International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.9, No.09 pp 472-482, 2016

Dual Function of *p*-Hydroxybenzoic Acid as Reducing and Capping Agent in Rapid and Simple Formation of Stable Silver Nanoparticles

Gusrizal Gusrizal[1,2]*, Sri Juari Santosa[2], Eko Sri Kunarti[2], Bambang Rusdiarso[2]

[1] Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Tanjungpura , Jl. Prof. Dr. H. Hadari Nawawi, Pontianak 78124, Indonesia
[2] Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada , Sekip Utara, PO BOX BLS 21 Yogyakarta 55281, Indonesia
*Corresponding Author E-mail: sjuari@ugm.ac.id (S.J.S), gusrizal@mail.ugm.ac.id (G.G)

Abstract : This paper highlights the fact that *p*-hydroxybenzoic acid has a dual function as reducing and capping agent in the formation of stable silver nanoparticles. The first step of the formation of silver nanoparticles was an adjustment of the pH of the *p*-hydroxybenzoic solution by the addition of sodium hydroxide solution. Silver nitrate was then added to pH adjusted *p*-hydroxybenzoic and the mixture was then heated for 1 hour in boiling water bath. Reduction of Ag^+ to form silver nanoparticle was monitored using a UV-visible spectrophotometer. Interaction of *p*-hydroxybenzoic acid with silver nanoparticles in capping silver nanoparticles was determined by Fourier transform infrared spectrophotometry. The size and morphology of silver nanoparticles were determined by transmission electron microscopy. The data showed that the pH of *p*-hydroxybenzoic acid had an effect on the reducing ability of *p*-hydroxybenzoic acid. At pH of *p*-hydroxybenzoic acid equal to 11, the reaction with a mole ratio of $AgNO_3$:*p*-hydroxybenzoic acid 1:20 resulted in the formation of silver nanoparticles with the average size of 26±11 nm. Stability observation for 18 weeks at normal laboratory condition showed that the silver nanoparticles were highly stable with the reduction of surface plasmon resonance peak intensity only 1% and no significant shift of the maximum peak. The results suggest the *p*-hydroxybenzoic acid can be used as reducing agent in the formation of stable silver nanoparticles without additional another capping agent. These silver nanoparticles show a promising application in the development of antibacterial product and chemical sensor.

Keywords : Silver nanoparticles; *p*-hydroxybenzoic acid; surface plasmon resonance.

Introduction

In recent years, nanomaterials have attracted the interest of researchers due to its properties as a consequence of the particles size. Nanoscale materials (1-100 nm) show properties that are not found in a bulk material with the same chemical composition.[1] By reducing the noble metals to the nanoscale, surface plasmon resonance absorption can be observed. This absorption results from collective oscillation of conduction electron of the metal nanoparticles.[2,3]

Silver nanoparticles have received increasing attention of researchers owing to its cytotoxicity, antibacterial, anti-inflammatory, and antiviral activities and are already established for medical application.[4,5,6] In food industries, silver nanoparticles have been applied as food contact materials that preserve the food by inhibiting the growth of microorganisms.[7] In the development of textile fabrics containing antibacterial agents, silver nanoparticles coated textile fabrics have been produced.[8] Immobilized silver nanoparticles have been used as a water disinfectant in the water treatment process.[9] Silver nanoparticles have also been used as a chemical sensor for metal ion,[10,11] fluoride ion,[12] and herbicide detection.[13]

Nanoscale materials can be obtained in two approaches: top-down and bottom-up approaches.[14] In the first approach, the material is removed from the bulk material leaving only the nanostructures material, but in the second approach, atoms are assembled to generate the nanostructures material. Nanochemist uses a bottom-up approach in producing metal nanoparticles. In this approach, a metal source, reducing agent, and capping agent should be available. Atoms of metals are produced from reduction of metal ions by reducing agent and the capping agent is used to prevent the aggregation and precipitation of metal atoms produced.[2]

In the formation of silver nanoparticles, Ag^+ ion is reduced to Ag^o by a reducing agent. The common method that has been widely used in the formation of silver nanoparticles was developed by Turkevich *et al.*[15] and Lee and Meisel.[16] The first method used sodium citrate, both as reducing and capping agent, and the second method used sodium borohydride as reducing agent and polyvinyl alcohol as a capping agent. Since the size of silver nanoparticles can be affected by reducing and capping agents, researchers have been trying to explore the using of different reducing and capping agents.

Carbohydrates such as galactose, maltose, lactose[17] and glycerol[18] were used as reducing agents without other capping agents. In addition, glucose was used as reducing agent with polyvinylpyrrolidone as capping agent.[19] Gelatin, tween-20[11] and chitosan[20] were used as a capping agent for silver ion reduced by glucose. Furthermore, dextrose was used in the reduction of silver ion with polyvinylpyrrolidone as capping agent.[21] Amino acids have also been used in the formation of silver nanoparticles. L-tyrosin has been used as reducing and capping agents.[10] Plant extracts have also been used in the formation of silver nanoparticles.[22,23] The activity of plant metabolites as reducing and capping agents is ascribed to the content of steroids, sapogenins, tannins, carbohydrates, and flavonoids.[24,25] Antioxidant from plant metabolites, mainly consist of phenolic compounds, has been used in the reduction of silver ion.[26]

Previous studies using plant metabolites indicate that Ag^+ ions are able to be reduced by phenolic compounds and three isomers of hydroxybenzoic acid show an activity as antioxidant.[27] In addition, hydroxybenzoic acids can be absorbed onto the surface of silver nanoparticles. The molecules of hydroxybenzoic acid are attached through its carboxylate group to the silver.[28,29,30]

A molecule of hydroxybenzoic acid contains hydroxyl phenolic and carboxylate functional groups. It is, therefore, reasonable to expect that hydroxybenzoic acid would be suitable for the formation of silver nanoparticles simultaneously act as reducing and capping agents. The hydroxyl phenolic group that can be oxidized makes the hydroxybenzoic acid plays a role as a reducing agent. The carboxylate group of hydroxybenzoic acid can be a ligand for coordinating with silver nanoparticles surfaces produced by reduction of silver ion, and it will be then capped with hydroxybenzoic acid. Coordination of silver nanoparticles with hydroxybenzoic acid stabilizes the size of silver nanoparticles from agglomeration.

In this paper, the dual function of *p*-hydroxybenzoic acid as reducing and capping agent in the formation of stable silver nanoparticles is reported for the first time. Silver nanoparticles were characterized initially after the formation and their stability at 18 weeks. Formation of silver nanoparticles was studied using UV-visible spectroscopy to monitor the appearance of surface plasmon resonance absorption. Further characterizations of silver nanoparticles were determined by Fourier transform infrared (FTIR) spectroscopy and transmission electron microscopy (TEM).

Experimental

Materials

Silver nitrate (Merck), *p*-hydroxybenzoic acid (Sigma-Aldrich), and sodium hydroxide (Merck) were used in this work. All chemicals were used as received without any further purification. Double distilled water was used in all experiments. Surface plasmon resonance spectra were obtained by measurement using UV-visible spectrophotometer (Shimadzu UV-1700 PharmaSpec). Fourier transmission infrared spectrophotometer (Shimadzu FTIR Prestige-21) was used to investigate the interaction of silver nanoparticles surface with *p*-hydroxybenzoic acid. The sample for FTIR measurement was obtained by centrifugation at 13.000 rpm of resulting silver nanoparticles colloid using IEC MB Centrifuge. Transmission electron microscope (JEOL JEM-1400) was used to determine the size and morphology of silver nanoparticles.

Formation of silver nanoparticles

Silver nanoparticles were produced by reduction of silver nitrate with *p*-hydroxybenzoic acid. Silver nitrate concentration varied from 0.5 to 2.5×10^{-4} M. Concentration of *p*-hydroxybenzoic was 1.0×10^{-3} and 1.0×10^{-2} M. The pH of *p*-hydroxybenzoic acid was adjusted to 11 and 12 by the addition of sodium hydroxide solution and measurement of pH were performed on Horiba F-52 pH meter. Typically, 5 mL of silver nitrate solution was added to 5 mL of pH adjusted *p*-hydroxybenzoic acid solution in a test tube. The mixture of the reaction was heated in boiling water bath for 1 hour and then cooled in tap water and stored at room temperature.

Characterization of silver nanoparticles

The UV-visible spectrophotometer was used to obtain the spectra of surface plasmon resonance absorption. All UV-visible measurements were performed using 1 cm optical path length quartz cuvette and at room temperature within 200-800 nm wavelength range. To obtain the stability data of silver nanoparticles, UV-visible measurements were performed periodically for a period of 18 weeks.

The solid sample for FTIR analysis was prepared using silver nanoparticles recovered from colloidal solution via centrifugation at 13.000 rpm and then were dried at 65 ^{o}C in an oven. FTIR spectra were obtained by the KBr plate method with the scanning range of 400-4000 cm^{-1}.

The sample for TEM analysis was prepared by immersing the copper grid to silver nanoparticles colloid and then dried at room temperature. The image was taken with an accelerating voltage of 120 kV. The size of particles was calculated using the scale provided in the micrograph.

Results and Discussion

Effect of pH on the formation of silver nanoparticles

In this work, the formation of silver nanoparticles was performed by mixing of silver nitrate with *p*-hydroxybenzoic acid. Since *p*-hydroxybenzoic acid reduced the silver ion, the color of the solution changed to yellow indicated the formation of silver nanoparticles. This color is due to the surface plasmon resonance absorption.[2,3]

Our initial experiment showed that the formation of silver nanoparticles could be achieved using *p*-hydroxybenzoic acid whose pH adjusted to 11 and 12 and heating the mixture of silver nitrate and *p*-hydroxybenzoic in boiling water bath. The formation of silver nanoparticles was not detected if pH of *p*-hydroxybenzoic was adjusted to 13. It is clear that the pH of *p*-hydroxybenzoic acid plays the crucial role in the reduction of Ag^+.

Effect of pH on the formation of silver nanoparticles by reduction of Ag^+ using a compound containing carboxylate and phenolic groups in their structures has been described.[31] At high pH, hydroxyl and carboxylate groups of *p*-hydroxybenzoic acid were available for reduction of Ag^+. Sodium hydroxide was used for ionization of functional groups of *p*-hydroxybenzoic acid and formation of Ag_2O (Equation 1). In water solution, Ag_2O was hydrolyzed as described in Equation 2.[32] It can be seen that Ag_2O was the source of Ag^+ and the concentration of Ag^+ depended on the concentration of OH^-. In our experiment using *p*-hydroxybenzoic acid with pH=13 the formation of silver nanoparticles was not detected. It is predicted that there is an excessive concentration of OH^- so that Ag_2O is insoluble and Ag^+ is not available for the reduction process.

$$2Ag^+ + 2OH^- \longrightarrow Ag_2O\downarrow + H_2O \qquad (1)$$

$$Ag_2O\downarrow + H_2O \rightleftharpoons 2Ag(OH)_2\downarrow \rightleftharpoons 2\,Ag^+ + 2OH^- \qquad (2)$$

In addition to the formation of Ag_2O, at high pH, the functional groups of *p*-hydroxybenzoic were deprotonated. The pK_{a1} and pK_{a2} of *p*-hydroxybenzoic acid are 4.57 and 9.46 respectively.[33] In consideration to this p*Ka*, at our experimental pH both of hydroxyl phenolic and carboxylate groups of *p*-hydroxybenzoic acid were ionized. The Anion of *p*-hydroxybenzoic acid formed a complex with Ag^+ through carboxylate group. The complexation of *p*-hydroxybenzoic acid with Ag^+ was an initial step in the formation of silver nanoparticles prior to the reduction step in which metallic silver was formed. Oxidation occurred at the ionized hydroxyl phenolic group of *p*-hydroxybenzoic acid. Complexation of Ag^+ and *p*-hydroxybenzoic acid become the center of oxidation and reduction process. Litvin *et al.*[31] and Adegboyega *et al.*[34] suggested that the formation of Ag_2O and complexation of Ag^+ with *p*-hydroxybenzoic acid contributed to the formation of silver nanoparticle at high pH.

Effect of reaction time on the formation of silver nanoparticles

To obtain the optimum reaction time, the formation of silver nanoparticles was monitored at regular time interval using UV-visible spectrophotometer for 2 hours experiment. For a comparison, *o*-hydroxybenzoic acid was also used as reducing agent. Figure 1 shows that the formation of silver nanoparticles with *p*-hydroxybenzoic acid as reducing agent was faster than those with *o*-hydroxybenzoic acid. Using 1.0×10^{-3} M *p*-hydroxybenzoic acid and 2.0×10^{-4} M silver nitrate, the length of time to achieve maximum absorbance was 1 hour. On the other hand, a reaction using 1.0×10^{-2} M *o*-hydroxybenzoic acid and 3.0×10^{-4} M silver nitrate took 2 hours.

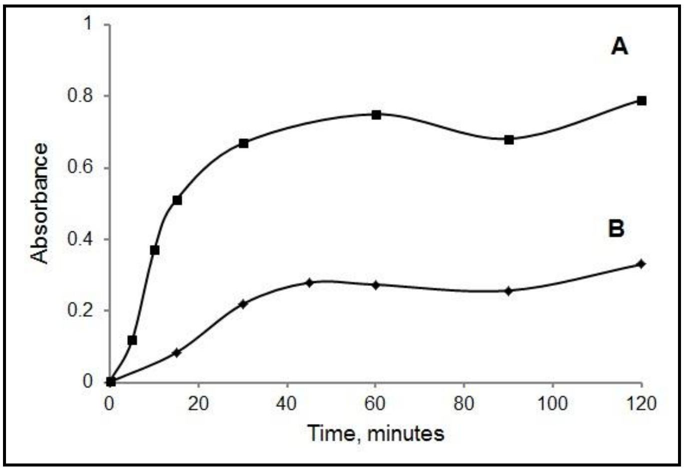

Figure 1. Reaction time of silver nanoparticles formation (A). [*p*-hydroxybenzoic acid] = 1.0×10^{-3} M, [$AgNO_3$] = 2.0×10^{-4} M, pH = 11, (B). [*o*- hydroxybenzoic acid] = 1.0×10^{-2} M, [$AgNO_3$] = 3.0×10^{-4} M, pH = 11

Reducing ability of *p*-hydroxybenzoic acid

UV-visible spectroscopy has been widely used to monitor the formation of metal nanoparticles. The characteristic of metal nanoparticles spectra is the presence of a band in the visible range. This band is a result of the interaction of light incident on the surface of metal nanoparticles with the conduction electrons of metal. Under the effect of the electric field of the light beam, conduction electrons of metal are shifted and the electron motion leads to the appearance of a dipole that oscillates with the frequency of the exciting electric field. The resonance light absorption and scattering are observed if the frequency of incident light oscillation coincides with the intrinsic frequency of conduction electrons. This absorption and scattering of light are referred as surface plasmon resonance spectra.[3] The colloid of silver nanoparticles exhibits a yellow color and a peak around 400 nm. The color and position of λ_{max} are dependent on the size of the particle. The position of λ_{max} shifts to longer wavelength with increasing the size of the particle. The full width at half maximum (FWHM) of the peaks determines the dispersity of the silver nanoparticles. Polydisperse silver nanoparticle will have a larger FWHM.[35]

Intensity or extinction of spectra is a cumulative contribution of absorption and scattering of light. The contribution of absorption and scattering of light depends on the size of particles. Extinction of silver nanoparticles with the particle size larger than 50 nm is dominated by scattering whereas extinction of particles smaller than 30 nm is the only contribution of absorption. For the particles with 50 nm in size, the contribution of absorption and scattering in extinction becomes equal.[36]

In this work, UV-visible spectroscopy was used to evaluate the ability of *p*-hydroxybenzoic acid in the reduction of Ag^+. To evaluate the reducing ability of *p*-hydroxybenzoic acid, the concentrations of $AgNO_3$ were varied from 0.5 to 2.5 x10^{-4} M. Figure 2 shows the spectra of silver nanoparticles produced by the reaction with a different initial concentration of $AgNO_3$. All reactions of *p*-hydroxybenzoic acid and silver nitrate at various concentrations yielded yellow color with absorption peak around 410-430 nm confirming the formation of silver nanoparticles. Increasing the initial concentration of $AgNO_3$ enhanced the intensity of spectra. Increasing the intensity of peak suggested an increase in the number of silver nanoparticles formed.[37]

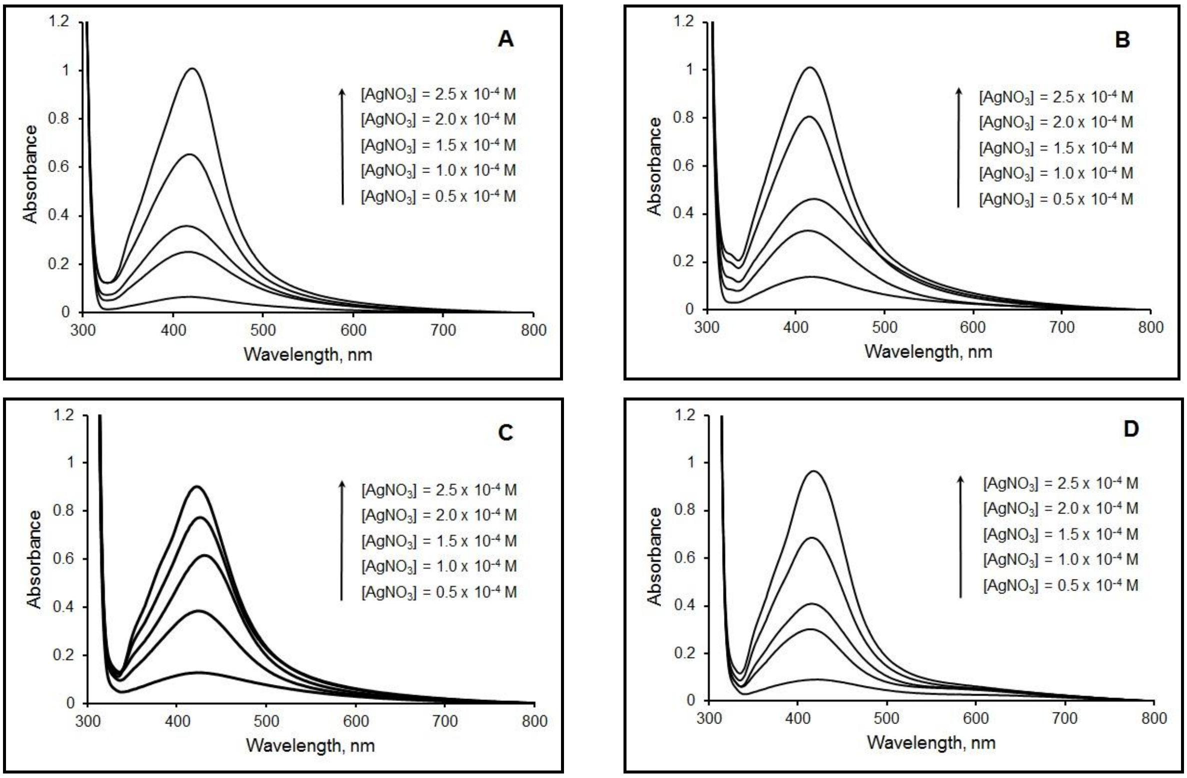

Figure 2. UV-visible spectra of silver nanoparticles using different initial concentration of $AgNO_3$. $[AgNO_3]$ = 0.5-2.5x10^{-4} M, (A). [*p*-hydroxybenzoic acid] = 1.0x10^{-3} M, pH = 11, (B). [*p*-hydroxybenzoic acid] = 1.0x10^{-3} M, pH = 12, (C). [*p*-hydroxybenzoic acid] = 1.0x10^{-2} M, pH = 11, (D). [*p*-hydroxybenzoic acid] = 1.0x10^{-2} M, pH = 12

The ability of *p*-hydroxybenzoic in reducing Ag^+ can be measured from the determination of the concentration of the resulted silver nanoparticles, indicated by the intensity of maximum peak, in relation to the initial concentration of $AgNO_3$. Plotting the intensity of the maximum peak as a function of the initial concentration of $AgNO_3$ yields a straight line model described by the equation of $y = ax + c$, where y is the intensity of the maximum peak, a or slope of the straight line represents the reducing ability, x is the initial concentration of $AgNO_3$, and c is a constant. From the data presented in Figure 2, the equation of each reaction was $y = 4575x - 0.2$ ($R^2 = 0.96$), $y = 4445x - 0.1$ ($R^2 = 0.98$), $y = 3870x$ ($R^2 = 0.98$) and $y = 4269x - 0.2$ ($R^2 = 0.98$) for data in Figure 2A, B, C, and D, respectively. The slope of the linear line indicated the reducing ability of *p*-hydroxybenzoic towards Ag^+. The maximum reducing ability of *p*-hydroxybenzoic acid was achieved if the reaction was performed at pH of *p*-hydroxybenzoic acid equal to 11 and concentration of *p*-hydroxybenzoic used was 1.0x10^{-3} M. Increasing the pH of *p*-hydroxybenzoic acid to 12 slightly decreased the reducing ability of *p*-hydroxybenzoic when 1.0x10^{-3} M *p*-hydroxybenzoic acid was used. On the other site, increasing the pH of *p*-hydroxybenzoic acid to 12 slightly increased the ability of *p*-hydroxybenzoic in reducing Ag^+ when 1.0x10^{-2} M *p*-hydroxybenzoic acid was used.

An isomer of *p*-hydroxybenzoic acid, *o*-hydroxybenzoic acid was also used as reducing agent to obtain a comparison data. Similar experimental parameters were used for reduction of Ag^+. Because the intensity of the resulted spectra was too low, more concentrated initial concentrations of $AgNO_3$, i.e. from 1.0 to 8.0x 10^{-4} M, were used in the reaction with 1.0x 10^{-3} M *o*-hydroxybenzoic acid. The profile of UV-visible spectra of the resulted silver nanoparticles was similar to that obtained by using *p*-hydroxybenzoic acid as reducing agent (spectra is not provided here). Equations showing the relation between the initial concentration of $AgNO_3$ and intensity of spectra for the reaction with 1.0x10^{-3} M *o*-hydroxybenzoic acid at pH=11 and pH=12 were $y = 778x + 0.4$ ($R^2 = 0.92$) and $y = 884x + 0.3$ ($R^2 = 0.97$), respectively.

Increasing the concentration of *o*-hydroxybenzoic acid to 1.0x10^{-2} M enhanced the intensity of spectra. The equations showing the relation between the initial concentration of $AgNO_3$ and intensity of spectra for reaction with 1.0x10^{-2} M *o*-hydroxybenzoic pH=11 and pH=12 were $y = 2585x$ ($R^2 = 0.97$) and $y = 4465x – 0.1$ ($R^2 = 0.97$).

The reducing ability of *o*-hydroxybenzoic acid significantly increased by increasing the pH and concentration of *o*-hydroxybenzoic acid. The highest reducing ability of *o*-hydroxybenzoic acid for Ag^+ was achieved if the reaction performed with the pH of the *o*-hydroxybenzoic acid solution was 12 and at the concentration of 1.0x10^{-2} M. It is predicted that the chemical structure of *o*-hydroxybenzoic affects the reaction. Internal hydrogen bonding in *o*-hydroxybenzoic acid appears to inhibit the dissociation of functional groups of *o*-hydroxybenzoic acid so that the higher pH and concentration of *o*-hydroxybenzoic acid are needed. It has been described that protonation of functional groups of hydroxybenzoic acid contributed in the reduction of Ag^+.[34]

By comparing the slopes of the linear lines correlating the initial concentration of $AgNO_3$ and the intensity of spectra of the reaction using *p*-hydroxybenzoic acid as well as *o*-hydroxybenzoic acids as described above, it could be shown that the reducing ability of *p*-hydroxybenzoic acid in the reduction of Ag^+ was higher than that of *o*-hydroxybenzoic acid. According to Simić *et al.*[27] there is a correlation between the reducing ability with the oxidation potential of *p*-hydroxybenzoic acid and *o*-hydroxybenzoic acid. The oxidation potential of *p*-hydroxybenzoic and *o*-hydroxybenzoic acid is 0.87 and 0.94 V, respectively. These oxidation potential data supported that *p*-hydroxybenzoic acid has higher reducing ability than *o*-hydroxybenzoic acid.

Effect of the concentration of *p*-hydroxybenzoic acid on the particle size

Our data showed that *p*-hydroxybenzoic acid could act as reducing agents in reaction with Ag^+. To obtain the effective mole ratio between $AgNO_3$ and *p*-hydroxybenzoic acid, the formation of silver nanoparticles was performed at a constant initial concentration of $AgNO_3$ (2.0x10^{-4} M) while the concentration of *p*-hydroxybenzoic acid was varied from 0.5x10^{-4} M to 1.0x10^{-2} M. It means that the mole ratio of $AgNO_3$ to *p*-hydroxybenzoic acid changes from 4:1 to 1:50. Figure 3 shows the correlation between the intensity of UV-visible spectra of resulting silver nanoparticle at 420 nm and initial concentration of *p*-hydroxybenzoic acid. The data suggested that the highest concentration of silver nanoparticles can be reached at a mole ratio of $AgNO_3$:*p*-hydroxybenzoic acid 1:20.

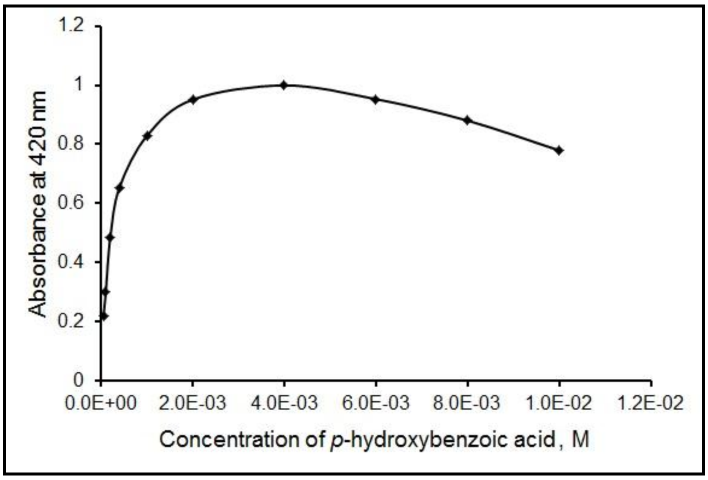

Figure 3. Plot of silver nanoparticles absorbance at 420 nm and concentration of *p*-hydroxybenzoic acid used in formation of silver nanoparticles. [*p*-hydroxybenzoic acid] = 0.5x10^{-4}–1.0x10^{-2} M, [$AgNO_3$] = 2.0x10^{-4} M, pH = 11

To understand the effect of an excess concentration of *p*-hydroxybenzoic acid on the particle size, silver nanoparticles resulted from the reaction of $AgNO_3$ and *p*-hydroxybenzoic acid at mole ratio 1:20 and 1:50 were characterized by measurement with UV-visible spectroscopy and TEM.

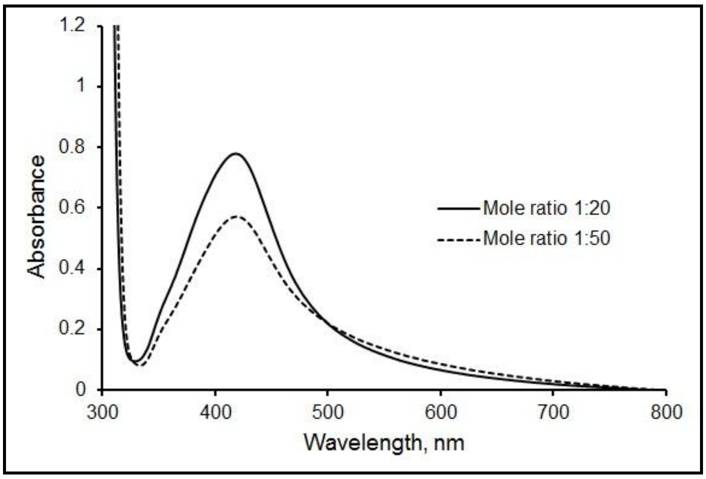

Figure 4. UV-visible spectra of silver nanoparticles resulted from reaction with different mole ratio of $AgNO_3$ to *p*-hydroxybenzoic acid

As shown in Figure 4, there was a decrease of peak intensity and an increase of the FWHM from 100 to 111 nm if higher *p*-hydroxybenzoic concentration acid was used. In addition, the increase in the intensity of the spectra at the wavelength around 500-700 nm was also observable. It indicates that using an excess of *p*-hydroxybenzoic acid concentration yields an increase of polydispersity and particle size, and lowering the reducing ability of *p*-hydroxybenzoic. The similar evidence was also observed by Agnihotri *et al*,[35] Evanoff and Chumanov.[36] They found that there was a relation between particle size and dispersity to FWHM and the intensity of spectra at longer wavelengths. The previous study also showed that increasing the concentration of reducing agent that also acts as capping agent increased the size of resulted particles.[37] Another study also described that a peak broadening resulted from increasing the concentration of capping agent.[13]

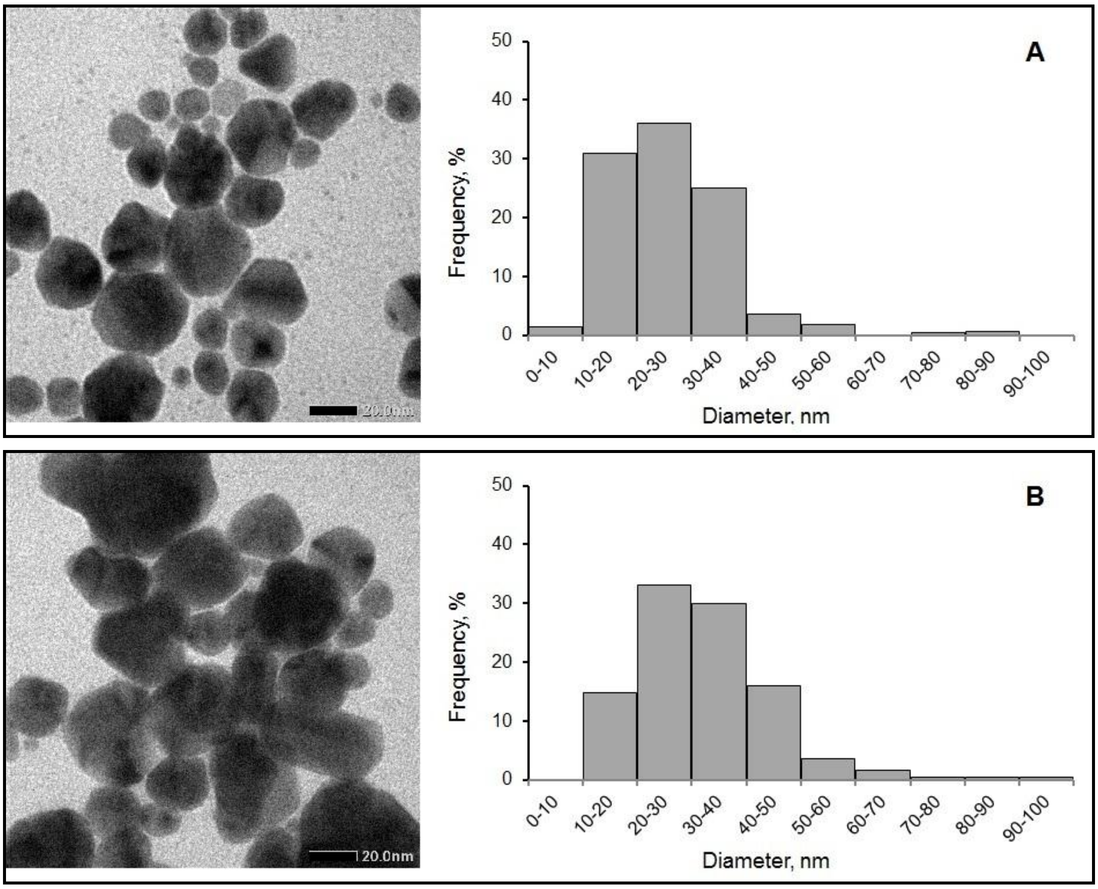

Figure 5. TEM image and particle size distribution of resulting silver nanoparticles. Scale bar 20 nm, (A). Mole ratio of $AgNO_3$:*p*-hydroxybenzoic acid 1:20 (B). Mole ratio of AgNO3:*p*-hydroxybenzoic acid 1:50

The size and morphology of the resulted silver nanoparticles observed using TEM is shown in Figure 5. It shows that reduction using a mole ratio of $AgNO_3$:*p*-hydroxybenzoic acid 1:20 resulted in the particles ranged in size from 9 to 84 nm with an average size of 26±11 nm. The reaction of $AgNO_3$ and *p*-hydroxybenzoic acid at mole ratio 1:50 produced the particles with size ranged from 11 to 90 nm and average size of 32±12 nm. It confirms the above finding using UV-Visible data that reaction using an excess concentration of *p*-hydroxybenzoic acid increase the size and heterogeneity of the particles.

p-hydroxybenzoic acid as a capping agent

To understand the role of *p*-hydroxybenzoic acid as capping agent, the stability test was performed to silver nanoparticles produced by the reaction of 1.5×10^{-4} M $AgNO_3$ with 1.0×10^{-3} M and 1.0×10^{-2} M *p*-hydroxybenzoic acid. The resulted silver nanoparticles were stored in a capped bottle at normal laboratory condition. The colloid of silver nanoparticles visually appeared to be identical even after storing for 18 weeks. As shown in Figure 6, silver nanoparticles formed by reduction with 1.0×10^{-3} M *p*-hydroxybenzoic acid showed only a 1% decrease in the peak intensity and no observable shift of λ_{max} from 410 nm after a period of 18 weeks. The FWHM was found to be 100 nm and it was also still maintained after a period of 18 weeks. A decrease of 1% in the peak intensity was also observed for silver nanoparticles formed from reduction of Ag^+ with 1.0×10^{-2} M *p*-hydroxybenzoic acid. The peak experienced a small red shifted from 422 nm to 424 nm and the FWHM increased slightly from 97 nm to 102 nm. This data indicated that the silver nanoparticles were relatively stable over a period of 18 weeks.

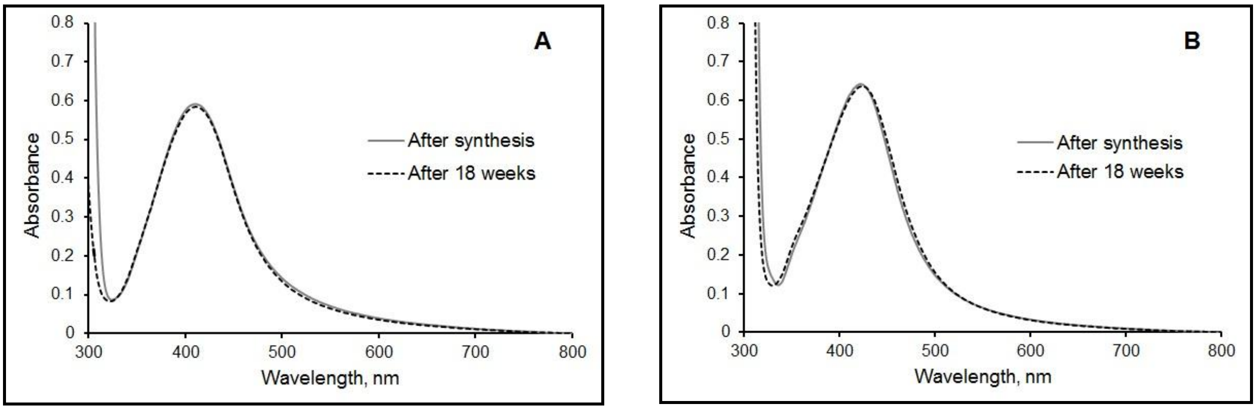

Figure 6. UV-visible spectra of silver nanoparticles after a period of 18 weeks. (A). [*p*-hydroxybenzoic acid] = 1.0×10^{-3} M, [$AgNO_3$] = 1.5×10^{-4} M, pH = 11, (B). [*p*-hydroxybenzoic acid] = 1.0×10^{-2} M, [$AgNO_3$] = 1.5×10^{-4} M, pH = 11,

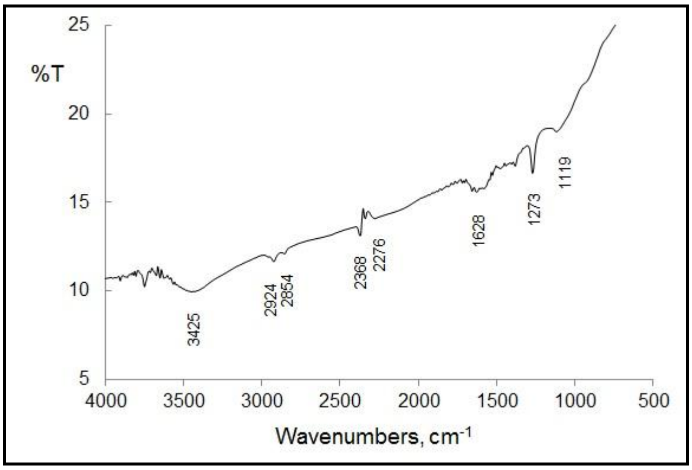

Figure 7. FTIR spectra of silver nanoparticles. [*p*-hydroxybenzoic acid] = 1.0×10^{-3} M, [$AgNO_3$] = 2.0×10^{-4} M, pH = 11

Since no additional capping agent was used in this experiment, it is clear that *p*-hydroxybenzoic acid not only acts as reducing agent but also act as capping agent which is able to maintain the size of nanoparticles from agglomeration. The action of a *p*-hydroxybenzoic acid as capping agent should result from the interaction of silver nanoparticles surface with the functional groups of *p*-hydroxybenzoic. FTIR spectra of *p*-hydroxybenzoic acid capped silver nanoparticles (Figure 7) is used to trace the type of interaction between silver nanoparticles and *p*-hydroxybenzoic acid. The spectra of *p*-hydroxybenzoic acid capped silver nanoparticles showed no observable band around 1700 cm^{-1} (C=O vibration) whereas the spectra of *p*-hydroxybenzoic acid (spectra not shown here) showed a strong peak at that wavelength. In the spectra of *p*-hydroxybenzoic acid, the intensity of the peak around 1700 cm^{-1} was equal to that of around 1600 cm^{-1} (C=C aromatic vibration). It indicates that carboxylate group of *p*-hydroxybenzoic acid participates in the complexation of *p*-hydroxybenzoic acid with the surface of the silver nanoparticles. This conclusion is supported by the previous study showing that three isomers of hydroxybenzoic acid, i.e. *o*-hydroxybenzoic acid, *m*-hydroxybenzoic acid, and *p*-hydroxybenzoic acid were attached to the silver nanoparticles surface through its carboxylate group.[28.29,30]

Figure 4 and Figure 5 suggest that the mole ratio of $AgNO_3$ and *p*-hydroxybenzoic acid has an effect on the size and heterogeneity of resulted silver nanoparticles. The reaction with an excess concentration of *p*-hydroxybenzoic acid increased the size and heterogeneity of resulted silver nanoparticles. It is predicted that the action of a *p*-hydroxybenzoic acid as a capping agent is occurred by creating an assembly of the *p*-hydroxybenzoic molecules to provide the space for silver nanoparticle growth. Considering the effect of steric and electron density of *p*-hydroxybenzoic acid, the higher concentrations of *p*-hydroxybenzoic acid produce a larger assembly that providing a larger space for the growth of silver nanoparticles.

Conclusions

Formation of stable silver nanoparticles using *p*-hydroxybenzoic acid was described at two different pH medium, i.e. at pH=11 and 12. At pH of *p*-hydroxybenzoic acid equal to 11, the effective mole ratio of $AgNO_3$ and *p*-hydroxybenzoic acid for the reaction was 1:20. The resulting silver nanoparticles were mostly spherical with an average size of 26±11 nm and stable over a period of 18 weeks. The results suggest that *p*-hydroxybenzoic acid shows the dual function, act as capping agent as well as reducing agent, in the formation of the silver nanoparticles.

Acknowledgments

The authors thank Directorate General of Higher Education, Ministry of Research, Technology, and Higher Education of the Republic of Indonesia for BPPDN Scholarship that financially supports this study.

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