FROM MARINE ORIGIN TO NANO WORLD: UTILISATION OF AQUATIC RESOURCES IN DEVELOPMENT OF METAL NANOPARTICLES

Abstract

Metal nanoparticles synthesised using green technologies can be utilised for biomedical and other environmental applications. Better ways of utilisation of aquatic resources will help to design new technologies of sustainable nature. Aquatic organisms are rich in bioactive and reducing compounds and can effectively be used for the green synthesis of metal nanoparticles. The compounds like chitosan, collagen, gelatin and fish oil from fish waste help in synthesising nanoparticles under both thermal and non-thermal treatments. The extractives from some aquatic organisms can reduce the metal ions without any chemical supplementation. The synthesis of metal nanoparticles was also possible using the phytoconstituents from seaweeds. The marine microbes can synthesise the nanoparticles both intracellularly and extracellularly. These technologies can be scaled up to meet different industrial applications. This review explains the production strategies of metal nanoparticles using different marine compounds and also their applications.

Keywords: Aquatic, Nanoparticles, Organisms, Applications, Seaweed

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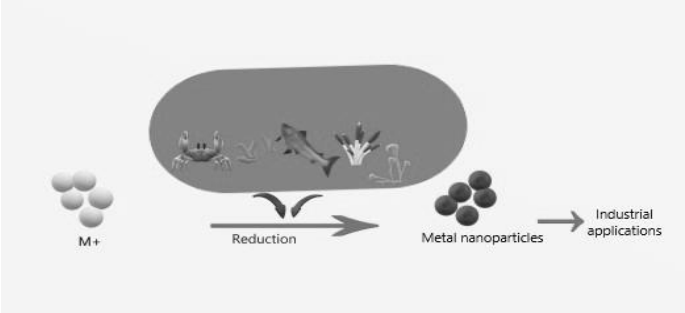
Graphical Abstract



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I. INTRODUCTION

The word "nano" came from the Greek "nanos" which means dwarf and it is the prefix for 10^{-9}. Those particles that have size of 1-100nm are termed as nanoparticles (Khan et al. 2014). The peculiar physical and chemical characteristics of these materials arise from the special electronic structures and increased surface area resulting in higher reactive efficiency. The applications of nanoparticles include photochemical, chemistry, biomedical and electronics field (Di Guglielmo et al. 2010). Nanoparticles of inorganic metals or hybrids of inorganic and organic nanoparticles provides them unique characteristics and increases the applicability than pure large sized counterparts (Kim and Jon, 2012). The properties of nanostructures are determined by shape and size, interparticle distance and nature of protective outer shell (Baraton, 2003).

Generally, the methods employed for production of metal nanoparticles can be divided into two, top down approach and bottom up approach**.** The top down method includes the mechanical reduction of metals to nanostructured colloids and further stabilisation using stabilising agents. This includes typical solid–state processing of the metals. The breaking up of bulk particles use the physical processes like crushing, grinding or milling. The problem with this approach is imperfection on the structure of surface which affects the physical properties and surface chemistry. Also the challenging use of apparatus involved adds on to the difficulty. Ball milling and metal vapour technique are examples of methods used for synthesis of nanoparticles using top down approach.

The "bottom-up" method includes the building up of a nanoparticle from the bottom. Atom-atom, molecule to molecule or cluster to cluster approach can be involved in this. The chemical reduction of metal salts is the most common way of synthesis. Other techniques like electrochemical pathways and decomposition of organometallics in a controlled manner are also included in this. The use of stabilisers including polymers, donor ligands and surfactants helps to control formation of nanoclusters and further aggregation.

The mechanism of nanoparticle formation includes two stages of nucleation and growth. Nucleation is the embryonic stage where metal salt will get reduced to metal atoms which are zerovalent. These atoms will collide with other ions or atoms or clusters and irreversible 'seeds' will be formed which can be of size below 1nm. The seed formation depends on the difference in redox potential of metal salt and reducing agent and also on the bond strength between metal-metal atoms. The deactivation of formed nanoparticles occurs by formation of bulk. This happens mainly by two ways, Ostwald ripening or aggregation. In Ostwald ripening the diffusion of smaller particles on to larger particles will cause the increase in particle size with increase in time. This happens because of the tendency of the system to reduce total energy by reducing size. The aggregation of particles is caused not by diffusion based interaction, but both increases the size of particle. In order to prevent the nanostructured particles from agglomeration, protective/stabilising agents are necessary. The stabilisation can be electrostatic stabilisation where repulsion between electrical double layers prevents the formation of larger clusters. The other type of stabilisation is steric stabilisation which includes formation of a protective layer on metallic surfaces by organic molecules. Another classification of synthesis of nanoparticles given by Iravani et al. (2018) is given in figure 1.

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In this article, the use of different materials originated form the aquatic organisms or direct use of those organisms for reducing or stabilising metal nanoparticles are highlighted to emphasize the importance and scope of marine life in nanotechnology.

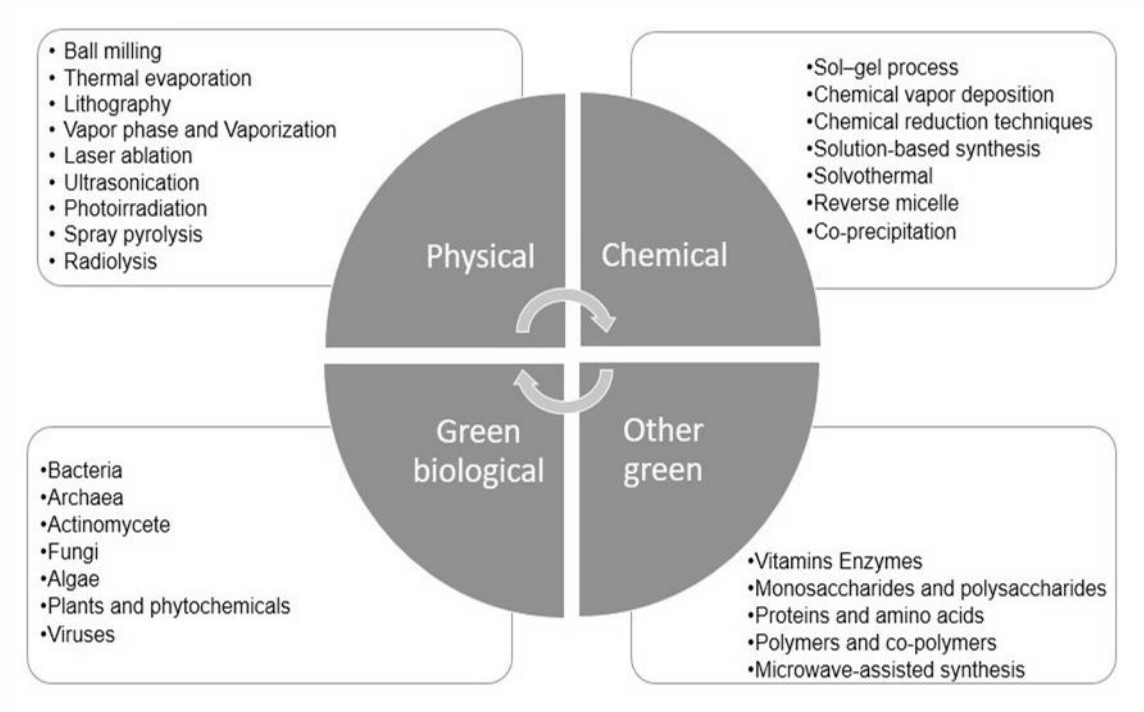

Figure 1: Methods of nanoparticle synthesis by Iravani et al. (2018)

II. SYNTHESIS OF NANOPARTICLES BY MARINE ANIMALS OR ANIMAL DERIVED COMPOUNDS

1. **Chitosan:** Chitin is a polymer of acetyl amino-D-glucose and is distributed in nature in crustaceans, insects and fungi. Chitosan (Figure 2.) is a copolymer of b (1-4) 2-acetamido 2-deoxy-b-D-glucopyranose and 2-amino-2- deoxy-b-D-glycopyranose (Rinaudo et al. 2006). Chitosan is produced by the deacetylation of chitin either using chemicals like acids or alkali or using enzymes. The polymer can be characterised by the number of monomer units which defines the molecular weight and the degree of deacetylation of chitosan. It is soluble in acidic solutions and partially soluble in alkaline solutions and the solubility is affected by degree of deacetylation (Filar and Wirick, 1977). The physicochemical properties of chitosan are explained in table 1.

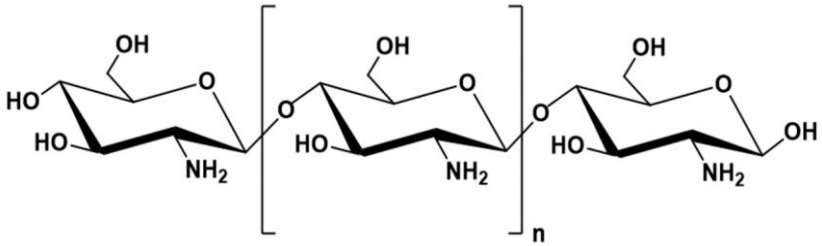

Figure 2: Structure of chitosan

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Table 1: Physicochemical characteristics of chitosan (Li et al. 1992)

Property	Comment
Degree of deacetylation	• UV spectrophotometry • IR spectrometry • Dye adsorption • Metachromatic titration • Gas chromatography
Molecular weight	• Chromatography • Light scattering • Viscometry
Viscosity	Affected by, • Ionic strength • Deacetylation time • Molecular weight • Concentration
Solubility	Usually dissolved when pH is less than 6 but affected by • Solvent mixing • Deacetylation • Salvation • Chemical modification
Coagulating ability	Binding for • Metal ions • Anionic polymers • Amino acids • Proteins • DNA • Cells • Dyes • Solids

2. **Chitosan- Gold Nanocomposites:** The synthesis of gold nanoparticles in chitosan involves three reactions. In acidic solution, the chitosan chain will get broken and open chain will be formed at part of the end groups (Reaction I). All the polymers from hydrolysed chitosan will exist in dynamic equilibrium and this process is reversible. On addition of Au3+ ions into the system, the oxidation of –CHO groups in the end of chain occurs and form –COOH groups (Reaction II). This step is irreversible and the dynamic equilibrium will get broken, and the reaction will proceed towards a positive reaction. In this manner chitosan chains will be continuously degraded and gold nanoparticles will be formed. The degradation of chitosan is higher at higher temperatures and increased concentration of gold ions (Sun et al. 2008). The presence of -NH2, -CH2OH and -CHO groups in chitosan, which are associated with chitosan degree of deacetylation (DD) and

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molecular weight (MW), decide its chelating, reducing and stabilizing characteristics and finally influence the size and shape of the synthesized AuNPs. The synthesis of nanoparticles can also be done using other reducing agents like sodium citrate. The chitosan nanocomposites exhibit pink to wine red colour and the plasmonic peak will be at ~520nm under UV spectral analysis.

3. **Protocol for the Synthesis of Gold Nanoparticles:** In the synthesis of gold nanoparticles, chitosan act as both reducing and stabilising agent. The chitosan is dissolved in 1% acetic acid solution and gold precursor is added under continuous stirring and heat (Usually 70^{o}C to 100^{o}C). Continue stirring is applied for required time till a wine red colour is obtained which indicates the formation of nanoparticles. The time of complete reduction of all gold ions depends on the concentration of gold precursor, concentration of chitosan, temperature of treatment and intrinsic characteristics of chitosan. The solution can be stored under refrigerated conditions after cooling to room temperature (Sreelakshmi et al. 2022).

4. **Properties and Applications:** The antioxidant activity of chitosan gold nanocomposites were 80 times higher than the potential of ascorbic acid (Esumi et al. (2003). The chitosan gold nanocomposite exhibit antitumour activity as they induce different cell death modalities in HeLa and MCF-7 cells which depends on reactive oxygen species production (Martínez-Torres et al. 2018).

   The effect of these nanoparticles against both gram negative and gram positive bacteria like P. aeruginosa, E. Coli, S. aureus and Bacillus spp. etc is well established (Regiel-Futyra et al. 2015, Katas et al. 2019). These nanoparticles can be efficiently used for drug delivery (Bhumkar et al. 2007) and gene delivery (Bhattarai et al. 2008). They can be used for development of sensors for glucose (Du et al. 2007), acetylcholamine, heavymetals (Sugunan et al. 2005) silver ions (Zhao et al. 2019), caffeic acid (De Carlo et al. 2019) organophosphate pesticides (Du et al. 2007), antibiotics (Lai et al. 2017) and Salmonella (Cinti et al. 2017).

5. **Toxicity:** The chitosan gold nanoparticles produce no treatment related toxicity on oral administration and the LD 50 value in rats was found to be 2000mg/Kg (Pokharkhar et al. 2009).

6. **Chitosan Silver Nanocomposites:** The Chitosan-silver composites (Figure 3.) can be made with silver in ionic or metallic form. The stabilisation and dispersion of nanoparticles in chitosan depends on the chemical bond between nitrogen in the amino groups of chitosan and the lone pairs in silver orbitals (Latif et al. 2015). Insitu chemical reduction method is the commonly used technique for preparation of chitosan silver nanocomposites where chemicals like NaBH were used as reducing agent. Different techniques in preparation of silver nanoparticles using chitosan as reducing agent are shown in Table 2. The chitosan silver nanocomposites exhibit yellow to brown colour and the plasmonic peak will be at ~400nm on UV spectral analysis.

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Table 2: Different methods of silver nanoparticle synthesis using chitosan

Reducing and Stabilising agent	Size of AgNps	References
Thermal method		
Chitosan	20-150	Bal et al., 2012
Chitosan	10-50	Wang et al., 2012
Chitosan	55-278	Jena et al., 2012
Chitosan	80-120	Saravanan et al
Carboxymethyl chitosan	12-18	Fouda et al., 2013
Gamma ray method		
Chitosan	20-25	Yoksan et al. 2010
Chitosan	7-30	Yoksan et al., 2009
UV-irradiation method		
Chitosan	3.16-10.97	Shameli et al. 2010
Electrochemicalmethod		
Chitosan	<50 2-16	Pishbin et al. 2013 Reicha et al. 2012

7. **Properties and Applications:** Silver nanoparticles exhibit fungicidal activity (Wang et al. 2015) and antibacterial activity against both gram negative and gram positive bacteria (Wei et asl., 2009, Tran et al. 2010). They have antixodant activity (Hajji et al. 2019) and exhibit antiproliferative activities against carcinoma cells (Tran et al. 2010). They can be used for development of gels or nanofibres with wound healing properties (Li et al. 2016) and as sensor for glucose detection (Jiang et al. 2012). These nanoparticles are effective plasmonic substrates for Surface-enhanced Raman spectroscopy detection of nonresonant analytes at the single-molecule level (Potara et al. 2012). These can form a protective film over steel and act as cathodic type inhibitor against corrosion (Solomon et al. 2017) and also has the ability to remove pesticides from water (Saiffudin et al. 2011).

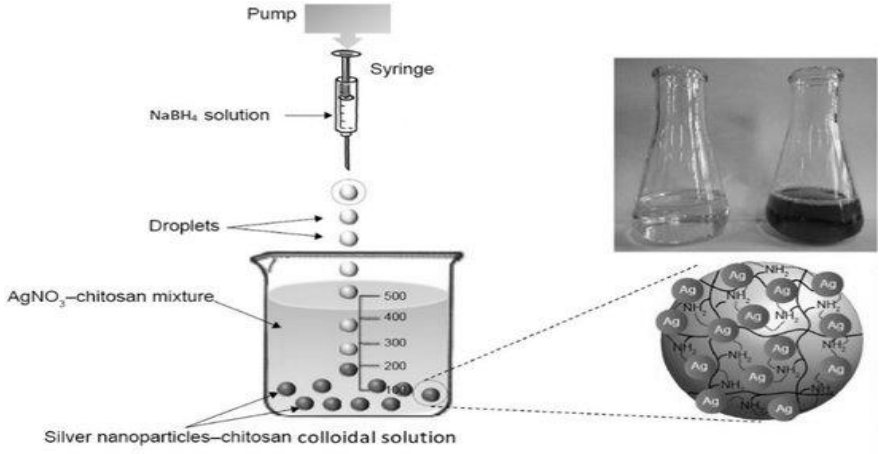

Figure 3: Reduction of AgNPs using NaBH4 and its stabilisation using chitosan (Wang et al. 2015)

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III. OTHER METAL NANOCOMPOSITES

Chitosan can effectively be utilised for the formation of nanoparticles using metals like zinc, palladium, platinum and copper, either as pure chitosan or as modified forms like carboxymethyl chitosan (Adlim et al. 2004, Zain et al. 2014).

1. **Collagen:** Collagen constitutes the major component of connective tissue membranes that joins the myotomes in fish muscle. It constitutes about 0.2 to 1.4% of total protein in fish and is highly responsible for the integrity of fillets. The content of collagen increases during maturation of fish without significant changes in extend of crosslinking. The hydroxyproline and proline residues effects the properties of collagen significantly (Sikorski et al. 1984).

   Collagen comprises 30% of extracellular matrix proteins in living organisms and hence is the most abundant protein in extracellular matrix. It functions as a structural component and helps in signalling through the biomolecular interactions. Collagen can interact with many biomolecules including sugars, proteins, polyphenols, proteoglycans and drugs. This helps in selective probing of a number of molecular interactions. As collagen is rigid and naturally exists as a protein scaffold, it can be an ideal protein target for making a protein-nanoparticle scaffold. The structural stability of collagen offers less deviations in structure and function on combination with nanoparticles (Figure 4.) and thus helps in targeted interactions with biomolecules (Unser et al. 2017).

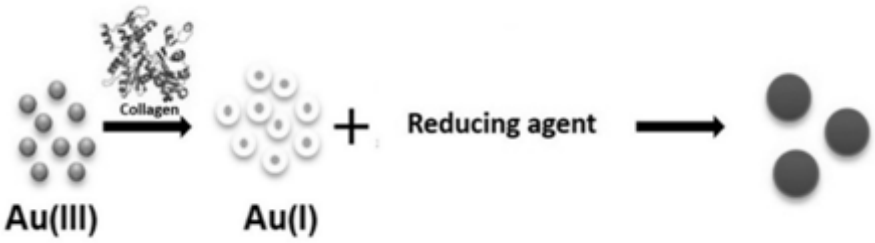

Figure 4: Gold nanoparticle synthesis assisted by collagen

In formation of gold nanoparticles, collagen plays dual role by making complexes on binding to the metal ions besides acting as a capping ligand. The concentration of collagen can be positively correlated with the formation of nanoparticles but also increases aggregation (Kumari et al. 2012). Collagen AuNP composites with nanoparticle dose of <20 ppm and size >20 nm are non-toxic 3T3 fibroblasts and HaCat keratinocytes. Sponges made of these composites have high biocompatibility and wound healing capacity (Akturk et al. 2016). The presence of collagen helps in giving uniform shape to nanoparticles and also the positive charged linear structures will help to assemble nanoparticles on to collagen and thus forming nanoparticle films or matrices (Wei et al. 2007). The silver nanoparticles stabilised with collagen have peculiar shape, size and positive zeta potential which impart antibacterial activity to nanoparticles and collagen hydrogels.

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2. **Applications and Properties:** The collagen nanoconjugates with gold chloride can be used as colorimetric sensor for large range of targets, eg; the collagen conjugates with 10nm gold nanoparticles can be used as sensor for detecting glucose and heparin (Unser et al. 2017). The collagen silver nano-composites are non-toxic to human fibroblasts and keratinocytes (Alarcon et al. 2012). The collagen copper nanocomposites also have antibacterial efficiency and can be used for wound healing purpose and is non-toxic (Premalatha and Kothai, 2017). The antimicrobial collagen nanocomposites are biocompatible, biodegradable and are cost effective (Grigore et al. 2017).

3. **Gelatin:** Gelatin is the edible protein derived by hydrolysis of collagen. The applicability of gelatin is vast because of the non-toxicity, lower cost, biocompatilbility and affinity to proteins. The gelatin can stabilize nanoparticles electrostatically and in a steric manner. The gelatin (Figure 5) exists in an ordered α helix conformation at room temperature and remains in unfolded condition at elevated temperatures. The functional groups of gelatin like suspended double bond, -SH, -NH and –COOH makes further modifications of gelatin with other molecules. This makes gelatin ideal for development of nanomaterialised devices. The degradation of gelatin can be caused by the incubation parameters like temperature, pH, salt ions and time which inturn affects the reducing and stabilizing property of gelatin. (van den Bosch & Gielens, 2003). The studies on synthesis of metal nanoparticles using fish gelatin is given as table 3.

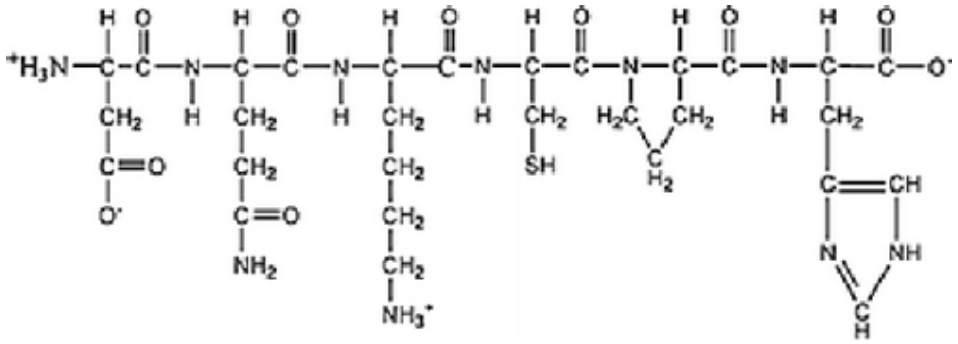

Figure 5: Typical gelatin polypeptide (Liu et al. 2011)

4. **Gelatin Gold Nanocomposites:** The synthesis of AuNPs can be affected by gelatin in a time dependent manner. The introduction of gelatin in early stage (nucleation) will slow down the process of reduction using other reducing agents and hence the nanoparticles formed will be larger but fewer. Similarly, the gelatin addition in a later stage, ie; growth stage, will yield smaller but larger number of nanoparticles. Gelatin will stabilize AuNPs on sustained heating and diffusion barrier caused by gelatin cause a reduction in growth of nanoparticles and colour of the resultant solution changes accordingly. Reduction time, heating time and heating temperature are the major factors affecting the synthesis of nanoparticles (Wang and Gunasekharan, 2012). The synthesis of gold nanoparticles with gelatin as both reducing and stabilizing agent will result in larger irregular particles which disintegrates to smaller particles with increase in time of application of heat.

The concentration of gelatin affects the stability of nanoparticles. A decreased concentration causes a shift in plasmonic peak towards right which can be attributed to the reduced stability leading to aggregation. This is because few gelatin molecules will

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be available to bind with the nanoparticles thus reducing the stability. Also the change in concentration of gelatin cause a change in dielectric constant of the medium and this reduces the dipole oscillations in the electrons. The peculiar plasmonic peak of nanoparticles is also affected by the sharp edges which shows the influence of shape on characteristics exhibited by nanocomposites (Mock et al. 2002).

Both acidic and basic functional groups are present in gelatin polypeptides. The amine groups changes to –NH3+ and thus giving a net positive charge to gelatin below the isoelectric point of gelatin ie, Ph 4.7. Similarly, the carboxyl groups and the sulfhydryl groups impart a net negative charge to gelatin at pH higher than 4.7. This changes the electron donating capacity of gelatin and the reduction of Au III to Au II will get affected. Gelatin cannot act as a reducing agent for AuNPs at pH < 1 or pH > 9 which is attributed to gelatin structure. The glutamic acid and proline interacts well with gold ions and the glutamic acid plays a key role in reducing the ions and stabilising the nanoparticles. The plasmon resonance peak of gelatin gold nanocomposite will remain stable even at altered pH and salt concentration (Liu et al. 2011).

5. **Gelatin Silver Nanocomposites:** The synthesis of silver nanoparticles in gelatin depends on temperature. At higher temperatures the nanoparticles will dissociate forming smaller particles which are stabilized by the amide pendents of gelatin. (Darroudi et al. 2011). On preparation of AgNPs in gelatin using UV irradiation, the increased time of UV application can be attributed to the degradation of gelatin leading to agglomeration of nanoparticles and a broader spectrum.

Table 3: Reports on synthesis of metal nanoparticles using gelatin

Reducing agent	Stabilizing agent	Temperature	Time	Metal	Size of nanoparticle (nm)	reference
Gelatin	gelatin	60^{o}C	48hrs	silver	3.78	Darroudi et al. 2011[a]
Gelatin	gelatin	80^{o}C	40min	Gold	50-80	Liu et al. 2011
Laser ablation	gelatin	-	-	silver	8.95-33.76	Darroudi et al. 2011[b]
Formic acid	gelatin	Room temperature	1min	silver	13-25	Jeong and Park (2014)
	gelatin	UV irradiation	48h	silver	20.99	Darroudi et al. 2011
Formic acid	gelatin	UV irradiation	3h	silver	9-20	Xu and Zhou, 2008
Sodium citrate	gelatin	-	15min	gold	10-15	Neupane et al. 2011

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6. **Synthesis of Nanoparticles Using Fish Oil:** Fish oil is an important source of fatty acids and vitamins especially omega 3 fatty acids, vitamin A and and vitamin D. Omega-3 fatty acids regulate every system in the body, including immune system, cardiovascular system and nervous system. Fish oil can act as both reducing agent and surfactant in formation of nanoparticles (Asmathunisha and Kathiresan, 2013). This property is exhibited mainly because of the carboxylate groups and amines present in fish oil.

7. **Protocol for the Synthesis of Silver Nanoparticles:** Heating Fish oil and silver salt (silver nitrate or silver myristate) at 100-140^{o}C for 5h in inert atmosphere can form silver nanoparticles (Khanna and Nair, 2009)

8. **Synthesis of Nanoparticle Using Marine Sponge:** Marine sponges are known to be gold mines because of the variety of molecules they exhibit. They possess bioactive properties like antitumour, anti-inflammatory, anti-viral and antioxidant properties because of the presence of a variety of biomolecules. Manoalide, dysitrotonic acid, spongothymidine, xestospongin etc. are some of the bioactive molecules present in sponges. Apart from these activities the sponges can synthesis nanoparticles extracellularly. The presence of organic amines in sponge extracts helps to reduce the metal ions and form metal nanoparticles. The nanoparticles are synthesised successfully using extracts of Acanthella elongata (Inbakandan et al. 2010, Inbakandan et al. 2012), Haliclona spp (Hamed et al. 2015) and Axinella sinoxia (Hamed et al. 2017). The silver nanoparticles produced using extract of Heliclona exigua can be used for dental care and for the oral cancer cell lines the half maximal inhibitory concentration value estimated for these AgNPs is 0.6µg/ml (Inbakandan et al. 2016).

9. **Protocol for the Synthesis of Gold Nanoparticles Using Marine Sponge Acanthella Elongate:** For the synthesis of gold nanoparticles, the sponge is ground in water and collect the filtrate. Ten millilitres of filtrate can be added to 100ml auric chloride solution and stirred for 4hrs at 45^{o}C for the formation of nanoparticles.

10. **Seaweed in Synthesis Of Metal Nanoparticles:** Over last 70 years there was a dramatic development in seaweed culture mostly in Asia and also recently in Americas and Europe. Seaweed, also known as marine macroalgae is the common name for uncountable plants in sea, rivers and other water bodies. Seaweeds are among the primary producers of sea and are suppliers of oxygen for other living organisms. They have the capacity to remove pollutants and thus have bioremediation capacity. They possess high survival capabilities under any environmental conditions. They possess good antioxidant, antimicrobial and other bioactive properties. They are widely used in food, cosmetics, fertilisers and for production of hydrocolloids like agar, carrageenan etc. (Chan et al. 2006).

    Based on the photosynthetic pigments, storage compounds and composition of cell wall, seaweeds are classified into three phyla namely, rhodophyceae (red seaweeds), pheophyceae (brown seaweeds), and chlorophyceae (green seaweeds). They can be easily distinguished based on the colour of thallus. Green seaweeds are seen in freshwater than in marine water. Both red and brown seaweeds are common in marine waters. Polysaccharides, polyphenolic compounds, proteins and other chelating agents in seaweeds can act as reducing agents for metal ions. Thus the extracts of seaweeds can

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form better alternative for the other plant sources used for the preparation of metal nanoparticles.

11. **Protocol for Synthesis of Metal Nanoparticles Using Seaweed:** The algal extract is prepared in water or organic solvents. The extract is heated and metal precursor is added to it. The solution is incubated for specific period under controlled conditions for the formation of nanoparticles. Details of synthesis of nanoparticles using different seaweed are given in tables 4 and 5.

Table 4: Synthesis of silver nanoparticles using seaweeds

C	Size	Temperature	Active agent	Application	Reference
Ulva lactuca	48.59	RT	Phenolic compounds, amines and aromatic rings	Photocatalytic degradation	Kumar et al. 2013 [a]
Ulva lactuca	10-30	100	phytochemicals	antibacterial	Raja et al. 2012
Ulva fasciata	40.05	RT	Ethyl acetate	antibacterial	Rajesh et al. 2012
Urospora spp.	20-30	70	phytochemicals	antibacterial	Suriya et al. 2012
Codium capitatum	3-44	RT	proteins		Kannan et al. 2013
Enteromorpha	2-32	RT		antimicrobial	Yousefzadi et al. 2014
Caulerpa scalpelliformis		RT	Aminoacids and proteins	mosquitocidal	Murugan et al. 2015 [a]
Ulva lactuca		RT	Polyphenol	Malaria control	Murugan et al. 2015 [b]
Acanthophora spicifera	48	60	Phenolic compounds	Antibiofilm activity	Kumar et al. 2012 [a]
Gracillaria corticata	18-46	60	Polyphenols and tannins	Antifungal activity	Kumar et al. 2013 [b]
Gelidiella acerosa	22	RT	Aromatic compounds/ alkanes/amines	Antifungal activity	Vivek et al. 2011
Hypnea musciformis	2-55.8	28	Cyclic peptides	Photocatalytic degradation	Ganapathy et al. 2014
Hypnea musciformis	40-65	-	In situ oxidation of hydroxyl groups and by the intrinsic carbonyl groups	Larvicidal activity	Roni et al. 2015
Pterocladiella capillacea	11.4	RT	Proteins	Anticancer activity	El Kassas et al. 2014
Sargassum	30	RT	Proteins	Anticancer	Devi et al.

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longifolium				activity	2013
Padina tetrastromatica	20	RT	Aromatic compound or alkanes or amines	-	Jegadeeswaran et al. 2012
Sargassum ilicifolium	33-44	60	-	Antibacterial and in vitro cytotoxicity	Kumar et al. 2012 [b]
Sargassum tenerrimum	20	60	Hydroxyl/methoxy groups, ketones, polysaccharides	Antibacterial activity	Kumar et al. 2012 [c]
Sargassum polycystum	50-100	RT	Carboxylic, amine, phosphate, and hydroxyl groups	Anticancer activity	Thangaraju et al. 2012
Turbinaria conodies	96	RT	Polyphenols, polysaccharides, primary amines	Antimicrobial activity	Rajeshkumar et al. 2013
Turbinaria conodies	2-17	RT	Free hydroxyl and carboxylic acid groups	Antimicrofouling activity	Vijayan et al. 2014
Cystophora moniliformis	75				Prasad et al. 2012
Padina gymnospora	25-40			Antibacterial activity	Shiny et al. 2013
Sargassum muticum	43-79	RT	Sulfated and hydroxyl groups	Antibacterial and insecticidal property	Madhiyazhagan et al. 2015
Sargassum cinereum	45-75	RT		Antibacterial	Mohandass et al. 2013
Sargassum swartzii	35	60	Alcohol, carboxylic and amide 1 group	Anticancer	Dass et al. 2014

Table 5: Synthesis of other metal nanoparticles using seaweed

Seaweed Name	Metal	Size	Temperature	Active agent	Application	Reference
Stoemchospermum marginatum	Gold	18.7–93.7 nm	RT	Terpenoids, polyphenols and phenolic compounds	Antibacterial activity	Arockiya Aarthi Rajathi et al. 2012

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Padina gymnospora	Platinum	35 nm	50	Sulfated polysaccharide	Hemolytic assay	Shiny et al. 2014
Padina gymnospora	Gold	53nm	75	Fucoxanthin or flavanoids	Anticancer activity	Singh et al. 2013
Sargassum muticum	Magnetic Iron-oxide	18 nm	25	Sulfated polysaccharides, hydroxyl, and aldehyde groups		Mahadevi et al. 2013
Sargassum muticum	Zinc oxide	30-57	450	Sulfated polysaccharides		Azizi et al. 2014
Sargassum muticum	Gold	5.42	45	Fucoxanthin or polysaccharides, polyphenol, and other biomolecules	Anticancer activity	Namvar et al. 2014
Sargassum myriocystum	Zinc oxide	76–186 nm	80 C	Alginic acid, ascorbic acid, protein, carbohydrates, flavanoids, tannins, mannitol, and lipids	Antibacterial activity	Stalin dhas et al. 2012
Turbinaria Connoides	gold	12-57			Catalyst	Ramakrishna et al. 2016
Sargassum tennerimum	gold	5-45			Catalyst	Ramakrishna et al. 2016

12. **Synthesis of Nanoparticles using Marine Microorganisms:** Marine microorganisms include microalgae, bacteria and fungi are the microbes living in marine environment and are either prokaryotic or eukaryotic organisms. They can synthesis metallic nanoparticles using intracellular or extracellular pathway. The microbially synthesised nanoparticles can be used as therapeutic agents.

13. **Intracellular Synthesis:** The mechanism of synthesis of nanoparticles intracellularly can't be explained exactly because of different biomolecules involved in synthesis in different microbes. Hypothetically, the positive charged metal ions are trapped on the surface of cell wall or cytoplasm which contains negative charged ions of enzymes or

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proteins. The trapped metal ions will get reduced forming nanoparticles. The synthesis of nanoparticles occurs on cell wall (eg: synthesis of silver nanoparticles by fungus, Verticillium sp.), on cytoplasmic membrane (eg: synthesis of gold nanoparticles in Rhodococcus Spp) or both (eg: gold nanoparticles synthesis by algae, Tetraselmis kochinensis).

The protocol for synthesis of nanoparticles using microorganisms intracellularly include the following steps,

- Culturing the microorganisms in growth media
- Washing the microorganisms
- Centrifugation to remove the components of growth media
- Inoculation of the collected mass with metal precursor
- Incubation under specific atmospheric conditions
- Cell lysis and centrifugation for collection of nanoparticles

14. **Extracellular Synthesis:** The synthesis of nanoparticles extracellularly depend on the microbial proteins or secreted enzymes. In fungus and bacteria the enzyme α-NADPH dependent nitrate reductase plays a key role in formation of nanoparticles. The cofactor NADH is secreted to the enzyme and the electron is transferred from NADH to NADH dependent reductase. Thus the enzyme acts as an electron carrier which is finally transferred to metal ions which get reduced to form nanoparticles. The fungus Fusarium oxysporum and the bacteria Rhodopseudomonas capsulata can synthesis gold nanoparticles in this manner. The microalgae, Chlorella vulgaris reduces silver ions extracellularly, but the mechanism is unknown.The synthesis of nanoparticles using microorganisms extracellularly can be done using two methods,

- **Using cell extract**
  - Culturing the microbes in growth media
  - Washing and centrifugation of cell biomass
  - Collection of extract using either lysis of biomass or incubation of biomass in distilled water or using dry biomass extraction
  - Centrifuge to collect the cell free extract and inoculate with metal precursor
  - Incubation for nanoparticle formation
  - Centrifugation for collection of nanoparticles

- **Using cells**
  - Culturing the microorganisms in growth media
  - Washing the microorganisms
  - Centrifugation to remove the components of growth media
  - Inoculation of the collected mass with metal precursor
  - Incubation under specific atmospheric conditions
  - Cell lysis and centrifugation for collection of nanoparticles

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The synthesis of nanoparticles using marine microbes and the potential applications reported are given in table 6.

15. **Future prospects:** There are more reducing sources available from aquatic resources that are to be investigated. Further studies on utilization of underutilized or unutilized resources for development of nano based structures will help to reduce the environmental pollution. Along with the studies on effect of different sources as reducing agents, the mechanism of formation of nanoparticles using these technologies and their characteristics are not well-known. In the case of use of extracts, the composition of extracts and the components responsible for the reduction of meal ions are to be investigated further. The applications of the nanoparticles formed is to be studied because the characteristics of nano particles synthesized has immense effect on the performance of nanoparticles.

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Table 6: Synthesis of metal nanoparticles using different microorganisms of marine origin (Patil and Kim, 2018)

Marine microorganism	Species	Type of nanoparticle	Location of nanoparticles	Reaction condition	Size (nm)	Applications
Actinobacteria	Rhodococcus sp.	gold	intracellular	Active biomass	5-15	-
	Streptomyces sp. Al-Dhabi-87	Silver	extracellular	supernantent	10-17	antimicrobial
Bacteria	Desulforibrio caledoiensis	Zinc sulfide	extracellular	supernatent	30	photocatalysis
	Enterococcus sp	Cadmium sulfide	extracellular	supernatent	50-180	antimicrobial
	Escherichia coli VM1	silver	extracellular	supernatent	10-15	anticancer
	Idiomarina sp.PR 58-8	silver	intracellular	Active biomass	26	
	Marinobactor pelagius	gold	extracellular	Active biomass	2-6	
	Ochrobactrum anthropi	silver	intracellular	Active biomass	38-85	antibacterial
	Saccharophagus degradans ATCC 43961	Manganese dioxide	extracellular	supernatent	34	
	Vibrio alginolyticus	silver	intracellular	Active biomass	50-100	
Cyanobacteria	Plectonema boryanum UTEX 485	silver	intracellular	Active biomass	<10	
	Spirulina platensis	gold	extracellular	Dry biomass	6-10	
Fungi	Aspergillus sydowii	gold	Intracellular, extracellular	Active biomass	8.7-15.6	
	Aspergillus terreus	silver	extracellular	supernatent	1-20	
	Aspergillus terreus	gold	intracellular	Active biomass		cytotoxicity

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IV. CONCLUSION

The aquatic environment acts as a store house for a wide variety of with different applications. The compounds extracted from fish waste as source of nanoparticle synthesis will help in reducing pollution and improved environmental management. Also the cost of production of nanoparticles can be reduced. The efficient synthesis of nanoparticles using seaweeds creates improved use of seaweed stocks for industrial applications. The techniques for development of nanoparticles using the marine organisms like sponges, seaweeds, microorganisms etc. lead to further investigations for up gradation of aquaculture of the specific species.

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