REVIEW PAPER

Zinc selenide nanoparticles: Green synthesis and biomedical applications

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ABSTRACT
Nanotechnology has become one of the most widely used technologies in translational research and may significantly impact the future of healthcare. Because of their distinctive physicochemical characteristics, nanoparticles (NPs) have diverse applications in all areas of science including biomedicine, agriculture, biolabeling, catalysis, electronics, sensors, and fiber optics. Recently, green synthesis technology, as a reliable and eco-friendly method, has been taken into consideration for synthesizing a wide range of nanomaterials of desired sizes, shape, and functionalities. In this regard, zinc selenide nanoparticles (ZnSe-NPs) as a semiconductor nanostructure with low toxicity and high luminescence features have potential applications in different research fields like optoelectronic devices, laser solar cells, and, particularly in medical and biological sciences. ZnSe NPs can be synthesized by various chemical methods, including sol-gel, solvothermal, hydrothermal, wet chemical, and green and biological synthesis approaches. In this study, we have reviewed the green chemical or biological ZnSe nanoparticles synthesis as eco-friendly methods. Also, we have discussed the biological applications of ZnSe nanoparticles, including antibacterial activity, cytotoxicity effect, biomedical imaging and, drug delivery.

Keywords: *Green synthesis, Nanoparticles, Semiconductor, Zinc selenide, ZnSe NPs*

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INTRODUCTION

Observation of novel properties in some materials at nanometer levels is the basis for the advent of nanotechnology. The field of nanotechnology has received considerable attention due to its vast applications in various areas of science, especially in medicine. The development of nanomedicine depends on the manipulation of nanomaterials at the nano-scale. To date, using nanomaterials in biological systems is increasing parallel to synthesizing more biocompatible nanostructures with unique and fascinating properties for biomedical applications, including antibacterial activity, drug delivery, anti-cancer, and bioimaging agents [1, 2]. One important group of these metal-based nanoparticles is quantum dots (QDs) or semiconductor nanocrystals. QDs-based nanotechnology has emerged as a versatile and applicable field in materials science. In the past few years, these semiconductor nanostructures have been studied widely due to their promising applications, emanated from their specific features such as high physicochemical stability, size-dependent optical property, continuous absorption bands as well as photophysical and electronic properties. Among various applications of QDs in the vast areas of science, including medicine, chemistry, environment, energy, etc., the roles and potential applications of this kind of nanomaterials in biological science are seriously growing nowadays. Owing to their high specific surface area and quantum size effect, QDs show various size-dependent properties compared to their bulk materials. In recent years, there has been

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extensive enthusiasm in synthesizing nanocrystals (NCs) such as CdS, CdTe, CdSe, ZnS, ZnTe, ZnO, and ZnSe. Some of these components are intrinsically toxic, could be released from nanocrystals, and would be detrimental to biological systems. Zinc selenide (ZnSe) is a critical II-VI semiconductor with a wide bandgap of ~ 2.7 eV at room temperature, demonstrating tunable blue-ultraviolet (UV) luminescence via its quantum confinement effects. ZnSe nanostructures have prominent applications in fundamental researches areas and construction of nano-scale electric and optoelectronic devices such as laser diodes, green-blue light-emitting diodes, and photodetectors. ZnSe, as a low toxic semiconductor nanocrystal and environmentally friendly material, is considered an up-and-coming candidate for applying in biological systems. ZnSe QDs have been prepared by different synthetic methods such as wet chemical [3], solvothermal [4], reverse micelles [5], sol-gel [6], thermal evaporation [7], hydrothermal Synthesis method [8], and microemulsion [9]. However, most of these synthetic routes commonly involve hazardous chemicals. On the other hand, using high pressure and temperature during some of these synthesis processes limit the purity of final products. Because water-solubility of synthesized nanomaterials is an essential requirement for applying in biological environments, it is necessary to choose surface modifiers that influence the synthesis of biocompatible nanomaterials water-solubility and desirable properties of final nanostructures for employing the biological purposes. Recently there has been an attractive trend to synthesize and develop less toxic and bio-friendly materials based on the green chemistry criteria and sustainable technology. According to the principles of green chemistry, besides applying environmentally benign chemicals, the synthesis route also needs to be energetically efficient. Despite many research articles about green synthesis methods of ZnSe NPs, there is no review article on their green synthesis approaches up now. In this review article, we will describe some state-of-the-art research works emphasizing the green chemical and biological synthesis approaches of ZnSe nanostructures using environmental-friendly and economical methods. Various synthesis methods of metallic nanoparticles are illustrated in Fig. 1.

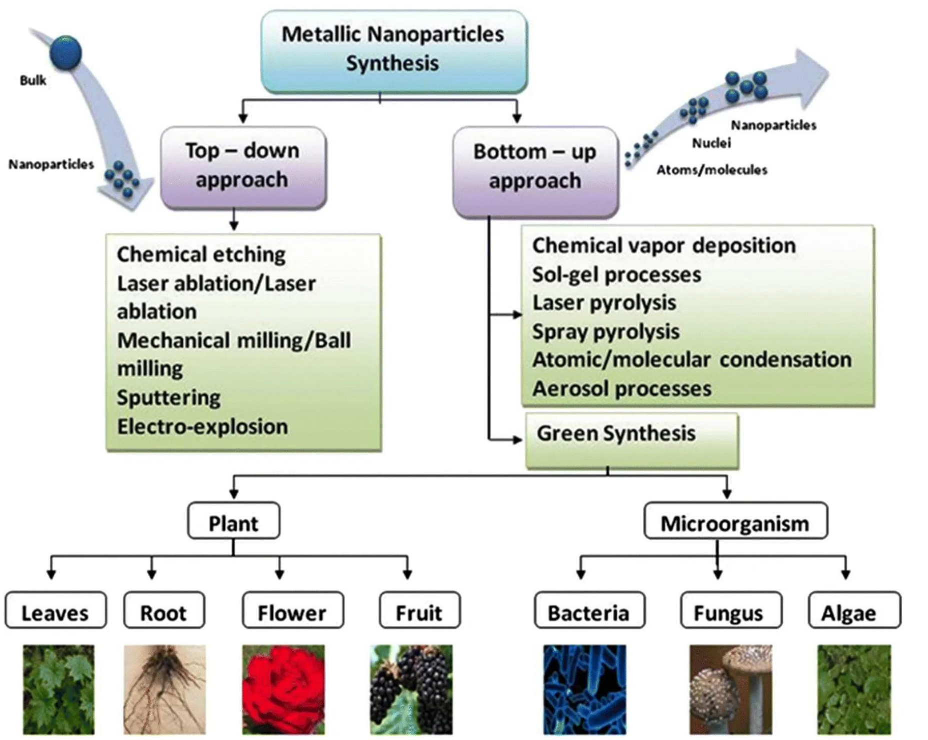

Fig 1. Pictorial representation of different approaches for synthesizing metallic nanoparticles. Reprinted from [10] with permission

Green-based synthesis strategy

Even though there are different physical and chemical synthetic strategies for the fabrication of NPs, some drawbacks of traditional methods that commonly have been utilized to synthesize various NPs included high energy consumption and low reaction efficiencies during the synthetic processes, limiting these methods' application. In addition, the use of sophisticated equipment confined the simplicity of experiments. It is worth mention the solubility of synthesized nanostructures is a crucial issue for biological applications. In traditional methods synthesizing the organic soluble materials and tedious and time-consuming surface modification techniques are required for final solubility. Recently NPs synthesis based on green chemistry or biological synthesis methods has been received significant attention. The benefits of these synthetic methods include energy efficiency, less toxicity, facility, ease of characterization, safety, use of non-toxic and biodegradable passivating materials under ambient environmental conditions, and production of biocompatible nanoparticles. Commonly biological synthesis route is considered a bottom-up approach. Generally reducing the use of toxic and dangerous substances is the aim of applying green chemistry strategies. Today, nanostructures are widely used in various sciences, including medicine, biology, and many other fields. In the green chemistry NPs synthesis routes, selecting suitable, non-toxic and environmentally reducing agents, capping (stabilizing) agents, and the solvent is essential. It is worth mentioning the prominent role of capping agents as the physical barrier is aggregation and overgrowth prevention, therefore commonly used to control the size and NPs morphology [11-13]. Polymers, small molecules, dendrimers, long-chain hydrocarbons, and polysaccharides are widely used as capping agents [14]. Among the capping agents listed above, polysaccharides polymer, small molecule and, biomolecules are considered green capping, and some of them as reducing agents. One of the main strategies in green-based synthesis methods is the use of plants extracts in the synthesis process**.** Plants extracts contain several biomolecules and secondary metabolites such as phenolic compounds, alkaloids, enzymes, peptides, etc., that act as stabilizing, reducing agents and hamper the need for capping and reducing agents. In addition to plants, applying fungal, bacterial, actinomycetes, yeast and, virus extracts are noticeable [15-17]. These organisms can be used for *in vivo* and *in vitro* NPs production. One of the other benefits of plants and the microbial extract is the capability to the mass generation of NPs. Given the advantages mentioned above, the production of NPs mainly used in different fields of medicine by the biological synthesis method will be beneficial [18]. Generally, NPs synthesis by applying enzymes, vitamins, plants and microbial extract, polysaccharides, polymer, and biomolecules is considered green synthesis strategies [19].

Green synthesis of ZnSe-NP

So far, different green strategies have been applied for synthesizing a wide variety of metallic nanoparticles.

Biopolymer-mediated synthesis of ZnSe-NPs

Polymers as stabilizing and capping agents offered to be suitable macromolecules for the facile synthesis of NPs. Passivation of NPs surfaces with polymers prevents uncontrolled growth and accumulation of as-synthesized NPs. On the other hand, surface modification of NPs with polymers improves their biomedical applications. Polymers have a tremendous steric hindrance effect that gives exceptional stability to the NPs. Moreover, the higher viscosity of the polymer solution has an enhancing role in controlling the growth of metallic and semiconductor forms. Furthermore, NPs, after hybridization with polymers, have good compatibility and capability, which are crucial for their final technical applications. It is highly desirable to find ways to directly synthesize more biocompatible nanostructures using the polymer in the one-pot process and without any additional stabilizer as an alternative approach. In addition to increasing the water solubility, hydroxyl groups of the polymers enhance the metal ion complexation and make appropriate conjugation sites for better functionalization of the as-synthesized NPs; consequently, no further stabilizers are needed. In general, after encapsulation of NP with a polymer, synthesized nanocomposites show mixed properties of the polymer and NPs resulting in more applicable NPs. Some polymers like starch as a biodegradable, safe and, renewable polysaccharide polymer and polyethylene glycol (PEG), a low toxic and highly biocompatible poly (ether) biopolymer, are used for the synthesis of ZnSe NPs up now [16, 20]. Because of their high aqueous solubility, polymers seriously prevent the oxidation of reaction reagents and as-prepared ZnSe NPs; hence less cytotoxic NPs will be produced.

Vitamin-mediated synthesis of ZnSe-NPs

In various studies for the synthesis of different

Tabale 1. Green synthesis methods of ZnSe-NP

Number	Method	size (nm)	Morphology	Ref
1	Polymer-mediated synthesis	2.3 - 4.2	Spherical	[16]
		2.3 - 4.2	Spherical	[19]
		210	Microsphere	[20]
2	Vitamin-mediated synthesis	40 - 60	Spherical	[22]
		2.8	Spherical	[23]
3	Biomolecule-mediated synthesis	3.8	Cubic	[29]
		2 - 3	Spherical	[39]
		3.4	Cubic	[30]
		100-500	Nanospheres	[40]
		4	Cubic- flower-shaped NCs	[31]
		3	Cubic	[41]
		3 to 4	Cubic	[42]
		3	Cubic	[43]
		3.4	Cubic	[44]
4	Bacteria- mediated synthesis	-	-	[37]
		30	Cubic sphalerite	[35]

NPs, including gold, silver, copper, etc., some kinds of vitamins, especially ascorbic acid (vitamin C), have been applied as green reducing and capping agents. Ascorbic acid molecules can cover the surface of NPs and forbid uncontrolled particles growth [15, 21]. So far, in several studies synthesis of ZnSe NPs by using ascorbic acid as a water-soluble vitamin has been investigated [22, 23]. It should be noted in addition to ascorbic acid, synthesis of other NPs by using vitamins such as the B group has been reported [24, 25].

Biomolecule-mediated synthesis of ZnSe-NPs

Different biomolecules like proteins, peptides, lipids, polysaccharides, etc., can be used as biocompatible, eco-friendly, low toxic, bio reducing, capping agents, and serve as possible biocatalysts in NPs production process. These molecules can be immobilized on nanoparticles surfaces to prevent accumulation and also develop nanoparticle–biomolecule conjugates. Especially in clinical application, these conjugated structures based on their nature would be beneficial [26-28]. Until now, various types of nanostructures, including ZnSe, have been synthesized by applying peptide and hydrocarbon-based biomolecules [29-31].

ZnSe-NPs synthesis by microorganisms

Nowadays, different microorganisms due to extracellularly or intracellularly NPs synthesis ability have been studied. The tests showed bacteria, yeast, fungi, and plants can generate nano-scale particles [32]. The results revealed that some microorganisms could synthesize different forms of NPs, such as Metallic, Oxide, Sulfide, Compound, and other states. Also, it showed that synthesis conditions, the type of microorganism and, growth medium could affect the shape, size, and monodispersity of particles [33, 34]. Generally, nanomaterial synthesis by microorganisms is considered cost-effective, low toxic, and eco-friendly [35]. Due to the advantages of NPs production based on microorganism employment, the synthesized NPs can be used in different medical fields including gene therapy and drug delivery, to treat various cancer types [36]. In several studies, ZnSe synthesized by *Veillonella atypica,* a gram-negative bacteria, has been reported [35, 37]. Also, for the first time, Çakıcı *et al*. synthesized ZnSe nanoparticles via the biosyntheses method by applying a particular bacterium [38]. The ZnSe NPs synthesized by green synthesis methods are listed in Tab. 1.

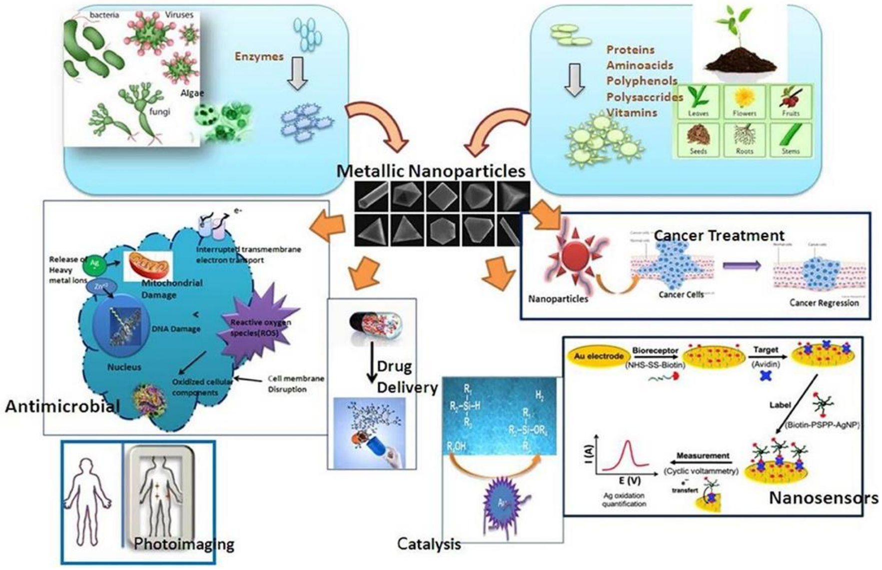

Fig. 2. Graphical illustration of different applications of green synthesized nanoparticles. Reprinted from [45] with permission

Biological applications of ZnSe-NPs

Nanoparticles, produced by green procedures, have received extensive attention for their applications in biomedical and physicochemical areas, including antibacterial activity, bioimaging, gene delivery, drug delivery, nanobiosensing, and catalysis [10, 32, 45]. Diverse applications of green synthesized nanoparticles are summarized in Fig. 2.

Antibacterial effect

So far, in several studies, this important application of ZnSe-NPs has been investigated. In our previous study antimicrobial activity of diverse doses of ZnSe-NPs on several gram-positive (*Staphylococcus epidermidis, Staphylococcus lugdunensis, Enterococcus faecalis, Staphylococcus aureus)*, and gram-negative (*Escherichia coli, Pseudomonas aeruginosa,* and *Enterobacter aerogenes*) planktonic bacteria were tested. The results showed that most gram-positive series had average susceptibility, but gram-negative bacteria exhibited high resistance to ZnSe NPs. Also, antibacterial activity was tested on *Staphylococcus aureus* biofilm form. The biofilm assay results indicated that biofilm form was quite resistant to different ZnSe-NPs concentrations [46]. In another work, the effect of ZnSe and ZnSe@ZnS core-shell QDs was performed against *Staphylococcus aureus* and *Escherichia coli* bacteria using broth dilution and disc diffusion tests. In a previous study, *Escherichia coli*, a gram-negative bacteria, was resistant, unlike Staphylococcus aureus, a gram-positive bacteria. Due to the non-toxic effects of fluorescence ZnSe NPs towards *E. coli,* it can be expected that these NPs can be used as microbial cell labeling agents to monitor microbial cell metabolism [47]. In a report by Soheyli (2018) *et al*. Fe-doped ZnSe(S)/ZnSe(S) core/shell QDs significantly affected gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*). Also, the antibacterial activity of this synthesized core/shell doped-QDs has been compared with some introduced antibiotics (*Ciprofloxacin, Tetracycline, and Penicillin*) based on the agar disk diffusion method. Each bacteria-related disc's growth inhibitory zone demonstrates that QDs antimicrobial activity against gram-positive was higher than gram-negative and even exhibited better antibacterial effect than tested antibiotics [48]. Fakhri et al. synthesized ZnSe QDs by wet chemical method and tested their activity on some gram-positive (*Bacillus megaterium, Staphylococcus aureus*) and gram-negative bacteria (*Micrococcus luteus, Pseudomonas aeruginosa*). The Minimum inhibitory concentration (MIC) value in their research confirmed the antibacterial activity of ZnSe QDs [49]. It should be mentioned NPs size and shape are important parameters that affect NPs activity against bacterial structures, especially biofilm ones. Generally, the exact mechanisms of NPs antibacterial activity are not precise, but the attempt to search nanostructures with this capability continues [50].

Cytotoxicity effect

One of the significant applications of NPs in medicine is their cytotoxicity effect and ability to remove cancer cells. On the other hand, non-toxic NPs can be a desirable option for use in biological imaging and further biological utilization. According to this importance, in several studies, cytotoxicity of ZnSe nanostructure on cancerous and normal cell lines has been searched. In a study, we examined in vitro cytotoxicity effect of different concentrations of ZnSe-NPs by MTT assay on Neuro 2A (neuroblastoma) and HeLa (cervical cancer) cells line. Results showed that cytotoxic dose for Neuro 2A and HeLa cells lines was up to 3.9 mg/mL and 7.8 µg/mL, respectively. Due to the non-cytotoxicity effect of ZnSe-NPs in low concentration, these NPs can be a proper candidate for biological activity [22]. Also, the cytotoxicity effect of ZnSe QDs on human skin fibroblast cells (WS1) and human colorectal carcinoma cells (HCT-116) have been studied by Mirnajafizadeh et al. the results of proliferation assay by using of Cell Counting Kit-8 (CCK-8) showed that both of cell lines were utterly viable at concentrations up to 0.5 g/L of ZnSe QDs [51]. Kumar Mahto et al. developed surface-modified CdSe/ZnSe QDs by two ligands, mercaptopropionic acid (MPA) and gum arabic (GA)/tri-n-octyl phosphine oxide (TOPO). Also, they examined the cytocompatibility of MPA-coated QDs and GA/TOPO-coated QDs on BALB/3T3 fibroblast cells by using Live/Dead Cytotoxicity Kit. The results showed surface modification could affect cytocompatibility of QDs [52]. To ZnSe cytotoxicity assay, Kiplagat et al. used the MTT method for testing the cytotoxicity effect of InP/ZnSe nanocrystals on KMST6 and MCF-12A, two type's noncancerous human cell lines. The results demonstrated that InP/ZnSe nanocrystals were low toxic and suitable for biological applications [53].

Labeling and biomedical imaging

Nowadays, the employment of NPs, especially QDs, as bioimaging agents is attractive. However, there are limitations due to the necessity of applying non-toxic ones in medical applications. Therefore, researchers have tried to develop biocompatible luminescent nanostructures. ZnSe-NPs with high luminescence efficiency can be a proper choice as a bioimaging agent. In a study by Saikia et al., ZnSe alloyed QDs (5 nm) as a novel, biocompatible, and highly luminescent imaging probes has been developed. To non-toxicity confirmation, they tested the cytotoxicity effect of synthesized QDs on mammalian leukocyte cultures by MTT assay [54]. Considering the impact of Mn-doped ZnSe QDs with magnetic and fluorescent properties loading into mesoporous silica (MSN) NPs, a fluorescent/MRI bioimaging has been done by Zhou et al. the fluorescence imaging results exhibited MSN@QDs better performance compared with both single and MSN-free single QDs [55]. In another investigation, cysteamine-coated ZnSe QDs as a fluorescent and positively charged structure were prepared by Moura et al. for bio labeling and bioimaging purposes. They tested the toxicity effect of QDs against macrophage cells, and viability assay was evaluated by the MTT method. Also, for testing labeling purposes, they applied human red blood cells and candida albicans cells. Biological labeling results obtained by fluorescence microscopy confirmed the process has been efficient [56]. In addition to the above studies, some research has been reported on using Mn/ZnSe d-dots as labeling agents of different cell lines with favorable results [57-59].

ZnSe-NPs as drug carrier agents

In recent years, NPs have been introduced as a powerful tool in the drug delivery systems. So far, the ability of different NPs as drugs transfer agents has been evaluated [60]. In an experiment, fluorescent chitosan-ZnSe/ZnS NPs potency as a drug carrier agent has been examined. Here (ZnSe/ZnS QDs) applied as fluorescent labeling, a cross-linking agent and, chitosan as an anti-cancer drug delivery vehicle was loaded by 5-fluorouracil (5-Fu). Also, an MTT assay test for cytotoxicity assessment and drug release test was performed. The results suggested that these ZnSe nano-based structures can be a proper candidate for biological labeling and drug transfer [61]. In addition to investigating the ability of drug delivery by ZnSe nanoparticles, their potential to transfer protein structures also has been considered. In a study, CdSe/ZnSe NPs coated with thiolated PAMAM dendrimer to transfer the S100A4, an EF-hand calcium-binding protein in NT2 cells investigated.

PAMAM dendrimers can facilitate penetration into the cell [62].

CONCLUSION

Considering their specific chemical and physical properties, semiconductor nanoparticles are being used in a wide variety of biomedical applications. Among these, ZnSe as an II-VI semiconductor with a wide bandgap and high luminescence efficiency have been synthesized by various methods and different forms. Generally, synthetic processes are essential because they can affect some characteristics of NPs like morphology, surface properties, size, etc. ZnSe NPs, dependent on their properties, can be used in many fields of science, including sensors, electronics, energy, and especially biology and medicine. Herein, we described some green chemical and biological synthesis approaches of ZnSe NPs using environmental-friendly and economical methods and reviewed the potential biological applications of ZnSe nanostructures.

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CONFLICT OF INTEREST

The authors declare no Conflict of interest.

Ethics Approval

For this type of study, the ethical approval was not.

AVAILABILITY OF DATA AND MATERIAL

Not applicable.

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