

Preparation and Characterization of Gold Nanoparticles in Chitosan

Suspension by one-pot Chemical Reduction Method

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Abstract. Gold nanoparticles were synthesized in chitosan suspension by one-pot chemical reduction method without any chemical reducing reagent. In aqueous acidic medium, chitosan exhibits polycationic nature due to protonation of amine groups of each glucosamine repeating units. The adsorption of AuCl_4^- ions occurs on chitosan through electrostatic attraction and further reduction of Au^{3+} to Au^0 proceeds through a three electron transfer mechanism by oxidation of protonated amine groups of chitosan chain. The change of color from dirty yellow to intense red indicates the formation of gold nanoparticles in chitosan suspension which acts as both reducing and stabilizing agent. Characterization techniques such as UV-VIS, FTIR, Zeta potential measurement, FESEM and TEM were carried out in order to examine the reduction and stabilization phenomena rendered by chitosan.

Introduction

Chitin and Chitosan are described as a family of linear polysaccharides consisting of varying amounts of $\beta(1 - 4)$ linked residues of N-acetyl-2-amino-2-deoxy-D-glucose and 2-amino-2-deoxy-D-glucose residues which is also called as N-acetyl glucosamine and glucosamine residues respectively. Chitin is insoluble in most organic solvents but chitosan is readily soluble in dilute acidic solutions below pH 6.0. The solubilisation of chitosan occurs by protonation of the amine group on the C-2 position of the D-glucosamine repeating unit thereby the chitosan polysaccharide exhibits polycationic nature possessing metal chelating property which can able to bind metal ions in aqueous solution [1].

In recent years, research work was focused on simultaneous formation and conjugation of metal nanoparticles with chitosan under thermal treatment. Metal chelating chitosan with functional groups such as amine and hydroxyl groups can able to reduce gold precursors in aqueous suspension. Chitosan exhibits polyelectrolyte behaviour due to protonated amine groups which can reduce gold precursors and also stabilize the formed gold nanoparticles [2]. Chitosan stabilized gold nanoparticles also possess the same functional groups on the gold nanoparticle surface which can be further functionalized with biomolecules for various biological applications such as drug delivery, biosensor and also in other applications such as catalysis, antioxidants, film packaging, hydrogels, adhesives, water treatment and membranes [3].

Silver/chitosan nanocomposites were prepared by using basic chitosan suspension as a reductant in the absence of other chemicals. In Murugadoss and Chattopadhyay methodology, stable chitosan-Ag nanocomposites were prepared in the basic chitosan suspension at 95°C under reflux condition [4]. In Bhumkar *et al* [5] methodology, gold nanoparticles prepared with higher concentration (> 0.1% w/v) at pH below 5 were stable showing no signs of aggregation. Gold nanoparticles were prepared with different concentration of chitosan suspension, where it acts as both reducing as well as stabilizing agent. Zeta potential value of gold nanoparticles stabilized by 90% deacetylation

degree (DD) and 45 kDa at 0.2% w/v concentration is about +62.7mV which possess excellent stability.

In our work, efforts were made to prepare gold nanoparticles through one pot chemical reduction method in chitosan suspension having medium molecular weight with 90% DD. The reaction was carried out under reflux condition at 100 °C and at the pH range of 5- 6.

Experimental

Materials. Gold precursor stock solution was prepared by dissolving 10 mg of Hydroaurochloric acid (M.W. 393.83) in 100 ml Millipore water. The stock solution of medium molecular weight chitosan with 95% degree of deacetylation was obtained by dissolving 0.2 g of chitosan flakes in 50 ml diluted 1% acetic acid solution. The chitosan solution was allowed to stir overnight, ultra sonicated for certain minutes using 14 mm diameter probe dipped in the suspension with overall operating condition at 24 kz, 400W. After complete dissolution of chitosan, the suspension was filtered through whatmaan filter paper having pore size of 2.5 μm to obtain clear homogenous solution.

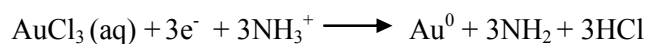
One Pot preparation of Gold Nanoparticles in Chitosan Suspension. In this wet chemical reduction method, gold nanoparticles were prepared through reduction of gold precursors in chitosan suspension at 100 °C with pH in the range of 5-6. In reaction vessel, 25 ml of 0.1 mg/ml gold precursor solution was added slowly in to 25 ml of 0.2 % w/v chitosan solution under rapid stirring and refluxed for certain duration. Total volume of the final suspension was then centrifuged and washed with Millipore water for several times and obtained residue was suspended in Millipore water for analysis purpose.

Characterization Studies. UV-Visible absorption spectrum was taken using Perkin-Elmer Lambda-25 spectrometer in the wavelength range of 200-700 nm. FT-IR spectrum was recorded on Perkin-Elmer RX-1 model FTIR Spectrophotometer in the range of 400-4000 cm^{-1} . Particle size distribution

was analyzed through DLS technique using Nanotracs instrument. Zeta potential measurements were recorded on Zetasizer Nano S, Malvern in the range of -100 mV to +100 mV. FESEM analysis was made using Ultra 55, Carl Zeiss instrument. TEM analysis was performed using JEOL Model 1200EX instrument operated at an accelerating voltage of 120 kV.

Results and Discussion

Reduction and Stabilization of Gold Nanoparticles in Chitosan Suspension. During reaction process, there was a color change from dirty yellow to colorless and finally the incurrence of ruby red color indicates the formation of gold nanoparticles. Since chitosan was dissolved in aqueous acidic media, the amine groups of the D-glucosamine repeating units get protonated [1, 2]. When diluted Hydrochloric acid was added in to chitosan solution, negatively charged gold (III) ions were adsorbed on various chains of chitosan due to electrostatic attraction with positively charged protonated amino groups [3,5]. Generally, the reduction of $\text{Au}^{3+}(\text{aq})$ to Au^0 follows three electron transfer mechanism [6]. But adsorbed gold (III) ions on various chitosan chains get reduced to neutral gold atoms through oxidation of three protonated amine groups without influence of external chemical reagent and further agglomerates to form gold nanoparticles. Thus, the overall reaction for the reduction mechanism can be expressed as follows:



UV-Visible characterization can confirm the gold nanoparticles formation in chitosan suspension. In Fig. 3a, curve 1 depicts the absorption spectrum of the Au Nps - chitosan suspension shows a strong and broad absorption peak at 521nm which is the peculiar characteristics of surface plasmon resonance of gold nanoparticles. The curve 1 depicts the absorption spectrum for blank chitosan shows sharp absorption peak at 225 nm. The absence of chitosan absorption peak in curve 2 clearly

states that all the amino groups of chitosan are actively involved in the reduction process leaving no traces of blank chitosan in the Au Nps- chitosan suspension.

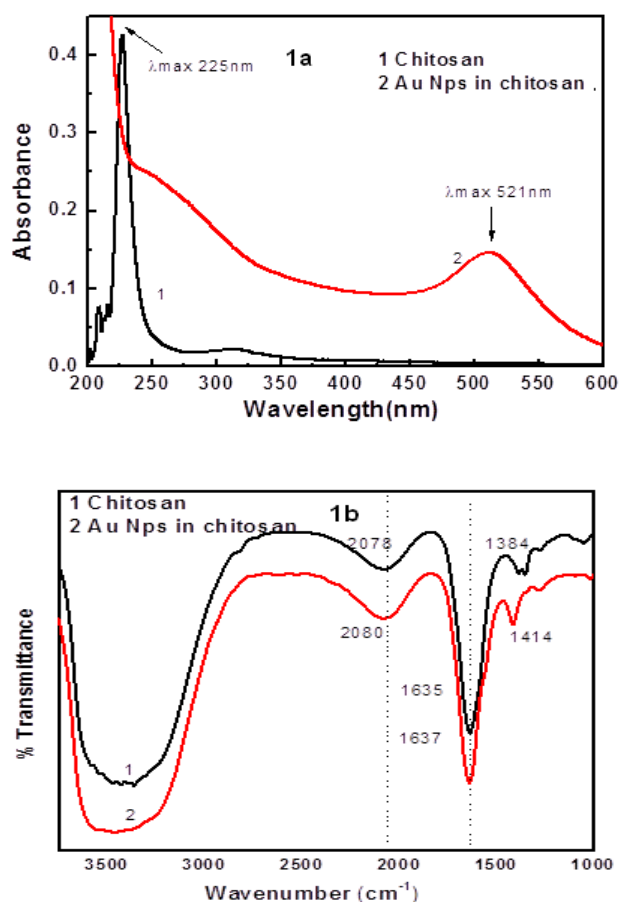


Fig. 1. Comparison of blank chitosan with Au Nps- chitosan suspension by UV-Visible Spectra (1a) and FTIR Spectra (1b).

After reduction, the formed gold nanoparticles strongly adsorb to chitosan due to affinity binding of oxidized amine groups with gold surface. FT-IR spectra can reveal the specific interaction of amine group with gold nanoparticles through analysis of spectral shift [7]. In Fig 3b, curve 1 and 2 depicts the fingerprint pattern of FTIR spectra for blank chitosan and Au Nps- chitosan. In both curves there exists an overlapping band from $3300\text{--}3500\text{ cm}^{-1}$ which attributes to the stretching vibration of hydroxyl, amino and amide groups with slight widening for Au Nps-chitosan when compared to

blank chitosan. For blank chitosan, a peak at 1635 cm^{-1} ascribed to bending vibration of -NH_2 group, shifted towards 1637 cm^{-1} for Au Nps- chitosan which clearly indicates that the gold nanoparticles are completely bounded with chitosan through affinity interaction with amino group leaving no traces of blank gold nanoparticles in the suspension.

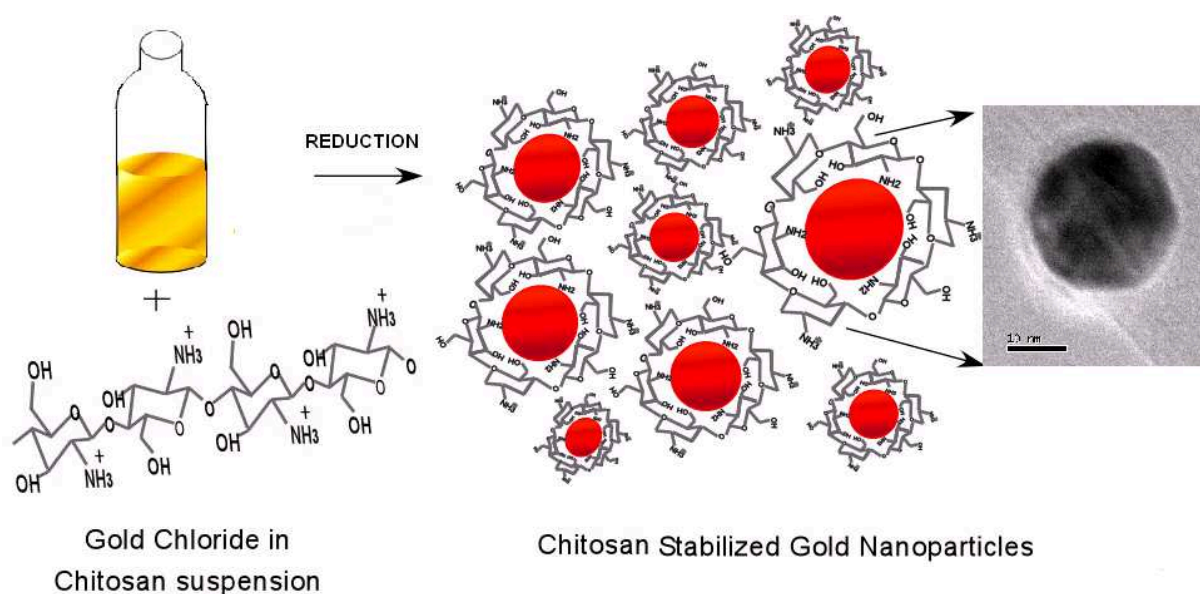
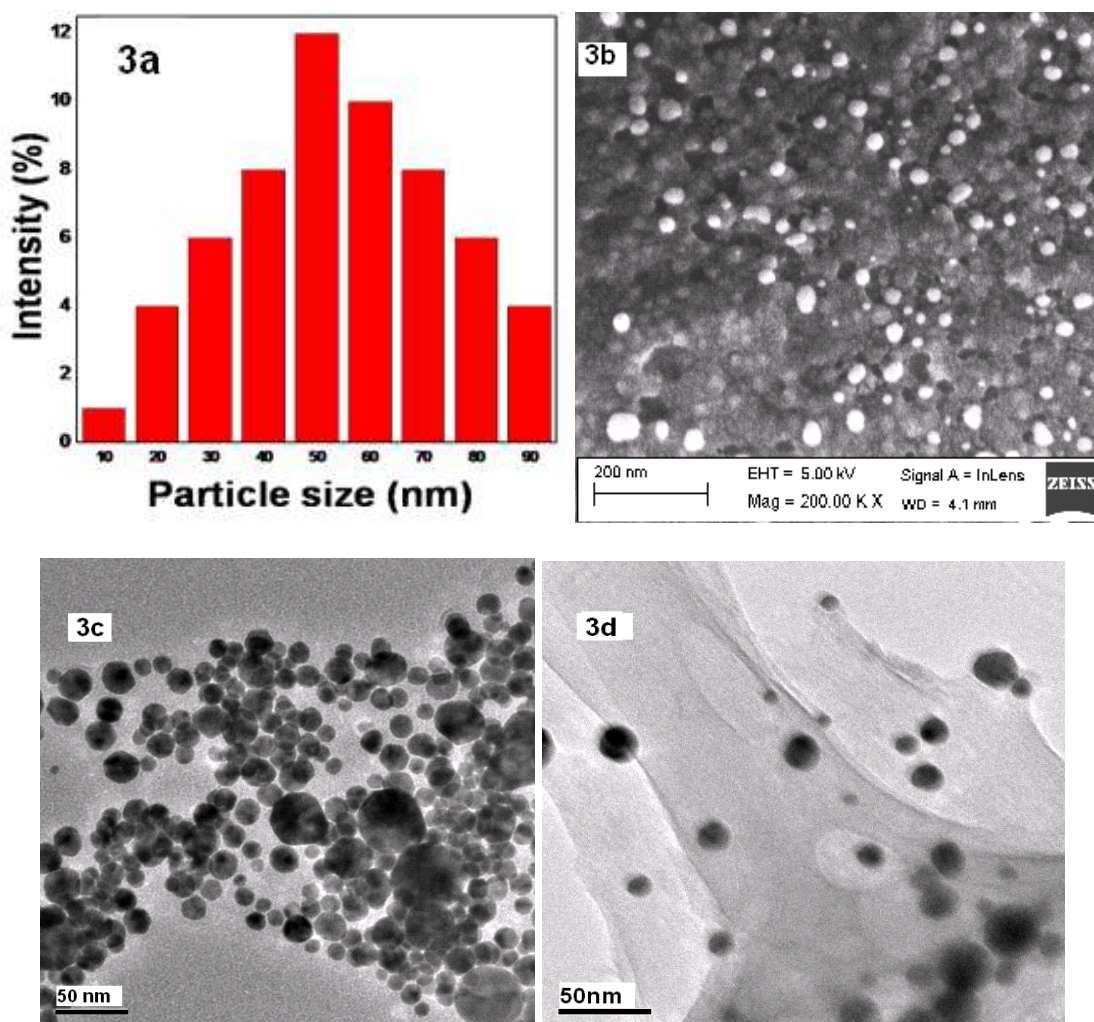


Fig. 2. Scheme for One-Pot preparation of gold nanoparticles in chitosan suspension.

The formed gold nanoparticles in chitosan suspension possess excellent stability and the particle aggregation is less likely to occur even after several months. The zeta potential of suspended gold nanoparticles was measured to determine the surface charge & stability of the nanoparticles in aqueous dispersion [5]. In colloids, usually particle aggregation is less likely to occur when the zeta potential is more than $+30\text{ mV}$ or less than -30 mV . The average zeta potential was measured about $+33\text{ mV}$ clearly indicates that the stability arises due to electrostatic repulsion between positively charged amine groups of chitosan layer rendered on each gold nanoparticle surface. Thus the reduction and stabilization phenomenon occurs in the same suspension without any other external chemical reagents and the methodology is simple which can be termed as one pot chemical reduction method which is depicted in Fig. 2.

Morphological Studies of Chitosan Stabilized Gold Nanoparticles. In Fig. 3a, particle size distribution of the chitosan stabilized gold nanoparticles shown with respect to intensity variation having wide range of distribution from 10 to 90 nm. The Fig. 3b depicts the FESEM secondary electron imaging of film casted Au Nps- chitosan suspension on glass substrate, gold nanoparticles possess uniform size distribution in the range of 5- 50 nm. By closely examining the FESEM picture, gold nanoparticles on the top layer of chitosan appears bright than the gold nanoparticles lying on the bottom layer indicates good dispersion even though shrinkage occurs during film formation.



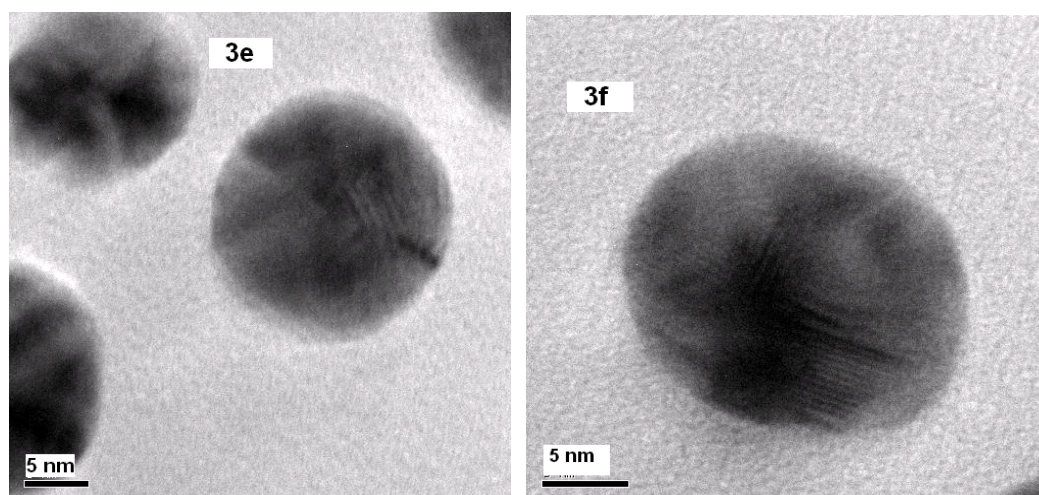


Fig. 3. Size and morphological studies of chitosan stabilized gold nanoparticles by DLS (3a), FESEM (3b) & TEM (3c, 3d, 3e, 3f).

In Fig. 3c, the TEM image of drop casted Au NPs- chitosan suspension is shown at scale bar of 50 nm indicates that the gold nanoparticles are well dispersed having spherical morphology. Even though particles are closed arranged in different fashion there is no signs of agglomeration. Thus the electrostatic repulsion between NH_3^+ groups on gold nanoparticles makes it more stable in aqueous medium and the formed AuNps in chitosan suspension possess positive charge which is already confirmed through zeta potential analysis.

The size and morphology of the chitosan stabilized gold nanoparticles purely depends upon varying chain length of polymeric chitosan. When the chain length of chitosan is longer in compare to other chains, more number of gold ions adsorbed along the entire length which leads to bigger size nanoparticles and also slight variation in spherical geometry [8]. In Fig. 3e, single gold nanoparticle is closely viewed at scale bar of 5 nm possess elongated spherical geometry with crystalline atomic lattice pattern in central region and amorphous in peripheral region.

Protonated amine groups of chitosan chain after reducing Au^{3+} ions gets conjugated to gold nanoparticle surface through Au-N bond formation which is already confirmed through FTIR studies. Chitosan is a biological macromolecule which is not easily observed in TEM studies due to

its lower atomic number. But in Fig. 3d, the polymeric chains of chitosan are observed along with adsorbed gold nanoparticles and it has been confirmed that the formed gold nanoparticles are completely bound to chitosan. In Fig. 3e, the closer view of gold nanoparticles at 5 nm scale bar saying that the chitosan layer is conjugated around each gold nanoparticle surface. The layer formation is due to simultaneous adsorption and reduction of gold precursors mediated by protonated amine groups which subsequently stabilizes the gold nanoparticle through Au-N bond formation [9]. After stabilization the unreacted amine groups of conjugated chitosan gets exposed to the surrounding environment possessing smooth morphology of robust gold core and mild chitosan shell layer.

Conclusion

Chitosan stabilized gold nanoparticles was prepared by simple one pot chemical reduction method. The formation of gold nanoparticles in chitosan suspension was confirmed through existence of broad UV-Visible absorption peak at 521 nm. The protonated amine groups play a major role in the reduction of gold precursors and subsequent stabilization of gold nanoparticles through Au-N bond formation was analyzed through peak shift of FTIR spectrum. The FESEM image indicates the formation of well dispersed gold nanoparticles in the size range of 5-50 nm. The TEM image shows the formation of spherical nanoparticles with varying sizes. The gold nanoparticles are seen to be conjugated with chitosan showing no signs of agglomeration. It is evident that the chitosan layer is formed during subsequent reduction and stabilization and positive charge is rendered on nanoparticle surface due to presence of unreacted form of protonated amine groups which is measured through zeta potential analysis.

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Reference

- [1] D. Wei, Y. Ye, X. Jia, C. Yuan, W. Qian, Chitosan as an active support for assembly of metal nanoparticles and application of the resultant bioconjugates in catalysis, *Carbohydr. Res.* 345 (2010) 74-81.
- [2] H. Huang, X. Yang, Synthesis of chitosan-stabilized gold nanoparticles in the absence/presence of tripolyphosphate, *Biomacromolecules* 5 (2004) 2340-2346.
- [3] A. Sugunana, C. Thanachayanont, J. Dutta, J.G. Hilborn, Heavy-metal ion sensors using chitosan-capped gold nanoparticles, *Sci. Technol. Adv. Mater.* 6 (2005) 335-340.
- [4] A. Murugadoss, A. Chattopadhyay, A 'green' chitosan–silver nanoparticle composite as a heterogeneous as well as micro-heterogeneous catalyst, *Nanotechnology* 19 (2008) 015603.
- [5] D.R. Bhumkar, H.M. Joshi, M. Sastry, V.B. Pokharkar, Chitosan Reduced Gold Nanoparticles as Novel Carriers for Transmucosal Delivery of Insulin, *Pharm. Res.* 24(8) (2007) 1415-1426.
- [6] M. Brust, M. Walker, D. Bethell, D.J. Schiffrin, R.J. Whyman, Synthesis of thiol-derivatised gold nanoparticles in a two-phase Liquid–Liquid system, *J. Chem. Soc., Chem. Commun* (1994) 801-802.
- [7] S. Govindan, E.A.K. Nivethaa, R. Saravanan, V. Narayanan, A. Stephen, Synthesis and characterization of chitosan–silver nanocomposite, *J. Appl Nanosci.* 2 (2012) 299-303.

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- [8] K. Tokarek, J.L. Hueso, P. Kustrowski, G. Stochel, A. Kyziol, Green synthesis of Chitosan-stabilized copper nanoparticles, *Eur. J. Inorg. Chem.* 28 (2013) 4940-4947.
- [9] G. Veerabhadram, M. Venkatesham, D. Ayodhya, A. Madhusudhan, N. Veera Babu, A novel green one-step synthesis of silver nanoparticles using chitosan: catalytic activity and antimicrobial studies, *Applied Nanoscience* 4 (2014) 113-119.