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Zinc oxide nanoparticles: A comprehensive review on its synthesis, anticancer and drug delivery applications

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ABSTRACT

Zinc oxide nanoparticles (ZnONPs), are becoming a more useful tool in a number of fields, including electronics, food packaging, optics, and most significantly, medicinal applications. These nanoparticles show a special capacity to target cancer cells specifically, dissolving into Zn^{2+} ions in an acidic environment. This leads to the generation of reactive oxygen species that specifically induce cytotoxic effects in malignant cells. Furthermore, ZnONPs work well as carriers to deliver certain anticancer medications straight into tumour cells. Growing interest in ZnONPs has prompted the creation of a variety of production methods, including chemical, physical, and environmentally benign biological approaches. This review explores the biomedical uses of ZnONPs and their production techniques, with an emphasis on the anticancer properties of the compounds. Detailed investigations into the mechanisms by which ZnONPs combat various cancers, influenced by their size, shape, and surface properties, are discussed. Additionally, their role in enhancing cancer treatment through the combined use of chemotherapy and photodynamic therapy, triggered by exposure to ultraviolet (UV) or near-infrared (NIR) light, is examined. The paper further explores the drug delivery capabilities of ZnONPs, including drug loading, stimulus-responsive controlled release, and therapeutic advantages. Finally, the future prospects of ZnONPs research and applications are considered, highlighting potential advancements and innovations.

1. Introduction

In recent decades, the term "nano" has garnered considerable attention from scientists worldwide. This term has profoundly altered the thinking of individuals who previously held the belief that only substantial measures are necessary to address important challenges. The development of nanotechnology commenced with a seminal address delivered by the eminent theoretical physicist, Richard Feynman, titled "There is plenty of room at the bottom," at the California Institute of Technology (Caltech) in 1959. Nanotechnology involves the production of materials with dimensions in the nanoscale range and their use in various fields [1]. Nanomaterials exhibit numerous enhanced chemical, thermal, optical, electrical, magnetic, and biological attributes in comparison to their bulk counterparts, mostly due to their significant surface area to volume ratio. Because nanoparticles can be utilised in a wide variety of therapeutic and theranostic applications, they are currently receiving a great deal of attention from researchers in the field of biology. Additionally, nanoparticles have the potential to be utilised as targeted gene and drug delivery systems in the field of

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biomedicine. In addition, nanoparticles can be utilized for the detection of bioactive chemicals or ions by fluorescence quenching, as well as for cancer photothermal therapy. This involves generating heat to destroy tumor cells by absorbing near-infrared radiation [2]. Nanoparticles of carbon, platinum (Pt), and silver (Ag), as well as metal oxides like iron oxide (Fe_3O_4), zirconia (ZrO_2), titania (TiO_2), and zinc oxide (ZnO), are very useful in the field of medicine [3]. Nanoparticles (NPs) can be produced in many nanostructure configurations, such as nanorods, nanotubes, nanoflowers, nanobelts, and so on. Adding stabilizers and biocompatible polymers to the nanoparticles (NPs) also keeps them from sticking together and lowers the chance that non-specific proteins will bind to their surface in living things, which also makes them less harmful. ZnONPs are a type of metal oxide nanoparticles that have gained attention for their use as a semiconductor nanomaterial. The biocompatible qualities of these materials allow them to be utilised in a wide variety of applications, including but not limited to electronic and optoelectronic devices, photocatalysis, cosmetic products, and especially in the field of biomedicine [4,5].

Zinc is a vital mineral for the human body and may be found in several sites such as the brain, muscles, bones, and other important organs [6]. This is achieved by activating many enzymes that play a role in the synthesis of nucleic acids and proteins within our bodies. Furthermore, it serves as an antioxidant, helps in the digestion of proteins, promotes blood coagulation, and provides support for bone metabolism. A shortage of this nutrient triggers the release of vitamin A from the liver. Under normal conditions (pH = 7.4), zinc is non-toxic due to its dependence on the amount of Zn^{2+} ions for toxicity [7]. Like elemental zinc, ZnONPs are also compatible with normal mammalian cells because they dissolve slowly. However, they cause oxidative stress and damage to cancer cells because they dissolve rapidly into Zn^{2+} ions at a slightly acidic pH. This means that ZnONPs exhibit cytotoxicity that is responsive to pH [8]. Additionally, ZnONPs have a wide variety of applications in the medical field, including tissue engineering, drug delivery systems, bioimaging, and the development of antibacterial, antioxidant, and antidiabetic medicines. It is possible to easily synthesise ZnONPs by making use of a wide variety of affordable starting materials.

Despite the burgeoning interest and extensive applications of ZnONPs in biomedical research, several challenges persist that curtail their broader implementation, particularly in oncology and drug delivery systems. Firstly, the synthesis methods of ZnONPs vary widely, each with its unique advantages and limitations, affecting the reproducibility and scalability of production. In addition, the specific toxicity of ZnONPs towards cancer cells, despite the fact that it is encouraging, calls for a more in-depth comprehension of the mechanisms by which these nanoparticles exercise their cytotoxic effects. These mechanisms include the influence of the nanoparticles' size, shape, and surface charge. Additionally, the dual functionality of ZnONPs as both therapeutic agents and drug delivery vehicles raises complex questions regarding their pharmacokinetics, biodistribution, and long-term safety. These challenges underscore the need for comprehensive studies that not only elucidate the underlying mechanisms of ZnONP action but also optimize their design for clinical applications. The main goal of this review is to thoroughly assess and compare the various synthesis methods of ZnONPs, emphasizing their efficiency, challenges, and scalability in clinical applications. Furthermore, the review aims to delineate the mechanisms behind ZnONPs' selective toxicity towards different cancer cell types, with a particular focus on how nanoparticle physicochemical properties—such as size, shape, and surface charge—affect their biological interactions and therapeutic efficacy. In addition, the study aims to thoroughly examine the role of ZnONPs in drug delivery. This includes assessing their ability to load drugs, release them in response to stimuli, and improve therapeutic results. The review also

aims to create guidelines for the development of ZnONPs as efficient vehicles for drug delivery. The integration of ZnONPs with other therapeutic modalities, such as photodynamic and chemotherapy, will also be explored to assess potential synergistic effects and the improvement of cancer treatment protocols. Finally, this review will address the existing research gaps and regulatory challenges, discussing the future trajectory of ZnONP applications in the medical field and the translation of laboratory findings into clinical practices.

Currently, there are just a few review publications available that provide a comprehensive explanation of the intricate mechanisms involved in the medicinal uses of ZnONPs, specifically in relation to their anticancer properties and drug transport capabilities. In this review paper, we will specifically examine the many techniques used to synthesize ZnONPs and explore their toxicity mechanisms on both cancerous and normal cells. In addition, we looked at the implications that their future applications could have in the field of drug delivery systems. We have completed a comprehensive analysis of the data gathered from both prior and ongoing research, as well as a review of the publications, in order to acquire a comprehensive understanding of their prospective applications in the aforementioned biological fields. In addition, the research and application of ZnONPs are put under the microscope in terms of their prospective orientation.

2. Synthesis of ZnONPs

Typically, there are two primary methods used for the production of nanoparticles: the top-down technique and the bottom-up approach. The top-down technique involves the reduction of bigger materials into smaller particles at the nano-scale. Conversely, the bottom-up strategy involves utilizing smaller entities such as atoms and molecules to construct NPs. In this discussion, we will focus exclusively on the techniques employed for synthesizing colloidal ZnO nanopowders. Several techniques have been utilized in the past to produce ZnONPs, including combustion, thermal decomposition, sol-gel process, and hydrothermal procedures. The most recent methods employed for the production of ZnONPs include ultrasonication, co-precipitation, and green synthesis [9,10]. In this section, we have examined the different techniques employed for the synthesis of ZnONPs, as illustrated in Figure 1.

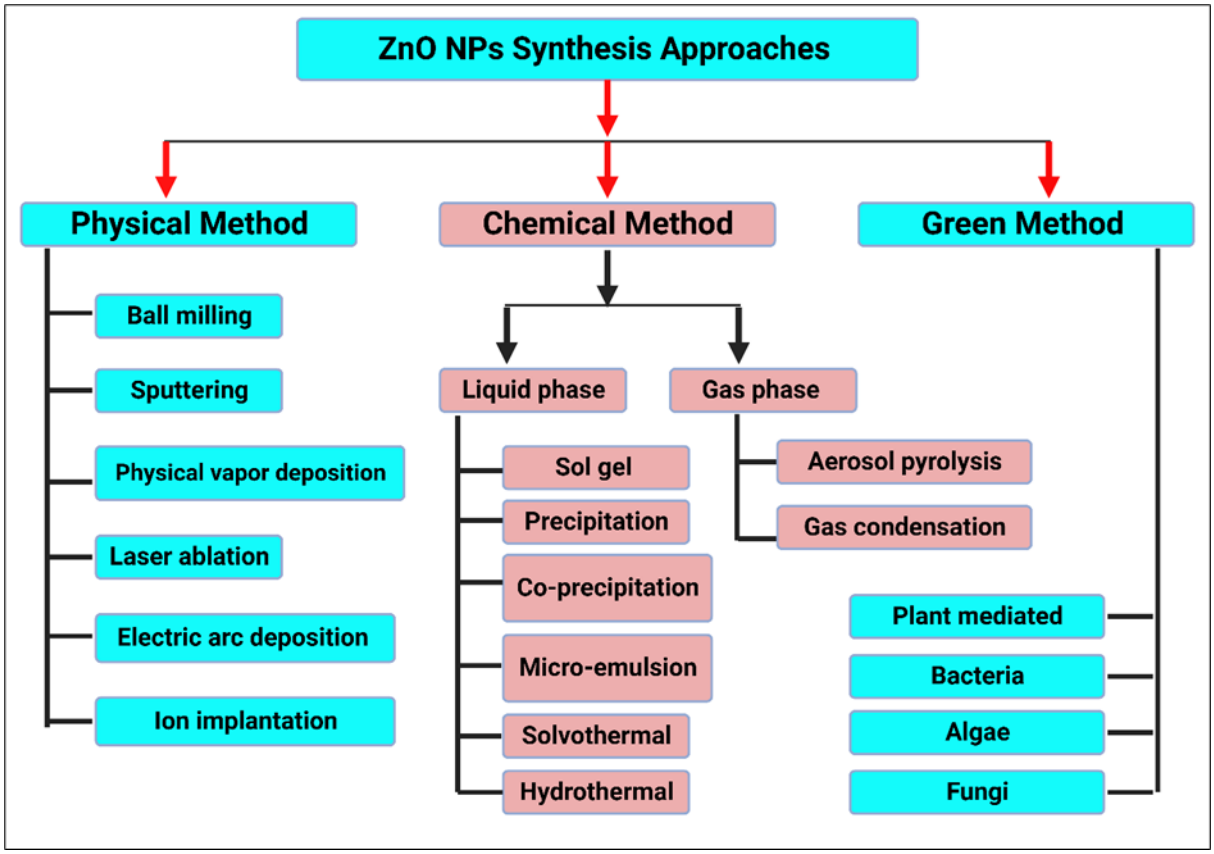

Figure 1. Summary of the top-down and bottom-up methods in the synthesis of ZnONPs.

2.1. Combustion method

For the production of metal oxide nanoparticles that are of an exceptionally high purity, the combustion process is a straightforward, efficient, quick, cost-effective, and energy-conserving technique. The self-propagating mode and the volume combustion mode are two distinct methods that can be employed to conduct combustion processes. These reactions can take place in the liquid, solid, or gas phases. In the first mode, the combustion process begins in a particular region by increasing the temperature to the point of ignition. Subsequently, the combustion process spreads over the entire reaction mixture through the process of self-sustained combustion throughout the initial mode. When the second mode is utilised, the entire reaction mixture is heated to the ignition temperature in a consistent manner. This causes the combustion process to begin concurrently in all areas of the mixture. In the year 2019, Shaat et al. [11] were able to create ZnONPs that were irregularly spherical and had an average size that ranged from 17.33 to 28.03 nm. The method of solution combustion was utilised, and the precursor ingredients that were utilised were zinc acetate dihydrate and urea. An initial strong agitation of the reaction mixture was performed in order to guarantee that all of the components were completely dissolved. The resulting precipitates were then subjected to heating in a Muffle furnace at a temperature of 500 °C for a duration of 15 minutes, resulting in the production of the nanopowder. In a further study conducted in 2020, Chandekar et al. [12] synthesized elliptical La-doped ZnONPs utilizing zinc nitrate hexahydrate, citric acid, and lanthanum nitrate hexahydrate as precursor materials. The reaction mixture underwent thermal treatment at a temperature of 550°C in a furnace for a duration of 4 hours, leading to the production of nanopowder particles with dimensions ranging from 46 to 74 nanometers.

2.2. Thermal decomposition

Thermal decomposition is the process of chemically breaking down the primary structural constituent. This is an economical, time-efficient, and user-friendly method for producing metal oxide nanoparticles on a large scale without the need for complex equipment or costly raw ingredients. Zinc oxide nanoparticles (ZnONPs) can be produced using either the solution or solid-phase thermal decomposition method. Following the initial thermal decomposition, both solution-based and solid-phase methods offer distinct pathways for synthesizing ZnONPs. In the solution-phase method, precursors are dissolved in a solvent that thermally decomposes to form nanoparticles. This approach allows for better control over the particle size and distribution, making it suitable for applications requiring uniformity in particle characteristics. On the other hand, the solid-phase method involves heating solid precursors, often leading to higher purity and crystallinity, which is crucial for applications in electronics and photonics. Both methods benefit from thermal decomposition's ability to scale up production, facilitating the manufacture of ZnONPs for commercial and industrial uses [13]. Little et al. [14] performed a later study in 2020 where they produced ZnONPs with a crystallite size ranging from 27 to 35 nm. This was achieved using the thermal breakdown of an aqueous solution of zinc oxalate, which involved a three-step process. First, the solution was heated to 100 °C in order to remove all of the water from it. After that, it was heated to 200 °C in order to completely remove all of the water. In the end, the powder was subjected to a heating procedure that lasted for six hours and was carried out at a temperature of 450 °C. This was done in order to accomplish complete disintegration.

2.3. Sol-gel method

The sol-gel process is a wet chemical approach that gives the resulting nanoparticles significant surface area and surface stability. There are two main steps in this method: (1) breaking down metal alkoxides through hydrolysis to make a colloidal solution (sol) in an acidic or basic environment; and (2) polymerizing the hydrolyzed substances to make a gel-like structure that is full of liquid. Moreover, the temperature is elevated in order to achieve material densification and facilitate the elimination of liquid, so promoting crystallization. Ba-Abbad et al. [15] made spherical nanoparticles of zinc oxide doped with samarium (Sm^{3+}) in 2017. They did this by mixing zinc acetate, oxalic acid, and samarium nitrate in an alcohol solution. The gel was synthesized by introducing oxalic acid into a combination of zinc acetate and samarium nitrate while stirring at a temperature of 65 °C. Ultimately, the gel was subjected to oven drying at a temperature of 80 °C for the duration of the entire night, followed by calcination at 400 °C for a period of 2 hours. The versatility of the sol-gel process allows for precise control over the chemical composition, porosity, and morphology of the final nanoparticles. This control is achieved through the manipulation of process variables such as temperature, pH, and the nature of the solvent and catalyst. The resulting nanoparticles are notable for their high surface area, which is crucial for applications where interaction with other substances is key, such as in catalysis and sensor technologies. Furthermore, the sol-gel process enhances the surface stability of nanoparticles, making them suitable for use in environments that might degrade less robust materials. This stability is critical in applications ranging from medical implants and drug delivery systems to protective coatings and environmental sensors. The process is also scalable, offering a cost-effective route to large-scale production of high-purity nanoparticles tailored for specific applications [16]. Figure 2 illustrates the sol-gel synthesis of ZnONPs, providing a clear visual representation of the process.

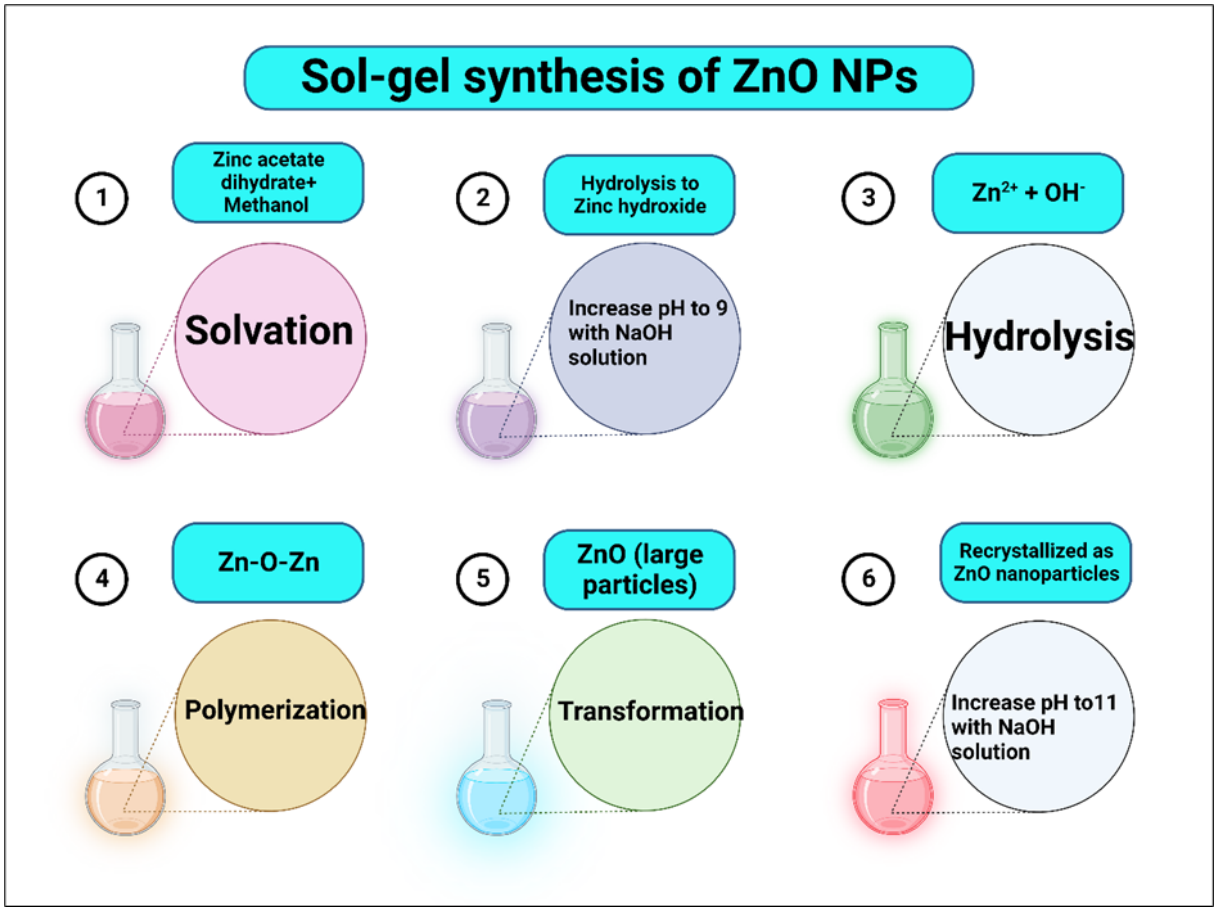

Figure 2. Pictorial representation of the Sol-gel synthesis of ZnONPs

2.4. Hydrothermal methods

The hydrothermal method is a widely used, cost-effective technique for synthesizing single crystalline metal oxide nanoparticles. This approach utilizes an aqueous solution under high pressure and temperature conditions, typically in a sealed vessel such as an autoclave. The process allows for the dissolution of precursors that would not normally dissolve under standard conditions, facilitating the growth of well-defined, single crystalline structures. One of the key advantages of the hydrothermal method is the ability to control the size, shape, and crystallinity of the nanoparticles by adjusting parameters such as temperature, pressure, reaction time, and the concentration of the solution. This precise control makes it particularly valuable for applications where the properties of the nanoparticles directly influence performance, such as in electronics, photonics, and catalysis.

The cost-effectiveness of the hydrothermal method stems from its use of water as a solvent, which is not only inexpensive but also minimizes the environmental impact compared to organic solvents. Furthermore, the high efficiency of this method allows for the direct synthesis of nanoparticles without the need for subsequent thermal treatments or purifications, which can further drive-up costs. Overall, the hydrothermal method is favored for its efficiency, environmental friendliness, and the high quality of the nanoparticles produced, making it a cornerstone technique in the field of materials science and nanotechnology.

In 2019, Agarwal et al. [17] used an aqueous ethanolic solution of zinc acetate dihydrate and citric acid monohydrate to make ZnO nanorods that were arranged in flower-like and floral shapes. The average crystallite size of these assemblies was measured to be 21 and 43 nm. Initially, the reaction mixture was

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agitated with a solution of sodium hydroxide and subjected to heat in an autoclave at a temperature of 150 °C for 17 hours and 19 hours, respectively. This process produced ZnO nanorods with dimensions of 2-3 micrometers in a flower-like structure, as well as floral assemblies of 5 micrometers. Ultimately, the solid substances were gathered, cleansed, and subjected to heat in an oven at a temperature of 80 °C for a duration of 12 hours.

2.5. Ultrasonication

The ultrasonication method is a simple, rapid, and extensively employed technique for synthesizing nanoemulsions that exhibit exceptional homogeneity and stability. It employs sound radiation with a frequency exceeding 20,000 Hz to either separate aggregated particles or decrease their size within the nanoscale range. The formation of bubbles, their subsequent expansion and collapse, and the subsequent high temperatures (5000 K) and pressures (1000 bar) that arise from the flow of ultrasound waves through the emulsion are all observed. It is the cavitation phenomena that is responsible for the necessary physical transformation, and in certain cases, the chemical transformation that occurs in conjunction with the physical transformation in some cases. A study by Yang et al. [18] in 2016 made spherical ZnO quantum dots (2.4 nm) by ultrasonically treating a solution of Zn (Ac)2 and LiOH in ethanol at temperatures ranging from 20 to 60 °C.

2.6. Co-precipitation

The co-precipitation method is a very practical, straightforward, and cost-effective technique for producing various metal oxide nanoparticles with a high output and purity. In this process, solid precipitates are made by mixing the precursor solution, which is made up of metal salts, with a precipitating agent, like an alkali. The precipitates are then washed to remove impurities and dried to obtain a solid powder. Occasionally, the metal hydroxides that have been created are subjected to calcination in order to obtain metal oxide nanoparticles. Adam and his colleagues [19] used the same method in 2018 to make spherical ZnONPs (140 nm) from a water solution that had zinc acetate dihydrate and sodium hydroxide in it. Initially, they agitated the solution for 2 hours at 60 °C to produce a solid substance. This substance was then rinsed with acetone and subsequently dehydrated in an oven at a temperature of 75 °C for 2 hours to yield ZnO nanopowder. Human ingenuity and precise chemical techniques were demonstrated in a 2020 study by Katiyar et al., where they successfully synthesized quasi-spherical ZnONPs measuring approximately 60 and 50 nm. They accomplished this through the co-precipitation method, employing zinc nitrate hexahydrate dissolved in ethanol as the precursor, and using KOH or NaOH as the precipitating agents. After forming the precipitates, they were isolated, thoroughly rinsed, and then air-dried at a temperature of 60 °C to produce the desired zinc oxide nanoparticles. This method highlights the potential of co-precipitation for generating nanoparticles with controlled size and shape, which are crucial for their application in fields such as medicine, electronics, and environmental science [20].

2.7. Microwave-assisted combustion

Microwave-assisted combustion is a rapid and efficient synthesis technique widely recognized for producing various metal oxide nanoparticles. This method leverages microwave energy to generate heat instantaneously and uniformly throughout the chemical medium. The rapid heating effectively reduces the synthesis time compared to traditional combustion methods and ensures a uniform heat distribution, which is crucial for achieving consistent particle sizes and shapes. The primary advantage of microwave-assisted combustion lies in its enhanced selectivity and chemical yield. The precise control of microwave energy allows for targeted heating, minimizing unwanted side reactions and improving the purity of the final product. This selectivity is particularly beneficial when synthesizing nanoparticles with specific properties required for high-performance applications in catalysis,

electronic devices, and energy storage systems. Furthermore, this method's efficiency and speed facilitate the large-scale production of nanoparticles, making it an attractive option for industrial applications. The reduced energy consumption and shorter reaction times also contribute to a lower environmental impact, aligning with sustainable manufacturing practices.

Saloga et al. [21] made oleate-coated ZnONPs (2.6–3.8 nm) in 2019 using a microwave-assisted hydrolysis method. The whole process took about 5 minutes. The first step was to synthesize zinc oleate by reacting sodium oleate with zinc (II) chloride. Following this, they heated the mixture with tetrabutylammonium hydroxide in a solvent system consisting of methanol and tetrahydrofuran at temperatures ranging from 125 to 200 °C using a microoven. In 2020, Ahammed et al. [22] produced spherical ZnONPs with a size range of 70–90 nm. The starting material was an aqueous solution of zinc nitrate hexahydrate. Ascorbic acid was used as a cap, and poly (vinyl alcohol) was used to keep the mixture stable. The reaction mixture was subjected to microwave irradiation at 400W for 3 minutes. The resulting precipitates were then dried in an oven at a temperature of 105 °C for a period of 3 hours. Subsequently, the dried precipitates were subjected to calcination at a temperature of 500°C for a duration of 4 hours in order to obtain the ZnONPs.

3. Anticancer activity

Cancer is a prominent contributor to global mortality [23]. Chemotherapy treatments that are already in use, like alkylating agents, antimetabolites, bioactive natural products, and other similar chemicals, don't always completely fight cancer. In addition, many therapy regimens are unable to differentiate between malignant and normal cells, resulting in widespread toxicity and negative consequences such as bone marrow suppression, neurotoxicity, and cardiomyopathy [24]. Thus, it is necessary to seek alternative chemotherapeutic drugs that exhibit a high level of selectivity towards cancer cells. Multiple researchers have reported the particular toxicity of ZnONPs towards cancer cells.

3.1. Cervical cancer

Zinc oxide nanoparticles (ZnONPs), with a size of approximately 10 nm, present a promising avenue for anticancer therapy, particularly in targeting human cervical carcinoma cells (HeLa). Recent studies have demonstrated that these nanoparticles can induce cytotoxic effects through mechanisms such as reactive oxygen species (ROS) generation, mitochondrial dysfunction, and the activation of apoptosis pathways. The diminutive dimensions of 10 nm ZnONPs enable heightened absorption by cells and interaction with biological constituents, potentially resulting in more efficacious suppression of cancer cell proliferation. This nano-dimensional approach to cancer therapy could revolutionize the treatment modalities for cervical carcinoma, offering a targeted, less invasive alternative to conventional treatments. The investigation into the specific cellular interactions and molecular mechanisms of ZnONPs provides a critical foundation for developing advanced therapeutic strategies in oncology.

In 2016, a study by Pandurangan et al. [25] explored how zinc oxide nanoparticles (ZnONPs) affect cancer cells. They tested small ZnONPs (10 nm) on human cervical cancer cells, using concentrations from 0.001 to 0.06 mg/mL over 48 hours. They found that the nanoparticles reduced the cancer cells' survival rate by 5% to 50%, depending on the dose. Interestingly, these nanoparticles didn't harm normal kidney cells from dogs, which maintained 95% viability even at the highest concentration.

In another study in 2017, Manshian et al. [26] compared the effects of pure ZnONPs and iron-doped ZnONPs (10-20 nm in size) on cancer. They discovered that adding iron (2-10%) slowed the release of zinc ions, reducing the negative effects on both normal and cancerous cells. Both pure and 2% iron-doped

ZnONPs were particularly effective against cancer cells, causing oxidative stress, mitochondrial damage, and triggering autophagy—a process that can lead to cell death. They also found that treating tumors in mice with 2% iron-doped ZnONPs was more effective and safer than using a higher concentration of iron. However, using pure ZnONPs without iron had severe side effects, including death.

In 2018, Wu and Zhang conducted a study on human cervical cancer cells (HeLa) to evaluate the anticancer properties of chitosan-coated (100 nm, spherical) and uncoated zinc oxide nanoparticles (ZnONPs, 30 nm, spherical). The cells were treated with various concentrations of these nanoparticles, ranging from 0.1 to 75 μg/mL, over a 24-hour period. Both the coated and uncoated ZnONPs showed minimal toxicity at low doses, with less than a 10% reduction in cell viability at up to 1 μg/mL. However, the chitosan-coated ZnONPs, which carry a positive charge, were more cytotoxic because they were more easily absorbed by the cells. This absorption increased the production of reactive oxygen species (ROS), leading to cell apoptosis. At the highest tested concentration of 75 μg/mL, these nanoparticles were able to completely kill the cancer cells [27].

In 2019, Gowdhami et al. [28] studied rod-shaped zinc oxide nanoparticles (ZnONPs, 20-50 nm) that were synthesized using environmentally friendly methods. They evaluated the effectiveness of these nanoparticles in inhibiting the growth of cervical cancer cells (SiHa) by combining them with an aqueous extract of the seaweed Gracilaria edulis. The study found that the ZnONPs had a cytotoxic effect on the cancer cells that increased with the dosage, using concentrations ranging from 20 to 200 μg/mL over a 24-hour period. The half-maximal inhibitory concentration (IC50) was determined to be 35 μg/mL, with nearly complete cell death occurring at the highest concentration of 200 μg/mL. Importantly, these ZnONPs showed no toxicity towards normal, healthy peripheral blood mononuclear cells even at 200 μg/mL. The researchers also observed that the ZnONPs triggered cell death in the cancer cells through mitochondria-dependent apoptosis and necrosis.

3.2. Breast cancer

Zinc oxide nanoparticles (ZnONPs) have emerged as a significant player in the realm of nanomedicine, particularly in the context of breast cancer treatment. Their unique physicochemical properties enable selective cytotoxicity against malignant cells without harming healthy tissue. Studies have shown that ZnONPs can induce apoptosis in breast cancer cells by generating ROS and disrupting mitochondrial function. Moreover, the size-dependent penetration capabilities of ZnONPs enhance their efficacy, allowing them to effectively target and accumulate within tumor sites. This targeted approach not only minimizes systemic toxicity but also improves the therapeutic index of anticancer treatments. The ongoing research into the mechanisms of ZnONPs' action against breast cancer underscores their potential as a transformative tool for advancing cancer therapy, warranting further investigation and clinical trials to fully harness their capabilities.

In 2015, Baskar et al. [29] developed a new material by combining small, spherical zinc oxide nanoparticles (ZnONPs, 28-63 nm) with a natural enzyme using a fungus called Aspergillus terreus. They tested this material on human breast cancer cells (MCF-7) to see if it could stop their growth.

In 2016, KC et al. [30] studied the effect of slightly smaller, quasi-spherical ZnONPs (about 18.67 nm) on a different type of breast cancer cell (MDA-MB-231). They treated the cells with varying amounts of nanoparticles, from 12.5 to 200 μg/mL, for 24 hours. At the lowest concentration, the nanoparticles were relatively safe and caused less than a 20% decrease in cell survival for both normal mouse cells and cancer cells. As the concentration increased, the nanoparticles began to break down the cancer cells' DNA, leading to their death. At the highest concentration, nearly all

the cancer cells were killed, while about 40% of the normal cells survived.

That same year, Krishna et al. [31] found that rod-like ZnONPs (250-500 nm) also had a toxic effect on MDA-MB-231 cancer cells, depending on the amount used. The cells were treated with doses ranging from 2.4 to 300 μg/mL for 24 hours. Cell survival was above 80% for lower doses up to about 9.4 μg/mL, but at the highest dose, 60% of the cells died. Importantly, even with daily doses given orally to mice at 2 g/kg for two weeks, there were no signs of toxicity from the nanoparticles.

In 2020, Khorsandi et al. [32] studied how small, spherical zinc oxide nanoparticles (ZnONPs, less than 80 nm) affected breast cancer cells (MCF-7) that respond to estrogen. They found that the toxicity of these nanoparticles towards the cancer cells depended on the concentration used, ranging from 5 to 20 μM/mL. Over 48 hours, the nanoparticles not only promoted cell death but also slowed down the movement of the cancer cells. At the highest concentration tested (20 μM/mL), about 55% of the cancer cells died, while normal breast cells (MCF-10A) remained unaffected. The study also revealed that the anti-cancer effects of the nanoparticles were due to the activation of several proteins including maspin, which blocks certain enzymes in the breast, and p53 and Bax, which promote cell death. At the same time, the nanoparticles reduced the levels of Bcl-2 and the estrogen receptor α, both of which are typically involved in cancer cell survival.

3.3. Lung cancer

Zinc oxide nanoparticles (ZnONPs) demonstrate significant potential in targeting and inhibiting the growth of human lung cancer cells, offering a new frontier in oncological treatments. The cytotoxic effects of ZnONPs are primarily attributed to their ability to induce oxidative stress within cancerous cells, leading to cellular apoptosis and reduced tumor viability. These nanoparticles preferentially accumulate in the acidic environments typical of tumor sites, enhancing their selectivity and effectiveness. Additionally, ZnONPs disrupt cellular signaling pathways critical for cancer cell proliferation and survival. Their nanoscale size facilitates deep penetration into the lung tissue, allowing for direct interaction with the tumor microenvironment. This tailored strategy not only promises to boost the therapeutic efficacy, but it also promises to lessen the unpleasant side effects that are associated with conventional chemotherapy. Therefore, ZnONPs are considered a promising choice for the treatment of lung cancer in future clinical settings.

In 2015, Selvakumari et al. [33] studied how spherical zinc oxide nanoparticles (ZnONPs, 16–19 nm) could potentially treat lung cancer using the A549 cell line, a type of human lung cancer cell. They treated these cancer cells with varying amounts of ZnONPs, from 7.8 to 1000 μg/mL, over a period of 72 hours. They observed that the survival rate of the cancer cells decreased as the concentration of nanoparticles increased. At the lowest concentration (7.8 μg/mL), cell viability dropped by 20%, and at the highest concentration (1000 μg/mL), over 90% of the cells died. Interestingly, the same high concentration did not significantly harm normal monkey kidney cells (VERO).

In 2019, Wu et al. [34] found that spherical zinc oxide nanoparticles (ZnONPs, 50 nm) are toxic to A549 lung cancer cells, with the level of toxicity varying based on the dose. This toxicity manifests as increased damage to the cell membranes and oxidative stress, along with a reduction in the energy-producing potential of the cell's mitochondria. The harmful effects were observed when cells were exposed to ZnONPs at concentrations ranging from 10 to 40 μg/mL for 24 hours. However, when graphene oxide (GO) was used alongside ZnONPs at concentrations of 1–10 μg/mL, it helped reduce these negative effects by decreasing the availability of the nanoparticles to the cells. The presence of ZnONPs significantly altered the cells' metabolic processes, including those involved in antioxidant defense (glutathione synthesis), energy production (tricarboxylic acid cycle), fat metabolism, and the making of nucleosides,

primarily due to the damage to the plasma membrane and oxidative stress.

3.4. Prostate cancer

ZnONPs are increasingly recognized for their potential in combating prostate cancer, showcasing a novel approach to targeted cancer therapy. The anticancer efficacy of ZnONPs against prostate cancer cells is primarily mediated through their ability to induce apoptosis and inhibit cell proliferation. This is facilitated by the oxidative stress generated by ROS production, which ZnONPs are known to enhance within the tumor microenvironment. Additionally, the small size of ZnONPs allows for efficient cellular uptake and deeper tissue penetration, thereby increasing their interaction with cancerous cells. Importantly, ZnONPs have demonstrated selectivity in inducing cytotoxic effects in malignant cells while sparing normal cells, which could significantly reduce the side effects associated with traditional chemotherapy. This selective toxicity, combined with their potential for functionalization with targeting moieties, underscores the promise of ZnONPs in developing more effective and less toxic treatments for prostate cancer.

In 2014, Priyadharshini et al. [35] studied how zinc oxide nanoparticles (ZnONPs, 66-95 nm, rod-like) made using environmentally friendly methods could affect prostate cancer cells. They used extracts from the seaweed Gracilaria edulis to see how these nanoparticles worked on human prostate cancer cells (PC3). They found that the toxicity of ZnONPs to cancer cells increased with the amount given, ranging from 10 to 100 μg/mL over 48 hours. Within this range, the nanoparticles initiated cell death (apoptosis) in the cancer cells but were less harmful to normal monkey kidney (vero) cells. The concentration at which half the cancer cells were killed (IC50) was 35 μg/mL, while it was 85 μg/mL for the normal cells.

In 2020, Rahimi et al. [36] also used green methods to create ZnONPs from the leaves of the Hyssop plant (Hyssopus officinalis). They tested these nanoparticles on the same type of prostate cancer cells. The results showed that the nanoparticles were toxic to the cancer cells in a way that depended on both the dose and the duration of exposure. The effective concentrations were quite low, between 4.88 and 6.25 μg/mL, with significant toxicity observed between 24 to 48 hours. The way these nanoparticles killed the cells involved both the early and late stages of apoptosis. After 24 and 48 hours, the concentrations needed to kill half the cancer cells were 8.07 μg/mL and 5 μg/mL, respectively.

3.5. Liver cancer

Zinc oxide nanoparticles (ZnONPs) hold substantial promise for enhancing the treatment of liver cancer, given their unique ability to selectively target and induce cytotoxic effects in hepatocellular carcinoma cells. The mechanism through which ZnONPs exert their antitumor effects involves the generation of ROS, which promote apoptosis and inhibit cell proliferation specifically in cancerous cells. This targeted action minimizes damage to surrounding healthy liver tissue, a critical consideration given the liver's central role in metabolism and detoxification. The small size of the nanoparticles makes it easier for them to be taken up and penetrate deeply into the tissue of the liver, which in turn increases their efficiency at the location of the tumour. The possibility of modifying the surface of zinc oxide nanoparticles (ZnONPs) in order to enhance their biocompatibility and target specificity opens up new doors for the development of personalised and less intrusive treatments for liver cancer. This focused strategy not only tries to improve treatment outcomes, but it also intends to lessen the systemic adverse effects that are typically associated with traditional chemotherapy.

In 2017, Ashokan et al. [37] studied zinc oxide nanoparticles (ZnONPs, 100 nm, rod-like) made using extracts from the leaves of the nutmeg tree (Myristica fragrans). They tested these nanoparticles on liver cancer cells (HepG2) to see if they could stop their growth. They found that the effectiveness of the nanoparticles depended on how much and how long they were used. At a lower dose of 10

μg/mL, about 90% of the cells were still alive, but at a higher dose of 35 μg/mL, 60-70% of the cells died. They measured the concentration needed to kill half the cells (IC50) as 22 μg/mL after 24 hours and 20 μg/mL after 48 hours.

In 2018, Ezhuthupurakkal et al. [38] also explored how zinc oxide (ZnO, 60-105 nm, rod-like) affected liver cancer cells (Huh-7 and HepG2). They observed that at a very low concentration (1 μg/mL), 60% of the cells survived, but increasing the dose to 20 μg/mL killed more than 95% of the cells in 24 hours. The ZnO increased the production of harmful oxygen molecules and DNA damage, and it decreased the energy output of the cells' mitochondria. It also increased levels of proteins that promote cell death while reducing levels of proteins that prevent it, indicating that the toxic oxygen molecules triggered cell death.

In 2020, Elje et al. [39] looked at the toxic and DNA-damaging effects of irregularly shaped zinc oxide nanoparticles (around 147 nm) on liver cancer cells using both traditional 2D and more complex 3D cell cultures. They found that the toxicity of the nanoparticles varied with the concentration, determining that the concentration needed for a half-maximal effect (EC50) was 10.1 μg/cm2 in 2D cultures and much lower, at 1.2 μg/cm2, in 3D cultures after 24 hours. They also noted a potential for DNA damage from the nanoparticles, although this was not clearly significant.

3.6. Colon cancer

Considering the one-of-a-kind anticancer capabilities that zinc oxide nanoparticles (ZnONPs) possess, there is reason to be optimistic about their prospective application in the treatment of colon cancer. Through the formation of ROS, these nanoparticles cause apoptosis and impede cell proliferation in colon cancer cells. This is accomplished by disrupting the functioning of the cells, which ultimately results in cytotoxic effects, particularly in malignant cells. The ability of ZnONPs to selectively target cancerous cells minimizes the collateral damage to the healthy cells lining the colon, which is critical for maintaining the integrity of the gastrointestinal tract. Furthermore, the small size of ZnONPs enables them to penetrate deeply into the tumor tissue, enhancing their therapeutic efficacy. This targeted approach not only promises to enhance the effectiveness of treatment but also to reduce the side effects and complications associated with traditional chemotherapeutic agents. The ongoing development and functionalization of ZnONPs to improve their selectivity and biocompatibility underscore their potential as a cornerstone in the next generation of colon cancer therapies.

In 2016, Namvar et al. [40] explored the anticancer effects of small, polygonal zinc oxide nanoparticles (ZnONPs, 3-8 nm) made using environmentally friendly methods. They incorporated extracts from the seaweed Sargassum muticum and hyaluronic acid to test the nanoparticles on human colon cancer cells (COLO205). They found that the effectiveness of the ZnONPs in killing cancer cells depended on the amount used, ranging from 1 to 100 μg/mL over 72 hours. At doses between 20 and 100 μg/mL, the nanoparticles killed about 70 to 80% of the cancer cells.

In a 2019 study, Majeed et al. [41] also used green methods to produce spherical zinc oxide nanoparticles (ZnONPs, 12-24 nm) from the leaf extract of the jackfruit tree (Artocarpus heterophyllus). They examined how these nanoparticles affected another type of human colon cancer cells (HCT-116). When exposed to concentrations ranging from 5 to 50 μg/mL for 24 hours, the ZnONPs significantly reduced the survival of the cancer cells in a dose-dependent manner, more so than in normal monkey kidney cells (Vero). The primary cause of death for the cancer cells was through a process leading to programmed cell death (apoptosis), with an estimated concentration to kill half the cells (IC50) being 20 μg/mL for the cancer cells and 30 μg/mL for the normal cells.

4. Drug delivery

Zinc oxide nanoparticles (ZnONPs) have been recognised as a highly favourable option for

precise delivery of drugs to specific targets. This is mostly due to their simple production using inexpensive starting materials, their biocompatible properties, and their ability to efficiently enter cells by endocytosis [42]. ZnONPs have the distinct benefit of being available in several nanostructure forms, including nanospheres, nanosheets, nanorods, nanobelts, and quantum dots [43]. There are a number of different types of interactions that are involved in targeted drug delivery that is mediated by nanoparticles [44]. These interactions include ligand-receptor recognition, hydrophobic interactions, and coulombic interactions. In addition, the release of drugs from nanoparticles is affected by a wide variety of stimuli-responsiveness parameters, including temperature, pH, enzymes, light, and biomolecules like glucose and GSH.

Up until now, many researchers have developed advanced methods using zinc oxide nanoparticles (ZnONPs) to deliver the cancer drug doxorubicin directly to cancer cells. In 2011, Muhammad et al. [45] studied how ZnO quantum dots (QDs) could be used to control the release of doxorubicin loaded inside another type of nanoparticle called mesoporous silica nanoparticles (MSN). They first packed the drug into the MSN's tiny pores. Then, they sealed these pores using ZnO quantum dots that were modified with a chemical group called amine (NH2-ZnOQDs). These quantum dots attached themselves to the outside of the MSN by forming strong chemical bonds with acid groups there.When these drug-loaded nanoparticles were introduced into HeLa cells, which are a type of cancer cell, the acidic environment inside the cells caused the ZnO quantum dot seals to break down. This breakdown triggered the release of doxorubicin right where it was needed inside the cells. Moreover, the zinc oxide nanoparticles themselves helped to kill the cancer cells, enhancing the overall anti-cancer effect.

In 2020, Chen et al. [46] created a pH-sensitive and biodegradable drug delivery system using mesoporous silica nanoparticles (MSN) that were coated with zinc oxide quantum dots (ZnOQDs). They started by loading the cancer drug doxorubicin (DOX) into the MSN's pores. These pores were then securely sealed with ZnOQDs, which formed covalent bonds to keep the drug contained. Once these nanoparticles entered 4 T1 cancer cells, the acidic environment inside the cells caused the ZnOQD seals to break down. This breakdown triggered the release of DOX directly into the cell's cytoplasm. Overall, this drug delivery system effectively combined the anticancer effects of both the drug and the nanoparticles, showing significant potential in treating 4 T1 cells.

Zinc oxide nanoparticle-based drug delivery systems (ZnONPs-based DDS) bring several advantages to medical treatments. Firstly, they keep drugs stable and prevent them from being released too early into the bloodstream, which helps to minimize the risk of side effects throughout the body. Secondly, they help drugs that typically don't dissolve well in water to become more soluble, improving how well these drugs work in the body. Thirdly, they can target specific cells or organs more directly, enhancing the effectiveness of the treatment. Lastly, these systems are usually safe and cause little or no harm to healthy tissues or organs.

Despite these benefits, most current research on ZnONPs-based DDS focuses on delivering the water-soluble cancer drug, doxorubicin. There's still a need for more real-world data on how effectively these systems can target specific cells in living organisms. Additionally, these systems often release drugs in response to the acidic environment inside the body.

There are mainly four types of zinc oxide nanoparticle-based drug delivery systems:

1. **Mesoporous Silica Nanoparticle-based DDS**: Uses acid-sensitive ZnONPs to block the pores of the nanoparticles after the drug is loaded inside.

2. **Porous ZnONPs**: Directly load drugs into the nanoparticle's pores.

3. **ZnO/Polymer Core-Shell Nanocomposite**: Encases drugs within a hydrophobic polymer shell.
4. **ZnONP/Drug Complex**: Forms stable bonds between the zinc ions in the ZnONPs and the oxygen-containing groups in the drug.

These systems are designed to improve how drugs are delivered and released in the body, targeting cancer cells more efficiently and with fewer side effects.

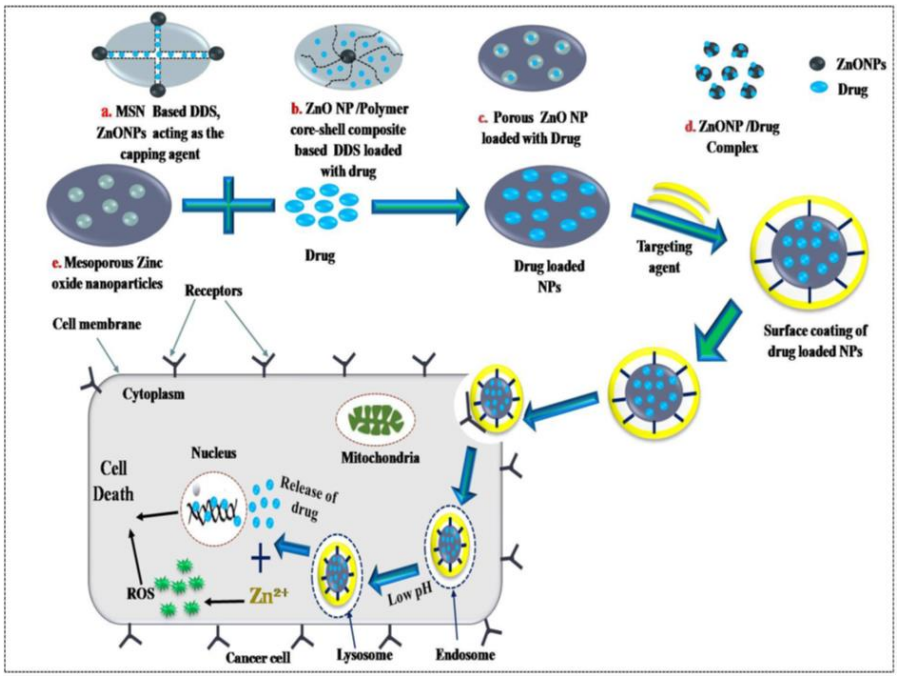

Figure 3. ZnONPs as anticancer drug delivery carrier [43].

6. Conclusion and future perspectives

This review article takes a detailed look at the various methods that can be utilised to synthesise zinc oxide nanoparticles (ZnONPs). These methods include chemical, biological, and physical approaches. This article also emphasises the substantial medicinal uses of zinc oxide nanoparticles (ZnONPs), with a particular emphasis on their utilisation in anticancer treatments and drug administration. There is a correlation between the pH of the surrounding environment and the cytotoxicity of zinc oxide nanoparticles, often known as ZnONPs. The breakdown of zinc oxide nanoparticles (ZnONPs) into zinc ions (Zn2+) yields this cytotoxic effect. Furthermore, this characteristic of ZnONPs is extremely useful in efficiently targeting and eliminating cancer cells in a specific manner. Additionally, ZnONPs have shown that they have the potential to be utilised in the field of individualised drug delivery systems. We are primarily focused on the mechanistic techniques that are the foundation of the biological uses of zinc oxide nanoparticles (ZnONPs). In the end, we investigated the potential adverse effects that ZnONPs could have on a wide variety of vital organs in our bodies by exposing them to a variety of different routes of exposure. Nevertheless, in order to further research the safer methods of synthesising ZnONPs and the medical applications of these compounds, it is required to analyse the following points:

- In the process of designing the chemical synthesis of ZnONPs, it is important to avoid the use of hazardous surfactants and expensive precursors.

- Investigation into the mechanism of ZnONP production by environmentally friendly synthesis techniques is recommended.
- For the purpose of creating ZnONPs that have a high level of stability, alternative green synthesis procedures should be utilised.
- The anticancer properties of zinc oxide nanoparticles (ZnONPs) ought to be thoroughly examined by employing suitable in vivo models.
- For the purpose of establishing zinc oxide nanoparticles (ZnONPs) as versatile drug delivery vehicles for the targeted distribution of hydrophilic, hydrophobic, and immunomodulatory medicines for the treatment of cancer, additional research needs to be conducted.
- In addition to the pH-responsiveness approach, the possibility for drug release should be investigated using ROS, ultrasonic, MW, and light-triggered methods.
- Additional risk studies of ZnONPs across the various exposure pathways are required in order to investigate the clinical application of this substance.

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