Green synthesis of zinc oxide nanoparticles: a review of the synthesis methodology and mechanism of formation

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ABSTRACT

Zinc oxide is of significant importance for many industries due to its versatile properties, which have been enhanced with the production of this material in the nanoscale. Nonetheless, the increase in concerns related to environmental impact has led to the development of eco-friendly processes for its production. Recent interest in obtaining metal and metal oxide nanoparticles using biological approaches has been reported in the literature. This method was termed ‘green synthesis’ as it is a less hazardous process than chemical and physical synthesis methods currently used in the industry to obtain these nanomaterials. Zinc oxide nanoparticles have been successfully obtained by green synthesis using different biological substrates. However, large scale production using green synthesis approaches remains a challenge due to the complexity of the biological extracts that poses a barrier onto the elucidation of the reactions and mechanism of formation that occur during the synthesis. Hence, the current review includes a summary of the different sources of biological substrates and methodologies applied to the green synthesis of zinc oxide nanoparticles and the impact on their properties. This work also describes the advances on the understanding of the mechanism routes reported in the literature.

**Keywords:** zinc oxide nanoparticles; green synthesis; biological extracts; mechanism route; environmentally friendly
1. Introduction

Zinc oxide is of great economic and industrial interest due a wide range of properties that allows its application in many different areas, such as the rubber industry, biomedical field and metal surface treatment (Gujel et al., 2017; Kathalewar et al., 2013; Pasquet et al., 2014; Xie et al., 2011; Zhang et al., 2013). Among the many attributes of zinc oxide, the main characteristics are its semiconductivity, antimicrobial activity, vulcanization activator and UV absorption (Ahmoum et al., 2019; Grasland et al., 2019; Kumar and Koteswara Rao, 2015; Lee et al., 2016; Pasquet et al., 2014; Xie et al., 2018; Zaki et al., 2018; Zhang et al., 2018). With the introduction of Nanotechnology, it is possible to enhance these properties once the surface area of the material increases with the reduction in particle size and by manipulating the morphology of the nanomaterial (Goh et al., 2014; Kumar and Rani, 2013).

Reports on the improvements of zinc oxide nanoparticles (ZnONPs) properties have increased in the last few years. Several studies have reported its use for photocatalysis (Dimapilis et al., 2018; Roshitha et al., 2019); as an antimicrobial agent (Ginjupalli et al., 2018; Khatami et al., 2018b; Saravanan et al., 2018; Shahriyari Rad et al., 2019); in energy cells (Lee et al., 2018; Mahmood et al., 2019); and in sensors (Arafat et al., 2018; Basha et al., 2016). Novel applications of ZnONPs in biomedical engineering is also an emerging field of study with ZnONPs been applied for tissue regeneration, implant coatings, bio imaging, wound healing, development of cancer therapies, among others (Iqbal et al., 2019; Khatami et al., 2018a; Mirzaei and Darroudi, 2016; Mishra et al., 2017; Oliveira and Zarbin, 2005; Ullah et al., 2017).

In the last few years, the development of products and processes that are environmentally friendly has been gaining attention due the concerns related to climate change, water pollution, finite natural resources, human health and others (Anastas and Eghbali, 2010; Duan et al., 2015; Sheldon, 2018). Hence, researchers have been developing methods to
improve the production of metal and metal oxide nanoparticles using greener technologies (Kharissova et al., 2013; Król et al., 2017; Muthuvinodini and Stella, 2019; Shah et al., 2015; Zikalala et al., 2018). This environmentally friendly synthesis of metal and metal oxide nanoparticles using biological substrates has been extensively investigated to replace chemical and physical methods commonly used in industry.

Even though a considerable amount of research has been reported in this field, the mechanism of formation of the nanoparticles obtained by green synthesis has still to be defined and understood due to the high complexity of the biological extracts. In this sense, this review summarizes the recent developments in the green synthesis of ZnONPs, focusing on contrasting the distinct methodologies applied as well as the investigation of the formation mechanism routes of the green synthesis with different biological extracts.

2. Synthesis of zinc oxide nanoparticles

ZnONPs can be obtained using chemical, physical or biological methods. Chemical methods include precipitation, microemulsion, chemical reduction, sol-gel and hydrothermal techniques, which may lead to high energy consumption when high pressure or temperature conditions are required in the process (Brintha and Ajitha, 2015; Król et al., 2017; Li et al., 2009; Naveed et al., 2017). Among the chemical methods, the most commonly used is the sol-gel synthesis, developed by Spanhel and Anderson (1991), which uses a zinc precursor salt (nitrate, sulphate, chloride, etc.) and a chemical reagent in order to regulate the solution pH and avoid the precipitation of Zn(OH)₂. After, this solution will be exposed to thermal treatment under temperatures up to 1000 °C to obtain the ZnONPs (Bekkari et al., 2017; Hasnidawani et al., 2016; Morandi et al., 2017).
Chemical stabilizers, such as citrates or polymers like polyethylene glycols, polyvinylpyrrolidone and amphiphilic block copolymers, can be added to the ZnONPs synthesis for controlling the size of the nanoparticles and avoid particle agglomeration (Lepot et al., 2007; Li et al., 2009; Naveed et al., 2017; Zhang and Mu, 2007). In addition, a significant factor to be considered on the chemical synthesis is that the concentration of the chemicals used in the process can influence the size and shape of the particles considerably. It is known that it is possible to obtain particles from few nanometers (5-10 nm) up to micrometric size using the same process but different concentrations and ratios of chemicals (Król et al., 2017; Naveed et al., 2017).

Although less used than the chemical method, ZnONPs can be synthesized via physical techniques by vapor deposition, plasma and ultrasonic irradiation (Dong et al., 1997; Kong et al., 2001; Król et al., 2017). Nonetheless, these techniques usually require a high amount of energy and robust equipment, which increases the cost of the products. Another approach to obtain ZnONPs is using biological synthesis, which has arisen as an alternative eco-friendly process.

**2.1 Green synthesis of zinc oxide nanoparticles**

The interest in synthesizing ZnONPs via biological methods has increased considerably in the last decade. The development of this new approach and the significant interest in it is mainly related to the absence of toxic chemicals or high amount of energy applied to the biological synthesis, which makes the process more cost-effective and eco-friendly (Khalid et al., 2017; Kharissova et al., 2013; Król et al., 2017; Makarov et al., 2014; Naveed et al., 2017).

Many reports in the literature indicate that the biological synthesis of metallic and metal oxides nanoparticles is more environmentally friendly than the conventional chemical or physical methods used nowadays (Kharissova et al., 2013; Makarov et al., 2014). Therefore,
these biological methods have become more known as green synthesis. It was also given this term because it goes in agreement with the twelve principles of the green chemistry, which are shown in Figure 1 (Anastas and Eghbali, 2010). Nowadays, these principles are considered the fundamentals to contribute to sustainable development, and comprise instructions to implement new chemical products, new synthesis, and new processes.

![Figure 1: Green chemistry principles](image)

Briefly, the green chemistry principles are based on 1) atom economy, to improve reaction efficiency, 2) energy efficiency, avoiding high energy consumption process, 3) safer chemicals, to minimize the toxicity of processes and products, 4) prevention, to minimize waste in every stage of the process, 5) renewable feedstocks, using chemicals made of renewable sources, 6) design for degradation, design biodegradable and non-toxic products, 7) less hazardous chemical synthesis, to design safer synthesis routes, 8) reduce derivatives, avoid the use of derivatives such as protectors or stabilizers, 9) pollution prevention, prevent the release of hazardous substances, 10) safer solvents and auxiliaries, to use the least possible solvent or chemical, 11) catalysis, use catalysis to improve processes like energy consumption or efficiency and 12) accident prevention, to minimize the risks of accident. For instance, the
The main advantages of the biological synthesis are the employment of renewable sources, safer solvents and auxiliaries while producing safer chemicals.

The large scale production of nanoparticles by green synthesis remains a challenge, and these syntheses have been performed only at a laboratory scale. However, it is likely that its industrial application will take place in a near future as no robust equipment is necessary and significant advancements have been achieved on understanding the biological extracts composition and their interaction with the metal ions (Kharissova et al., 2013; Makarov et al., 2014; Mirzaei and Darroudi, 2016).

Essentially, green synthesis uses biological substrates such as plants, bacteria, fungus and algae to replace chemical solvents and stabilizers to decrease the toxicity of both product and process (Kharissova et al., 2013; Król et al., 2017). In the case of ZnONPs synthesis, many different biological substrates have been successfully applied to obtain this metal oxide. In general, the biosynthesis of ZnONPs is a very straightforward process in which a zinc salt, such as zinc nitrate or zinc acetate, is added to a biological extract previously prepared. After the reaction, this solution is submitted to a thermal treatment, and the ZnO powder is obtained (Król et al., 2017; Mirzaei and Darroudi, 2016; Singh et al., 2016). The mentioned process is illustrated in Figure 2.

![Figure 2: Green synthesis of ZnONPs](image-url)
Nonetheless, published works indicate different methodologies for the green synthesis of ZnONPs. Table 1 summarizes several of the synthesis methods reported in the literature to date. Despite the fact that a wide variety of biological substrates with distinct composition have been applied for this purpose, the concentration of zinc salts, pH variations, reaction time and temperature vary considerably, resulting in particles with different sizes and morphologies.
Table 1. Reported methodologies of the green synthesis of ZnONPs

<table>
<thead>
<tr>
<th>Biological substrate</th>
<th>Zinc source</th>
<th>pH</th>
<th>Reaction time and temperature</th>
<th>Thermal treatment</th>
<th>Average size (nm)</th>
<th>Shape</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algae</td>
<td></td>
<td></td>
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<tr>
<td>Sargassum murticum (20 g L⁻¹)</td>
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<tr>
<td>C. peltata, H. Valencia and S. myriocystum (5 g L⁻¹)</td>
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<td></td>
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</tr>
<tr>
<td>Acinetobacter schindleri culture (NS)</td>
<td>Nitrate (0.9 g L⁻¹)</td>
<td>NS</td>
<td>48 h, 37 °C</td>
<td>6 h, 60 °C</td>
<td>20-100</td>
<td>Spheres</td>
<td>(Busi et al., 2016)</td>
</tr>
<tr>
<td>Bacillus licheniformis biomass (50 g L⁻¹)</td>
<td>Acetate (43.9 g L⁻¹)</td>
<td>NS</td>
<td>48 h, 37 °C</td>
<td>Not applied</td>
<td>Not applied</td>
<td>Spheres</td>
<td>(Tripathi et al., 2014)</td>
</tr>
<tr>
<td>Lactobacillus plantarum culture (NS)</td>
<td>Sulphate (17.9 g L⁻¹)</td>
<td>6</td>
<td>10 min, 80 °C – 12h, 37 °C</td>
<td>4 h, 40 °C</td>
<td>13</td>
<td>Spheres</td>
<td>(Selvarajan and MohanaasriniVan, 2013)</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa (0.05 g L⁻¹)</td>
<td>Nitrate (0.19 g L⁻¹)</td>
<td>NS</td>
<td>30 min, 80 °C</td>
<td>NS, 70 °C</td>
<td>Not applied</td>
<td>Spheres</td>
<td>(Singh et al., 2014)</td>
</tr>
<tr>
<td>Serratia ureilytica culture (NS)</td>
<td>Acetate (3.7 g L⁻¹)</td>
<td>NS</td>
<td>50-90 min, 50 °C</td>
<td>Not applied</td>
<td>170-600</td>
<td>Varied</td>
<td>(Rauf et al., 2017)</td>
</tr>
<tr>
<td>Staphylococcus aureus culture (NS)</td>
<td>Acetate (0.18 g L⁻¹)</td>
<td>NS</td>
<td>50-90 min, 50 °C</td>
<td>Not applied</td>
<td>10-50</td>
<td>Acicular</td>
<td></td>
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<tr>
<td>Bacteria</td>
<td></td>
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</tr>
<tr>
<td>Aloe vera leaves (NS)</td>
<td>Nitrate (NS)</td>
<td>NS</td>
<td>4-5 h, 150 °C</td>
<td>Not applied</td>
<td>7.5</td>
<td>Spheres</td>
<td>(Gunalan et al., 2012)</td>
</tr>
<tr>
<td>Aloe barbadensis mille leaves (50-500 g L⁻¹)</td>
<td>Nitrate (NS)</td>
<td>NS</td>
<td>5 h, 150 °C</td>
<td>Not applied</td>
<td>7-8 h, 80 °C</td>
<td>Spheres</td>
<td>(Sangeetha et al., 2011)</td>
</tr>
<tr>
<td>Canella sinensis leaves (NS)</td>
<td>Nitrate (50 g L⁻¹)</td>
<td>NS</td>
<td>5 h, 60 °C</td>
<td>Not applied</td>
<td>7-8 h, 80 °C</td>
<td>Spheres</td>
<td>(Nava et al., 2017a)</td>
</tr>
<tr>
<td>Citrus aurantifolia peel (20 g L⁻¹)</td>
<td>Nitrate (20 g L⁻¹)</td>
<td>NS</td>
<td>3 h, Tₐ</td>
<td>1 h, 60 °C</td>
<td>11</td>
<td>Polyhedron</td>
<td>(Nava et al., 2017b)</td>
</tr>
<tr>
<td>Citrus paradise peel (20 g L⁻¹)</td>
<td>Nitrate (20 g L⁻¹)</td>
<td>NS</td>
<td>3 h, Tₐ</td>
<td>1 h, 60 °C</td>
<td>19</td>
<td>Polyhedron</td>
<td>(Nava et al., 2017b)</td>
</tr>
<tr>
<td>Citrus sinensis peel (20 g L⁻¹)</td>
<td>Nitrate (20 g L⁻¹)</td>
<td>NS</td>
<td>3 h, Tₐ</td>
<td>1 h, 60 °C</td>
<td>12</td>
<td>Polyhedron</td>
<td>(Nava et al., 2017b)</td>
</tr>
<tr>
<td>Couroupita guianensis leaves (50 g L⁻¹)</td>
<td>Acetate (5 g L⁻¹)</td>
<td>NS</td>
<td>10 min, NS</td>
<td>Overnight, Tₐ</td>
<td>60</td>
<td>Nanoflores</td>
<td>(Sathishkumar et al., 2018)</td>
</tr>
<tr>
<td>Plants</td>
<td></td>
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<tr>
<td>Costus woodsonii leaves (30-60 g L⁻¹)</td>
<td>Nitrate (100 g L⁻¹)</td>
<td>NS</td>
<td>60 °C, NS</td>
<td>Not applied</td>
<td>20-25</td>
<td>Varied</td>
<td>(Khan et al., 2019)</td>
</tr>
<tr>
<td>Eclipta alba leaves (100 g L⁻¹)</td>
<td>Acetate (0.2-1.1 g L⁻¹)</td>
<td>4-8</td>
<td>5-75 min, 20-100 °C</td>
<td>Not applied</td>
<td>3-9</td>
<td>Spheres</td>
<td>(Singh et al., 2018)</td>
</tr>
<tr>
<td>Hibiscus sabdariffa leaves (20 g L⁻¹)</td>
<td>Acetate (14.3 g L⁻¹)</td>
<td>NS</td>
<td>30 min, Tₐ</td>
<td>4 h, 30-100 °C</td>
<td>190-400</td>
<td>Dumbbell</td>
<td>(Bala et al., 2015)</td>
</tr>
<tr>
<td>Lycopersicon sculentus peel (20 g L⁻¹)</td>
<td>Nitrate (20 g L⁻¹)</td>
<td>NS</td>
<td>3 h, Tₐ</td>
<td>1 h, 60 °C</td>
<td>9</td>
<td>Polyhedron</td>
<td>(Nava et al., 2017b)</td>
</tr>
<tr>
<td>Lycopersicon esculentum fruit (NS)</td>
<td>Nitrate (NS)</td>
<td>NS</td>
<td>5 min, 80 °C or microwave</td>
<td>4-5 h, NS</td>
<td>40-100</td>
<td>Spheres</td>
<td>(Sutrathar and Saha, 2017)</td>
</tr>
<tr>
<td>Menta pulegium L. leaves (50 g L⁻¹)</td>
<td>Nitrate (100 g L⁻¹)</td>
<td>NS</td>
<td>NS</td>
<td>Not applied</td>
<td>38-49</td>
<td>Spheres</td>
<td>(Shahriyari Rad et al., 2019)</td>
</tr>
<tr>
<td>Moringa oleifera leaves (100 g L⁻¹)</td>
<td>Nitrate (6 -206 g L⁻¹)</td>
<td>5</td>
<td>18 h, Tₐ</td>
<td>1 h, 500 °C</td>
<td>12-30</td>
<td>Spheres and rods</td>
<td>(Matinise et al., 2017)</td>
</tr>
<tr>
<td>Oak fruit hull (jaf) (200 g L⁻¹)</td>
<td>Acetate (33.4 g L⁻¹)</td>
<td>NS</td>
<td>4 h, 60-80 °C</td>
<td>6 h, 80 °C</td>
<td>34 nm</td>
<td>Spheres</td>
<td>(Sorbiun et al., 2018)</td>
</tr>
<tr>
<td>Stevia leaves (72.4 g L⁻¹)</td>
<td>Acetate (18.3 g L⁻¹)</td>
<td>NS</td>
<td>NS, 80 °C</td>
<td>4 h, 500 °C</td>
<td>50</td>
<td>Rectangular</td>
<td>(Khatami et al., 2018a)</td>
</tr>
<tr>
<td>Peganum harmala seed (60 g L⁻¹)</td>
<td>Nitrate (NS)</td>
<td>NS</td>
<td>1 h, NS</td>
<td>NS, 50 °C</td>
<td>40</td>
<td>Irregular</td>
<td>(Fazlizadeh et al., 2017)</td>
</tr>
<tr>
<td>Punica granatum leaves (NS)</td>
<td>Nitrate (18.9 g L⁻¹)</td>
<td>NS</td>
<td>3-4 h, 60 °C</td>
<td>3-4 h, 400 °C</td>
<td>10-30</td>
<td>Spheres</td>
<td>(Singh et al., 2019)</td>
</tr>
</tbody>
</table>

Concentrations were approximated to be reported with the same unit; NS: Not specified; Tₐ: Ambient temperature
For instance, Bala et al. (2015) observed that changing the temperature of the thermal treatment resulted in different morphologies and size of the ZnONPs. The thermal treatment at 30 °C showed irregular morphology and low crystallinity of the particles. In contrast, the nanoparticles obtained with thermal treatment at 60 °C and 100 °C presented high crystallinity and agglomerates of nanoparticles in morphologies such as cauliflower and dumbbell shape, respectively. These variations are probably related to the fact that higher temperatures increase the nucleation rate of crystal formation. In agreement, Parra and Haque (2014) chemically synthesized ZnONPs. They observed that the higher the temperature, the faster will be the crystal growth and nucleation rate, which results in the agglomeration of nanoparticles and larger particle sizes.

Another feature related to the agglomeration is that the interval of time of the heat treatment may affect the formation of clusters. Dhadapani et al. (2014) observed that increasing the time of the thermal treatment conducted at 50 °C from 30 to 90 min, increased agglomerates and particle growth. These findings corroborate with the results observed in different chemical synthesis process, where the increase of the time of nucleation led to the formation of larger particles of ZnONPs (Manzoor et al., 2015; Shaziman et al., 2015).

It is also known that the pH conditions during synthesis can significantly modify the particle size and morphology of metals and metal oxides, which will consequently modify the properties of the nanomaterials (Chitra and Annadurai, 2014; Das et al., 2015; Roselina et al., 2013; Velgosová et al., 2016). In relation to the zinc oxide, ZnONPs chemically synthesized by sol-gel method showed different morphology when varying the pH of the solution. For example, Alias et al. (2010) observed that larger agglomerates were formed when ZnO was synthesized using pH 6.0 or 7.0, compared to a decrease in particle size and agglomerations when the pH was increased to 11.0. To the best of our knowledge, only the works reported by Singh et al. (2018) and Nagarajan et al. (2013) have investigated the variation of the pH on
the green synthesis of ZnONPs. The findings of these authors corroborate with the work of Alias et al. (2010) since the increase of the pH from 4.0 to 8.0 led to a decrease on particle size and agglomeration. However, Singh et al. (2018) states that a neutral pH would be the best condition for the biosynthesis of ZnONPs proposed in their study. This conclusion was based on the fact that the formation of Zn(OH)$_2$ might take place in alkaline pH solutions, which can alter the production of ZnONPs.

Although the pH condition poses a notable influence on the synthesis of inorganic nanoparticles, much of the available literature on the green synthesis of ZnONPs does not consider or report the pH of the solutions of the biological extracts used for obtaining this nanomaterial, as reported in Table 1 (Bala et al., 2015; Gunalan et al., 2012; Nava et al., 2017b, 2017a; Raliya and Tarafdar, 2013; Sangeetha et al., 2011; Sathishkumar et al., 2018; Tripathi et al., 2014). Thus, considering that each biological substrate has different compositions and pH values, the evaluation of the pH solutions would be of great interest to better understand the differences on physical-chemical properties of the nanoparticles obtained through green synthesis.

Chinassamy et al. (2018) reported a further study on the green synthesis of ZnONPs by evaluating the effect of biological extract and zinc salt concentration, operating time and temperature, on the particle size and yield of the produced nanoparticles. From this work, the authors stated that using the minimum concentration of zinc precursor (~ 65 g L$^{-1}$) and maximum temperature (200 °C) and time (2 h) of reaction resulted in the highest yield. Furthermore, they concluded that the concentration of zinc nitrate is the factor that influences mostly the particle size among all the evaluated parameters.

Singh et al. (2018) also reported the variation of the concentration of both plant extract and zinc precursor, incubation time and temperature of reaction in a study of the biosynthesis of ZnONPs using Eclipta alba leaves extract. The authors observed that increasing zinc acetate
concentration (1.0 to 5.0 mM), particle formation became more uniform and smaller. Moreover, Singh et al. (2018) state that these findings are in agreement with the ZnO synthesis performed using *Citrus aurantifolia* in which the increase of zinc acetate concentration enhanced the homogeneity of the particles and reduced their size (Ain Samat and Md Nor, 2013).

Furthermore, it was observed that there was a direct correlation between the concentration of *Eclipta alba* extract and the intensity of the UV absorbance peaks observed, indicating a superior formation of ZnONPs (Singh et al., 2018). These features may be related to a higher concentration of antioxidants which are available during the synthesis when the plant extract concentration is increased. As it is believed that these compounds play an important role on the mechanism of formation of metal and metal oxides nanoparticles obtained by green synthesis (Król et al., 2017; Matinise et al., 2017; Shah et al., 2015; Singh et al., 2018).

Moreover, Singh et al. (2018) investigated the influence of the variation of the temperature from 20 to 100 °C on the yield and size of ZnONPs. In their study, it was concluded that the higher the temperature, the greater the yield of ZnONPs. However, the increase of the temperature of reaction resulted in particles with a larger size, which corroborates with a similar study of the properties of ZnO synthesized via sol-gel synthesis (Pelicano et al., 2016). Related to the time of reaction, it seems that rapid synthesis results in smaller particles, as the short reaction time will reduce the grain growth (Singh et al., 2018).

Although it is well understood that changing the parameters of the green synthesis will affect the physical-chemical properties of the ZnONPs, the mechanism route of the formation of these nanoparticles remains unclear. Several studies propose theoretical mechanistic routes for this biosynthesis (Matinise et al., 2017; Raliya and Tarafdar, 2013; Selvarajan and Mohanasrinivasan, 2013; Tripathi et al., 2014). Nonetheless, the composition complexity of
the biological substrates poses a challenge for analytical evaluation and definition of the chemical reactions that take place during the green synthesis.

2.2 Mechanism of formation of zinc oxide nanoparticles via green synthesis

Although many studies indicate the effectiveness of the green synthesis for the production of metal and metal oxides nanoparticles, this process has only been demonstrated at a laboratory scale (Agarwal et al., 2017; Kharissova et al., 2013; Mirzaei and Darroudi, 2016). Hence, the determination of the mechanism route of the formation of green synthesis is of great interest for establishing large scale process. Thus, in this review, we summarize the recent advances found in the literature related to the mechanism of formation of ZnONPs using different types of biological substrates.

2.2.1 Bacteria

The biosynthesis of metal and metal oxides nanoparticles using microbial culture or biomass may occur in an extra or intracellular environment (Kumar et al., 2007; Ovais et al., 2018; Pantidos and Horsfall, 2014; Rauf et al., 2017; Tripathi et al., 2014). In the case of the extracellular synthesis, studies suggest that the enzymes and proteins produced and released by the microorganisms can reduce the metal ions and stabilize the particles. Tripathi et al. (2014) reported that ZnONPs can be stabilized by enzymes secreted by bacteria cells (Bacillus licheniformis). In their study, zinc acetate and sodium bicarbonate react to form Zn(OH)$_2$, which is thermally degraded to form the ZnO nuclei. The enzymes presented in the bacteria will then stabilize the ZnONPs to avoid agglomeration and particle growth guaranteeing the nanoscale size of the metal oxide.

In addition, the work developed by Selvajaran et al. (2013) identifies that the enzymes produced by the microorganisms are responsible for the ZnONPs formation. However, authors
state that the solution pH and the electrokinetic potential of the bacteria may play a role in the synthesis route by reducing the metal ions and, consequently, triggering the biosynthesis of the nanoparticles rather than forming Zn(OH)\textsubscript{2}. The same concept was stated in a similar study using \textit{Staphylococcus aureus} to obtain ZnONPs \textit{via} extracellular biosynthesis (Rauf et al., 2017).

Further work reports the successful utilization of activated ammonia from ureolytic bacteria (\textit{Serratia ureilytica}) for ZnONPs production. The synthesis route proposed in this study for the formation of nanoparticles is based on the reaction of zinc ions with the microorganism culture media, rich in ammonia producing Zn(OH)\textsubscript{2} and [Zn(NH\textsubscript{3})\textsubscript{4}]\textsuperscript{2+}. These substances are then submitted to thermal decomposition at 50 °C to obtain the crystalline ZnONPs powder (Dhadapani et al., 2014).

Regarding the intracellular synthesis, the mechanism of formation definition can be more challenging due to the complexity of the cell compositions and processes. However, various studies believe that the cells internalize the metallic ions which will be reduced by the proteins and enzymes within the cell to form the nanoparticles (Hulkoti and Taranath, 2014; Klaus et al., 1999; Li et al., 2011). Klaus \textit{et al.} (1999), for example, used \textit{Pseudomonas stutzeri} to obtain silver nanoparticles with different shapes and identified their formation within the cell using transmission electronic microscopy (TEM). A recent study observed the formation of gold nanoparticles with sizes varying from 5-30 nm inside \textit{Lactobacillus kimchicus} using the same analysis (Markus et al., 2016). Rajeshkumar \textit{et al.} (2013), on the other hand, compared both intra and extracellular synthesis of silver nanoparticles and observed that it is more difficult to regulate particle morphology, dispersion and size when synthesizing the nanoparticles within the cells.

Similarly, it is also well known that microorganisms can internalize zinc (II) ions (Argawal et al., 2018; Grass et al., 2002; McDevitt et al., 2011; Sirelkhatim et al., 2015).
Therefore, the intracellular biosynthesis of ZnONPs could be a plausible mechanism route to obtain this nanomaterial. However, the literature indicates that the extracellular formation is the most common route to produce ZnONPs using bacteria cultures (Busi et al., 2016; Dhadapani et al., 2014; Selvarajan and Mohanasrinivasan, 2013; Tripathi et al., 2014). Figure 3 summarizes both extracellular and intracellular mechanism of the biosynthesis of nanoparticles using bacteria, based on the current literature. In contrast with the extracellular biosynthesis, the intracellular route requires an additional process of cell lysis to release the nanoparticles from inside the microorganism (Molnár et al., 2018). Hence, this process becomes more time consuming and expensive than the extracellular synthesis in which the metal ions are directly reduced or chelated by the proteins and enzymes outside the cells.

Figure 3: Green synthesis of nanoparticles using bacteria cultures

2.2.2 Fungus

The production of metal and metal oxides nanoparticles using fungal biomass or culture has a similar mechanistic route as the one described for the green synthesis using bacteria in Figure 3. Raliya et al. (2013) successfully synthesized ZnONPs using Aspergillus fumigatus cell culture. They suggested that the proteins and enzymes secreted by this microorganism are
responsible for the formation and encapsulation of the nanomaterial. In addition, Kalpana et al. (2018) also reported the extracellular biosynthesis of ZnONPs using Aspergillus niger cell-free filtrate.

In comparison to the bacteria synthesis, it is believed that the fungus may have superior potential for the green synthesis of nanoparticles since it can release higher concentrations of metabolites to the culture media than bacteria cells. Furthermore, fungus cells seem to be more resistant to process conditions and variations such as pressure, flow rate and stirring which enhance their potential use for large scale synthesis (Li et al., 2012; Narayanan and Sakthivel, 2010; Zielonka and Klimkochab, 2017).

2.2.3 Plants

Plants are the most common biological substrate used for the green synthesis of nanoparticles with metallic ions (Iravani, 2011; Kharissova et al., 2013). This might be related to the fact that vegetal substrates are believed to be more cost-effective, easy to process and less toxic than microorganisms. Also, there is no exposure to health risks or concerns about safety issues related to hazardous microorganisms during the process when using plant based substrates. In addition, plant extracts can be obtained in a straight forward manner by exposing the plant to a solvent, which is usually distilled water or ethanol (Ahmed et al., 2016). Different parts of the plant have been applied to this purpose such as leaves, roots, seeds and fruits (Fazlzadeh et al., 2017; Matinise et al., 2017; Nava et al., 2017a; Sangeetha et al., 2011).

It is known that the plants have high concentrations of active compounds like methylxanthines, phenolic acids, flavonoids and saponins (Altemimi et al., 2017; Guldiken et al., 2018; Maisuthisakul et al., 2008; Xu et al., 2017). These compounds are more known as antioxidants as they can neutralize reactive oxygen species (ROS) and free radicals and chelate metals (Flora, 2009). Hence, it is concluded that the antioxidants present in the plants are
responsible for the green synthesis of metal or metal oxides nanoparticles due to their capability to bioreduce or chelate metal ions and to act as stabilizers of the produced nanoparticles (Ahmed et al., 2017; Anjum et al., 2015).

Despite the knowledge of the phytochemical properties of the antioxidants, plant extracts are constituted of an enormous variety of these active compounds in different concentrations (Altemimi et al., 2017; Oz and Kafkas, 2017; Saxena et al., 2013; Sharma et al., 2017). This feature poses a problem to analytically determine the exact amount of all molecules that are extracted from the plant. Consequently, the definition of a precise mechanism route of the biosynthesis of metal and metal oxide nanoparticles using vegetal substrates is still a challenge to be surpassed.

Regarding the green synthesis of ZnONPs, published research suggest in theory that the compounds present in the plant extract react with a zinc salt to reduce or to form complexes with the metal (Fazlzadeh et al., 2017; Matinise et al., 2017; Nava et al., 2017b; Sangeetha et al., 2011; Singh et al., 2018). Nava et al. (2017b) proposed a mechanism route based on the chemical characteristics of the flavonoids, limonoids and carotenoids that constitute the fruit peels used for obtaining the ZnONPs. In this work, these antioxidants are believed to chelate the zinc ions and form metal coordinated complexes that are further thermally treated to degrade the complex and form zinc oxide with an average size of 9.7 nm.

Matinise et al. (2017) established a similar mechanism where the antioxidants of Moringa oleifera leaves also chelate the zinc (II) ions which formed zinc oxide after a calcination process. In this work, the plant extract and ZnONPs obtained with different temperatures treatment (100 °C and 500 °C) were analysed using Fourier transform infrared spectroscopy (FTIR). The vegetal extract exhibited absorption bands typical of bioactive compounds and the ZnO synthesized at 100 °C showed hydroxyl (–OH) stretching bands that might be an indication of the formation of zinc complexes with antioxidants during the
synthesis. This result correlates with the findings of other research groups, where FTIR absorption bands characteristic of bioactive compounds were identified in the green synthesized ZnONPs (Azizi et al., 2014; Bala et al., 2015; Fazlzadeh et al., 2017; Nava et al., 2017a; Sangeetha et al., 2011).

Conversely, when *Eclipta alba* leaves are utilized for the production of ZnONPs, studies have implied that zinc (II) ions are reduced by the plant active compounds to metallic zinc rather than forming coordinated complex with them. After the bioreduction of zinc, the metallic zinc reacts with dissolved oxygen present in the solution to form the ZnO nuclei. It is also proposed that the plant compounds act as stabilizers preventing agglomeration of particles and crystal growth (Singh et al., 2018). Likewise, Sutradhar and Saha (2017b) proposed the bioreduction of zinc (II) ions by ascorbic acid when using *Lycopersicon esculentum* extract to obtain ZnONPs. Gupta *et al.* (2018) biosynthesized ZnONPs using plant extract and suggested that the metabolites that compose the substrate are responsible for both the reduction of the metallic ions and particle stabilization.

Figure 4 illustrates the possible mechanism routes described in the literature for the green synthesis of ZnONPs using plant extracts, and some active compounds are cited as examples of the substances frequently found in plants. Also, two different mechanisms of ZnONPs formation are shown considering the ability of the active compounds in chelating and reducing the zinc (II) ions, as previously discussed. In addition, it is interesting to observe that, for metal oxide nanoparticles, the mechanism route of metal complexation requires a thermal treatment to obtain the nanoparticles. In contrast, the metal bioreduction produces the colloidal nanoparticle with the plant extract without further treatment.
2.2.4 Algae

Although algae are simple organisms, the phytochemical composition of algae can be related to the composition of plant extracts. Active compounds containing functional groups such as hydroxyl and carboxyl groups can be found in different species of algae and their antioxidant activity has been reported (Azizi et al., 2014; Kelman et al., 2012; Plaza et al., 2010; Zhang et al., 2010). In addition, different studies identified the presence of such active compounds by FTIR analysis in algae extracts when using them as substrates to green synthesize ZnONPs (Azizi et al., 2014; Ishwarya et al., 2018; Nagarajan and Kuppusamy, 2013).

Figure 4: Green synthesis of ZnONPS using plants extracts
Therefore, the mechanism of formation of ZnONPs when using algae substrates for the biological synthesis can be related to the mechanism of plants already described and summarized in Figure 4, where active compounds such as polyphenols and flavonoids act as reducing and stabilizer agents and/or chelating substances.

2.3 Properties of ZnONPs green synthesized

The main advantages of using a green route to obtain nanoparticles are it is an environmentally friendly, low cost and simple technique. Nonetheless, the green synthesis of nanoparticles can also enhance the properties of these nanomaterials due to the small size and shape obtained, and the specific properties of the biological substrates used (Gour and Jain, 2019; Kharissova et al., 2013; Khatami et al., 2018b).

In the case of ZnONPs, the green synthesis route has been shown to improve properties like antimicrobial activity (Sangeetha et al., 2011), photocatalytic efficacy (Raja et al., 2018) and biocompatibility (Mirzaei and Darroudi, 2016). Therefore, the green synthesized ZnONPs have great potential to substitute conventional ZnONPs and to be applied on the development of nanocomposites. For example, biosynthesized ZnONPs can be utilised in the production of nanocomposites for anticancer and antimicrobial coatings in the biomedical field and to improve the degradation of dyes, to name but two applications (Khatami et al., 2018b; Mishra et al., 2017; Namvar et al., 2016; Roshitha et al., 2019).

Nanoparticles of similar metal oxides to ZnO were also obtained via biosynthesis. For instance, SnO$_2$ and CdO$_2$ nanoparticles were successful obtained using plant extract showing interesting properties, such as antimicrobial effect and potential to be applied in solar cells (Dobrucka et al., 2018; Thovhogi et al., 2016). Moreover, recent studies observed the potential of producing binary composites based on ZnONPs using biological substrates. Rahmayeni et
al. (2019) synthesized ZnO-CoFe$_2$O$_4$ using *Nephelium lappaceum* L. peel extract to enhance the photocatalytic performance of ZnONPs. Honeycomb-like Ag-ZnO nanocomposite was also obtained for photocatalytic purposes with *Azadirachta indica* gum (Basavalingiah et al., 2019). Likewise, Fuku et al. (2016) obtained ternary nanocomposites of CuO, Cu and ZnO using pomegranate peels to produce a nanoplatelet structured electrode. Thus, the green synthesis has shown to be a promising alternative to easily obtain more complexes nanostructures.

3. Conclusions

Numerous studies report the possibility of obtaining ZnONPs through a green synthesis process using a variety of plants, fungus, bacteria and algae. Moreover, the studies cited here indicate that these substrates act as reducing and stabilizers or as chelating substances despite its source. It is interesting to notice that besides the difference between the compositions found in biological extracts, parameters such as conditions of temperature, time of reaction, pH and concentrations, significantly alter the final properties of the synthesized nanoparticles. Among these parameters and according to the literature cited, the concentrations of both biological extract and zinc source and also the pH of the solution play a major role on the final properties of ZnONPs obtained using green route.

Although the complexity of biological substrates still poses a challenge to evaluate the green synthesis of nanoparticles, further investigations on the mechanism of formation of the biological synthesis of ZnONPs are necessary to achieve a better understanding of the chemical processes and reactions that occur during the synthesis. It seems that with the designation of the mentioned mechanism, it will be possible to control and optimize the green synthesis process, which is essential for the large scale production of ZnONPs. Hence, the rapidly advancing understanding of green synthesis described herein, indicate
the enormous potential of ZnONPs for industrial production using biological extracts in the near future.

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