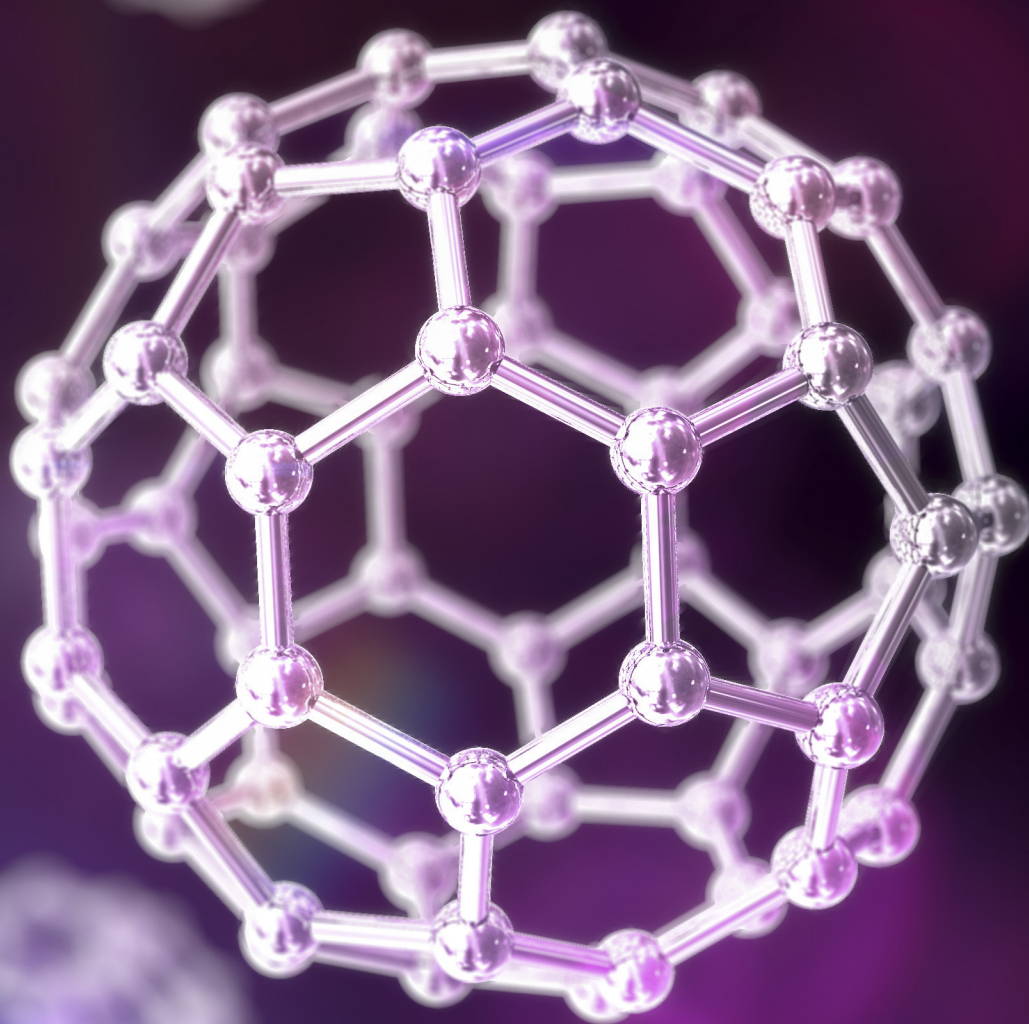


PLANT MEDIATED SYNTHESIS OF METAL NANOPARTICLES



Editors:
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Plant Mediated Synthesis of Metal Nanoparticles

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PREFACE

The term “Green” makes us recall something natural, eco-friendly, economical, non-hazardous and environmentally friendly. Nowadays, we are living in a world where we largely depend on medicines to cater to health issues. In this era, the pharmaceutical industry has become an important part of our lives. We know that the formation of medicines requires three main components *i.e.* reagent, solvent, and energy but the use of hazardous chemicals results in the formation of medicines with harmful effects. There is a strong need to generate medicines that do not harm the body and cure disease with ease. In this way, green synthesis is found to be very effective in producing pharmaceutical products having very little consumption of sources as well as less waste production with the development of new methods and resources of synthesis. This book aims to know the qualities of nanoparticles obtained from plants through green approaches of synthesis. The content of this book gives information about different methods of synthesis of green nanoparticles by using various metal salts and different characterization techniques used to identify them. Along with that, this chapter elaborates on the use of green nanoparticles alone in the form of medicines as well as with synergism with various preexisting drugs. The mechanism of action of these green nanodrugs is also explained. So, this book provides comprehensive knowledge of green nanomedicines that have the ability to replace harmful and costly medicines.

This book will be very helpful for postgraduate students and scholars who have selected nanoparticles as their area of research. This will provide them an insight into the topic and related targets.

We highly appreciate and thank the publisher for the utmost efforts they put in to give an excellent and uniform style to the text of the book.

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Green Nanoparticles: An Introduction

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Abstract: This chapter elaborates the basic introduction of nanoparticles obtained from different sources. This includes the information regarding different types of nanoparticles, methods of synthesis and important principles of green chemistry *etc.* along with all these parameters this chapter emphasize on production of environmental friendly green nanoparticles by using different parts of the plants such as stem, bark, leaves, root, flower, seed and fruit *etc.* Clear information can be gathered from the chapter regarding appropriate parameters and precautions to be taken while synthesis of green and sustainable materials with wide ranges of applications which includes essential industries like food, cosmetics, pharmaceuticals *etc.* Brief introduction has also been mentioned here about the various characterization techniques adopted for identification and monitoring of green nanoparticles.

Keywords: Applications, Green nanoparticles, Nanoparticles, Synthesis.

1. INTRODUCTION

Nanotechnology is science, engineering, and technology carried out at the Nano-scale, which ranges from up to 100 nm. Richards Feynman is the pioneer of nanotechnology. The study of incredibly small objects is known as nanoscience or nanotechnology, and it has applications in all other scientific domains, including chemistry, biology, physics, material science, and engineering. Richard Feynman proposed the theory and notion behind nanotechnology and nanoscience in a discussion on “There is Plenty of Room at the Bottom” at a meeting on December 29, 1959. Professor Norio Taniguchi coined the term nanotechnology. The ability to observe and control individual atoms and molecules is at the heart of nanotechnology and nanoscience. Atoms contribute to everything on Earth, especially the food we consume, the clothes we put on, buildings, houses, and our bodies. However, atoms are so minuscule that they are invisible to the naked eye.

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The microscope, which is used in the school science lab, can also observe the infection. In the early 1980s, the microscope required to examine the thing at the Nano size was invented. Scientists possessed the necessary tools at the time, such as the scanning tunneling microscopy (STM) and the atomic force microscope (AFM). Today's scientists and engineers are discovering a wide range of ways to purposefully create materials at the Nano size in order to benefit from them. Nanotechnology is a small solution to big problems. As it is the rule of nature that a thing with advantages has disadvantages too (Fig. 1).

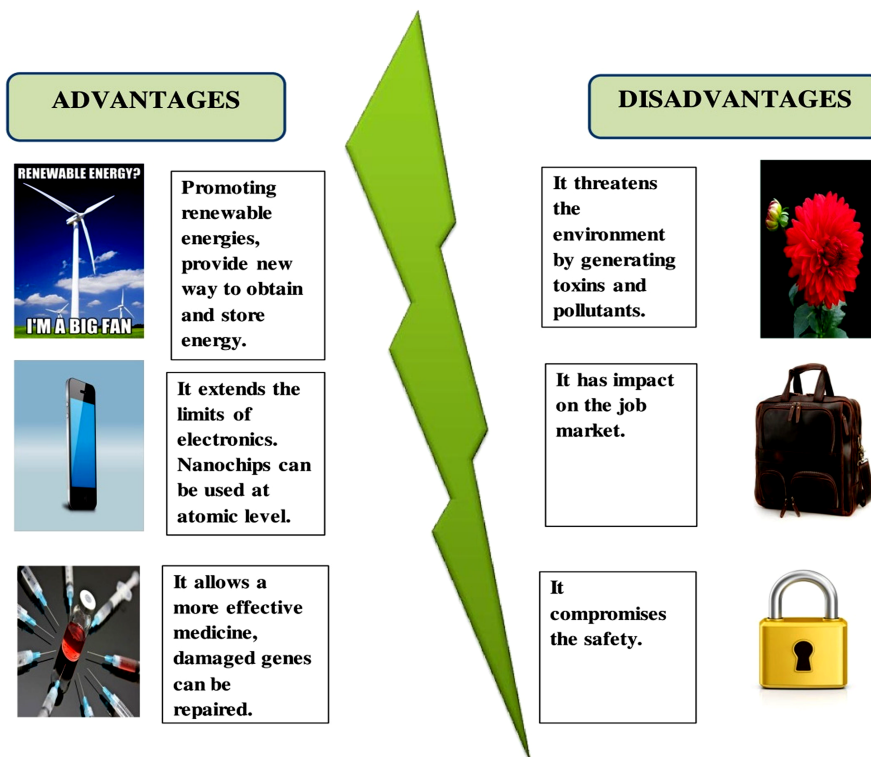


Fig. (1). Nanotechnology's benefits and drawbacks.

2. TYPES OF NANOTECHNOLOGY

There are many forms of nanotechnology, which are explained below. These forms depend upon different techniques for the formation of nanoparticles.

2.1. Descending (Top-down)

This is the most frequent trend, especially in the electronic field. The structure is miniature at the nanometer scale from 1 to 100 nanometers.

2.2. Ascending (Bottom-up)

This is a mounting or self-assembly process that allows you to build a larger mechanism than you started with (you start with a Nanometric structure- a molecule) [1].

2.3. Dry Nanotechnology

It is utilized to make structures that do not work with humidity out of coal, silicon, inorganic materials, metals, and semiconductors.

2.4. Wet Nanotechnology

Based on biological systems found in watery environments. It is concerned with genetic material, the membrane, enzymes, and other cellular components.

2.5. Green Nanotechnology

This branch of nanotechnology deals with the concept of green chemistry and green engineering. It refers to the utilization of plant products. It consumes less energy and fuel. NPs synthesized by biological methods are more valuable and preferred over Physicochemical methods. Physico-chemical methods require a large amount of investment, which may allow the use of toxic solvents and the production of hazardous substances at the end of the process. Whereas in chemical methods, the use of more than one chemical species may lead to toxicity, which also harms human health and the environment. NPs synthesized from green synthesis have different approaches to synthesis.

3. WHAT IS A NANOPARTICLE?

Nanoparticles are materials with diameters ranging from 1 to 100 nm. They fall in the transition zone between the molecule and their bulk counterparts [2, 3]. Because of their small size, they feature unique physiochemical qualities such as enormous surface area, high energy, and quantum confinement [4 - 6]. Since they have their very large scale specific surface area, high surface energy, and quantum confinement [7], they exhibit numerous unique properties (optical, magnetic, electrical, and so on). Because of their unique physiochemical properties, nanoparticles have a wide range of uses, including medicine [8], cosmetics [9], electronics [10], the food business [11], and the chemical industry [12]. Nanoparticles exhibit a wide range of chemical and morphological features [13].

CHAPTER 2

Synthesis, Characterization and Analysis of Green Nanoparticles

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Abstract: The chapter focus on detailed information regarding all recently adopted methods for synthesis of green nanoparticles such as Biological synthesis, Co-precipitation method, Template synthesis, Hydrothermal technique, Microwave assisted synthesis, Inert gas condensation, Laser ablation method, Micro-emulsion technique, Spark discharge method and Sputtering *etc.* Along with their advantages and disadvantages. Biological methods of synthesis have been described extensively here to synthesise environmental friendly nanoparticles with economical costs. Biological synthesis has been shown by using bacteria, fungi and yeast *etc.* Along with methods, this chapter gave detailed description about various spectral and analytical techniques such as FTIR, SEM, TEM, XRD, EDX, UV-Visible spectroscopy *etc.*

Keywords: Advantages, Characterisation techniques, Green synthesis, Nanoparticles.

1. INTRODUCTION

Nanoparticles (NPs) with a diameter between 1 and 100 nm serve as a link between molecular or atomic and bulk materials structures [1]. Due to their small sizes, huge surface areas with free dangling bonds, and more reactivity than their bulk cousins, they possess surprising and fascinating properties [2]. Although the potential of living entities to decrease metal precursors has been widely known to scientists since the nineteenth century, the methods are still unknown. Researchers have been interested in biological approaches because of the development of effective green synthesis by using natural stabilizing, capping, and reducing agents without the use of hazardous chemicals and significant consumption of energy [3]. Increasing urbanization, and industrialization along with the growth of population are degrading the earth's atmosphere and releasing a vast quantity of

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dangerous and undesired compounds. The moment has come to uncover the mysteries held inside nature and its natural products in order to develop the techniques used to create NPs. Also, because NPs are frequently used in regions where people come into touch with them, it is becoming more and more important to create synthesis methods that don't use harmful chemicals. As a result, green or biological synthesis of NPs presents a potential substitute for physical and chemical processes. When compared to particles made by physic-chemical methods, green synthesis produces different particles. For the fabrication of metal or metal oxide NPs, green synthesis substitutes costly chemical reducing agents with an extract from natural sources like leaves from trees, crops, or fruits. There is an enormous ability for the creation of NPs in biological entities. Environmentally benign, chemically free, and cost-effective, biogenic metal precursor reduction to matching nanoparticles (NPs) can be employed for large-scale production [4]. Furthermore, the biological synthesis of nanoparticles enables the recycling of pricey metal salts like silver and gold present in waste. Resources for these metals are few, and their prices are unstable [5]. The desired qualities may be obtained in green NPs. The biological components that stabilize NPs, mostly sugars, enzymes, proteins, and sometimes the complete cells enable nanoparticles to interact with some other biomolecules and hence enhance interactions with microbes to increase the antimicrobial activity. NPs make it simple to up-concentrate by centrifugation or separate the NPs from the reaction mixture [6]. When compared to NPs made chemically, biogenic silver demonstrated 20 times more antibacterial activity [7]. The selection of plant extracts used to make NPs is based on the inherent value of biological materials. The *Spirulina platensis* algae cells were chosen since they not only function as a reducing agent but also display medicinal and nutraceutical qualities [8]. In the procedures, unicellular bacteria and extracts of various multicellular eukaryotes turn precursors of metals into NPs of required forms and dimensions. Moreover, biological entities have stabilizing and capping substances that are needed in the form of growth inhibitors to stop the aggregation or agglomeration process [9]. The type of biological entities, their concentration, and organic reducing agents all have an impact on the size and structure of NPs. Moreover, factors like pH, salt concentration, temperature, and duration of exposure in the growing medium have a significant effect on the shape and size of NPs [10]. *In vitro* or *in vivo* bio-reduction of metal precursors occurs for the creation of nanomaterials. Enzymes, carbohydrates, proteins, and phytochemicals including terpenoids, flavonoids, and phenolics among others, mostly serve as the most effective stabilizing and reducing agents [11].

It has been claimed that NPs can be produced *in vivo* utilizing bacteria, fungi, yeast, algae, and plants. For *In vitro* synthesis, which entails purifying bio-reducing agents and carefully blending them into an aqueous solution of the

necessary metal precursor, biological extracts are typically used. At room temperature, the reaction happens on its own, although occasionally more heating and stirring are required [12]. Plant extracts are found to be the most effective agents among the various biological moieties discussed above since they are widely accessible, ideal for the mass production of NPs, and their waste products are environmentally acceptable, in contrast to some microbial extracts [13]. Despite extensive physico-chemical research in the field of nanotechnology, green fabrication is still normally used to create silver and gold nanoparticles. To clarify the biogenesis and prospective uses of different metallic and semiconductor nanoparticles, a relatively small number of research has been conducted. This article gives an overview of the synthesis of several semiconductor and metallic NPs such as Cu, CdS, PbS, Ru, Pd, Fe, CuO, Fe₃O₄, CeO₂, TiO₂, and ZnO nanoparticles with an emphasis on their uses in pharmaceuticals.

2. GREEN PRODUCTION OF NANOPARTICLES AND THEIR IMPACTS

In general, the synthesis of NPs can be performed in either a “top-down” or “bottom up” manner (Fig. 1). NPs are manufactured through size reduction in the “Top-down” strategy, which is accomplished through a variety of physical and chemical techniques [14]. In “Bottom-up” synthesis, NPs are made from small building blocks like atoms and molecules, with reduction/oxidation serving as the primary process. This more environmentally friendly method produces NPs with a uniform chemical composition and few flaws. Microorganisms and extracts of plants are frequently utilized in the biological production of NPs [15].

3. TECHNIQUES OF NANOPARTICLE SYNTHESIS

The synthesis method depicted (Fig. 2) can be used to create a variety of nanostructures; a nanostructure is a structure having at least one or two dimensions in the range of 1-100 nm. Many methods can be employed to create both dry particles and NPs that are dispersed in a liquid.

3.1. Biological Synthesis

Nanotechnology and biotechnology are connected through green chemistry methods known as the biological production of nanoparticles. Biological nanoparticles are stable, yet, despite this, they are not mono-dispersed, and the rate of synthesis is slow. The polydispersity of nanoparticles and an ensuing slowdown in the rate of synthesis are caused by the fluctuating concentration of fabrication macromolecules involved in the particles' nucleation throughout time.

CHAPTER 3**Biological Potential of Nanoparticles as Effective Pharmacological Agents****Pallvi Aggarwal^{1,*}**¹ Department of Chemistry, Pt. CLS Govt. College, Karnal Haryana, India

Abstract: Green nanoparticles are being used in the field of pharmaceutical industry to convert them into pharmacologically effective agents that are used to cure many dangerous diseases. Inventions have been done in this area to prepare non-toxic and pharmacologically beneficial drugs to save life of humans and to preserve the environment to its originality. This chapter gives information about the different types of nanoparticles that are used in the form of drugs such as Dendrimers, Micelles, Drug conjugates, Protein nanoparticles, Nano-gels, Nanotubes of carbon and Nano-diamonds *etc.* It has been found that incorporation of green nanoparticles into drug reduces the toxicity level of the drug and used to cure Cancer, Infectious disorders, Auto-immune disorders, Cardiovascular Diseases, Neurodegenerative diseases, Disorders of the eye and lungs *etc.* Along with this, the chapter describes the various route of administration of nanoparticles with appropriate mechanisms.

Keywords: Diseases treatment, Nanoparticles, Pharmacological agents, Route of administration.

1. INTRODUCTION

For thousands of years, people all over the world have relied on natural products derived from plants as a means of treating illness. Herbal therapy has been the foundation of modern medicine for centuries, with much of the modern pharmaceutical industry built on ancient practices and expertise. Today, about 25% of the most important medicinal substances and their variants come from natural sources. In order to find new therapeutics, it is necessary to start with natural substances that have never been used before [1].

The interest in creating synthetically adaptable lead compounds that mirror their equivalent chemistry has been a new development in natural product-based drug discovery [2]. Natural goods have unique qualities, such as lower toxicity, greater

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chemical and biological specialization at the macromolecular level, and exceptional chemical variety. Because of this, they are promising starting points for finding new drugs [3]. Furthermore, quantitative studies have aided in visualizing drug molecule interactions, leading to the creation of next-generation drug innovations like target-based drug finding and drug transport.

Natural product-based drug discovery and drug transport methods have several benefits, but pharmaceutical firms are reluctant to spend more on them [4]. Instead, they prefer to explore the catalogs of available chemical substances in search of new medicines. Cancer, diabetes, coronary, inflammation, and infectious illnesses are just some of the diseases for which natural substances are being investigated as potential treatments. The primary reason for this is the numerous benefits natural medicines provide over synthetic alternatives, including reduced toxicity and adverse effects, cheap cost, and high curative potential.

Natural substances indeed have therapeutic potential, but the issues of biocompatibility and toxicity raise significant barriers to their use. As a result, many natural substances are being rejected from clinical trials [5]. (Fig. 1) explains the targeted drug delivery system using nanotechnology.

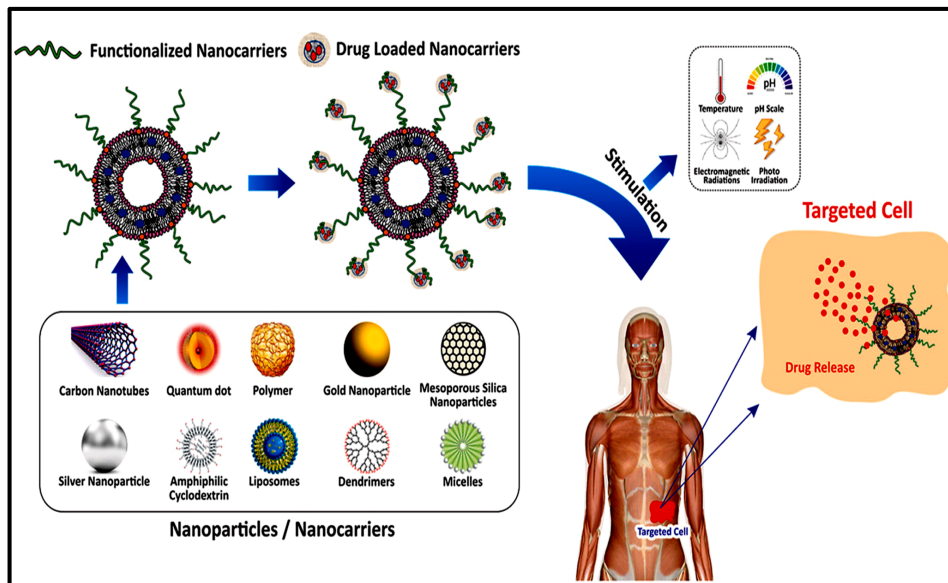


Fig. (1). Targeted drug delivery system using nanoparticles [119].

Issues with target-specific delivery, solubility, absorption, efficacy as a stimulant, and possible adverse effects are just some of the problems that can arise when using large-sized materials in medication administration. Therefore, the use of

novel drug delivery methods to focus medications on particular bodily regions may be a choice to address these pressing concerns. Therefore, nanotechnology plays a crucial part in the development of cutting-edge pharmaceuticals, as well as in the realm of targeted drug administration and regulated drug release [6].

This is especially true in nanomedicine and nano-based drug delivery systems [7], where such particles are of significant interest and have been shown to cross the gap between the biological and physical disciplines through their application in nanotechnology. Nanomaterials have vast applications in the field of biosensors, microfluidics, drug transport, microarray assays, and tissue engineering and also have an impact on cutting-edge nanomedicine [8].

Nanomedicines use nanotechnology to implement therapeutic substances at the nanoscale. Nanoparticles have been the driving force in the biomedical area including nanobiotechnology, medication transport, biosensors, and tissue engineering [9]. Because atoms and molecules are the building blocks of nanoparticles, these particles tend to be very tiny and different in shape, consequently, they have greater mobility within the human body than larger elements [10]. Nanoparticles can be used as transport agents for drugs by enclosing them or adding medicinal drugs and releasing them more accurately and at a regulated rate into target regions [11].

The term “nanomedicine” was coined to describe the use of nanotechnology in the healthcare industry and the field of biological studies [12]. About a hundred nanomedicine apps and goods have been given the approval for widespread distribution by the US Food and Drug Administration in the last couple of decades [13]. These findings demonstrate the significant impact of nanotechnology on modern biological research [14, 15]. Throughout the globe, nanotechnology has received a great deal of interest. A recent study by Forbes ranked nanotechnology as one of the top five emerging technologies to keep an eye on in the next decade [16].

Due to the substantial constraints and issues that impacted traditional pharmacological agents and earlier formulas and delivery systems, nanotechnology plays an important role in the field of medicine and medication administration. Conventional drug delivery systems have the issue of leaving non-biodegradable material within the patient's body, which can cause poisoning [17]. This is because it is difficult to remove the leftover components of such systems. Similarly, most traditional DDSs exhibit a rapid surge of drug release soon after drug delivery, and conventional DDSs typically have poor drug solubility [18]. Nano-pharmaceuticals offer potentially useful approaches to the aforementioned issues [19]. Nanoparticles (NPs) are much smaller than the smallest component of

Mechanism of Action of Nanoparticles

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Abstract: The current trend highly favors the synthesis and production of metallic nanoparticles such as gold, silver, copper, zinc, iron, magnesium *etc.* utilizing plant components that have superior properties. Simultaneously nanoparticles have been produced utilizing diverse plant extracts sourced from leaves, roots, seeds and other plants. Plant-extract-based nanoparticles are in high demand due to their outstanding characteristics and versatile applications. These nanoparticles are extensively employed in numerous medical procedures, devices and biological applications they do present drawbacks attributed to nanotoxicity. This review offers a comprehensive view of the mechanism of action, production, and medical applications of nanoparticles particularly focusing on their potential health and environmental impacts. This study discusses medicinal plants, plant- extract-based nanoparticles and their potential uses for antiviral, anticancer, antibacterial, and antifungal treatment.

Keywords: Antibacterial, Anticancer, Mechanism of action, Nanoparticles.

1. INTRODUCTION

The term nanoparticles refers to the clusters of atoms, ions, or molecules that typically have sizes between 1 to 20 nm. It balances the gap between small molecules (with discrete energy states) and bulk material, making this size range interesting (with continuous energy states). Nanoparticles are different kinds of material because they frequently exhibit properties that are fundamentally dissimilar from those of discrete molecules or bulk solids. There are many similarities between nanoparticles and bulk surfaces because clusters have a significant proportion of surface in inner atoms. Most of the elements in the periodic table may form nanoparticles and they can be divided into different categories based on the types of atoms and bonds. Nanoparticles can be weakly

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bonded with rare gas and molecular nanoparticles (like argon and Water) covalently bonded semiconductor nanoparticles (like carbon fullerenes and Cadmium) and electrostatically bonded ionic nanoparticles [1]. There is a lack of research on the subject of nanoparticle toxicity in the field of nanomedicine. However, its main focus is on the mechanism of action. Over the past few decades, nanoparticles have been employed more and more in industry [2]. Small nanoparticles are commonly referred to as nanoclusters or sub-nanoclusters for those smaller than one nm [3]. Construction materials, paints, and stained glass all made use of nanoparticles before their nature and properties were discovered and understood. Transition metal nanoparticles have been utilized as heterogeneous catalysts for more than a century, and they have been a significant source of income for the photochemical industry. Many techniques can be used for nanoparticles: like photocatalysis, and antimicrobial and antibacterial activities [4]. Now a time, researchers are becoming more and more interested in atmospheric nanoparticles. The formation of larger particles which affect the global temperature, atmospheric chemistry, visibility, and regional and global movement of contaminants and biological nutrients needs the presence of nanoparticles as key precursors [5]. Nanoparticles are already present in nature for a very long time, and they are not always produced in modern synthesis laboratories. The deliberate reinforcement of ceramic matrix with natural asbestos nanofibers more than 4500 years ago is more interesting, even though the use of clay minerals as natural nanomaterials does not appear to be highly advanced. Metal nanoparticles used as color pigments in luster and glass technologies gave the most stunning results. Research on nanoparticles is primarily driven by the so-called quantum size effect [6]. Nanoparticles that are commonly referred to as bio-nanoparticles and found in nature include ferritin, the tobacco mosaic virus, and cowpea chlorotic mottle virus. Surface-activated nanoparticles are predicted to play a significant role in the future because they have the ability to be chemically modified at the surface to add additional activity [7]. Recently there has been a rise in research into how nanoparticles affect biological systems that are regularly present in the environment. The majority of these nanoparticles are made of transition metals, including silicon, carbon, copper, aluminum, silver, and metal oxides [8]. Nanoparticles are deposited mostly through a diffusion mechanism depending on the delivery method, sedimentation, and inertial compaction may also be involved [9]. This chapter describes different mechanisms of action of nanoparticles on the basis of these applications.

2. APPLICATION AND MECHANISM OF DIFFERENT NANOPARTICLES

2.1. As Antimicrobial Agent

The mechanisms of antibiotic activities are well established. Some of them prevent the formation of bacterial cell walls, proteins, nucleic acids, or enzymes or nucleic acids, proteins or enzymes. Penicillin is one example of an antibiotic with a limited range of applications.

The fact that nanoparticles have many antimicrobial modes of action that may kill a variety of bacteria is one of their greatest advantages [10] such as:

- Increased membrane permeability and cell membrane deterioration.
- Bacterial cell wall or wall damage.
- Nanoparticles release ions that interact with intracellular and extracellular components that can produce reactive oxygen species (ROS) through photocatalysis which can harm cellular components.
- DNA synthesis and enzyme activity inhibition.
- Disruption of energy transfer.

2.2. Silver Nanoparticles

Nowadays, there have been a large number of articles and publications showing the synthesis of silver nanoparticles by a variety of species, including bacteria, fungi, lichens, algae, and higher plants; the mechanism behind this process has never been fully investigated. Using the range of microorganisms, silver nanoparticles can be generated both within and outside the cell. Because of the presence of proteins- enzymes on bacterial cell walls and secreted proteins, Ag^+ is reduced to Ag^0 during the extracellular synthesis. Silver nanoparticles' extracellular synthesis was demonstrated to be typical for both Gram-positive Bacillus species, particularly *B. pumilus*, *B. persicus*, *B. licheniformis*, *B. indicus* and *B. cecembensis*, as well as *planomicrobium*, *streptomyces* and *Rhodococcus* species as well as for Gram-negative bacteria like *Klebsiella pneumonia*, *Escherichia coli*, and *Acineto*. The process of synthesis has also been identified in a number of other microbes, including the fungus *Rhizopus stolonifera*, *Aspergillus niger*, *Fusarium oxysporum*, *Fusarium* species, and *A. flavus*.

Enzymes are the primary component of silver nanoparticles whether they occur intracellularly or extracellularly. The majority of researchers concur that NADH-dependent nitrate reductase plays a key role in the formation of silver nanoparticles. This enzyme serves as an electron shuttle, transferring electrons

CHAPTER 5

Synergic Effects of Nanoparticles with other Drugs and Their Combined Effects

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Abstract: As discussed in previous chapters that the formation of nanoparticles results in various advantages for multidrug delivery when combined with particular drug as compared to free drugs. So this article emphasize on use of nanoparticle and drug combination to reduce the toxicity and improve the efficiency which is given in the form of advantages. Drug combination analysis has been explained in the form of isoboles and dose equivalent *etc.* Synergic effects of drug-nanoparticle combination on bacteria, fungi, virus, diabetes and cancer has been shown by proper mechanism. Beside these harmful effects of nanoparticles alone has been discussed in this section. Drugs are combined with various metal nanoparticles such as gold, silver, copper *etc.* has been shown to act as excellent agents to cure diseases effectively.

Keywords: Drug-nanoparticle combination, Mechanisms, Nanoparticles, Synergic effects.

1. INTRODUCTION

The Greek word “*synergos*” means “working together”, so the term synergic effect defines the combined effect of at least two substances making a more significant impact than both could show separately. In biological and pharmacological systems, two terms *i.e.* “synergy” and “synergism” are being used. Synergy refers to the interaction in biological systems while synergism is related to pharmacology where it describes the condition among a set of different drugs that result in the enhancement of their efficacy. At the bimolecular level, the synergic effect is examined by various enzymes. On the other hand, at the cellular level, it is illustrated by hormones present in the body [1]. For example, during the birth of a child, oxytocin is produced incrementally to induce labor contraction which results in muscle contraction until the neonate is pushed outside the birth

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canal. Similarly, in the world of medicines, drug synergism occurs when the positive effects of two or more different drugs are amplified after administration jointly. For example, if two drugs A and B produce an impact of 15% and 25% separately. However, after combination, the synergic effect is found to be 80%, which is 40% greater than the individual. There are various examples in the pharmacology that explain this effect more significantly such as the synergic effect of aminoglycoside and penicillin in combination on the bacterial cell wall is more prominent than individual drugs. Similarly, aspirin and caffeine when together, provide greater relief in pain. Moreover, when the Probenecid drug is administered with penicillin, it delays renal excretion with greater potency [2].

The formation of nanoparticles gives several advantages for multidrug delivery in combination as compared to free drugs. The appropriate concentration of nanocarriers has the ability to normalize the biodistribution, pharmacokinetics, and stability of dissimilar drugs (chemically) that independently show different behavior. That's why formulations that are long circulating continuously release the drugs at the required amount.

This efficiency of Synergism has also been found in nanoparticles when combined with different drugs. In the case of the association of antibiotics with nanoparticles, the synergic effect is associated with the production of hydroxyl radicals, a change in protective cellular functions, and the potential of anti-biofilm. This combination is more effective against bacteria as compared to the use of antibiotics alone. It has been found that the combination of a drug with nanoparticles reduces system toxicity, improves drug solubility, enhances the time of blood circulation, and targets specific cells with controllable release profiles [3]. The first clinical treatment was found in the 1990s when >25 nano therapies and Doxil for cancer were clinically tested. The first multi-drug containing nanoparticles are now subjected to phases 2nd and 3rd. Formulations of nanoparticles where drugs themselves behave as carrier gives a number of opportunities after adjusting through top-to-down fabrication or solvent-mediated self-assembly. Here the insoluble drugs have the ability to form colloids with tunable size and homogenous composition. Researchers have shown the ability of nanoparticles to combine with certain drugs that leads to the formation of the hydrophobic drug to which the antibody "trastuzumab" (anti-HER2) is absorbed with the hydrophilic drug "Doxil" [4]. Efforts have been made to create more effective formulations with a size of approximately 100 nm to enhance the biodistribution and target the solid tumors. It has been found that the compact particles penetrate the tumor area with more effectiveness and also come out of the body through renal excretion rather than slower clearance which decreases the chance of liver toxicity [5, 6]. A combination of drugs is more effective if they can block the parallel pathways that feed into the same cellular behavior.

Additionally, drugs can behave synergistically by increasing the uptake or decreasing the elimination or degradation of any other drug [7].

2. ADVANTAGES OF CONJUGATION OF NANOPARTICLES WITH DRUGS

Several benefits are found with drugs when they are combined with nanoparticles. The effects depend upon the nature and chemical compositions of both nanoparticles and drugs. Some of these are given [8, 9]:

- Exhibit multiple targets and mechanisms of action.
- Reduction in the emergence of resistant pathogens.
- Improvement in self-assembly into nanostructures for delivery systems.
- Facilitation for intracellular targeting.
- Decrease in the individual dosages that consequently minimize host toxicity.
- Prolonged circulation and stabilization of drugs in the body.
- Increasing the spectrum of antimicrobial coverage during therapies.

The synergic studies focus on different metal-based nanoparticles such as copper sulfide (CuS), iron oxide ($\text{Fe}_3\text{O}_4/\text{Fe}_2\text{O}_3$), platinum (Pt), gold (Au), copper oxide (CuO), copper (Cu), iron (Fe), zinc (Zn) and zinc oxide (ZnO). This combination of NPs and drugs serves as effective agents against different species of microbes along with other diseases.

3. QUANTITATIVE METHODS FOR ASSESSMENT OF DRUG SYNERGISM

3.1. Drug Combination Analysis

3.1.1. Isoboles

It is the most common method to assess the interaction between the agonist drugs is a method of isoboles. This is actually a method that is based on a graphical procedure and uses the dose-effect relation of individual drugs and combinations which are expected to generate a specific effect level. Often the used effect level is 50% of the maximum effect and the dose of individual drug gives this effect. In this method, the ED_{50} dose of each drug is used and its intercept value is on the Cartesian coordinate system where doses are represented on x-axes, and y-axes.

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