Carbohydrate Polymers xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Green synthesis of silver nanoparticles by a novel method: Comparative study of their properties

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ARTICLE INFO

Article history:
Received 21 January 2012
Received in revised form 25 February 2012
Accepted 8 March 2012
Available online xxx

Keywords:
Silver nanoparticles
Green synthesis
Starch
Polysaccharide

ABSTRACT

This study presents a novel green method using starch for the synthesis of silver nanoparticles (AgNPs). In this method, the fungal supernatant was added to a mixture of starch and silver nitrate solutions. The characteristics of AgNPs synthesized by three independent processes—a modified polysaccharide method, a microbial method, and our novel method—were compared. Dynamic light scattering (DLS) analysis revealed that the average sizes of AgNPs synthesized by the microbial method, the modified polysaccharide method, and our novel method were 84, 20, and 15 nm, respectively. The most stable AgNPs were generated by our novel method. The X-ray diffraction (XRD) spectrum of the products from the novel method showed that these AgNPs have high crystallinity. Fourier-transform infrared spectroscopy (FTIR) spectra demonstrated that the functional groups present in the products prepared by the novel method are a combination of the functional groups present in the AgNPs synthesized by the other two methods.

1. Introduction

Recently, silver nanoparticles (AgNPs) have become the focus of intensive research owing to their wide range of applications in areas such as catalysis, optics, antimicrobials, and the biomaterial production (Batabyal, Basu, Das, & Sanyal, 2007; Das et al., 2011; Le et al., 2010).

The chemical synthesis of nanomaterials uses organic solvents and toxic reducing agents. These methods also suffer from other disadvantages, including low yield, high-energy requirements, and a need for difficult and wasteful purifications (El-Rafie et al., 2011; Vasileva, Donkova, Karadjova, & Dushkin, 2010). The application of AgNPs as biosensors and in other molecular techniques requires the use of biocompatible materials for their synthesis. Thus, it is essential to develop an efficient green synthetic method (Hebeish, El-Rafie, Abdel-Mohdy, Abdel-Halim, & Emam, 2010; Singh, Sinha, & Mandal, 2009). Some of the previously developed green methods report the use of reagents, such as biological materials, Tollens' reagent, and polysaccharides, and techniques such as irradiation (Sharma, Yngard, & Lin, 2009).

A green method for the synthesis of nanoparticles should be distinguished by its use of an appropriate solvent, reducing agent, and stabilizing agents. Various types of microorganisms have been reported to synthesize AgNPs either intra-or extracellularly (Li et al., 2012; Narayanan & Sakthivel, 2010). There are reports on the cell-associated biosynthesis of silver nanoparticles using *Fusarium* species in the literature (El-Rafie, Mohamed, Shaheen, & Hebeish, 2010; Ingle, Rai, Gade, & Bawaskar, 2009). The organic polymers have been used as templates for the preparation of AgNPs (Abdel-Halim & Al-Deyab, 2011; Konwarh, Karak, Sawian, Baruah, & Mandal, 2011; Xia, Cai, Jiang, & Yao, 2011). It is also known that polysaccharides can serve both as reducing and capping agents. For instance, starch is a naturally abundant polysaccharide, which is generally non-toxic, available from renewable agricultural sources and suitable for film formation (Yoksan & Chirachanchai, 2010). Starch–Ag NPs were synthesized using starch as a capping agent and β-D-glucose as a reducing agent (Chairam, Poolperm, & Somsook, 2009; Vasileva et al., 2010). Also, AgNPs were synthesized by autoclaving a solution of $AgNO_3$ and starch (Batabyal et al., 2007). Starch undergoes hydrothermal hydrolysis in an autoclave to produce glucose. This led us to utilize starch instead of pure glucose for the synthesis of silver nano/micro materials (Batabyal et al., 2007).

In this study, a mixture of silver ions, a starch solution, and a supernatant of a fungal culture are used to synthesize high-quality AgNPs. Such a novel method for the synthesis of AgNPs has not yet been reported.

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doi:10.1016/j.carbpol.2012.03.030

2. Materials and methods

2.1. Microorganism and materials

The fungus *Fusarium oxysporum* (strain 24) was obtained from the National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran. The pure culture was maintained on a potato dextrose agar (PDA), and the resulting medium was stored at 4 °C. All chemicals, including starch, β-D-glucose, and silver nitrate were of analytical grade and were purchased from Sigma Aldrich.

2.2. Fungal growth conditions

F. oxysporum was grown aerobically in a potato dextrose broth (PDB) medium. The final pH of the medium was adjusted to 6.2 using 1 N HCl. The PDB medium (100 mL) was transferred into a 250-mL flask. The flask was inoculated and then incubated at 28 °C with agitation on an orbital shaker operating at 200 rpm for 72 h.

2.3. Green synthesis of silver nanoparticles

In all experiments, a carefully weighed amount of $AgNO_3$ was added to the flasks to produce an overall Ag^+ ion concentration of 1 mM in the aqueous solutions. Furthermore, all reactions were carried out in the presence of light.

2.3.1. Modified polysaccharide synthesis

In this method, silver nitrate reduced by β-D-glucose was used as a reducing agent, and a starch solution (1%, w/w) was used as a stabilizer to prevent the aggregation of the nanoparticles. Initially, 2 mL of a silver nitrate solution (25 mM) was mixed with 50 mL of a starch solution (1%, w/w), and subsequently, 4 mL of β-D-glucose (25 mM) was added. Finally, the resultant solution was autoclaved at 121 °C and 15 psi for 15 min.

2.3.2. Microbial synthesis

After the cultivation period, the fungal culture was centrifuged at 6000 rpm for 20 min and the supernatant was collected. The fungal supernatant was added to the silver nitrate solution (1 mM) with a volume ratio of 1:100. Finally, the mixture was incubated at 50 °C and agitated at 200 rpm for 24 h.

2.3.3. Novel synthesis

In this method, 2 mL of a starch solution (1%, w/w) was added to 45 mL of a silver nitrate solution (1.11 mM), and the final pH was adjusted to 6.8 using a phosphate buffer. Then, the culture supernatant was added to the above solution with the volume ratio of 1:100 (supernatant:solution). Finally, the reaction mixture was incubated at 50 °C and agitated at 200 rpm for 24 h to complete the biosynthesis of the nanoparticles.

2.4. Dinitrosalicylic acid (DNS) method

To evaluate starch hydrolysis (quantification), the reaction mixture containing 1.25 mL of a 1% starch solution, 0.25 mL of a 0.1 M phosphate buffer (adjusted to a different pH), 0.25 mL of distilled water, and 0.25 mL of the culture supernatant was prepared (Ramachandran et al., 2004). After 10 min of incubation at 50 °C, the reducing sugars (glucose equivalents) were estimated by Miller's dinitrosalicylic acid (DNS) method (Miller, 1959). The absorbance of the colored solution was measured at 575 nm using a spectrophotometer (Cary 100, Varian). A glucose solution of known concentration was used as the standard for plotting a standard curve. The blank solution consisted of 1.25 mL of a 1% starch solution, 0.5 mL of a 0.1 M phosphate buffer (adjusted to a different pH), and 0.25 mL of distilled water.

2.5. Characterization of silver nanoparticles

2.5.1. UV–visible spectral analysis

The color changes of the reaction mixtures are evidence for AgNP formation. Therefore, 1-mL samples were withdrawn at various intervals, and the absorbance was measured by a double beam UV–visible spectrophotometer (Cary 100, Varian) at a resolution of 1 nm in the range 200–800 nm.

2.5.2. Dynamic light scattering (DLS)

The size distribution and average size of the synthesized AgNPs were determined by dynamic light scattering (DLS). DLS (Malvern, UK) measurements were carried out for size ranges from 0.1 nm to 10 μm.

2.5.3. Fourier-transform infrared spectroscopy (FTIR)

The characterization of functional groups on the surface of AgNPs was performed by Fourier-transform infrared spectroscopy (FTIR) (Perkin-Elmer, Germany), and the spectra were scanned in the 500–4000 cm^{-1} range at a resolution of 4 cm^{-1}.

2.5.4. X-ray diffraction (XRD)

X-ray diffraction was carried out on a Philips PW-1730 system operating at the Co Kα wavelength of 1.7889 Å, 30 mA, and 40 kV. Samples were prepared by casting silver solutions on a silicon substrate.

2.5.5. Transmission electron microscopy (TEM)

The morphology of the synthesized AgNPs was observed by a Zeiss EM-900 transmission electron microscope (TEM) operating at 50 kV. TEM samples were prepared by drop-casting a dispersion of AgNPs on carbon-coated copper grids, which were allowed to dry at room temperature.

3. Results and discussion

To optimize the pH and temperature of the starch hydrolysis process, the effects of the incubation temperature (25 °C, 35 °C, 50 °C, or 60 °C) and pH (5.5, 6.0, 6.4, 6.8, or 7.8) were investigated (Ramachandran et al., 2004). Fig. 1A and B shows the glucose concentration produced at the individual incubation temperatures and pH values, respectively.

As shown in Fig. 1A, the glucose concentration increased with increasing temperature to 50 °C, after which the concentration decreased with increasing temperature. Fig. 1B shows that starch hydrolysis was optimal at pH 6.8. These results are consistent with the study of Chary and Reddy (Chary & Reddy, 1985).

After identifying the optimum conditions for starch hydrolysis, the properties of the synthesized AgNPs, such as size and size distribution, were compared as well as the productivity of the preparatory methods.

3.1. UV–visible analysis

The preliminary detection of AgNPs was carried out by visual observation of the color changes of the reaction solutions. These changes were attributed to the excitation of surface plasmon resonance (SPR) in the metal nanoparticles (Natarajan, Selvaraj, & Murty, 2010). Typically, UV–visible absorption is used to investigate SPR. Initially, the influence of pH on the synthesis of AgNPs by the novel method was investigated by measuring UV–visible spectra of the reaction solutions at different pH values. As shown in Fig. 2, the peak area and height of the UV–visible spectrum obtained for the reaction solutions at pH 6.8 were considerably higher than those at other pH values, which is an indication of the higher productivity of the method at this pH. Hence, the pH of 6.8

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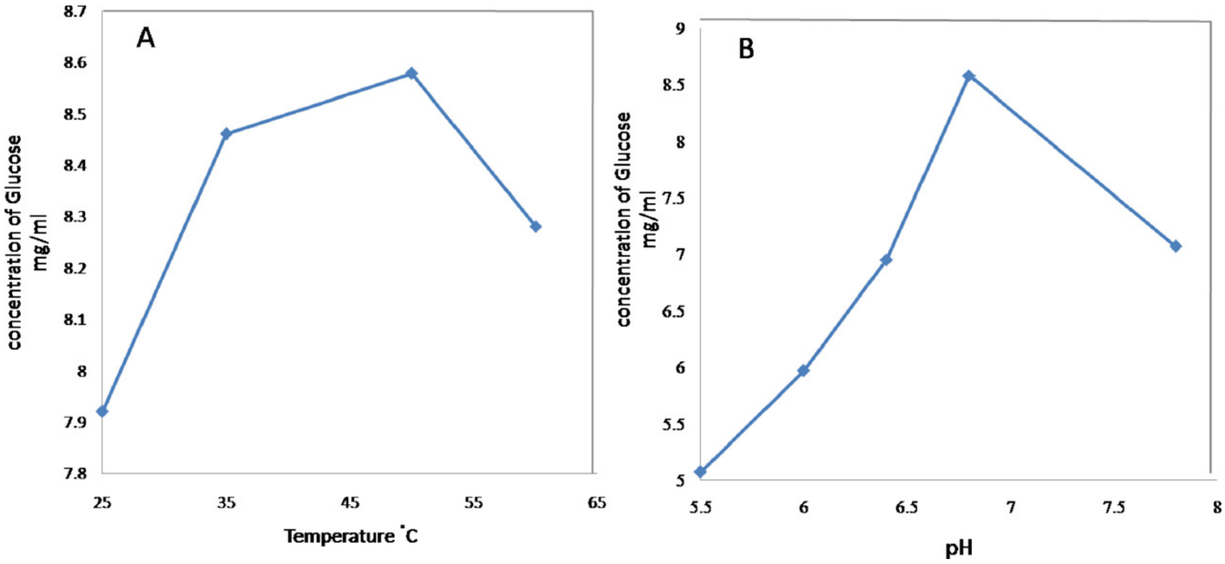

Fig. 1. Concentrations of produced glucose in reaction mixture of starch and fungal supernatant at different (A) temperatures and (B) pH from starch hydrolysis by α-amylase which present in fungal supernatant (analyzed by DNS method).

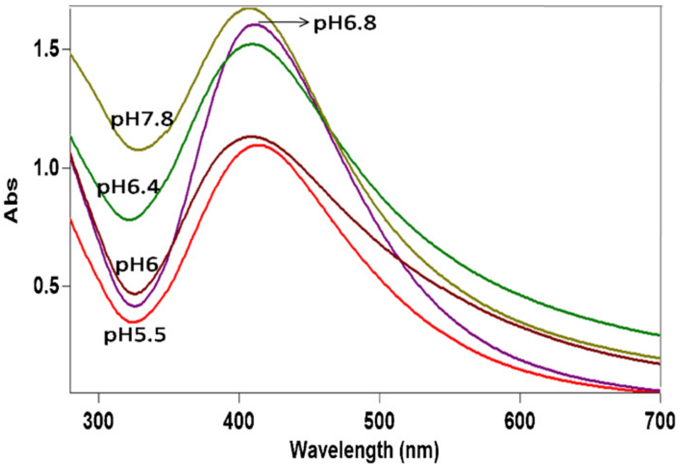

Fig. 2. UV–visible spectra of colloidal AgNPs produced at constant temperature of 50 °C and different pH of 5.5, 6, 6.4, 6.8 and 7.8 by combinatorial method.

was selected as the optimum pH for the synthesis of AgNPs using our method. These results revealed that the efficiency of the synthesis of AgNPs depends on the concentration of the generated glucose as the reducing agent, and consequently, on starch hydrolysis.

The UV–visible spectra of the AgNPs synthesized by the modified polysaccharide, microbial, and novel methods are presented in Fig. 3A. As shown in the figure, a strong SPR is observed near 416, 427, and 412 nm for the modified polysaccharide, microbial, and novel methods, respectively. These bands are considered to be in the ideal wavelength range for AgNP colloidal solutions (Vigneshwaran, Nachane, Balasubramanya, & Varadarajan, 2006). SPR is affected by the size and shape of the synthesized AgNPs. On the other hand, the lower-wavelength region implies the formation of smaller nanoparticles (Prathna, Chandrasekaran, Raichur, & Mukherjee, 2011). Thus, Fig. 3A indicates that the use of the novel method results in the formation of the smallest AgNPs.

The absorbance intensity provided insight into the reduction of Ag^+ and productivity of each method (Maliszewska, Szewczyk, & Waszak, 2009). As shown in Fig. 3A, the largest plasmon resonance peak was observed for the modified polysaccharide method. Therefore, it has higher productivity than the others. However, our method was more productive than the microbial method. Also, the plasmon band observed for the novel method was symmetrical. This can be attributed to a narrower size distribution of the particles formed using this method.

The stability of the AgNPs synthesized by the novel method was assessed by measuring absorbance intensities of the solutions following reaction completion over a period of 3 months at room temperature. Fig. 3B shows no significant change in the absorbance

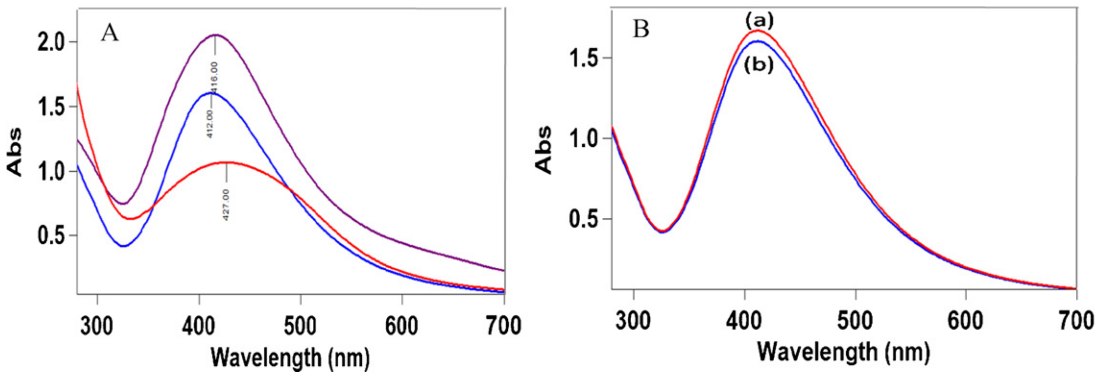

Fig. 3. (A) Comparison of capability of microbial, modified Polysaccharide and novel combinatorial methods in AgNPs synthesis, (B) UV–visible spectra of colloidal AgNPs synthesized by novel method (a) after reaction termination; (b) after 3 months storage at room temperature in shelf.

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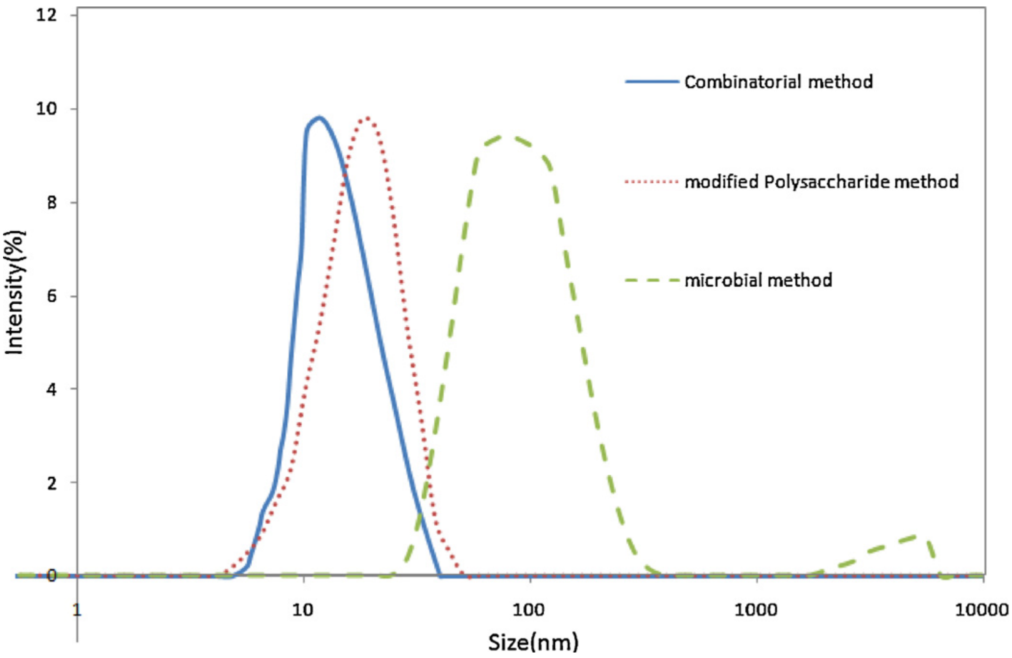

Fig. 4. DLS analysis of colloidal AgNPs generated by microbial, modified Polysaccharide and combinatorial methods.

intensity and wavelength of the colloidal AgNPs after this period. This observation verified that the colloidal AgNPs solution was extremely stable with no evidence of particle agglomeration.

3.2. Particle size analysis

The average particle size, size distribution, and polydispersity index (PDI) of the synthesized AgNPs were determined by DLS, and the results are shown in Fig. 4. As indicated, the average sizes of the AgNPs produced by the microbial, modified polysaccharide, and novel methods were 84, 20, and 15 nm, respectively. The PDIs of the AgNPs were 0.3, 0.24, and 0.16 for the microbial, modified polysaccharide, and novel methods, respectively. A comparison of the average particle size and PDI for all methods revealed that our method produced the smallest AgNPs with the highest monodispersity. These results are in agreement with the UV–visible analysis.

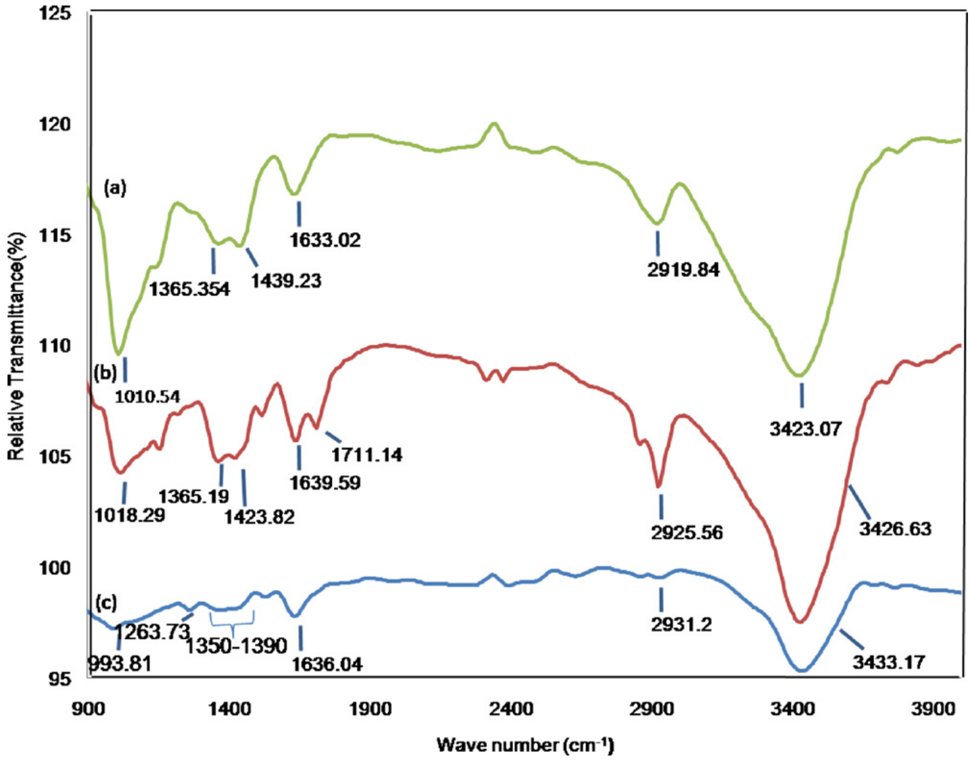

Fig. 5. Comparison of FTIR spectra for AgNPs synthesized by (a) microbial; (b) modified Polysaccharide; and (c) combinatorial methods.

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3.3. Fourier-transform infrared spectroscopy (FTIR)

As shown in Fig. 5, the FTIR spectrum of the AgNPs synthesized by the microbial method shows absorption bands associated with the O—H stretch of the carboxylic acid groups, the C—H stretch of the methylene groups of the protein, the N—H bend of primary amines, and the N—H vibration of the amide III groups at 3433.17, 2931.20, 1636.04, and 1263.73 cm^{-1}, respectively (Bozanic, Trandafilovic, Luyt, & Djokovic, 2010; Kumar & Mamidyala, 2011; Mahitha et al., 2011). Also, the C—N absorption band at 1350–1390 cm^{-1} overlaps with the residual NO_3^-, and the weak broad band at 990–1100 cm^{-1} may represent the C—OH bending vibrations due to proteins. Thus, the carbonyl group of the amino acid residues and proteins has a stronger ability to bind with the metal and could possibly perform dual functions in the formation and stabilization of the AgNPs synthesized by the microbial method (Mahitha et al., 2011).

The FTIR spectrum of the AgNPs synthesized by the modified polysaccharide method shows O—H (carboxyl), C═O, and C—OH stretching bands, corresponding to a number of bands at 3423.07, 1633.02, and 1010.54 cm^{-1}, respectively. The absorption bands at 2919.84, 1439.23, and 1360 cm^{-1} are related to the C—H stretching bands in starch. The FTIR spectrum reveals that the stabilization of AgNPs could be due to the O—H, C—H, and C—O functional groups of starch.

The FTIR spectrum used to evaluate the novel method is the combination of FTIR results obtained from two other samples. The main difference between the spectra associated with the novel method and those from the other two methods was the appearance of the peak at 1711.14 cm^{-1}. This reveals the possible oxidation of a hydroxyl group from starch to a carboxyl group (Kassaee, Akhavan, Sheikh, & Beteshobabrud, 2008). Also, a comparison of the FTIR spectrum of the samples prepared by the novel method with that of the products from the microbial method shows the disappearance of the absorption band from residual NO_3^-, which may indicate the complete reduction of Ag^+ to Ag^0. Finally, the results revealed that in the combinational method, stabilizing and reducing agents are more effective than those in the other two methods owing to the presence of different functional groups, such as O—H, N—H, C—N, C—H, C—O, and C═O.

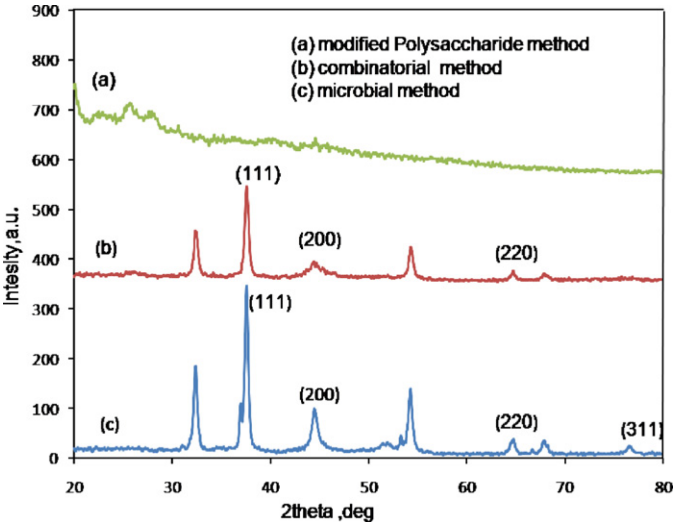

Fig. 6. FTIR analysis of AgNPs generated by microbial, modified polysaccharide and combinatorial methods.

3.4. X-ray diffraction (XRD)

AgNPs are embedded within soluble starch in the modified polysaccharide method; thus, the diffraction intensity of silver is very low, and these nanoparticles could be considered as a non-crystalline species (Fig. 6a). The broad peaks observed at 22, 25, 28, and 40 in the products of the modified polysaccharide method are due to the low crystalline soluble starch. AgNPs produced by the novel method (Fig. 6b) have peaks with 2θ values of 37.605, 44.505, and 64.692, whereas AgNPs formed by the microbial method (Fig. 6c) have peaks with 2θ values of 37.590, 44.524, 64.715, and 76.600 that are assigned to the (1 1 1), (2 0 0), (2 2 0), and (3 1 1) reflection planes of face-centered-cubic (fcc) silver, respectively (Kassaee et al., 2008; Vasileva et al., 2010). As shown, the XRD peaks of the AgNPs from the novel method are broader than those of the AgNPs from the microbial method. This confirms that the size of the AgNPs produced by the novel method is smaller than that of the AgNPs produced by the microbial method. This may be due to the generation of smaller nanoparticles by the novel method (Eid, 2011).

3.5. Transmission electron microscopy (TEM)

Fig. 7 shows TEM images of AgNPs formed by the modified polysaccharide and novel methods. The shape of the AgNPs from both methods is predominantly spherical. As shown, the size distribution of the products of these two methods is relatively the same, but the size of the AgNPs produced by the novel method is

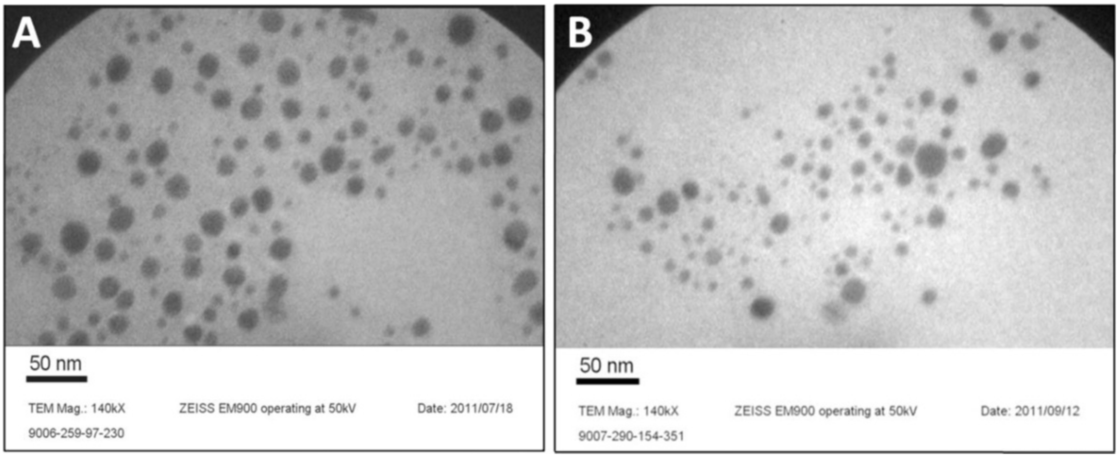

Fig. 7. TEM images of colloidal AgNPs synthesized by (A) modified polysaccharide; (B) combinatorial methods.

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Table 1
Comparison of AgNPs synthesis by modified polysaccharide, microbial and combinatorial methods.

Method	Reducer	Stabilizer	Wavelength Of SPR band (nm)	Average size (nm)	Satiability	Crystallinity	Size distribution (PDI[a])
Modified polysaccharide	β-D-Glucose	Starch	416	20	Two months	Low	0.24
Microbial	Supernatant	Supernatant	427	84	One months	High	0.3
Combinatorial	Supernatant & starch	Supernatant & starch	412	15	Three months	High	0.16

[a] PDI, polydispersity index

smaller than that of the AgNPs from the modified polysaccharide method.

The characterization of the AgNPs synthesized using the modified polysaccharide, microbial, and novel methods is presented in Table 1.

4. Conclusion

This study presents a novel and environmentally benign method for the synthesis of AgNPs with excellent characteristics. The structural analyses (UV–visible, DLS, TEM, FTIR, and XRD) of the AgNPs synthesized by this method were compared with those of the AgNPs prepared by the modified polysaccharide and microbial methods. The results show that the novel method generated the smallest AgNPs (average size of 15 nm) with the narrowest size distribution. The AgNPs synthesized by this method were highly stable and did not show any aggregation even after storage for three months. Unlike the modified polysaccharide method, the novel method could produce AgNPs with high crystallinity. The FTIR spectra of the products from the microbial method revealed that the carbonyl groups of the amino acid residues and proteins were the major agents that are involved in the stabilization of the AgNPs. In the modified polysaccharide method, O—H, C—H, and C—O functional groups of the starch molecules act as stabilizers. All the groups mentioned above are present in the products from the novel method and these components amplified the stability of the resultant AgNPs.

Acknowledgments

The authors wish to acknowledge Tarbiat Modares University of Iran (TMU) and Iranian Nanotechnology Initiative Council for their financial support towards major research projects.

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