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A Review on Phytofabricated Metallic Nanoparticles and their Characterization Techniques

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Abstract : Metallic nanoparticles (MNPs) have innumerable applications in various fields of science and technology. Green chemistry based NP synthesis offers several advantages over conventional approaches. The biological approach is inexpensive, facile, rapid, and environmentally benign method for NPs synthesis. Characterization of NPs is generally based on their dimension, surface area, and dispersity. The most common techniques used for this purpose are UV-Vis, SEM, TEM, FTIR, XRD, DLS, AFM, Raman spectroscopy, *etc.* These techniques are considered highly useful as they provide important information about these materials for diverse biological and environmental applications. Until now, numerous plant materials have been utilized to generate numerous MNPs, such as silver, gold, copper, titanium, platinum, palladium, zinc, and iron NPs. Among these MNPs, the noble ones have attracted significant researchers' attention because they are non-corrosive and resistant to oxidation in moist air. They have been useful in providing remedies with less or no adverse effects for several acute diseases like malaria, hepatitis, cancer, and many others. They have a wide range of applications, such as diagnostics and therapeutics, antimicrobial agents, anti-inflammatory agents, cancer treatment, sensors, *etc.* Several techniques have been used to characterize size, crystal structure, elemental composition and a variety of other physical properties of nanoparticles. In many cases, there are physical properties that can be evaluated by more than one technique. Different strengths and limitations of each technique complicate the choice of most suitable method. Often, a combined characterization approach is needed. It is needed that researchers from different fields overcome these challenges by reliable characterization of nanomaterials. We present here a systematic review, focusing on phytofabricated MNPs and their characterization techniques.

Key words: Metallic nanoparticles, characterization techniques, phytofabrication, nanomaterials.

I. INTRODUCTION

Nanotechnology is the creation and careful manipulation of materials on an atomic or molecular level, and the use of these materials at the nano level for various purposes.[1] "Nano" is acquired from a Greek word 'nanos' which means dwarf or extremely small. The word nano indicates one billionth (10^{-9}) of a meter and NPs are groups of atoms or molecules in the range of 1–100 nm. NPs can be divided into two different types, inorganic and organic NPs. Inorganic NPs include noble metal (silver and gold) NPs, magnetic (iron oxide) NPs, and semi-conductor (titanium oxide and zinc oxide) NPs, on the other hand organic NPs include carbon nanotubes, dendrimers, and liposomes. Extensive research in nanotechnology has opened several advanced and practically applicable research frontiers, like nanobiotechnology, nanomedicine, and theranostics. This research integrates the fundamental and applied aspects of nanotechnology and biology. Nanomaterials are synthesized either by using traditional physicochemical methods or by biosynthetic routes using microbes, plants, algae, *etc.*[2, 3] NPs are important because of their small size. Large surface area to volume ratio imparts them properties that are considerably different from those of the same material in bulk. Due to unique properties, NPs are used for various purposes which extend from bio-imaging, catalysis, bio-sensing,[4] diagnostics,[5] wound dressings, medical implants,[6] gene or drug delivery, cosmetics, food and electronic components.[1, 7] NPs-bound drugs are claimed to have certain advantages over traditional forms of drugs.[8] These drugs have greater *in vivo* half-life, longer persistence in the body and ability to carry higher concentration of drugs to the target site.[9] Targeted drug delivery using aptamer and antibody functionalization has been used to deliver drug molecules with NPs as carriers.[10] Noble MNPs (AgNPs and AuNPs) are of greater interest because of chemical stability, good conductivity, catalytic activity, and ability to conjugate many biomolecules.[11, 12]

NPs can be synthesized by 'top down' or 'bottom up'approach.[13] 'Top down' fabrication requires a huge amount of material and reduces large-size material to the nanoscale level, whereas the 'bottom up' approach creates NPs by adding atoms and molecules. The top down approach has a shortcoming of generating defects in the NP's surface, which affects the surface chemistry and physical characteristics of synthesized NPs. Being an effective approach, bottom up is preferred to avoid such drawbacks. Traditionally, physical, chemical, and physicochemical methods have been used to generate and stabilize the MNPs.[14] Laser ablation,[15] ion sputtering, mechanical milling,[16] colloidal lithography,[17] and high energy irradiation[18] are the physical approaches (gold standard) whereas chemical methods (electrochemical and photochemical reduction) are simple to prepare MNPs.[19] These methods are valuable, but carcinogenicity and environmental toxicity are limiting MNP production for invasive biomedical applications.[20] Therefore, there is need for clean, reliable, non-toxic, environmentally benign approaches to synthesize NPs.[21] To address these issues, green synthesis of MNPs involving a bottom up approach came into the spotlight. This method is considered highly effective, because it offers an inexpensive, easily scalable, and environment friendly approach for NPs fabrication. More importantly, no requirement of high temperature, pressure, energy, and toxic chemicals make it a method of choice. However, the detailed mechanism behind NP synthesis is still under investigation.[22]

In the last few decades, there has been extensive research in green chemistry based NPs synthesis focusing on the exploitation of living entities, such as bacteria,[23] fungus,[24] plants,[25] and viruses,[26] for NP production. Extracts from plants such as *Allium sativum*,[27] *Aloe vera*,[28] *Curcuma longa*,[29] *Eclipta prostrata*,[30] *Emblica officinalis*,[31] *Azadirachta indica*,[32] *Piper longum*,[33] *Terminalia catappa*[34] and various others[35] have been used for generating noble MNPs. Biogenesis wards off the problems faced by the traditional physicochemical approaches and results in NPs of many shapes, (spherical,[36] hexagonal,[37] tetrahedral,[38] quasispherical,[36] decahedral,[39] triangular,[28] cubic,[40] spheroidal,[41]) sizes, and composition. The biosynthetic route is proficient as it can be used to generate MNPs of well-defined shape and size at a large scale.[42] Among various biological routes of MNP production, microbe-mediated synthesis has not gained much importance due to its complex and special requirements of highly aseptic conditions, strain isolation, culture preparation and maintenance. However, the use of plant extracts for MNP synthesis has become a better method of choice due to its ease of improvement and lack of any special requirement.[25] Extracts from different plants contain different concentrations and combinations of reducing agents (alkaloids, phenolic acids, terpenoids, flavones, steroids, polysaccharides, amino acids, oximes, tannins, polyols, *etc.*) and due to these compounds plant extracts as a whole have been effectively utilized for the synthesis of MNPs.[43] Reviews focusing on green MNPs synthesis have been published with great emphasis on different biological sources; [2, 35] however, their diverse characterization techniques have not been well documented. We therefore, present this review with the aim of giving a brief description of the green synthesis of MNPs using natural biological factories, especially plant extracts and techniques used for their characterization.

2. PLANT-MEDIATED SYNTHESIS OF METALLIC NANOPARTICLES

Plants accumulate specific metals by reducing their ionic forms in different tissues away from penetration site and later these metals can be extracted by an approach called phytomining. Detailed study of metal bioaccumulation has shown that these metals get accumulated in the form of nano-sized particles.[44] This study paved the way for researchers to develop plant mediated nanoparticle synthesis. Different plant parts such as leaves, fruit, peel, roots, seeds, callus, stems, and flowers have been utilized to synthesize MNPs (silver, gold, platinum, palladium, magnetite, zinc, copper, titanium oxide, nickel, *etc.)*[45] The general steps of plant-mediated MNP synthesis are shown in Fig. 1.

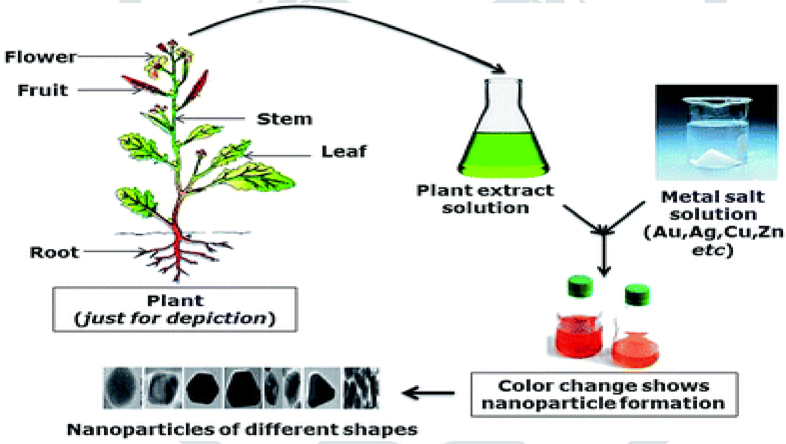

Fig. 1. Basic steps in plant-mediated metallic nanoparticle synthesis. [106]

2.1. Role of plant metabolites in metallic nanoparticle synthesis

There are several plant biomolecules which play a crucial role in the reduction of metal salts to form nanoparticles and stabilize them by acting as capping agents. A few commonly known biochemicals present in plant extracts and their role in MNP synthesis are discussed.

Terpenoids belong to a diverse organic polymer class which displays strong anti-oxidant activity. They are produced in plants from 5 carbon isoprene units and have been demonstrated to be associated with MNP production. The data from FTIR spectroscopy laid a platform to describe the mechanism of terpenoid-mediated metal reduction into MNPs. Singh *et al.* in their work suggested that deprotonation of the eugenol–OH group leads to the formation of resonance structures which can undergo a further oxidation process, which is eventually followed by active reduction of metal salts to form MNPs.[46]
Sugars present in plant extracts can also assist in MNP synthesis. The linear aldehyde containing monosaccharides, such as glucose, have been known to act as reducing agents. However, keto groups containing monosaccharides, such as fructose, can act as reducing agents only after they have undergone a series of tautomeric transitions. According to studies, the aldehyde group present in sugar gets oxidized into a –COOH group through nucleophilic addition of a hydroxyl group which in turn results in metal ion reduction and, thus, MNP formation.[44] For instance, *Cinnamon camphora* leaf extract has been utilized for the formation of AgNPs and AuNPs.[47]
Flavonoids belong to a large group of polyphenolic compounds which consists of numerous classes, such as flavonols, isoflavonoids, chalcones, flavones, and anthocyanins. Researchers have found that the enolic groups in flavonoids can undergo tautomeric transitions to give keto groups by releasing a reactive hydrogen atom which can reduce metal ions into their respective MNPs. For instance, the transformation of an enol group of luteolin flavonoid and rosmarinic acid present in *Ocimum basilicum* extract into a keto group played a crucial role in the reduction of silver ions into AgNPs.[39]

Proteins/peptides are polymers of amino acids, organic compounds with both acidic (–COOH) and basic (–NH_2) functional groups present in them. According to FTIR spectroscopy data, nascent MNPs are very often found in association with proteins/peptides which are present in plant extracts. Both carbonyl and/or amino groups of the main chain or side chain can mediate the binding of metallic ions such as the amino group of lysine, arginine, and histidine or the carboxyl group of aspartate and glutamate. Other groups which can bind metal ions include the thioether of methionine, the thiol of cysteine, and the hydroxyl group of serine, threonine, and tyrosine. It has been experimentally proven that these amino acids chelate metallic ions and reduce them to form MNPs.[44] In one example, a cyclic peptide present in the latex of *Jatropha curcas* has been utilized for the formation of AgNPs.[48]

Tannins belong to a class of hydrophilic polyphenolic compounds and are found in different parts of plants, such as pods, bark, roots, leaves, and plant galls. They act as powerful reducing agents (anti-oxidants) by donating electrons to the reactive oxygen species (ROS). Moreover, they are capable of chelating metal ions, leading to MNP formation.[49] For example, tannins present in a leaf extract of *Calliandra haematocephala* have assisted in the formation of AgNP by providing electrons to ionic silver (Ag^+).[50]

2.2. Factors influencing synthesis and geometry of metallic nanoparticles

There are several factors like pH, temperature, reactant concentration, and reaction time which are known to influence the nucleation and formation of MNPs. These parameters are extremely important to discuss since they directly influence the geometry and stability of MNPs and the reproducibility of the biosynthetic method.

Impact of pH and temperature The pH and temperature of the reaction mixture play a pivotal role in MNPs synthesis from plant extracts. The varying hydrogen ion concentration and fluctuating temperature can generate MNPs of variable size, shape, and texture.[29] Studies have shown that the lower the pH value the larger the size of the MNPs produced and *vice versa*. For instance, rod-shaped AuNP produced from the biomass of the *Avena sativus* plant were larger when the reaction mixture was kept at a pH value of 2, whereas on increasing this value to 3 and 4 the MNP size became relatively smaller.[51] This study suggested that in the pH range 3 and 4 more functional groups were available in the reaction mixture for particle nucleation whereas at pH 2, the availability of functional groups was less and hence, agglomeration of particles led to the formation of larger AuNPs.

Green technology based MNPs synthesis usually requires a temperature below 100 °C. Raju *et al.* studied AuNP synthesis from *Semecarpus anacardium* leaf extract over a range of temperatures[52] and it was concluded that higher temperature will lead to highly spherical NPs whereas lower temperature supports nanotriangle formation.[53] Rapid NP growth rate is observed at high incubation temperatures. AgNP synthesis from *Chrysosporium tropicum* over a temperature range revealed that with an increase in temperature from 25 to 30 °C, there was an increase in absorption and NP production. When the incubation temperature was kept at 25 °C, most of the AgNPs were of smaller size, whereas with subsequent increment in temperature to 30 °C, the formation of larger particles with a well-defined shape was reported. This observation suggests that there is a direct and distinct relationship between absorbance and the reaction mixture temperature.[54]

Influence of reduction time Incubation time plays a vital role in determining the shape and quality of MNPs. Prathna *et al.* studied the effect of incubation time on AgNP synthesis from *Azadirachta indica* leaf extract and revealed that with an increase in incubation time, there is an increase in particle size.[32] Variation in length of incubation time can influence particle properties in several ways; first, incubation for a long duration can lead to aggregation of NPs, which eventually results in large-sized particles; second, NPs may have a varying shelf life; and, third, in some cases their size may also decrease due to prolonged incubation time.

Influence of reactant concentration Different plant extracts tend to have different biomolecular concentrations and studies have revealed that varying the concentration of biomolecules influences the geometry of NPs. It has been found that by increasing the concentration of *Aloe vera* extract in the reaction mixture there is an increase in spherical AuNP population and the percentage of gold nanotriangle formation was reduced.[28] Moreover, AgNPs of various, shapes such as hexagonal, triangular, and spherical, have been synthesized by varying the amount of *Plectranthus amboinicus* plant extract in the reaction mixture.[55]

Based on the above discussion, it is evident that these factors greatly influence the size, shape, texture, and stability of MNPs. However, it cannot be denied that these experimental factors are prone to change with different plant extracts, as they contain different biochemicals and, hence, should be optimized for each synthetic procedure.

3. CHARACTERIZATION OF METALLIC NANOPARTICLES

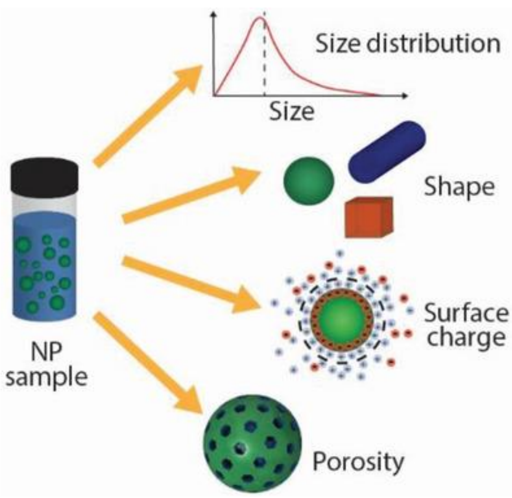

Fig. 2. Nanoparticle Characterization: What to measure? [107]

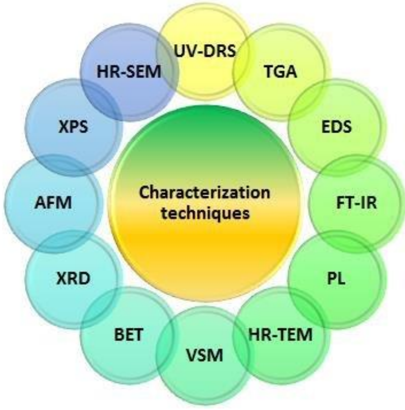

Fig. 3. Characterization-techniques for NPs. [108]

Fig. 4. Different techniques for characterization of metallic nanoparticles. [109]

Synthesis of MNPs is followed by their characterization based on properties like size, shape, surface area, and dispersity. In the case of both chemical and green approaches, the metal ions in aqueous metal salts get reduced and a color change is observed in the reaction mixture as a result, which is the very first indication that MNPs are being formed. However, a detailed characterization is required to ensure their formation.

The most commonly used techniques for characterization of MNPs are as follows: UV-visible spectroscopy (UV-Vis), dynamic light scattering (DLS), atomic force microscopy (AFM), scanning electron microscopy (SEM), Fourier transformation infrared spectroscopy (FTIR), transmission electron microscopy (TEM), powder X-ray diffraction (XRD), Raman spectroscopy, Auger electron spectroscopy (AES), photo correlation spectroscopy (PCS), inductively coupled plasma spectrometry (ICP), scanning probe electron microscopy (SPM), X-ray photoelectron spectroscopy (XPS), low energy ion scattering (LEIS), scanning tunneling microscopy (STM), time of flight secondary ion mass spectrometry (TOF-SIMS), and energy dispersive X-ray spectroscopy (EDS).

UV-visible spectroscopy is usually the first technique which is used to confirm MNP formation. It covers the range of both UV and visible radiations of the electromagnetic spectrum, where UV radiation lies in the range of 190–380 nm and visible radiation extends from 380 to 800 nm. This technique utilizes visible light of wavelength range 300–800 nm for identification, characterization and analysis of MNPs of various sizes.[35] For instance, AgNPs and AuNPs show absorption in wavelength ranges of 400–450 nm and 500–550 nm, respectively.[56]

DLS is usually used to determine the surface charge, quality, size distribution and polydispersity index of prepared MNPs suspended in a liquid medium. The mean hydrodynamic radius of MNP is calculated by using the Stokes–Einstein equation. [57]

However, the determination of attached functional groups, such as hydroxyls, carbonyls, amines, and the surface chemistry study of prepared MNPs are depicted by **FTIR**.

TEM and **SEM** are electron microscopy techniques that are routinely used to determine the surface topology and other sub-micron-level characteristics of MNPs.[58] TEM is used for the morphological characterization of MNPs and has thousand-fold higher resolution compared to SEM, whereas SEM provides morphological information at submicron level and elemental information when integrated with EDS.[59]

XRD is used in phase identification: *i.e.* examination of the overall oxidation state of particles as a function of time and determination of crystal structure of MNPs.[60] Penetration of X-rays into the nanomaterial results in a diffraction pattern which is matched with standards to obtain structural data.[35] XRD allows calculation of average MNP size (*D*) with the use of the Debye–Scherer equation. The line width of the (111) XRD peak gives the MNP size.[61] Measurement of zeta potential enables a determination of the stability of prepared MNPs and greater values of zeta potential represent highly stable MNPs.[58]

AES, XPS, SPM and **TOFSIMS** are the techniques which allow primary surface analysis of MNPs. XPS and AES assist in the study of the composition and depth of the coating on particles, surface enrichment and depletion at NP surfaces. XPS analysis offers an advantage over other techniques as it can be used to determine the NP size when other techniques can't be used due to inappropriate conditions. Like FTIR, TOFSIMS is also used to attain molecular information about MNPs' surface chemistry.[58]

Thermal Gravimetric Analysis (**TGA**) and Differential Thermal Analysis (**DTA**) help in the analysis of the thermal stability of prepared MNPs and the identification of crystalline conditions. However, inductively coupled plasma spectrometry (ICP) allows the characterization of MNPs under actual environmental conditions. It helps in the quantification and precise size measurement of MNPs in the range 10–200 nm.[62]

AFM, STM and other scanning probe microscopy allow the characterization of MNPs under realistic conditions.[63] AFM helps in the three-dimensional characterization of MNPs with subnanometer resolution and offers unique advantages over other techniques: *viz.* the determination of MNPs' physical properties, such as electrical, mechanical, and magnetic properties and, direct visualization of MNPs in hydrated form.

Raman spectroscopy helps in the detection of the vibrational modes of molecules, and therefore it has been widely used to determine the vibrational signals of various chemical groups linked to the MNP surface during the process of particle fabrication. For instance, surface enhanced Raman scattering (**SERS**) is useful in measuring single molecular attachment on an NP's surface.[64]

The low energy ion scattering (**LEIS**) process involves the scattering of low energy ionic beams, typically obtained from an inert gas (Ar, Rd), from the surface of particles, which leads to an energy loss in the ions. This loss in energy during the scattering process is examined and used for elemental analysis of the outermost surface of the material. LEIS is particularly useful because it offers high sensitivity towards the outermost layers of the MNP.[65]

X-ray diffraction (XRD) is one of the most extensively used techniques for the characterization of NPs. Typically; XRD provides information regarding the crystalline structure, nature of the phase, lattice parameters and crystalline grain size. The latter parameter is estimated by using the Scherrer equation using the broadening of the most intense peak of an XRD measurement for a specific sample. An advantage of the XRD techniques, commonly performed in samples of powder form, usually after drying their corresponding colloidal solutions, is that it results in statistically representative, volume-averaged values. The composition of the particles can be determined by comparing the position and intensity of the peaks with the reference patterns available from the International Centre for Diffraction Data (ICDD, previously known as Joint Committee on Powder Diffraction Standards, JCPDS) database. However, it is not suitable for amorphous materials and the XRD peaks are too broad for particles with a size below 3 nm.

Complete characterization of synthesized MNPs with these techniques is considered very useful because it allows a proper understanding of the different aspects of MNPs, which play an important role in their widespread applications.

Table 1 Summery of parameters and characterization techniques [110]

S. N.	Parameters	Characterization techniques
1.	Size (structural properties)	TEM, XRD, DLS, NTA, SAXS, HRTEM, SEM, AFM, EXAFS, FMR, DCS, ICP-MS, UV-Vis, MALDI, NMR, TRPS, EPLS, magnetic susceptibility
2.	Shape	TEM, HRTEM, AFM, EPLS, FMR, 3D-tomography
3.	Elemental-chemical composition	XRD, XPS, ICP-MS, ICP-OES, SEM-EDX, NMR, MFM, LEIS
4.	Crystal structure	XRD, EXAFS, HRTEM, electron diffraction, STEM
5.	Size distribution	DCS, DLS, SAXS, NTA, ICP-MS, FMR, superparamagnetic relaxometry, DTA, TRPS, SEM
6.	Chemical state–oxidation state	XAS, EELS, XPS, Mössbauer
7.	Growth kinetics	SAXS, NMR, TEM, cryo-TEM, liquid-TEM
8.	Ligand binding/composition/density/ arrangement/mass, surface composition	XPS, FTIR, NMR, SIMS, FMR, TGA, SANS
9.	Surface area, specific surface area	BET, liquid NMR
10.	Surface charge	Zeta potential, EPM
11.	Concentration	ICP-MS, UV-Vis, RMM-MEMS, PTA, DCS, TRPS
12.	Agglomeration state	Zeta potential, DLS, DCS, UV-Vis, SEM, Cryo-TEM, TEM
13.	Density	DCS, RMM-MEMS
14.	Single particle properties	Sp-ICP-MS, MFM, HRTEM, liquid TEM
15.	3D visualization	3D-tomography, AFM, SEM
16.	Dispersion of NP in matrices/supports	SEM, AFM, TEM
17.	Structural defects	HRTEM, EBSD
18.	Detection of NPs	TEM, SEM, STEM, EBSD, magnetic susceptibility

19.	Optical properties	UV-Vis-NIR, PL, EELS-STEM
20.	Magnetic properties	SQUID, VSM, Mössbauer, MFM, FMR, XMCD, magnetic susceptibility

4. TYPES OF PHYTOFABRICATED METALLIC NANOPARTICLES (MNPS):

4.1. Silver nanoparticles Silver is a rare but naturally occurring noble metal which has been widely used for various purposes across civilizations. It has been used as jewellery and fine cutlery in many societies and it was believed that Ag offered health benefits to the users. The medicinal and preservative properties of silver have been known since ancient times. Out of all the metals with antimicrobial properties, silver was found to have the most effective antibacterial action and as result AgNPs have been synthesized by using plant sources like *Tribulus terrestris* L., *Abutilon indicum*, *Cymbopogon citratus*, *O. tenuiflorum*, *S. tricobatum*, *Syzygium cumini*, *Centella asiatica*, *and Citrus sinensis* and used as antimicrobials in numerous commercially available formulations.[66] Another application of AgNPs has been in crop safety from plant diseases, thereby improving crop yield.[67] Recently, Logeswari *et al.* revealed the fungicidal effect of AgNPs and it was found that they can control phytopathogens in much a safer way compared to traditional fungicides.[68] Huang *et al.* studied the anti-cancerous and anti-protozoal activities exhibited by AgNPs.[47]

4.2. Gold nanoparticle Gold is another noble metal, very well known for exerting remedial proerties against numerous diseases. There are several reports where a colloidal gold solution was used as a potable solution in order to cure various infections.[69] AuNPs are of particular interest because they offer several unique properties, such as biocompatibility, high surface reactivity, small size, different shapes, and resistance to oxidation.[70] Although, AuNPs are biologically inactive, they can be modified to have photochemical and chemical functionality.[71] AuNPs have various applications such as biosensors,[72] antimicrobial agents,[73] theranostics,[74] cancer hyperthermia,[28] gene and drug delivery platforms.[75] AuNPs (cages, spheres, and rods) with their characteristic absorption in the near infra-red region could be used for the destruction of bacteria and cancerous cells *via* a photo-thermal heating mechanism.[76] Synthesis of AuNPs using plants as natural factories has provided a better, environmentally benign approach.[1] For example, *Aloe vera* plant extract has been utilized to obtain gold nanotriangles of a size between 20 to 50 nm.[28] Das *et al.* exploited *Nyctanthes arbortristis* flower extract to synthesize spherical AuNPs of a diameter of approximately 20 nm.[77] There are several independent reports of AuNPs synthesis of various shapes (spherical, triangular, quasispherical, cubic hexagonal, decahedral, icosahedral, and rod shaped)[35] by using a variety of plant sources, such as *Anacardium occidentale*,[78] *Camelia sinensis*,[79] *Cymbopogon* sp.,[80] *Geranium*,[81] *Vitex negundo* L.,[82] *Terminalia catappa*,[34] *Memecylon edule*,[83] and *Cinnamomum camphor*.[84]

4.3. Copper and copper oxide nanoparticles CuNPs possess noteworthy bactericidal activity against common pathogens such as *E. coli* and *S. aureus*[85] and cytotoxic activity.[86] Furthermore, CuO-NPs have been found to exhibit antioxidant and bactericidal behaviour.[87] The fact that both CuNP and CuO-NPs possess strong antibacterial activity has enabled them to be used as an effective antibacterial coating on hospital equipment. Extracts from plants such as *Syzygium aromaticum*[88] and *Euphorbia nivulia*[89] have been utilized to synthesize CuNP, and CuO-NP synthesis was made possible by using *Sterculia urens* extract.[85]

4.4. Titanium dioxide and zinc oxide nanoparticles Titanium is an abundant and widely distributed element in the earth's crust and its natural metallic form doesn't exist as it exhibits great affinity for oxygen and other metals. Its unique properties, like potential oxidation strength, high photo-stability, optical properties, anti-corrosive and photo-catalytic properties, and non-toxicity, have resulted in potential applications of TiO_2-NPs in various fields, such as photo-catalysis, dermatological therapies, cosmetics, and skin care products. Currently preparation of TiO_2-NP is under investigation as a novel treatment for dermatitis, such as acne vulgaris, hyper-pigmented skin lesions, atopic dermatitis, and recurrent condyloma accuminata.[90] The antiseptic and bactericidal properties of TiO_2-NPs have been studied against *A. hydrophila*, *P. mirabilis*, *E. coli*, *S. aureus,* and *P. aeruginosa* pathogens and the NPs were found to be highly effective against *E. coli* and *S. aureus*.[91] TiO_2-NPs have been synthesized using different plant extracts, such as *Psidium guajava*,[91] *Aloe vera*,[92] *Nyctanthes arbortristis*, and *Citrus sinensis*.[93, 94] ZnO nano formulation has also been studied recently as an important component in biomedical and cosmetic products[1] and it has been synthesized using diverse plants sources, such as a flower extract of *Cassia auriculata*[48] and leaf extracts of *Hibiscus rosa-sinensis*,[95] *Pongamia pinnata*, *Camellia sinensis*,[96] and *Aloe vera*.[97] ZnO-NPs exhibit potent bactericidal action, and have been employed in waste water treatment, food packaging, and personal care products. Vimala *et al.* studied ZnO-NP as a drug delivery platform for doxorubicin and observed cytotoxicity against breast (MCF-7) and colon (HT-29) cancer cells.[98]

4.5. Palladium and platinum nanoparticles Palladium is a noble metal with remarkable optical, catalytic, mechanical and electronic properties. PdNPs have been widely used in catalysis,[99] drug manufacture and environmental pollutant processing,[100] electrochemical applications,[101] and/or as a sensor for the detection of various analytes. The medicinal application of PdNP is seen in dental treatment, target-based pro-drug activation, photo-thermal therapy, anti-tumor and antibacterial agents, and for prostate cancer and choroidal melanoma brachytherapy.[102] However, PtNPs produced from *Ocimum sanctum* extract were found to have a catalytic property and therefore have the potential to be used in the production of hydrogen fuel components and chemical sensing.[103]

4.6. Indium oxide nanoparticle Indium oxide (In_2O_3) is an important n-type semiconductor with several unique properties, like the ability to form strong interactions with poisonous gas molecules, high visible light transparency and electrical conductivity.[104] In_2O_3-NPs have been produced using Aloe vera extract and extensively studied as promising materials for gas-sensing applications.[105] But, to the best of our knowledge, plants other than Aloe vera have not been exploited for its synthesis; therefore, more research work needs to be focused in this direction.

5. CONCLUSION AND FUTURE PERSPECTIVES

Two of the main parameters studied in the characterization of NPs are size and shape. We can also measure size distribution, degree of aggregation, surface charge, surface area, and to some extent evaluate the surface chemistry.5 Size, size distribution and organic ligands present on the surface of the particles may affect other properties and possible applications of the NPs. The crystal structure of NPs and their chemical composition are thoroughly investigated as a first step after nanoparticle synthesis. Credible and robust measurement methods for NPs will greatly affect the uptake of these materials in commercial applications. However, there are many challenges in analysis of nanomaterials because of interdisciplinary nature of the field, absence of suitable reference materials, calibration of analytical tools, difficulties related to the sample preparation for analysis and interpretation of data. In addition, there are unmet challenges in the characterization of NPs such as the measurement of their concentration *in situ* and on-line, especially in a scaled-up production, as well as their analysis in complex matrices. Waste and effluent from mass production will also need to be monitored.6 With the scale-up of nanoparticles, more reliable quantification techniques will be required. For this reason, it is crucial to characterize the nanomaterials prepared in several ways to the maximum extent. Nanotechnology as a pioneering technology has been anticipated to give hope for different fields of science, especially in biomedicine and therapy. The green synthesis of MNPs using plant tissue has been extensively studied in the last couple of decades, and it is still a fascinating area of research. The crude plant extracts induce MNP production in an inexpensive, facile, clean, and non-toxic manner. Utilization of these MNPs has been attempted for the treatment of several infectious and life-threatening diseases like malaria, HIV, hepatitis, cancer, *etc*. However, there has not been much work in this field at the pilot or commercial scales. Therefore, more research should be directed towards developing facile methods by understanding the kinetics and mechanism of MNP for large-scale synthesis using plant sources. There is an inevitable need to understand the safety and risk factors associated with the nanoparticles. In future, more research should be performed towards the use of phytofabricated MNPs for the functionalization of various biomolecules (antibodies, peptides, aptamers, *etc*). It is also anticipated that these MNPs might have lower or no toxicity. Hence, they could be good candidates for advanced clinical research.

Conflict of interest

Authors report no conflict of interest in this work.

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