



Role of Biogenic Capping Agents in the Synthesis of Metallic Nanoparticles and Evaluation of Their Therapeutic Potential

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OPEN ACCESS

Edited by:

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Reviewed by:

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Specialty section:

This article was submitted to Biomedical Nanotechnology, a section of the journal Frontiers in Nanotechnology

Received: 25 October 2021 Accepted: 20 December 2021 Published: 31 January 2022

Citation:

Sidhu AK, Verma N and Kaushal P (2022) Role of Biogenic Capping Agents in the Synthesis of Metallic Nanoparticles and Evaluation of Their Therapeutic Potential. Front. Nanotechnol. 3:801620. doi: 10.3389/fnano.2021.801620 The biomedical properties of nanoparticles have been the area of focus for contemporary science; however, there are issues concerning their long-term toxicities. Recent trends in nanoparticle fabrication and surface manipulation, the use of distinctive biogenic capping agents, have allowed the preparation of nontoxic, surface-functionalized, and monodispersed nanoparticles for medical applications. These capping agents act as stabilizers or binding molecules that prevent agglomeration and steric hindrance, alter the biological activity and surface chemistry, and stabilize the interaction of nanoparticles within the preparation medium. Explicit features of nanoparticles are majorly ascribed to the capping present on their surface. The present review article is an attempt to compile distinctive biological capping agents deployed in the synthesis of metal nanoparticles along with the medical applications of these capped nanoparticles. First, this innovative review highlights the various biogenic capping agents, including biomolecules and biological extracts of plants and microorganisms. Next, the therapeutic applications of capped nanoparticles and the effect of biomolecules on the efficiency of the nanoparticles have been expounded. Finally, challenges and future directions on the use of biological capping agents have been concluded. The goal of the present review article is to provide a comprehensive report to researchers who are looking for alternative biological capping agents for the green synthesis of important metallic nanoparticles.

Keywords: biomolecules, capping agents, green synthesis, nanoparticles, therapeutic applications

INTRODUCTION

Nanoparticles are generally defined as small-sized particles with core-shell structure falling in the nanoscale range of 1–100 nm with broad properties like catalytic, optical, magnetic, mechanical, superior reactivity, electric, and thermal conductivity (Agarwal et al., 2017; Salem and Fouda, 2021; Ting and Chin, 2020). Over the last decades, the chemical and physical methods have been widely used for the large-scale synthesis of nanoparticles, but due to the usage of toxic solvents, harmful chemical capping agents, high energy input, low output yield, larger size, and their catastrophic effect on the environment, recently, the green synthesis involving biological capping agents has fetched more attention in the nanoscience field (Javed et al., 2020; Kumar et al., 2020; Priya et al., 2021). In this era of the 21st century, nanoparticles have been extensively used in the biomedical sector, including bioimaging [magnetic resonance imaging (MRI), computed tomography (CT), and positron emission tomography (PET)], biomarkers, drug delivery, cell labeling, gene delivery,

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hyperthermia, photoablation therapy, magnetic tissue engineering, bone regeneration, wound healing and dressing, antipermeability agents (in diabetic retinopathy), intracellular analysis, catalysis (artificial enzymes), medical devices (catheter modifications, prostheses, and vascular grafts), treatment of inflammation, cancer, pathogenic infections, and dental caries (biofilm inhibition) (Figure 1) (Burdusel et al., 2018; Magro et al., 2018; McNamara and Tofail, 2017; Khan et al., 2019; Kravanja et al., 2019; Shivaramakrishnan et al., 2017; Singh et al., 2020). As the biocompatibility and stability of nanoparticles in the biological fluids are the crucial factors, the surface of nanoparticles can be modified with different functional groups for their potent use in the biomedicine field (Ocsoy et al., 2018). Capping agents are widely employed to functionalize and stabilize

the synthesized nanoparticles. Myriad chemical capping agents are utilized in the controlled synthesis of nanoparticles. However, being chemical in nature, they leave no meaning to green synthesis. To address the problems associated with the chemical capping agents, size, shape, agglomeration, and monodispersity of nanoparticles in the chemical and physical methods, green synthesis methods are based upon the usage of biodegradable, well-dispersed, biosoluble, biologically acceptable reducing, stabilizing, or capping agents so that they can be suitable in the living systems (Singh et al., 2015; Javed et al., 2020; Ocsoy et al., 2018). Capping agents are defined as "binding molecules" that are used in minute quantity for the synthesis of nanoparticles (Sharma et al., 2021). These molecules mainly modulate the surface chemistry, morphology, and size



distribution of the nanoparticles and shield them to prevent agglomeration and enhance the reduction kinetics of nanoparticles by forming complex structures with the metallic ions in the precursor salts (Li et al., 2013; Niu and Li, 2014; Campisi et al., 2016) (Figure 2). Majority of nanoparticle synthesis methods, including the biological method, rely on the use of chemical capping agents like surfactants (CTAB and SDS), polymers (PVP, PEG, and PGA), and thiols (dodecanethiol and thioglycerol) (Niu and Li, 2014). These molecules show a strong interaction with the surface of metal nanoparticles and thus serve as good stabilizing agents. However, a point value considered here is that these chemical capping agents are tough to detach from the surface of the nanoparticles and are nonbiodegradable, besides some conjointly being toxic to the biological systems. Thus, there may be a pressing want to go looking and searching for green capping agents in order to secure the biological system and the environment (Sharma et al., 2019). Keeping this in mind, green synthesis nowadays is focusing more on the naturally occurring reducing and stabilizing agents like carbohydrates (glucose, lactose, sucrose, fructose, cellulose, starch, and chitosan), proteins (collagen, enzymes, and

albumin), amino acids (protein and nonprotein), lipids, honey, nucleic acid (DNA), and biological extracts (plants, bacteria, viruses, and fungal extracts) (Al-Zaban et al., 2021; Caschera et al., 2020; Chowdhury et al., 2014; Javed et al., 2020; Nogueira et al., 2019; Ocsoy et al., 2018; Ogundare and Zyl, 2018; Rather et al., 2019; Verma and Kumar et al., 2019). These are the best candidates for the fabrication of nanoparticles, and the conjugation of nanoparticles with these biomolecules could bring many remarkable biofunctions and protracted their biomedical applications (Miao et al., 2018). In this review, we mainly highlight the use of biological capping agents in the synthesis of nanoparticles, their therapeutic potential, and the efficiency of the capped nanoparticles.

BIOGENIC CAPPING AGENTS

The use of capping agents for controlling growth, aggregation, and physical and chemical properties is the key area of concern in the synthesis of nanoparticles. An appropriate choice of capping agents is required to regulate the stability and functionalization of





the nanoparticles in the medium in which they are suspended (Javed et al., 2020; Maris;ca and Leopold, 2019). Different classes of green capping agents have been enlisted below with their roles in the synthesis of nanoparticles (**Figure 3**).

PROTEINS

Proteins are an excellent source of distinctive functional groups that can be utilized in modulating the surface of the nanoparticles (Marişca and Leopold, 2019). From the literature study, it has been deduced that collagen is one of the most abundant natural animal proteins used for the capping of nanoparticles. Collagen is a widespread structural protein in mammals that is crucial for the formation of tissue and regeneration of organs and helps in maintaining the structural integrity of the extracellular matrix (Vijayakumar and Vaseeharan, 2018; Craciunescu et al., 2019; Municoy et al., 2021). It is the best material extensively used in nanoscience due to its biocompatibility, biodegradation, weak antigenicity, and the property of self-assembly and cross-linkage (Municoy et al., 2021). Using this ability of the collagen, Craciunescu et al. (2019) have explained the synthesis of silver nanoparticles via type 1 collagen as a reducing and stabilizing agent. In this method, three different concentrations of AgNO₃ (0.1, 0.5, and 2.5 mM) were mixed with 0.3% (w/w) collagen gel in three variants. From the transmission electron microscopy (TEM) results, it has been observed that the intermediate concentration of AgNO₃ (0.5 mM) yields the smallest-sized particles with an average diameter of 20 nm. Furthermore, the effect of collagen on the size and stability of AgNPs has also been compared with the suspension of silver nanoparticles in sodium citrate. The average diameter of AgNPs in collagen was ~30 nm, whereas in the citrate suspension, the mean diameter of spherical

NPs was 130.87 nm. Moreover, in the collagen-based suspension of AgNPs, no aggregation among the nanoparticles was observed, but in citrate suspension, the aggregates were seen during the storage at 4°C. This shows that nondenatured triple helical type 1 collagen molecules play a pivotal role in controlling the size and provide stability to the AgNPs by interacting with its amino acid residues and metal ions (Figure 4). In the same year, Vijayakumar and Vaseeharan highlighted the synthesis of ZnO collagen-coated nanoparticles by using the coprecipitation method. Electron microscopy results reveal that Cl-ZnO nanoparticles showed agglomerated hexagonal wurtzite shape with size in the range of 20-50 nm. Similarly, from the biomedical application point of view, this work also intensifies that biopolymer-capped zinc oxide nanoparticles had a greater ability for antimicrobial and anticancer activities. It has been studied that in the field of nanoscience, the physical properties shape, and surface charge) of the (size, nanoparticles influence their interactions with the living cells. Keeping this in mind, Marisca and Leopold (2019) put forward the novel simplified synthesis of gold nanoparticles by using hydrogen peroxide as a reducing agent and collagen as a stabilizing agent. Electron microscopy results demonstrate that Cl-AuNPs were anisotropic (raspberry-like), stable, and nonagglomerated and fall in the range of 60-80 nm.

Besides collagen, different types of albumin (protein component of blood plasma) like ovalbumin (OVA), rat serum albumin (RSA), bovine serum albumin (BSA), and human serum albumin (HSA) are also considered eco-friendly proteins for the capping of the nanoparticles. Out of these proteins, BSA or HSA has been broadly used as a stabilizing agent due to its nontoxicity, low immunogenicity, biodegradability, high stability at 60°C temperature, high drug binding ability, selective drug delivery capability, and availability of natural binding sites (exposed ionic groups at C and N termini) (Nghiem et al., 2010; Pradeepa et al., 2017; Bolaños et al., 2019; Elsupikhe et al., 2020; Khodashenas et al., 2020). Pradeepa et al. (2017) elucidated the synthesis of silver nanoparticles in a foam matrix at room temperature by using HSA as a coating and stabilizing agent. The scanning electron microscopy (SEM) characterization of HSA-Ag nanoparticles indicates that particles were of face-centered cubic size, 20-30 nm in diameter, and without any aggregation. Similarly, Majeed et al. (2019) also demonstrated the anticancer property of BSA-capped and -uncapped AgNPs against bone, breast, and intestinal colon cell lines. The method of synthesis of silver nanoparticles was quite different from Pradeepa et al. (2017), as in this work, a completely green approach was used for the extracellular synthesis of silver nanoparticles by using the Bacillus cereus strain. TEM characterization results showed that uncapped AgNPs were spherical in shape and 5.37-17.19 nm in size, while on the BSA-capped AgNPs were larger in contrary, size (11.26-23.85 nm) but showed little agglomeration due to the interaction of AgNPs and BSA. This speculates that the coating of surface nanoparticles with biogenic capping agents prevents their aggregation among themselves. In the same year, Wang et al. (2019) synthesized in situ concentrated silver nanoparticles by using BSA as a stabilizer and costabilizer with other ligands (PVP and citrate). Interestingly, it has been found that though the PVP has been popularly used as a stabilizing agent for AgNPs, its capacity of stabilization was 40 times less than that of BSA. Various interactions like α -helix capping, peptide bond chelating, and different binding sites (thiol groups, guanidine side chains, and arginine and lysine residues) for functional groups, which are present in BSA (Figure 4), provide AgNPs more stability. In addition, BSA-capped AgNPs (10 ± 5 nm) were not only stable in the colloidal solution but also remained stable in the solid phase (after solvent water evaporation), 0.9% NaCl (physiological condition) without showing any agglomeration.

AMINO ACIDS

Amino acids are the "building blocks of proteins" comprised of amine (-NH₂), carboxyl functional groups (-COOH), and specific side chains in their structure. Due to the existence of side chains, these amino acid residues can interact with other compounds and hence act as eco-friendly, biocompatible capping agents (Figure 4) (Ghiyasiyan-Arani et al., 2018; Basnet et al., 2020; Hameed et al., 2020; Báez-Cruz et al., 2021). Maruyama et al. (2015) reported the synthesis of gold nanoparticles using 20 different types of amino acids (alanine, arginine, asparagine, aspartic acid, cysteine, glycine, glutamine, glutamic acid, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine). Out of these, histidine was considered the best reducing agent for gold chloride acid (HAuCl₄, 0.5 mM) and capping agent for the synthesized Au nanoparticles. TEM results showed that with an increase in the concentration of His (25-100 mM), there is a direct decrease in the size of the nanoparticles (4-7.4 nm). This means that an increase in the histidine concentration prevents the

aggregation of the Au nanoparticles. Another study based on the biological application of nonprotein amino acid as a stable, noncytotoxic capping agent for gold nanoparticles has been put forth by Verma and Kumar (2019). They, for the first time, utilized taurine (2-aminoethanesulfonic acid) as a capping agent in the synthesis of gold nanoparticles via an in situ wet chemical reduction method. They found that the average size of taurine-capped AuNPs with λ_{max} 515 nm was 6.9 nm, whereas at λ_{max} 545 nm, the size was 46 nm. These nanoparticles $(10^8 \text{ and } 10^{11} \text{ particles/ml})$ were further tested for a cytotoxicity study (MTT assay) on normal liver carcinoma cells (HepG2) (the major organ that accumulates nanoparticles during hepatotoxicity), and it was found that taurine-capped gold nanospheres were nontoxic, biocompatible, and safer as their size has no effect on cell death. This concluded that taurine is a harmless novel capping agent and is the best substitute to noxious chemical capping agents (CTAB and trisodium citrate) used in the synthesis of gold nanoparticles. Hameed et al. (2020) employed a biomimetic method for the synthesis of gold nanoparticles by using simply reducible aryldiazonium gold (III) salt AuCl₄ with L-tyrosine, L-tryptophan, and L-cysteine amino acids as capping agents. TEM analysis showed that capped nanoparticles were spherical in shape and fall in the range of 27.2 ± 5.4 nm (for tyrosine AuNPs), 14.6 ± 7.7 nm (for tryptophan AuNPs), and 8.6 ± 2.6 nm (for cysteine AuNPs). The cytotoxicity study of amino acid-capped AuNPs (less than 6.25% concentration) was observed against normal human dermal fibroblast cells. This concentration of tyrosinefabricated nanoparticles was considered safer, but in the case of cysteine and tryptophan nanoparticles, 53%-68% less viable cells were observed. Moreover, these capped nanoparticles also behaved as cell imaging agents as observed from a cellular uptake study in the viable HeLa cells.

CARBOHYDRATES

Carbohydrates have also been used as fabricating agents for the large-scale production of nanoparticles with beneficial properties (Ghiyasiyan-Arani et al., 2018). Fructose, lactose, glucose, dextrose, starch, cellulose, sucrose, and chitosan are used as reducing and stabilizing agents due to their sustainability, abundance, low cost, harmless, renewability, biodegradable, and compatibility with biological systems (Ghiyasiyan-Arani et al., 2018; Caschera et al., 2020; Garza-Cervantes et al., 2020; Rather et al., 2019; Shanmuganathan et al., 2019). Maiti et al. (2019) illustrated the synthesis of silver nanoparticles by using carboxymethyl cellulose (CMC) as a primary reducing and capping agent, whereas dextrose was used as an additional reducing agent. The study showed that AgNPs were triangular in shape with 9.5 nm in size, and from the biological point of view, the antimicrobial activity of nanoparticles was also studied. It has been found that the antimicrobial property of CMC-capped AgNPs was 128-512 fold higher in comparison with native silver nitrate solution. Additionally, AgNPs were also employed as an ophthalmic medicine on a rat model, and it was concluded that CMC-capped nanoparticles were safer to use

in treatment as no loss of vision, no opacity in the corona, and any corneal reflex change were observed in the rat after 7 days of application on the skin. Besides, Rather et al. (2019) explained the impact of capping agents (glucose, fructose, starch, and sucrose) on the bactericidal property, size, and absorption bands [surface plasmon resonance (SPR)] of Ag and Cu nanoparticles. It has been found that sugars showed a variable effect on the absorption peaks of silver and copper nanoparticles; as in the case of AgNPs, the maximum plasmonic band (λ_{max}) (434 nm) was shown in sucrose-capped nanoparticles, but in CuNPs, λ_{max} (641 nm) was observed in starch-mediated capped nanoparticles. Interestingly, the same type of sugars not only alters the surface plasmon resonance behavior but also alters the size of the nanoparticles. In the case of silver, the starch-mediated nanoparticles exhibited a size of 76 nm, whereas Cu fructose-mediated nanoparticles showed a maximum size of 164 nm. The main reason behind the variable size distribution of metallic nanoparticles by the same reducing agent is due to the structure and number of monomeric units present in the sugar molecules. Out of fructose, glucose, sucrose, and starch, both fructose and glucose showed easy stabilization of nanoparticles due to the presence of only one anomeric carbon atom; however, in the case of starch (polysaccharide) and sucrose (disaccharide, nonreducing sugar with no free anomeric carbon atom), strong alkali treatment was required for their fragmentation. Moreover, antimicrobial studies put on view that starch-based AgNPs were more effective than CuNPs using glucose, fructose, and sucrose. The larger zone of inhibition (1.82 cm) for Ag was observed for starch-based NPs, whereas for CuNPs, a larger zone of inhibition was seen in fructose-based nanoparticles (1.10 cm). In the end, we can conclude that different linkages of sugars affect the morphological, antimicrobial, and optical properties of the nanoparticles. From the literature studies, it has been inferred that silver nanoparticles are extensively used in the treatment of microbial infections, but how the capping agents modulate their antimicrobial activity has not been well illustrated (Nguyen et al., 2019; Radzig et al., 2013). Keeping this in view, Nguyen et al. (2019) compared the effect of chitosan and gelatin capping agents on the silver nanoparticle-based hydrogels and membranes. TEM characterization of GelAg and CsAg hydrogels showed that gelatin was the best stabilizer as nanoparticles were spherical, uniform, and monodispersed in gelatin hydrogels, whereas chitosan hydrogel particles were asymmetric and agglomerated in nature. Second, the average size of silver nanoparticles capped by chitosan was larger in size (20-40 nm) than gelatin-stabilized AgNPs (6-12 nm). The gelatin was considered a good stabilizer, but in antimicrobial assays, it has been found that chitosancapped Ag hydrogels had lower MIC values and maximum antibacterial potency. On the other hand, in the case of Ag nanoparticle-coated membranes, GelAgNPs exhibited good antimicrobial properties than CsAgNPs. At last, we can infer that both stabilizing agents showed different behaviors in different application designs of AgNPs for wound treatment. Garza-Cervantes et al. (2020) explained the synthesis of the silver biocomposite using ascorbic acid as a reducing agent and microfibrillated cellulose (obtained from orange peels) as a capping agent and also studied the antimicrobial activity. TEM

results showed that AgNPs were spherical in shape, capped with cellulose matrix, and fall in the average size of 140.79 ± 85.41 nm. Similarly, Caschera et al. (2020) also presented the fabrication of silver nanoparticles by using carboxylated nanocellulose (ONC, obtained from raw bagasse fibers) as a capping agent. The TEM, SEM, and atomic force microscopy (AFM) characterization of Ag–ONC nanocomposite showed that these exhibited spherical shape with diameter 4–10 nm with uniformly tight attachment to ONC fibrils. Accordingly, we can conclude that these molecules prevent the agglomeration of the nanoparticles due to the availability of hydroxyl and carbonyl groups in their structure that form hydrogen bonds with the surface of nanoparticles, also promote the nucleation of monodispersed nanoparticles, and hence provide stability (**Figure 4**) (Ghiyasiyan-Arani et al., 2018; Caschera et al., 2020; Garza-Cervantes et al., 2020).

LIPIDS

Any organic compound that is insoluble in water but soluble in organic solvents has been loosely classified as a "lipid." These chemical characteristics can be found in fatty acids, phospholipids, sterols, sphingolipids, terpenes, and other compounds. Lipids are a complex and ubiquitous category of molecules that perform a variety of important biological tasks, including acting as structural components of cell membranes, storing energy, and engaging in signaling cascades (Fahy et al., 2011). Being the natural component of the cell membranes, lipids serve as the structural framework. Phospholipids make up the majority of cell membranes, which operate as a barrier to protect cell components from the outside world (Simons, 2016). Lipids have proved to be appropriate candidates for several biomedical applications where they form a biocompatible protective barrier on the nanomaterial surface, similar to their biological functions (Luchini and Vitiello, 2019).

Several fatty acids and edible oils have been tried and found to be excellent capping agents for the synthesis of a wide variety of metallic nanoparticles. Oleic acid is one of the most commonly employed fatty acids. In one study, Bi₂WO₆ nanoplates had been synthesized by a simple one-step hydrothermal technique by using oleic acid (OA) as a biological and cost-effective capping agent. From the SEM images, the particles were predicted to possess an average thickness of 90 nm along with an approximate width of 100-300 nm. It was determined from the FTIR research that oleic acid was chemically adsorbed via hydrogen-bonded carbonyl functional groups (Figure 4) on the Bi₂WO₆ surface, and also, there might be physical adsorption of oleic acid onto the nanotubes through van der Waals forces (Rahimi et al., 2016). In a similar study, Lu et al. (2008) compared the synthesis of magnetic Co nanoparticles using three long-chain acids-oleic, elaidic, and stearic acids-as capping agents. They discovered that elaidic acid, oleic acid, and a mixture of elaidic/stearic or oleic/ stearic acids result in the narrow size distribution of synthesized nanoparticles with spherical shape. Overall, the oleic acid gave superior results, which is believed to stabilize the colloidal suspension, control particle growth, and prevent the final product from being oxidized by the air. The superior

TABLE 1 | List of various biogenic capping agents used in the synthesis of nanoparticles.

Biomolecules	Nanoparticles synthesized	Size (nm)	Applications	References
Collagen	Ag	~30 nm	Periodontitis treatment	Craciunescu et al. (2019)
Collagen	ZnO	20–50 nm	Antibacterial, antibiofilm, and	Vijayakumar and
Collagen	Au	60–80 nm	anticancer Hyperthermia, sensing, and	Vaseeharan (2018) Marisca and Leopold (2019)
Human serum albumin (HSA)	Ag	20–30 nm	imaging Anticancer	Pradeepa et al. (2017)
Bovine serum albumin (BSA)	Ag	11.26–23.85 nm	Anticancer	Majeed et al. (2019)
	Ag	10 ± 5 nm	_	Wang et al. (2019)
Histidine	Au	4–7.4 nm	_	Maruyama et al. (2015)
Taurine (nonprotein amino acid)	Au	6.9 nm, 46 nm	_	Kumar et al. (2020)
L-tyrosine	Au	27.2 ± 5.4 nm	Cellular imaging	Hameed et al. (2020)
L-tryptophan		14.6 ± 7.7 nm		
L-cysteine		8.6 ± 2.6 nm		
Carboxymethyl cellulose (CMC)	Ag	9.5 nm	Antimicrobial, ophthalmic, and skin infections	Maiti et al. (2019)
Fructose	Ag	21 nm	Antibacterial	Rather et al. (2019)
Glucose	5	38–39 nm		× ,
Sucrose		38–39 nm		
Starch		76 nm		
Fructose	Cu	164 nm	Antibacterial	Rather et al. (2019)
Glucose		110 nm		× ,
Sucrose		27 nm		
Starch		97 nm		
Chitosan	Ag	20–40 nm	Antibacterial	Nguyen et al. (2019)
Cellulose	Ag	140.79 ± 85.41 nm	Antibiotic microbial-resistant (AMR) treatment	Garza-Cervantes et al. (2020)
Carboxylated nanocellulose (ONC)	Ag	4–10 nm	Antimicrobial	Caschera et al. (2020)
Oleic acid	Co	8.5 nm	_	Lu et al. (2008)
	SnS	10.67–17.74 nm	-	Oluwalana and Ajibade (2019)
	Bi ₂ WO ₆	Thickness of 90 nm, width of 100–300 nm	Catalytic activity	Rahimi et al. (2016)
Stearic acid	CdSe	4–25 nm	_	Qu and Peng (2002)
Almond oil	Mn ₃ O ₄	7 nm	_	Bastami and Entezari (2010)
Olive oil	Mn ₃ O ₄	7 nm	_	Bastami and Entezari (2010)
Olive oil	CdS	4.75 nm	_	Nyamen et al. (2014)
Olive oil	PbS	18.74 nm	_	Nyamen et al. (2014)
Sunflower oil	ZnO	3 nm	_	Pereira et al. (2012)
Sunflower oil	Fe ₂ O ₃ /Fe ₃ O ₄	7 nm	_	Pereira et al. (2012)
Coconut oil	Au	38.49 nm	_	Kumari and Philip, (2013)
	Ag	21 nm	_	Kumari and Philip (2013)
Corn oil	Fe	10–15 nm	_	Koltypin et al. (2004)
Rapeseed oil	Fe	20–30 nm	_	Koltypin et al. (2004)
Palm oil	Ag	2–2.5 nm	_	Zamiri et al. (2010)
	Au	8.92–19.73 nm	_	Sadrolhosseini et al. (2017)
Palmitic acid	Fe ₃ O ₄	8–10 nm	_	Cahyana and Reza (2018)
Sodium 9,10-epoxy-12-	AgNPs	23 nm	Environment remediation	Costa et al. (2017)

performance of oleic acid is mostly believed to be attributed to its nonlinear conformation. Oluwalana and Ajibade (2019) synthesized SnS nanocrystals at three different temperatures (150°C, 190°C, and 230°C) using bis(phenylpiperazine dithiocarbamate) tin (II) in oleic acid (OA) and octadecylamine (ODA). It was found that both the temperature and capping agent show a predominant effect on the size and shape of nanocrystals. The shape of the nanocrystals synthesized using oleic acid-capped SnS was spherical (OASnS), while the particles synthesized using octadecylamine (ODASnS) were cubic in nature. The size of the nanoparticles changes with the increase in temperature; monodispersed SnS with a size range of 10.67-17.74 nm was obtained at 150° C for OASnS, while the biggest-sized nanocrystals were obtained at 230° C.

Edible VOs such as rapeseed, corn, castor, sunflower, olive, coconut, and palm oils have been used as inexpensive and eco-friendly capping agents for the synthesis of various nanoparticles.

Thermodynamically, nanoparticles demonstrate a tendency to agglomerate and form larger particles after synthesis. VO and FA are amphiphilic molecules used to effectively control the aggregation of nanoparticles. The polar or hydrophilic end (carboxylic group) of FAs interacts with the nanoparticles, while the nonpolar aliphatic tails or hydrophobic end sterically disperses the particles (Figure 4). However, in an evolving nanotechnology industry, the use of edible oils as solvents poses an inherent challenge that may be related to the pricing and raw material rivalry (Mensah et al., 2018). In a research by Sadrolhosseini et al. (2017), gold nanoparticles were fabricated using palm oil as a capping agent at different temperatures (40°C, 64°C, 75°C, 83°C, and 92°C) to investigate the effect on the overall size of the particles. The use of palm oil has resulted in the formation of spherical gold nanoparticles with a size range of 8.92-19.73 nm. The increase in temperature is considered to cause the agglomeration of nanoparticles resulting in the formation of larger-sized particles. Cahyana and Reza (2018) reported the synthesis of magnetic iron oxide nanoparticles by the thermal decomposition reaction between rust, solvent (n-octadecane), and palmitic acid (as a capping agent). Fe₃O₄ nanoparticles with a size range between 8 and 10 nm were synthesized successfully using palmitic acid as a capping agent. In another study, silver nanoparticles were synthesized deploying an epoxidized derivative of ricinoleic acid, sodium 9,10-epoxy-12-hydroxytetradecanoate (SEAR), as a reducing and stabilizing agent. The concentration of SEAR was found to be an important factor contributing to the size of nanoparticles. Concentrations below the critical micelle concentration (CMC) of SEAR produced AgNPs of 23 nm in size with a standard deviation of 10 nm, whereas AgNPs of 50 nm (σ = 21 nm) were obtained at a concentration of above SEAR CMC (Costa et al., 2017). Table 1 illustrates the various biogenic capping agents (proteins, amino acids, carbohydrates, and lipids) in the synthesis of nanoparticles.

FUNGAL EXTRACT AS A CAPPING AGENT

Fungi are considered one of the attractive sources for the biological synthesis of nanoparticles due to the presence of a large quantity of proteins, high yield of nanoparticles, ease of handling, and production of less toxic effluents. Fungi act as both reducing and stabilizing agents in the synthesis of metal nanoparticles (Ninganagouda et al., 2013). The biomolecules present in the fungi confer a coating onto the nanoparticles, which increase their stability, inhibit agglomeration, and aid in enhancing the biological activity of nanoparticles (Guilger-Casagrande and Lima, 2019; Priya et al., 2021). These biomolecules pose a high affinity toward binding onto the surface of the nanoparticles, with mostly proteins and amino acid residues coating the nanoparticle surfaces (Basavaraja et al., 2008). The free amino groups or cysteine residues are believed to interact and bind the nanoparticle surfaces. The cell wall enzymes are also assumed to provide negative carboxyl groups adding to the electrostatic attraction between the nanoparticles and these biomolecules (Figure 4) (Gole et al., 2001; Durán et al., 2011; Husseiny et al., 2015). Furthermore, the nucleophilic (OH⁻) ions present on the surface of the nanoparticles are thought to supply electrons required for the reduction of the metal ions resulting in the production of nanoparticles demonstrating narrow size distribution (Gurunathan et al., 2009). Several studies have demonstrated the role of biomolecules from fungal extract,

acting as a capping agent. Gudikandula et al. (2017) synthesized biogenic silver nanoparticles using two white-rot fungi Trametes ljubarskvi and Ganoderma enigmaticum. FTIR analysis revealed bands at 3,385 and 1,638 cm⁻¹ representing primary amine (N-H) stretching and amide (N-H) bending vibration, respectively. C-N stretching vibrations of the aromatic and aliphatic amine were attributed to the bands observed around 1,383 and 1,032 cm⁻¹, confirming the presence of proteins on the surface of nanoparticles for their stabilization (Figure 4). Elgorban et al. (2016) synthesized biological silver nanoparticles using Aspergillus versicolor and established the presence of a capping from SEM and EDS analysis. While noticing the strong signal at about 3 keV corresponding to Ag, the workers also reported signals for oxygen and carbon, indicating the presence of organic compounds getting adsorbed on the nanoparticles from the culture filtrate. Kadam et al. (2019) synthesized ZnO nanoparticles employing zinc-tolerant endophytic fungi identified as Cochliobolus geniculatus. To confirm the presence of proteins as capping agents on the surface of the synthesized nanoparticles, the particles were treated with 1% SDS for 10 min, followed by centrifugation at 8,500 g for 10 min. The obtained supernatant was further denatured using 3X Laemmli's dye, followed by heating for 5 min and centrifugation for 1 min at $8,500 \times g$ (4°C). The sample thus obtained was separated by SDS-PAGE, which demonstrated the presence of numerous protein bands in the range of 97-36 kDa. The occurrence of capping protein on the ZnO nanoparticle surface was evaluated by recording fluorescence spectra at an excitation wavelength of 280 and 325 nm, respectively. UV-vis spectroscopy analysis of nanoparticles exhibits a typical signature peak at 280 nm. The protein shows a strong absorption at 282 nm due to $\pi \to \pi$ transitions arising from aromatic amino acids tyrosine and tryptophan of proteins present on the surface of the particles. Further, FTIR analysis also revealed the presence of several bands specifying the protein coating on the surface of the nanoparticles. Cuevas et al. (2015) synthesized copper and copper oxide (Cu, Cu₂O, and CuO) nanoparticles by a white-rot fungus Stereum hirsutum. The FTIR and XRD studies of all three synthesized nanoparticles portray the presence of proteins and carbohydrates on the surface of these nanoparticles. Besides, the typical FTIR bands corresponding to C-N stretching vibrations of aromatic and aliphatic amines, bending vibration movements in amides I, II, and III, bands at $900-1,105 \text{ cm}^{-1}$ indicating the presence of pyranose ring stretching, glycosidic ethers, and beta-glycosidic linkages of some polycarbohydrates were also observed (Figure 4). The XRD analysis demonstrates the presence of peaks indicating the existence of polycarbohydrate as surface capping on the particles. Another study conducted by Das et al. (2009) for the synthesis of AuNPs by the viable biomass of Rhizopus oryzae postulated the possible involvement of phosphate bonds in the interaction of proteins on the nanoparticle surface. EDX studies indicate the C, N, and O signals representing the presence of biomolecules on the surface of nanoparticles along with strong peaks corresponding to Au. A shift in FTIR bands from 1,034 to 1,025 cm⁻¹ observed could be assigned to the phosphate bond

intervention in this interaction process of proteins on the surface of the nanoparticles. Majority of the studies involving the use of fungi as reducing and stabilizing agents put forward the fact that proteins along with carbohydrates to a certain extent are present in the extract acting as the sole capping agent. Yeast has also been employed widely as a reducing-cum-capping agent for the synthesis of monodispersed nanoparticles. In one such study, Shu et al. (2020) synthesized silver nanoparticles using yeast extract for the reduction of AgNO₃ solution. The shape and size as revealed by the SEM image is spherical with an average particle size of 13.8 nm. Data obtained through EDX, FTIR, and zeta potential (helps in depicting the surface electrostatic potential at the junction between the diffuse and compact layers of the nanoparticles) measurement clarifies the presence of surface coating, dispersion ability of the colloidal solutions, and longterm storage stability of the synthesized particles. Proteins and amino acids are regarded as the main molecules acting as capping agents. The zeta potential of AgNPs decreased drastically from -12.1 mV at pH 7.0 to -24.4 mV at pH 11.0, which defines the presence of negatively charged groups on the surface of AgNPs. Overall, it is assumed that the surface amino acid carries a net negative charge at the alkaline pH providing storage stability for more than a year without the formation of any precipitate.

ALGAL EXTRACT AS A CAPPING AGENT

Algae have been exploited industrially and commercially for ages as food, feed additives, fuels, fertilizers, pharmaceuticals, and cosmetics (Sharma and Sharma, 2017). Recently, a drastic shift has been noticed in this field, and now algae are looked at as one of the green resources for the synthesis of various nanoparticles. Algae are a rich source of proteins, peptides, secondary metabolites, and pigments, which make them an attractive nanofactory (Negi and Singh, 2018). Working with algae is advantageous as they are fast-growing, can grow in a simple, inexpensive medium, offer ease of scale-up, and are easy to culture and harvest (Fabris et al., 2020). Various classes of algae like red algae (Rhodophyceae), blue-green algae (Cyanophyceae), green algae (Chlorophyceae), and brown algae (Phaeophyceae) (Abdel-Raouf et al., 2017; Aboelfetoh et al., 2017; Khan et al., 2018; Fatima et al., 2020) have been widely employed for the synthesis of metallic nanoparticles. Like plants and fungi, algal extracts are expected to act as both reducing and capping agents in the synthesis of metallic nanoparticles (Chaudhary et al., 2020).

The biomolecules present in the extract reduce the metal ions and coat the surface of the nanoparticles. Proteins, amino acids, and secondary metabolites such as terpenoids, alkaloids, and phenolic compounds secreted extracellularly by algae are considered main candidates for surface capping (**Figures 3** and **4**). Moreover, the alkaline pH of the algal extract is also considered to play a crucial role in the stabilization of the synthesized nanoparticles. The higher pH enhances the reducing power of the surface functional groups preventing particle agglomeration. Alkaline pH is also believed to interact with the amino group of proteins and amino acids which aid the stabilization of the particles (Parial et al., 2012; Namvar et al., 2015). Gold nanoparticles were more stable in the pH range of 5-9, whereas their agglomeration was observed at pH 3 (Namvar et al., 2015). In a study carried out for the green synthesis of silver nanoparticles using green algae Caulerpa serrulata, the FTIR study reveals the existence of various bands depicting the stretching of (O-H) and C=O groups, (C-H) stretching of alkanes, stretching vibration of the alkene C=C group, and C=C stretching. This strongly recommends the presence of organic biomolecules as capping agents on the surface of the synthesized AgNPs (Figure 4). These workers also investigated the effect of varied extract concentration, temperature, and pH on the size and shape of synthesized NPs. The results showed that AgNPs synthesized at higher temperature (95°C), at higher pH values (>6.65), and at higher extract concentration are superior being crystalline and spherical with an average particle size of 10 ± 2 nm (Aboelfetoh et al., 2017). Abdel-Raouf et al. (2017) synthesized the gold nanoparticles by using Galaxaura elongata. The results of the FTIR study showed the presence of three bands at about 332.49, 2,158.12, and 1,635.09 cm⁻¹ for the ethanolic extract and at 3,414.96, 2,158.28, and 1,640.60 cm⁻¹ for nanoparticles synthesized using algal powder, which could be assigned to amide bands of proteins, as well as carbonyl stretching and free N-H stretching vibrations, respectively, in the amide linkages of the proteins (Figure 4). The study thus indicates the role of proteins in the extract acting as capping and stabilizing agents. Another study conducted by the same researchers dealt with the synthesis of highly stable silver nanoparticles employing marine brown alga Padina pavonia. The synthesis was carried out using algal powder and organic extracts (ethanol and chloroform). The workers noticed the synthesis of the Ag nanoparticles using powder form in the stunningly shorter duration of 2 min as compared with the 3 h taken by ethanol and chloroform extracts. The HPLC and GC-MS analysis for the algal extracts proved the presence of high values of natural functional compounds, such as alkaloids, terpenes, amino acids, and fatty acids, which were considered to act as stabilizers and were responsible for preventing particle agglomeration (Abdel-Raouf et al., 2017). Sinha et al. (2015) carried out the biological synthesis of silver nanoparticles using freshwater green alga Pithophora oedogonia by the reduction of silver nitrate. It was observed that the synthesis process was extremely fast, and the reduction of silver ions to silver nanoparticles using algal extract happened within a few minutes. The preliminary phytochemical analysis of the extract revealed the presence of saponins, carbohydrates, steroids, proteins, tannins, and terpenoids. FTIR analysis of the synthesized nanoparticles demonstrated the presence of various bands concluding the involvement of long-chain fatty acids, terpenoids, and secondary amide derivatives to be possible biomolecules acting as capping and stabilizing agents (Figure 4).

Araya-Castro et al. (2021) attempted a novel protein-assisted method for the synthesis of biogenic CuO NPs using separated protein fractions from an aqueous extract of *Macrocystis pyrifera* (brown algae) through size exclusion chromatography (HPLC-SEC). The study was carried out to prove the potential of proteins present in the extract acting as reducing and capping agents in the synthesis process. TEM images inferred the synthesis of spherical nanoparticles ranging from 2 to 50 nm in diameter in all the protein fractions. EDX analysis of nanoparticles depicted a strong signal for copper (Cu) and carbon (C) which is assumed to be from the surface coating of proteins in the synthesized nanoparticles. Another interesting study was conducted by Xie et al. (2007) to synthesize singlecrystalline gold nanoplates by the reduction of chloroauric acid with the extract of green alga Chlorella vulgaris. The study suggests proteins as the principal biomolecules involved in providing the dual function of Au reduction and the size- and shape-controlled synthesis of the nanogold crystals. Further, a protein with a molecular weight of around 28 kDa was extracted and purified by using reversed-phase HPLC; the isolated protein was examined for the specific reduction of chloroauric acid in an aqueous solution. The sole use of this protein resulted in the production of triangular nanoplates with hexagonal shapes, demonstrating a yield of 90%. Later, this protein was named as gold shape-directing protein (GSP).

BACTERIAL EXTRACT AS A CAPPING AGENT

Bacteria depict an extraordinary ability to reduce a variety of metal ions and hence are emerging as one of the best candidates for the synthesis of nanoparticles. Some bacterial species have developed the mechanism to tolerate metal stress, including heavy metal toxicity. For instance, many bacterial species can survive and grow at extremely high metal ion concentrations (Iravani, 2014). Bacteria can not only tolerate high concentrations of metals but can also convert these metal ions into nontoxic metal nanoparticles. Due to this reason, bacteria are looked at as alternative nanofactories for the synthesis of metal nanoparticles. The use of bacteria offers certain obvious advantages like a high degree of adaptability to extreme conditions, fast growth rate, low energy consumption, and superior process controllability (Tsekhmistrenko et al., 2020). Nanoparticle formation in bacteria can be intracellular or extracellular. Extracellular synthesis is more convenient because of the easy separation of the nanoparticles. In addition to this, these particles are observed to be more resistant to environmental oxidation after their synthesis (Gahlawat and Choudhury, 2019). Bacterial extracts like other organisms are considered to act as both reducing and stabilizing agents in the synthesis of nanoparticles. Out of the biomolecules present in the bacterial extract, biosurfactants have been explored widely for nanoparticle synthesis. Biosurfactants are amphiphilic molecules that are capable of playing a vital role in the process of nanoparticle stabilization (Kumar et al., 2010). These molecules get adsorbed on the surface of the metallic nanoparticles, preventing their aggregation and leading to their stabilization (Figure 4). One of the modes of action is through adsorption onto metallic nanoparticles. The mechanism of surfactant adsorption is determined by the type of surfactant (polymeric,

ionic, nonionic, etc.) and the thickness of the layer adsorbed on the surface of the particles (Płaza et al., 2014).

Joanna et al. (2018) synthesized biogenic silver nanoparticles using Bacillus subtilis grown on agro-industrial wastes, such as brewery effluents and molasses. The synthesized nanoparticles were evaluated for their antimicrobial activity and were compared with the chemically synthesized silver nanoparticles. Biosurfactants present in the cell-free extract had assumed to cap the nanoparticles. The presence of biosurfactant coating considerably increased the stability of biogenic AgNPs and improved their antimicrobial activities. These nanoparticles have demonstrated broad-spectrum activity against bacteria and fungi. Salazar-Bryam et al. (2021) investigated the effect of pH on the synthesis of silver nanoparticles stabilized by glycolipid surfactants: rhamnolipids. A comparison of physical properties and nanoparticle stability was carried out for the particles synthesized at pH 5, 7, and 9. Dynamic light scattering techniques were studied to evaluate particle size-larger particles were formed at pH 5 (78-190 nm), whereas smallersized particles were formed at pH 9 (5.6-28.1 nm). Similar results were obtained for nanoparticle stability (analyzed based on the zeta potential), where the highest potential was noticed at pH 9, demonstrating enhanced particle stability. Spherical morphology of the particles was confirmed using field emission gun scanning electron microscopy. More particle aggregation was observed at low pH of 5, which could be because of the protonation of the carboxylic acid group of the rhamnolipids, which overweigh the electrostatic repulsion forces resulting in increased particle size. The rhamnolipids acted as capping agents, reducing the formation of aggregates, determining the particle size, and also stabilizing the colloidal suspension. Lipopeptide biosurfactants have also been reported in nanoparticle synthesis. Similar results were obtained by Reddy et al. (2009) while synthesizing Ag and Au nanoparticles deploying lipopeptide biosurfactants: surfactin produced by Bacillus subtilis. While comparing the effect of pH 5, 7, and 9 on the particle size and stability, they too inferred that the nanoparticles synthesized at pH 9 were smaller in size and were stable for 2 months. The silver nanoparticles synthesized by Brevibacterium casei using a glycolipid biosurfactant were more stable than the nanoparticles synthesized without a surfactant. These workers checked the aggregation of the nanoparticles by placing them at room temperature for different time intervals. The use of surfactants enhanced the stability up to 2 months, whereas control particles synthesized in the absence of surfactants aggregated within 7 days. The data concludes that the surfactant acts as a stabilizing agent and prevents particle agglomeration (Kiran et al., 2010). Xu et al. (2018) confirmed the presence of protein and polysaccharide capping onto selenium nanoparticles synthesized by Lactobacillus casei. SEM, TEM, and EDX images of the prepared SeNPs showed the presence of spherical particles consisting of carbon (C), nitrogen (N), oxygen (O), phosphorus (P), sulfur (S), and Se elements. Furthermore, the FTIR analysis demonstrates the stretching vibration peak of O-H and N-H at 3,323 cm⁻¹, corresponding to polysaccharide and protein residues. Stretching vibration of C-H in the sugar ring or peptide chain could be assigned to the band observed at 2,923 cm⁻¹. The bands

at 1726 and 1,467 cm⁻¹ can be related to the stretching vibrations of C=O and COO-, respectively, while the bands at 1,631 cm⁻¹ could be associated with the stretching vibrations of C=C and C=N. The concentration of Se, protein, and polysaccharide in the purified SeNPs was 7.6%, 70.88%, and 14.17%, respectively. This data denoted the presence of protein and carbohydrate coating on the surface of the prepared nanoparticles.

PLANT EXTRACT AS A CAPPING AGENT

Plants have gained immense importance in the field of nanotechnology due to their cost-effectiveness, less toxicity, rapidity, availability, and nonpathogenicity; the economic protocol includes mild conditions for biosynthesis, large-scale production, and a broad range of biological activities (Jadoun et al., 2021; Sivakumar, 2021; Tadele et al., 2021). Different parts of plants, including the leaf, stem, root, fruit, bark, flowers, rhizomes, callus, seed, bud, and latex, have been extensively utilized in the green synthesis of nanoparticles due to the presence various biomolecules. of The plant-based bioconstituents like steroids, sapogenins, tannins, terpenoids, polyols, alkaloids, polysaccharides, flavonoids, phenolics, proteins, amino acids, enzymes, and vitamins (Figures 3 and 4) function as strong chelating, reducing, and stabilizing agents, which provide stability, prevent agglomeration, and help in tuning the shape and size of the nanoparticles (Adelere and Lateef, 2016; Nayak et al., 2020; Din et al., 2021; Jadoun et al., 2021; Restrepo and villa, 2021; Sivakumar, 2021; Tadele et al., 2021). A research study carried out by Shaheen and Ahmad (2020) identified the different biogenic capping agents [phenols (ascorbic acid, resorcinol, 4-nitrophenol, umbelliferone, vanillic acid, tannic acid, phenol, rutin, and ellagic acid), flavonoids, alkaloids, acetic acid, D-alanine, and aromatic and aliphatic amines (octodrine)] by HPLC and GC-MS from the leaves of Nasturtium officinale for the synthesis of Mo3 nanoparticles. The data concluded by this report confirmed the presence of phytochemicals on the surface of Mo3 nanoparticles. SEM results of synthesized nanoparticles showed different shapes of particles with C and O elements at different magnifications. Moreover, FTIR analysis speculates that vibration peaks at 461.5 cm^{-1} represent Mo₃ particles along with stabilizing agents at 1,637.62, 1,484.94, and 1,021.6 cm⁻¹, which correspond to the primary amine, aromatic, and aliphatic amine functional groups. This work concluded that phytochemicals not only help in the reduction process but also minimize the aggregation of nanoparticles. Furthermore, Navak and his coworkers highlight the contribution of bioactive compounds in the synthesis of silver nanoparticles by using Dillenia indica L. leaf extract. In this study, the FTIR study showed the various peaks at 541, 668, 778, 862, 1,044, 1,119, 1,266, 1,318, 1,384, 1,427, 1,459, 1,597, 2,853, 2,923, and $3,416 \text{ cm}^{-1}$. The peaks at 3,416, 1,597, 668, 778, and 862 cm^{-1} correspond to free -COOH groups, as well as stretching modes of nitro (containing oxygen molecules) and functional groups of esters. These stretching vibrational peaks clearly indicate the presence of biomolecules as stabilizing agents. TEM results

demonstrate that AgNPs were spherical in shape with size in the range of 10.01-23.24 nm. Moreover, zeta potential measurements exhibit that exterior parts of nanoparticles were negatively charged with a sharp peak at -39.5 mV zeta potential. This negative value states the repulsion among the synthesized nanoparticles, which in turn were stable for a long time. An interesting work done by Yulizar et al. (2020) for the first time showed the synthesis of cerium nanoparticles (CeO₂) by using Datura metel leaf extract (DME). Various literature studies showed that secondary metabolites play a key role in the reduction of metal ions and redox reactions for the formation of a nanosized mass of metal oxide nanoparticles (Navak et al., 2020), so these workers initially performed phytochemical screening to confirm the presence of secondary metabolites (alkaloids and flavonoids). FTIR analysis of DME showed the strong absorption bands at 1,030, 1,408, and 1,610 cm⁻¹ that corresponded to C-N stretching vibrations (indicates the presence of aliphatic amine groups of alkaloids), C=O carbonyl groups, and C-C aromatic groups (indicates flavonoids). In addition, SEM and TEM characterization of CeO₂ nanoparticles showed that particles were spherical in shape, agglomerated, and were 5-15 nm in size. This result rendered that secondary metabolites play a multifunctional role of a weak base source, as well as oxidizing, capping, and chelating agents without the addition of any base chemical compound in the preparation of nanoparticles. Similarly, Sahayaraj et al. (2020) also showed that the presence of secondary metabolites in the plant extract not only contributes as fabricating agents but also enhances the dispersity and stability of the nanoparticles. These workers synthesized AgNPs by using the dry leaf aqueous extract of the Pongamia glabra tree, and FTIR characterization of silver bionanoparticles demonstrates that amines (vibration band at 771 cm⁻¹) and amides (peak at 1,630 cm⁻¹) were responsible for the reduction of metal ions and capping agents. SEM results exhibit that synthesized silver nanoparticles were well dispersed, with largely spherical shape (67.8%) and few squares (38.2%) with 29.4 nm in size, and were highly dense in nature due to the electrostatic interactions between the capping molecules on the surface of AgNPs. Further, nanosilver ions were also stabilized as there was no direct contact among them. Interestingly, a fascinating study has been conducted by Kambale et al. (2020) that showed the effect of different plant leaf extracts [Brillantaisia patula (BR-PA), Crossopteryx febrifuga (CR-FE), and Senna siamea (SE-SI)] on the optical, structural, surface morphology, and biological properties of the silver nanoparticles. It has been observed that Ag nanoparticles synthesized from the aqueous extract of BR-PA, CR-FE, and SE-SI showed different UV-vis absorption bands at 434, 489, and 522 nm due to the surface plasmon resonance (SPR). The main reason for distinctive absorption peaks has been correlated with the interaction of phytoconstituents with the surface of the nanoparticles. EDX analysis showed the existence of carbon and oxygen atoms on the surface of AgNPs, supporting the difference in the SPR absorption bands of three types of AgNPs from BR-PA, CR-FE, and SE-SI extracts due to the difference in the phytochemical composition of the extracts that has been validated by FTIR analysis. The sharp

round bands at around $3,325-3,198 \text{ cm}^{-1}$, $2,917-2,833 \text{ cm}^{-1}$, $1,380-1,360 \text{ cm}^{-1}$, and $1,050-1,025 \text{ cm}^{-1}$ correspond to alcoholic (O–H), alkane (C–H), phenolic (O–H), and (C–O) stretching vibrations, whereas $1,640-1,620 \text{ cm}^{-1}$ stretching vibrations were with respect to the carbonyl group (C=O) from carboxylic, aldehyde, ketone, or ester-containing compounds. The particle size distribution from TEM results indicates that AgNPs were 45 nm, 115 nm, and 47 nm, which correspond to BR-PA, SE-SI, and CR-FE extracts. This confirms that the difference in the size, UV-vis, and FTIR behavior of the synthesized nanoparticles was due to the presence of various types of phytoconstituents in the extract.

BIOMEDICAL APPLICATIONS OF CAPPED NANOPARTICLES

Antimicrobial agent: It is a known fact that different metallic nanoparticles like Au, Ag, ZnO, Pd, Cu, Pt, Ti, and Fe possessed antimicrobial activity against bacteria, fungi, viruses, etc. (Puja and Kumar, 2019). Generally, the basic mechanism for the antimicrobial action of these nanoparticles is based upon the oxidative and nonoxidative stress, metal ion release, electrostatic interaction between the metal ion and the plasma membrane, and inhibition of ATP-dependent protein synthesis, and inducing DNA damage will in turn lead to ROS production, lipid peroxidation, and protein oxidation (Singh et al., 2018; Puja and Kumar, 2019). Various studies elucidate the antimicrobial property of biogenic-capped nanoparticles against pathogenic microorganisms. For instance, the research of Vijayakumar and Vaseeharan on zinc oxide showed that 75 µg/ml concentration of Cl-ZnO NPs exhibited greater antibacterial and antibiofilm activity against Gram-positive (Streptococcus mutans) and Gram-negative (Proteus vulgaris) bacteria and fungi (Candida albicans). Additionally, Garza-Cervantes et al. (2020) also showed that cellulose-capped AgNPs were effectively used as antimicrobial agents for killing antibiotic microbialresistant and -sensitive bacteria. It has been found that Ag-cellulose composite showed an antimicrobial efficiency five times higher than the optimum clear zone value obtained for Gram-negative strains, whereas in the case of Gram-positive strains, these showed 10 times more efficiency. Moreover, Caschera et al. (2020) also reported the antibacterial activity of nanocomposites against Gram-positive (Bacillus subtilis and Staphylococcus aureus) and Gram-negative bacterial strains (Escherichia coli and Pseudomonas aeruginosa). Antibacterial assay results speculate that Ag-ONC nanocomposites have low solubility in the agar media but had a good antibacterial activity (12-14-mm inhibition zone) against both types of bacteria.

Anticancer: In a similar trend, biologically synthesized nanoparticles also exhibited antitumor and cytotoxic activity. Besides an antibacterial study, Vijayakumar and Vaseeharan also highlighted the cytotoxicity activity of collagen-capped ZnO NPs (75 μ g/ml) against normal murine macrophages (RAW 264.7) and human liver cancer cells (HepG2). This intensifies that biopolymer-capped zinc oxide nanoparticles had a greater ability for anticancer activities. Furthermore, the

research work of Pradeepa et al. (2017) for the first time showed the antiproliferative effect of silver nanoparticles against breast cancer cell lines (MCF-7). MTT assay results speculate the cytotoxic effect of synthesized AgNPs in MCF-7 cell lines; moreover, apoptosis (cell shrinkage, impaction of nuclei, and fragmentation of chromatin) was also observed in the treated cell lines. This clearly indicates that AgNPs inhibit the growth of cancer cells and can be used as therapeutic agents for cancer treatment. Similarly, Majeed et al. (2019) comparatively showed the cytotoxic analysis of both the capped and uncapped nanoparticles against MCF-7, HCT-116, and MG-63 cell lines. It has been inferred that a low concentration of capped AgNPs (80 µg/ml) showed half-maximal inhibition (IC50), whereas uncapped AgNPs showed IC50 at 100 µg/ml against MCF-7 and MG-63 cells. In the case of HCT-116 cell lines, 60 µg/ml of BSA-AgNPs and 80 µg/ml of AgNPs showed IC50. Besides this, capped AgNP-treated cell lines also showed maximum release of lactate dehydrogenase (LDH) enzyme in comparison with uncapped nanoparticle-treated cancerous cell lines. This confirmed that cAgNPs were used as antitumor agents as they kill the cancer cells by increasing programmed cell death (apoptosis).

Anti-inflammatory: Due to the increase in the risk of chronic inflammatory diseases like fibromyalgia, gastrointestinal, kidney, cardiovascular, diabetes, neurodegenerative, musculoskeletal, or rheumatic diseases, there has been a paradigm shift from the usage of anti-inflammatory drugs (aspirin, acetylsalicylic acid, diclofenac, ibuprofen, etc.) to the nanoparticulate drug delivery systems which facilitate the solubility, bioavailability, and targeted delivery of drugs (NDDS) (Ovinlove et al., 2015; Placha and Jampilek, 2021). Various nanoformulations of include hydrocortisone, budesonide, drugs methylprednisolone-encapsulated chitosan and BSA nanoparticles, dexamethasone incorporated into solid lipid nanoparticles, celecoxib-loaded silk fibroin nanoparticles, and hyaluronan nanocapsules; experimental evaluation on treated mouse models and cell cultures showed that nanoformulated drugs have more anti-inflammatory activity [decrease in immune markers (histamine, IgE, helper T cells, prostaglandin E2, and EGFa), IL-1, TNF-a, IL-10, NO, and IL-6; increase in antioxidant activity (ROS scavenging)] than normal drugs (Gai et al., 2018; Crivelli et al., 2019; Choi et al., 2020; Ramos Campos et al., 2020; Nedelcu et al., 2021).

Biosensors, medical diagnosis, and imaging: Different nanoparticles like silver, gold, superparamagnetic iron oxide, titanium dioxide, manganese dioxide, cerium, zinc oxide, liposome, and solid lipid nanoparticles are widely used in diagnostic, biomedicine, and medical imaging like MRI, ultrasound imaging, computed tomography, positron emission tomography, and single-photon emission computed tomography as highly sensitive probes for targeting and imaging small molecules like DNA, proteins, cell tissue, and tumors (Carrasco-Esteban et al., 2021; Lee and Jun, 2019; Mirahadi et al., 2018; Silva et al., 2021).

Wound healing: Wound dressing is usually required for the maintenance of the environment, eliminating the growth of other invading microorganisms, and the removal of excess fluid and dead cells around the wound. To overcome the problems (narrow

antimicrobial spectrum, skin allergies, irritation, and cytotoxicity) related to the use of conventional drugs [glucocorticoid steroids and nonsteroid anti-inflammatory drugs (ibuprofen, naproxen, diclofenac, ketoprofen, indomethacin, and acetylsalicylic acid)], various types of metallic nanoparticles have been used in the healing process (Chugh et al., 2021). It has been found that silver, zinc oxide, and gold nanoparticles coated with biomolecules like polysaccharides and biological extracts help in the wound healing mechanism. These nanoparticles trigger apoptosis, tissue necrosis, platelet and stem cell activation, angiogenesis, tissue scar formation, and bacterial clearance and reduce cytokines (IL-6 and IL-10) and reepithelialization (Wang et al., 2017; Ovais et al., 2018; Batool et al., 2021; Chugh et al., 2021).

Drug delivery: Chemotherapeutic agents have been widely accepted for the treatment of diseases, but these have some limitations like high toxicity, short lifespan in the body, low solubility, and large volume of distributions (Wang et al., 2017). Nowadays, nanoparticles are utilized in targeted drug delivery systems in order to increase the efficiency of chemical agents (antineoplastic drugs). Silver, gold, zinc, copper, iron oxide, titanium dioxide, cerium dioxide, and iron-platinum have achieved considerable attention in this field due to their small size and ability to cross biological membranes (Andleeb et al., 2021; Desai et al., 2021; Nikzamir et al., 2021; Roy et al., 2021).

CONCLUDING REMARKS

Capping agents play a crucial role in controlling particle size, morphology, agglomeration, surface energy, grain growth, dispersion, and electrostatic and steric hindrance by providing specific functional groups on the surface of nanoparticles. Keeping in mind the limitations of chemical capping agents, green chemistry has been applied for reducing, stabilizing, and fabricating synthesized nanoparticles. In this review, functions of various biomolecules like proteins, amino acids, lipids,

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carbohydrates, and biosurfactants (as shown in Table 1) and biological extracts along with their medical applications have been emphasized. Natural substances used to synthesize and/or cap the nanoparticles offer various advantages over synthetic medications, including fewer adverse effects; therefore, biological materials have long piqued the interest of researchers. Biological molecules (collagen, BSA, amino acids, lipids, and carbohydrates) coating onto the nanoparticles not only reduce their side effects but also improve their characteristics like small size, long-term stability, and biocompatibility (speed up the uptake and retention of nanoparticles) and enhance antimicrobial and anticancer activity All the capping agents discussed in this paper provide surface functionalization and reduce the size of nanoparticles by preventing agglomeration in addition to increasing their storage stability. Comparison between these agents to determine the best one is difficult. Thus, the choice of the suitable green capping agent can be made by considering the surface functional groups introduced by them and their availability and cost. However, these capping agents offer several advantages; storage stability (in the case of biological extracts), homogenous fabrication, toxicity levels, and somehow agglomeration of nanoparticles based upon biogenic capping agents are the biggest issues that need considerable attention. With the pace of time, the demand for nanoparticles in the biomedical sector has been increasing day by day; therefore, for large-scale production, further research is urgently required to address the issues especially related to yield, stability, uniformity, reproducibility, and kinetics. Moreover, intensive research is also mandatory for exploring the mechanism of biomolecules as capping agents in the green way-mediated biosynthesis processes.

AUTHOR CONTRIBUTIONS

AS, NV, and PK designed and wrote the manuscript and revised and finalized the draft. AS and PK supervised the whole project and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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