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Silver-carboxymethyl glucomannan nanocomposites: Synthesis and its antibacterial activities

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ABSTRACT

Silver-O-carboxymethyl glucomannan nanocomposites (Ag-CMG nanocomposites) were synthesized via reduction of $[Ag(NH_3)_2]OH$ by $NaBH_4$ and HCHO reductant agents in the presence of water-soluble carboxymethyl glucomannan as a stabilizer. The characteristics of Ag-CMG nanocomposites were investigated by transmission electron microscopy (TEM) and UV-vis spectra. The results shown that both $NaBH_4$ and HCHO reductant agents could be used effectively for preparing the Ag-CMG nanocomposites, and carboxymethyl glucomannan substrate could be used as a good stabilizer for nano-system. The average particle size of silver nanoparticles was ranged between 2÷25nm and 10÷20nm when reductive agents were $NaBH_4$ and HCHO, respectively. The characteristic surface plasmon resonance band of silver nanoparticles in the two cases centered at about 384÷425nm. In vitro antibacterial activities of Ag-CMG nanocomposites were evaluated against *Escherichia coli (Ec); Proteus mirabilis (Pro); Shigella flexneri (Shi); Salmonella typhi (Sal) and Pseudomonas aeruginosa (Pseu),* which are Gram-negative bacteria, and *Staphylococcus aureus* ATCC 1128 (*Sta*); *Bacillus cereus* ATCC 9946 (*Bc*); which are Gram-positive bacteria. The results shown that the Ag-CMG nanocomposites could inhibit the growth and multiplication of the tested bacteria, especially to Ag-CMG with smaller particles.

KEYWORDS

Sliver;
Carboxymethyl glucomannan;
Nanoparticles;
Antibacterial;
Nanocomposites.

INTRODUCTION

In recent years, silver nanoparticles have attracted much attention due to their diminutive size and novel material properties. With their nanometer scale size, which was responsible for different properties concerning the bulk material renders them suitable for applications. Therefore, many approaches have been used to prepare silver nanoparticles for a rapidly growing list of catalysis, electronic, non-linear optics and biomaterial applications. As biomaterials, colloidal silver solutions have an increased interest due to their antibacterial properties, with large applications including pharmacology, human and veterinary medicine, food industry, water purification[11]. For a long time silver has been known to have a disinfecting effect and has found applications

in traditional medicines culinary items[13] silver has known to be a metal that come into use even before neolithic revolution. Thus nanoparticles of silver have aptly been investigated for their antimicrobial properties[9]. Nanoparticles of silver have thus been studied as a medium for antibiotic delivery[8]. Antimicrobial activity of silver nanoparticles have been studied by various researchers especially on *E. coli, S. aureus*[5, 6].

Due to the above important applications, the synthesis of silver nanoparticles still need to study for enlarge ability of its applications. Reality, a large variety of chemical processes is involved in the preparation of silver nanoparticles with a well controlled size. Many reports have addressed silver ions reduced by reductants and stabilizing or protecting agents to prevent these nanoparticles from agglomeration[3, 4, 7]. In process for preparing of silver nanoparticles, the stabilizer play an important role in preventing naoparticles from aggregating. So polysaccharides such as chitosan have been used for preparation of nanocomposites. Due to the interaction between the amino groups in chitosan and metal nanoparticles, chitosan was chosen as a protecting agent in synthesis of silver nanoparticles. Paul et al. investigated the effect of the reducing sugar of dextran, another kind of polysaccharide, on the formation and stability of dextran-coated ultra-small superparamagnetic iron oxides; they demonstrated that reduction of the terminal reducing sugar could have a significant effect on particle size, coating stability, and magnetic properties[10].

Although, the synthesis of silver nanoparticles using carboxymethyl glucomannan substrate as a stabilizer has not been reported elsewhere. For this reason, the aims of this work were to synthesize and characterize of silver-CMG nanocomposites and to evaluate the its antibacterial activities in vitro.

EXPERIMENTAL

Materials

Carboxymethyl glucomannan was synthesized using the method mentioned in reference[1]. Sodium hydrogen carbonate, silver nitrate, ammonia, formic aldehyde and sodium borohydride were purchased from Merck (Germany). All other chemicals and reagents used in experiments were of analytical grade.

Bacterial strains both gram positive: *Staphylococcus aureus* ATCC 1128 (Sta); *Bacillus cereus* ATCC 9946 (Bc); and gram negative: *Escherichia coli* ATCC 25922 (Ec); *Proteus mirabilis* BV 108 (Pro); *Shigella flexneri* DT 112 (Shi); *Salmonella typhi* DT 220 (Sal); *Pseudomonas aeruginosa* VM 201 (Pseu) which were multi drug resistant strains provided by Hanoi Pharmaceutical University-Vietnam cultures. These bacteria were maintained at 4°C on nutrient agar slants.

Synthesis and characterization of silver-carboxymethyl glucomannan nanocomposites

A solution of CMG (1g/100ml) in distilled water with stirring until a clear solution was obtained. $[Ag(NH_3)_2]OH$ aqueous solution was prepared by reaction of $AgNO_3$ solution with an excess amount of NH_3 solution. The preparation of silver-CMG nanocomposites was quite simple; in general, silver nanoparticles were obtained by chemical reduction of silver complexes to yield the corresponding zero valent silver nanoparticles with $NaBH_4$ or aldehyde. In a typical procedure, a 0.1ml of 1mM $[Ag(NH_3)_2]OH$ aqueous solution was mixed with 100ml of 1.0g/100ml of CMG, the mixture was stirred for 30 min, then aqueous solution of $NaBH_4$ (0.5ml, 0.2mM) was added quickly to the mixture, the reaction was carried out at room temperature and kept stirring for another 30min until the entire reduction of silver complexes (in the case HCHO (0.5ml, 0.2M) reductive agent was used, the reaction was carried out at 70°C). The silver-CMG nanocomposites were collected by precipitation and washing many times with 90% ethanol solution. Then, silver-CMG nanocomposites were redissolved in distilled water for obtaining colloidal silver solutions. The nanocomposites obtained were kept at room temperature for further characterization.

Transmission electron microscopy of Ag-CMGs were carried out with a JEOL-2000EX TEM operating at 80 kV. Specimens for inspection by TEM were prepared by slowly evaporating one drop of prepared nanocomposites at room temperature on a 400 mesh copper grid, which was covered by a carbon supported film. UV-vis absorbance spectra of Ag-CMGs were collected using a UV-vis spectroscopy, Cintra 40 GBC, Shimadzu Co., Japan.

Evaluation of antibacterial activity in vitro of Ag-CMGs

The Ag-CMG nanocomposites were tested against multi drug resistant strains by diffusion method according to the method of[12]. Zones of inhibition were measured after 24 h of incubation at 35°C. The comparative stability of discs containing penicillin was made.

RESULTS AND DISCUSSION

Synthesis and characteristics of Ag-CMG nanocomposites

The structure of the carboxymethyl glucomannan is like carboxymethyl cellulose, which possesses a lot of negative carboxyl groups. So when $AgNO_3$ was mixed with carboxymethyl glucomannan solution, Ag^+ ions could be bound to carboxymethyl glucomannan macromolecules probably via electrostatic interactions. This reason would make carboxymethyl glucomannan precipitate and caused difficulty for synthesizing silver nanoparticles. However, this could be overcome by using diamine silver (I) precursor complex ($[Ag(NH_3)_2]OH$) instead of $AgNO_3$. By forming complex with ammonia molecule, the electrostatic interactions of positive silver ions with negative carboxyl groups was reduced. Therefore, the system was in homogeneous state, so the reductive reaction of silver ions occurred easily for forming silver nanoparticles. Due to the high viscosity of carboxymethyl glucomannan, nanosystem obtained was quite stable for severe months without aggregations observed.

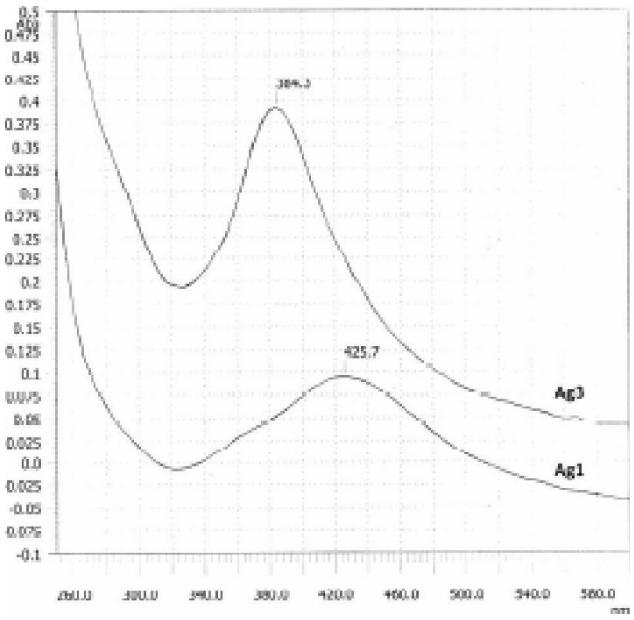

Figure 1 : UV-vis spectra of Ag-CMG nanocomposites: Ag1 (1mM) and Ag3 (20mM), using NaBH4 reductive agent.

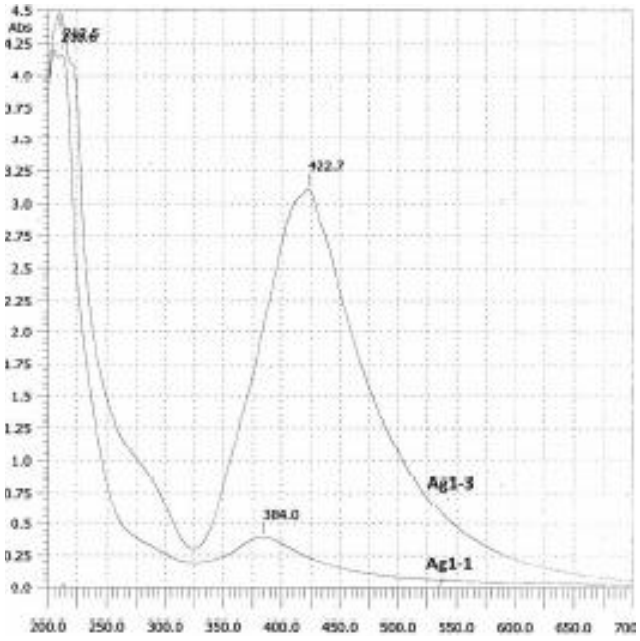

Figure 2 : UV-vis spectra of Ag-CMG nanocomposites: Ag1-1 (1mM) and Ag1-3 (20mM), using HCHO reductive agent.

The UV-vis absorption spectra of the Ag-CMGs were shown in the Figure 1 and Figure 2.

Figure 3 and Figure 4 shown typical TEM images of Ag-CMGs nanocomposites. The images revealed monodisperse of silver nanoparticles, which were homogeneously distributed in the nanocomposites. The particles were found to be spherical with a narrow size distribution; the average particle diameter was about 2÷20 nm ($NaBH_4$ reductant agent) and 5÷30nm (HCHO reductant agent). Silver nanoparticles in these nanocomposites were very stable and no aggregation of silver nanoparticles were observed by TEM after 6 months.

As shown in Figure 3, when $NaBH_4$ was used as a reductant agent, virtually highly dispersed silver nanoparticles were obtained at 1mM $[Ag(NH_3)_2]OH$. And at a relatively low concentration of $[Ag(NH_3)_2]OH$, silver nanoparticles with a bimodal size distribution were obtained (the particle size was about 2÷5nm). At 10mM and 20mM $[Ag(NH_3)_2]OH$, the particle size increased dramatically, some silver clusters could be observed in addition to spherical silver nanoparticles. Therefore, it was possible to control the size and size distribution by

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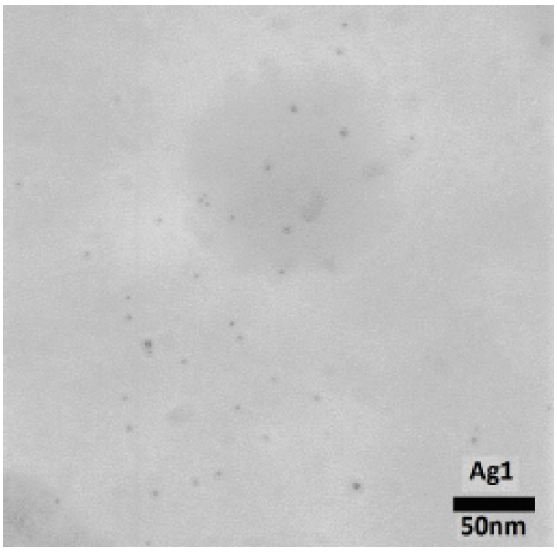
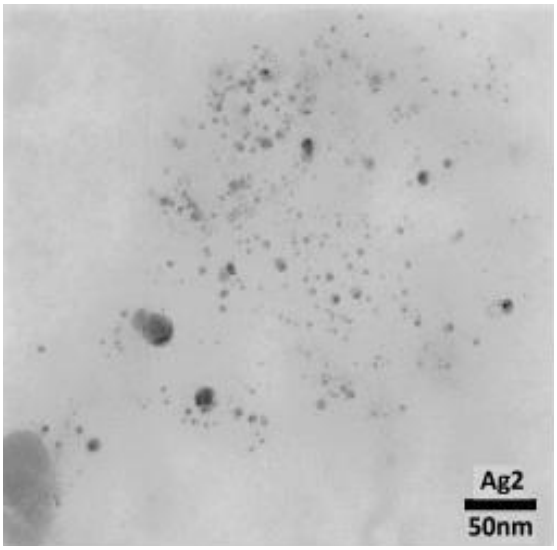




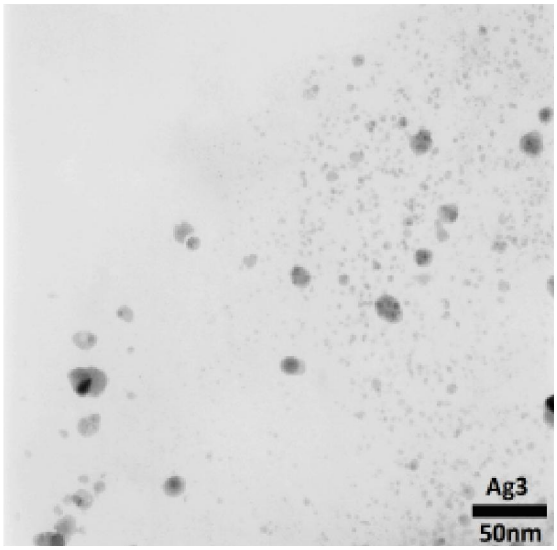
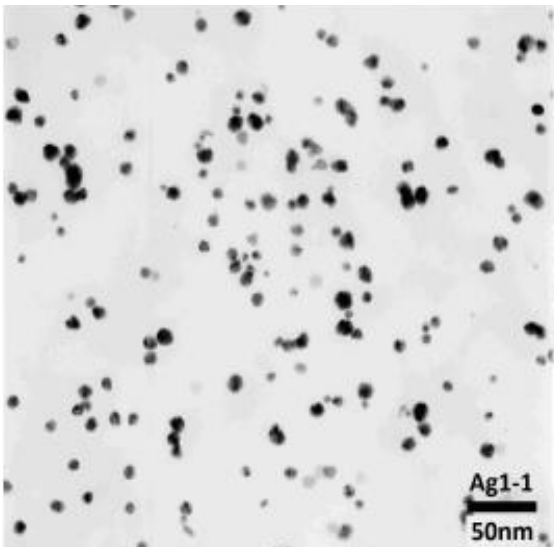
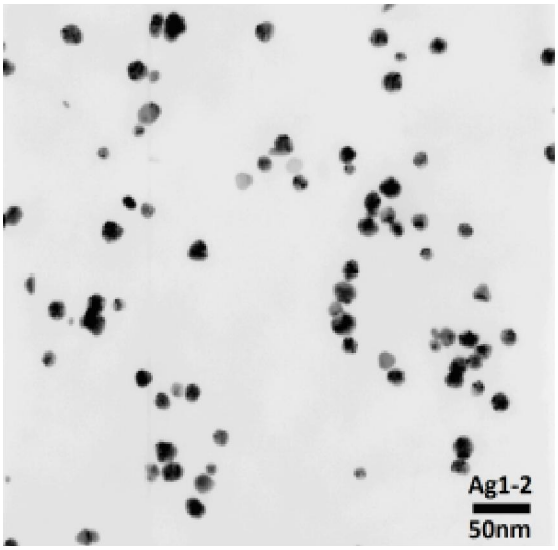


Figure 3 : TEM images spectra of Ag-CMG nanocomposites: Ag1 (1mM); Ag2 (10mM) and Ag3 (20mM), using $NaBH_4$ reductive agent.





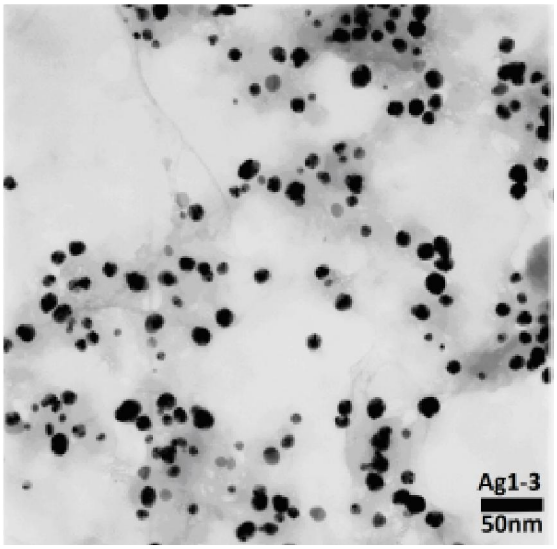


Figure 4 : TEM images spectra of Ag-CMG nanocomposites: Ag1-1 (1mM); Ag1-2 (10mM) and Ag1-3 (20mM), using HCHO reductive agent.

adjusting the concentration of silver ions in solution. Meanwhile, when HCHO was used as a reductant agent, virtually highly dispersed silver nanoparticles was also obtained at 1mM $[Ag(NH_3)_2]OH$ (the particle size was ranged between 10÷15nm). At 10mM and 20mM $[Ag(NH_3)_2]OH$, the particle size of silver nanoparticles increased slightly. In this case, the particle size was about 15÷20nm and there were no clusters to be observed. Thus, the average particle size of silver nanoparticles obtained from reducing silver ion by HCHO agent was slightly affected with the concentration of $[Ag(NH_3)_2]OH$ added.

Antibacterial activity of silver-carboxymethyl glucomannan nanocomposites

The antibacterial activities of penicillin and various Ag-CMGs with the bacterial strains were shown in TABLE 1.

TABLE 1 : Zone of inhibition of Ag-CMG nanocomposites and penicillin

Samples	Diameter of inhibition (mm)						
	Bc	*Sta*	*Ec*	*Shi*	*Pro*	*Pseu*	*Sal*
Ag1	11.87	10.46	11.55	11.73	12.84	13.88	11.89
Ag3	10.93	9.080	10.94	11.26	12.40	13.02	10.07
Ag1-1	10.77	11.13	10.64	12.37	12.17	12.63	9.790
Ag1-3	10.67	10.47	10.40	11.93	11.07	9.60	9.070
Penicillin	17.17	21.37	16.72	12.56	18.12	10.33	17.31

It was found that these samples shown effectively antibacterial activities against seven bacterial trains, which were used in the test, although differences existed among them. Generally, the samples had more effective inhibition on *Shi* and *Pseu* than the others. However, the antibacterial activities of Ag-CMG nanocomposites for seven above bacteria were less than that of penicillin. The results also shown that the size particles of silver nanoparticles increased the antibacterial activity decreased. The mechanism of the bactericidal effect of Ag-CMGs against bacteria was not very well-known. Silver nanoparticles might attach to the surface of the cell membrane and disturbed its power function such as permeability and respiration. It was reasonable to state that the binding of the particles to the bacteria depended on the surface area available for interaction. Smaller particles having the larger surface area available for interaction would give more bactericidal effect than the larger particles.

CONCLUSIONS

Silver-carboxymethyl glucomannan nanocomposites have been synthesized via the reduction of silver ions by both $NaBH_4$ and HCHO agent in the presence of carboxymethyl glucomannan as a stabilizer. The Ag-CMGs were characterized by TEM and UV-vis spectra. The results shown that the average particle size of silver nanoparticles was ranged between 2÷25nm and 10÷20nm when reductive agents were $NaBH_4$ and HCHO, respectively. The characteristic surface plasmon resonance band of silver nanoparticles in the two cases centered at about 384÷425nm. The antibacterial activity of Ag-CMG nanocomposites against seven bacterial strains of both gram positive and gram negative strains was tested in vitro. The results of this study clearly demonstrated that the Ag-CMGs nanocomposites could inhibit the growth and multiplication of the tested bacteria, especially to Ag-CMG with smaller particles.

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