

## Preparation and Characterization of Chitosan-Silver Nanoparticle

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**ABSTRACT:** Silver nanoparticle was synthesized using chitosan as both a reducing and a stabilizing agent in the presence of ethanol. Silver nitrate was used as a source of silver. The chitosan-silver nanoparticle obtained was characterized using UV spectrophotometry, IR spectrophotometry and Thermogravimetric analysis to investigate their surface plasmon resonance, functional groups and thermal behaviour respectively. The IR spectra of chitosan-silver nanocomposite revealed the presence of hydroxyl, amine, and other functional groups appearing at various wave numbers. Surface plasmon resonance appearing at 430 nm was observed indicating the reduction of silver ion to a zerovalent state. Thermogravimetric analysis at a temperature of 700 °C indicated a change in thermal stability of the silver nanoparticle as compared to chitosan alone. These novel characteristics of silver- chitosan based nanoparticle could potentially be exploited for various applications in medicine, agriculture, environmental science and in industries.

**Keywords:** chitosan, surface plasmon resonance, nanoparticle, silver, reducing agent.

### Introduction

The field of nanotechnology has emerged as one of the most active areas of research in materials science over the last decades. Nanoparticles are discrete particles or clusters with dimension between 10-100 nm. They are specially produced or formed as a by product in industrial processes. Nanoparticle synthesis, study of their size and properties is of main importance in the advancement of nanoscience and technology. Nanocomposite refers to filled polymers containing dispersed nanoparticles. Nanometer-size metallic particles show unique and considerably changed physical, chemical and biological properties compared to their macro scaled counterparts, due to their high surface-to-volume ratio. Thus, these nanoparticles have been the subject of substantial research in recent years (1,2). Metallic nanoparticles exhibit size and shape-dependent properties that are of interest for applications ranging from catalysts and sensing to optics, antibacterial activity and data storage. (1, 3, 4, 5, 6, 7). In nanoparticle synthesis it is very important to control not only the particle size but also the particle shape and morphology as well. For instance, the antibacterial activity of different metal nanoparticles such as silver colloids is closely related to their size; that is, the smaller the silver nuclei, the higher the antibacterial activity. Moreover, the catalytic activity of these nanoparticles is also dependent on their size as well as their structure, shape, size distribution, and chemical/physical environment. Thus, control over the size and size distribution is an important task. Generally, specific control of shape, size, and size distribution is often achieved by varying the synthesis methods, reducing agents and stabilizers (8,9). Metal nanoparticles can be prepared by two routes, the first one is a physical approach that utilizes several methods such as evaporation/condensation and laser ablation, thermal decomposition, sonochemical analysis, microwave irradiation, photochemical (1,7, 10 a and b, 11,12, 13). The second one is a chemical approach in which the metal ions in solution is reduced in conditions favoring the subsequent formation of small metal clusters or aggregates (14, 15, 16). Chemical methods are further divided into those using non-deleterious solvent and naturally occurring reducing agent such as polysaccharides, plants extract or employs biological micro-organism such as bacteria and fungus as reductants and those working in reverse micellar systems where the aggregation process takes place in the aqueous core of reverse micelles and growing particles are surrounded by the surfactant molecules (1). Various methods have been introduced to synthesize silver nanocomposite, including chemical (4, 17), radiochemical reducing methods (18, 19) among others. Silver nanoparticles are usually obtained by reducing silver nitrate with sodium borohydride (NaBH<sub>4</sub>), EDTA or radiation (17). However recent developments have discouraged the use of borohydride due to its high cost. Furthermore, borohydride is relatively unstable, meaning that its production, transport and usage require careful control, and thereby further expense. Borohydride reduction method also produces large quantities of explosive hydrogen gas. Notwithstanding the inherent hazards of hydrogen gas, the additional safety protocols required for dealing with the gas on a large scale contribute to the high cost of presently available nanoscale zerovalent metals (20). Consequently, the cost of producing nanoscale zerovalent metals is relatively high, and in some cases, too high to be commercially viable. This calls for a newer, safer and less expensive method of producing nanoparticle. Recently, biosynthetic methods employing naturally occurring reducing agents such as polysaccharides, biological micro-organism such as bacteria and fungus or plants extract, i.e. green chemistry, have emerged as a simple and viable alternative to more complex chemical synthetic procedures to obtain silver nanoparticles AgNPs. (17,21,22). The abundant nature of polysaccharide biopolymers isolated from marine organisms and other animals such as snails are a new class of potentially inexpensive and environmentally friendly solid adsorbents that exhibit a high specificity toward metal ions. Chitosan, apolysaccharide (d-glucosamine) is a chemical derivative obtained by deacetylation of chitin (23). Chitin, after cellulose, is the second most abundant biopolymer on earth (24). Chitin is a white, hard inelastic, nitrogenous polysaccharide found in the exoskeleton of invertebrate (25). Chitin has a homogeneous chemical structure made up of 1-4 linked 2-acetamido-2-deoxy- D-glucopyranose. Chitosan is the form of chitin which has been deacetylated to at least 50% of the free amine form, which has a heterogeneous chemical structure made up of both 1-4 linked 2-acetamido-2-deoxy- -D-glucopyranose as well as 2-amino-2-deoxy- -D-glucopyranose (26). Chitosan is a non-toxic, biodegradable polymer of high molecular weight, and is very much similar to cellulose, a plant fibre. Chitosan possesses positive ionic charges, which give it the ability to chemically bind with negatively charged fats, lipids, cholesterol, metal ions, proteins, and macromolecules. In this respect, chitin and chitosan have attained increasing commercial interest as suitable resource materials due to their excellent properties including biocompatibility, biodegradability, adsorption, and ability to form films, and to chelate metal ions (27). In the preparation of silver nanoparticles (AgNPs), stabilizers are crucial agents that should be present for the control of nanoparticle formation as well as their dispersion stability. Polymers are often used as particle stabilizers due to the fact that they are effective in preventing agglomeration and precipitation of the particles. This is important in synthesizing nanoparticles with homogenous distributions. For example chitosan/gelatin, poly (vinylpyrrolidone) (PVP), poly (ethylene glycol) (PEG), poly

(methacrylic acid) (PMAA), polymethylmethacrylate (PMMA) (1,20). The dispersions of silver nanoparticles display intense colors due to the plasmon resonance absorption. The surface of a metal is like plasma, having free electrons in the conduction band and positively charged nuclei. Surface plasmon resonance is a collective excitation of the electrons in the conduction band; near the surface of the nanoparticles. Electrons are limited to specific vibrations modes by the particle's size and shape. Therefore, metallic nanoparticles have characteristic optical absorption spectrums in the UV-Vis region (28). The aim of this research is to produce silver nanoparticle using a natural based material, chitosan with minimal use of reagents.

### Materials and Methods

All reagents used were of analytical grade and used as received without further purification. AgNO<sub>3</sub> (99.98%) obtained from Merck (Darmstadt, Germany) was used as the silver precursor. Snail shell was obtained from a local market in Abeokuta, Ogun State. Ethanol (96% vol / vol) and glacial acetic acid (99%) were obtained from Sigma Aldrich (St. Louis, MO, USA). Deionised water was used for aqueous preparation.

#### Preparation of Chitosan

Fifty grams of finely grounded snail shell was measured into a flask to which 10M sodium hydroxide was added, placed on a magnetic stirrer with continuous stirring at 80°C for 6 h. The resulting residue was later washed with deionised water to bring the pH to neutral. The neutral residue was later dried at 100°C for 1 h. The dried neutral residue was demineralised using 1M hydrochloric acid. Effervescence occurred due to the liberation of carbon dioxide. The powder suspension was placed on a magnetic stirrer with continuous stirring at 30 °C for 3 h. This was washed repeatedly with deionised water to a neutral pH and dried at 90 °C for 1 h. The dry residue was decolourised by refluxing in 75 cm<sup>3</sup> acetone in a paraffin wax bath at 60 °C for 3 h on a magnetic stirrer. The near white powder was air dried in ambient condition. The powder was finally deacetylated with 12.5 M sodium hydroxide under constant stirring on a magnetic stirrer at 30°C for 4 h. The near white powder was washed repeatedly with deionised water to a neutral pH. Chitosan was dried at 90°C for 1h and kept inside a polythene container for storage Adewuyi (30).

#### Preparation of Chitosan-Silver

Chitosan-Silver was prepared using the method of Saifuddin (21). One milliliter of 20.0 mM AgNO<sub>3</sub> solution and 100 mL of chitosan solution (prepared by adding 3.0 g of chitosan in 100 mL of 2% (v/v) acetic acid) were mixed and stirred to a homogenous state with the addition of 25 cm<sup>3</sup> of ethanol. Heating was done at 60-80 °C with the system allowed to cool after 10 m to room temperature before reheating. The reaction took 1 h 30 m. The reduction of Ag<sup>+</sup> was monitored by taking aliquots after 30 m and 60 m and measuring the absorbance using UV Vis Spectrophotometer at 200-800 nm. Chitosan solution was used as the reference. The colour changes gradually from light to dark brown.

#### Characterization

The infrared spectra of both chitosan and chitosan-silver nanoparticle were recorded on Buck Demo spectrophotometer model 500(4000-200 cm<sup>-1</sup>). The concentration of chitosan-silver nanoparticle was determined using Thermo Elliot UVZ 164701 UV Vis Spectrophotometer. Thermal degradation processes were investigated using TG 209 *FI Libra* - Thermo-Microbalance (TGA-Thermogravimetric Analyzer) at a heating rate of 5 °C in argon atmosphere (20 ml min<sup>-1</sup>).

### Results and Discussion

#### UV Vis spectrum

The UV-Vis spectrum of chitosan-silver nanoparticle is represented in figure 1. A maximum peak occurring around 400-430 nm revealed surface plasmon resonance which is due to the excitation of silver atoms. Surface plasmon resonance is an in-phase oscillation of electrons lead by the coherent excitations of all free electrons in the conduction band. Surface plasmon resonance (SPR) is generated when the size of a metallic nano-crystal is smaller than the wavelength of incident radiation. The SPR is a dipolar excitation between the negatively charged electrons and the positive charge lattice in the particle. (31). This was evident in the change in colour of the solution to brown during the preparation of chitosan-silver nanoparticle.

#### Infra-Red Spectrum

The absorption bands at 3500 cm<sup>-1</sup> and 2946 cm<sup>-1</sup> as observed in both spectra (figures 2 and 3) are attributed to the stretching mode of hydroxyl and extension vibration of N-H which is a broad and strong band ranging .The peak observed at 1380cm<sup>-1</sup> is due to C-N stretching, peak at 2744 cm<sup>-1</sup> and 2946 cm<sup>-1</sup> can be attributed to asymmetric and symmetric -CH<sub>2</sub> groups. The peak at 1081 cm<sup>-1</sup> is the characteristic of a C-O stretching vibration. The absorption band at 900 cm<sup>-1</sup> corresponds to the characteristic absorption of -D-glucose unit. The appearance at 1467 cm<sup>-1</sup>, 1379 cm<sup>-1</sup>, 2888 cm<sup>-1</sup> and 2942 cm<sup>-1</sup> in the spectrum of chitosan -silver indicates the binding of silver to the functional groups of the chitosan (which was 1461 cm<sup>-1</sup> and 1381cm<sup>-1</sup> initially for amine, 3500 cm<sup>-1</sup> and 2946 cm<sup>-1</sup> for hydroxyl group). The shifting occurs to the coordination between silver and electron rich groups (nitrogen and oxygen).

#### Thermogravimetric Analysis

The thermal degradation of both chitosan and chitosan-silver are represented by figures 4 and 5 respectively. Figure 4 showed a first endothermic peak which was observed around 530°C with a minimum initial oxidation temperature peak at 440 °C. The weight loss associated with this was 0.03% which is due to evaporation. A second effect was observed at 700 °C with a loss of 43.56 %. Figure 5 showed a first endothermic peak around 700°C with a minimum initial oxidation temperature peak at 594°C. The mass loss associated with this was 1.62% due to evaporation of chemically bounded water. A second effect was observed around 711°C with an exothermic peak 680°C. This effect was associated with a mass loss of 44.32% which probably corresponds to the thermal degradation of elemental silver. This result shows slight change in thermal stability of the nanoparticle due to presence of silver in the composite films compared to the chitosan alone as previously reported by Shin (18). Silver ion was reduced to zerovalent silver according to the following stoichiometry

\*Ethanol being a protic solvent have the ability to exchange electrons (since chitosan did not dissolve in water) which donates electrons to the silver ion.

### Conclusion

Chitosan-silver nanoparticle was prepared using chitosan in a reduction reaction. This was confirmed by the maximum surface plasmon resonance peak around 430 nm. It can be concluded that chitosan works as a good stabilizer. Chitosan-silver may be used in various applications. For example it could be made into film for wrapping food that are highly susceptible to microbial growth or directly used as a surface coating on perishable fruits and vegetables to enhance safety and extend food shelf life. Also, it could be used as point of source for water remediation, groundwater and soil contaminated with pollutants.

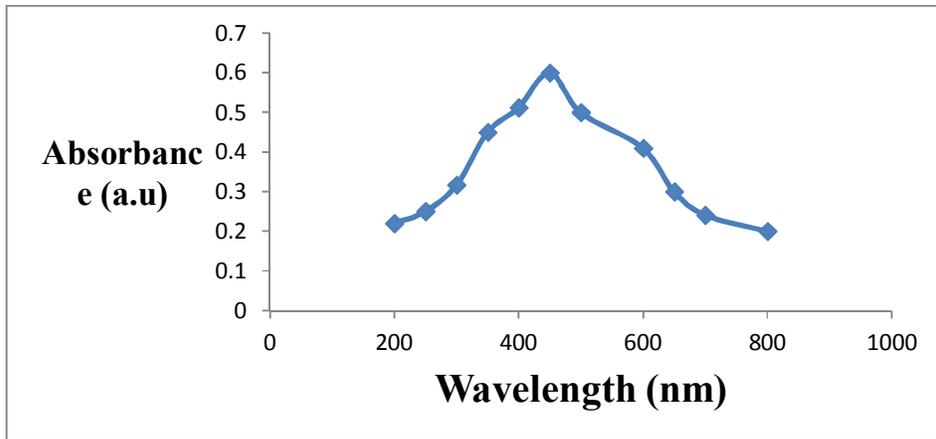


Figure 1: Characteristic optical absorption spectrum of chitosan- silver in the UV-Vis region indicating plasmon resonance

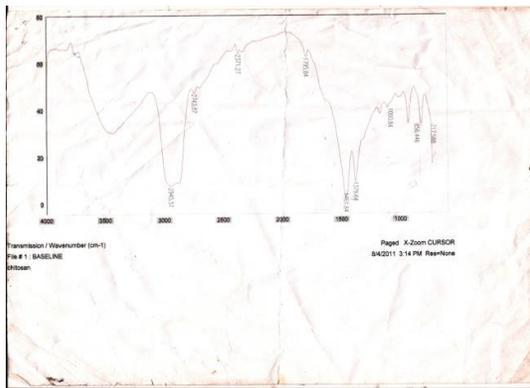


Figure 2: IR spectrum of chitosan indicating the various functional groups appearing at various wavelengths

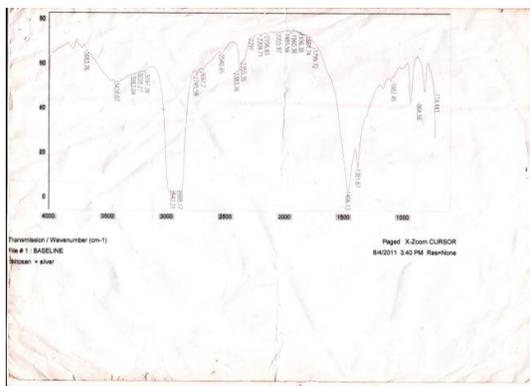


Figure 3: IR spectrum of chitosan-silver indicating shift in functional groups appearing at various wavelengths

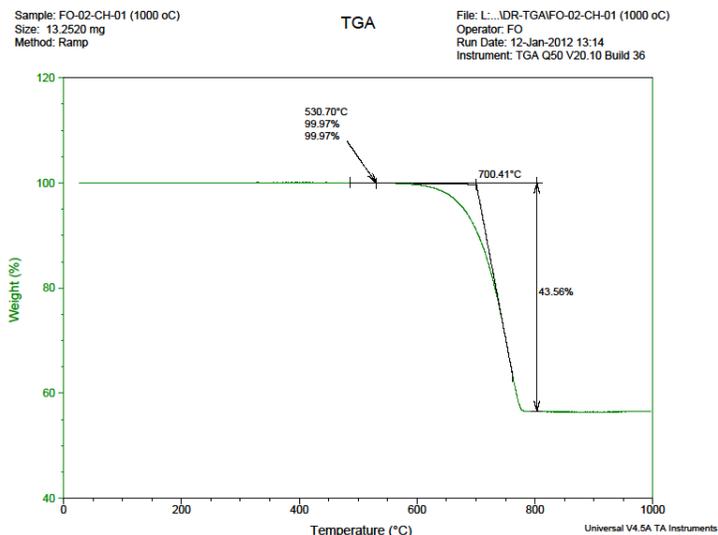


Figure 4: Thermogravimetric profile of chitosan indicating its thermal stability

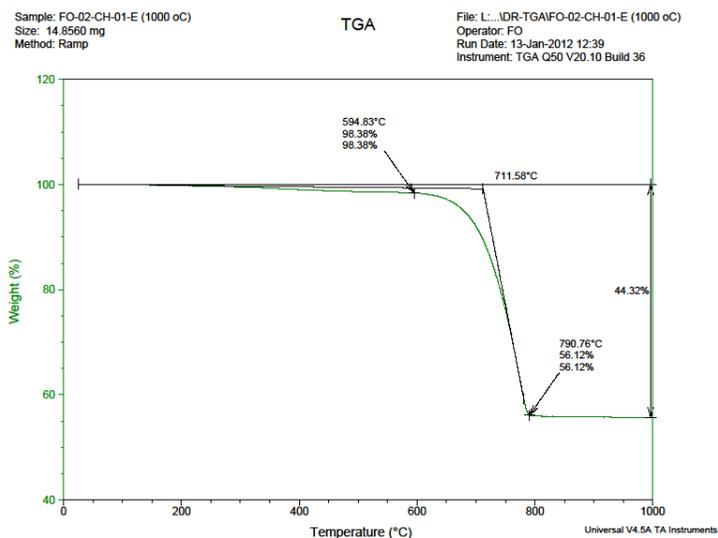


Figure 5: Thermogravimetric profile of chitosan-silver indicating change in its thermal stability due to the presence of elemental silver

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