

# Plant mediated green synthesis of Zinc Oxide Nanoparticles for burn wound therapy

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*In thinking about nanotechnology today, what's most important is understanding where it leads, what nanotechnology will look like after we reach the assembler breakthrough.*

*---K. Eric Drexler*

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## Abstract

In this changing world, we all are surrounded by the surmountable risk of getting injured. Amongst various risk factors, major burns are the most distressing and catastrophic. Burn wounds are not easy to heal via natural healing process and ultimately ended up with scar formation. If the degree of burn is high then the loss of tissue and its function is very common. To fasten-up the natural burn wound healing; zinc, an essential trace element is found to be very much effective. But due to its' particle size limitation, less contact with wounded cells and tissues, and high inherent toxicity restrict its use. Needlessly, zinc is an element with dual action i.e. both antimicrobial and wound healing it is a prime choice to apply its aptitude in burn wound healing. To overcome the documented limitations zinc has converted to nanoparticle form. Zinc oxide nanoparticles, in particular, have attained ample of interest due to their unique properties and potential antimicrobial activity along with wound healing activity which makes it promising for the healing of topical burn wounds. Plant mediated green synthesis of nano-metal oxide particles is gaining a lot of significance due to its simplicity, eco-friendliness and extensive antimicrobial activity and recommended as an appealing substitute to not only physical methods but also chemical methods avoiding the use of the high rate of toxic chemicals and extreme surroundings. This study includes ZnO NPs role in burn wound healing with Phyto-mediated synthesis methods to provide evidence of their potential applications. Additionally, it provides an overview of traditional methods used for the synthesis of ZnO nanoparticles and characterization techniques to obtain information concerning the size, shape and optical properties along with toxicity and safety concern of ZnO NPs and its biomedical applications.

## Keywords:

Burn wounds, ZnO NPs, Plant-based Green Synthesis, Wound healing

## Introduction

As the most devastating of all injuries, burn injuries are one of the major global public health crises. Following traffic accidents, falls, and interpersonal violence, burns are the fourth most common kind of trauma worldwide. Amongst the various countries of the world, the low and middle-income countries are most prone towards the burn injuries that are a consequence of lack of necessary infrastructure to downgrade the incidence, prevalence and severity of the burn injuries. As per WHO approximately 1,80,000 deaths occur annually due to the burns and complications associated to them. The morbidity rate is also very high in non-fatal burn injuries. Burns are preventable and burn wound healing could be fasten-up by using agents which accelerate the process of re-epithelization and proliferation. Zinc is one of such agents, which is categorized as an essential trace element present at a concentration of less than 50 mg/kg, in the human body. It acts as a vital cofactor for numerous proteins encoded by the

human genome (approx. more than 10% of proteins transcribed by the human genome). Transcription regulation, DNA repair, apoptosis, metabolic processing, extracellular matrix (ECM) regulation and antioxidant defence are few of the reckoning processes amongst the vast array of functions that zinc has in the body. Likewise, zinc also possesses bactericidal activity which is very important for curing the wounds and prevention of the sepsis.

To essentially heal and rejuvenate the topical burn wounds, zinc ions in the form of their oxide (ZnO) is used but due to several limitations of their larger particles size, it wouldn't be as effective as it could be. To resolve this snag ZnO are converted to nanoparticles which demonstrate well known antimicrobial property along with wound healing activity. This marked distinguished dual action makes them an attractive alternative to antibiotics and other wound healing substances. Nanotechnology is evolving fast and it has brought tremendous development in the field of science and technology. Nanomaterials are classified as organic (such as carbon nanoparticles) and inorganic (such as magnetic nanoparticles, noble metal nanoparticles such as gold and silver and semiconductor nanoparticles such as zinc oxide and titanium dioxide) according to their components, natural and artificial according to their origin, zero-dimensional (0D), one-dimensional (1D), two-dimensional (2D), and three-dimensional (3-D) according to dimensions of nanomaterial. Nanosized particles attracted a variety of researchers and industrialists to exploit the properties of these small particles for improvement of performance and stability of biomedical therapeutics. With due course of time, several techniques have been evolved to synthesize the nanoparticles but those conventional techniques (physical and chemical methods of Nanoparticles (NPs) synthesis) requires toxic chemicals which are ultimately responsible for environmental pollution. The synthesis ZnO NPs by chemical methods such as hydrothermal, precipitation, microemulsion, so-gel synthesis etc. involves toxic chemicals which adsorbed on the surface that may have adverse effects in medical application and often require higher temperature and pressure. As an Eco-friendly alternative, Plant-based-green-synthesis, an amalgamation of the power of green technology and biotechnology bounced up as an advantageous biosynthetic approach for NPs formation because they reduce the level of toxicity in the environment, they are cost-effective, possess requisite photocatalytic property, non-pathogenic and biological activity. Conversion to nanoparticles changes the physicochemical, electrical, and optical properties of zinc oxide particles. Relatively they have a broad range of properties such as optical, thermal conductivity, catalytic reactivity, mechanical, magnetic, photochemical and medicinal properties. Additionally, there is growing attention to the biosynthesis of metal nanoparticles using organisms and Phyto-mediated synthesis seems to be promising as they are suitable for the large-scale synthesis of nanoparticles and have extensive antimicrobial activity. Plant mediated NPs synthesis also imparted an extra potential of active phytochemicals to NPs synthesized by this method by getting adhered to their outer surfaces. NPs synthesized by plants are more stable and more varied in shape and size compared to those produced by other organisms. The Phyto-mediated synthesis gives a wide variety of morphologies of ZnO-NPs selectively based on the plant extract used.

Now, let us try to dive deep in the topic to clear the ambiguity related to green-synthesis of ZnO NPs and their role in burn wound healing.

## Pathophysiology of burn wounds

There are many possible risk factors which will lead to burn wounds like heat, freezing, chemicals, electricity, friction or radiations. Wounds caused by burning are variable as per the tissue site and location. Profound burns could lead to damage of muscular, epithelial, dermal and epidermal, vascular and osseous and bone tissues. The complications associated with burn wounds are serious and may prove to be fatal if not treated properly (Kara, 2018). Electrolytic imbalance, shock, respiratory failure, and infections are common amongst the complications as a result of burn wounds. Apart from these physiological snags, psychological and emotional distress can't be dissociated from the trauma of burn wounds (Evers et al., 2010).

### Classification of burn wounds

Internationally, the burn wounds are classified as:

- Superficial
- Superficial partial
- Deep partials, and
- Deep burns

This division can also be categorized in Degree of Burn i.e. Degree I – III (Jeschke et al., 2020). The table given below will help us to understand this classification with more clear demarcation.

*Table 1: Classification of Degree of Burns*

Degree	Aetiology	Skin layer involved	Look	Pain rating	The time required to heal
Superficial/ Degree I	Sun exposure, hot liquids with low viscosity and short exposure	Epidermis only	Pink to red, moist, no blisters	Moderate-Severe	3–7 days
Superficial partial IIa	Hot liquids, chemical burns with weak acid or alkali, flash	The superficial (papillary) dermis	Blister, red, moist, intact epidermal appendages, blanches of pressure	Severe	1–3 weeks, long-term pigment changes may occur
Deep partial IIb	Flame, chemical, electrical, hot liquids with high viscosity	Deeper layer (reticular) dermis	Dry, white, non-blanching, loss of all epidermal appendages	Minimal	3–6 weeks, with scars

Deep III	Flame, electrical, chemical, blast, self-immolation	Full-thickness of skin and into the subcutaneous fat or deeper	Leathery, dry, white or red with thrombosed vessels	No	Does not heal by primary intention, requires a skin graft
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The burns wounds would ultimately lead to cell/ tissue death or necrosis and this necrosis held responsible for the progression of the wound. As per the recent studies, apoptosis may also contribute to the wound progression (Kara, 2018).

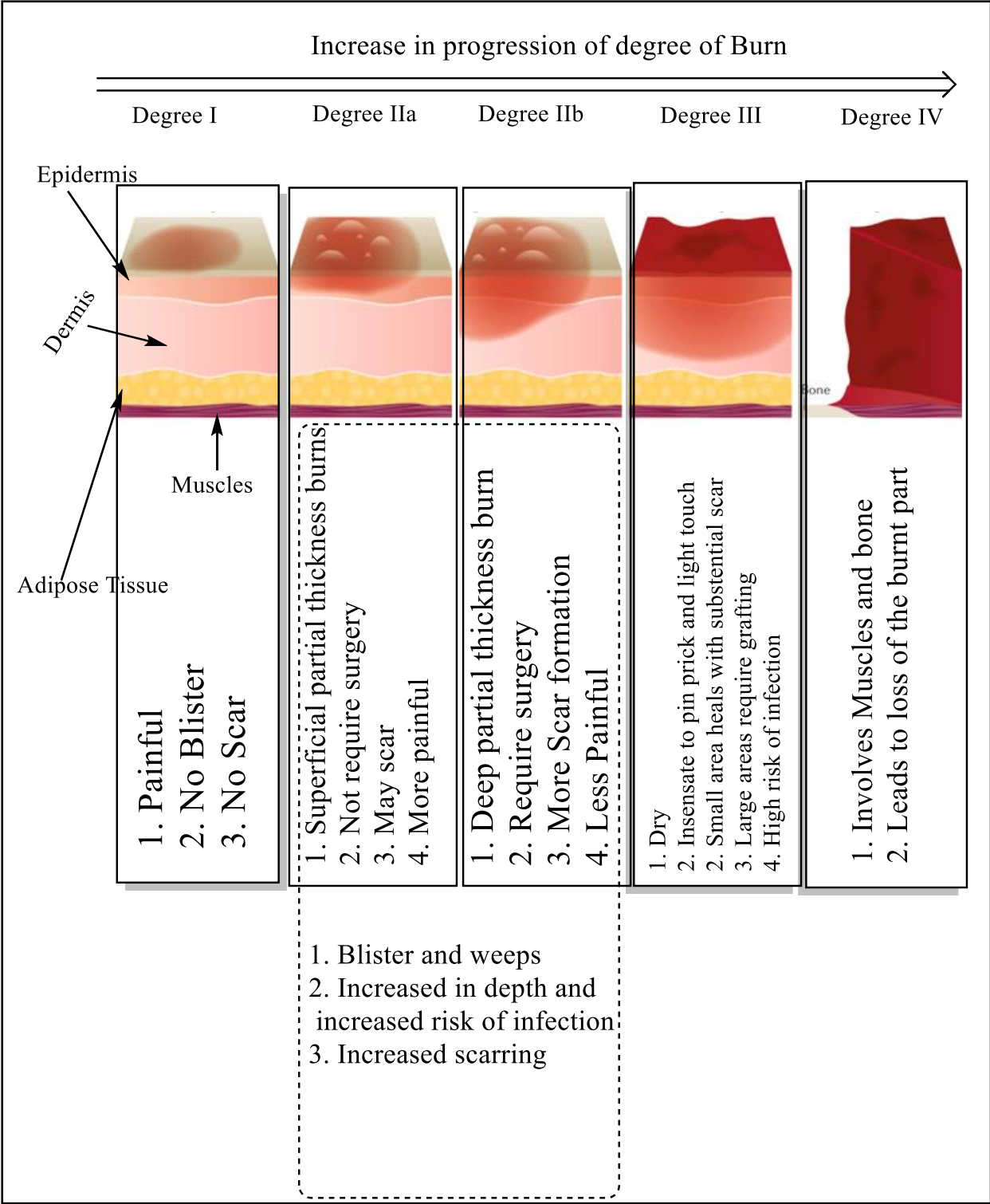


Figure 1: Burn wounds characterization for various degrees of Burns

Physiological responses related to burn wounds

All started with an extremely dysregulated inflammation immediately after the injury (Hettiaratchy & Dziewulski, 2004). At molecular level tissues start producing antigens, immunomodulatory agents along with the production of toxic metabolites this whole scenario is boosted up by the intervention of autacoids like Histamine, 5-HT, Bradykinin, NO, several ROS (oxygen free radicals) and an arsenal of mediators of an arachidonic acid pathway like

Prostaglandins, Thromboxane, apart from them cytokines like interleukins and TNF would also release at the site (Arturson, 1980).

The burn wound immediately after an injury get divided into 3 zones which are classified by Jackson (V. K. Tiwari, 2012).

- The most central zone is known as Zone of coagulation have the most damage.
- The zone of stasis or ischemia comes after that and decreased perfusion which is salvageable is it's characteristic.
- The zone of hyperemia is the outermost region where the inflammatory vasodilation is prominent without structural damage.

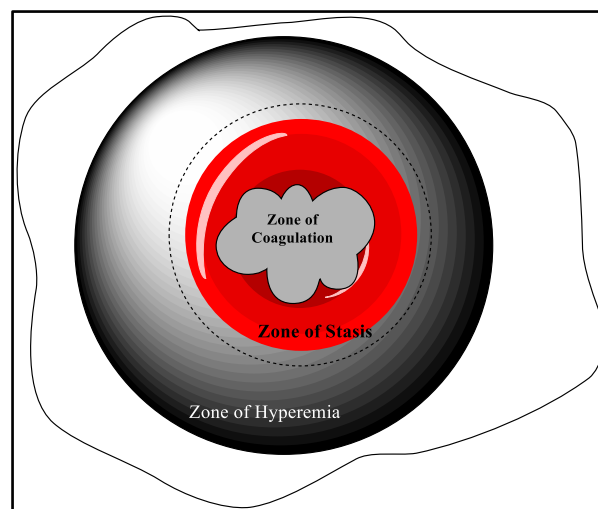


Figure 2: Jackson classification of Zone of burn wounds

Cellular autophagy started in the zone of injury within 24 hours which is followed by delayed onset apoptosis (24-48 hours). Healing without any aid by the body's response is known as natural healing, that is a dynamic and overlapping process (Kara, 2018).

Natural healing process initiated with monocytic and neutrophils infiltration to the wound site due to localized vascular dilation this marked an inflammatory phase. Proliferative phase overlaps the inflammatory phase with activation of different cytokines and growth factors. The activation of keratinocytes and fibroblasts as a result of the proliferation of growth factors and cellular signalling molecules to improve or promote the wound healing and restore the vascular perfusion of the tissues. At the last phase, the remodelling pledges the healing of the wound with deposition of elastin and collagen and myofibroblasts proliferation due to continuous conversion of fibroblast. With due course of time, re-epithelialization and contraction of myofibroblast repaired the wound and leave behind a scar which due to malposition of collagen fibres (Evers et al., 2010).

### The process leading to microbial growth and sepsis formation at the site of injury

The inflammatory response of the tissue towards the burn wound leads to the release of Damage associated molecular patterns (DAMPs) endogenously like mitochondrial DNA, dsRNA and Pathogen associated molecular patterns (PAMPs) which include lipopolysaccharides and peptidoglycans, increase the vascular leakage and induces the metabolic change. The immune and inflammatory response could lead to immunosuppression due to increased level of IL-4,

IL-6, IL-10, TNF, and PGE<sub>2</sub> (derived from Monocytes and T-lymphocytes); and a dip in the levels of Major histocompatibility proteins (Class II) and antigenic expression, IL-2 and IFN $\gamma$  and T-cell proliferation. Along with the sudden dip in T-cell proliferation rate, the decreased rate of Phagocytosis and bactericidal activity of Macrophages and Neutrophils eventually allow the microbes especially bacteria to invade the wounds and start multiplication (Jeschke et al., 2020). The summation of the above process is the development of sepsis and multiple organ failure if the burn wound covers a large area as per the Wallace rule of 9 (Arturson, 1980).

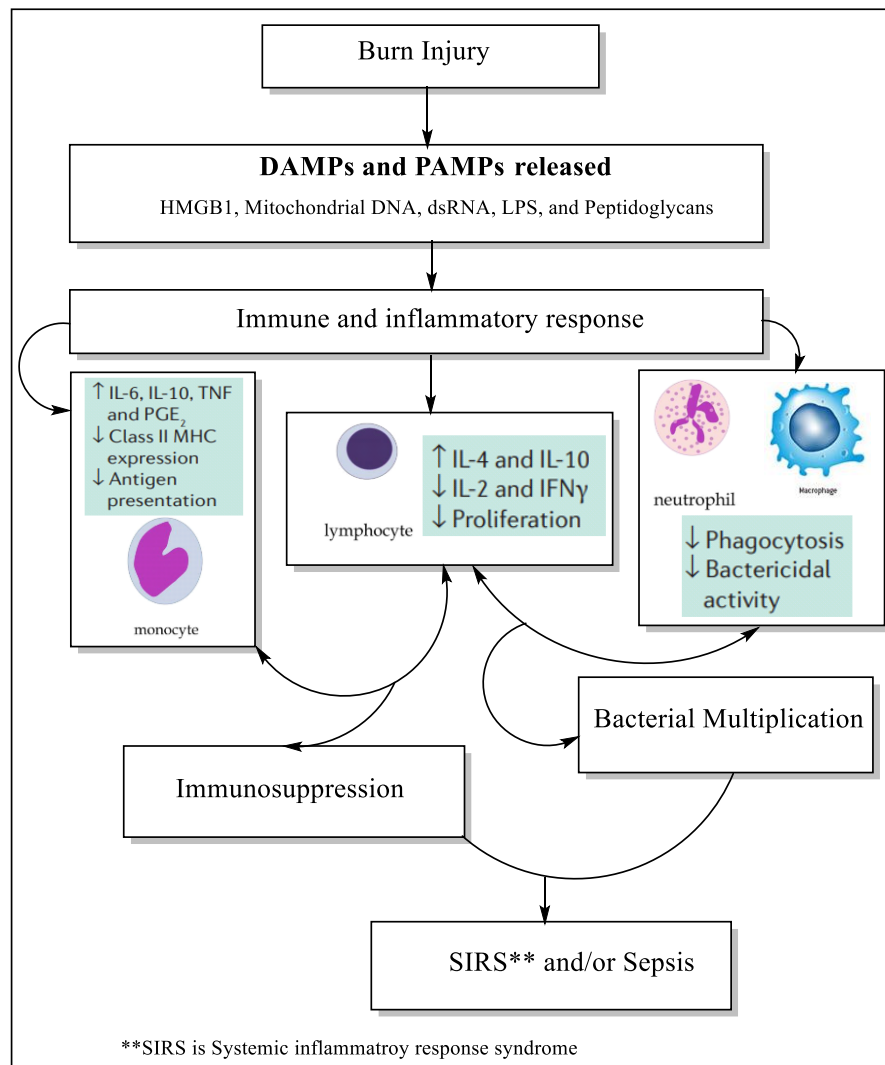


Figure 3: Pathophysiology of Burn Wounds



## Traditional Approaches/Methods used in the synthesis of ZnO Nanoparticles/nanomaterials

ZnO nanoparticles are multifunctional inorganic nanoparticles and due to their unique properties and potential applications various physical, chemical and biological methods have been applied for their synthesis. Zinc is an active element and gets easily oxidized to zinc oxide due to its reduction potential which is very useful for the synthesis of ZnO nanoparticles. Zinc is essential for the proper functioning of macromolecules and enzymes as it has both catalytic and structural role. Zinc finger motifs have a special scaffolding that enables the protein subdomains to interact with DNA or other proteins. While zinc lacks redox activity and is considered to be fairly non-toxic, there is increasing evidence that free zinc ions can lead, for example, to neuron degradation (Król et al., 2017). To reduce its cytotoxic effect synthesis of zinc oxide nanoparticles are performed. Zinc oxide has special optical, chemical sensing, piezoelectric and electric conductivity properties. It has high excitonic binding energy (60 meV) at room temperature, a direct wide bandgap (3.3 eV) in the near-UV spectrum and n-type electrical conductivity. Main forms of ZnO are hexagonal wurtzite and cubic zinc blende. Among which wurtzite is most common and stable at surrounding conditions and can exist in different growth morphology which can be prepared by using different physical, chemical and biological methods.

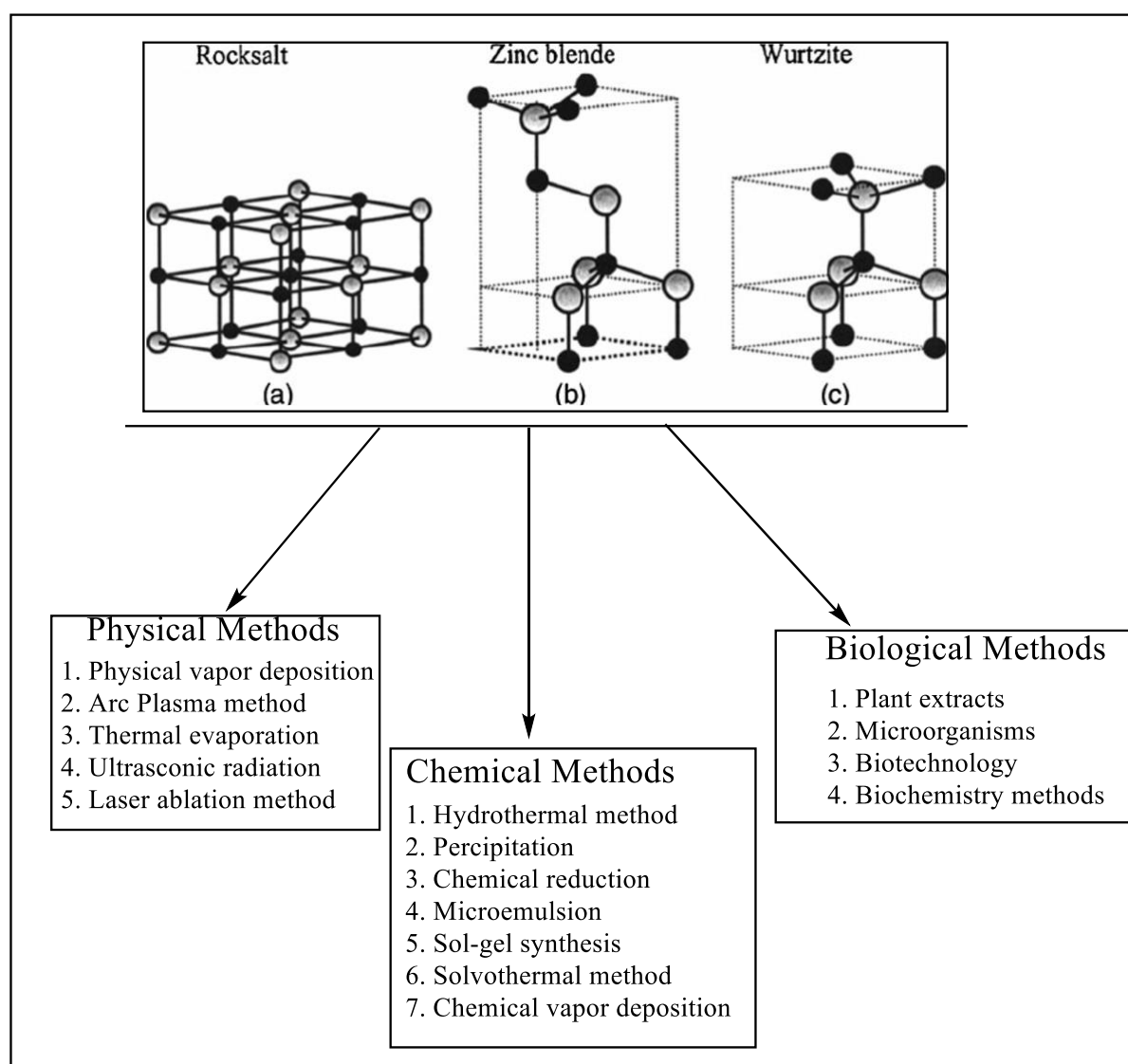


Figure 4: Different methods of synthesis of ZnO NPs

## Methods of zinc oxide nanomaterials synthesis

Generally, synthesis of nanoparticles can be done using two types-the bottoms up and top-down methods and are used on large scale industrial application. The first one generates nanostructures using chemical, biological methods or controlled deposition and growth. The second one converts bulk material to nano-sized particles which has disadvantage such as it can damage the crystal structure of nanoparticles which eventually affect the physicochemical properties. Both methods have certain advantages and disadvantages. Metallic nanoparticles should have a specific size and shape as well as the aggregation level must be small. The choice of the synthesis method is very crucial in the formation of ZnO nanoparticles as it determines the physicochemical properties. Therefore, green synthesis approach has gained a lot of interest in the production of zinc nanoparticles. Various physical and chemical methods for the synthesis of ZnO nanocomposites are as follows:

### Physical methods

There are various physical methods used for the synthesis of nanoparticles such as thermal evaporation, plasma arcing, spray pyrolysis, lithographic techniques, ball milling, sputter

deposition, molecular beam epitaxis, ultra-thin films, pulsed laser desorption, diffusion flame synthesis and layer by layer growth. ZnO nanoparticles can be synthesized by laser ablation, thermal evaporation, physical vapour deposition, arc plasma method or ultrasonic irradiation. Physical methods give high quality of the ZnO final product but require non-cost-effective vacuum infrastructure and high temperatures and also limited in terms of large-scale production.

ZnO NPs can be prepared by the laser ablation method in which colloidal solution of NPs can be obtained in a variety of solvents using bulk zinc metal. It is easy to use and give yield purity but the efficiency of the method depends upon process parameters such as the wavelength of the laser, ablation time and fluence. The size of the ZnO nanoparticles was not affected by the ablation time and fluence when ZnO NPs synthesis was performed by laser ablation in neat water (Kim et al., 2011). Another method such as thermal evaporation method is catalyst-free, simple and low cost. In this method, different shape or size of NPs can be obtained. It is a mechanism in which source material condensed or powdered vaporized to elevated temperature and vapour condenses under certain conditions and give desire product (Dai et al., 2003a).

### Chemical methods

Chemical methods used for the synthesis of nanoparticles are chemical solution deposition, sol-gel process, chemical vapour deposition soft chemical method, hydrolysis co-precipitation method, wet chemical method, electrodeposition, catalytic route and Langmuir Blodgett method. Chemical methods used for the synthesis of zinc nanocomposites are classified according to the physical state, i.e. liquid phase (wet chemical methods), solid-phase or vapour phase. In contrast to the physical-based methods, a bottom-up approach and wet chemical methods are efficient and suitable for ZnO nanostructures due to their low growth temperature, the potential for mass production and cost-effectiveness (Diallo et al., 2015). Finer tailoring of the size and shape of the nanocrystals achieved by wet chemical methods Some of the Chemical methods which are commonly used are as follows:

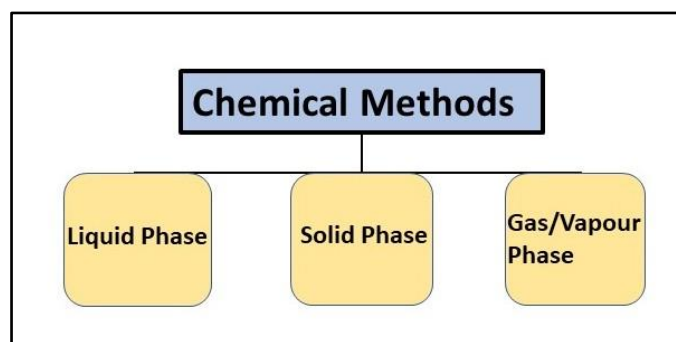


Figure 5: Classification of Chemical Methods of ZnONPs Synthesis

### Hydrothermal Method/ Solvothermal Method

Solvothermal and hydrothermal methods are performed similarly only the difference are the former uses solvents and the latter uses water other than solvents. These methods have the advantage of providing different morphologies of nanomaterials. Compared to another solution-based synthesis, the solvothermal method provides many benefits such as an easier fabrication of large-scale samples, catalyst-free growth, better homogeneity, easier composition control, low processing temperature, low cost, narrow grain size-distribution and less hazardous (Aneesh et al., 2007). In this method, zinc acetate dihydrate solution are prepared

in solvents such as water, 1-Hexanol, ethylene glycol, cyclohexane or methanol. To this NaOH/butyl alcohol added and maintained at 100-200 °C for 6-18 hours in an autoclave under certain pressure conditions. Further, it is cooled and resultant white solid product of ZnO nanoparticles washed with methanol/ethanol. Process parameters such as reaction temperature, the concentration of precursor and time play a very important role in controlling the size and morphology of nanoparticles in these methods as particle size increases with growth temperature and decreases with concentration of the precursors. Average particle size obtained by this method ranges from 5-25 nm. Water, 1-hexanol and ethylene glycol produce flower-like, hexagonal rod-like and spherical-like ZnO nanoparticles respectively (Talebian et al., 2013).

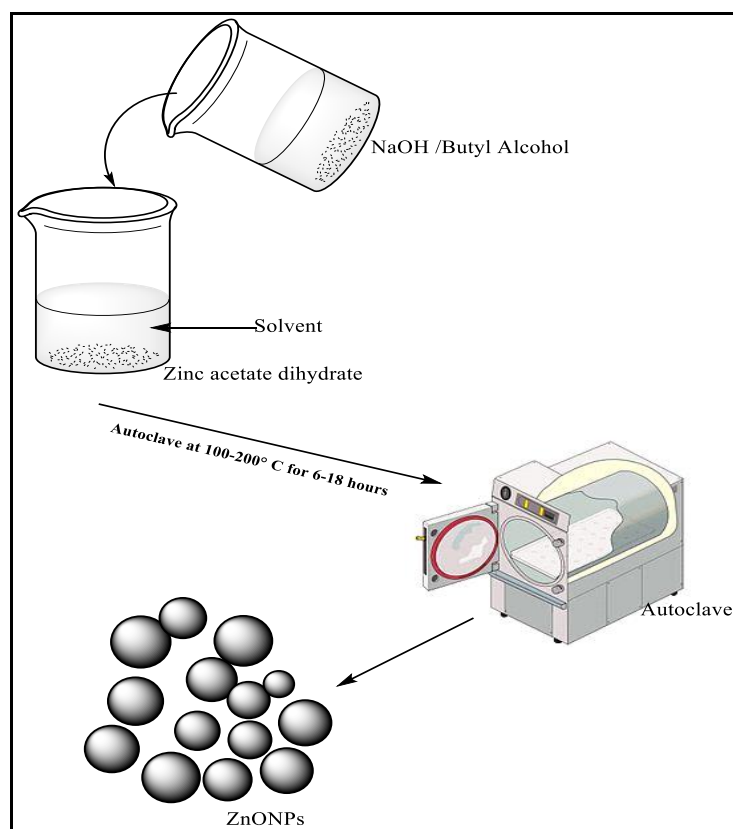


Figure 6: Hydrothermal Method of ZnO NPs synthesis

## Precipitation

Precipitation is the most common and successful method for different ZnO nanoparticle synthesis. It involves a reaction of zinc salts, such as  $\text{Zn}(\text{NO}_3)_2$ ,  $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ ,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  etc. with basic solutions containing LiOH,  $\text{NH}_4\text{OH}$  and NaOH (De la Rosa et al., 2007a). The synthesis starts with a reaction between zinc and hydroxide ions followed by the process of aggregation. Formation of a stable colloid suspension of ZnO nanoparticles is usually performed in an alcohol solution (Hu et al., 2003a). As  $\text{Zn}(\text{OH})_2$  prepared from aqueous solutions, precipitation of a stable colloid suspension of ZnO nanoparticles is performed in an alcohol solution. Solution concentration, pH, washing medium or calcination temperature are some of the process parameters of the precipitation method. The proper choice of Zinc NP precursor leads to successful precipitation as use of Zinc acetate, chloride, sulphate and nitrate as precursor gave ZnO NPs with sizes 25 nm 10–30 nm, 80–100 nm and 500 nm

respectively. However, higher  $\text{Zn}^{2+}$  concentration leads to the formation of larger and aggregated particles with different shapes (Wang & Muhammed, 1999a).

### Microemulsion

The synthesis of ZnO nanoparticle done by reversed microemulsion (water dispersed in oil, W/O) stabilized by the surfactants and uses various types of zinc precursor and other reagents. The microemulsion can effectively control the particle size and protect the nanoparticles from excess aggregation. The ZnO nanostructures with various morphologies and crystalline sizes can be obtained with different concentrations of PEG 400 in  $\text{Zn}(\text{NO}_3)_2$  solution which can adsorb or encapsulate the nuclei surface to control crystal growth (Li et al., 2009). Normally, the method of preparing ZnO particles through microemulsion involves salt of zinc being incorporated in aqueous core of a micelle and it is precipitated to get precursor particles. Anionic sodium bis-2-ethylhexylsulfosuccinate (AOT) and Triton X-100 are commonly used surfactants. The size and shape of zinc nanoparticles are dependent on both concentration and type of polyethylene glycol (PEG-200, 400, 600 and 1000). Another method used where zinc substituted surfactant used as both microemulsion stabilizer and metal source and oxalic acid for precipitation of Zinc oxalate and ZnO NPs produced after calcination but large and irregularly shaped particles are formed destabilization of microemulsion by solid oxalic acid (Elen et al., 2011a). The ZnO NPs can be obtained in a size range of 12-50nm by microemulsion method.

### Sol-gel method

The sol-gel process involves the preparation of a colloidal solution (sol) that is converted to gel and solid materials. The procedure consists of hydrolyzation, condensation and polymerization reactions. Typical precursors are metal alkoxides ( $\text{M}(\text{OR})_x$ , where M = metal, i.e. Zn) or corresponding chlorides, in an aqueous or organic medium (usually alcohol) (Król et al., 2017). Zinc acetate hydrate in alcohol is commonly used precursor for ZnONPs. The nature of the solvent and alkyl group, the concentration of precursor, molar ratio of water to alkoxide or temperature are several parameters which influence the growth of ZnO nanoparticles (Hench & West, 1990a). The use of zinc acetate solution as precursor give ZnO NPs with the size of 2-7 nm while in addition to that use of surfactant such as triethanolamine give spherical shape ZnO NPs with the size of 3-4 nm (Meulenkamp, 1998). Another surfactant ethyl diamine to the zinc acetate dihydrate and oxalic acid dihydrate give rod-like nanoparticles with 20–320 nm in diameter highest molar ratio. The ZnO NPs with varied size between 20-50 nm obtained with the addition of tetramethylammonium hydroxide (TMAH) solution to the alcoholic solution of zinc 2-ethylhexanoate. The drawback of the sol-gel method is the use of high-cost precursors but it can be used for large scale and also has faster nucleation and growth.

### Ultrasonic assisted wet chemical method

To overcome the limitation of the wet chemical method such as coalescence process produces non-uniform distribution and bigger grain size nanoparticles affecting the physicochemical properties, a newer method called Ultrasonic assisted wet chemical method is found to be useful. Ultrasonication during the reaction between the cation and anion influences variances in chemical reactions, structural orientation, surface morphology, optical properties and electrical conductivity as well. Applied ultrasonic vibration lead to changes in the properties of nanomaterials largely by ion-by-ion heterogeneous growth and nucleation process. The reaction of Sodium hydroxide to the aqueous solution of zinc sulphate carried out in an ultrasonic bath at a temperature of 55 °C. The final product centrifuged several times with

water and ethanol for removing impurities and dried under illumination of the Infra-red (IR) lamp for 4 hrs (Arote et al., 2019).

The chemical method has the drawback of using toxic chemicals and solvents as reducing agents and require further treatment of waste end product. Whereas, the Green nano-synthesis uses eco-friendly reagents or biogenic methods, avoids waste and reduces pollution risk and Nanomaterials synthesized by biochemical interaction with active compounds of natural extracts is gaining interest as it potentially reduces the precursors of the nano-material to be synthesized.

## Plant-based Green synthesis of ZnONPs

### Green synthesis of Metallic nanoparticles:

Green synthesis of nanomaterial consists of synthesizing Nanoparticles using biological routes either by using microorganisms, viruses, plants and their products like enzymes and lipids (Akbar et al., 2020a). Plant mediated synthesis of Metallic ion-containing nanoparticles (NPs) is one of the easily affordable and accessible biological methods of metallic NPs synthesis. It is a Green Chemistry approach that acts as a liaison between Nanotechnology and Biotechnology/ Natural products (Kalpana & Devi Rajeswari, 2018a).

Green synthesis is better than physical and chemical production of NPs due to its potentials like the requirement of ambient temperature, neutral pH, low costs, environmentally friendly due to use of non-toxic, safe reagents, consumes less energy, generation of benign products and by-products, and generally a single-step-process (Handore et al., 2014). Plants have evolved to make use of primary and secondary metabolites like sugars, phenols, terpenoids, alkaloids, proteins, etc. to synthesize nano and microscale inorganic materials via bio-reduction of metal ion and contributes to their stability (Chandra et al., 2019a). It is also suggested by recent studies that using plants or their extracts in the preparation of nanomaterials is non-hazardous. Various plants have been used to successfully transformed metal ions of Au, Ag, Co, Cu, Pd, Pt, Magnetite, Zn etc. to their greener nanoparticles. (Ankamwar et al., 2005).

The basic procedure behind plant-based green synthesis is based on the Bottom-up approach of synthesis NPs which is as follows:



Figure 7: Basic procedure involved in Green Synthesis of NPs



## Advantages of plant-mediated nanoparticles synthesis over physical and chemical methods.

Table 2: Differentiation between Physical/chemical and Green synthesis methods

S. No.	Physical/Chemical Methods	Green Synthesis Method
1	Costly and low production rate	Cost-effective due to easy availability of plant materials and the presence of active bio-components which act as reducing and capping agent. Large scale production of nanoparticles
2	Require highly restricted laboratory environment	Does not require well-equipped laboratory and restricted conditions for the synthesis of nanoparticles
3	Require high or low temperature/pressure and inert environment.	Nanoparticles can be synthesized at normal temperature and pressure conditions
4	Consumption of a great deal of energy during the synthesis process	Does not require complex energy system for synthesis
5	Chemical methods use toxic chemicals for synthesis or stabilization of nanoparticles that can absorb on the surface of nanoparticles resulting in contamination of defective surface formation of the final product	The final product is mostly safe and stable due to the utilization of natural secondary metabolites of plants
6	Production of toxic by-products harmful for the environment	Eco-friendly and safe system

### Green synthesis of ZnO Nanoparticles (ZnONPs):

The secondary metabolites such as flavonoids, alkaloids, phenols and terpenoids have been exploited for the synthesis of ZnONPs as these nanostructures are more biologically active due to possible add-on of several pharmacologically significant residues on their outer surfaces. Zinc oxide nanoparticles synthesis via plant extract as the bio-reducing agent has been popularized in the recent past due to its cost-effectiveness, safety and relative ease of handling (Akbar et al., 2020b). The bio-reductants are the primary or secondary metabolites (Phytochemicals) present in different parts of plants and extracted out when the extraction is carried out using some solvents like phenolics, tannins, alkaloids, saponins, terpenoids, amino acids, proteins, enzymes, vitamins, and polysaccharides. These bio-reductants themselves possessing medicinal values and are environmentally benign.

The general protocol for ZnONPs synthesis is as follows: (Akbar et al., 2020b)

- The desired plant is collected and verified
- The active molecules containing part of the plant is collected, washed thoroughly with tap water two or three times to remove any dirt or any other impurities and then rinsed with double distilled water.

- This clean part then dried either shade dry or oven dry for few hours until the Limit of Drying is reached.
- The dried part then chopped and sliced and further boiled in sterile deionized water.
- Go for size reduction and then extract them with any suitable extraction technique like Soxhlation, percolation, etc.
- Filter the final extract.
- At last, prepare the zinc oxide nanoparticles by mixing mM solution of Zinc Salt with a few mL of the plant extract. Here, the zinc salt act as a precursor for the synthesis of zinc oxide nanoparticles.



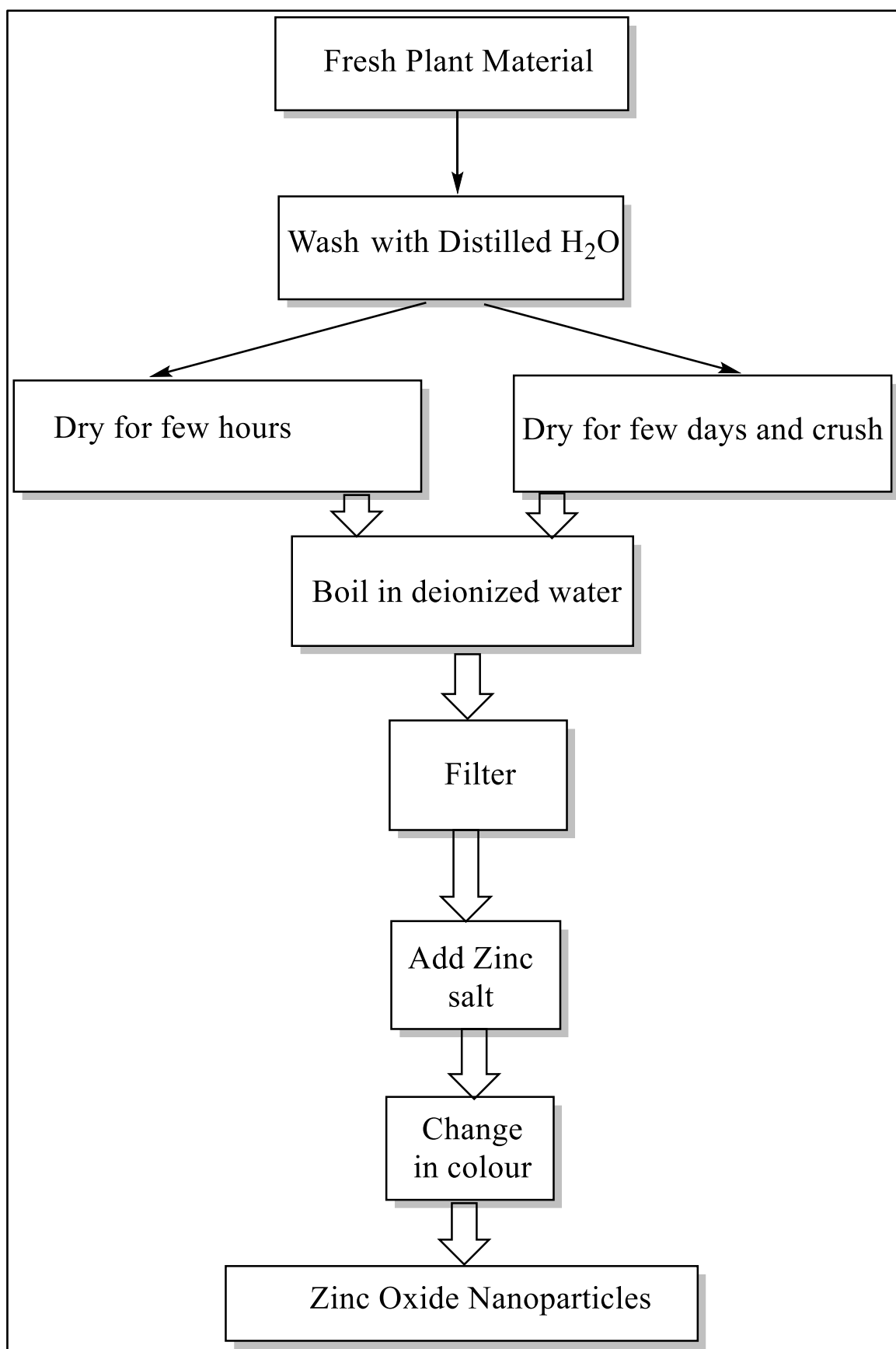


Figure 8: Protocol for Plant-Based-Green-Synthesis of ZnO NPs

### The mechanism involved in plant-mediated nanoparticles (Bottom-up approach):

The mechanism involved generally three phases which are as follow:

1. Activation phase: it involves the reduction of metal ions. The reduction is followed by nucleation of the reduced atoms.
2. Growth phase: It involves the association of small adjacent nanoparticles into larger sized particles spontaneously. The metal ions further reduced in this process.
3. Termination phase: that determines the final particle shape and size.

Note: a process called Ostwald ripening occurs during the growth phase which provides the thermodynamic stability to the NPs (P. Singh et al., 2016).

**Ostwald ripening:** a phenomenon observed in solid solutions or liquid sols that describes the change of an inhomogeneous structure over time, i.e., small crystals or sol particles dissolve and redeposit onto larger crystals or sol particles.<sup>[1]</sup>

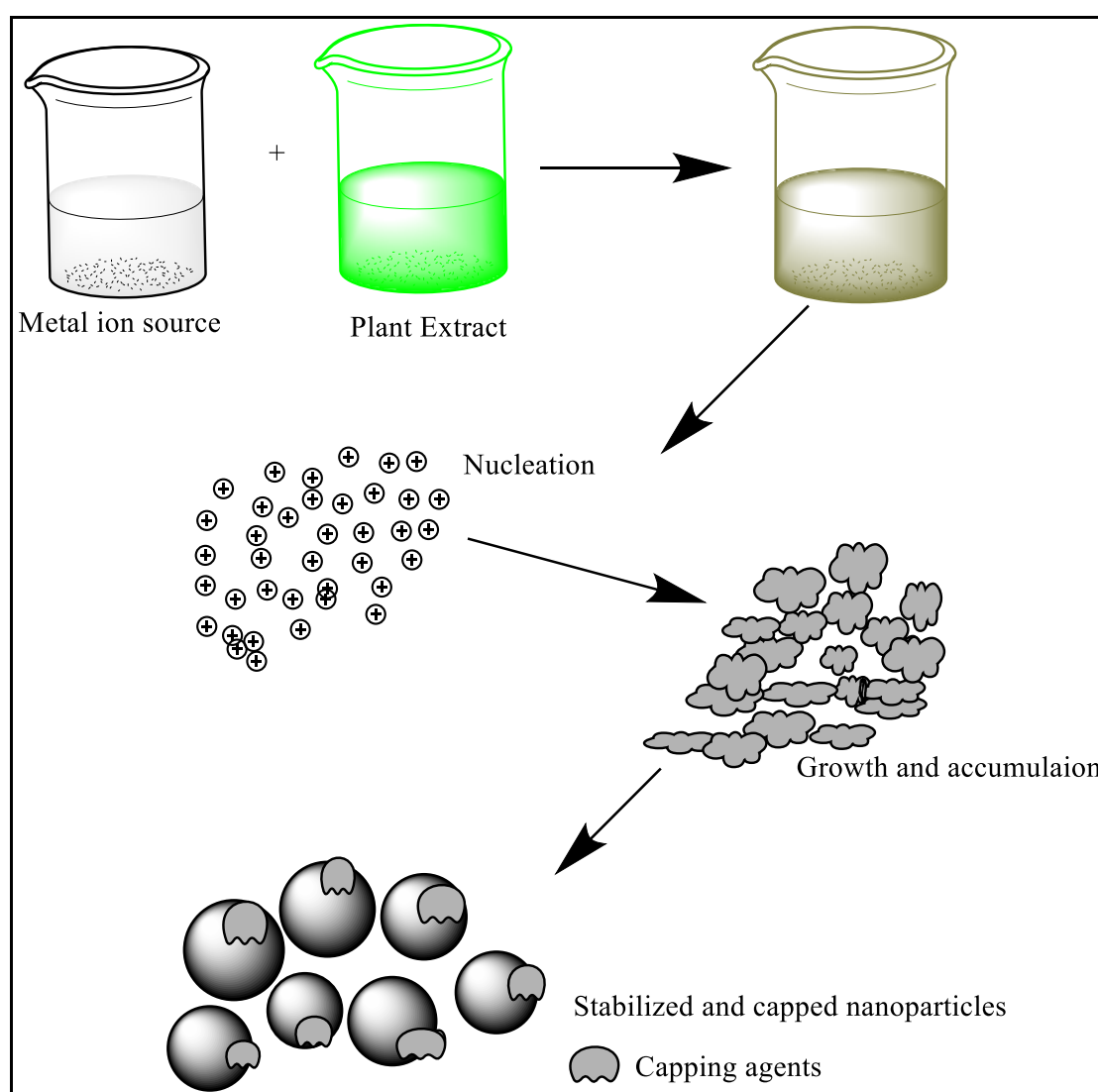


Figure 9: Illustration of Plant-based-green-synthesis of ZnO NPs

<sup>1</sup> IUPAC, *Compendium of Chemical Terminology*, 2nd ed. (the "Gold Book") (1997). Online corrected version: (2006–) "[Ostwald ripening](https://doi.org/10.1351/goldbook.O04348)". doi:10.1351/goldbook.O04348

## Applications of ZnO Nanoparticles as an antimicrobial agent and in the treatment of Infections and inflammations.

ZnONPs are of great importance in the field of science and technology. They are exceptional not only due to their physicochemical properties but also shows unique biomedical potential. The area of application of ZnONPs is diverse from the usage as a semiconductor integrated chip used in electronic to most recent activities showcasing its utilization as the antimicrobial, anticancer, antioxidant and photocatalytic agent. Here is the description of some of the applications of the ZnONPs used in different fields of biomedical sciences.

### Biomedical Applications of ZnO NPs

**Drug Delivery:** The benefits of using ZnO NPs for drug delivery were derived from their two main basic properties. First of all, due to their smaller size, nanoparticles can penetrate through smaller capillaries and are absorbed by the cells, allowing an efficient accumulation of drugs at the target sites. Second, the use of biodegradable materials for the preparation of nanoparticles allows the prolonged discharge of drugs within the site targeted over days or even weeks. The role of synthesized ZnO NPs in drug release by using the drug metronidazole benzoate was studied by observing its diffusion through egg membrane. Results revealed that the presence of ZnO NPs with the drug has many effects on the biological membrane (Kalpana & Devi Rajeswari, 2018b).

### Antimicrobial potential

Zinc oxide powder is generally utilized to induce antimicrobial effect but when zinc oxide nanoparticles are used, it results in an enhancement of its antimicrobial potential due to the photocatalytic ability of nanoparticles under UV and a huge increase in the surface area available for the microbes to be exposed.

Zinc oxide nanoparticles have been extensively investigated for antibacterial, antifungal, and drug delivery applications. Zinc oxide nanoparticles show stronger antimicrobial action than bulk zinc oxide.

Nanoparticles disrupt bacterial membranes probably by the production of reactive oxygen species, such as superoxide and hydroxyl radicals. Moreover, ZnO nanoparticles have positive zeta potential at their surface. This depends on the nature of the surface of different bacteria. Moreover, the antibacterial activity is reported to be dependent on the concentration of ZnO nanoparticles and the impact of the type of surfactant used. Also, ZnO nanoparticles could be attributed to the damage of the bacterial cell membrane and extrusion of the cytoplasmic contents thereby resulting in the death of the bacteria (Akbar et al., 2020c).

### Anti-oxidant activity

It was found that ZnO nanoparticles showed lower radical scavenging activity as compared to the standard. The variation in the DPPH radical scavenging activity was observed with increasing concentration of ZnO NPs

The efficiency of the antioxidant activity can be assessed by the IC 50 value. The IC 50 Value is Defined is the amount of sample required for 50% scavenging of the free radicals. The better the antioxidant potential relates to lower IC 50 Value of ZnO nanoparticles, IC 50 values were estimated from the % inhibition versus concentration sigmoidal curve, using linear regression analysis (Dobrucka & Długaszewska, 2016a).

## Antibacterial Activity of ZnO Nanoparticles.

Bacteria are generally characterized by a cell membrane, cell wall, and cytoplasm. The cell wall lies outside the cell membrane and is composed mostly of a homogeneous peptidoglycan layer (which consists of amino acids and sugars). The cell wall maintains the osmotic pressure of the cytoplasm as well as the characteristic cell shape. Gram-positive bacteria have one cytoplasmic membrane with a multilayer of peptidoglycan polymer, and a thicker cell wall (20–80 nm). Whereas gram-negative bacteria wall is composed of two cell membranes, an outer membrane and a plasma membrane with a thin layer of peptidoglycan with a thickness of 7–8 nm. NPs size within such ranges can readily pass through the peptidoglycan and hence are highly susceptible to damage.

Antibacterial activity is known according to The American Heritage Medical Dictionary 2007, as the action by which bacterial growth is destroyed or inhibited. It is also described as a function of the surface area in contact with the microorganisms. While antibacterial agents are selective concentration drugs capable of damage or inhibit bacterial growth and they are not harmful to the host. These compounds act as chemotherapeutic agents for the treatment or prevention of bacterial infections (Saunders Comprehensive Veterinary Dictionary 2007) An antibacterial agent is considered as bactericidal if it kills bacteria or as bacteriostatic if it inhibits their growth.

As a matter of concern of this review, we are now trying to focus on the latent possible use of ZnONPs in the treatment of wounds occurred as a result of Burn injuries. The probable potential of ZnONPs in burn wound healing is enormous. To understand this application of ZnONPs let us try to decode the pathophysiology behind the burn wounds and later in this chapter we will put forward the conceivable mechanism of action of ZnONPs as burn wound healer (Chandra et al., 2019b).

## Mechanism of action

As a rule of thumb, smaller the particle size larger will be the surface area and ZnONPs exploits this enhanced principle particle surface to increase the particle surface reactivity against the microorganisms ranging from hundreds of nanometers to few micrometres. The nanoparticles of ZnO are bio-safe materials which possess photocatalytic and protoxidizing activities. They impact microbiological systems in a variety of ways. The mechanism which is most promising against microbes can be broadly classified as (Sirelkhatim et al., 2015):

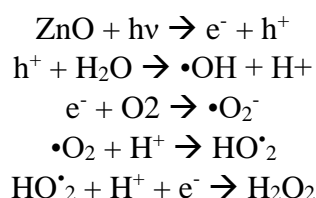
- Reactive oxygen species (ROS) generation like  $\text{H}_2\text{O}_2$ ,  $\text{OH}^-$ , and  $\text{O}_2^{-2}$
- Enhanced permeability of the plasma membrane
- Loss of Proton motive force and Nanoparticles internalization to disrupts mitochondrial functions.
- Oxidative stress and gene expression for apoptosis and cell necrosis.
- ZnO surface abrasive texture due to surface defects in nanoparticle geometry.

By reducing the size in the nano-range we can modify the mechanical, electrical, chemical, structural, morphological, and optical properties of ZnO. Bacteria possess cytoplasmic membrane with several layers of peptidoglycan whose thickness ranges from 20-80 nm and 7-8 nm in gram-positive and gram-negative bacteria respectively. To cross this nanosized barrier,

Nanocarriers are proved to be the best as they readily pass through the peptidoglycan layer and by various mechanisms sources the microbial killing (V. Tiwari et al., 2018).

In general, the bacterial cells owned a net negative charge. This would lead to electrostatic attraction of the  $\text{Zn}^{2+}$  ions which gets solubilized into the medium contains the bacteria because of their nano-sized dimensions (Salah et al., 2019). These solubilized NPs generate ROS which disrupts the cell wall and cytoplasmic membrane eventually lead to internalization of the ZnONPs. Once they internalized in the bacterial cells, generation of ROS get boosted which turn down the mitochondrial functions by unsettling the proton motive force hampers the electron transport chain and oxidative phosphorylation. The increased oxidative stress initiates the gene expression to produce apoptotic markers and ultimately leads to cell death (Swain et al., 2014).

As per the reports, aqueous ZnONPs suspension yields augmented level of ROS. We can understand the production of ROS on the ZnO surface as per the following chemical equations:



Superoxide and hydroxyl radicals are not able to penetrate the membrane due to their negative charges therefore, they are mostly found on the outer surface of the bacteria. Contrary to this  $\text{H}_2\text{O}_2$  molecules can pass through this barrier and subsequently leading to oxidative damage to the cell.

It is also proposed by several researchers that ZnONPs also show their activity via the release of  $\text{Zn}^{2+}$  ions which has a significant effect on the active transport (inhibition of active transport). As well as unrestingly damage the enzyme systems and amino acid metabolism (Sirelkhatim et al., 2015).

These nanoparticles are bactericidal and thus causing membrane dysfunction by disruption of membrane. Through damaged membrane internalization of ZnO becomes easy. The internalization causes declination in the ATP levels and hence reduces the cell energy. The direct liaison between ZnONPs, the cytoplasmic membrane, and ROS generation can be visualized as NPs attraction in the cytoplasmic area or the periplasmic space, therefore, disrupts the cellular activities and resulting in membranes disturbance and disorders (Dobrucka & Długaszewska, 2016b).

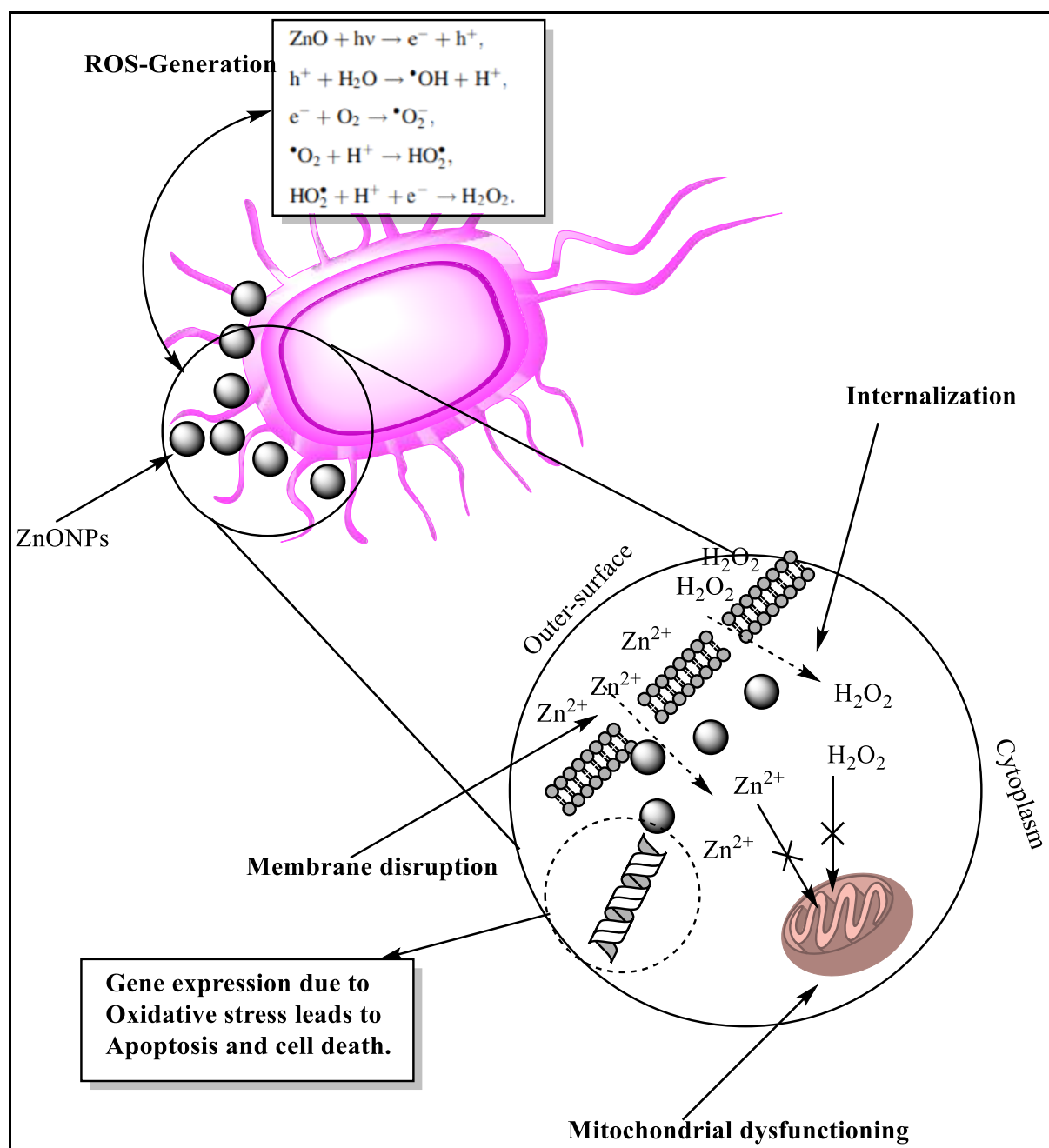


Figure 10: Mechanism of Action of ZnO NPs in treatment of Burn Wounds

### Burn wound healing mechanism of ZnONPs

One of the essential trace elements required by the body for numerous critical enzymatic, physiological and structural functions is Zinc. After several researches, it was found that wound healing is impaired with zinc-deficiency. So, to develop a therapeutic approach to enhance the rate of wound healing we can consider ZnONPs as a panacea tool.

As we all know that, there is an intimate association of wound healing, inflammation and immune response. Thus, ZnO particles enhance the skin re-epithelization via its anti-inflammatory action due to suppression of inflammatory markers genes like IL-6, IL-1 $\beta$ , IL-10, and TNF- $\alpha$  (Nagajyothi et al., 2015). The downregulation of COX-2 which is cardinal for inflammation also gets fostered by ZnO anti-inflammatory activity. It also alters the expression of inflammatory mediators by NF-kB pathway inhibition.

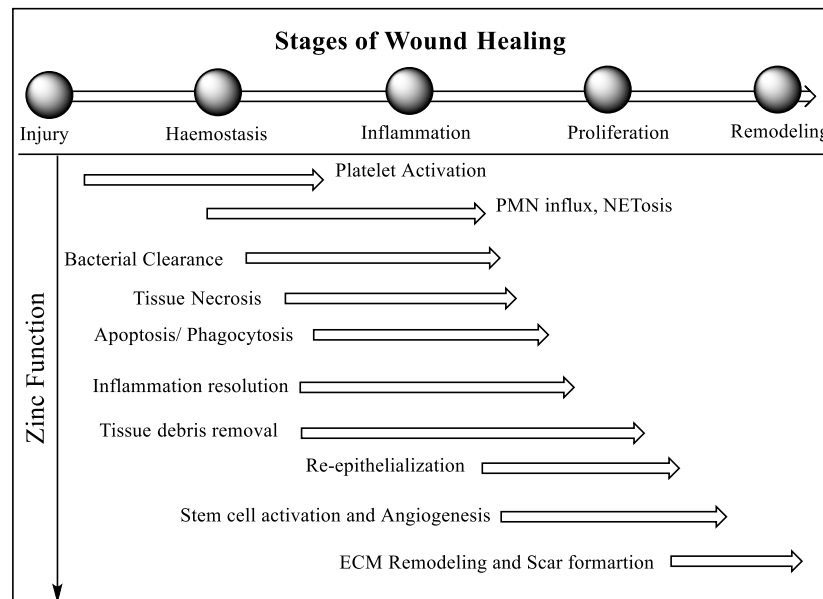


Figure 11: Phases of Burn wound healing

Zinc is a major player in regulating each and every phase of the wound healing process; starting from membrane repair, oxidative stress, coagulation, inflammation and immune defence, tissue re-epithelialization, angiogenesis, to fibrosis/scar formation (Lin et al., 2017).

Proficiently zinc enhances platelet activity and aggregation (Sekhon & Sen Gupta, 2018). As per the recent studies, zinc mediates its effect on platelets Protein kinase C (PKC)-mediated tyrosine phosphorylation of platelet proteins. Fascinatingly zinc mechanism of action on pathophysiological thrombus formation during tissue injury is still chiefly unidentified. Via cytokines and chemokines, the platelets are being able to recognized as immune cells capable of pathogen recognition (Bao et al., 2010).

Polymorphonuclear leukocytes (PMN) or neutrophils are one of the first responders to tissue injury and bacterial infection (Hasegawa et al., 2000). Neutrophilic migration along with other leukocytes towards the infected/damaged sites is drafted by gradients of chemokines and cytokines that gradually increased after the injury or damage, this process is known as chemotaxis. Due to zinc deficiency, the chemotactic movement of neutrophils gets disrupted while supplementation reverses the effects. Neutrophils secrete inflammatory cytokines and phagocytized the pathogens at the site of infection/ injury as a protective response (Fraker & King, 2004). Supplementation of zinc locally enhances the neutrophilic phagocytosis of opsonized zymosan particles (which is a Toll-like receptor-2 (TLR2) agonist) (Lin et al., 2017).

Zinc also capable of healing the wounds by a process called as “Nutritional Immunity”, it is a process in which there is sequestration and availability restriction of essential trace elements takes place by the action of host cells thereby inhibiting pathogenic growth (Zazzo et al., 1989). To combat this process, bacteria have developed their own strategies such as *calprotectin*. Due to mutation some bacteria also get evolved to express high zinc-affinity-zinc uptake receptors as compare to calprotectin. Sometimes bacteria also proposed some receptors that bind and repurpose calprotectin, thus letting them dodge nutritional immunity.

On the aspect of macrophages, the crucial part is to maintain the critical balance between M1/M2 macrophage populations (Dierichs et al., 2018). The effect of zinc on macrophage



phenotype is fully elucidated and shows a functional advancement of wound healing behaviours.

Negatively regulation of NF- $\kappa$ B signalling via PPAR- $\alpha$ , A20, I $\kappa$ B kinase- $\beta$  (IKK $\beta$ ) and phosphodiesterase (PDE) by zinc is another form of action of this essential trace element in treating burn wounds (Maywald et al., 2017). Both precursor and mature B-cells can reduce antibody production if zinc is deficient at the site of the wound. Wound clearance gets hindered due to falling B-cells populations and circulating antibodies that would negatively affect phagocytosis.

ROS-mediated augmentation of human dermal fibroblast migration is one of the hallmark actions of zinc. In the same fashion, keratinocyte migration and re-epithelialization of the epidermis is also amplified by zinc ions. Simultaneous re-epithelialization, endothelial cells migration and proliferation of new blood vessels at the wound site is called neovascularization, or angiogenesis. This process is essential to supply oxygen and nutrients for the growth of cells in the wound bed and zinc has shown to improve the process of angiogenesis (Agren et al., 1991).

ECM-remodelling matrix metalloproteinase (MMPs) family proteins are amongst the most crucial protein for wound healing process (Xue et al., 2006). Various cell types like inflammatory cells, keratinocytes, endothelial cells and fibroblasts secrete an array of MMPs. The process of growth-factor modulation, cleavage, degradation and composition of ECM, cell-cell junctional adhesion molecules processing, cytokines and cell surface receptors and cell-matrix signalling all are linked with MMPs which are zinc-dependent endopeptidases and helpful in remodelling the burn wounds (Chen et al., 2013).

As a whole, we can say that ZnONPs heals the burn wound with a complex interplay between intracellular and extracellular agents and pose a potential reflex to use them in to treat burn wound safely and effectively. Every coin has two faces, on a similar note ZnONPs also show some lag-behind in the form of cellular toxicity which we will try to discuss below.

## Toxicity and safety concern of ZnONPs

Zinc oxide (ZnO) is the most commonly used nanoparticle due to its varied applications ranging from personal care products, sensors, antibacterial creams and biomedical applications. These applications exhibit their potential toxicity. Therefore, it is necessary to understand their toxicity mechanism and forms on various levels. The primary aim is to summarize the cytotoxicity, genotoxicity, neurotoxicity and developmental toxicity of ZnO nanoparticles in various kinds of cells in vitro and in vivo.

After exposure of ZnO nanoparticles by any means it ranges to the circulatory framework, through its nanoparticles is disseminated in everywhere throughout the body, the fundamental targeted organ of ZnO nanoparticles where the compatibility high concentration of ZnO nanoparticles is found are lung, liver, kidney, bones brain and spleen.



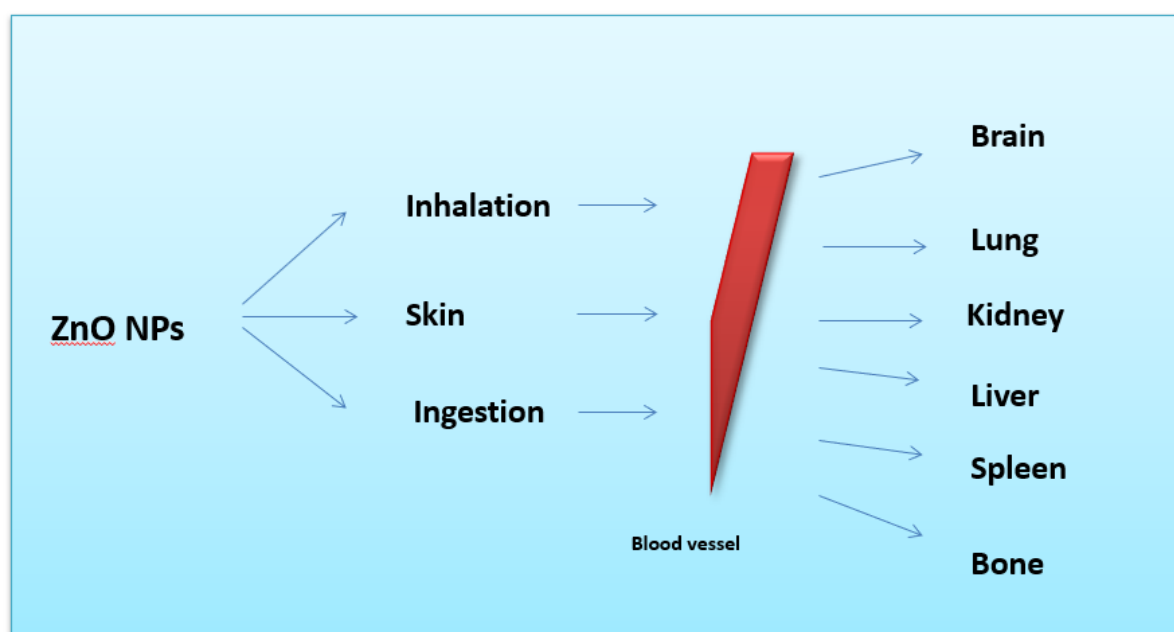


Figure 12: Toxicity of ZnO NPs in various organs

### Mechanism of Toxicity of ZnO Nanoparticles:

Three noteworthy toxicity activities of ZnO nanoparticles include:

- (I) Release of Zinc ions ( $\text{Zn}^{2+}$ ) from ZnO nanoparticles
- (II) Production reactive oxygen species (ROS)
- (III) Mechanical harm due to direct collaborations of ZnO nanoparticles with the cells

### Release of Zinc ions ( $\text{Zn}^{2+}$ ) from ZnO nanoparticles

Potent dissolvability of Zinc Oxide nanoparticles into free  $\text{Zn}^{2+}$  is a prominent reason for ZnO nanoparticles toxicity. The cellular hydrated zinc ion conjugate in the lysosomal acidic environment with unblemished Zinc Oxide nanoparticles which prompt mitochondrial damage and disruption of cellular zinc homeostasis prompting potential damage of the cell. Coordinate internalization or spilt  $\text{Zn}^{2+}$  from encompassing environment into the cell dictates huge barrier of the dynamic transport of membrane, DNA harm and catalyst framework interruption in charge of nanotoxicity of ZnO nanoparticles and this toxicity rely upon the concentration of solubilised  $\text{Zn}^{2+}$  in the medium (S. Singh, 2019a)

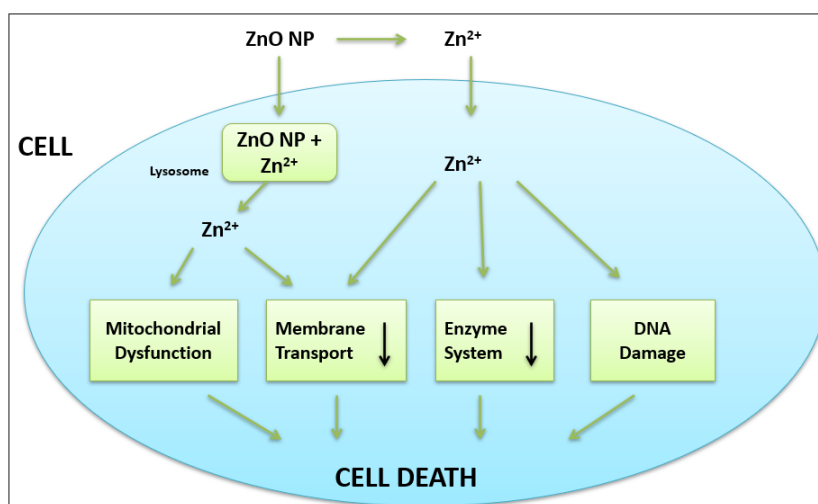


Figure 13: Mechanism of toxicity of ZnO NPs

### Production of reactive oxygen species (ROS)

The generation of ROS initiated by internalized ZnO nanoparticles as a principal component in charge of ZnO nanoparticles toxicity. When Zinc oxide nanoparticles enter inside the cell, cell guard instruments are initiated and cellular defence mechanism begins to create ROS and when the ROS production surpasses the antioxidant protective limit of the cell it builds a creation of powerful required incendiary cytokines promoting inflammation. This inflammation creates mitochondrial perturbation causing the harm to the membrane, cellular components, DNA and increment arrival of lactate dehydrogenase coming from necrosis or apoptosis and cell demise. Extracellular ROS age in light of the communication of ZnO nanoparticles with cell membranes in totality causes cell lethality. Extracellularly created ROS fundamentally follow up on electronic move chain in mitochondria internal membrane, it additionally causes toxicity by membrane harm or cell lysis. Additionally, it has been demonstrated that orally administered ZnO nanoparticles cause significant depletion of the concentrations of the antioxidants GSH, SOD and CAT indicating that ZnO nanoparticles deteriorate the antioxidant system in the brain through developing oxidative stress (Babele et al., 2018a).

### Mechanical damage due to direct interactions of ZnO Nanoparticles with the cells:

It was found that ZnO nanoparticles can connect the cell divider and causes mechanical harm like a change in cell morphology, distortion of membranes, complication or spillage of intracellular structures, mitochondrial harm and outflow of specific organelles. In a few cells, ZnO nanoparticles build the penetrability by obliteration of lipid and proteins of the cell membrane, punctures the cell divider to empower them to enter the ZnO nanoparticles inside the cells.

### Cytotoxicity studies

As ZnO nanoparticles mediated cytotoxicity has been shown in both in vivo and in vitro in different mammalian cells demonstrating the synopsis of every known cytotoxic examination which has been conducted till date. An established portion of the in vivo cytotoxic investigations on zinc oxide nanoparticles shows that ZnO nanoparticles enable a cytotoxic activity which is associated with expanded incendiary reactions. The disintegration or arrival

of  $\text{Zn}^{2+}$  from ZnO nanoparticles is essential for its in vivo cytotoxic activity (Almansour et al., 2017a).

### Genotoxicity studies

After the aggregation of ZnO nanoparticles inside the cell either in  $\text{Zn}^{2+}$  ion form or in nanoparticles, it has been causing the DNA harm by three routes:

- By creating ROS in cell
- Directly entering in core and aggregate or cross-connected with DNA strand
- At time of cell division when the membrane separates and offers to ascend to open the door for chromosome distortions.

Most examination on the hereditary toxicity of zinc oxide nanoparticles in the writing uses standard genotoxicity tests. Comet measure and micronucleus test are mostly used now and again utilized to assess ZnO nanoparticles genotoxicity.

### Neurotoxicity studies

The focal nervous system is made out of two sections: the brain and the spinal cord. This two are fragile organs in the human body which must be protected from the damage to xenobiotics. A few nanoparticles like Zinc can enter the brain employing blood-brain barrier (BBB) entrance or translocation along the olfactory nerve pathway and likewise cause damage by the enlistment of oxidative pressure, incendiary reactions, and cytotoxicity. As ZnO nanoparticles can infiltrate the BBB, they may subsequently impact the BBB capacity and cerebrum physiology and cause chronic symptoms. Collectively there are as of now a few reports, however, relatively few, which examined the neurotoxicity of Zinc nanoparticles both in vitro and in vivo (Adamcakova-Dodd et al., 2014a).

### Developmental toxicity studies

There is some limited proof, that ZnO nanoparticles may influence foetal advancement. Various investigations have indicated the maternally intervened impacts of ZnO nanoparticles exposure to the conceptive soundness of their posterity. Passing the limit dose of toxicity amid the sensitive part of the embryonic and foetal periods could then be a wellspring of various formative abnormalities with deadly effect on exposed animals and their offspring.

## Characterization techniques to evaluate the Plant-Based-Green-synthesized ZnONPs:

To qualify as a nanomaterial, it is necessary to characterize physical, optical and structural properties by using various characterization techniques as properties of nanomaterials differ as to the size changes concerning the bulk material. Commonly used structural and chemical characterization techniques are as follows:

### UV-visible spectroscopy

UV-visible spectroscopy refers to absorption spectroscopy on reflectance spectroscopy in the ultraviolet-visible spectral region. Molecules containing non-bonding electrons or  $\pi$  electrons absorb energy in the form of UV or visible light to excite these electrons to higher antibonding molecular orbital. The more easily excited the electrons the longer the wavelength of light it can absorb. It is employed to determine and quantify the amount of the substance present in the sample. UV-visible spectra can be used to examine the size and shape-controlled

nanoparticles in aqueous suspension. In UV-visible spectroscopy, the decrease in intensity of original extinction peak give information about particles destabilization and peak broadening or secondary peak occur at longer wavelength can be resulted from the formation of aggregates. Thus, it can be used to determine the stability of NPs solution and the extent of aggregation of NPs (Jose Chirayil et al., 2017).

ZnO particles are favourable for UV emitter due to its large bandgap ( $\sim 3.37$  eV at 300 K) and large exciton binding energy ( $\sim 60$  meV). The optical property of ZnO nanoparticles is determined via UV-visible spectroscopy in the range of 200 nm-800 nm. The decrease in the particle size influences the intensity of absorption peak and shifts towards lower wavelength (blue shift). ZnO nanoparticles usually show absorption peak in the range of 360 nm-380nm, a characteristic band for pure ZnO which indicates excitation of valence electrons of ZnO nanoparticles absorbing light in UV region. Increase in the metal concentration beyond threshold value lead to peak broadening and decreases the absorbance which implies a decrease in nanoparticle synthesis (Chandra et al., 2019b).

### Fourier Transform Infrared Spectroscopy (FT-IR)

FTIR is an essential technique for the characterization and identification of compounds. In FT-IR spectroscopy sample is exposed to IR radiations and it selectively absorbs radiation of a specific wavelength which causes a change in the dipole moment and leads to transfer of vibrational energy level from ground to the excited state. Vibrational energy gap determines the frequency of absorption peak. This technique is useful to identify the functional group involved in biomolecules in plant extracts which play an important role in the reduction and stabilisation of the green synthesis of nanoparticles.

The spectra of biosynthesized ZnO NPs analysed in the range 400-4000  $\text{cm}^{-1}$  using FTIR spectrophotometer. Metal oxide shows absorption bands in the fingerprint region below 1000  $\text{cm}^{-1}$  attributed to interatomic vibration. ZnO shows vibrational peaks in the region between 400 and 600  $\text{cm}^{-1}$ . The differences in the particle size may lead to different wavenumber and frequencies. The fundamental mode of vibration above 3000  $\text{cm}^{-1}$  corresponds to O-H stretch and hydrogen-bonded groups in alcohol or phenolic or water molecules present in the extract. All other peaks are attributed to the phytochemical components present with ZnO-NPs from synthesis.

### Photoluminescence Spectroscopy

Photoluminescence is a process of photon excitation followed by photon emission in which a substance absorbs photons (electromagnetic radiation) and then re-radiates photons. The emission of a photon takes place by excitation to a higher energy state and then return to lower energy state if a photon has an energy greater than bandgap energy. Thereby electron raises from the valence to conduction band. In the process of photoexcitation, electron energy ultimately falls back to the valence band and energy it loses is converted back into a luminescent photon which is emitted from the material. Photoluminescence spectroscopy is used for

- Bandgap determination,
- Impurity levels and defects detection,
- Surface structure and excited states,
- Recombination mechanisms.

The quantity of photoluminescence emitted from a material is directly related to the relative amount of radiative and non-radiative recombination rates. Nonradiative rates are associated with impurities and the amount of photoluminescence and its dependence on the level of photo-excitation and temperature. Impure samples result in three types of transition, conduction band to acceptor level, donor level to the valence band and donor level to acceptor level.

Surface defects such as carboxyl or hydroxyl are known to quench the exciton luminescence in ZnO and also prevent efficient charge transfer between ZnO and adsorbed molecules at the interface (Xiong et al., 2006). Impurities lead to primary non-radiative recombination centres during the relaxation of photo-excited valence electrons which in turn decreases the radiative combination intensity with smaller particle size. Annealing in vacuum effectively decreases impurities in samples. The photoluminescent properties, revealed that besides the intense sharp near-UV band edge emission, a strong broad emission band peak at 2.20 eV was detected for nanoparticles associated to crystal defects characterized by steady-state and time resolve photoluminescence measurements (Alves et al., 2019).

### Dynamic Light scattering (DLS)

Dynamic light scattering (DLS) is routinely used for nanoparticle size determination, size distribution and zeta potential in solution. It is a non-invasive and non-destructive technique for the characterization of particles ranging from nanometre to micrometre size. DLS measures time-dependent fluctuations of light scattered by nanoparticles in suspension undergoing Brownian motion and relates its velocity to the size of nanoparticles according to the Stokes-Einstein equation (Jose Chirayil et al., 2017). DLS can provide information about the average size, size distribution by measuring the timescale of light intensity fluctuations. The resolving power in constant instrument settings depends on the ratio of size and mass of the species in a mixture and also the dispersion characteristics and the total concentration of material. Zeta potential is an important parameter for evaluating surface charge, understanding the state of the nanoparticle surface and determining their long-term stability. Nanoparticles usually have a low degree of stability with zeta potential values between +30 mV and -30 mV (Król et al., 2017).

### Energy Dispersive X-ray Spectroscopy (EDS)

Energy Dispersive X-ray Spectroscopy (EDS) is used to determine the elemental chemical composition and it is usually integrated with either SEM (Scanning Electron Microscope) or EPMA (electron probe microanalyzer). EDS consist of an X-ray detector, liquid nitrogen for cooling, and software to collect energy spectra. A crystal in the Energy Dispersive Spectroscopy detector absorbs the energy of incoming X-rays by ionization, induces free electrons in the crystal that are conductive and creates a bias in electrical charge. The energy of individual X-rays converts into electrical voltages of proportional size by X-ray absorption. The electrical pulses correlate to the characteristic X-rays of the element and can be used for elemental identification. The EDS analysis is used to determine the elemental composition of the ZnO nanoparticles and to confirm the presence of ZnO nanoparticles.

### X-ray Diffraction Technique

X-ray Diffraction (XRD) is a promising non-destructive technique for the identification and characterization of crystalline solids. Different lattice planes cause simultaneous reflections of the X-ray beam incident on a crystal. This may lead to constructive or destructive interference

depending on the angle of incidence of X-rays and wavelength of X-rays. The angle and intensities of the diffracted X-rays are used to determine crystal structure. Crystalline solid has its unique characteristic X-ray diffraction pattern which is referred to as a “fingerprint” for its identification. Sharp and narrow diffraction peaks imply high crystallinity and small size of the biosynthesized ZnO nanoparticles. The broadening in the X-ray diffraction pattern implies the nano-sized material. Plant extract influences the crystalline structure of the NPs, while the process of formation of Zinc oxide phases for various plants in the XRD spectrum is the similar but intensities of the peaks differ which may be due to the chemical structure of the plant extract. The XRD pattern for ZnO nanoparticles shows major peaks of diffraction angles are  $31.61^\circ$ ,  $34.23^\circ$ ,  $36.35^\circ$ ,  $47.63^\circ$ ,  $56.32^\circ$ ,  $62.79^\circ$ ,  $66.97^\circ$ ,  $67.02^\circ$ ,  $69.37^\circ$ , and  $76.18^\circ$  which correlating to reflection planes are 100, 002, 101, 102, 110, 103, 200, 112, 201, and 202 respectively (Narendra Kumar et al., 2019).

### Microscopy Methods

**SEM (Scanning Electron Microscope)** has the advantage of its versatile applications such as determination surface morphology and size of the nanoparticles, ease of sample preparation, different modes of imaging and easy interpretation of the images. SEM images have a characteristic 3D occurrence used to examine the structure and good image resolution appear in the range of 0.5 nm. Several changes in the sample induced when a monochromatic electron beam with a fine spot size of approximately 5 nm and having energy from a few 100 eV to 50 KeV is passed over the surface of the specimen. The derived particles from the sample create an image of the specimen. Small dimensions of excitation beam of the order of a few nanometres cause the spatial resolution of the analysis. SEM images are useful in obtaining surface topological information of different NPs because of higher magnification and larger field depth and depend on the electron density of the surface. The ZnO nanoparticles size, surface morphology, aggregation can be obtained in the SEM images.

**TEM (Transmission Electron Microscopy)** uses energetic electrons to determine morphological, compositional, and crystallographic studies from the sample. The optimum resolution obtained for TEM images is much better than that for the light microscope as the wavelength of electrons is smaller than that of the light. Therefore, the finest details of internal structure can be resolved. High-resolution TEM (HR-TEM) is a promising tool to study properties of materials on the atomic scale such as NPs and allows for direct imaging of the atomic structure of the sample. TEM cannot give an accurate representation of the relative dispersion of the NPs in solution as nanoparticle suspensions are being dried for imaging before exposure under the high vacuum conditions. Hence, DLS is used to analyse particle dimension in solution (Murdock et al., 2008).

### Thermal analysis of Nanoparticles

The thermal analysis determines properties like enthalpy, mass changes, thermal capacity and the coefficient of heat expansion. Thermogravimetric analysis (TGA) is a technique used to determine the change in mass of a sample as a function of temperature and/or time in a controlled atmosphere and rapid assessment of the thermal stability of substances. Change in the mass of a sample can occur from thermal decomposition, evaporation, drying, sublimation, desorption or adsorption. These changes in mass are shown as step changes in the TGA curve or peaks in the DTG curve (Dongargaonkar & Clogston, 2018). The thermogravimetric measurement displays the thermogravimetric (TGA) curve as a mass versus temperature or time curve. The properties of nanomaterials are determined according to how they change with



temperature by thermal analysis and the results such as descending TGA thermal curve indicates the weight loss that has occurred. Thermogravimetric analysis (TGA) can also be used to interpret the effects of concentration surfactants or precipitating agents on the formation and decomposition process of ZnO nanoparticles.

### Differential Scanning Calorimetry (DSC)

It is another thermal method. This technique used to determine properties like melting/crystallization behaviour, polymorphism and degree of crystallinity, glass transitions, decomposition behaviour, purity determination, and specific heat, etc. In DSC, a controlled temperature program is applied by which phase changes can be characterized and the specific heat of a material can be determined. One of the DSC results of ZnO NPs obtained by precipitation method showed high melting temperature (1975 °C) of zinc oxide which concludes that NPs cannot be decomposed at low temperature (Kahouli et al., 2015a). DSC analysis carried out at temperatures 50–600 °C showed three peaks—a small low-temperature endothermic peaks were associated to the loss of a volatile surfactant molecule adsorbed on the ZnO surface, a large high-temperature peak was associated to the conversion of zinc hydroxide to zinc oxide nanoparticles and a small high-temperature one, confirmed the formation of zinc NPs by conversion from ZnO (Kumar & Rani, 2013a).

### Inductively Coupled Plasma Spectroscopy

Analytical techniques based on mass spectrometry (MS) give both molecular and elemental information and it is a very sensitive technique with a low limit of detection. Inductively coupled plasma mass spectrometry (ICP-MS) is the type of mass spectrometry useful for characterization of most metal-based nanoparticles. It is applied with increasing frequency to quantify directly the total concentration of the NPs and (bio)imaging/mapping elements. The ICP-MS is a fast and reliable technique for quantification of NPs in solution as it can easily vaporize, atomize and ionize NPs at 5–100 nm size in solution (Jose Chirayil et al., 2017). ICP-MS has better speed, precision, and sensitivity compared to atomic absorption spectroscopy.

## Conclusion

Nanoparticles development using green, cost-effective and eco-friendly approach has excited the researchers all around the world. The multifaceted feature of Green sources which act as both stabilizing and reducing agents supplemented for the controlled synthesis of nanoparticles with definitive shape and size that can be used for various applications. Replacement of use of toxic chemicals with eco-friendly phytochemicals as a reducing agent in the process of Green synthesis of nanoparticles provides an operative alternative to physical and chemical methods of nanoparticle synthesis. ZnONPs synthesised by plant-mediated-green-synthesis show improved biocompatibility, restored cell penetration capacity to ZnO NPs as an effect of the coating of various phytochemical groups which acts as ligands for nanoparticle-cell interaction. The wonderful dual action of ZnO as antimicrobial and as a wound-healing agent is found to be beneficial for burn wound healing. The actions like inactivation of p53 proteins, blocking of iNOS, improved cell proliferation by cytokine and chemokine mediated action, blocking of caspase-1, antimicrobial and inflammatory NF- $\kappa$ B pathway modulation are some of the potent glimpses of ZnONPs action in treating burn wound.

We can conclude on this account that further evidence-based studies are required to ultimately leverage the potential burn wound healing capacity of ZnONPs and future development in

green technology will eventually help in up-scaling the plant-mediated synthesis of these types of nanomaterials with essential biomedical applications.

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