

Establishment of Optimum Conditions for Preparation of Silver Nanoparticles Using Carboxymethyl Chitosan

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SILVER nanoparticles (AgNPs) with tailored characteristics are prepared using carboxymethyl chitosan (CMCs) which plays a dual role ; as a reducing agent for conversion of Ag⁺ to Ag⁰ and as a stabilizing agent to prevent aggregation of Ag⁰ and / or its clusters. The preparation involves a thorough investigation into factors affecting formation of AgNPs characteristics and dependence of them on these factors. Factors studied encompass concentrations of silver nitrate and CMCs as well as pH, time and temperature of the synthesizing medium. Sophisticated tools such as FT-IR, UV-vis spectral analyses and TEM reveal major characteristics of AgNPs formed as well as size, distribution and shape of AgNPs. According to current work, the most appropriate condition of converting silver ions into AgNPs are 0.25% solution of CMCs and 6ml of 0.1N AgNO₃ (0.102 g / 100 ml) and carrying out the reaction at 70 oC for 45 min at pH 11.

Keywords: CMCs, Silver nanoparticles and Chemical reduction method.

The preparation of metal nanoparticles is a major research area in nanoscale science and engineering ⁽¹⁾. This is rather emphasized by virtue of the unusual chemical and physical properties of the metal nanoparticles vis – á – vis these properties when the metal is in the bulk state. Among the unique properties of the metal nanoparticles are catalytic activity, novel electronic, optic and magnetic properties and their potential application in biotechnology.

Numerous studies have been reported on the preparation of new organic/inorganic hybrid materials and nanocomposites such as metal-polymer nanoparticles ⁽²⁾. AgNPs have also attracted much attention due to their diminutive size and novel material properties. With their nanometer scale size, which is responsible for different properties when compared with the bulk

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material, renders them suitable for applications⁽³⁾. Indeed, many approaches have been undertaken to prepare AgNPs for a rapidly growing list of catalysis, electronic, non-linear optics and biomaterial applications⁽⁴⁻⁸⁾. In the preparation of AgNPs, the stabilizer plays an important role in retaining the stabilization of nanosystem.

The antimicrobial properties of silver ions have been known since ancient times and silver ions are widely used as bactericide in surgical prostheses and dental implants^(1,9,11). Many methods have been used for the preparation of nanoparticles including microbial, chemical and physical methods. The most popular among them is the chemical reduction of silver salts by sodium citrate. AgNPs show optical properties, which are not observed neither in molecules nor in bulk metals⁽¹²⁾. The formation of the AgNPs can be monitored using UV-vis absorption spectroscopy since it exhibits the typical surface plasmon absorption maxima at 418-420 nm from the UV-vis spectrum⁽¹³⁾.

Most of the synthetic methods reported to date for preparation of AgNPs rely heavily on the use of organic solvents and toxic reducing agents like hydrazine⁽¹⁴⁾, N, N dimethylformamide and sodium borohydride⁽¹⁵⁾. All these chemicals are highly reactive and pose potential environmental and biological risks. Earlier reports have dealt with natural polymers like chitosan⁽¹⁶⁾, heparin⁽¹⁷⁾ and soluble starch⁽¹⁸⁾ as reducing and stabilizing agents for preparation of AgNPs.

CMCs was used in various publications for preparation of silver, palladium, gold and other nanoparticles⁽¹⁹⁾. However, to the authors' knowledge no systematic study for preparation of the AgNPs using CMCs has been published so far. It is the essential aim of the current work to present detailed and integrated investigations pertaining to the preparation of AgNPs with well-defined size using CMCs as reducing agent for silver ions and in the same time as stabilizing agent for the synthesized AgNPs. Preparation of AgNPs is carried out under a variety of conditions including concentrations of silver nitrate and CMCs, time, temperature and pH of the preparation medium. FTIR, UV-vis spectra, TEM images are employed to characterize AgNPs obtained under the said different conditions.

Materials and Methods

Materials

Chitosan DA 95 % (Aldrich Chemical Company), monochloroacetic acid (Morgan Chemical Ind. Co, Egypt), sodium hydroxide, glacial acetic acid (Adwic, Egypt). Isopropyl alcohol, citric acid anhydrous and silver nitrate were all of laboratory grade reagents.

Methods

Preparation of activated chitosan

The fully deacetylated chitosan was prepared according to a method described elsewhere⁽²⁰⁾. Initially, 1 g of chitosan (DA = 95%) was dissolved in

1% glacial acetic acid solution to obtain a homogeneous solution. The solution was filtered through centered glass funnel and dropped into 20% aqueous sodium hydroxide solution, whereby the chitosan precipitated immediately in gelatinous form. The chitosan gel was thoroughly washed with distilled water till neutrality, then with ethanol / water mixture "8/2, V/V" and finally with isopropyl alcohol and air-dried.

Preparation of CMCs

Activated chitosan was suspended in sodium hydroxide solution and placed in a refrigerator for alkalization. Alkali chitosan and isopropanol were mixed at different liquor ratios for 30 min at 0°C. Then, 4 g monochloroacetic acid dissolved in 10 ml isopropanol was added and the solution was placed in a refrigerator for 30 min at 0°C and then refluxed at 70°C for 2 hr, with continuous stirring. The reaction mixture was adjusted to pH 7 by adding diluted HCl, filtered and the obtained product was repeatedly washed by acetone and 95% (V/V) ethyl alcohol and then with methanol, and finally dried in an oven at 60°C.

Preparation of AgNPs

A definite weight of CMCs was dissolved in distilled water by making use of heating magnetic stirrer. After complete dissolution, the pH of the solution was adjusted within the range 6–12.5, followed by raising the temperature of the reaction medium to the desired temperature (40–80°C). Certain amount of silver nitrate solution was then added dropwise (keeping in mind that the total volume of the reaction medium is 100 ml). The reaction mixture was kept under continuous stirring for different durations (15–120 min). Short time after addition of silver nitrate, the reaction medium acquires a clear yellow color indicating the formation of AgNPs. The progression of the reaction was monitored by UV–vis absorption; aliquots from the reaction bulk were withdrawn at given time intervals and evaluated for their UV – vis spectra.

Characterization

The characterization of the prepared product in each step was evaluated as per the following standard procedures:

FTIR spectroscopy: FTIR spectra were recorded using FTIR Raman model Nexus 670(Nicollet-Madison, WI).

Ultra violet–visible (UV–vis) spectra: UV-vis spectral analysis was done by means of a 50 ANALYTIKA, JENA Spectrophotometer from 200 to 500 nm.

Transmission electron microscop (TEM): TEM was used to assess the potential impact of the modification on the elemental and structural properties of the synthesized AgNPs, whether alone or over and within the fabric surface. The TEM analysis was done using JEOL-JEM-1200 (Japan) .

Results and Discussion

The chitosan derivatives obtained by its carboxymethylation are old in the art with three types: N-CMCs (NCMC), O-CMCs (OCMC), and N,O-CMCs (NOCMC).

Characterization of CMCs by FTIR analysis

FTIR analysis was used to confirm functional groups of chitosan and CMCs. The main bands (Fig. 1a and b) observed in the infrared spectrum of chitosan were: (i) a strong and broad band due to the axial stretching of O-H and N-H bond centered at 3400 cm^{-1} ; (ii) a band centered at 2890 cm^{-1} corresponding to the axial stretching of C-H bonds⁽²¹⁾; (iii) a band centered at 1659 cm^{-1} which is attributed to the axial stretching of C=O bonds of the acetamide groups, named as amide I band; (iv) a band at 1590 cm^{-1} due to the angular deformation of the N-H bonds of the amino groups; (v) a band at 1378 cm^{-1} due to the symmetric angular deformation of CH₃; (vi) the amide III band at 1316 cm^{-1} ; (vii) the band corresponding to the polysaccharide skeleton, including the vibrations of the glycoside bonds, C-O and C-O-C stretching, in the range $1153\text{--}897\text{ cm}^{-1}$.

As presented in Fig. 1b, the carboxymethylation induces structural changes in the chitosan. This can be clearly identified by comparing the infrared spectra of chitosan and CMCs. Thus, the broader band centered at 3400 cm^{-1} revealed the more hydrophilic character of CMCs compared to the parent chitosan while the occurrence of the strong peak at 1425 cm^{-1} could be assigned to the symmetrical stretch vibration of COO⁻ groups. The asymmetrical stretch vibration of COO⁻ groups ($1910\text{--}1550\text{ cm}^{-1}$) overlaps with the deforming vibration of NH₂ at 1600 cm^{-1} to obtain a very strong bond.

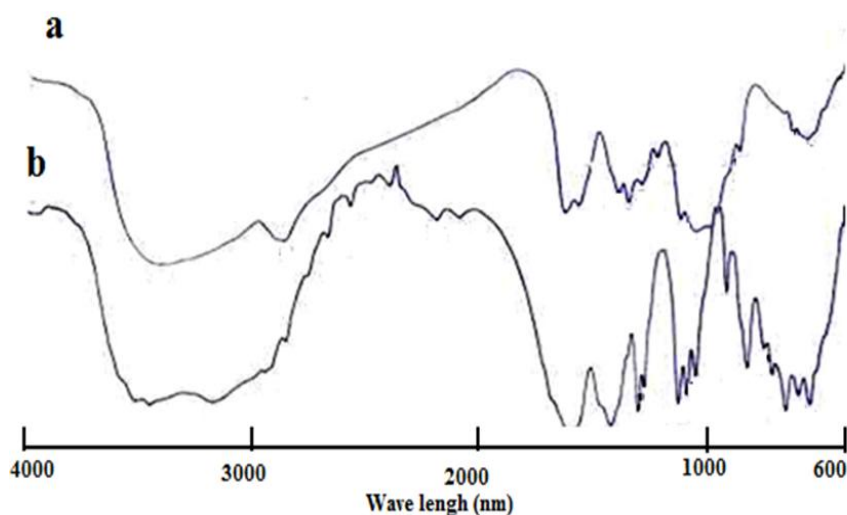


Fig. 1. FTIR of a) chitosan; b) carboxy methylchitosan (CMCs) .

Effect of pH on AgNPs formation

Figure 2 shows the UV- vis spectra of the silver colloid obtained using CMCs as reducing and stabilizing agent at different pH's. The results reveal a number of observations which may be summarized as follows: a) increasing the pH of AgNPs preparation medium with the range of pH 7 – pH 11 is accompanied by appreciable changes in the electronic absorption spectra; b) a band at higher energy (*i.e.* 217 nm) appears at pH 7; the intensity of this band decreases by increasing the pH to 9; c) further increase in the pH of the reaction medium up to 11 leads to disappearance of this band, d) concurrently another band at 405 nm starts to appear and reaches its maximum intensity at pH 11 and; e) when pH 11 is targeted, the band becomes stronger and symmetrical, with a pronounced bell shape at λ_{max} 410 nm. This band could be assigned to the plasmon resonance of silver nanoparticles. The behavior caused by the observations stated above could be attributed to: (i) the formation of various ionic states of silver such as Ag^+ , Ag^{+2} and Ag^{+3} ⁽²²⁾, bound to negatively charged surface of CMCs polymer which appears as the absorption band at 220 nm, (ii) increasing the pH enhances the reduction of Ag^+ to Ag^0 , (iii) maximum intensity of the plasmon peak (405 nm) at pH 11 indicate a full reduction of Ag^+ ⁽²³⁾, and, therefore, reflecting the dual role of CMCs efficient as stabilizing and reducing agent in alkaline medium.

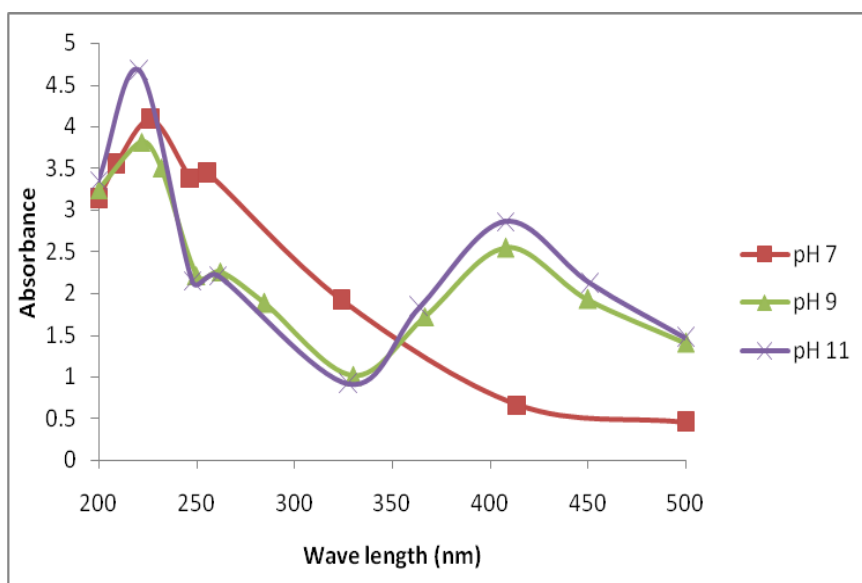


Fig. 2. UV-vis absorbance spectra of silver nanoparticles colloidal solutions at pH 7, pH 9 and pH 11.

Effect of temperature of AgNO₃ preparation on formation of AgNPs

Figure 3 depicts variation in AgNps formation with temperature of AgNPs preparation. The results feature that: i) at 40°C there is no sign of AgNO₃

formation indicating no response at 40°C among reactants pertaining to formation of AgNPs, instead of this silver nitrate reacted with sodium hydroxide to form silver hydroxide, ii) at 80 °C the AgNPs preparation medium acquired a red color meanwhile the UV – vis spectral analysis of the preparation solution could not exhibit the characteristic peaks of AgNPs, instead of this nitrate seem to undergo oxidation, iii) temperature up to 60 °C is accompanied by formation of deep yellow color and the UV –vis absorption band at 400 nm becomes stronger and narrower implying higher conversion of Ag⁺ to Ag⁰ with smaller nanoparticles.

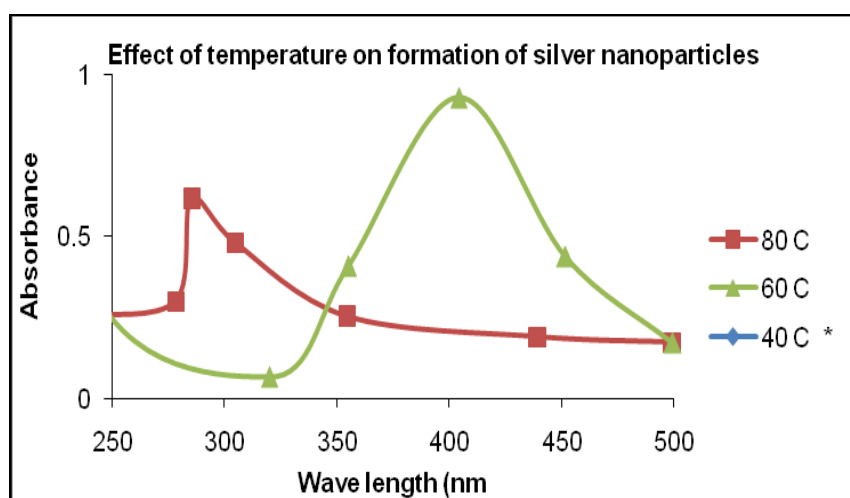


Fig. 3. UV – vis spectra of AgNO₃ colloidal solutions at different temperatures (40°C, 60°C and 80°C) of AgNPs synthesis.

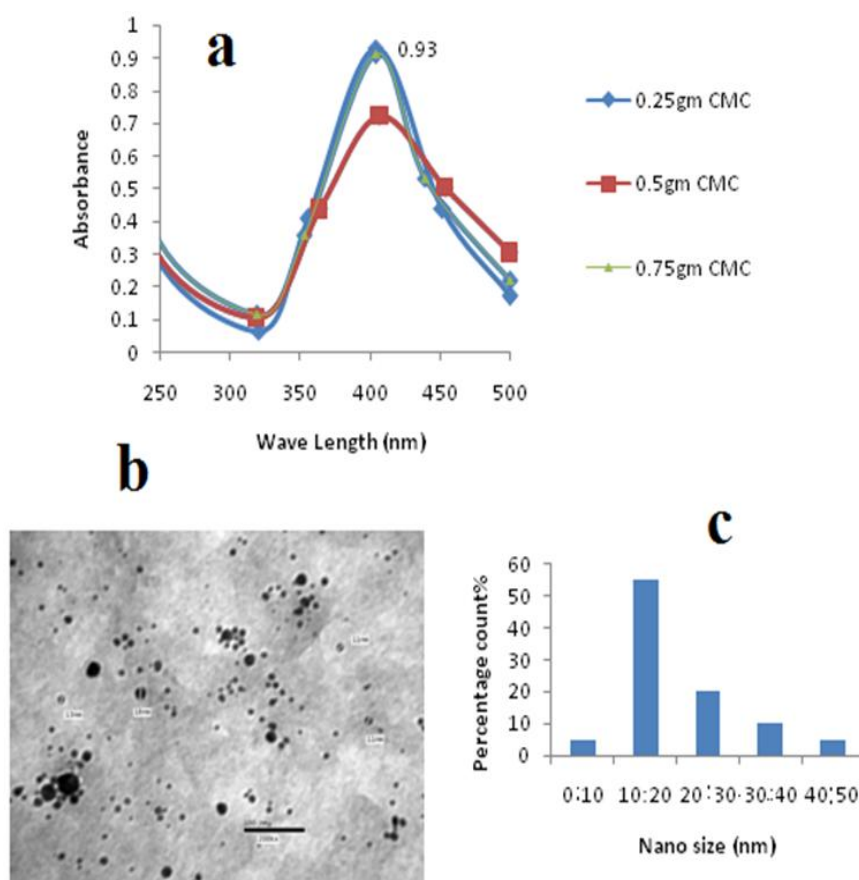
Effect of CMCs concentration

Figure 4 a shows the UV–vis absorption spectra of silver nanoparticles prepared using CMCs of different concentrations (0.25 – 0.75%, w/v), at initial pH of 11 and temperature of 60 °C for 30 min. It is obvious that regardless of the CMCs concentration used, similar Plasmon bands are formed at wavelength 408nm with the formation of the ideal bell shape which is characteristic of the formation of AgNPs. It is as well emphasizes that the lowest amount of CMCs concentration; namely 0.25g which is appropriate for forming AgNPs is also enough for full reduction of the Ag⁺ to Ag⁰ nanoparticles.

It is further noticed that the lower the amount of CMCs, the higher is the formation of silver nanoparticles. Besides, the homogeneity of silver nanoparticles increases. On the contrary increasing the starting amount of silver nitrate decreases the size of AgNPs formed. This could be ascribed to energy occurrence from the occlusion of particles in small space forming high heat thereby producing more silver nanoparticles. Similarly decreasing the weight of CMCs is accompanied by increased formation and homogeneity of nanosized

silver particles along with attraction among reactants because the space available for formation and attraction will ease formation of silver nanoparticles.

TEM image (Fig. 4b & c) depicts AgNPs as size spherical particles. The corresponding size distribution histogram clearly illustrates that the nanosize of the formed particles seem to be in the range of 10 -20 nm.



Reaction conditions: 0.25% (w/v) CMCs; 2ml (0.1 N) AgNO₃; temperature, 60 °C; pH 11; duration, 30 min.

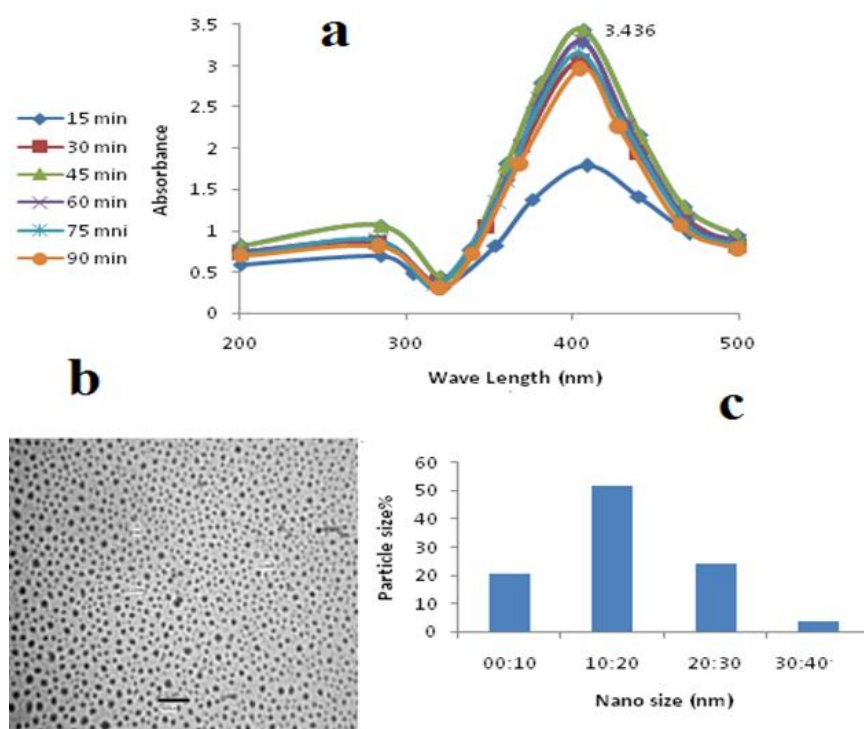
Fig. 4. a) UV – vis spectra b) TEM imag, c) histogram; at different weights (0.25 g, 0.5 g, 0.75 g) of CMCs, of silver nanoparticles formed after 30 min.

Effect of reaction duration

Figure 5 a shows the UV-vis. absorption spectra of silver nanoparticles colloidal solutions prepared at different durations. The results highlight the

following important findings: (i) at the early stage of reaction duration (after min 15) the plasmon band is broaden and simple test for silver ion using NaCl solution indicates low conversion of silver ions to metallic silver nanoparticles at this duration. (ii) prolonging the reaction duration up to 45 min leads to outstanding enhancement in the plasmon intensity indicating that large amounts of silver ions are reduced and used for cluster formation⁽²¹⁾. further increase in the reaction duration up to 60 min is accompanied by marginal decrement in the absorption intensity which could be attributed to some aggregation of the formed silver nanoparticles. (iii) increasing the reaction duration further, 75 and 90 min leads to significant decrease in the absorption intensity.

Figure 5 a & c discloses the TEM images and the particle size distribution histograms of silver nanoparticles formed 90 min. TEM image exhibits small size spherical particles. The corresponding size distribution histogram clearly illustrates that the size of the formed nanoparticles seem to be in the range of 10-25 nm and increases with cubic structure at 90 min, increasing the reaction duration up to 60 and 90 min leads to significant decrease in the absorption intensity due to aggregation as previously mentioned.



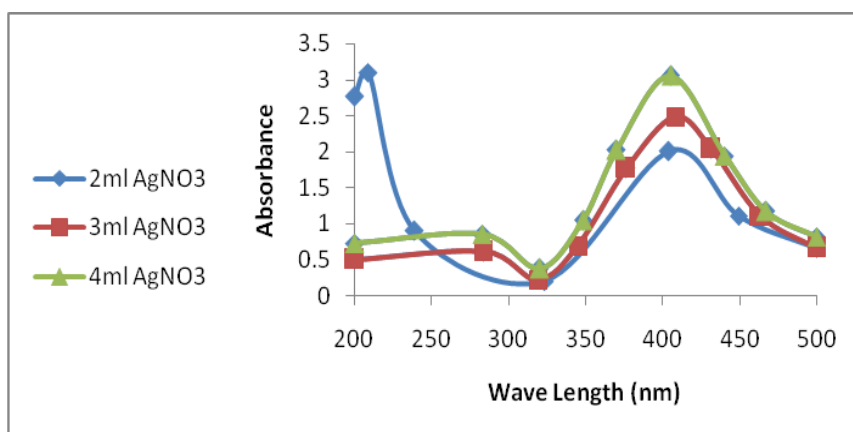
Reaction conditions: 0.25% (w/v) CMCs; 2ml (0.1 N) AgNO₃; temperature, 60 °C; pH 11.

Fig. 5. a) UV – vis spectra b) TEM image, c) histogram; of the effect of time on silver nanoparticles formation.

Effect of silver nitrate concentration

Results of the foregoing sections made it possible to prepare silver nanoparticles with concentration of ca. 100 ppm. This concentration is rather low for industrial applications. Interest in preparation of silver nanoparticles solutions, which acquire higher concentrations of the nanosized silver particles are, therefore, stimulated.

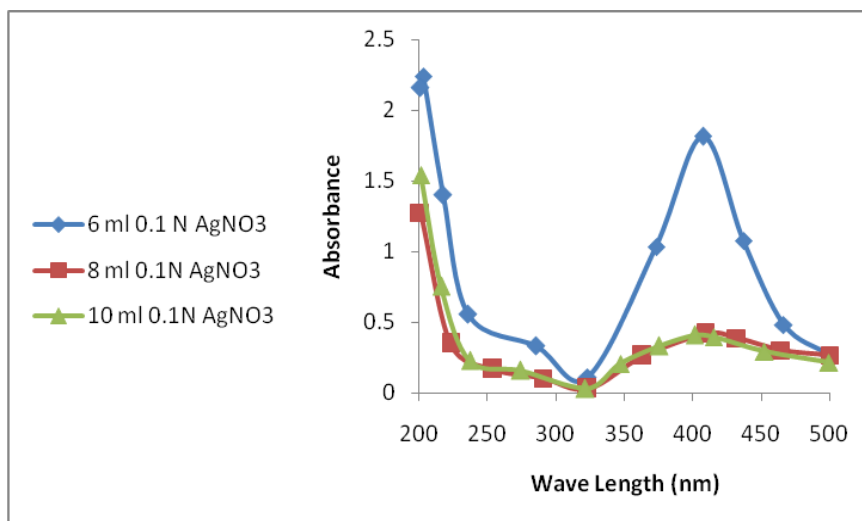
In this study, silver nitrate (AgNO_3) was incorporated at different concentrations in the reaction medium. Figure 6 shows the UV-vis spectra of Ag° resulting from incorporating different amounts of AgNO_3 having 0.25% CMCs. Figure 6 reveals that similar absorption spectra are obtained at wavelength 405 nm and that the intensity of the absorption peak increases by increasing the amount of AgNO_3 concentration in the reaction medium until reaching 4 ml of 0.1 N AgNO_3 .



Reaction conditions: 0.25% (w/v) CMCs; temperature, 60 °C; pH 11; duration, 45 min.

Fig. 6. Effect of silver nitrate concentration 2, 3 and 4 ml 0.1 N AgNO_3 .

TEM image and the histogram represented in Fig. 8 a show homogeneity in the produced silver nanoparticles with particle size (10-20 nm). Addition of more amount of silver nitrate (6, 8 and 10 ml of 0.1 AgNO_3) results in more concentrated solution while necessitate one hundred time dilution to be calculated. Unfortunately very sharp peak around 405 nm (Fig. 7) could be obtained with 6 ml of 0.1 N AgNO_3 while higher amounts (8 and 10 ml of 0.1 N AgNO_3) decrease the peak. At the same time their TEM images as shown in Fig. 8 (b, c & d) imply that the produced silver nanoparticles are out of antibacterial range which is known between (1-100 nm), as evident from the histogram of Fig. 8, the most abundant size is over 100 nm, results appear in Fig. 8a give preponderance to optimizing 6 ml 0.1 N AgNO_3 as the best amount forming AgNPs.



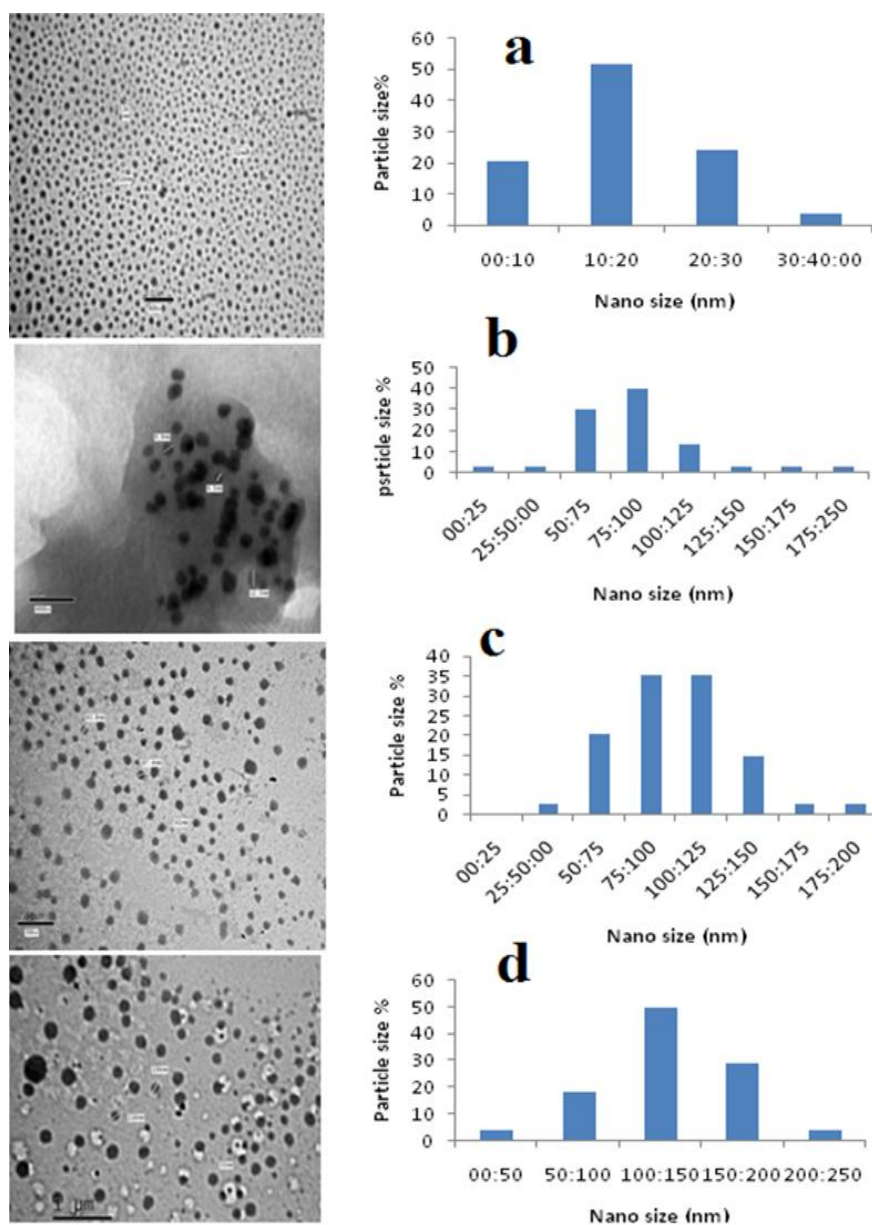
Reaction conditions: 0.25% (w/v) CMCs; temperature, 60 °C; pH 11; duration, 45 min.

Fig. 7. Effect of silver nitrate concentration 6, 8 and 10 ml 0.1 N AgNO₃.

Conclusion

A highly facile, simple, safe, cost effective green and innovative technique for the preparation of AgNPs was established. The innovation emphasizes the use of CMCs as a reducing and stabilizer agent during the preparation of AgNPs. State of the art facilities were employed for monitoring characterization and properties of AgNPs. Characteristics of AgNPs could be tailored through a thorough investigation into factors affecting the formation of AgNPs, specifically, CMCs and silver nitrate concentration as well as pH, temperature and duration of the synthesizing medium of AgNPs. Preparation of AgNPs colloids with excellent size distribution and nanosize within the range of 10-100 nm could be achieved under the following optimum conditions: 0.25 g CMCs; 6 ml of 0.1N AgNO₃, pH, 11; temperature, 60°C; duration, 45 min. Under these conditions AgNPs colloidal solution with a concentration of 600 ppm could be achieved. The colloidal solution is stable and remains without aggregation for more than six months. AgNPs colloidal solution with such unique characteristics will certainly be feasible for industrial applications. This methodology may also be adopted for preparation of other metal nanoparticles.

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Reaction conditions: 0.25% (w/v) CMCs; temperature, 60 °C; pH 11; duration, 45 min.

Fig. 8. TEM images and histograms of different concentrations of AgNO₃ a) 4ml of 0.1N AgNO₃, b) 6ml of 0.1N AgNO₃, c) 8ml of 0.1N AgNO₃ and d) 10ml of 0.1N AgNO₃.

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دراسة العوامل المثلى لتحضير جسيمات الفضة النانومترية بواسطة كربوكسى ميثيل الكيتوزان

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تم تصميم جسيمات الفضة النانومترية باستخدام كربوكسى الكيتوزان و التي تلعب دورا مزدوجا؛ كعامل مختزل لتحويل أيونات الفضة الموجبة إلى جسيمات الفضة النانومترية الفضة وكعامل استقرار لمنع التجمع أو تكوين مجموعات لها . تمت دراسة شاملة للعوامل التي تؤثر على تشكيل جسيمات الفضة النانومترية . العوامل التي تمت درستها شملت تركيزات نترات الفضة و الكربوكسى ميثيل كيتوزان، فضلا عن الأس الهيدروجيني، الوقت ودرجة الحرارة وسط التفاعل . أدوات متطورة مثل الأشعة تحت الحمراء و التحليل الطيفي استخدمت لإكتشاف السمات الرئيسية كالشكل و حجم الجسيمات وتوزيعها ، وفقا للعمل الحالي، أنسب الظروف لتحويل أيون الفضة إلى جسيمات الفضة النانومترية هي : 0.25% من الكربوكسى ميثيل كيتوزان و 6ML من ملح نترات الفضة 0.1 N وهو ما يكافئ ودرجة حرارة 70 سليزية لمدة 45 دقيقة والأس الهيدروجيني 11 .