPD card no. 36-1451 for wurtzite zinc oxide) [8]. No other diffraction peaks appear in the XRD patterns. The estimated size of ZnO nanocrystal using the Scherrer formula using the diffraction intensity of the ZnO (101) was 25.1 nm, 54.8 nm, and 116.3 nm for samples obtained by precipitation method, solution combustion synthesis and commercial method, respectively. These results are summarized in Table 1. Similar crystallites mean diameter of 24.9 nm, 25.6 nm, 26.0 nm, 26.0 nm, 52.4 nm, 54.3 nm and 58.0 nm were previously reported by Umar et al. [8], Anbuvannan et al. [16], Jangir et al. [17], Pushpanathan et al. [18], Omri et al. [19], Kayani et al. [20] and Karimian et al. [21], respectively.

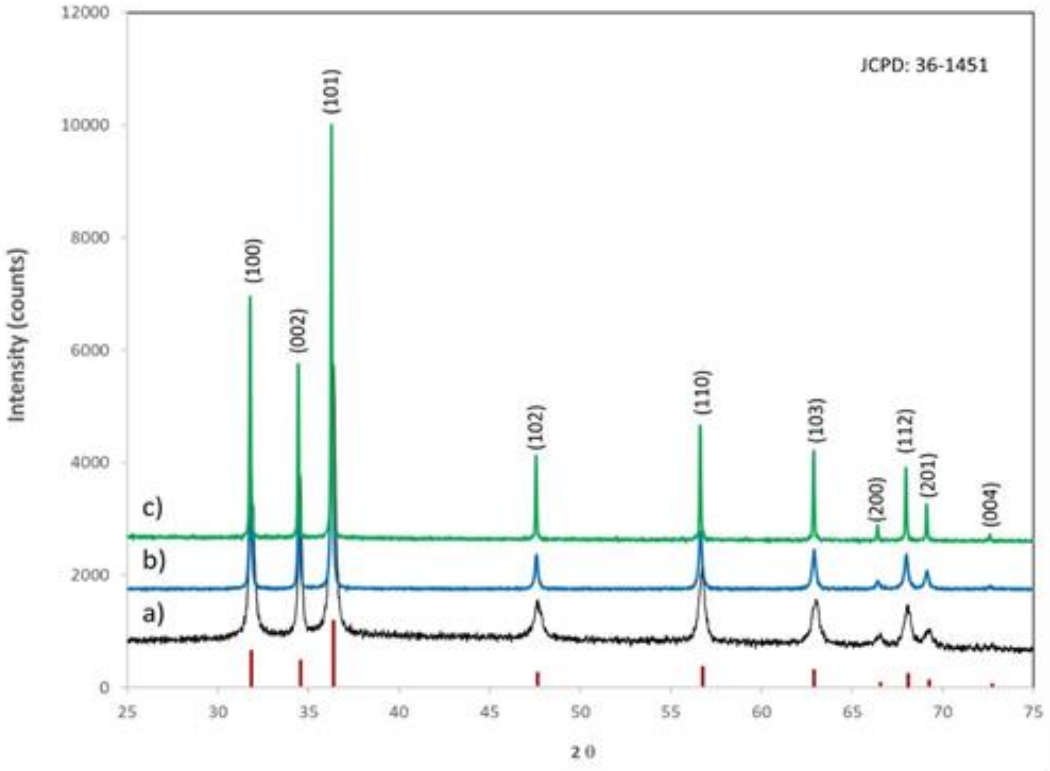

Fig. 2: XRD patterns of ZnO nanoparticles prepared by precipitation method a) solution combustion synthesis b) and commercial product c).

Table 1: Mean diameter, crystallite size and Zn/O ratio of each sample.

Sample	Zn/O	Mean diameter (nm)	Crystallite size (nm)
Chemical precipitation	0.81	15.5 ± 6.8	25.1
Solution combustion synthesis	1.36	73.4 ± 6.0	54.8
Commercial*	1.20	184.4 ± 82.0	116.3

*Reference material

The morphology of the obtained materials was analyzed by TEM analysis, Figure 3 shows typical TEM micrographs with the histograms of particle size obtained by evaluating at least 100 particles. It is clear that the three samples show a different distribution of nanoparticle sizes. The smallest particles were those synthesized by chemical precipitation (~15.5 nm). The largest were those obtained commercially. The nanoparticles of the sample obtained by chemical

precipitation show a particle size distribution ranging from 2.0 to 52.0 nm with a mean size of 15.5 nm ± 6.8 nm, (Figure 3a). On the other hand, the commercial sample show the larger nanoparticles with particles ranging from 48 nm to 390 nm nm in size with the estimated mean diameter of 184.4 nm ± 82.0 nm (Figure 3c). In the case of the nanoparticles obtained by by solution combustion synthesis, the mean size was 73.4 nm ± 6.0 nm, with a particle distribution of 66.5 nm to 86.5 nm nm (Figure 3b). Similar crystallites with mean diameter of 14.0 nm ± 2.0 nm, 15.0 nm, 85.0 nm, from 70.0 to 80.0 nm were were previously reported by Jangir et al. [17], Zandi et al. [22], Umar et al. [8] and Ramani et al. [23] respectively.

If we compare the two synthesis methods, it is observed that the precipitation method produces smaller nanoparticles with a wider size distribution. The TEM analysis allows us to observe the diverse morphologies of the synthesized ZnO nanoparticles. The precipitated sample presents a wide range of shapes for the nanoparticles, some of which are hemispherical, while the solution combustion synthesis sample presents almost a unique population of square-shaped nanoparticles. We attribute this difference in morphology to the synthesis method used.

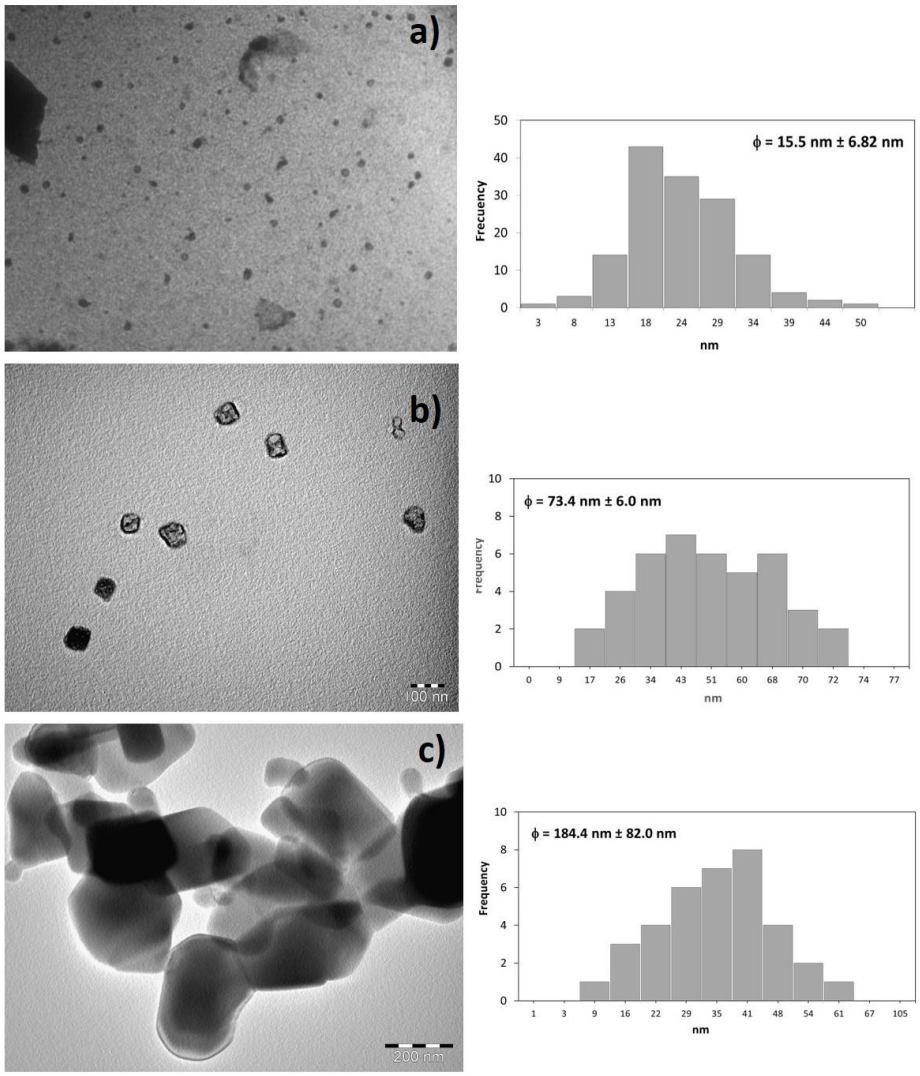

Fig. 3: TEM images and particle size distribution of ZnO nanoparticles prepared by precipitation method a) solution combustion synthesis b) and commercial product c).

Finally, the antibacterial effect of ZnO nanoparticles was analysed with the Kirby Bauer disk diffusion method [13,14]. The antibacterial activity of ZnO nanoparticles was tested against gram-negative bacteria *E. coli* (ATCC® 10536) and gram-positive bacteria *B. Subtilis* (ATCC® 6633). The antibacterial activities of ZnO nanoparticles at different concentrations 0.5 mg/mL, 1.0 mg/mL, 1.25 mg/mL and 1.5 mg/mL against both gram-negative and gram-positive bacteria is shown in Figure 4. The inhibition zones in millimetres of both bacteria are shown in Table 2. Similar results have been reported for the *E. coli* bacteria by Manyasree et al. [6] for a higher concentration (20 mg/mL) of larger ZnO nanoparticles (32 nm).

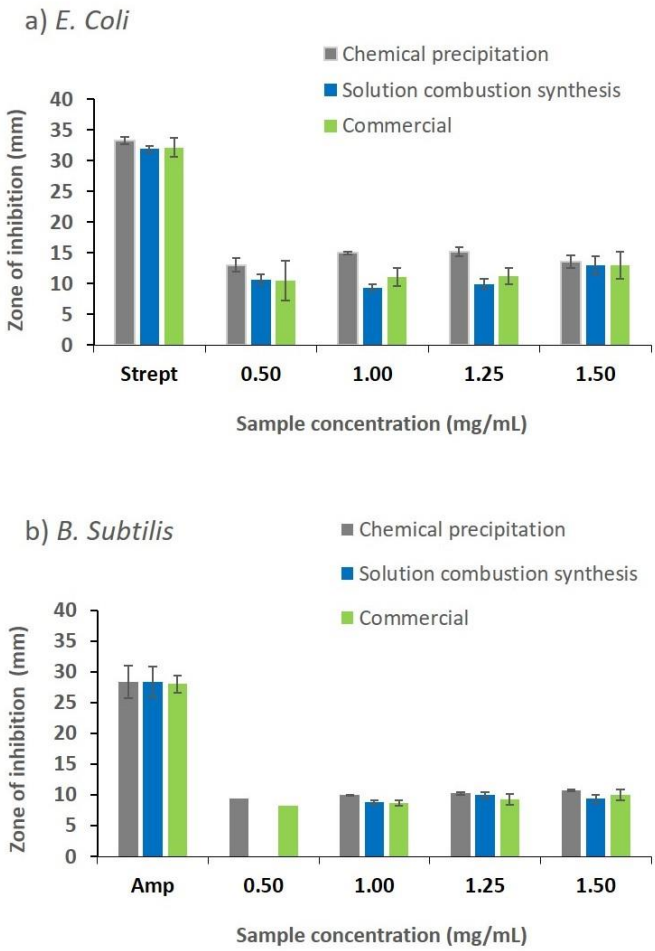

Fig. 4: Inhibition zone (mm) of ZnO Nanoparticles prepared by different method against *E. Coli* and *B. Subtilis*.

The Gram-negative bacterial strain *E. coli* was more sensitive to the antimicrobial action of ZnO nanoparticles obtained by chemical precipitation than the gram-positive *B. Subtilis*. Similar results were previously reported by *Jeeva et al.* [24]. However, since the ZnO nanoparticles obtained by solution combustion synthesis are smaller than the commercial ones, it would be expected that their bacterial activity would be higher. Considering that smaller particles would more easily penetrate the membrane structures of Gram-negative bacteria than Gram-positive bacteria [25,26]. Gram positive bacteria consist of a single cytoplasmic membrane surrounded by

multiple peptidoglycan layers as well as a thick cell wall with dimensions 20–80 nm. On the contrary, Gram-negative bacteria consist of a slender peptidoglycan layer of 7–8 nm thickness present in between the outer cell cell membrane and the inner plasma membrane [26,27,28].

The results show the following trend regarding the inhibition of bacteria: ZnO $_{(\text{chemical precipitation})}$ > ZnO $_{(\text{commercial sample})}$ > > ZnO $_{(\text{synthesis by combustion in solution})}$. This means that not only the size of the nanoparticle [25,29], but also the shape and the Zn/O ratio may be contributing to the complex inhibition mechanism of the bacteria.

Table 2: Inhibition zone (mm) of ZnO Nanoparticle Sols against *E. Coli* and *B. Subtilis*.

	mg/ml	**Chemical precipitation**	**Solution combustion synthesis**	**Commercial***
E. Coli	0.50	13.03 ± 1.14	10.66 ± 0.82	10.46 ± 3.29
	1.00	14.96 ± 0.20	9.37 ± 0.45	11.05 ± 1.51
	1.25	15.22 ± 0.76	9.92 ± 0.83	11.26 ± 1.30
	1.50	13.56 ± 1.01	12.93 ± 1.49	12.94 ± 2.23
B. Subtilis	0.50	9.39	n.d.	8.18
	1.00	10.06 ± 0.01	8.77 ± 0.35	8.67 ± 0.48
	1.25	10.25 ± 0.18	9.96 ± 0.48	9.27 ± 0.85
	1.50	10.75 ± 0.14	9.40 ± 0.64	9.94 ± 0.88

*Reference material

4. Conclusion

It is possible to obtain ZnO nanoparticles by two synthesis methods. The morphology shows semispherical and semi-square nanoparticles if the chemical precipitation and solution combustion synthesis was used. The production methods also influence the size distribution, obtaining smaller nanoparticles with chemical precipitation method. The prepared particles have mean diameters of 15.5 nm ±6.8 nm and 73.4 nm ± 6.0 nm. ZnO nanoparticles obtained by chemical precipitation present a better response in the inhibition of the *E. coli* strains than *B. Subtilis*.

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