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Green Synthesis of Zinc Oxide Nanostructures

Tuğba Isık, Mohamed Elhousseini Hilal and Nesrin Horzum

Abstract

ZnO-based nanomaterials have been proven to be of great use for several leading applications since the beginning of nanoscience due to the abundance of zinc element and the relatively easy conversion of its oxide to nanostructures. Nowadays, ZnO as nanoparticles, nanowires, nanofibers as well as plenty of other sophisticated nanostructures takes place among the pioneer nanomaterials employed in the photovoltaic systems, fuel cells, and biomedical fields. Nevertheless, optimizing energy consumption and being eco-friendly are the challenging requirements that are still to be overcome for their synthesis. Green chemistry has been strongly presented recently in the scientific arena as an adequate potential alternative; worldwide investigations have been held on subjects involving bacteria, fungus, or algae-based synthesis as efficient options, and some of the intriguing scientific findings on this subject are reported hereafter.

Keywords: biosynthesis, hydrothermal, microwave, nontoxic, sonochemical

1. Introduction

There are many conventional zinc oxide (ZnO) nanostructure synthesis routes employing the chemical and physical methods, which require particular set-up, high cost, high temperature-pressure conditions, and nonecological chemicals [1]. However, high-energy consumption of these routes and released toxic chemicals after the process can be hazardous to the environment and human health. In recent years, the green synthesis approach has been gaining attention, which eliminates the use of toxic chemicals and applies environmentally friendly routes. These strategies handle the use of plant-extracts, microorganisms, biomolecules, and ionic liquids by applying hydrothermal, microwave-assisted, sonochemical, and low-temperature processes (**Figure 1**).

The aim of these developments is to allow the use of toxic chemicals and reduce energy consumption by using simple, rapid, and safe routes. Green synthesis strategies for the ZnO nanostructures could be summarized as biosynthesis (natural extract-based, microorganism-based, and biomolecule-based) and nontoxic chemical synthesis (water-based, calcination, solvent-free, and ionic liquid).

2. Biosynthesis of ZnO nanostructures

2.1 Natural extract-based ZnO nanostructures

Natural extracts (mainly phytochemicals) obtained from plants, leaves, fruit peels, flowers, and seeds have been utilized for the green synthesis of metal oxide

nanoparticles for years. After the plants are collected from different sources, they are washed with water and basic extraction procedures are applied to obtain plant extracts in which leaves are ground and immersed in water by stirring at room temperature for a while. Then, the solutions are filtered and the eluted extract solution is separated for further use in ZnO synthesis (**Figure 2**). The eluent solution could be used directly for ZnO synthesis or could be dried for the concentration of solid extracts. Afterward, zinc precursors and plant extracts are reacted under various pH and temperature conditions [2]. If the extract is used as an aqueous solution, the zinc precursors are added into the solution. Otherwise, the zinc precursor and powder form of leaf extract are mixed in distilled water. The key mechanism is the oxidation and reduction of metal ion ‘zinc’ by phytochemicals, which are found in natural extracts. The leaf extracts behave as reducing and capping agents. Under favor of plant extracts, the synthesis procedure can be accomplished without using any chemical stabilizers. Finally, the obtained powders are washed with methanol or ethanol and annealed at high temperatures to attain purity [3].

The green synthesized ZnO nanoparticles have been used in various fields such as biomedical application due to their significant antibacterial activities, photocatalysis, and metal ion adsorption purposes [4]. Moreover, nanoparticles synthesized by the green route exhibit better antibacterial performances due to the functional

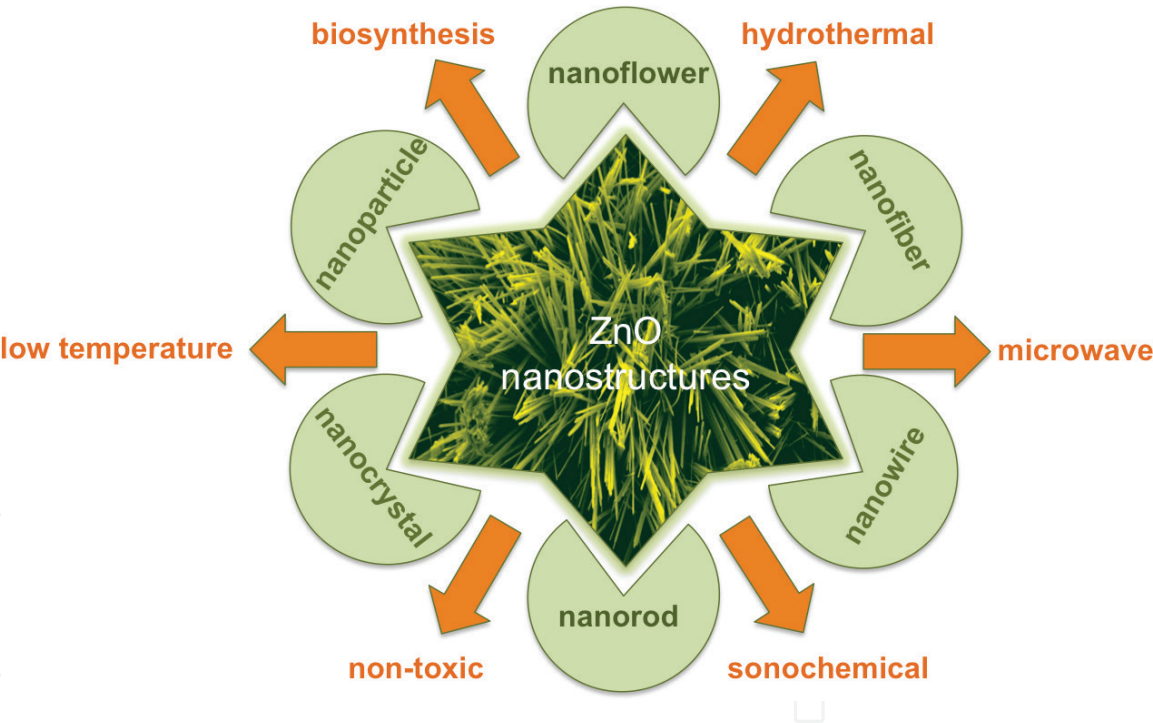


Figure 1.
Green synthesis strategies of ZnO nanostructures with various morphologies.

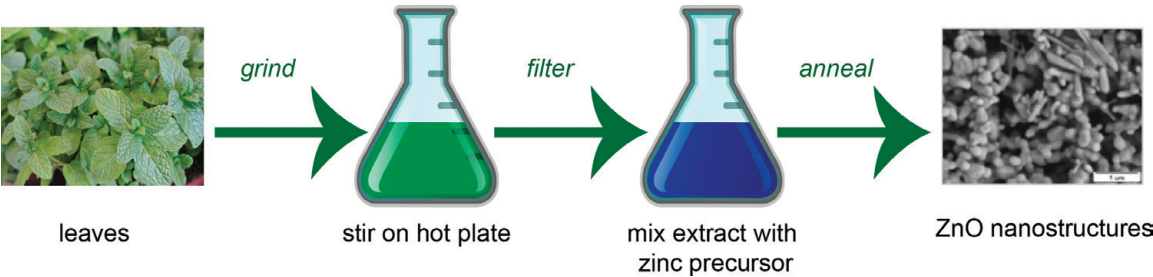


Figure 2.
Synthesis route of ZnO nanostructures from leaf extracts.

groups on their surfaces that come from phytochemicals. Here, we will describe the main applications of natural extract-based green synthesized ZnO nanoparticles.

2.2 Biomedical applications

The advantage of using natural extracts for the synthesis of ZnO nanoparticles is that coating of nanoparticles with various pharmacologically active biomolecules on the metal oxide surface allows the conjugation of nanoparticles with receptors of the bacterial membrane. These molecules might be flavones, aldehydes, amides, polysaccharides, etc. and the green synthesized nanoparticles exhibit better biomedical activity than the chemically synthesized ones [1]. Inorganic metal oxides have widely emerged as antibacterial, antioxidant, antifungal, and anticancer agents in the last decades. Moreover, because of their specific targeting and nominal toxicity, the metal oxide nanoparticles could be used in personalized medicine applications. In the area of nanoscaled metal oxides, ZnO has shown promising activity in the biomedical field due to its unique electronic, optical, and medicinal properties. The ZnO nanoparticles show antibacterial activity against a broad spectrum of pathogenic bacteria, and these nanoparticles adopt various mechanisms such as reactive oxygen species (ROS) generation, cell membrane integrity disruption, biofilm formation, or enzyme inhibition [5]. Under UV irradiation, ROS such as superoxide ions, hydroxyl ions, singlet oxygen species, and peroxide molecules are formed. The formed peroxide ions could easily penetrate through the cell membrane and result in cell death. **Figure 3** shows the possible ROS generation mechanism and its effect on the bacterial cell wall.

Cell membrane integrity disruption is another significant mechanism for the antibacterial effect of ZnO nanoparticles. Penetration of ZnO nanoparticles results in cell death by the loss of phospholipid bilayer integrity and leakage of intracellular components of the cell. While the Gram-positive bacteria have a thick layer of peptidoglycan, teichoic acid, and lipoteichoic acid in their cell membrane, Gram-negative bacteria have a triple layer of peptidoglycan. The different structure of cell membranes of these two types of bacteria results in a different mechanism of nanoparticle penetration through the cell membranes. In this part, we focused on the biomedical activity of ZnO nanoparticles, and in **Table 1**, the used plant extracts, zinc precursors, biomedical applications and related biomolecules are summarized.

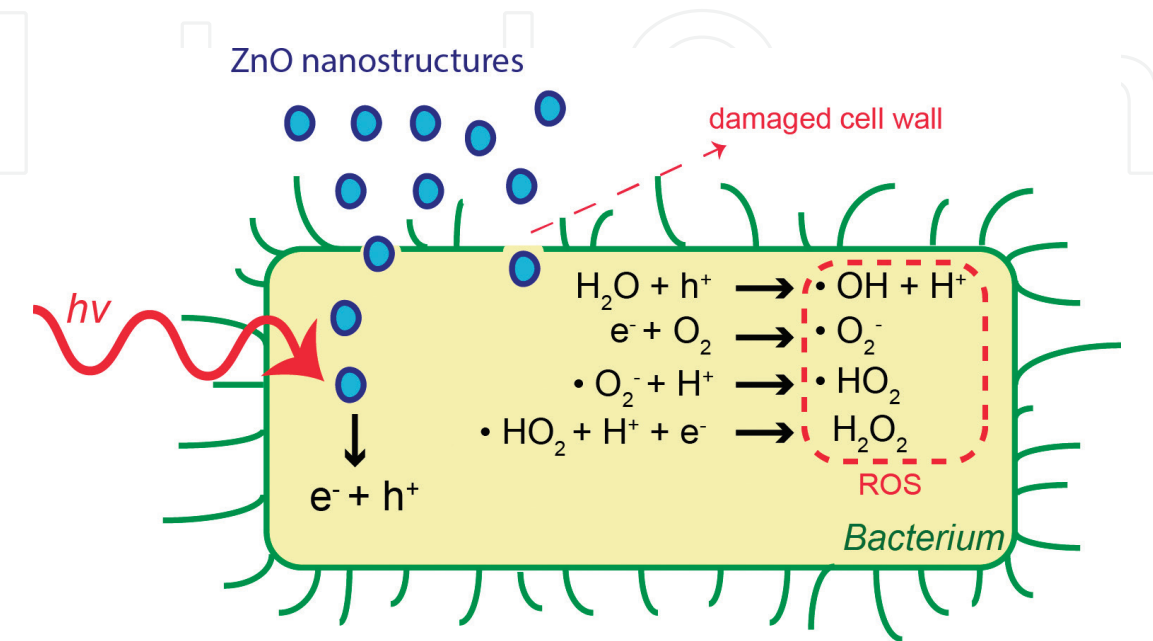


Figure 3.
ROS mechanism of ZnO nanoparticles [6].

Plant type	Zinc precursor type	Size of ZnO (nm)	Treated biomolecule	Biomedical field	Ref.
<i>Momordica charantia</i>	Nitrate	21	<i>R. microplus</i> , <i>P. humanus capitis</i> , <i>An. stephensi</i> , and <i>Cx. Quinquefasciatus</i>	Antiparasitic	[30]
<i>Rosa canina</i>	Nitrate	11	<i>S. typhimurium</i> , <i>S. aureus</i> , <i>L. monocytogenes</i> , and <i>E. coli</i>	Antibacterial	[31]
<i>Ulva fasciata</i>	Chloride	16	<i>B. cereus</i> , <i>S. aureus</i> , <i>S. thermophilus</i> , <i>E. coli</i> , and <i>P. aeruginosa</i>	Antibacterial	[32]
<i>Eclipta alba</i>	Acetate	6	<i>E. coli</i>	Antibacterial	[33]
<i>Terminalia arjuna</i>	Acetate	21	<i>E. coli</i> and <i>S. aureus</i>	Antibacterial	[34]
<i>Stevia</i>	Acetate	10–90	<i>E. coli</i> and <i>S. aureus</i>	Antibacterial Antiparasitic	[35]
<i>Glycosmis pentaphylla</i>	Acetate	32–36	<i>B. cereus</i> , <i>S. aureus</i> , <i>S. paratyphi</i> , <i>S. dysenteriae</i> , <i>C. albicans</i> , and <i>A. niger</i>	Antibacterial	[36]
<i>L. leschenaultiana</i>	Acetate	—	<i>L. sericata</i>	Antiparasitic	[37]
<i>Adhatoda vasica</i>	Acetate	10–12	<i>S. epidermidis</i> , <i>E. coli</i> , <i>P. aeruginosa</i> , <i>T. rubrum</i> , and <i>M. audouinii</i>	Antimicrobial	[38]
<i>Vitex negundo</i>	Nitrate	38	Human Serum Albumin	Protein binding	[39]
<i>Anchusa italica</i>	Acetate	8–14	<i>B. megaterium</i> , <i>S. aureus</i> , <i>E. coli</i> , <i>S. typhimurium</i>	Antimicrobial	[40]
<i>Jacaranda mimosifolia</i>	Gluconate	2–4	<i>E. faecium</i> and <i>E. coli</i>	Antibacterial	[41]
<i>Heritiera fomes</i> and <i>Sonneratia apetala</i>	Chloride	40–50	<i>S. aureus</i> , <i>S. flexneri</i> , <i>V. cholera</i> , <i>S. epidermidis</i> , <i>B. subtilis</i> , and <i>E. coli</i>	Antibacterial Anti-inflammatory	[42]
<i>Nyctanthes arbor-tristis</i>	Acetate	12–32	<i>A. alternata</i> , <i>A. niger</i> , <i>B. cinerea</i> , <i>F. oxysporum</i> , and <i>P. expansum</i>	Antifungal	[43]
<i>Nocardiopsis</i> sp	Nitrate	500	<i>E. coli</i> and <i>P. mirabilis</i>	Antibacterial	[44]
<i>Ceropegia candelabrum</i>	Nitrate	12–35	<i>S. aureus</i> , <i>B. subtilis</i> , <i>E. coli</i> , and <i>S. typhi</i>	Antibacterial	[45]
<i>Pongamia pinnata</i>	Acetate	21	<i>C. maculatus</i>	Anti-pesticide	[46]
<i>Capsicum annuum</i>	Nitrate	30–40	<i>E. coli</i> and <i>S. aureus</i>	Antibacterial	[47]
<i>Euphorbia petiolata</i>	Nitrate	—	<i>E. coli</i>	Antibacterial	[48]
<i>Tradescantia pallida</i>	Acetate	25	HeLa cervical cancer cell	Anticancer	[49]
<i>Punica granatum</i>	Nitrate	5	<i>P. vulgaris</i> , <i>E. coli</i> , and <i>S. aureus</i>	Antibacterial	[50]
<i>Swertia chirayita</i>	Nitrate	5	<i>S. enterica</i> , <i>E. coli</i> , and <i>S. aureus</i>	Antibacterial	[51]

Plant type	Zinc precursor type	Size of ZnO (nm)	Treated biomolecule	Biomedical field	Ref.
<i>Vitex negundo</i>	Nitrate	38	<i>E. coli</i> and <i>S. aureus</i>	Antibacterial	[52]
<i>Aegle marmelos</i>	Nitrate	20	<i>P. aeruginosa</i> , <i>E. coli</i> , <i>S. aureus</i> , <i>A. niger</i> , and <i>F. solani</i>	Antimicrobial	[53]
<i>Azadirachta indica</i>	Acetate	9–25	<i>S. pyogenes</i> , <i>E. coli</i> , and <i>S. aureus</i>	Antibacterial	[54]
<i>Vaccinium arctostaphylos</i>	Nitrate	15	Lipid, insulin, and fasting blood sugar	Antidiabetic	[55]
Coffee powder	Acetate	25–30	Proteinase K	Enzymatic	[56]
<i>Ziziphus nummularia</i>	Nitrate	17	HeLa cancer cell and <i>Candida</i> spp.	Anticancer Antifungal	[57]
<i>Chelidonium majus</i>	Nitrate	10	<i>P. aeruginosa</i> , <i>E. coli</i> , <i>S. aureus</i> , <i>C. albicans</i> , <i>A. niger</i> , and <i>T. rubrum</i>	Antibacterial	[58]
<i>Prunus yedoensis</i> <i>Matsumura</i>	Nitrate	10–40	<i>B. linens</i> and <i>S. epidermidis</i>	Antibacterial	[59]
<i>Sechium edule</i>	Acetate	36	<i>B. subtilis</i> and <i>K. pneumoniae</i>	Antibacterial	[60]
<i>Catharanthus roseus</i>	Acetate	50–90	<i>S. aureus</i> , <i>S. pyogenes</i> , <i>B. cereus</i> , <i>P. aeruginosa</i> , <i>P. mirabilis</i> , and <i>E. coli</i>	Antibacterial	[61]
<i>Albizia saman</i>	Nitrate	15–80	<i>D. indica</i>	Genotoxicity	[62]
<i>Parthenium hysterophorus</i>	Nitrate	16–45	<i>B. subtilis</i> , <i>S. aureus</i> , <i>K. pneumonia</i> , <i>E. coli</i> , and <i>Enterobacteraerogens</i>	Antibacterial	[63]
<i>Sarcopoterium spinosum</i>	Acetate	26–115	<i>E. coli</i> , <i>P. aeruginosa</i> , <i>S. aureus</i> , <i>S. pneumoniae</i> , <i>B. subtilis</i> , <i>E. faecalis</i> , <i>C. glabrata</i> , and <i>C. albicans</i>	Antibacterial	[64]
<i>Ruta graveolens</i>	Nitrate	28	<i>K. aerogenes</i> , <i>P. aeruginosa</i> , <i>E. coli</i> , and <i>S. aureus</i>	Antibacterial	[65]
<i>Pichia kudriavzevii</i>	Acetate	10–61	<i>B. subtilis</i> , <i>S. epidermidis</i> , <i>S. aureus</i> , <i>E. coli</i> , and <i>S. marcescens</i>	Antibacterial	[66]
<i>Azadirachta indica</i>	Sulfate	84	<i>Xanthomonas oryzae</i> pv. <i>oryzae</i>	Antibacterial	[67]
<i>Coptidis Rhizoma</i>	Nitrate	3–25	<i>B. megaterium</i> , <i>B. cereus</i> , <i>E. coli</i> , and <i>B. pumilus</i>	Antibacterial	[68]
<i>Polygala tenuifolia</i>	Nitrate	33–73	RAW 264.7 cells	Antibacterial	[69]
<i>Sargassum muticum</i>	Acetate	10–15	WEHI-3 cell	Anticancer	[70]
<i>Scadoxus multiflorus</i>	Acetate	31	<i>Aedes aegypti</i> , <i>A. niger</i> , and <i>A. flavus</i>	Antifungal	[71]
<i>Eclipta prostrata</i>	Nitrate	29	Hep-G2 cell	Anticancer	[72]
<i>Azadirachta indica</i>	Nitrate	30–60	<i>E. coli</i> and <i>S. aureus</i>	Antibacterial	[73]

Plant type	Zinc precursor type	Size of ZnO (nm)	Treated biomolecule	Biomedical field	Ref.
<i>Calotropis gigantea</i>	Acetate	25	DNA	Anticancer	[74]
<i>Andrographis paniculata</i>	Nitrate	57	α -Amylase and BSA	Antioxidant	[75]
<i>Tamarindus indica</i> , <i>Moringa oleifera</i>	Nitrate	27–54	α -Amylase and α -glucosidase	Antidiabetic Antioxidant	[76]
<i>Bauhinia tomentosa</i>	Sulfate	22–94	<i>B. subtilis</i> , <i>S. aureus</i> , <i>E. coli</i> , and <i>P. aeruginosa</i>	Antibacterial	[77]
<i>Egg albumen</i>	Acetate	16	<i>C. albicans</i>	Anticandidal	[78]
<i>Moringa oleifera</i>	Acetate	40–45	<i>A. saloni</i> , <i>S. rolfii</i> , <i>S. aureus</i> , and <i>E. coli</i>	Antibacterial	[79]
<i>Cassia fistula</i>	Nitrate	5–15	<i>K. aerogenes</i> , <i>E. coli</i> , <i>P. desmolyticum</i> , and <i>S. aureus</i>	Antibacterial	[80]

Table 1.
Biomedical activities of plant extract-based synthesized ZnO nanoparticles.

Sathishkumar et al. synthesized ZnO nanoflakes using *Couroupita guianensis* Aubl leaf extract and demonstrated the bactericidal activity against various types of bacteria. They reported that the main constituent of the extract, phenol, reduced the zinc acetate precursor into ZnO nanostructures. In history, Aloe (*Aloe barbadensis* Miller) extract has been used in therapeutic applications due to its antifungal, antidiabetic, anticancer, and antibacterial properties. Ali et al. also synthesized small-sized ZnO nanoparticles using *Aloe vera* extract and showed their antibacterial activity and cell damage of *Escherichia coli* and MRSA cells before and after ZnO nanoparticle treatment [7]. On the other hand, Gunalan et al. synthesized ZnO nanoparticles with *Aloe vera* extract and compared their results with the chemically synthesized ZnO nanoparticles. The results proved the enhanced antibacterial activity against various pathogens, and variation in the particle size is responsible for the significant bactericidal activity [8]. The effect of *Aloe vera* extract on the synthesis of ZnO nanoparticles and their antibacterial activity was investigated. Moreover, the antioxidant activity of the particles was evaluated by using five different free radical scavenging assays and the anticancer activity was tested against three cancerous cell lines [9].

In a very recent work, Zare et al. presented the effect of *Cuminum cyminum* leaf extract on the synthesis of ZnO nanoparticles by using zinc nitrate precursors. The resulting nanoparticle diameter is around 7 nm, and the nanoparticles show high sensitivity to Gram-negative bacteria [10]. The nanoparticle formation of zinc nitrate precursors has been investigated by using several types of plant extracts such as *Limonia acidissima* [11, 12], *Cochlospermum religiosum* [13], *Tabernaemontana divaricata* [14], *Conyza Canadensis*, *Citrus maxima* [15], *Aristolochia indica* [16], *Echinacea* [17], *Mentha* [18, 19], *Salvadora oleoides* [20], *Boswellia ovalifoliolata* [21], and *Costus pictus* [22]. The synthesized ZnO nanostructures have shown an enhanced antibacterial effect for a broad spectrum of bacterial cultures (see **Table 1**). Zinc acetate precursor is another choice for the plant-based ZnO nanoparticle synthesis, and Santoshkumar et al. synthesized ZnO nanoparticles using *Passiflora caerulea* extract against urinary tract

infection pathogens. The ZnO nanostructures have a particle size of around 37 nm and show good zone of inhibition to various pathogens [23]. *Hibiscus sabdariffa* [24] and *Acalypha indica* leaves [25] were used in ZnO nanoparticle synthesis, and the nanoparticles showed an enhanced antibacterial activity against *E. coli* and *S. aureus*.

ZnO nanoparticles are currently under investigation due to their utilization in cancer treatment and diagnostic applications [26]. Since the treatment of cancer by chemotherapy is limited because of the adverse effect of tumor drugs and drug resistance by cancer cells, natural plant-based drug researches have focused on overcoming these limitations. Vijayakumar et al. investigated the anticancer activity of ZnO nanoparticles, which were synthesized by *Laurus nobilis* extract-mediated synthesis. The nanoparticles showed anti-lung cancer activity against human A549 lung cancer cells [27]. Toxicity is an important parameter for the in-vivo and in-vitro activity of nanoparticles because some nanoparticles can generate free radicals even under dark conditions. *Anacardium occidentale* extract was used in the synthesis of ZnO nanoparticles, and the resulting nanostructures exhibited concentration-dependent cytotoxicity against pancreatic cancer cells [28]. Yuvakkumar et al. utilized the Rambutan peels (*Nephelium lappaceum*) for the synthesis of ZnO nanoparticles and explored the effect of these particles on HepG2 liver cancer cells [29].

On the other hand, zinc acts as an actuator for several enzymes, and blood sugar regulation is significantly affected in the presence of zinc element. Thus, the enzymatic and anti-diabetic activity of ZnO nanoparticles must be mentioned in their biomedical applications. Bayrami et al. synthesized ZnO nanoparticles by using *Vaccinium arctostaphylos* extract via a microwave-assisted method. The biosynthesized ZnO nanoparticles showed an enhanced efficiency for the treatment of diabetic problems and reduced the fasting blood glucose level effectively [55]. Rehana et al. demonstrated the antidiabetic activity of ZnO nanoparticles by using several types of plant extracts. The results showed that *Tamarindus indica* extract-based ZnO nanoparticles exhibited enhanced activity for α -amylase and α -glucosidase due to the presence of amino acids in the plant extract [76]. As an environmentally benign material, coffee powder extract was utilized in the biosynthesis of ZnO nanoparticles. Koupaei et al. studied the reduced effect of ZnO nanoparticles on proteinase K activity [56].

2.3 Photocatalytic application

Photocatalytic degradation of organic pollutants is a promising approach for the removal of dyes in wastewaters. ZnO nanoparticles have been involved in photocatalytic applications due to their optical and electronic properties (**Table 2**). When the ZnO nanoparticles are irradiated with UV light, valence band electrons are excited to the conduction band, which leaves holes behind. Then the generated holes create hydroxyl radicals by oxidizing H_2O and OH^- and the excited electrons are captured by oxygen in the air. The resulting anionic radicals are highly reactive and degrade the organic dyes into carbon dioxide and water (**Figure 4**).

Nava et al. addressed the effect of different amounts of *Camellia sinensis* extract on the synthesis of ZnO nanoparticles. The synthesized nanoparticles were studied in photocatalytic degradation of methylene blue (MB) dye where the nanoparticles presented MB degradation over 84% in 120 min [81]. In another study, *Parkia roxburghii* extracts have been used for the synthesis of ZnO nanoparticles, and they were found to be efficient in degradation with nearly 98% efficiency in 8 min for both MB and Rhodamine B (RhB) dyes [82]. The degradation of Congo Red dye has

Plant type	Zinc precursor type	Size of ZnO (nm)	Treated analyte	Efficiency (%) – time (min)	Ref.
<i>Lycopersicon esculentum</i>	Nitrate	9–20	MB	97 – 180	[86]
<i>Monsonia burkeana</i>	Chloride	20	MB	48 – 45	[87]
<i>Ulva lactuca</i>	Acetate	10–50	MB	90 – 120	[88]
<i>Conyza canadensis</i>	Nitrate	—	MO MB	94 – 45 85 – 20	[89]
<i>Allium sativum</i>	Nitrate	14–70	MB	100 – 180	[90]
<i>Garcinia mangostana</i>	Nitrate	21	MB	99 – 180	[91]
<i>Plectranthus amboinicus</i>	Nitrate	50–180	MR	92 – 180	[92]
<i>Calotropis procera</i>	Nitrate	15–25	MO	81 – 100	[93]
<i>Citrus paradisi</i>	Sulfate	12–72	MB	56 – 360	[94]
<i>Lantana camara</i>	Acetate	340–520	MB RhB	92 – 25 75 – 40	[95]
<i>Chlamydomonas reinhardtii</i>	Acetate	13	MO	92 – 120	[96]
<i>Lycopersicon esculentum</i>	Nitrate	7–20	MB	97 – 150	[97]
<i>Corymbia citriodora</i>	Nitrate	64	MB	83 – 90	[98]
<i>Catharanthus roseus</i>	Acetate	38	PR	100 – 480	[99]

Table 2.
Photocatalytic activities of plant-extract based synthesized ZnO nanoparticles (MB = methylene blue, MO = methyl orange, MR = methyl red, RhB = Rhodamine B, and PR = phenol red).

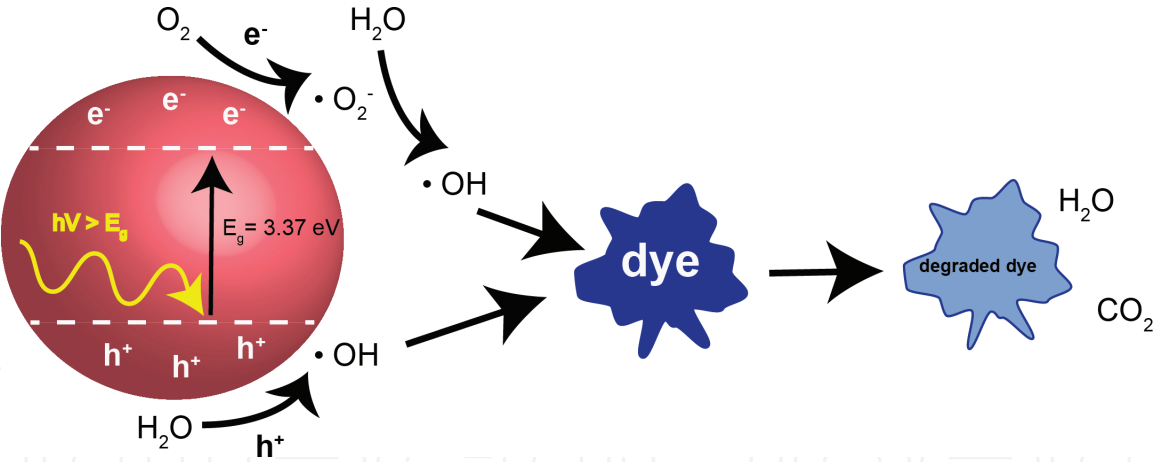


Figure 4.
Schematic diagram of dye degradation by ZnO nanostructures.

also been studied for the ZnO photocatalysis applications. Prasad et al. and Vidya et al. studied the degradation of Congo Red in aqueous solutions by ZnO nanoparticles. The dye was degraded with 90% efficiency in 35 and 60 min by using lemon juice [83] and *Artocarpus heterophyllus* [84] extracts in the nanoparticle synthesis, respectively. The aqueous leaf extract of *Coriandrum sativum* was used for the nanoparticle synthesis and the resulting materials have been used for the photocatalytic degradation of anthracene with 96% efficiency in 240 min [85].

2.4 Adsorption/sensing application

Heavy metal ion pollutants in wastewaters create a problem worldwide because of their serious effects on both human health and environment. ZnO nanostructures have also been used as an adsorbent material due to their low toxicity and

became more effective than the other adsorbent materials [100]. The plant-based synthesis of ZnO nanostructures enhances metal ion adsorption capacity due to the chemical interactions between ions and functional groups of plant extracts. Fazlzadeh et al. synthesized ZnO nanoparticle-loaded activated carbon by using *Peganum harmala* for the removal of chromium from aqueous systems. *Peganum harmala* acted as a stabilizing agent and enhanced the chromium uptake up to 68.48 mg g⁻¹ [101]. The lead ion removal was studied by Azizi et al. using *Zerumbone* extract-mediated ZnO nanoparticles. They reported that the lead ion adsorption capacity reached up to 19.65 mg g⁻¹ [100]. Sensing application of ZnO nanostructures is another field where the glucose sensing mechanism of ZnO nanostructures was studied by Muthuchamy et al. They fabricated a glucose sensor using ZnO nanoparticles and peach juice as a carbon source. The fabricated ZnO sensor showed high sensitivity (231.7 $\mu\text{A mM}^{-1} \text{cm}^{-2}$) and low detection limit (6.3 μM) [102]. Sharma et al. also studied silymarin detection capability of ZnO nanostructures by using *Carica papaya* extract. The results showed that ZnO-modified sensors have 2-fold greater electrochemical signals than the neat ones [103].

2.5 Microorganism-based ZnO nanostructures

Synthesis of ZnO nanostructures by using microorganisms has gained considerable interest, and numerous microorganisms can be utilized for their synthesis. Bacteria, fungus, and algae are the possible microorganisms in a green synthesis of ZnO nanostructures. Because of their easy genetic manipulation and easy handling, bacteria are preferred microorganisms [104]. Jayaseelan et al. used *Aeromonas hydrophila* bacteria as green capping agent, and the synthesized ZnO nanoparticles showed antibacterial activity against *Pseudomonas aeruginosa* and *Aspergillus flavus* [104]. In another study, *Pseudomonas aeruginosa* was used as a capping agent in ZnO nanoparticle synthesis, and the resulting particles demonstrated antioxidant activity [105]. Kundu et al. used *Rhodococcus pyridinivorans* as metabolically versatile Actinobacteria in the fabrication of self-cleaning, UV-blocking, and antibacterial textile fabrics with ZnO nanoparticles. Besides, the ZnO nanoparticles showed photocatalytic activity against malachite green and anticancer activity against HT-29 cancerous cells [106]. Tripathi et al. synthesized ZnO nanoflowers by using *Bacillus licheniformis* and assessed their photocatalytic activity against methylene blue [107]. However, bacterial utilization in ZnO green synthesis could be somewhat problematic because of the uncontrolled growth of bacteria and unavoidable contaminations [108].

Fungus-based green synthesis of ZnO nanoparticles is generally preferred over the bacteria based synthesis because of their large-scale production and better tolerance property [109]. Raliya et al. synthesized ZnO nanoparticles via an environmental method by using *Aspergillus fumigatus* as a stabilizing agent and investigated the effect of ZnO nanoparticles on phosphorus mobilizing enzymes in rhizosphere and gum contents in clusterbean grains [110]. In another study, ureolytic bacterium (*Serratia ureilytica*)-mediated green synthesis of ZnO nanoparticles was reported. The cotton fabrics were coated with the synthesized nanoparticles and their killing efficiency against *S. aureus* and *E. coli* bacteria was revealed [111].

Algae are the members of a diverse group of aquatic photosynthetic organisms and they have been utilized sometimes in the synthesis of ZnO nanostructures. *Sargassum muticum* extract, which is a brown marine macroalga, was used in the biosynthesis of ZnO nanoparticles [112]. Nagajaran et al. used the seaweed extracts of green *Caulerpa peltata*, red *Hypnea Valencia* and brown *Sargassum myriocystum* in the synthesis of ZnO nanoparticles. The results revealed that among three

seaweeds, only *S. myriocystum* could stabilize and reduce ZnO nanoparticles of size 36 nm. Also, the nanoparticles showed antimicrobial activity against a wide spectrum of bacterial cultures [109].

2.6 Biomolecule-based ZnO nanostructures

Synthesis of ZnO nanoparticles with controlled morphologies and using environmentally friendly chemicals could be possible in biomolecule-based synthesis routes, and utilization of amino acids, polysaccharides, gums, and enzymes is highly preferable. Gharagozlou et al. synthesized ZnO nanoparticles by using alanine amino acid, and a Schiff base complex was obtained at the end of the study [113]. Bovine skin gelatin has also been used in the synthesis of ZnO nanostructures, and Alnarabiji et al. demonstrated the environmentally friendly synthesis route for ZnO nanoparticles [114]. Arabic gum and *gum tragacanth*-based green synthesis of ZnO nanoparticles have been demonstrated by Fardood et al. [115] and Daraoudi et al. [116], respectively. Thus, they demonstrated an alternative method for the synthesis of ZnO nanoparticles instead of conventional ZnO reduction methods by using hazardous polymers or surfactants [115, 116]. Casein is another biomolecule that can be used as a capping and reducing agent in the ZnO nanoparticle synthesis. Somu et al. synthesized ZnO nanoparticles, which show heavy metal ion adsorption, dye adsorption, and antibacterial activity in wastewater treatment at the same time [117]. The resulting nanoparticles effectively remove Cd(III), Pd(II), and Co(II) ions, methylene blue, and Congo red dye from the wastewaters. Also, they demonstrated high antibacterial activity against *E. coli* cultures. Subramanian et al. synthesized ZnO nanoflowers, which comprise nanorods and ellipsoids as subunits, by using L-lysine as a capping and precipitating agent [118].

3. Synthesis of ZnO nanostructures using nontoxic chemicals

Combating the major drawbacks of common ZnO nanostructure synthesis methods, mainly identified as the generation of pollutants, toxic materials, and side products during reactions, green chemical techniques using only nontoxic and biologically compatible materials were developed. Gharagozlou et al. [113] reported a novel method to synthesize ZnO nanoparticles without any pollutant or combustible side product in the process. Water was used as a solvent with a biologically compatible nitrogen source, amino acid instead of toxic amines, alanine and sodium salicylaldehyde-5-sulfonate, and zinc acetate to prepare the zinc Schiff-base complex and then subsequently heated to obtain ZnO nanoparticles. This work showed that the solid-state decomposition process applied at moderate temperatures has yielded nanoparticles ranging from 5 to 110 nm with fewer defects yet interestingly high crystallinity.

Biomimetic and bioinspired synthesis has also been regarded among the most attractive strategies in fabricating novel functional materials, and biological materials like eggshell membrane, oyster shells, nacre, diatoms, cuttlefish bone, DNA chains, and sea urchin spines have been actually employed as templates or bioreactive substrates. Silk fibroin fibers (SFFs) extracted from silkworm *Bombyx mori* cocoons were used for their capping and directing functions to control the morphology of ZnO crystals. Acting at the same time, zinc ions are anchored on the SFF and in-situ react with OH⁻ generating ZnO nanoparticles. It was observed that several petals composed of ZnO nanoparticles form ZnO flowers and bestrewed the SFF substrates due to the electron-donating groups (amino and carboxyl groups) contained in SFF [119]. Another ZnO nanoparticle synthesis method through

biological roots such as the use of natural biopolymers was to be presented as more cost-effective compared to both physical and chemical methods available [120]. A recent work has focused on integrating ZnO nanoparticles with biopolymers that are excellent vehicles for cross-linking molecules. Thereby, collagen was used for zinc acetate in basic solution, to give a precipitate that was processed and thermally heated at 350°C giving birth to ZnO nanoparticles, yielding an interesting antibio-film, anti-cancer, and ecotoxicity material [121].

Extrusion dripping is another novel technique using environmentally friendly, cost-effective, degradable, and renewable biomass materials. Generally, the process yields monodispersed spherical particles by controlled dripping of working solution into a biopolymer solution after extruding it through a narrow tube, thanks to the effect of viscous-surface tension forces and impact-drag forces that help to preserve the spherical shape of the drop [122]. Goes et al. have reported that spherical uniform sized ZnO nanoparticles were obtained by dropwise addition of alginate solution to zinc nitrate solution under a long slow magnetic stirring to ensure the ion-exchange to happen and stabilize ZnO nanoparticles; the heated ZnO outcome was used to fabricate a polymeric nanocrystalline microfilm, exhibiting interesting photodegradation results, as ZnO on the surface is likely to accept photons and generate holes promoting the oxidative decomposition of the dye [123].

Chemical bath deposition and soft-template sol-gel methods are two wet organic solvent-free routes studied by a group led by Leone et al [124]. to obtain a nanostructured ZnO employed as a reservoir of clotrimazole for pharmaceutical purposes. Identifying the synthesis of carriers and active pharmaceutical ingredient loading as the main steps in which the waste of organic solvents occur, ZnO nanostructures have been introduced as green alternative carriers for their intrinsic biological properties, low toxicity, and high biocompatibility [125]. For the chemical bath deposition approach, a nanosheet-like zinc carbonate hydroxide hydrate was transformed into ZnO using solutions containing urea and different zinc salts [126]. As for the sol-gel method, pluronic F127 was used as a soft template forming an opalescent solution with zinc acetate in water, and then dried and calcined at 500°C to sacrifice the template and obtain the ZnO nanostructure [124].

In our previous work, we successfully enhanced the antibacterial activity of ZnO nanowires by modifying the cooling route. Zinc acetate was calcined in a muffle oven, followed by a rapid cooling; the three resulting samples were compared to a free cooled batch synthesized under the same conditions, revealing noticeable effects on ZnO nanowire morphology in addition to the improvement of surface area due to the limiting time for crystallite growth [127].

3.1 Ionic liquid as solvents for ZnO nanostructures

Ionic liquids (ILs) are an area of chemistry, which has received important attention in both academia and industry, because of the cost effectiveness coupled with being environmentally friendly [128]. ILs usually act as solvents and reactants as well as templates for inorganic nanomaterial synthesis and scavenging agents [129]. As a subdivision, room temperature ionic liquids (RTILs) are particularly doted of special considerations as nontoxic solvents with a wide liquid temperature range, remarkable chemical stability, negligible vapor pressures, and high fire resistance [130]. ILs have been great templates for the synthesis of nanomaterials as it was shown that only by modifying the structure of their cations or anions, it is possible to alter their properties in order to control the size, morphology, and thus the properties of nanomaterials [131]. Sabbaghan et al. have synthesized different morphologies of ZnO nanostructures using zinc

acetate as the metal source in a basic media to react with different symmetrical imidazolium-based ILs, yielding nanoparticles, nanoparticle-like, spherical-like, nanosheet in different sizes ranging from 16 to 30 nm and different band gaps between 2.98 and 3.17 eV, demonstrating through this work the relation morphology-IL [128].

ZnO nanostructures with nanosheet morphology have been successfully fabricated in another work by refluxing the mixture of zinc acetate and the ionic liquid in water according to Menshutkin reaction [132]. A comparative study has shown that when zinc is used with ILs as a template, ZnO nanoparticles with smaller crystallite size were formed compared to the yield without ILs. This work revealed as well that the template and the pH control the direction of growth of ZnO crystals and the shape of nanomaterials obtained. The final band gap values of ZnO with different morphology ranged around 2.88–3.16 eV [133]. Alammari et al. have studied the effect of five different ILs on ZnO morphologies, and claimed that the habitus and morphology come as the system naturally tends to reduce the total surface energy during formation; it is to note that the anion of the IL is proposed to be interacting with the ZnO surface during the growth [134]. Yet, the best performance was for ZnO nanoparticles that are obtained by use of IL with a long alkyl chain, reaching 95% in 9 h for methyl orange decomposition, proposing that along with high surface area, oxygen vacancies and polar plans that act like electron traps are the main factors for such interesting photocatalytic activity [135, 136]. It was also reported by Amde et al. [137] that common techniques for the determination of fungicide concentration in water are usually non-environmentally friendly, organic solvent, and time consuming; the group has prepared ZnO nanofluids by a green two-step method, dispersing the as-synthesized sol-gel ZnO nanoparticles in 1-hexyl-3-methylimidazolium hexafluorophosphate, hand shaking it to attain a homogeneous distribution, then sonicating it to break NPs clusters. The as-prepared ZnO nanofluids are applied in a modified, simple, versatile, and inexpensive liquid-liquid microextraction technique; this technique, called single drop microextraction not only reduces the amount of extraction solvent radically but also offers other functionalities such as high enrichment factor, different extraction modes, and full automation of the process [138]. It is proclaimed that the preferences of ZnO-based nanofluids for the investigation were driven from the fact that ZnO dotes on surface charges that enable to form stable suspensions, unlike many metallic nanofluids, and without the need of any additional stabilizer intervention.

4. Processes of synthesis of ZnO nanostructures

ZnO nanostructures can be synthesized by following different approaches, and each has its own distinctive advantages and downsides. In this section, we report some interesting methods and findings having the common main aim to avoid drawbacks like the use of toxic reagents, promoter, and stabilizer organic additives, lowering the reaction time, as well as high temperature and pressure. These methods have plenty of scopes to provide both qualitative and quantitative support for nanosized ZnO synthesis along with being simple, fast, efficient, and convenient.

4.1 Hydrothermal synthesis of ZnO nanostructures

Hydrothermal method has gained particular interest as an efficient method for high quality and mass production of ZnO nanostructures [139]; it is indeed

environmentally friendly as there is no need to control pH and subsequently no release of unwanted by-products. A work led by Guo et al. [140] reported a controllable hydrothermal synthesis of ZnO nanorods reacting with zinc carbonate hydroxide hydrate powder and H_2O_2 at various temperatures for different periods of time. The group claims that the formation mechanism of ZnO starts by the formation of ZnO_2 when subjecting it to hydrothermal treatment at 170°C for more time (3–6 h), and then the thermally unstable ZnO_2 would decompose into ZnO and O_2 . ZnO nanorods exhibited an optical band gap of 3.3 eV [140].

It has also been reported that flower shaped ZnO nanoparticles were synthesized by hydrothermal method, where zinc nitrate and hexamethylenetetramine solutions were prepared separately in double distilled water, while NaOH solution was added dropwise to adjust the pH to 10. The obtained milky solution was refluxed at 80°C for 7 h, washed, and dried. XRD asserted the formation of hexagonal crystal structure ZnO that had a flower-shape, formed by agglomeration during the hydrothermal process. Dynamic light scattering (DLS) data have affirmed the average diameter of ZnO between 600 and 800 nm. The effect of different pH values from 2 to 10 on the removal performance of ZnO has been studied and the results showed a maximum increase of 80% removal efficiency when pH reached 6; increasing the temperature of the process also improved the removal efficiency from 68 to 97%. The contact time had a sharp rise at the value of 15 min of initiation of the experiment, and higher stirring had an enhancing effect as well. Jamal Al-Sabahi and his group have treated for the first time the degradation of HPAM polymer in oil produced water with supported ZnO nanorods synthesized via a microwave-assisted hydrothermal method in an aqueous solution [141–143]. Placing a prepared microscope glass substrate (25 mm \times 75 mm) on a hotplate (350°C), 10 mM zinc acetate dihydrate solution is sprayed, and then the plate is immersed in an equimolar solution of zinc nitrate hexahydrate and hexamethylenetetramine, then heated in a domestic microwave oven for 45 min, and then cooled down for 15 min; the produced ZnO nanorod-covered substrate was afterward annealed in air at 350°C for 1 h. The morphology was of a typical ZnO array and the average length was about 4 μm while the average diameter reached around 95 nm [144].

Singh et al. have adopted a cost-effective and environmentally friendly method to fabricate a 3D self-assembled wool ball like spherical ZnO with high porosity by combining a urea-glycerol assisted hydrothermal approach with calcination under air atmosphere [145], where hydrated zinc carbonate was synthesized hydrothermally in a Teflon-lined stainless steel autoclave reacting zinc nitrate with urea in a triplet (glycerol, ethyl alcohol, and water (7:7:10)) solvent system. After drying the outcome, the white powder intermediate product was calcined at 450°C for 3 h to get hierarchical 3D porous ZnO. The group carried out a series of experiments to investigate the effect of synthesis parameters over the morphology, while 7 h and 140°C were about the optimum duration of synthesis and temperature to get the best ZnO with W-ball like spherical morphology. This ZnO photocatalyst has shown 98% of highly toxic Rhodamine B degradation in 60 min of UV photolysis catalysts at a pH of 4.

A green hydrothermal method was used recently to fabricate ZnO nanorods without any organic solvent or surfactant, by starting with ZnO powder and H_2O_2 aqueous solution in the sealed autoclave. Then the precipitates were washed and dried. In this work, Lam et al. reported as well that the hydrothermal treatment of ZnO at 100°C promotes the slow conversion of ZnO_2 to ZnO and O_2 without using any toxic reactant, nor releasing any pollutant by-product. XRD indicated the high purity of the ZnO wurtzite phase [146]. This environmentally friendly method has

generated highly performing ZnO nanorods that completely degrade the resorcinol in aqueous solution after 120 min. The same group has used a similar hydrothermal method to fabricate ZnO nanotubes (NTs) with a diameter of around 10 nm, a wall thickness of 3.5 nm, and average lengths of up to 200 nm by scrolling of the ZnO₂ layer nanosheet, which transforms to ZnO NTs with a symmetrical layer in both shell-tube structures. XRD asserted the hexagonal phase and then confirmed the purity of ZnO NTs that showed a ferromagnetic behavior because of the grain boundaries and developed free surfaces [147]; the band gap energy was measured to be around 3.21 eV, and the degradation of methylparaben over the surface of ZnO NTs had an efficiency of 87.6% in 105 min [148].

4.2 Microwave-assisted synthesis of ZnO nanostructures

Microwave-assisted sol-gel synthesis is based on subjecting samples to frequencies ranging from 300 MHz to 300 GHz [149]; besides its high selectivity of specific morphologies, dramatic reduction of reaction time (minutes), and remarkable increase of product yield, it generates localized superheating at the reaction sites promoting metal ion reduction in the solution [150]. It presents a promising green method for metal oxide nanostructures production. Azizi et al. [151] have worked on a green microwave-assisted combustion approach to synthesize ZnO-nanoparticles, presenting combustion as a fast, low cost, homogenous and highly pure outcome, as well a distinguished surface area at low temperature. Using fruit, seed, and pulp extracts of *Citrullus colocynthis* (L.) as biofuels with zinc nitrate as the zinc source, an in vitro cytotoxicity study has been made showing that smaller nanoparticles were more efficient penetrating in the cells membranes, while the optical band gap increased with the rear of the particle size from 3.25 to 3.40 eV. An interesting nontoxic and eco-friendly single-step, and green synthesis method of ZnO nanoparticles with excellent reproducibility was reported using coffee powder extract as a reducing material under microwave heating at 540 W for 5 min and the precipitate was dried in a hot air oven. SEM asserted a size of 80–120 nm for the nanoparticles. Afterward, a thin nanocomposite film was prepared using the as-prepared ZnO nanoparticles with natural graphite powder. The nanocomposite film showed a remarkable photovoltaic efficiency of 3.12% [152]. ZnO sub-micrometer particles and nanowires were synthesized by microwave assisted sol-gel reaction. Zinc acetate and *N,N*-dimethylacetamide were stirred into a beaker, and then, the solution was cooled rapidly to 15°C. After only a couple of minutes of the microwave, a white suspension was obtained. The average diameter of the particles prepared given by DLS analyses was around 275–352 nm [153]. Salari et al. reported a microwave-assisted synthesis of biogenic nanoparticles using *Lavandula vera* leaf extract as a reducing agent in the presence of zinc sulfate; the method led to simple and fast formation of microstructures that exhibited high antioxidant cytotoxic activity [154]. In a separate work, vertically aligned ZnO nanorods were grown at 90°C on a Si substrate by microwave synthesis and compared with nanorods made by the traditional heated waterbath method changing the pH from 10.07 to 10.9. The microwave synthesis was performed at a power of 100 W [155]. The same group observed that the increase of ammonia led in both methods to sparser and longer nanorods with the larger diameter, as well as an increase of oxygen percentages in the samples. The microwave synthesized samples exhibit a uniform distribution of nanorods as well as a better crystalline structure with fewer defects than the heated water bath-grown samples, which can be beneficial for band-edge transition optoelectronic devices.

4.3 Sonochemical synthesis of ZnO nanostructures

Microwave synthesis and ultrasound sonication have proven numerous advantages and actually become amid the most frequently sought nanosized material synthesis methods [156, 157]. Zak et al. have used ultrasonication to synthesize ZnO nanostructures at room temperature without any specific conditions or organic solvents, starting from an as-prepared zinc solution where zinc acetate was dissolved in ammonia solution, and sodium hydroxide was dropped in the solution. Deionized water was wisely added till attaining a concentration of 1 M zinc. The ultrasonication performed at different durations was sufficient to stimulate the formation of nanostructures. XRD asserted the presence of hexagonal pure ZnO and the band gap energies estimated by UV-Vis spectra are 3.3, 3.22, and 3.2 eV for ZnO seeds, nanorods, and nanoflowers, respectively [158]. Ultrasound was conducted as well for the synthesis of different ZnO nanostructures without any organic solvents, surfactants, or templating agents; zinc acetate and sodium hydroxide in ionic liquids (ILs) are reported to be a green, fast, and effective, yet highly selective route to 0D, 1D, and 2D nanostructures of ZnO [134]. A facile calcination-free ultrasound assisted approach has been reported by Bhatte et al. involving zinc acetate as a metal source and 1, 3-propane diol as a solvent, base, stabilizer, and template for the growth of nanocrystalline ZnO [159]. The mixture of both materials has been sonicated under 22 kHz frequency for 2 h with a 5 s interval on-off pulse. After sonication, the formed ZnO was collected, washed and dried. XRD confirmed the successful formation of ZnO without any impurities [159].

4.4 Low temperature synthesis of ZnO nanostructures

For the large-scale production of pure ZnO nanocrystallites, low thermal processes are one of the most efficient methods minimizing the generated waste yet implementing sustainable processes: simple, cheap, and nonpolluting, addressing the key issues that draw much consideration in a green solid-state synthetic method, by eliminating the use of nontoxic materials and reducing energy consumption. ZnO₂ nanocrystallites were employed as the precursor for ZnO production, because of their facile preparation, the absence of unwanted by-products, and low-temperature decomposition reaction [160]. Zinc acetate and hydrogen peroxide were used first to synthesize ZnO₂ nanocrystallites hydrothermally at 100°C for 12 h in an alkaline aqueous solution; the product was subjected to 180°C in air for 12 h yielding pure ZnO phase shaped nanocrystallites of 8–10 nm and blue shift at around 350 nm in the UV-Vis spectra [161].

ZnO nanosized structures were also synthesized starting from zinc acetate and urea in a 1:1 stoichiometry, where the decomposition of urea helped the formation of ZnO. Then two different heating methods were applied: microwave hydrothermal (MH) method and waterbath heating. XRD proved the purity of the wurtzite phase for both methods, while FE-SEM showed a difference in shape regularity in favor of the MH process; thus, the MH method contributes to the production of spherical and uniform particles after a short processing time by enhancing the interface mobility and the diffusivity in the medium [162].

Raja et al. have reported a laboratory procedure based on sol-gel for the preparation of nano-ZnO particles. Zinc acetate solution was stirred at room temperature while adding sodium hydroxide until reaching a pH of 14 and the solution went through a microemulsion. The suspension obtained was transferred for thermal treatment at 180°C for 3 h, and then the white precipitate was collected, washed, centrifuged, and dried under vacuum to reveal well-shaped uniform ZnO nanoparticles of 35 nm on average [163].

Another group has proposed a simple, eco-friendly approach to synthesize ZnO nanoparticles by using carboxylic curdlan (cc) as a reducing and stabilizing agent. A solution of zinc acetate was blended with cc aqueous solution and stirred at 70°C for 6 h, and then the outcome was freeze-dried [164]. The carboxyl group is in charge of chelating and reducing zinc ions for the sake of the formation of ZnO nanoparticles, thanks to the numerous negatively charged carboxyl groups it contains. The average diameter of cc-ZnO nanoparticles is around 58 nm exhibiting a band gap energy of 3.3 eV. Furthermore, the interaction between the as-prepared nanoparticles and bovine serum albumin (BSA) at room temperature was investigated, which suggested the formation of a certain complex revealed by a blue shift of the fluorescence peak by about 8 nm with increasing nanoparticle concentration, due to the binding of cc-ZnO nanoparticles and BSA [164].

Conflict of interest

The authors declare no conflict of interest.

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