Synthesis, characterization and applications of chitosan based metallic nanoparticles: A review

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How to Cite
Verma, D.K. et al. (2021). Synthesis, characterization and applications of chitosan based metallic nanoparticles: A review. Journal of Applied and Natural Science, 13(2), 544 - 551. https://doi.org/10.31018/jans.v13i2.2635

Abstract
Chitosan as a natural biopolymer has been produced to be the important host for the preparation of metallic nanoparticles (MNPs) because of its excellent characteristics like: good stabilizing and capping ability, biocompatibility, biodegradability, eco-friendly and non-toxicity properties. Chitosan can play an important role for synthesis of metallic nanoparticles, as chitosan is a cationic polymer. It attracts metal ions and reduces them and also Capps and stabilizes. So basically, chitosan can be responsible for the controlled synthesis of metallic nanoparticles. Chitosan has a very good chelating property. This property is due to its –NH$_2$ and –OH functional groups. Size and shape of metallic nanoparticles are much affected by chitosan concentration, molecular weight, time of reaction, degree of acetylation of chitosan, pH of the medium, method of synthesis and type of derivative of chitosan etc. Metallic nanoparticles’ properties and applications are much associated with their size and shape. Optimization of the metallic nanoparticle size and shape has been the subject of curiosity for nanotechnology scientists. Chitosan can solve this problem by applying the optimization conditions. But a very little work is reported about: how chitosan can affect the size and shape of metallic nanoparticles and how can it reduce metal salts to prepare metallic nanoparticles, stabilized in chitosan metrics. This is very first report as a review article highlighting the effect of chitosan on synthesis of metallic nanoparticles and optimization conditions. This review will also be beneficial for scientists working on food sensing application of nanoparticles. Various synthesis methods and applications of chitosan based metallic nanoparticles have also been reported in details.

Keywords: Chitosan, Cationic polymer, Metallic nanoparticles (MNPs), Nanotechnology, Synthesis

INTRODUCTION

Size and shape of the nanoparticles are one of the very important properties of metallic nanoparticles. Applications of metallic nanoparticles are decided on the basis of size, shape and the method of synthesis of nanoparticles. So to optimize the conditions for controlled synthesis of any metallic nanoparticle has always been the topic of curiosity between nanotechnology scientists. All metallic nanoparticles (MNPs) are especially attractive owing to their unique properties and applications (Huang et al., 2008). It has been accepted that the size, morphology, dispensability and physicochemical properties of MNPs are strongly associated with their applications, which are affected by the synthesized approach (Sengupta et al., 2004). Chitosan is a biopolymer synthesized from marine crustacean shells. However, commercially available chitosan is produced from deacetylation of chitin, which is a natural biopolymer found in crab, coral shrimp, mushroom lobster, jellyfish, butterfly, ladybug and fungi (Potara et al., 2009, Lupusoru et al., 2017). Chitosan is a cationic polymer...
composed of randomly distributed N-acetyl glucosamine and D-glucosamine, containing in composition, sequence, and molecular chain length (Fig. 1).

The degree of acetylation of chitosan is characterized by the molar fraction of N-acetylated units (DA) or as a percentage of acetylation (DA %). Different methods for conversion of chitin to chitosan like Chemical Extraction (Chemical Deproteinization, Chemical Demineralization), Biological Extraction of Chitin, Enzymatic Deproteinization, Fermentation Chemical Deacetylation, (Younes et al., 2015) have been used. Chitosan enables the electrostatic interaction with negatively charged biopolymer and the interaction with cell membranes. Chitosan has been developed in diverse forms like films, foams, (Lin et al., 2009, Marpu and Benton 2018, Kumar et al., 2017) fibers, (Wang et al., 2017a) hydrogel and nanoparticles (Thirumavalavan et al., 2013). Chitosan forms inter- and intra-molecular hydrogen bonding owing to amine and hydroxyl groups. Therefore, it has a rigid crystalline structure (Khedri et al., 2018). Incorporation of metallic nanoparticles in chitosan increases its application on huge scale. Some of the applications included are in catalysis, nonlinear optics, adsorptions, heavy metal ion sensing, environmental remediation, antimicrobial activity, catalytic activity, removal of dye, antioxidant, drug delivery, bio imaging, anti-cancer activity, Sensing and wound healing (Ahmad et al., 2011, Sannegowda et al., 2015, Liu et al., 2017, Misra et al., 2016, Akbari-Sharbat et al., 2015) (Fig. 2).

This review is focused on two major topics mainly. First is the synthesis, characterization, and applications of chitosan-based metallic nanoparticles/ nanocomposites and the second is the effect of chitosan on the size and shape of metallic nanoparticles.

Fig. 1. Chitin and Chitosan structure (Younes & Rinaudo, 2015).

Fig 2. Applications of chitosan based metallic nanoparticle.
PREPARATION AND CHARACTERIZATION

Preparation of chitosan based metallic nanoparticles/nanocomposites
Chitosan based metallic nanocomposites have been synthesized by various methods for different metal nanoparticles (Fe, Cu, Au, Ag, Zn, V, Ti, Cr, Ni, Co, etc.) (Table 1). Some of the important methods for biopolymer chitosan based metallic nanoparticle preparation includes; co-precipitations, green synthesis, in-situ precipitations, ex-situ precipitations and hydrothermal method etc. Chitosan (CS) is important for metallic nanoparticles synthesis because it works like a capping as well as a reducing agent. Chitosan as a cation, it also makes complex with anions to make nanocomposites and nanoparticles. CS-stabilized Au nanoparticles were synthesized in the absence of a reducing agent (Vo et al., 2014). Stabilization of Au and Ag nanoparticles within CS Adopting/demonstrating green synthesis method for making AuNPs and AgNPs has been studied (Sanpui et al., 2008). It has been found that size and stability of metallic nanoparticles also depend on chitosan molecular weight and concentration. Chitosan-coated AuNPs were synthesized and analyzed for the stability of the different size of AuNPs with respect to CS-molecular weight and concentration (Lupusoru et al., 2017). CS-stabilized AuNPs were prepared in the presence of Thiamine pyrophosphate (TPP) to see the effect of Chitosan (CS) concentration on the size and shape of AuNPs without any additional reducing agents. CS-capped AuNPs have been used in the sensing ability of heavy metal ions based on SPR changes. Au-CS nanocomposites were found suitable for using selective electrochemical sensors to determine antioxidants and determination of polyphenol index in wines (Sanpui, et al., 2008). Copper-Chitosan Nanoparticles were synthesized by green method and further studied its Antibacterial Activity (Manikandan & Sathiyabama et al., 2015). Preparations of Magnetic Chitosan nanoparticles support for Cellulase immobilization. The immobilized cellulase retained 50% of its initial activity after 10 cycles (Zang et al., 2014). Chitosan-titanium oxide fibers and zero-valent nanoparticles were prepared by hydrothermal method (Ali et al., 2018). Zinc encapsulated chitosan NPs were prepared to promote maize crop yield (Chaudhary et al., 2019). In-situ method has been found the most famous and easiest method for formation of chitosan based metallic nanocomposites. Nickel-chitosan nanocomposite coated cellulose filter paper was synthesized using the in-situ method (Kamal et al., 2016). Chromium-loaded Chitosan Nanocomposites were also synthesized by in-situ method (Chattopadhyay, et al., 2014). Different methods and metallic nanocomposites synthesized using chitosan as a biopolymer as capping and complexing agent are shown in Table 1.

Characterization of chitosan based metallic (CS-M) nanoparticles
Various techniques have been explored to analysis the

Table 1. Various methods used for the synthesis of chitosan based metallic nanoparticles/nanocomposites.

| S. No | Biopolymer | Metal | Method | Characterization | Reference |
|-------|------------|-------|--------|------------------|----------|
| 1     | Chitosan   | Au    | Complex formations | Potentiometry, electronic spectroscopy and X-ray absorption spectroscopy (XAS) | (Vo et al., 2014) |
| 2     | Chitosan   | Ag\Au | Green synthesis | SEM, UV | (Sanpui et al., 2008) |
| 3     | Chitosan   | Cu    | Green synthesis | SEM, XRD, TEM, FTIR, VSM | (Manikandan and Sathiyabama 2015) |
| 4     | Chitosan   | Fe    | Co-precipitations | FTIR, Vibrating-sample magnetometer (VSM), DLS, SEM, TEM, XRD, UV | (Dung et al., 2009) |
| 5     | Chitosan   | Pd    | Hydrothermal method | FTIR, SEM, XRD, VSM | (Baran 2020) |
| 6     | Chitosan   | TiO₂  | in-situ method | FTIR, XRD, FESEM | (Ali et al., 2018) |
| 7     | Chitosan   | Zn    | in-situ method | FTIR, XRD, DLS, XRD | (Choudhary et al., 2019) |
| 8     | Chitosan   | Ni    | in-situ method | FESEM, XRD, FTIR, UV | (Kamal et al., 2016) |
| 9     | Chitosan   | Co    | - | XRD, FTIR, SEM | (Chattopadhyay et al., 2014) |
| 10    | Chitosan   | Cr    | Suspensions method | SEM | (Tahir et al., 2019) |
| 11    | Chitosan   | V     | adsorption/desorption method | pH | (Guzman et al., 2002) |
| 12    | Chitosan   | Ag, Au, Pt, Cu | Chemical Reductions | TEM, FTIR, UV-Vis spectroscopy | (León et al., 2017) |
Effect of chitosan on synthesis and optimization of metallic nanoparticles
Metallic nanoparticles properties are much affected by their size and shape. These properties decide the application of metallic nanoparticles in various fields. Chitosan plays an important role in deciding the size and shape of metallic nanoparticles. The positive charge on chitosan makes it suitable to be used as a core shell for nanoparticles and covering metallic particles. Chitosan possesses –OH and -NH₂ groups. These groups are responsible for the chelating property of chitosan. A chemical bond is developed between chitosan and Ag⁺ ion. This has been confirmed experimentally by FTIR and NMR spectroscopy (Modrzejewska et al., 2009). So chitosan has been used as a reducing and capping agent in synthesis of metallic nanoparticles. Gold, silver, copper, iron and other transition metal series element nanoparticles have been synthesised by using chitosan as a reducing and capping agent (Pestova et al., 2005). Usually, metal salt is used as precursor, chitosan as reducing and capping agent. Chitosan is used in a range of (1-2) % solution in acidic or basic medium. The amount of the chitosan affect the size and shape of metallic nanoparticles a lot.

The size and shape of the metallic nanoparticles reduced and stabilized by chitosan are affected by many factors: 1. Degree of chitosan deacetylation, 2. molecular weight, 3. reaction time, 4. concentration, 5. Temperature of the reaction and pH of the solution (Abrica-González et al., 2019). The time of capping of Au nanoparticles with chitosan decides the colour of the solution corresponding to the size of Au nanoparticles. The intensity of the Au nanoparticles decides the shape of nanoparticles which is affected by the capping time duration. Chitosan stabilized Au nanoparticles are used in food sensing application (Javed et al., 2020). Chitosan and its derivative have different effect on the size and shape of nanoparticles. Generally, on increasing the chitosan concentration metallic nanoparticle size increases but in case of carboxymethyl guar gum on increasing the carboxymethyl chitosan concentration the size of Fe₃O₄ nanoparticles decreases. This may be due to more amount of carboxymethylchitosan stabilizes more metallic nanoparticles. Sonication time was studied from 30 to 60 minutes for coating of Fe₃O₄ nanoparticles by chitosan. It was found that on increasing time of sonication size of nanoparticles decreases, but increasing further time can remove the capping of chitosan. Effect of temperature was also studied in this case. It was found that on increasing temperature from (25 to 50 and 80) °C, the size of Fe₃O₄ nanoparticles decreases (Zeinali et al., 2016). Ag nanoparticles have been synthesized by chitosan using different molecular weight. Ag Nanoparticle size was found decreased with increasing in stirrer speed and decrease in temperature from (25 to 4) °C. The UV spectrum of Ag- chitosan nanoparticles shows that a broad peak spectrum is obtained on increasing the molecular weight. This explains that on increasing the higher molecular weight of chitosan a range of size of Ag nanoparticles is obtained. But on decreasing the molecular weight of chitosan small Ag nanoparticles of the small range of nanoparticles are obtained. The best antimicrobial activities of Ag nanoparticles are obtained smallest size corresponds to smallest molecular weight of chitosan (Honary et al., 2011).

APPLICATIONS OF METALLIC CHITOSAN NANO-PARTICLE

Metal removal by chitosan metallic nanoparticles
Chitosan metallic nanoparticles are being used in metal removal from waste water treatment by the adsorptions batch method. In this process first, chitosan based metallic nanoparticle is synthesized by in-situ or ex-situ methods. Then such made nanoparticles solutions are used to make films by solution casting method. These films are dried in Teflon or plastic plate or boxes. Then these films are cut in fixed dimensions and then dipped in different concentration stock solution of metals. To check the effect of metal and chitosan concentration on adsorption, optimum conditions are applied. Cr (IV) was removed by adsorption technique using chitosan based magnetic nanoparticles. Optimum conditions were applied to see the effect of pH and time of adsorption (Zimmermann et al., 2010). In most cases, it has been found that an increase in the ratio of chitosan and biopolymer concentration increases the adsorption of heavy metals (Al-Sayed et al., 2019). Fe₃O₄-C18-chitosan-DETA (FCCD) particles were used in the removal of Dy³⁺, Nd³⁺, and Er³⁺ at 25°C and 7pH of the medium. These nanoparticles were synthesized by the surface deposition-stepwise grafting method successfully (Liu, E et al., 2017). Kinetics data was used to calculate the order of the adsorption reaction, it was found a pseudo-second-order reaction, and the Langmuir equation fitted well to the adsorption isotherms. A comparative study was done to see the effect of chitosan on metallic nanoparticle for the adsorption of Pb (II) and Cd (II). Very fine results were obtained. Pb (II) and Cd (II) were removed maximum up to (79.24 and 36.42) mg g⁻¹ respectively by Fe₃O₄/CS NPs. The magnetic chitosan nanoparticles were prepared by a simple one-
very attractive. Adsorption efficiency was found up to ions from its stock solution in water. Results were found assisted hydrothermal method for the removal of Cu (II) were synthesized by a one-step microwave- assisted hydrothermal method for the removal of Cu²⁺ ions from its stock solution in water. Results were found very attractive. Adsorption efficiency was found up to 100% and 96.7% after 500 min at 6.5 pH with starting Cu²⁺ ions concentration (50 mg/L and 100 mg/L). Langmuir isotherm models were found fit for adsorption data. Langmuir adsorption equilibrium constant, a maximum adsorption capacity, rate constants were also determined by using adsorption data. It was found in good agreement with pseudo second order model (Meng et al., 2015) (Table 2).

### Antibacterial activity
Chitosan stabilized metallic nanoparticles have huge attention of scientist as chitosan itself shows antimicrobial activity alone and when metal nanoparticles are attached with it. It shows an excellent antimicrobial property. Scientists have suggested many mechanisms for antimicrobial activity of chitosan. One of the mecha-

### Table 2. Application of chitosan metallic nanoparticles in metal removing.

| Chitosan/Metal | Removal metal | Reference |
|---------------|--------------|-----------|
| Chitosan–Fe–S | Cu(II)       | (Wen et al., 2015) |
| CS–Fe(III) HF | As(V)        | (Seyed et al., 2014) |
| CS–Fe₂O₃     | Pb(II)       | (He et al., 2019) |
| CS–Fe        | Cr(VI)       | (Zimmermann et al., 2010) |
| Magnetic-chitosan | Cr(VI)     | (Thinh et al., 2013) |
| MCS–Fe       | Cr(VI)       | (Yu et al., 2013) |
| CTS/MMT–Fe₂O₃ | Cr(VI)       | (Pina et al., 2015) |
| Chitosan /Fe  | Adsorption of rare-earth metal ions | (Liu et al., 2017) |
| Fe₂O₃/CS NPs | Removal of heavy metal ions | (Fan et al., 2017) |
| Fe₂O₃/chitosan | Adsorption of thorium (IV) ion | (Broujeni and Rouhi, 2018) |
| Fe₂O₃/CS | Mercury(II) Removal | (Kyzas & Deliyan 2003) |
| MnFe₂O₄/CS | Adsorption of Cu²⁺ | (Meng et al., 2015) |

### Table 3. Applications of chitosan based metallic nanoparticles as: Antimicrobial, antioxidant, anticancer etc.

| Biopolymer/metal | Bacteria                                                                 | Reference                                                                                      |
|------------------|--------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|
| Chitosan cellulose/ Ag/ ZnO | *Escherichia coli, Pseudomonas aeruginosa, Lactobacillus ferment, Enterococcus faecium, Staphylococcus aureus, Bacillus licheniformis, Bacillus subtilis, Bacillus cereus, Vibrio parahaemolyticus, Proteus vulgaris* | (Ghasemzadeh et al.,2016., Thaya et al.,2016) |
| Chitosan-gopoly (acrylamide)/ ZnS | *Escherichia coli* | (Gupta et al., 2015) |
| 6-O-chitosan sulfate/ Au | *Escherichia coli* | (Ehmann et al.,2015) |
| Chitosan / Cu | Antibacterial activity | (Manikandan & Sathiabrama, 2015) |
| Chitosan / Fe2O3 | Antioxidant | (Ma et al., 2008) |
| Chitosan/ Ag | Antibacterial activity/ Antifungal fungal activity | (Badawy et al., 2019) |
| Chitosan/ ZnO | Antibacterial activity/ Antifungal fungal activity | (AbdElhady et al., 2012) |
| Chitosan/ Ag | Anticancer activity | (Tran et al.,2010) (Arjuman et al., 2016) |
| Chitosan/ Au | DNA Carrier | (Abrica-González et al., 2019) |
| Chitosan-nylon-6/Ag | blended membranes Packaging material *Escherichia coli, Staphylococcus aureus* | (Ma et al., 2008) |
| CS/Ag | *Escherichia coli, Acinetobacter baumannii, Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa, and Streptococcus pneumoniae* | (Meng et al., 2015) |

step in situ co-precipitation method (Liu, et al., 2017). Magnetic particles stabilized by chitosan are being used extensively. A super paramagnetic nanoparticles (36emu/g) of (8 to 14) nm size were used in removal of Cu (II) ions up to 35.5 mg/g using the Langmuir isotherm model (Meng et al., 2015). Two chitosan derivatives were made, one by crosslinking through glutaraldehyde and second by functionalizing with crosslinking and functionalizing with magnetic nanoparticles. Second derivative was found better adsorption capacity than without magnetic nanoparticles (Kyzas and Deliyanni, 2013). Chitosan-modified Mn ferrite nanoparticles were synthesized by a one-step microwave-assisted hydrothermal method for the removal of Cu²⁺ ions from its stock solution in water. Results were found very attractive. Adsorption efficiency was found up to 100% and 96.7% after 500 min at 6.5 pH with starting Cu²⁺ ions concentration (50 mg/L and 100 mg/L). Langmuir isotherm models were found fit for adsorption data. Langmuir adsorption equilibrium constant, a maximum adsorption capacity, rate constants were also determined by using adsorption data. It was found in good agreement with pseudo second order model (Meng et al., 2015) (Table 2).
nisms says that chitosan is very good chelating agent and when it comes in contact of the microbial cell; it binds to the metals of the cell. So cell function is stopped and bacteria dyes. The chitosan-CMC Ag Nanocomposite demonstrates good antimicrobial activity against Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa (Ghasemzadeh, et al., 2016, Thaya et al., 2016). Chitosan stabilized Ag nanoparticles have been found antimicrobial again, both gram-positive (Bacillus sp. and Staphylococcus) and gram-negative (Pseudomonas) bacterial (Meng et al., 2015, Ma, et al., 2008). A chitosan stabilized Cu nanoparticles have been prepared to see the antimicrobial activity against gram-positive and gram-negative bacteria. Results show that chitosan stabilized Cu nanoparticles were more effective in gram-negative bacteria than gram-positive. This may be due to differences in cell wall composition (Manikandan et al., 2015) (Table 3).

Conclusion

Chitosan stabilized metallic nanoparticles appear to be a suitable polymeric complex for many applications viz. antimicrobial activity, adsorptions (purification), biosensing, environmental remediation, catalytic activity, antioxidant activity, food packaging preservation, drug delivery, bio imaging anti-cancer activity and wound healing property. Chitosan is not only responsible for reducing, stabilizing and capping nanoparticles, but it is also responsible for the size and shape of nanoparticles. Less work is done so far about the effect of chitosan on the shape and size of nanoparticles. Chitosan has been found responsible for deciding the size and shape of metallic nanoparticles. Even the variation in molecular weight can affect the size of metallic nanoparticles. It is important to notice that chitosan is responsible for size and shape deciding, reaction time, type of method, sonication time, temperature of reaction, etc. This review will be beneficial for those scientists who are working on optimization condition of metallic nanoparticles stabilized by chitosan and their applications.

Conflict of interest

The authors declare that they have no conflict of interest.

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