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Review Article

Green Synthesized Zinc Oxide Nanoparticles: A Comprehensive Review

Akash Nayaka M.*, Nimisha Jain

Department of Pharmaceutics, Krupanidhi College of Pharmacy, Bangalore.

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ABSTRACT

Zinc Oxide nanoparticles refer to a white powder compound that is insoluble in water and has excellent chemical, electrical, and thermal stabilities. It is widely used in various applications such as solar cells, photocatalysis, chemical sensors, biomedical field, and ceramic industry due to its optical, electrical, and photocatalytic properties. They are typically less than 100 nanometers in diameter, with a broad size distribution range of 30-150 nanometers. Zinc oxide nanoparticles (ZnO NPs) have recently attracted considerable attention due to their wide bandgap, high exciton binding energy, and various potential applications, such as antibacterial, antifungal, anti-diabetic, anti-inflammatory, wound healing, antioxidant, and optical properties. Traditional physical and chemical methods for synthesizing ZnO nanoparticles often involve toxic chemicals and extreme conditions, raising concerns about their safety and environmental impact. To address these issues, green synthesis techniques, utilizing natural resources such as plants, fungi, bacteria, and algae, have been explored as eco-friendly alternatives. The distinctive properties of ZnO nanoparticles have led to them being extensively been utilized in the cosmetic industry especially in skin whitening and anti - wrinkle products. Additionally, they are also extensively employed in sunscreens and other UV protective formulations in view of the fact that they get effectively absorbed or scatter the harmful ultraviolet radiation. While evaluating their skin penetration and life-long accumulation of DNA as well as systemic toxicity, more data is required to prove safety of their application. For further studies, mechanism action should be known properly, formulations would be done in a condensed and safe manner and safety problems are solved, then the ZnO nanoparticles can help to reach their full potential in cosmetics and other applications of nanotechnology.

INTRODUCTION

Zinc oxide (ZnO) is an n-type semiconducting metal oxide that has achieved significant attention in current years due to its diverse applications in

***Corresponding Author:** Akash Nayaka M.

Address: *Department of Pharmaceutics, Krupanidhi College of Pharmacy, Bangalore.*

Email ✉: akashnayaka2001@gmail.com

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electronics, optics, and biomedicine. Among diverse inorganic metal oxides, such as titanium dioxide (TiO2) and copper oxide (CuO), ZnO stands out because it is cost-effective, safe, and easy to produce. The US Food and Drug Administration (FDA) has classified ZnO as a Generally Recognized as Safe (GRAS) substance. ZnO nanoparticles (NPs) demonstrate great semiconducting properties, owing to their large band gap of 3.37 eV and high exciton binding energy of 60 meV. These characteristics contribute to their catalytic activity, optical performance, UV filtration capabilities, anti-inflammatory effects, and wound healing properties. Due to their UV-filtering abilities, ZnO NPs are widely used in cosmetic products, particularly in sunscreen lotions. They have multiple biomedical applications, including drug delivery, anti-cancer and anti-diabetic treatments, antibacterial and antifungal uses, as well as agricultural applications. Nevertheless, the challenge of cytotoxicity in targeted drug delivery remains a concern that needs addressing. Studies have revealed that ZnO NPs exhibit strong antibacterial properties at low concentrations against both gram-negative and gram-positive bacteria, often surpassing the effectiveness of chemically synthesized ZnO NPs. Beyond biomedical uses, ZnO NPs are utilized in industries such as rubber manufacturing, paint production, and water purification, particularly for removing sulfur and arsenic. They also find applications in protein adsorption and dentistry. ZnO NPs show piezoelectric and pyroelectric properties and have proven useful in controlling resistant aquatic weeds that are difficult to control with traditional methods. Likewise, ZnO NPs can be synthesized in various morphologies, including nanoflakes, nanoflowers, nanobelts, nanorods, and nanowires.(1) (2)

Delivery system and enhanced efficacy

Although Zinc Oxide (ZnO) has been extensively studied across various research fields, its use as a drug delivery system has only gained significant attention in the past decade, and it is still considered to be in its early stages of development (Figure 1.1). In recent years, numerous studies have focused on advancing ZnO-based materials as drug carriers, driven by their exceptional biocompatibility, which has renewed interest in this remarkable material.(3)

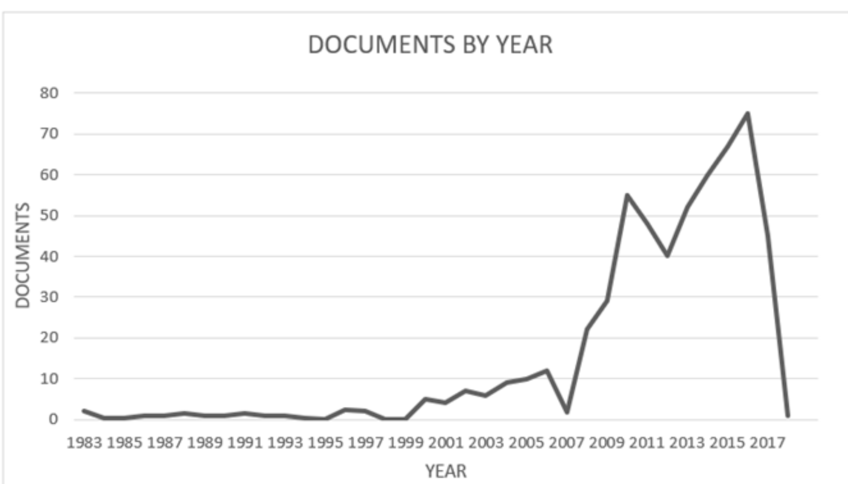


Figure 1.1 Analysis of the number of documents related to the use of Zinc Oxide as drug delivery system, in the range 1983-2018.

Enhanced stability

Zinc Oxide nanoparticles help preserve the stability of active ingredients by shielding them from degradation, oxidation, or evaporation, ensuring that they retain their potency and effectiveness over the product's shelf life.[4]

Improved penetration

Zinc Oxide nanoparticles possess distinctive properties, such as their tiny size and high surface area, which allow them to penetrate the skin more efficiently. These nanoparticles can bypass the skin's natural barrier and deliver active ingredients to targeted layers like the epidermis or dermis, where they can produce their therapeutic effects.[5] The role of ZnO as a skin penetration enhancer has also been studied. This application consists in the simultaneous skin administration of ZnO with another compound (i.e. drug) and not in its direct use as a drug carrier. Shokri N. has been one of the pioneering scientist in this field. He dedicated significant research works to the study of ZnO as a skin enhancer in topical formulations. In 2014, ZnO and ZnO nanoparticles were compared as absorption enhancers for Ibuprofen, so demonstrating that both compounds could act as enhancers for transdermal delivery of Ibuprofen, with a higher efficiency for the ZnO nanoparticles. In the same year, Shokri and coworkers also investigated the enhanced skin permeation of solvents and surfactants due to the co-utilization of ZnO nanoparticles in topical products. ZnO resulted able to increase the permeation of hydrophobic, oily and hydrophilic solvents and surfactants, which indicated a potential role in the co enhancement of drug absorption. The aim of the research work that was published two years ago by the same authors consisted in the evaluation of the skin permeation of albumin when co-administered with ZnO nanoparticles in comparison with Calcium Phosphate. In their work, the nanoparticles were not used as drug carriers, but were simultaneously administered separately with the Albumin, but separated from it. Their enhancer effect depended on their position and stay in the skin layers, which helped the drug to cross the skin. Results showed that the enhancer effect of Calcium Phosphate nanoparticles was weaker than that of ZnO, because of the different skin distribution and solubility of the particles. [3]

Increased Bioavailability

Zinc Oxide nanoparticles can increase the bioavailability of active ingredients by enhancing their solubility and dissolution rate. By loading or encapsulating active ingredients within metal nanoparticles, these ingredients can overcome challenges like poor water solubility, making them more easily absorbed and utilized by the skin.[6] *L'Oreal S.A*, one of the biggest world-famous brands has achieved maximum patents ranking sixth in the market use of ZnO nanoparticles in their many products, whereas Shiseido has limited the use of ZnO in emulsion-based formulas used in gels, lotions, and creams with the ultimate goal of enhancing the moisturizing power. [3]

Synergistic effect

In certain instances, Zinc Oxide nanoparticles may offer direct benefits for the skin, including antioxidant and antimicrobial properties. When paired with active ingredients, they can produce synergistic effects, enhancing the overall effectiveness of the cosmetic formulation.[7] Nanotechnology has expanded into a broad range of clinical applications. In particular, metal nanoparticles (MNPs) display unique antimicrobial properties, a fundamental function of novel medical devices. The combination of MNPs with commercial antimicrobial drugs (e.g., antibiotics, antifungals, and antivirals may offer several opportunities to overcome some disadvantages of their individual use and enhance effectiveness. MNP conjugates display multiple advantages. As drug delivery systems, the conjugates can extend the circulation of the drugs in the body, facilitate intercellular targeting, improve drug stabilization, and possess superior delivery. Concomitantly, they reduce the required drug dose, minimize toxicity, and broaden the antimicrobial spectrum. In this work, the common strategies to combine MNPs with clinically used antimicrobial agents are under scored. [7]

Targeted Delivery

Zinc Oxide nanoparticles can be modified with ligands or antibodies that selectively bind to specific skin receptors or cells. This enables targeted delivery of active ingredients to particular areas or cell types, boosting their effectiveness and minimizing potential side effects.[8] *Gupta S et al.,* reported that recent advances have shifted our focus to inorganic nanoparticles for specific targeting and control of their cellular actions. Being inorganic, they remain stable for long periods. Inorganic nanoparticles generally possess versatile properties suitable for cellular delivery, including wide availability, rich functionality, good biocompatibility, potential capability of targeted delivery (e.g., selectively destroying cancer cells but sparing normal tissues) and controlled release of carried drugs. These show advantages not only in the cosmetics area, such as in anti-aging and anti-acne treatments, and hydration and skin care products, but also in the treatment of skin diseases such as skin cancer and vitiligo, and for transdermal delivery of substances. Nanosized particles solve the cosmetic drawback of these opaque sunscreens, microsized TiO_2 and ZnO have been increasingly replaced by TiO_2 and ZnO nanoparticles (NPs). [8] Going through the literature, it has emerged that few research works have been dedicated to the evaluation of ZnO as a material for drug delivery to the skin. During the International Conference and Exhibition on Nanomedicine and Drug Delivery (May 29-31, 2017 Osaka, Japan) Markus and colleagues presented a poster focused on the development of a drug carrier for topical delivery composed by ZnO nanocomposites functionalized by hyaluronic acid or carboxymethyl chitosan. The resulting flower-shaped nanocomposites were further loaded with a vegetal extract and functionalized with ginsenoside Rh2. The properties of the drug delivery system were deeply characterized, which resulted a promising carrier for the topical delivery of active substances to treat skin disorders and prevent skin cancer. [3]

Photo-protection

Metal nanoparticles and their oxides can also offer photoprotection in cosmetic formulations. They function as physical sunscreens by absorbing or scattering harmful UV radiation, thus limiting its penetration into the skin.
Zinc Oxide (ZnO) Nanoparticles: ZnO nanoparticles are widely used in sunscreens due to their ability to provide broad-spectrum UV protection, reflecting and scattering both UVA and UVB rays. They are often favoured for their enhanced photostability and lower risk of skin irritation.[4]. *Jose manuel et al* reported that “The use of sunscreens is essential for preventing skin damage and the potential appearance of skin cancer in humans. Inorganic active components such as zinc oxide (ZnO) have been used commonly in sunscreens due to their ability to block UVA radiation. This ultraviolet (UV) protection might be enhanced to cover the UVB and UVC bands when combined with other components such as titanium dioxide (TiO_2). In this work we evaluate the photoprotection properties of organic nanoparticles made from lignin in

combination with ZnO nanoparticles as active ingredients for sunscreens. Lignin nanoparticles were synthesized from Agavetequilana lignin. Two different pulping methods were used for dissolving lignin from agave bagasse. ZnO nanoparticles were synthesized by the precipitation method. All nanoparticles were characterized by SEM, UV–Vis and FT-IR spectroscopy. Nanoparticles were mixed with a neutral vehicle in different concentrations and in-vitro sun protection factor (SPF) values were calculated. Different sizes of spherical lignin nanoparticles were obtained from the spent liqors of two different pulping methods. ZnO nanoparticles resulted with a flake shape. The mixture of all components gave SPF values in a range between 4 and 13. Lignin nanoparticles showed absorption in the UVB and UVC regions which can enhance the SPF value of sunscreens composed only of Zinc oxide nanoparticles. Lignin nanoparticles have the added advantage of being of organic nature and its brown color can be used to match the skin tone of the person using it".[9]

Green synthesis

Green synthesis of ZnO NP's using plant extract

Plant parts such as leaves, stems, roots, fruits, and seeds are commonly used for the synthesis of ZnO nanoparticles due to the unique phytochemicals they contain. Utilizing natural plant extracts for this process is eco-friendly, cost-effective, and does not require the use of intermediate base groups. It is a quick method that eliminates the need for expensive equipment or precursors, resulting in a highly pure and abundant product free of impurities. Plants are a preferred source for nanoparticle synthesis as they enable large-scale production of stable nanoparticles with varying shapes and sizes. Bio-reduction involves the reduction of metal ions or metal oxides to zero-valence metal nanoparticles through the action of phytochemicals like polysaccharides, polyphenolic compounds, vitamins, amino acids, alkaloids, and terpenoids secreted by the plants.[10]

Green synthesis of ZnO NP's using bacteria

NP synthesis using bacteria is considered an environmentally friendly approach, but it comes with several drawbacks. The strategy of screening microbes is time-consuming and directs careful monitoring of the culture broth to prevent contamination. Similarly, there is limited control over the size and shape of the nanoparticles produced. Additionally, the cost of the media required to grow bacteria can be quite high.

Green synthesis of ZnO NP's using microalgae and macroalgae

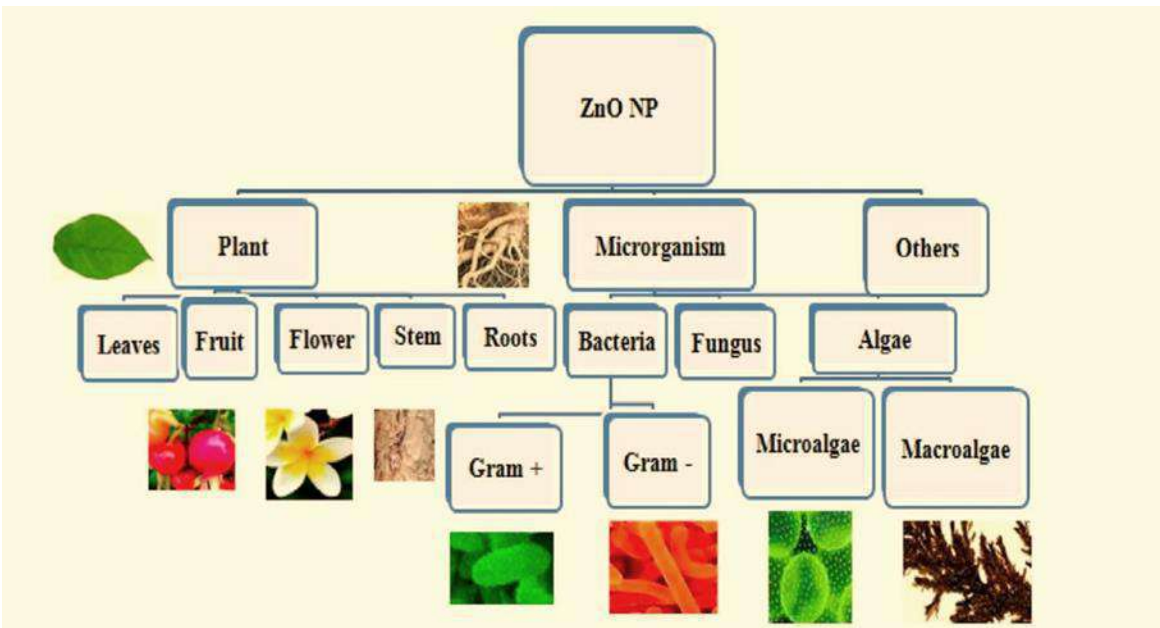
Algae are photosynthetic organisms that can be seen in both unicellular forms, such as Chlorella, and multicellular forms, such as brown algae. They do not own the basic structures typically associated with plants, like roots and leaves. Marine algae are categorized based on the pigments they contain: Rhodophyta has a red pigment, Phaeophyta has a brown pigment, and Chlorophyta has a green pigment. While algae have been broadly used for the synthesis of gold (Au) and silver (Ag) nanoparticles, their application in the synthesis of zinc oxide (ZnO) nanoparticles is less analyzed and has been reported in rarer studies. Microalgae, in particular, attract attention due to their ability to degrade toxic metals and transform them into less harmful forms.[11]

Green synthesis of ZnO NP's using fungus

Extracellular synthesis of nanoparticles using fungi is highly advantageous due to its potential for large-scale production, straightforward downstream processing, and cost-effectiveness. Fungal strains are preferred over bacteria due to their superior tolerance and ability to bioaccumulate metals.

Green synthesis of ZnO NP's using other green sources

Biocompatible chemicals serve as another green source for nanoparticle synthesis. This method is fast, cost-effective, and avoids the production of by-products during the nucleation and synthesis of nanoparticles. It enables the formation of nanoparticles with controlled shape and size, along with a well-dispersed nature. Nanoparticles synthesized through the wet chemical method exhibit unique properties, such as enhanced antibacterial efficiency of up to 99.9% when applied to cotton fabric.



Bacteria mediated synthesis of ZnO NP:

S.no	Bacterial strain	Family	Size (nm)	Shape	Reference
1	*Aeromonas hydrophila*	Pseudomonadaceae	57.72(AFM) 42-64(XRD)	Spherical, oval	(12)
2	*Lactobacillus sporonges*	Bacillaceae	5-15(TEM) 11(XRD)	Hexagonal unit cell	(10)
3	*Pseudomonas aeruginosa*	Pseudomonadaceae	35-80(TEM) 27(XRD) 81(DLS)	Spherical	(13)
4	*B.licheniformis*	Bacillacea	200 with nanopetals 40 in width and 400 in length (TEM)	Nanoflowers	(18)

Plant mediated synthesis of ZnO NP:

S. No	Plant (Family)	Common name	Part taken for extraction	Size (nm)
1	*Azadirachta indica*(melliacea)	Neem	Fresh leaves	18(XRD)
2	*Cocus nucifera* (Arecacea)	Coconut	Coconut water	20-80(TEM) 21.2(XRD)
3	*Centella Asiatica* (umbellifera)	Pennywort	leaves	13(XRD)
4	*Gossypium* (Malvacea)	Cotton	Cellulosic fibre	13(XRD)
5	*S. album* (Santalacea)	Sandalwood	leaves	38.17(XRD)10-30(DLS)

Synthesis of ZnO NP using algae:

S. No	Algal strain	Family	Size (nm)	Shape	Reference
1	*Chalmydomonas reinhardtrii*	Chlamydomonaceae	55-80(HR-SEM) 21(XRD)	Nanorod, Nanoflower	(17)
2	*Sargassum muticum*	Sargassaceae	30-57(FE-SEM)	Hexagonal wurtzite	(15)
3	*S. myriocystum*	Sargassaceae	46.6(DLS) 20-36(AFM)	Spherical, radial, triangle	(18)

UV protection and sunscreen activity

In non-commercial research on sunscreens containing nanoparticles (NPs), safety concerns primarily focus on skin penetration studies. However, the safety and effectiveness of NP-based sunscreens are also influenced by the physicochemical properties of the nanoparticles, their coatings, formulations, skin interaction, and how these components interact with UV radiation. Currently, the lack of comprehensive physicochemical characterization of commercial NP sunscreens hinders study design in this area. Replacing micro-sized TiO_2 and ZnO particles with nanoparticles enhances the desired cosmetic transparency of the sunscreen but may reduce broad UV-A protection. Using micro- and nanosized ZnO dispersions (20–200 nm) alongside nanosized TiO_2 particles (~20–35 nm) could help address this issue. When the skin is exposed to NP sunscreens, TiO_2 and ZnO nanoparticles can penetrate into the deepest layers of the stratum corneum (SC) and hair follicles, potentially serving as long-term reservoirs. Within the skin, NP aggregation, as well as physicochemical interactions between the particles, skin, and UV light, affect the overall UV attenuation efficacy, a complex process that is still underexplored. Even in the absence of light, the production of reactive oxygen species (ROS) can result in cytotoxicity and genotoxicity. Anatase TiO_2 exhibits the highest photocatalytic activity compared to rutile TiO_2 and ZnO nanoparticles. Coating the nanoparticles can reduce toxic effects, especially with silica-based coatings, but it cannot entirely eliminate them. ZnO nanoparticles in sunscreens are rarely observed in viable skin layers, even after long-term exposure, and usually in low concentrations. TiO_2 has attracted more scientific attention than ZnO. The development of NP-based sunscreens requires careful attention, best achieved through close collaboration between scientific institutions and sunscreen manufacturers. To minimize risks and optimize the efficacy of NP sunscreens, further research should focus on sub-chronic (sunburned) human skin exposures, as well as the photo-stabilization and size optimization of nanoparticles. Additionally, it is preferable to prevent ROS production rather than simply quenching its effects.(16)

Anti-microbial activity of ZnO NP's

The antibacterial properties of ZnO nanoparticles (NPs) are believed to result from their ability to induce oxidative stress, disrupt cell membranes, and interfere with respiratory enzymes through interaction with Zn(I) ions. This interaction generates reactive oxygen species (ROS) and free radicals, causing irreversible damage to bacterial mitochondria, DNA, and membranes. NPs can penetrate cell membranes more easily than larger particles, allowing them to directly interact with intracellular components, which enhances ROS production and leads to further cellular damage. The size of the nanoparticles also influences their uptake by cells via endocytosis, with smaller ZnO NPs being more efficiently absorbed and distributed throughout the cytoplasm and organelles, including mitochondria. Once inside, these NPs can cause mitochondrial dysfunction, resulting in additional ROS production and triggering cell death pathways such as apoptosis or necrosis. The main mechanism of ZnO NP toxicity is oxidative stress caused by ROS, with smaller nanoparticles generating higher levels of ROS, overwhelming the cell's antioxidant defenses. This imbalance leads to oxidative damage to lipids, proteins, and DNA. Elevated ROS levels can also activate inflammatory pathways, and due to their higher ROS production, smaller ZnO NPs induce stronger inflammatory responses, causing the release of pro-inflammatory cytokines. Chronic inflammation can result in tissue damage and contribute to diseases such as cancer. Additionally, the ROS generated by smaller ZnO NPs can cause DNA damage, leading to mutations and chromosomal abnormalities. This genotoxicity can result in cell cycle arrest, apoptosis, or uncontrolled cell proliferation, all of which play a role in carcinogenesis.(17)

Safety concerns

Although ZnO nanoparticles hold great promise for use in cosmetology, their application also brings certain challenges and requires further investigation. A primary concern is the safety of metal nanoparticles in cosmetic formulations. While numerous studies have highlighted their potential benefits, it is essential to conduct comprehensive evaluations of their long-term effects, including possible skin penetration, accumulation, and systemic toxicity. More research is needed to better understand the biological interactions and potential risks related to the use of metal nanoparticles in cosmetic products.(18)

Future work

Standardization and Regulation:

Standardizing manufacturing processes, characterization methods, and safety assessment protocols for ZnO nanoparticles in cosmetic products is crucial. Specifying clear guidelines and regulations for their use will assist ensure uniform product quality, effectiveness, and safety.

Environmental Impact:

The possible release of ZnO nanoparticles from cosmetic products into the environment increases concerns about their impact on ecosystems and living organisms. Research should concentrate on understanding their behavior, fate, and potential ecological effects. Further, it is important to develop strategies for their sustainable use and disposal.

Optimization of Formulation:

Additional research is required to optimize the formulation of ZnO nanoparticles in cosmetic products. This involves exploring ways to improve their stability, control their release rates, enhance compatibility with other ingredients, and reduce any potential negative impacts on product aesthetics, such as undesired colour changes or texture modifications.

Efficacy and Mechanism of Action:

Further research is required to evaluate the effectiveness and mechanisms of action of ZnO nanoparticles in cosmetic applications. This research should include an investigation of their interactions with the skin, their capability to deliver active ingredients efficiently, and their impact on various skin conditions and concerns.

CONCLUSION

The inclusion of ZnO nanoparticles in cosmetic formulations has led to notable advancements in the beauty industry. ZnO nanoparticles exhibit antimicrobial properties that offer effective solutions for preventing microbial growth, reducing infections, and prolonging the shelf life of cosmetic products. Their mechanisms of action include the release of ions that interfere with vital cellular processes in microorganisms. Additionally, ZnO nanoparticles act as efficient carriers for active ingredients in cosmetic formulations. They improve stability, enhance skin penetration, enable controlled release, increase bioavailability, and allow for targeted delivery. By using ZnO nanoparticles as delivery systems, cosmetic products can achieve greater efficacy and optimize the effects of active ingredients. ZnO nanoparticles also provide photo-protection in cosmetics, functioning as physical sunscreens by absorbing or scattering harmful UV radiation. Zinc oxide, like titanium dioxide and iron oxide nanoparticles, delivers broad-spectrum UV protection, protecting the skin from both UVA and UVB radiation. Problems regarding the safety of ZnO nanoparticles in cosmetic formulations continue to exist. Further research is necessary to thoroughly evaluate their long-term effects, including skin penetration, accumulation, and potential systemic toxicity. Standardization, regulation, and thorough safety evaluations are important for ensuring consistent product quality and safety. Future efforts should focus on standardizing manufacturing processes, characterization techniques, and safety assessment protocols for metal nanoparticles employed in cosmetic products. Besides, it is crucial to understand their environmental impact and to develop sustainable strategies for their use and disposal. Endless research on optimizing formulations, understanding mechanisms of action, skin interactions, and their effects on various skin conditions will further advance the field. In conclusion, zinc oxide (ZnO) nanoparticles deliver promising prospects in the cosmetics industry. They provide antimicrobial properties, skin whitening effects, anti-aging benefits, efficient delivery systems, and photo-protection. With continued research and development, the integration and application of ZnO nanoparticles in cosmetics can be further improved, ensuring their effectiveness, safety, and contribution to inventive cosmetic formulations.

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