Original Article

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Silver nanoparticles fabricated by reducing property of cellulose derivatives

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Summary **The aim of this study was to synthesize silver nanoparticles (AgNPs) by using cellulose derivatives as a reducing agent. Methyl cellulose (MC), hydroxy ethylcellulose (HEC), and hydroxypropyl methylcellulose (HPMC) were compared for their reducing property. HPMC presented the highest reducing power, with equilibrium concentration (EC) of 84.6 ± 4.5 µmol Fe^{2+}/g, followed by MC and HEC, with the EC of 62.3 ± 1.4, and 38.1 ± 3.2 µmol Fe^{2+}/g, respectively. Using these cellulose derivatives as a reducing agent and silver nitrate as a precursor in fabrication of silver nanoparticles (AgNPs), three cellulose-AgNPs, HEC-AgNPs, MC-AgNPs, and HPMC-AgNPs, were obtained. The cellulose-AgNPs showed different maximum absorptions confirming AgNPs spectra at 415, 425, and 418 nm, respectively. Reaction parameters such as pH, temperature, and period of reaction affected intensity of the maximum absorptions and size of AgNPs. Using 0.3% cellulose solution at pH 9 and reaction at 70°C for 90 min, the particle size of MC-AgNPs, HEC-AgNPs, and HPMC-AgNPs was 97.7 ± 2.4, 165.6 ± 10.6, and 51.8 ± 1.6 nm, respectively. AgNPs obtained from different cellulose derivatives and various preparation parameters possess different inhibition potential against *Escherichia coli* and *Staphylococcus aureus*. The cellulose-AgNPs have higher effective against *E. coli* than *S. aureus*. HPMC-AgNPs showed significantly higher antibacterial activity than MC- AgNPs and HEC-AgNPs, respectively. These results suggest that the type of cellulose derivatives and the reaction parameters of the synthesis such as pH, temperature, and reaction period play an important role to the yield and physicochemical property of the obtained AgNPs.**

Keywords: Cellulose derivatives, HPMC, green synthesis, AgNPs, antibacterial activity

1. Introduction

Silver nanoparticles (AgNPs) receive increase interest in application for many fields (*1*) due to their efficiency in antibacterial (*2*), antifungal (*3*), antiviral (*4*), anticancer (*5*), and antioxidant activities (*6*). Synthesis of AgNPs can be generally performed by reacting silver salts with certain reducing agents from natural such as plant extracts (*7*) or chemically synthesized agents (*8*). The reducing agents from natural resources have gain increasing interest since there are less hazardous waste than those from chemical synthesis. Polysaccharides are one of the natural interesting groups that some of them, *e.g*. starch, dextran, and cellulose were used as a reducing agent for synthesis of metal nanoparticles. For example, our group previously reported the use of starch derivatives from rice for synthesis of AgNPs (*9*). Bankura *et al*. reported the use of dextran to synthesize gold nanoparticles (*10*). For cellulose, there are some reports on using cellulose derivatives such as methylcellulose and carboxymethyl cellulose to synthesize AgNPs (*11-13*). However, there is still less report on factors affecting the obtained AgNPs synthesized by using cellulose derivatives as a reducing agent as well as the comparison of reducing efficiency among many types of cellulose derivatives.

Cellulose is an organic polysaccharide consisting

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of a linear chain of D-glucose units. It is insoluble in organic solvents and water, however, it can be modified to yield cellulose derivatives in order to increase its water solubility (*14*). Cellulose derivatives can be categorized according to their chemical structure into ether and ester groups. Examples of cellulose ether derivatives are methyl cellulose (MC), hydroxyethyl cellulose (HEC), and hydroxypropyl methylcellulose (HPMC) whereas that of cellulose ester derivatives are cellulose acetate, cellulose acetate trimellitate, and cellulose acetate phthalate. Cellulose ester derivatives are less water soluble, therefore, they are commonly used as drug delivery systems in the form of membrane (*15*), nanocrystal (*16*), and fiber (*17*). Comparison between these two groups of cellulose derivatives, those in the ether form are of higher interest as they are more water soluble than those in the ester group. In the present study, three types of cellulose ether derivatives, MC, HEC, and HPMC were used. They are odorless and tasteless, white to slightly off-white, fibrous or granular, free-flowing powder. They were firstly compared for the reducing power by using ferric reducing antioxidant power (FRAP) assay. Synthesis of AgNPs then was prepared from these three cellulose derivatives using silver nitrate ($AgNO_3$) as a precursor. Effects of preparation parameters such as pH, temperature, and duration of reaction were investigated. Three kinds of cellulose-AgNPs, MC-AgNPs, HEC-AgNPs, and HPMC-AgNPs, were obtained from the respective cellulose derivatives. Physicochemical properties of the obtained cellulose-AgNPs were characterized using UV-visible spectrophotometer (UV-vis) and photon correlation spectrophotometer (PCS). The inhibitory activity of AgNPs from the most suitable reacting condition were selected to test against Gram-positive and Gram-negative bacteria. The antibacterial activity was evaluated by measuring the inhibition zones, minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC).

2. Materials and Methods

2.1. *Materials*

MC, HEC, and HPMC were purchased from S. Tong Chemicals Co., Ltd (Bangkok, Thailand). $AgNO_3$ was supplied by RCI Lab-scan Co., Ltd. (Bangkok, Thailand). Ferrous sulfate ($FeSO_4$) and 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) were purchased from Sigma-Aldrich, Inc (St. Louis, MO, USA). Tryptic soy agar (TSA) and tryptic soy broth (TSB) were supplied by Difco™ (Baltimore, MD, USA). All other chemicals and solvents were of AR grade or the highest grade available.

2.2. *Microbial strain*

The aerobic bacterial strains of *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 represented for Gram-positive and Gram-negative bacteria, respectively, were used.

2.3. *Reducing property of the cellulose derivatives*

The reducing power of MC, HEC, and HPMC was determined by using FRAP assay as described previously (*18*) with some modification. Briefly, the FRAP reagent was prepared by mixing 2.5 mL of 10 mM TPTZ solution in 40 mM HCl with 2.5 mL of 20 mM $FeCl_3$ and 25 mL of 0.3 M acetate buffer, pH 3.6. Cellulose derivative solutions having cellulose concentrations of 0.05-0.4% (w/v) were prepared. An amount of 20 µL of each cellulose derivative solution was mixed with 180 µL of FRAP reagent in 96 well plate. Blank samples were prepared by mixing acetate buffer and different concentration of MC, HEC, and HPMC. The test samples and blank were incubated for 10 min at room temperature, then the absorbance was determined at 595 nm using microplate reader (Bio-Rad, Model 680, Hercules, CA, USA). The reducing power of the samples was evaluated by calculating the amount of Fe^{2+} produced by cellulose derivatives samples using the calibration curve of $FeSO_4$. All experiments were run in triplicate.

2.4. *Synthesis of cellulose-AgNPs*

2.4.1. *Effect of pH*

Aqueous solutions containing 0.3% (w/v) of MC, HEC, and HPMC were prepared and the pH was adjusted to 3, 5, 7, 9, and 12 by using 1 M NaOH and 1 M HCl. Then, 10 mM $AgNO_3$ solution was added dropwise to the cellulose solutions at 50ºC with continuous stirring until the volume ratio of the cellulose solution and $AgNO_3$ solution was 100:1. The reaction was kept at this temperature under continuous stirring for 60 min. The obtained cellulose-AgNPs were cooled down to room temperature for further studies.

2.4.2. *Effect of temperature*

Aqueous solutions containing 0.3% (w/v) of MC, HEC, and HPMC were prepared and adjusted to pH 9. Then, 10 mM $AgNO_3$ solution was added dropwise to the cellulose solution with continuous stirring until the volume ratio of the cellulose solution and $AgNO_3$ solution was 100:1. The reaction temperature was studied at 28, 50, 70, and 90°C under continuous stirring for 60 min. The obtained cellulose-AgNPs were cooled down to room temperature for further studies.

2.4.3. *Effect of reaction period*

An aqueous solution containing 0.3% (w/v) of MC, HEC, and HPMC was prepared. Then, 10 mM $AgNO_3$

solution was added dropwise to the cellulose solution at 70ºC with continuous stirring until the volume ratio of the rice solution and $AgNO_3$ solution was 100:1. The reaction was kept at this temperature under continuous stirring for 15, 30, 60, 90, and 120 min. The obtained cellulose-AgNPs from each reaction period were cooled down to room temperature for further studies.

2.5. *Characterization*

2.5.1. *UV-vis*

The dispersion of cellulose-AgNPs obtained from each preparation condition was diluted to 100 fold with deionized water. Outer color appearance of cellulose-AgNPs was observed by visualization. Optical property of the cellulose-AgNPs solution was observed by using UV-vis (Shimadzu-2450, Kyoto, Japan) in the wavelength range of 200-700 nm.

2.5.2. *PCS*

The size, size distribution, and zeta potential of cellulose-AgNPs were investigated using PCS (Malvern Zetasizer Nano ZS, Malvern instrument, Worcestershire, UK) at 25ºC. Each sample was diluted to 100 fold with deionized water before measuring.

2.6. *Evaluation of antimicrobial activity*

2.6.1. *Well diffusion method*

A well diffusion used for investigating antibacterial activity was based on Kirby-Bauer method (*19*). The active test strains of *S. aureus* and *E. coli* grown in TSA at 37ºC for 24 h were diluted in TSB to a final concentration of 1.5×10^6 colony-forming units (CFU)/mL. Bacterial density was adjusted to 0.5 McFarland constant observed at 600 nm using UV-vis. The agar surface was spread with bacterial suspension by using sterile cotton swab. Aqueous solutions (40 µL) of HEC-AgNPs, MC-AgNPs, and HPMC-AgNPs were added into the 6-mm wells of the agar plates. The plates were incubated at 37ºC for 24 h. The antimicrobial activity was investigated by determining the diameter of the clear inhibition zone around the well expressed in millimeter (mm). All samples were done in triplicate.

2.6.2. *Broth dilution method*

In this experiment, the test bacterial suspensions were prepared as the same manner as the well diffusion method. Aqueous solutions containing 0.2 mg/mL of lyophilized cellulose-AgNPs were prepared in deionized water. These solutions were further diluted with TSB to obtained the 2-fold dilution series having cellulose-AgNPs concentration of 0.1, 0.05, 0.025, 0.0125, and 0.00625 mg/mL. Subsequently, 50 µL of these 2-fold dilutions were added into the 96-well plates containing 50 µL of TSB and 50 µL of the test bacterial suspension. The plates were incubated at 37ºC. After 24 h of incubation, the minimum cellulose-AgNPs concentration giving clear solution was recorded and this concentration was denoted as MIC. For MBC determination, the clear samples resulted from the MIC series were swabbed on the agar plates. The minimum cellulose-AgNPs concentration showing no bacterial growth in the agar plate was recorded and it was denoted as MBC (*20*). All samples were done in triplicate.

2.7. *Statistical analysis*

Data were analyzed using a One-way analysis of variance (ANOVA) and Duncan's multiple range test Statistic a software version 17 (SPSS Inc., Chicago, USA). The values were presented as means ± standard deviation which a *p*-value less than 0.05 was considered as a significant difference.

3. Results

3.1. *Reducing property of the cellulose derivatives*

The results showed that all three cellulose derivatives exhibited reducing power as a dose dependent manner as seen in Figure 1. Among them, HPMC showed the highest reducing activity of 84.6 ± 4.5 µmol Fe^{2+}/g sample whereas that of MC and HEC were 38.1 ± 3.2 and 62.3 ± 1.4 µmol Fe^{2+}/g sample, respectively. However, high concentration of cellulose derivative, such as 0.3 and 0.4 % (w/v), showed similar activity.

3.2. *Effect of pH*

The effect of pH on cellulose-AgNPs systems was clearly observed by color change. Increasing pH from

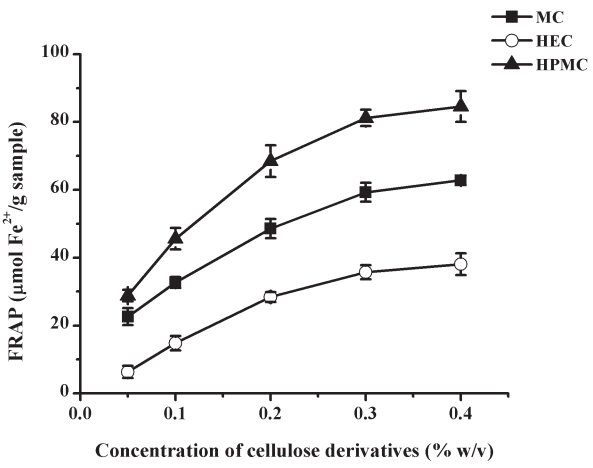

Figure 1. Reducing power of MC, HEC, and HPMC.

3 to 12, *e.g.* at pH 3, 5, 7, 9, and 12, the color of MC-AgNPs, after complete reaction period of 60 min, were light yellow, intense yellow, yellow-brown, brown, and dark brown, respectively. HEC-AgNPs systems were different. At pH 3 and 5, the systems of HEC-AgNPs appeared as mixtures of colorless solution with dark large particles precipitation. Whereas at pH 7, 9, and 12, HEC-AgNPs showed light yellow, yellow, and brown color, respectively. Interestingly, the systems of HPMC-AgNPs at pH 3, 5, 7, 9, and 12 showed light yellow, yellow, orange, red wine, and dark brown, respectively.

Measuring the effect of pH on the absorbance of MC-AgNPs, HEC-AgNPs, and HPMC-AgNPs, the results revealed that the maximum absorption of each system was at 415, 425, and 418 nm, respectively. The quantity of absorbance for each system at its respective maximum absorption was measured and the results are shown in Figure 2A. It was found that the absorbance of cellulose-AgNPs rapidly increased when pH was increased up to around pH 9. After pH 9, such as pH 9 and 12, the absorbance was not significantly different, indicating the systems reached the maximum absorption at about pH 9. Moreover, at pH 12, large precipitation was observed indicating that this pH causes instability of cellulose-AgNPs.

The size of the obtained cellulose-AgNPs was also affected by pH as shown in Figure 2B. The higher pH gave the smaller size until pH 9 that the systems showed the smallest size of the particles. The average particle size of cellulose-AgNPs obtained from the following preparing conditions; 0.3% (w/v) cellulose derivatives, period time at 60 min and 10 mM $AgNO_3$ was 117 ± 6, 183 ± 12, and 53 ± 2 nm for MC-AgNPs, HEC-AgNPs, and HPMC-AgNPs, respectively. The size distribution expressed by polydispersity index (PdI) of each system was not significantly different, *i.e.*, 0.18 ± 0.08, 0.21 ± 0.08, and 0.15 ± 0.04 for MC-AgNPs, HEC-AgNPs, and HPMC-AgNPs, respectively. These PdI values are acceptable as narrow size distribution. It was concluded that pH and type of cellulose derivatives showed the influence on absorption and size of cellulose-AgNPs. Among the obtained cellulose-AgNPs, HPMC-AgNPs showed the highest absorption and the smallest size. pH 9 is considered as the most suitable pH for preparing cellulose-AgNPs.

3.3. *Effect of temperature*

Keeping constant synthesized conditions such as cellulose and $AgNO_3$ concentration, pH of reacting medium, and reaction period, the effects of reaction temperature (at 28, 50, 70, and 90°C) was studied. The results showed that different temperature caused different manner of color changing. After complete fabrication at 90°C, the resulted systems of MC-AgNPs and HEC-AgNPs showed dark brown color whereas that of HPMC-AgNPs was red brown. Synthesizing of MC-AgNPs and HPMC-AgNPs at 28, 50, and 70°C, the color of the obtained systems was light yellow, intense yellow, and red wine, respectively. Whereas that of HEC-AgNPs was light brown, brown, and dark brown, respectively. After standing at room temperature for 24 h, aggregation and precipitation were observed in the cellulose-AgNPs systems obtained from reacting temperature of 28 and 50°C.

The intensity of absorbance of cellulose-AgNPs at their respective maximum absorption was recorded and the results are shown in Figure 3A. It was found that increasing reacting temperature caused the increase of the absorbance intensity of the obtained cellulose-AgNPs. The results revealed that at 90°C, all cellulose-

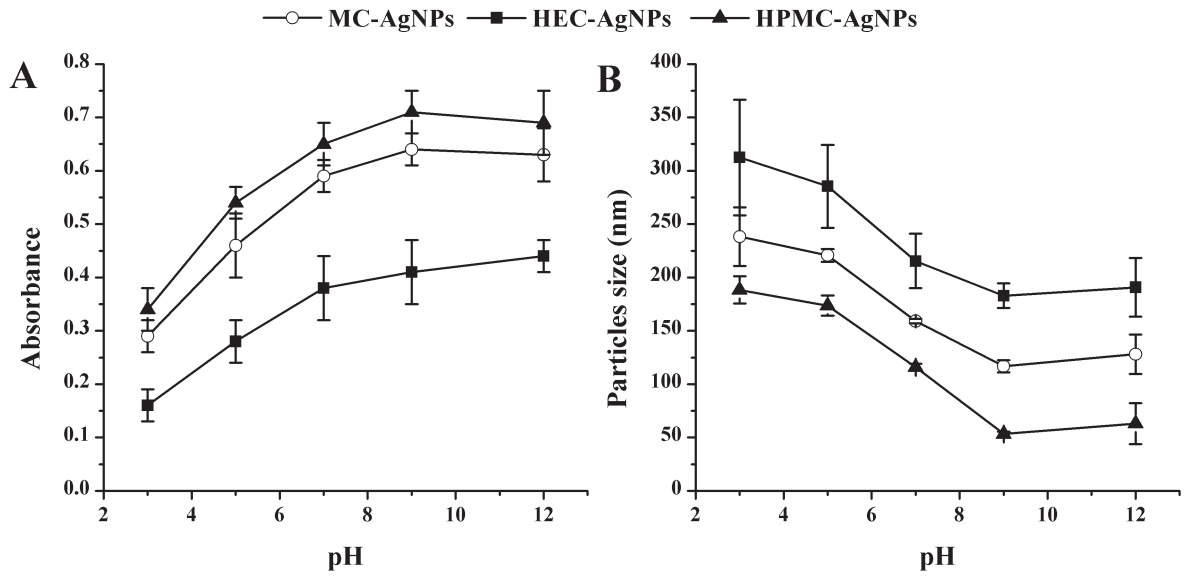

Figure 2. Effects of pH on intensity of maximum absorbance (A) and particle size (B) of cellulose-AgNPs.

AgNPs systems showed the highest intensity of absorption.

The effect of temperature on size and size distribution of the synthesized cellulose-AgNPs was investigated. The results explored that increasing synthesized temperature decreased the particles size of the obtained cellulose-AgNPs as shown in Figure 3B. The temperature at 90°C displayed the smallest size of cellulose-AgNPs. The average size of MC-AgNPs, HEC-AgNPs, and HPMC-AgNPs prepared at this temperature was 168 ± 2, 108 ± 3, and 45 ± 2 nm, respectively with PdI values of 0.19 ± 0.02, 0.21 ± 0.04, and 0.17 ± 0.06, respectively. From these results, the most suitable temperature for synthesizing cellulose-AgNPs was considered to be 90°C.

3.4. *Effects of reaction period*

The effect of reaction period on the synthesized cellulose-AgNPs was investigated using the most suitable pH (pH 9) and reacting temperature (90°C) obtained from the above results. The reaction period of 15, 30, 60, 90, and 120 min was studied. The obtained systems exhibited different physical properties including absorption intensity at different reaction period. The color of MC-AgNPs and MC-AgNPs was slightly brown whereas that of HPMC-AgNPs was slightly red. Standing at room temperature for 24 h, the systems of MC-AgNPs and HEC-AgNPs fabricated with reacting period of 15 and 30 min showed aggregation and large dark brown precipitates were found. Whereas the

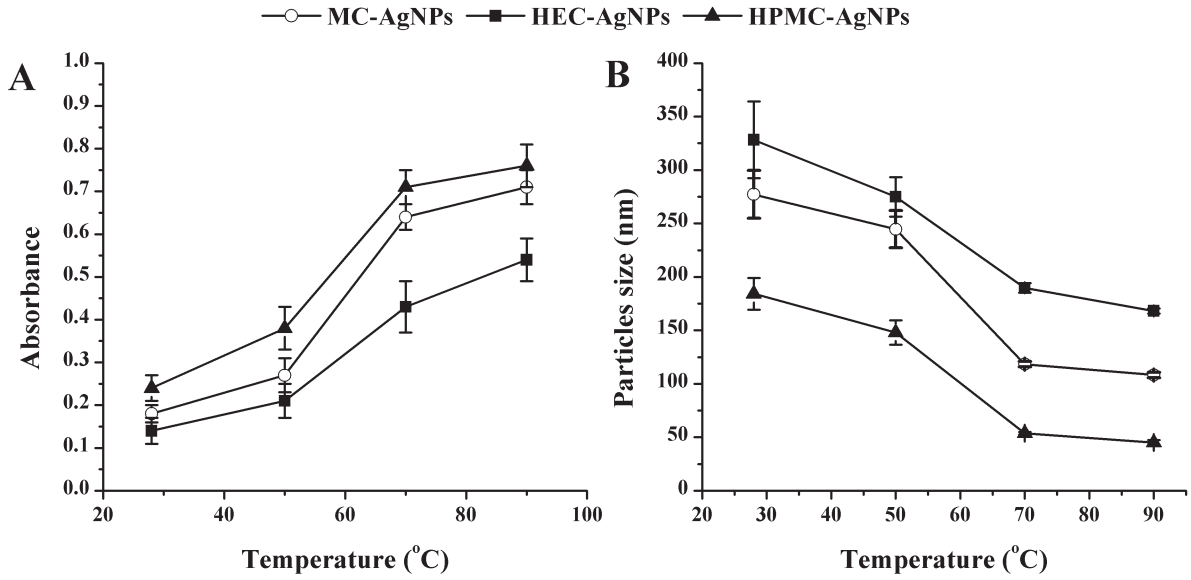

Figure 3. Effects of reaction temperature on intensity of maximum absorbance (A) and particle size (B) of cellulose-AgNPs.

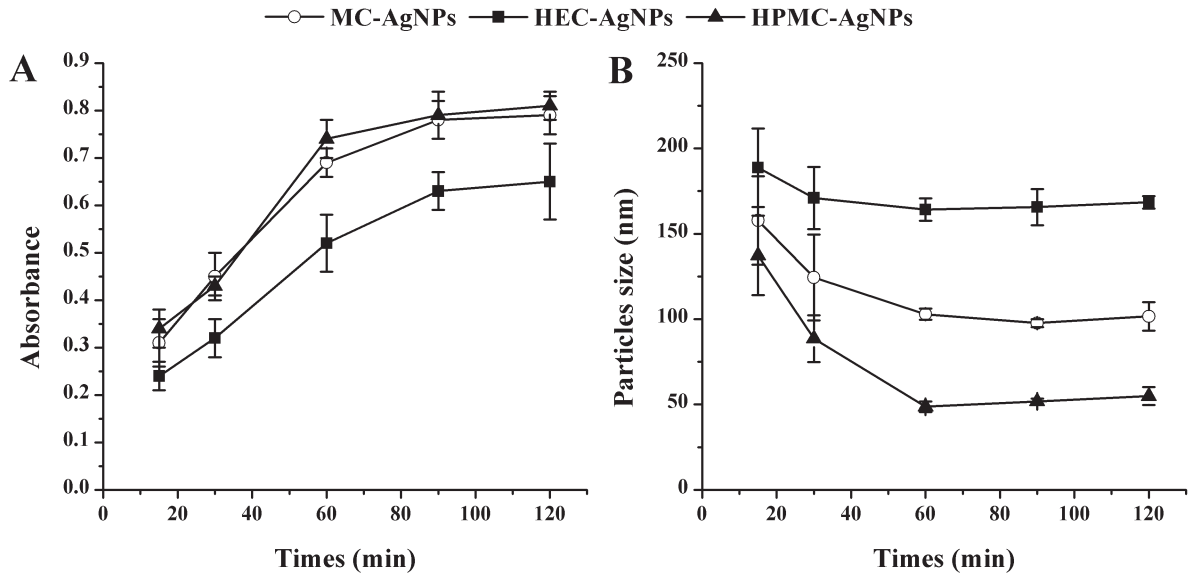

Figure 4. Effects of reaction period on intensity of maximum absorbance (A) and particle size (B) of cellulose-AgNPs.

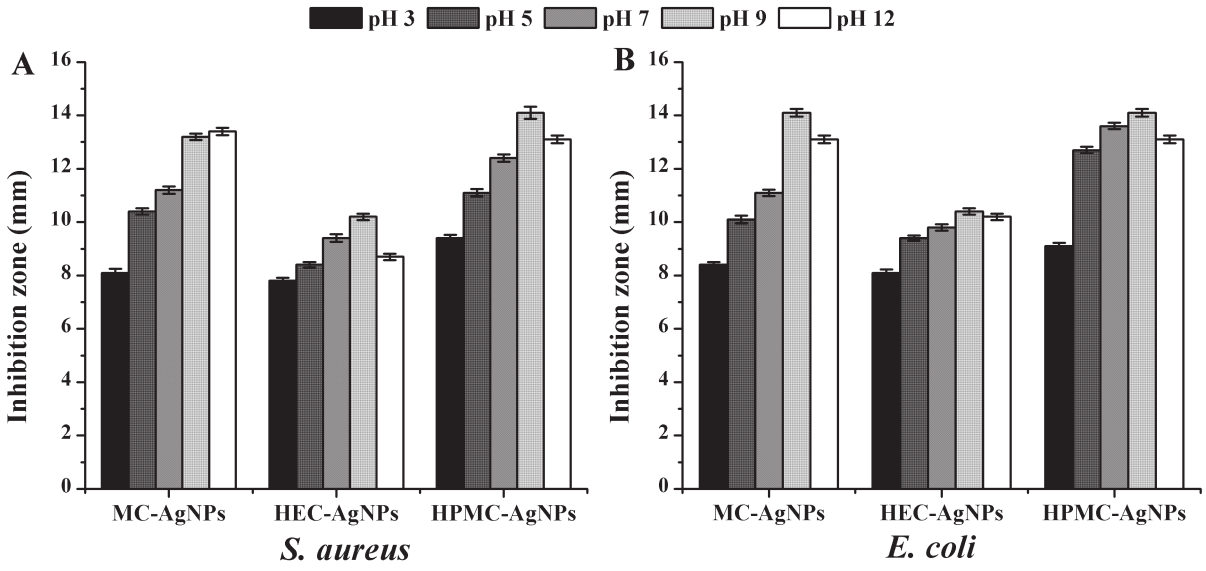

Figure 5. Antibacterial activity of cellulose-AgNPs obtained from different pH of reaction media.

aggregation and dark green precipitates were observed from HPMC-AgNPs system using reacting period of 15 min. Increased reaction period from 15 to 60 min caused significant increase of absorption intensity of the obtained cellulose-AgNPs systems as shown in Figure 4A. Reaction periods of 90 and 120 min gave the high absorbance with no significant different intensity.

The average size of MC-AgNPs and HPMC-AgNPs was significantly decreased when the reaction period was increased whereas that of HEC-AgNPs was slightly insignificantly decreased as shown in Figure 4B. The size change was clearly seen when short reaction period was used. It was found that reaction period of 60-120 min caused no effect on size of cellulose-AgNPs. The average particle size of MC-AgNPs, HEC-AgNPs, and HPMC-AgNPs obtained from the reaction periods of 60-120 min was 98 ± 2, 166 ± 11, 52 ± 2 nm, respectively with PdI values of 0.18 ± 0.08, 0.22 ± 0.06, 0.17 ± 0.04, respectively. Therefore, the suitable reaction period of cellulose-AgNPs was considered to be about 60-120 min.

3.5. *Antibacterial test*

The obtained cellulose-AgNPs showed antibacterial effect against *S. aureus* and *E. coli* whereas the pure cellulose solution could not inhibit the tested strains. The results suggested that the antibacterial activity of cellulose-AgNPs were depended on cellulose type and conditions of AgNPs synthesis. HPMC-AgNPs explored the highest inhibitory activity against *S. aureus* and *E. coli* followed by MC-AgNPs and HEC-AgNPs, respectively. The antibacterial activity of cellulose-AgNPs obtained from different pH condition against *S. aureus* and *E. coli* is shown in Figure 5A and 5B, respectively. The width of inhibition zone indicates the bacterial inhibitory potential. The results showed that the width of inhibition zone of cellulose-AgNPs was depended on pH of the synthesized reaction. Cellulose-AgNPs obtained from reaction at high pH, particularly at pH 9 showed the great inhibition zone for both strains. For reacting temperature condition, cellulose-AgNPs obtained from high temperature displayed the high antibacterial activity, which was clearly seen in the systems of MC-AgNPs and HPMC-AgNPs obtained from the reaction temperature at 50 and 70°C as shown in Figure 6A and 6B for inhibition of *S. aureus* and *E. coli*, respectively. The cellulose-AgNPs obtained from different reaction period showed different antibacterial activity as shown in Figure 7A and 7B for inhibition of *S. aureus* and *E. coli*, respectively. The results showed that increased reaction period yielded the cellulose-AgNPs with high antibacterial activity, except those obtained from 120-min reaction period that the activity was decreased. This might be due to the occurrence of particle aggregation and precipitation in the agar plates. From this experiment, it was noted that the inhibition zones of HPMC-AgNPs against both strains were relatively wider than those of MC-AgNPs and HEC-AgNPs, respectively, indicating that HPMC-AgNPs was the strongest antibacterial activity, followed by MC-AgNPs and HEC-AgNPs, respectively. To confirm these results, MIC and MBC values of the obtained cellulose-AgNPs were investigated. The results showed that the MIC values of MC-AgNPs, HEC-AgNPs, and HPMC-AgNPs against *S. aureus* were 0.05, 0.05, and 0.025 mg/mL, respectively and against *E. coli* were 0.05, 0.05, and 0.025 mg/mL, respectively. The MBC value of all cellulose-AgNPs against *S. aureus* was 0.1 mg/mL whereas that values of MC-AgNPs, HEC-AgNPs, and HPMC-AgNPs against *E. coli* were 0.1, 0.1, and 0.05 mg/mL, respectively. From these results, it was obviously seen that HPMC-AgNPs showed the highest antibacterial activity against both Gram-positive and Gram-negative bacteria whereas MC-AgNPs and HEC-AgNPs had similar potential.

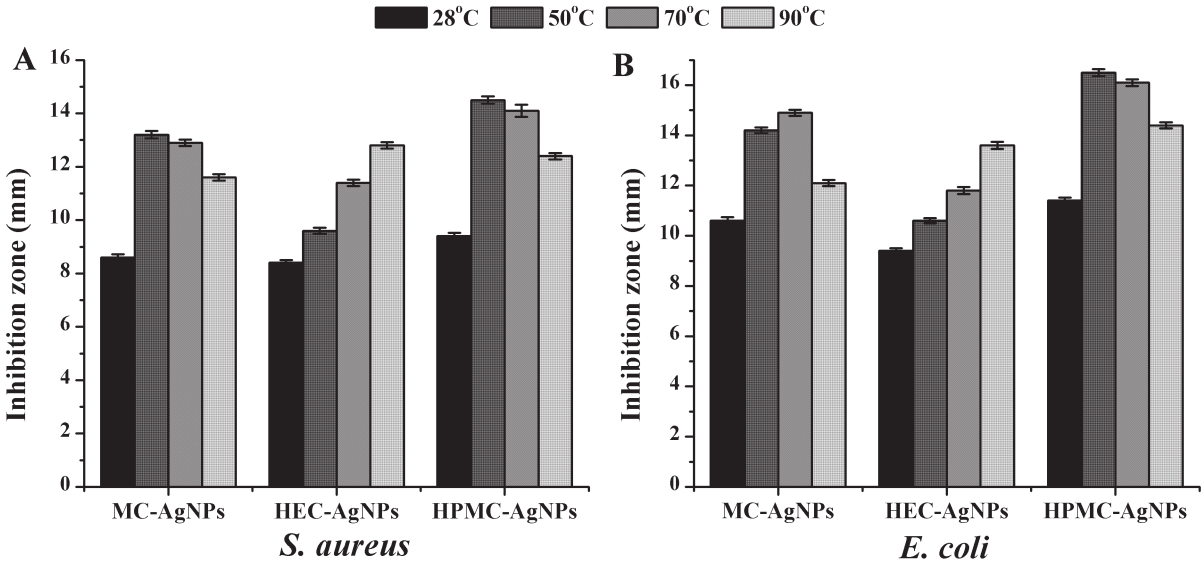

Figure 6. Antibacterial activity of cellulose-AgNPs obtained from different reaction temperature.

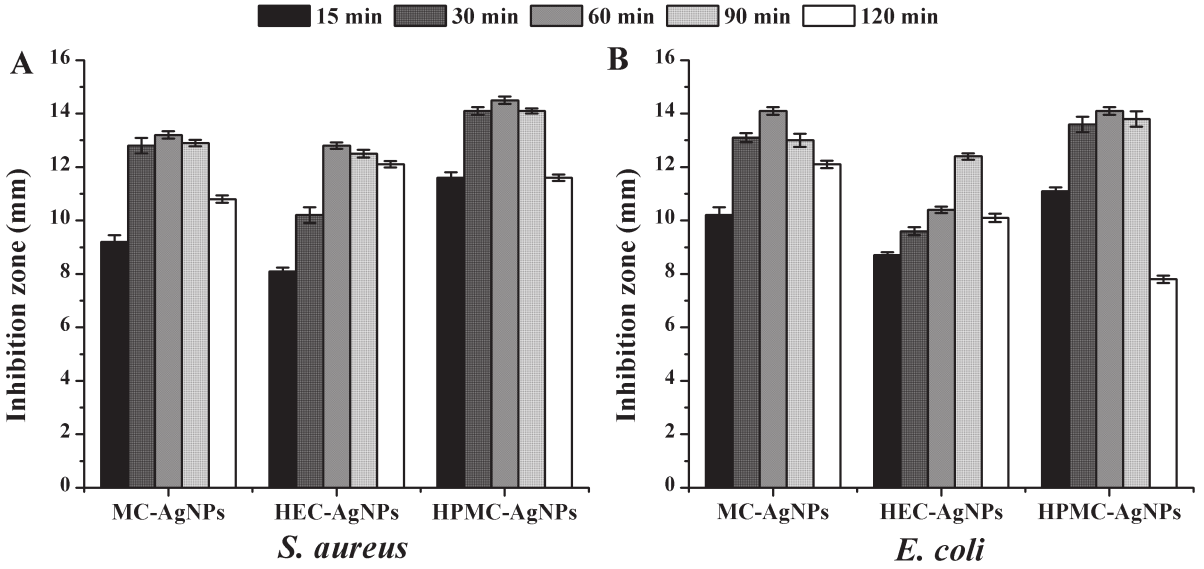

Figure 7. Antibacterial activity of cellulose-AgNPs obtained from different reaction period.

4. Discussion

AgNPs have received significantly increase attention because of their unique physical, chemical, and biological properties (*21,22*). Generally, AgNPs are synthesized by chemical reduction of metal ions from its salt, such as $AgNO_3$, by mostly chemical reducing agents (*23*). However, some chemical reducing agents are toxic to human beings and also to the environment. Therefore, many researchers attempt to synthesize AgNPs using reducing agents from natural such as microorganisms, fungi and plant (*24,25*). Cellulose derivatives are the most abundant resource in nature. They have long been used as a good candidate for fabricating hydrogels in pharmaceutical application owing to their excellent properties in hydrophilicity, biodegradability, and safety (*26*). Moreover, some cellulose derivatives have been demonstrated that they can stabilize the nanoparticles from aggregation (*12,27*). Therefore, at least there are two main actions of cellulose derivatives in the synthesis of AgNPs, one is as a reducing agent and the other is as a stabilizing agent. Three cellulose derivatives, MC, HEC, and HPMC that are chosen for AgNPs synthesis in the present study are non ionic substances and possess positive adsorption at the liquid/air and the other interfaces. To be able to synthesize AgNPs from $AgNO_3$ precursor, a substance should have reducing property, which can be used as an antioxidant. From the best of our knowledge, there is very less report on antioxidant or reducing property of cellulose derivatives. Previously, HPMC was reported to have antioxidant activity but the mechanism of action

was lipid peroxidation (*28*), which might not be related directly to the reducing property. Zimoch-Korzycka *et al*. reported free radical scavenging property of HPMC using 2,2-diphenyl-1-picrylhydrazyl as free radicals (DPPH) and reducing property using FRAP assay (*29*). In the present study, we are the first who demonstrate that the three selected cellulose derivatives; MC, HEC, and HPMC possess reducing activity and in relatively different levels. The reducing activity of the HPMC was found to be the highest among them.

All reactive agents for AgNPs synthesis need to be water soluble because the synthetic medium is aqueous. Therefore, MC, HEC, and HPMC have been selected as representatives of cellulose derivatives for AgNPs synthesis in the present study because they are well water soluble. Our results confirm that MC, HEC, and HPMC can be used as reducing agent in the synthesis of AgNPs. MC has been reported to form a protective colloid against droplets or particles agglomeration (*6*). The cellulose-AgNPs systems of MC, HEC, and HPMC obtained from the suitable conditions showed no aggregation or precipitation, indicating that these cellulose derivatives can act not only as a reducing agent but also a stabilizing or capping agent to protect the resulted AgNPs from aggregation. To obtain AgNPs, a reducing agent should have negative active charge to react with Ag^+ of $AgNO_3$ precursor. Considering the chemical structure of MC, HEC, and HPMC, it is shown that there are linear chains with β-(1→4) linkage but these three types of cellulose derivatives are different to each other in the substitution groups and carbon position (*27*). For being reacted with Ag^+ of $AgNO_3$ precursor, there is a negative charge group ($-COO^-$) in the structure of these cellulose derivatives (*12*).

AgNPs possess optical properties that are depended on size, shape, concentration, and agglomeration state as well as refractive index near the particle surface. These properties can cause UV-vis spectroscopy a valuable tool for identifying, characterizing and studying the obtained AgNPs (*30*). Therefore, the color change of the system after complete reaction can indicate the formation of AgNPs (*31*). Three process parameters of AgNPs synthesis, including pH of reacting media, reacting temperature, and duration of reaction or reaction period are investigated in the present study. The results show that these parameters significantly affected the obtained AgNPs.

Effect of pH on the particle size of the obtained AgNPs is clearly seen. The higher pH causes the smaller particle size. It was reported that slightly acid (pH 6) of Millipore water can retard the reduction of Ag^+ to Ag^o (*32*). Meanwhile, in alkaline or higher pH media, OH^- ions can maintain AgNPs stability by adsorbing these OH^- ions on the particles and providing high repulsive force between the particles. So that it can prevent aggregation of the obtained AgNPs, resulting in maintenance the small size of the AgNPs. Our results are in good agreement with the other groups that in alkaline pH, AgNPs are stable and aggregates formed at lower pH (*33*). Moreover, previous studies reported that alkaline solution increased solubility (*34*) of cellulose derivatives leading to a high viscosity (*35*). The increased viscosity can protect cellulose-AgNPs from aggregation and precipitation. Therefore, it is suggested that synthesis of AgNPs should be performed at alkaline pH.

Studying effect of temperature on the obtained cellulose-AgNPs, it was found that as the temperature increases the absorbance is also increased. Among the studied temperature, synthesis at room temperature at 28ºC gave the least yield of cellulose-AgNPs as the lowest absorbance at the identical wavelength was shown. It is considered that at low temperature, the reduction reaction could not finish completely within the limited time used. The particle size of cellulose-AgNPs decreases with increasing temperature. The smallest size of the particles is obtained when the synthesis was performed at 70ºC or above. Sarkar *et al*. (*36*) reported that increasing temperature, the kinetic energy of the AgNPs in the solution also increases; as a result, the collision frequency between the particles also rises, and this leads to the higher rate of reaction.

For the influence of reaction period, it was observed that within 60 min of reaction period, the absorbance intensity at the fixed wavelength steadily increases as a function of time, indicating that the continued reduction of silver ions. After 60 min, the increasing rate of the absorbance is decreased until 90 min that there is no further increase in the absorbance, indicating the complete reduction of the silver ions. The obtained particles size of cellulose-AgNPs is depended on duration of reaction which decreases with increased time. Among the three cellulose derivatives used, it is found that the particle size of HPMC-AgNPs is the smallest, followed by that of MC-AgNPs and HEC-AgNPs, respectively.

The antibacterial activity of HPMC-AgNPs is significantly higher than MC-AgNPs and HEC-AgNPs, respectively. It has been reported that AgNPs are well adsorbed onto the surface of bacterial cell membrane and can damage the cell by modifying the intracellular structures and inducing cellular toxicity (*37*). The results in the present study indicate that the obtained cellulose-AgNPs are slightly higher effective to Gram-negative bacteria than Gram-positive strains. It is considered that this is due to certain difference between Gram-positive and Gram-negative bacteria, which markedly differ in their cell walls. The cell wall of Gram-positive cells is much thicker with higher amount of peptidoglycan than Gram-negative. The thicker peptidoglycan layer might be therefore extensive practical importance in protecting the cell from penetration of silver ions into the cytoplasm. It is noted that pH, temperature, and period of reaction play an important role on the

antibacterial activity of AgNPs according to the particle size obtained. The smaller particle size gives the higher antibacterial activity. Among the three cellulose-AgNPs, HPMC-AgNPs are the smallest size and possess the most effective activity.

From our study, it can be concluded that HPMC is the most effective reducing agent for synthesizing AgNPs, followed by MC and HEC, respectively. Synthesized parameters such as pH, temperature, and period of reaction play an important role to the color, absorption intensity, particles size, and antibacterial activity of the obtained AgNPs.

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