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Biomimetic Synthesis of Nanoparticles: State-of-the-Art

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ABSTRACT

A state-of-the-art review of biomimetic nanoparticle synthesis is presented. The technique's origin has been traced to the studies, started over 150 years ago, on the hyperaccumulation of certain metals by different species of plants. How the initial intracellular method of nanoparticle synthesis evolved into the now widely used extracellular route has been described. The review then covers the gist of all the studies reported on the biomimetic synthesis of nanoparticles of different metals using extracts of different botanical species (plants). The synthesis mechanism is discussed, and the factors influencing the nanoparticles' extent, shapes, and sizes are identified.

INTRODUCTION

Barring some exceptions – such as the reactions in the atmosphere due to lightning – most chemical reactions occur in nature under very benign conditions of ambient temperatures and pressures (Huang et al. 2015, Bao et al. 2021). The natural synthesis routes are also almost always free from harmful emissions. Additionally, they are exceedingly efficient in terms of energy and material inputs (Jabbari et al. 2014, Ngo 2015, Miyauchi & Shimomura 2023).

These characteristics of biosynthesis in nature extend from the formation of simple products like carbon dioxide to very complicated and large biomolecules (Prescott et al. 2018, Kim 2020, Primrose 2020).

The science of biomimetics has emerged in recent decades in appreciation of the 'simplicity in complexity' of nature, especially in the astonishing manner in which organisms have been designed by nature to achieve multiple tasks in the most space-efficient, energy-efficient, and material-efficient manner - with little or no accompanying pollution (Gebeshuber 2022). What little pollution that does get generated-such as animal excreta and dead zoomass-is immediately worked upon by a wide variety of scavengers and is totally recycled. Biomimetics aims to mimic nature by synthesizing chemicals, designing systems, and problemsolving in the 'easy' and 'natural' way it occurs in nature.

BIOMIMETIC NANOPARTICLE SYNTHESIS

Biomimetic nanoparticle (BMNP) synthesis is one of the many branches of biomimetics. It mimics how living plants and other organisms convert ions of different elements, especially metals, into nanoparticles (Fig. 1). The focus of this paper is on plant-based synthesis because it is a simpler, cleaner, faster, and more versatile route than the ones based on other organisms (Ganaie et al. 2014, 2015, 2016, 2021). 'Plant'refers to vascular botanical species which form the largest component of the plant kingdom.

Plant-based BMNP synthesis constitutes one of the 'bottom-up' approaches to nanoparticle (NP) synthesis wherein one begins with elements or molecules and induces their aggregation into nanoparticles (Anuradha et al. 2009, Khandel et al. 2018). It contrasts with the 'top down' approaches in which one begins with bulks of elements or molecules and gradually disaggregates them to the nanoscale (Fig. 2). But if we put aside the biomimetic option, all other routes to NP synthesis, be it 'top down' or 'bottom-up,' are besieged with serious disadvantages.

The top-down approaches usually need expensive instruments and consume a lot of power, which leads to high operating costs and a proportionately large ecological footprint. On the other hand, the bottom-up approach often involves using toxic chemicals and generating problematic



Fig. 1: An overview of the biomimetic synthesis of nanoparticles and their applications.



Fig. 2: The bottom-up and top-down routes of nanoparticle synthesis.

emissions. Moreover, NPs thus generated are likely to carry toxic moieties. There is also a significant requirement for energy which may not be as great as necessitated in the top-

energy which may not be as great as necessitated in the topdown methods, yet is nevertheless substantial (Abbasi et al. 2015, Shah et al. 2015, Siddiqi et al. 2018).

The biological entities explored for BMNP synthesis are broadly of two types. One comprises unicellular organisms such as viruses, bacteria, yeast, etc., and the other comprises multicellular organisms such as algae, fungi, plants, and their parts (Fig. 2). Of all these, the synthesis based on vascular plants is found to be quicker and simpler than the synthesis employing unicellular and the other multicellular organisms (Ganaie et al. 2018a, 2018b). This paper surveys and analyzes past work on BMNP synthesis using plants.

A BRIEF HISTORY OF THE EVENTS LEADING TO THE EXTRACELLULAR NP SYNTHESIS ROUTE

The Discovery That Plants Can Hyperaccumulate Metals

A hyperaccumulator plant can grow in soil or water containing abnormally high concentrations of certain chemicals, absorbing those chemicals through its roots and storing them in its tissues to an abnormal extent without suffering from phytotoxicity (Reeves et al. 2017). Studies related to the bioaccumulation of nickel, copper, zinc, cobalt, lead, and manganese by various species of plants have been reported since the mid-nineteenth century (Abbasi 1988, 1989, Abbasi & Sharma 1981). Besides providing a means to bioremediate soils by extracting metal ions from them, hyperaccumulator plants also serve as a means for prospecting I as indicators of the contents of the soils on which they grow (Abbasi 1982, Hussain et al. 2022).

As noted by Reeves and Baker (Reeves 2000), the maiden in vitro study on hyperaccumulation of metal was reported by Braun in 1855 (Barun 1855). Later, Gautheret (1935) observed that silver ions were reduced to metal and were bioaccumulated in the tissues of the hydroponically cultured carrot. But the products could not be characterized because the equipment required to do so did not exist at that time. Now, with the advent of analytical instruments such as Fourier transform infrared (FTIR) spectroscopy, X-ray absorption spectroscopy (XAS), proton-induced X-ray emission (PIXE), energy dispersive X-ray spectroscopy (EDX), and transmission electron microscopy (TEM), it has become possible to study the formation and distribution of metal NPs within plant cells. The instruments can also determine the size of the metal NPs accumulated inside the cytosol of the plant cells. It is now well-established that hyperaccumulating plants have an enhanced rate of metal uptake, a faster rest-to-shoot translation, and a greater ability to sequester the accumulated metals. It is also believed that hyperaccumulator species so behave to defend themselves against grazers and other natural enemies. And that the heavy metals support and enhance the effect of organic defensive compounds (Sytar et al. 2021, Skuza et al. 2022).

Anderson et al. (1998) were the first to record that gold ions were hyper-accumulated in Indian mustard (*Brassica juncea*) when the latter was grown in containers filled with different gold compounds mixed with ammonium thiocyanate. They characterized the products using XAS and stated that ammonium thiocyanate had acted as a chelating agent and had induced the gold ions to accumulate in the leaf tissues.

The Discovery that NP Formation Drives Hyperaccumulation of Metals by Plants

Gardea-Torresdey et al. (2002, 2003) were among the first to report that gold and silver metals are stored inside the living plant cells of alfalfa (*Medicago sativa*) as NPs. They characterized the products using XAS and TEM and showed that the plant's roots were key in absorbing the metal ions. They observed that the absorbed metal ions accumulate inside the plant tissues. They also noted that the accumulated metal ions undergo aggregation at the nanoscale, which causes the formation of the NPs.

Attempts at Using Intracellular Synthesis to Obtain NPs

After the early work described above, several authors have shown that the storage of metals inside the cytoplasm of plant cells occurs as NPs. Yates et al. (2005) reported the formation of silver, cobalt, titanium, gold, zinc, and chromium NPs inside the living plants of the morning glory (*Ipomoea lacunosa*) and alfalfa (*Medicago sativa*).

Haverkamp et al. (2007) reported that AuNPs, AgNPs, and CuNPs were formed in the tissues of the *Brassica juncea* plants grown in an Au-Ag-Cu enriched soil culture over a period of 9 weeks. EDX analysis of the products confirmed the presence of all three elements, and the results indicated that it is possible to produce alloys of NPs.

Over the years, numerous reports have appeared on the formation of NPs in different plant species encompassing herbs, shrubs, and trees (Sytar et al. 2020). These include food plants, medicinals, and ornamentals of a very wide variety, representing diverse regions and genotypes (Armendariz et al. 2004, Rai et al. 2004, Gardea-Torresdey et al. 2005, Lopez et al. 2005, Beattie & Haverkamp 2011, Sharma et al. 2007, Huang et al. 2007, Manceau et al. 2008,

Łukaszewicz et al. 2009, Mishra et al. 2010, Cyganiuk et al. 2010, Cai et al. 2010, Anuradha et al. 2015, Roy et al. 2012, Tamuly et al. 2014, Marchiol et al. 2014). Many aquatic, amphibious, and terrestrial weeds have also been explored (Rai et al. 2004, Manceau et al. 2008, Kumar et al. 2015, Ganaie et al. 2017, 2021).

Difficulties Associated with the Use of Intracellular Synthesis for Manufacturing NPs.

The intracellular route has several major constraints. It requires steps like cell lysis/cracking through ultra-sonication to extract formed NPs from the plant biomass (Gürsoy et al. 2021). This makes the process highly cumbersome and can also cause changes in the shapes of the NPs. Moreover, enzymes or detergents may have to be used to separate NPs from unwanted cellulosic materials.

Special Attributes of Extracellular BMNP Synthesis Using Plants

In the first decade of this century, attempts were made to synthesize NPs extracellularly by employing plant extracts to get around the complications associated with retrieving the intracellularly synthesized NPs. It was also expected that extracellular synthesis of NPs may be more suitable for the large-scale manufacture of the NPs. As reviewed by these authors and others (Abbasi et al. 2012, Singh et al. 2016, Pareek et al. 2017, Gondwal & Joshi 2018, Khandel & Shahi 2018, Wei et al. 2018, Ahmed et al. 2021), there is a rapidly rising interest in the field of plant-based NP synthesis through extracellular route, due to the non-toxic, non-hazardous, and eco-friendly nature of this route. Further analysis by these authors has revealed that India is presently leading the world in the number of papers published in this field (Fig. 3), and the Indian scientists happen to have pioneered the plant-based extracellular NP synthesis.

In contrast to other organisms used for extracellular NP synthesis-notably algae, fungi, and bacteria -plants have a special advantage. The phytochemicals available in most plant extracts reduce metal ions much faster than other organisms (Jha et al. 2009). Phytochemicals also serve as stabilizing agents, facilitating the synthesis even further. Additionally, harvesting and using plants is much easier than other organisms requiring much greater care and effort to maintain and use (Malik et al. 2014).

SUMMARY OF PRIOR ART ON THE EXTRACELLULAR BMNPS SYNTHESIS USING PLANTS

Based on a very extensive prior art search using Web of Science, Science Direct, Google Scholar, and Research Gate, we have compiled most of the reports, which are close to 1500, that have appeared so far on this subject. We have prepared the gist of their contents, which are discussed in this and subsequent sections.

It is seen that 97.7% of all the reports are based on the use of aqueous extracts of the plant parts in synthesizing the



Fig. 3: Number of papers published on the bioinspired synthesis of nanoparticles using various plant parts.

NPs. In each case, those aqueous extracts were prepared by boiling a known quantity of a plant part — mostly leaves and flowers, in a few cases fruits — in water for a few minutes. Then the extract was mixed with one or more metal ion solutions, with or without some pH adjustment and/or heating/stirring, to generate mono-metallic or multi-metallic NPs.

The remaining 2.3% of the reports use organic solvents, principally methanol and ethanol, instead of water to extract biomolecules from the plants.

Most (85.5%) of the studies are on synthesizing AgNPs and AuNPs. Of the rest, 7.5%, 3.2%, and 2.4% of the studies have been on PdNPs, CuNPs, and PtNPs, respectively. The remaining 1.4% of the reports are on other metals and metal oxides. The focus on AgNPs and AuNPs stems from both possessing low cytotoxicity, high surface area-to-volume ratio, and stability. These attributes make them particularly preferred for immunotherapy and chemotherapy (Wei et al. 2019). AuNPs have also been found to be excellent contrasting agents in X-ray and CT scans. Additionally, they have gained prominence as radio sensitizers for early cancer detection and as antitumor agents (Sindhu et al. 2011, Daraee et al. 2016, Panahi et al. 2017). AgNPs have similar virtues and much less expensive parent metal than AuNPs (Mousavi et al. 2018).

First Attempts at Using Extracellular Synthesis to Obtain NPs

The first breakthrough in achieving an extracellular synthesis of NPs was achieved by Sastry and co-workers (Shankar et al. 2003). They employed a broth of geranium *(Pelargonium graveolens)* to synthesize gold NPs extracellularly. After this pioneering work, Sastry and co-workers demonstrated using the extracts of neem, lemon grass, gooseberry, and other species for synthesizing gold and silver NPs (Shankar et al. 2004a, 2004b, 2004c). Their work has been followed by a fast-increasing number of studies being carried out worldwide (Fig. 3).

The gist of the prior art on different categories of plants is given in the following sections. Of all the categories of plants, the ones with recognized medical properties-medicinal plants-have been the most widely studied. Food plants and ornamental plants, respectively, follow them.

BMNP Synthesis Using Medicinal Plants

More than 300 medicinal plants have been employed for generating NPs of silver, gold, palladium, platinum, cobalt, copper, and zinc oxide (Priya et al. 2022, Shahid et al. 2022, Singh et al. 2022, Johnson et al. 2022).

Of all the medicinal plants, neem (*Azadirachta indica*), tulsi (*Ocimum tenuiflorum*), and aloe vera (*Aloe vera*) are

the most extensively explored. Most (90%) of the authors have used leaf extracts of medicinal plants for synthesizing the NPs. The rest have used seeds, fruits/pods, or roots. TEM and SEM studies have revealed that the formed NPs were mostly spherical. Triangular, hexagonal, and rod-like shapes are also encountered but to a much lesser extent.

With the aid of FTIR, it has been seen that, in general, the leaf extracts of medicinal plants contain flavonoids, terpenoids, proteins, alkaloids, and polyols, which could have been involved in the formation of the NPs. The AuNPs and AgNPs formed using medicinal plants have been mostly explored for their antimicrobial, catalytic, and free-radical scavenging properties, besides their use in the early diagnosis and treatment of cancer, as detailed in Section 4.

Gist of the Reports on NP Synthesis Using Food Plants

Close to 275 food plants have been used for synthesizing NPs of Au, Ag, Pt, Pd, Cu, Co, and ZnO (Eze et al. 2022, Veeramani et al. 2022, Indira et al. 2022). Grapes, pomegranate, turmeric, onion, and garlic have been the most often explored. Interestingly 80% of the researchers have used the edible parts of these plants, while a much lesser number have used the leaves.

As was seen in medicinal plants, TEM and SEM studies reveal that the formed NPs are mostly spherical, and only a few plants generated cubical, triangular, rod-like, and hexagonal shapes. Polysaccharides, flavonoids, proteins, and terpenoids have been identified as the biomolecules most often associated with forming the NPs by food plants. The resulting NPs have been mainly explored as agents in antimicrobial, catalytic, and antioxidant applications.

BMNP Synthesis Using Ornamental Plants

More than 150 ornamental plants have been utilized to synthesize various NPs. Henna and rose have been the most explored. In 85% of the studies, plant leaves have been used to prepare extracts, while stem or bark have been used in the rest of the reports.

As revealed by TEM, the NPs synthesized using these plants have had triangular, polygonal, rod-like, spherical, and cubical shapes. As revealed by FTIR, the biomolecules involved have been terpenoids, flavonoids, alkaloids, proteins, and polyols. As in the case of NPs synthesized with other plants, the NPs of ornamentals have been mostly explored for their antimicrobial, catalytic, and antioxidant scavenging properties.

BMNP Synthesis Using Weeds

These authors have been among the first to utilize terrestrial,

i) The elements are in their ionic forms when absorbed by plants via their roots.

aquatic, and amphibious weeds for nanoparticle synthesis (Ganaie et al. 2014, 2015, 2016, 2017, 2021, Anuradha et

al. 2015, Abbasi et al. 2012). The following considerations

i) Unlike medicinal, feed, food, or ornamental plants, the

ii) For this reason, coupled with abundant availability that

iii) As weeds harm biodiversity and stress the environment

iv) The biomass from weed-infested tracts of land and water

potentially reduce the harm they cause.

weeds have no competing use. Hence their utilization

for NP synthesis does not infringe upon other demands.

too free of cost, the use of weeds helps bring down the

in multiple other ways, their large-scale utilization can

runs into billions of tonnes. Hence it can support very

Several common weeds have been explored to manufac-

ture gold, silver, palladium, platinum, copper oxide, and zinc

oxide NPs. All other weeds studied so far have been terrestrial

except for five aquatic weeds and 1 amphibious weed. These authors (Ganaie et al. 2014, 2015, Abbasi et al. 2015) have

utilized whole plants for synthesizing gold, silver, and bime-

tallic Au-Ag NPs, while Saikia et al. (2018) have used only

the stem. All other authors have used leaves for the synthesis.

ecules, and application of the NPs, the findings with the

weeds have been broadly similar to those with other plants.

MECHANISM OF NANOPARTICLE FORMATION

The initial work on the mechanism of plant-based NP

synthesis was with reference to intracellular NP formation.

Gardea-Torresdey et al. (1999), while studying the

accumulation of gold in the desert willow Chilopsis linearis,

hypothesized that the ionic form of the metal is evidently

getting taken up by the membrane of the plant root. It then

gets translocated in the plant, eventually converted to a

zerovalent form, and aggregated into nano-sized particles.

Concerning the shape of the NPs, associated biomol-

prompted the choice of weeds:

cost of production of the NPs.

large-scale NP manufacture.

WITH PLANT EXTRACT

The Accepted Mechanism

- ii) The absorbed ions are transported across the root membranes.
- iii) The ions are reduced to zerovalent forms by the biomolecules available in the cytosol of the plant cells.

iv) There is an aggregation of zerovalent atoms inside the plant cells at nanosize through biomolecules in the plant cells.

v) The NPs thus formed diffuse into other compartments of the plant cells, and their deposition/storage occurs therein.

The mechanism suggested that biomolecules are occurring in the plant cells' cytoplasm, which plays a key role in reducing and agglomerating metal ions to form NPs inside the living plant cells. When the first successful attempt to extracellularly synthesize the NPs was reported by Shanker et al. (2003), they also suggested that a mechanism similar to this is operative. According to them, the gold ions were reduced to gold atoms by reducing agents such as terpenoids in the geranium extract. The essential steps of the mechanism are depicted in Fig. 4.

About two decades have passed since this mechanism of NP formation by extracts of plants was proposed. It now stands almost universally accepted because no other plausible suggestion has emerged. (Singh et al. 2018, El Shafey, 2020)

How Different Biomolecules Act?

In the intervening years, several authors have identified the biomolecules that reduce metal ions into their atoms and/ or facilitate the stabilization of the aggregating atoms at a nano size. For example, Shahverdi et al. (2007) reported that *Cinnamon zeylanicum* extract contains eugenol (a terpenoid), which causes the reduction of silver and gold ions. Through FTIR analysis, the authors found that the presence of -OH groups in eugenol causes tautomeric transformation, forming a compound that probably acts as a reducing agent.

Li et al. (2007), explaining Ag NP formation from chili extract, suggested that in the first step, the silver ions attach to the proteins contained in the plant extract through electrostatic attraction. This reduces the silver ions to zerovalent silver, followed by Ag NP formation. According to Tan et al. (2010a), if metal ions are sequestrated into the peptides present in a plant extract, it may cause an inhibition in the reduction step.

Rajani et al. (2010) opined that a repulsive force must be available to stabilize the NPs in a reaction medium to overcome the attractive Van der Waals force, which causes coagulation. They proposed that carboxylate groups present in proteins may act as surfactants. Those groups may attach to the active sites of NPs, providing a repulsive force to prevent aggregation beyond nano-size and then stabilizing the formed NPs.

Lukman et al.(2011) gave the NP formation a 3-phase interpretation (Fig. 4): induction phase – where metal ions



Fig. 4: The generally accepted mechanism of plant-based biomimetic NP synthesis.

interact with the bioagents and undergo reduction, which then leads to nucleation, growth phase—where the nuclei aggregates with each other forming nanostructures, and termination phase — where the for the rate of reduction controls the formation of NPs. They also suggested that the reduction rate may determine whether the nuclei are homogenous or heterogeneous.

Dumur et al. (2011) state that the stabilization of NPs may occur via two basic modes. The interaction of metal particles with the biomolecules may be causing repulsive electrostatic forces, providing the particle surfaces with a charged coating. Non-ionic surfactants may generate steric repulsions between the particles and biomolecules, forming a coating around the surface of the NPs. Either mode may be preventing aggregation of the metal particles beyond nano size.

It is generally agreed that the NPs continue to grow until the stabilizing agents occupy the active sites of NPs. For this reason, the capping/stabilizing agents appear to play a crucial role in regulating the growth of the NPs.

Postulating the mechanism of AgNP formation, Ahmad et al. (2011) assumed that the secondary metabolites in the plant extracts cause a reduction of the Ag ion in the reaction medium. The energy released during glycolysis could transform the monovalent Ag ion to elemental Ag. The ascorbates in the plant extract may also be reacting with oxygen species present in the reaction medium, reducing them to form ascorbate radicals and free electrons. The free electrons may interact with Ag⁺ ions to reduce them to Ag⁰ NPs.

Gan & Li (2012) have attributed the formation of NPs of multiple shapes to Ostwald ripening. They suggest that during Ostwald ripening, the smaller particles may migrate and feed the adjacent particles, aggregating to larger particles of isotropic and anisotropic shapes.

Others, including Mittal et al. (2013) and Castillo-Lopez & Pal (2014), have given a mechanism of NP formation that essentially confirms the deductions made by Gardea-Torresdey et al. (1999) and Haverkamp & Marshall (2009). To wit, secondary metabolites or some other biomolecules found in the plant extracts cause the reduction of the metal ions. They also play the role of stabilizing agents. As for the shapes and sizes of the NPs, they are controlled by pH, temperature, metal-extract stoichiometry, and other conditions such as reaction duration, illumination, and stirring. These aspects are addressed below.

FACTORS WHICH INFLUENCE THE FORMATION AND THE MORPHOLOGY OF THE NPs

In all approaches to NP synthesis-chemical, physical, or biological-the main thrust is towards tailoring the process to obtain NPs of premeditated sizes and shapes. The same concern dominates the route of NP synthesis using plant extracts. Accordingly, several authors have studied the influence of numerous factors likely to regulate the synthesis. These most extensively explored parameters are temperature, pH, metal-extract stoichiometry, stirring, illumination, and reaction time.

Nature and Concentration of Phytochemicals

The composition and relative concentration of different phytochemicals can strongly influence the kinetics of NP formation and the shapes, sizes, and concentrations of the resulting NPs (Li et al. 2011, Mukunthan & Balaji 2012). The phytochemicals influencing NP formation in plants include flavones, terpenoids, sugars, ketones, aldehydes, carboxylic acids, and amides (Prathna et al. 2010).

The functional groups contained by flavonoids have a particularly marked ability to reduce metal ions. During NP synthesis, reactive hydrogen atoms are released due to tautomeric transformations in flavonoids converting enol-forms into the keto-form. The reduction of metal ions achieves this process into metal nanoparticles. For example, enol- to keto-transformation is the key factor in synthesizing biogenic silver nanoparticles from sweet basil (Ocimum basilicum) (Ahmad et al. 2010, 2021).

Glucose and fructose can also be responsible for forming metallic nanoparticles. Whereas glucose drives the formation of polydisperse nanoparticles, fructose-mediated gold, and silver nanoparticles are predominantly monodisperse (Panigrahi et al. 2004).

Nascent nanoparticles have repeatedly been found to be associated with proteins (Zayed et al. 2012). Amino acids cysteine, arginine, lysine, and methionine are proficient in

binding with silver ions (Gruen, 1975) and gold ions (Tan et al. 2010b).

In summary, the types and the relative concentrations of phytochemicals present in a plant extract play a key role in the first and the third steps of nanoparticle synthesis, which occur in these three phases : (1) the activation phase (bioreduction of metal ions/salts and nucleation process of the reduced metal ions), mediated by phytochemicals, (2) the growth phase (combination of smaller particles into larger ones) via a process acknowledged as Ostwald ripening, and (3) the termination phase which finalizes the shapes and the sizes of the NPs and in which phytochemicals cause stabilization of the formed NPs (Si & Mandal, 2007, Shah et al. 2015).

Effect of pH

In their study on intracellular NP formation, Gardea-Torresdey et al. (1999) observed that the pH had exerted a significant influence on the formation of the NPs.

A decade later, Sathishkumar et al. (2009) became the first to report that pH plays a major role in extracellular NP formation. They observed this while synthesizing AgNPs extracellularly with the aid of bark extracts of Cinnamon (Cinnamon zeylanicum). They reported that the pH of the reaction medium had a decisive effect on the reduction of the metal ions.

Dubey et al. (2010) have reported that when the reaction medium's pH increases above 7 units, the zeta potential value of the formed NPs tends to increase. On the other hand, a decrease in the pH below 7 causes the zeta potential value of the formed NPs to decrease. Philip (2010) reported that an increase in the pH of the reaction medium induces an increase in the reduction rate of metal ions, thereby facilitating NP formation. Veerasamy et al. (2011) have shown that acidic conditions in the reaction mixture suppress the formation of NPs even as alkaline pH facilitates it. Edison & Sethuraman (2012) also found that NP formation occurred more rapidly at neutral or alkaline than at acidic pH. They have linked it to the ionization of the phenolic groups occurring in the plant extract, which is suppressed at acidic pH due to the common ion effect. According to these authors, slower NP formation at acidic pH may also be due to the electrostatic repulsion of the anions occurring in the acidic pH range.

The sizes and size ranges are also strongly influenced by pH (Gontijo et al. 2020). Yang et al. (2019) found that the SPR peaks of the AgNPs became sharper at elevated pH values and attributed it to the decrease in the size of the NPs. They observed that the particles' shape was irregular at acidic pH, and the particles tended to aggregate. The decrease in the size of the NPs was attributed to the deprotonation of the functional groups carrying negative charges. The functional groups seemed to bind with the formed NPs, enhancing their stability as the electrostatic repulsion prevented aggregation. Dauthal & Mukhopadhyay (2013) also believed that the shift in the SPR peak of the formed NPs is due to their surface charges at different pH levels.

The studies of Tripathi et al. (2013), Sadeghi & Gholamhoseinpour (2015), and others (Ganaie et al. 2017, 2018b, Yazdani et al. 2021) have led to similar conclusions.

In summary, the pH of the reaction medium exerts a major influence in controlling the morphology, average size, size range, and stability of NPs during biomimetic extracellular synthesis.

Effect of Temperature

Several authors have shown that the kinetics of the reduction of metal ions and the shape and size of the formed NPs are strongly influenced by the temperature of the reaction medium. Chen et al. (2008) were the first to study the influence of temperature while synthesizing AgNPs extracellularly with the extracts of onion (*Allium cepa*) peels. They report that when the temperature of the reaction medium was increased, the size of the formed NPs tended to decrease. Similarly, in their work on AuNPs, Song & Kim (2008) have reported that as the reaction medium's temperature increases, smaller-sized NPs are formed in larger concentrations, as compared to larger NPs of smaller concentrations forming at lower temperatures.

In another study, Song et al. (2009) reported that when the reaction temperature was increased from 25°C to 95°C the rate of formation of AgNPs from Ag(i) by the leaf extracts of mokreyon (Magnolia kobus) and Japanese persimmon (Diospyros kaki) went up. There was a simultaneous decrease in the circumference of the NPs from 50 nm to 16 nm. The authors inferred that elevated temperatures might be suppressing the secondary nucleation process. This might be the reason for forming smaller-sized NPs of more consistent morphology. Chauhan & Upadhyay (2012) observed that an increase in the temperature of the reaction mixture was seen to cause a rapid depletion of the reactants, leading to the formation of smaller-sized NPs. A similar effect has been recorded by White et al. (2012), who report that an increase in the temperature of the reaction medium resulted in a reduction in the size of the formed NPs. It was attributed to the variation in the nucleation process and the growth rate of the NPs during the synthesis.

Interestingly Sun et al. (2014) found no significant change in the NP formation while investigating the effect of 25, 40, and 55°C temperatures. The possible reason, as suggested by the authors, was that almost complete (99.7%) NP formation had occurred at 25°C itself, which had left little scope for further increase in the NP formation. One of the exceptions is the report of Paarakh and Jose (2020), which claims an increase in the size of the *Murraya Koenig*-derived AgNps formation as the temperature was increased from room temperature to 55°C and 75°C.

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Even as higher temperature favors greater NP formation, this benefit is countered by the lower stability of the NPs. Due to this reason, it is deemed preferable to synthesize NPs at ambient temperatures. Moreover, the concentration of the metal ion precursor can influence both the nucleation and growth kinetics as the reaction temperature changes (Li et al. 2020).

Effect of Metal Ion-Extract Stoichiometry on the NP Formation

Shankar et al. (2005) were the first to explore the impact of changes in the metal-extract concentration ratio on NP formation while synthesizing AuNPs extracellularly with the aid of leaf extracts of cochin grass (Cymbopogon flexuosus). The authors reported that the change in the concentration of the extract relative to the metal ion concentration led to a change in the size of the NPs. They also observed that when the extract concentration was raised with respect to metal ion concentration, the size of the formed NPs decreased, and the ratio of the number of spherical NPs to the NPs of different shapes increased. The reported studies seem to support the hypothesis proposed by Dumur et al. (2011), according to which the formation of NPs occurs only when the precursor concentration is within a range suitable for nucleation. However, this range is seen to vary from species to species of the plants and the other physics-chemical conditions associated with the synthesis process.

According to Vilchis-Nestor et al. (2008), an increase in the extract concentration relative to the metal ion concentration caused the formed NPs to exhibit anisotropy. It was attributed to the faster reduction of metal ions in the NPs formation. Bar et al. (2009) also reported that NPs tended to aggregate more when the concentration of the plant extract was increased while keeping the metal ion concentration constant.

It was found by Vidhu et al. (2011) that sharpness in the SPR peaks of the NPs increased in proportion to the extract concentration relative to the metal ion concentration. The NP formation also occurred more rapidly. In contrast, with a lower concentration of the extract relative to the metal ion, the SPR peaks of the NPs were seen to be broader, and the reduction of the metal ions occurred more slowly. Khan et al. (2012) and Daniel et al. (2012) reported similar variations

accompanying an increase in the concentration of the plant extract relative to the metal ion solution.

In their work on AgNPs, Khalil et al. (2013) reported that at lower extract concentrations relative to the metal ion solution, quasi-spherical NPs were formed with an average size of 24-36 nm. In contrast, most of the formed NPs were in the 8-15 nm range at higher extract concentrations relative to the metal ion solution. It was attributed to the insufficient availability of biomolecules in the extracts. Their concentration was inadequate to reduce the metal ions or protect the formed NPs from aggregating.

According to Bindhu & Umadevi (2013), as the concentration of the extract increased, the full width at half maximum (FWHM) values of the spectral peaks of the formed NPs decreased. Concomitantly, the sharpness of the SPR peaks increased, and a blue shift of the peak was observed. This indicates a decrease in the mean diameter of the formed NPs. The blue shift and the greater sharpness in the peak indicated the formation of spherical NPs and the homogeneous distribution of the NPs.

Broadening of the SPR peaks was observed, along with the formation of larger-sized NPs, by Yang et al. (2019) when the concentration of the plant extract was increased relative to the concentration of the metal ion. The authors felt that reducing agents bound to the surface of the preformed nuclei might have intensified the subsequent reduction of the metal ions and would have enhanced bridging among the formed NPs, leading to the aggregation of the NPs. On the other hand, Yang et al. (2019) reported that with an increase in the metal ion concentration with respect to the extract concentration, an increase in the polydispersity of the formed NPs was seen owing to the occurrence of secondary particle growth by aggregation, coalescence, and Ostwald ripening. In several other studies by Anuradha et al. (2015), Abbasi et al. (2015), and Ganaie et al. (2015, 2017, 2018a), it has been shown that by controlling the metal-extract stoichiometry, it is possible to manipulate the rate of reaction, and the shapes and sizes of the NPs to suit the objective of the synthesis.

In general, smaller particles of spherical shape are obtained at higher extract: metal ion ratios. When the ratio decreases, anisotropic nanostructures form a mix of triangular, hexagonal, polygonal, rod-shaped, and cubical NPs. The concentrations of the plant extracts necessary for obtaining isotropic or anisotropic particles differ for different plant species. This is because plants' nature and concentration of biomolecules vary from species to species, even from variety to variety. reaction time while synthesizing AgNPs extracellularly with fruit extracts of capsicum (*Capsicum annum*). According to them, the optical density values of the metal-plant extract mixtures increased with time, reflecting a rising trend in forming NPs. They also noted that NP formation started after 5 hours of mixing the reactants and was almost completed by the 11th hour, as indicated by the gradual rise and then flattening of the optical density values of the SPR peaks.

The work of Li et al. (2016) was followed by Vilchis-Nestor et al. (2008) and Wang et al. (2009), who all noted that an increase in NP formation was observed with an increase in reaction duration. Dubey et al. (2010) opined that an increase in reaction duration was responsible for sharpening the SPR peaks of the formed NPs.

Several subsequent reports have shown that the contact time of the reactants plays a major role in NP synthesis.

Effect of Other Factors on the NP Formation

Apart from pH, metal-extract stoichiometry, temperature, and contact time, several other factors also play a role in determining the dimensions, shape, and longevity of the NPs. The investigations of Haverkamp & Marshall (2009) were among the first on the role of a source of metal ion solution while studying AgNP formation inside a living plant mustard (*Brassica juncea*). According to them, the plants supplied with AgNO₃ as a source of Ag (i) produced AgNPs of 10-35 nm. But the plants which were supplied with complex ions such as Ag $(NH_3)^{2+}$ and $Ag(S_2O_3)^{2+}$ as an Ag (i) source produced much smaller AgNPs of 2-3 nm.

Rai et al. (2007) have reported on the influence of halide ions while synthesizing AuNPs extracellularly with leaf extracts of lemon grass. They observed that the halide ions could transform triangular AuNPs to circular disk-like shapes and that iodide ions were more effective than other halide ions. The addition of iodide ions distorted the nanotriangles into spherical AuNPs.

Several authors (Saxena et al. 2010, Ali et al. 2011, Sheny et al. 2011, Prathna et al. 2011, Abdelrasoul et al. 2014, Raut et al. 2014) have reported the influence of duration of exposure to electromagnetic waves on NP formation. They also noted that when the reaction mixtures were kept for shaking, NPs of different sizes and shapes were formed as a function of the duration and intensity of shaking. According to Abdelrasoul et al. (2014), the irradiation of reaction mixtures by visible and UV light influenced the sizes and shapes of the formed NPs. A reckoner is presented in Fig. 5.

Effect of Reaction Duration

Li et al. (2016) were the first to report on the influence of In most c

ENERGY EFFICIENCY OF BMNPS

In most cases, BMNPs are synthesized at ambient

temperatures and pressures, without hazardous chemicals, and they have lower ecological carbon footprints than NPs synthesized by other routes. This imparts life-cycle energy efficiency to all those applications in which BMNPs are utilized. This aspect is illustrated below with a specific example of the role of BMNPs in enhancing the efficiency of microbial fuel cells (MFCs).

In recent decades, enormous effort has been made to develop microbial fuel cells (MFCs) with which energy can be generated with concomitant waste treatment (Yang et al. 2019, Goel 2018, Mian et al. 2019). However, as elucidated by us recently (Tabassum-Abbasi & Abbasi 2019), MFCs are both cost-ineffective and leave large ecological footprints. Hence there is no net energy gain and/or pollution control advantage from MFCs. This has made it imperative to remedy this situation.

One dimension of the MFCs that BMNPs can contribute is to catalyze the redox reactions occurring in the MFCs. So far, the other catalysts used for the purpose are either expensive and susceptible to poisoning – such as those based on platinum group metals – or are low in efficacy (Palanisamy et al. 2019, Noori et al. 2020). Moreover, making these catalysts itself leaves a significantly large carbon footprint. In contrast, BMNPs entail very little energy use and generate negligible emissions. The use of such catalysts can contribute towards reducing the ecological as well as monetary costs of MFCs. However, a very extensive survey has revealed that other than the work of these authors, only a solitary report by Harshiny et al. (2017) is available on the use of BMNPs in MFCs. Harshiny et al. (2017) generated iron oxide NPs using amaranthus (Amaranthus dubius) leaf extract and carbon-coated papers as electrodes in MFCs. They found that coating of the NPs led to a 31% enhancement in the accruable power density, attaining 145.5 mW.m⁻². The NP coating also enabled 68.5% COD removal efficiency compared to 63.1% achieved with the bare electrode, even as the NPs decreased anodic charge transfer resistance.

Available reports on the use of NPs synthesized with chemical or physical methods in the MFCs (Noori et al. 2020, Rajalakshmi 2019) show that NPs help in increasing MFC performance by reducing the biofouling caused by fungi such as *Cladasporium* spp. and *Aspergillus* spp., thereby reducing ohmic over potential and proportionately enhancing the MFC's energy output. They also enhance the oxygen reduction reaction (ORR), a major step in the MFC functioning (Li et al. 2016), thus serving as efficient electrochemical catalysts.

Recently those authors (Abbasi et al. 2021) have studied the role of AgNPs, and AuNPs derived from several weedy plant species in MFCs, to find that the NCs boost energy yield compared to substrates not spiked by them.

Besides stand-alone single-element NPs like AgNPs, the NPs of silver, gold, manganese oxide, titanium oxide, vanadium pentoxide, etc., either coated upon or, in general, modified with carbon paper or nanotubes, have been explored in enhancing the waste reduction and energy generation capabilities of MFCs (Naruse et al. 2011, Alatraktchi et al. 2012, Kalathil et al. 2013, Noori et al. 2016, Zakaria et al. 2018).



Fig. 5: A reckoner of the factors that influence the shapes and sizes of the nanoparticles.

From the foregoing, it appears clear that nanoparticles have the potential to enhance the performance of MFCs. If instead of the NPs generated using chemical or physical methods as in the studies quoted above, BMNPs are used (as pioneered by Harshini & Preeti (2017), it would reduce monetary and ecological costs. Future efforts ought to be oriented in that direction.

SUMMARY

- 1. Studies on the hyperaccumulation of metals by various species of plants have been carried out for more than 150 years. In vitro studies on metal accumulation gave the first clue that NPs can be formed intracellularly. Later, attempts were made to obtain intracellularly synthesized NPs, but several difficulties have prompted efforts toward an extracellular synthesis of the NPs. Sastry, Ahmed, and co-workers from the National Chemical Laboratory, Pune, India, were the first to successfully use a plant broth, that of geranium (Pelargonium graveolens), for synthesizing gold NPs extracellularly. Following that work, several reports have been published on synthesizing NPs of various metals using many different plant species. The non-toxic, non-hazardous, and simple nature of this synthetic route makes it particularly attractive.
- 2. Most reported studies on extracellular NP synthesis based on plant extracts have covered medicinal plants, food, and ornamental plants. In more recent years, there has been an increasing emphasis on the synthesis of NPs by utilizing weeds because weeds are freely and abundantly available and have no economically viable use at present.
- 3. The metals explored so far have largely been gold and silver. Palladium, copper, and platinum have been studied to a lesser extent in that order. Hence, many elements and their compounds have not been explored so far.
- 4. The NPs formed by the biomimetic route have been mostly explored for their antimicrobial, catalytic, and free radical scavenging properties.
- 5. The reaction medium's pH strongly influences the reduction of metal ions to their zerovalent forms. The NP formation occurred more rapidly at neutral and alkaline pH than at acidic pH. The NPs formed at elevated temperatures were generally smaller in size and larger in number than the NPs formed at ambient temperature. However, the former tended to lose stability and agglomerated much faster. Hence synthesizing NPs at ambient temperature appears preferable.

6. Studies on the effect of the metal-extract concentration ratio on NP formation reveal that the concentrations which favor the formation of isotropic or anisotropic particles vary with the plant species, in turn, with the type and the concentration of biomolecules present in the extract of a given plant. The contact time of reaction mixtures has also been found to play a key role in NP synthesis. The extent of this impact varied with the plant species.

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