Green synthesis of biopolymer–silver nanoparticle nanocomposite: An optical sensor for ammonia detection

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ABSTRACT

Biopolymer used for the production of nanoparticles (NPs) has attracted increasing attention. In the present article, we use aqueous solution of polysaccharide Cymopiosis tetragonolauna commonly known as guar gum (GG), from plants. GG acts as reductive preparation of silver nanoparticles which are found to be <10 nm in size. The uniformity of the NPs size was measured by the SEM and TEM, while a face centered cubic structure of crystalline silver nanoparticles was characterized using powder X-ray diffraction technique. Aqueous ammonia sensing study of polymer/silver nanoparticles nanocomposite (GG/AgNPs NC) was performed by optical method based on surface plasmon resonance (SPR). The performances of optical sensor were investigated which provide the excellent result. The response time of 2–3 s and the detection limit of ammonia solution, 1 ppm were found at room temperature. Thus, in future this room temperature optical ammonia sensor can be used for clinical and medical diagnosis for detecting low ammonia level in biological fluids, such as plasma, sweat, saliva, cerebrospinal liquid or biological samples in general for various biomedical applications in human.

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1. Introduction

Now-a-days, silver Nanoparticles (AgNPs) has gained tremendous popularity in the field of sensors because of outstanding significance optical, electronic and chemical properties [1–3]. The silver nanoparticles provide high surface energy, which promotes surface reactivity (e.g., adsorption, catalysis). One unique property of silver nanoparticles includes their utilization in fluorescence and surface plasmon resonance (SPR). SPR is associated with the coupled oscillation of free electrons on the conduction band accompanying enhanced local electromagnetic field, which is intensely sensitive to surrounding medium conditions. When a nanoparticle is exposed to an electromagnetic wave, the electrons in the particles oscillate at the same frequency as the incident wave. Spectral characteristics of silver nanoparticles are strongly dependent on their size, shape, interparticle spacing and environment [4]. Therefore, the geometry of noble metal nanoparticles would provide important control over linear and nonlinear optical properties [5–7].

These properties allow silver nanoparticles to be used in various imaging method and in chemical and biological sensing applications such as detection of human serum antibody [8], laser desorption/ionization mass spectrometry of peptides [9], colorimetric sensor for histidine [10], determination of fibrinogens in human plasma [11], real-time probing of membrane transport in living microbial cells [12], enhanced IR absorption spectroscopy [13], colorimetric sensors for measuring ammonia concentration [14], bio-labeling, optical imaging of cancer [15], biosensors for detection of herbicides [16], and glucose sensor for medical diagnostics [17].

The development of highly sensitive, reversible ammonia sensing technology is always an active area of research because of ammonia’s widespread use in industry and its toxic nature to organism of human body and animal bodies. Ammonia sensors benefit industrial air quality control by detecting leaks and unhealthy ambient ammonia concentrations before health damage occurs. These sensors also have wide applications for monitoring ammonia in environmental, biological and clinical samples [18–26]. Several techniques have been investigated for monitoring ammonia in gas samples, including ion mobility spectrometry [18], electrochemical sensors [19], metal oxide semiconductor detectors [20], pH sensors [21], deep UV or near IR absorption techniques [22,23], and optical ammonia sensor based on up-converting luminescent nanoparticles [24].

All these techniques only work for ammonia gas and cannot be used to measure ammonia dissolved in water. For the detection of ammonia in solution, only few literatures are available [25–28]. Among these techniques, mentioned above, optical sensors based on the unique surface plasmon resonance properties of metallic nanoparticles have shown to be very useful in the analysis of biomolecular, metal ions and liquid ammonia: they have received considerable attention due to their simplicity, high sensitivity,
low cost, rapidity and they do not need the use of complicated apparatus.

The concept of green nanoparticles preparation was first developed by Raveendran et al. [29] who used β-D-glucose as the reducing agent and starch as a capping agent to prepare starch silver nanoparticles. Silver nanoparticles preparation was also performed using natural polysaccharide chitosan [30], heparin [31], Acacia [32], Gum Kondagouli[33], and gum Arabic [34]. A green method for nanoparticles preparation should be evaluated from three aspects: the solvent, the reducing agent and the stabilizing agent.

In a recent research work of this lab, a very simple method is presented for the synthesis of silver nanoparticles to be used in colorimetric optical sensors based on localized SPR (LSPR) measurement for ammonia. Silver nitrate salts are reduced using guar gum which acts as capping and reducing agent. Commonly used reducing agents such as hydrazine or sodium borohydride are replaced by a more environmental friendly green method using natural polysaccharide. UV–vis spectrophotometer was used to monitor the kinetic of nanoparticles synthesis while their size and morphology were confirmed by transmission electron microscopy (TEM). Finally, the sensing properties of the nanoparticles solution were tested against increasing ammonia concentration between 1 and 100 ppm by monitoring the changes in LSPR position and amplitude with a UV–vis spectrophotometer.

2. Experimental

2.1. Materials

AgNO₃ was purchased from Aldrich and used without further purification. Guar gum was purchased from Merck, India. All aqueous solutions were made using ultrahigh purity water purified using a Mill-Q Plus system (Millipore Co.).

2.2. Preparation of silver nanoparticles

For the synthesis of silver nanoparticle, a natural polysaccharide: guar gum (GG) is used as a substrate which act as stabilizing and capping agent. 2.5% (w/v) of GG substrate was dissolved in 10 ml distilled water with the help of magnetic stirrer. The main significance about our synthesis is that we do not require maintaining the pH during the synthesis process. The synthesis takes place at solution pH which is 6. After complete dissolution, the temperature of the reaction medium is raised to 70 °C. Now 10 ml of 15 mM silver nitrate solution was then added drop wise. The reaction mixture was kept under continuous stirring for 90 min. Short time after addition of silver nitrate, the reaction medium acquires a clear yellow brown color showing the formation of silver nanoparticles. The progression of the reaction was controlled by recording UV–vis absorption at given time intervals and evaluated.

2.3. Characterization of synthesized silver nanoparticles

The nanostructured materials are characterized by X-ray diffraction (XRD) using Bruker D8 Advance powder diffractometer operating in the reflection mode with CuKα radiation. The morphology of the films was investigated by scanning electron microscope (SEM) using a FEI–SIRION operated at voltage of 20 kV. To avoid charging these samples were coated with gold. The size and shape of the nanoparticles were obtained with transmission electron microscope (TEM) using a TECHNAI T20 microscope operating at 200 kV and HRTEM using Madison, WI operating at 200 kV. The HRTEM samples were prepared by dispersing the colloidal solution in methanol using an ultrasonicator or directly dispersing the colloidal solution on a carbon coated copper grid.

UV–vis spectroscopy model: U-2900 (Hitachi) was used to ensure the formation of nanoparticles.

2.4. Sensing study of Ammonia detection

For the sensing study 25% of ammonia solution is used. Ammonia concentration is varied by diluting ammonia solutions of different concentrations (1 to 100 ppm in deionised distilled water) were prepared right before experiment.

3. Result and discussion

3.1. Synthesis and characterization of GG/AgNPs NC

As mentioned above, GG is rich in various functional groups, and their capping on silver nanoparticles provides surface reactivity. In addition no colors change and Visual aggregation for more than 6 months is observed. Besides this, the absorbance spectra of silver nanoparticles showed hardly any change in the λmax and intensity values, even after six months of storage. This observation is supported by the complete absence of peaks at 335 and 560 nm in UV–vis absorption spectra, showing no nanoparticles aggregation or nanocluster formation (Fig. 1a). Hence a silver nanoparticle formed by this method is highly stable and well dispersed in nature.

Silver nanoparticles absorb radiation in the visible region of the electromagnetic spectrum (380–450 nm) due to the excitation of Surface plason Vibrations, and this is responsible for the striking yellow–brown color of silver nanoparticles in various medium [35–37]. The UV–vis absorption spectrum of the silver nanoparticles solution we prepared is shown in Fig. 1b. A plasmon absorbance of the silver nanoparticles was observed between 440–450 nm, and the plasmon band is symmetric, which shows that the solution does not contain many aggregated particles, a conclusion that agrees to the electron micrograph observations (below).

3.1.1. Effect of reaction duration

The formation of silver nanoparticles was evidenced with the change in color of the solution after addition of AgNO₃ from the colorless to light yellowish color, then to the dark brown color. The formation of silver nanoparticles was monitored through UV–vis spectrophotometer at time intervals of 20, 40, 50, 60, 70, 80 and 90 min (Fig. 1c). The spectrum obtained at 90 min shows the absorption maximum as 450 nm. The intensity of the peak with respect to the height increases gradually with increase of time. Fig. 1c shows the UV–vis absorption spectra of silver nanoparticles colloidal solutions prepared at different durations. The data reveal several important findings which can be presented as follows: (i) at the early stage reaction duration (after 20 min) the plasmon band is broadened and simple test for silver ion using NaCl solution shows low conversion of silver ions to metallic silver nanoparticles at this duration, (ii) prolonging the reaction duration up to 70 min leads to outstanding enhancement in the Plasmon intensity showing that large amounts of silver ions are reduced and used for cluster formation, and (iii) further increase in the reaction duration up to 90 min is accompanied with marginal decrement in the absorption intensity which could be attributed to some aggregation of the formed silver nanoparticles.

The silver nanoparticles formation and the size can be easily being monitored by observing peak due to SPR and blue shift position. Within a few minutes of reaction, the solution became colorless to light yellow color, indicating the synthesis of silver nanoparticles. As the reaction proceeded, the light yellowish color changes to the dark brown color. The effect of different reaction times on silver nanoparticles synthesis results in a blue shift of the absorption peak i.e. there is decrease in wavelength (increase in frequency) and shifts the color from the red end of the spectrum.
to the blue end, indicating that the particle size of silver nanoparticles decreases, with the reaction time changing from 0 to 90 min, when all the other reaction conditions are identical. Also there is much more amounts of silver nanoparticles were synthesized at the 90 min. It is suggested that small particle size and large quantity of particles are acquired also by controlling reaction time.

3.1.2. Effect of polysaccharide concentration

The UV–vis absorption spectra of silver nanoparticles prepared using GG in different concentrations (1.2–3.5%, w/v), at initial pH of 6 and temperature of 70 °C for 90 min. The data reveal that regardless of the GG concentration used, similar plasmon bands are formed at wavelength 440 nm with the formation of the ideal bell shape which is characteristic for the formation of Ag\(^{0}\) nanoparticles. It is clear also that there is a gradual increase in the absorption intensity, by increasing the GG concentration up to 3.5% which could be ascribed to the enhancement in the stabilization efficiency of the formed silver nanoparticles. It should be also mentioned that the least amount of GG in the reaction medium (1.2%) is enough for full reduction of the Ag\(^{+}\) to Ag\(^{0}\) nanoparticles.

Thus, we found that the morphology and size distribution of the silver nanoparticles varied with the concentration of both the polysaccharides and the precursor metal salts. For example, when the concentration of polysaccharide or Ag\(^{+}\) ions was increased, the size of the silver nanoparticles also increased. All spectra exhibit an absorption band between 400 and 450 nm, a typical plasmon resonance band of silver nanoparticles [35,36]. Both the increases in absorption intensity and the shifts in plasmon absorption show that the size of silver nanoparticles formed varies with the concentration of polysaccharide and AgNO\(_3\). When the concentration of AgNO\(_3\) was increased from 2.5 to 15 mM, larger particles and Ag clusters were obtained while with a further increase in the concentration of polysaccharide, larger spherical Ag nanoparticles were obtained (Fig. 1d). These changes in particle size and morphology may be controlled by the polysaccharide as the controller of nucleation and stabilizer.

Stability study of silver nanoparticles was performed up to six months. And it was found that stability is found to be nearly constant even up to six months. During the preparation of silver nanoparticle suspension, the individual particles have a tendency to form large sized agglomerates through Vander Waals force or Coulomb's force. To prevent the agglomeration of small particles, we added GG to the suspension as the stabilizer. The GG can form a protective layer on the particle’s surface. A strong physical adsorption of the GG onto the surface of the silver nanoparticles is also an indication of better stabilization.

3.1.3. FTIR

Evidence for the interaction of hydroxyl groups in the stabilization of the silver clusters was obtained from the Fourier transform infrared (FTIR) spectra of the GG-containing Ag clusters. The FTIR spectra of GG exhibited characteristics bands at 3426 and 2926 cm\(^{-1}\) because of the O–H stretching vibrations of the polymer associated with C–H stretching vibrations. Additional characteristics absorption bands of GG appeared at 1418 and 1026 cm\(^{-1}\) because of C–H bending and O–H bends vibrations, respectively.
The band at 3426 cm\(^{-1}\) shifted to 3457 cm\(^{-1}\) in the presence of Ag; also, the band was broader in Ag/GG compared to GG. These observations clearly show the interaction of Ag with the \(-\text{OH}\) group of GG (Fig. not provided).

The polar groups \(\text{O--H}\) of polysaccharide have the good ability of coordination reaction with metal ions (e.g., with silver ions). When \(\text{O--H}\) groups and silver ions form coordination bonds, the interactions among the resultant Ag particles and oxygen atoms of \(\text{O--H}\) groups become stronger with increasing amount of Ag. This can lead to corresponding changes both in the positions and in the strengths of IR spectra of GG. Thus, the stretching peaks of both \(\text{O--H}\) and \(\text{C--O}\) become wider gradually. And so does the bending absorption peak of \(\text{C--O--H}\) at 1350 cm\(^{-1}\) influenced by the Ag particles.

3.1.4. XRD

The nature of the silver formed in this approach has been evaluated with XRD methods. The XRD of GG and AgNPs are compared. XRD of GG shows amorphous nature, while the XRD profile of AgNPs presented in Fig. 2a and b exhibits characteristic peaks at scattering angles (2\(\theta\)) of 38.06, 44.22, 64.48, and 77.32 corresponding with scattering from the (1 1 1), (2 0 0), (2 2 0), and (3 1 1) planes, respectively. These diffraction peaks firmly represent a face centered cubic structure of crystalline silver nanoparticles (from JCPDS, No. 4-0783) [38,39]. All the peaks in the XRD pattern can be indexed as a standard Ag crystal (JCPDS, No. 4-0783) [40], which also shows a high purity of as synthesized silver crystal.

3.1.5. SEM

The SEM micrograph of GG/AgNPs NC was found to be spherical shape grain like morphology (Fig. 2c). Thus, it confirms the formation of GG/AgNPs NC formation. The presence of Ag has been confirmed by EDS spectrum (Fig. 2d).

3.1.6. TEM

The spherical shape was further confirmed with the high-resolution bright field TEM images of Ag NPs (Fig. 2e). The mean
particle size is ~8 nm, calculated from 542 nanoparticles (Fig. 2f). The lattice fringes in processed high resolution TEM images (Fig. 3a) show that the Ag NPs are single crystals and also reveal that the particles have a lattice d-spacing of 2.37 Å in the (1 1 1) plane, matching the d-spacing in the (1 1 1) plane of Ag with a face-centered cubic (fcc) structure. Fig. 2e and f shows the TEM images and the particle size distribution histograms of silver nanoparticles formed after 90 min. TEM image shows small size spherical particles. The corresponding size distribution histogram clearly illustrates that the size of the formed particles seem to be between 4 and 12 nm.

The stabilization phenomena have been observed clearly with a TEM experiment. Fig. 3b displays a typical TEM image of the silver nanoparticles. It appears that the silver nanoparticles are encapsulated by the GG polymeric networks or silver–guar gum core–shell structure. The formed GG polymer networks ease the excellent stability of the nanoparticles through electrostatic and steric affects and the hydroxyl groups of the polymeric chains (Fig. 3b), which further promote the stabilization of silver nanoparticles. Because of the coordination ability of the polymer with silver metal nanoparticles through the hydroxyl-functional groups of the polymeric

![Image](image_url)

**Fig. 3.** (a) High resolution TEM image of Ag NPs, showing the lattice fringes in the (1 1 1) plane of the particles (indicated on one particle by white bars) and measured lattice d-spacing. (b) Reaction mechanism for formation of silver nanoparticles.

![Image](image_url)

**Fig. 4.** (a) The plot of changes in spectral absorbance of GG/Ag NPs colloidal solution as a function of various ammonia concentration. (b) Variation of absorbance at 413 and 440 nm. (c) Ratio of the absorbance peak at 413 and 440 nm which displays a linear relationship as a function of ammonia concentration (1–50 ppm) with a correlation factor $R^2$ equal to 0.966. (d) Ratio of the absorbance peak (Abs$_{413}$/Abs$_{440}$) for a silver nanoparticles solution exposed to increasing ammonia content showing a saturation beyond 50 ppm.
chains and networks, they surround and protect the particles over longer periods. On the other hand, a strong physical adsorption of the GG polymer onto the surface of the silver nanoparticles is also an indication of better stabilization.

3.2. Reaction mechanism for formation of silver nanoparticles

For the synthesis of silver nanoparticles, the generally accepted mechanism suggests a two-step process, i.e., atom formation and then polymerization of the atoms. In the first step, a portion of metal ions in a solution is reduced by the available reducing groups of the GG. The atoms thus produced act as nucleation centers and catalyze the reduction of the remaining metal ions present in the bulk solution (Fig. 3b) and subsequently the atoms coalesce leading to the formation of metal clusters. The surface ions are again reduced and in this way the aggregation process does not cease until high values of nuclearity are attained, which results in larger particles. The process is stabilized by the interaction with the polymer and thus preventing further coalescence [41].

HRTEM clearly shows the spherical morphology of nanoparticles. Compared to other methods the formation of silver nanoparticles is faster. The amount of hydroxyl groups in GG can help the reduction of silver ions. The nanoparticles could have grown through reduction of silver ions inside the nanosopic GG templates, with the GG hydroxyl groups acting as passivation contacts for the stabilization of the nanoparticles.

3.3. Ammonia sensing of GG/AgNPs NC

The sensing study of ammonia solution was performed by optical measurement [14]. The absorbance spectra as a function of ammonia concentration increasing from 0, 1, 10, 25, 35, 45, 50, 70 and 100 ppm is shown in Fig. 4(a). For each measurement, fresh colloidal solutions of silver nanoparticles are taken. It may be noted from Fig. 4(a) that the LSPR peak intensity at 440 nm decreases and another peak appears at 413 nm as the ammonia content is increased. Shifts of LSPR band are usually due to the change in inter-particles distance and the dielectric constant of the surrounding medium [14]. It has been shown that the absorption spectra can be analyzed and the concentration of ammonia can be detected. Change in absorbance at two different wavelength as a function of ammonia concentration are provided in Fig. 4(b). As analyzed in Ref. [14], we have plotted absorbance ratio \(\text{Abs}_{413}/\text{Abs}_{440}\) as a function of ammonia concentration in Fig. 4(b). The absorbance ratio is almost linear with ammonia concentration in the range of 1 to 50 ppm. The correlation factor \(R^2\) is found to be 0.966 which is in good agreement with that reported by other researchers [14]. Beyond 50 ppm, the ratio remains constant Fig. 4(d), which indicates that the silver nanoparticles based optical sensor is active for low detection of ammonia content in the solution.

4. Conclusions

The present study reports the facile synthesis of silver nanoparticles from silver nitrate using polysaccharide as a template. The adapted method is compatible with green chemistry principles as the GG serves as a matrix for both reduction and stabilization of the silver nanoparticles synthesized. At a given gum concentration, the efficiency of nanoparticle synthesis increases with silver nitrate concentration and reaction time, a property attributable to the large reduction capacity for the GG. As the particle size of the nanoparticles can be controlled, this method can be carried out for the large-scale production of monodispersed and spherical nanoparticles of around 8 nm due to the availability of low cost biopolymer.

Further the application of nanoparticles was studied on the aqueous ammonia sensing properties of GG/AgNPs NC. The silver NPs dispersion in polymer matrix played a more important role in calorimetric sensing applications. Colorimetric assays based on the unique LSPR properties of metallic nanoparticles have showed to be very useful due to their simplicity, high sensitivity, low detection limit of 1 ppm, low cost, fast response time and great reproducibility. Keeping these significance properties in mind, in the near future we can use our room temperature optical ammonia sensor for clinical and medical diagnosis for detecting low ammonia level in human.

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