SYNTHESIS OF SILVER-CHITOSAN NANOCOMPOSITES COLLOIDAL BY GLUCOSE AS REDUCING AGENT

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ABSTRACT

Silver-chitosan nanocomposites colloidal was successfully performed by chemical reduction method at room temperature using glucose as reducing agent, sodium hydroxide (NaOH) as accelerator reagent, silver nitrate (AgNO$_3$) as metal precursor and chitosan as stabilizing agent. Compared to other synthetic methods, this work is green and simple. The effect of the amount of NaOH, molar ratio of AgNO$_3$ to glucose and AgNO$_3$ concentration towards Localized Surface Plasmon Resonance (LSPR) absorption band of silver nanoparticles was investigated using UV-Vis spectrophotometer. The stability of the colloid was also studied for the first 16 weeks of storage at ambient temperature. The formation of silver nanoparticles was confirmed by the appearance of LSPR absorption peak at 402.4–414.5 nm. It is also shown that the absorption peak of LSPR were affected by NaOH amount, ratio molar AgNO$_3$/glucose and concentration of AgNO$_3$. The produced silver nanoparticles were spherical with dominant size range of 6 to 18 nm as shown by TEM images. All colloidals were stable without any aggregation for 16 weeks after preparation. The newly prepared silver-chitosan nanocomposites colloidal may have potential for antibacterial applications.

Keywords: *nanocomposite; colloidal; silver nanoparticles; chitosan; glucose*

ABSTRAK

Koloid nanokomposit perak-kitosan telah berhasil dibuat dengan metode reduksi kimia pada suhu ruang menggunakan glukosa sebagai reduktor, natrium hidroksida (NaOH) sebagai akselerator, perak nitrat (AgNO$_3$) sebagai prekursor logam dan kitosan sebagai bahan penstabil. Metode ini bersifat "green" dan sederhana dibandingkan dengan metode sintetis lain. Pengaruh jumlah NaOH, rasio molar AgNO$_3$/glukosa dan konsentrasi AgNO$_3$ terhadap pita serapan Localized Surface Plasmon Resonance (LSPR) nanopartikel perak diselidiki menggunakan spektrofotometer UV-Vis. Stabilitas koloid juga dipelajari untuk 16 minggu pertama penyimpanan pada suhu ruang. Pembentukan nanopartikel perak dikonfirmasi oleh munculnya puncak serapan LSPR pada 402,4-414,5 nm. Hasil penelitian menunjukkan bahwa intensitas serapan maksimum LSPR dipengaruhi oleh jumlah NaOH, rasio molar AgNO$_3$/glukosa dan konsentrasi AgNO$_3$. Berdasarkan gambar TEM nanopartikel perak yang dihasilkan berbentuk bulat dengan ukuran didominasi antara 6–18 nm. Semua koloid yang dihasilkan stabil tanpa agregasi selama 16 minggu setelah pembuatan. Pembuatan dengan cara baru dari koloid nanokomposit perak-kitosan ini mungkin memiliki potensi untuk aplikasi antibakteri.

Kata Kunci: *nanokomposit; koloid; silver nanopartikel; kitosan; glukosa*

INTRODUCTION

In recent years, studies on the synthesis of nanocomposite materials for medical applications have been drawn a lot of attention. Nanocomposites are a new class of composites that are particle-filled polymers for which at least one dimension of the dispersed particles is in the nanometer range [1]. For application, nanocomposite may be used in different forms, such us colloids, powders, fibers, membranes and film. Incorporation of silver nanoparticles into biopolymer chitosan resulting in silver-chitosan nanocomposites is interesting to study due to their high antibacterial activity [2-3]. They can be synthesized using various methods, such as chemical reduction [3-4], electrochemical [5], γ-radiation, [6] photochemical [7] and laser ablation [8]. However, the most popular

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preparation of silver colloids is chemical reduction or "wet" chemical reduction of silver ions [9].

In many research, polymers have been used as stabilizers to provide stability for the metal nanoparticles against oxidation, agglomeration, and precipitation [10]. Preparations of silver nanoparticles in various polymers have been published to produce well-dispersed silver nanoparticles, such as polyvinylpyrrolidone (PVP) [11], polyethylene glycol [12], poly(methyl methacrylate) [13] and poly (vinyl alcohol) [14]. Natural polymers have also been used because they are non-toxic and biocompatible. Cellulose [15], chitosan [16], and starch [17] have been used as matrices or stabilizers for preparation of metallic nanoparticles. Chitosan can act as stabilizing agent for silver nanoparticles synthesis and dispersing material in nanocomposites.

Chitosan, a polysaccharide biopolymer derived from naturally occurring chitin, displays unique polycationic and chelating agent due to the presence of active amino and hydroxyl functional groups. Chitosan also has good biocompatibility, biodegradability and antimicrobial activity [18]. Meanwhile, silver nanoparticles are of particular interest for applications in medical devices and healthcare products because of their antibacterial activity and low toxicity to humans cells [19-20]. Incorporation of silver nanoparticles in chitosan is expected to give enhanced antibacterial activity.

The formation mechanism of silver nanoparticles in colloidal solutions from the reduction of silver ions consists of two stages: nucleation and crystal growth. Recently, the use of environmentally friendly weak reducing agent has been widely investigated, such as glucose [17,21-22]. The use of glucose as a reducing agent requires accelerator agent, such as NaOH [22]. Accelerator agent will increase the rate of Ag nanoparticles formation [22,24]. However, in the silver colloidal synthesis using chitosan as stabilizer agent, chitosan only soluble in aqueous solution of organic and mineral acids [23] that will reduce their role as stabilizing agent. Previous studies were employed heating treatment instead of introducing NaOH on the synthesis of silver nanoparticles using glucose as reducing agent [17,21-22]. New strategy on the formation of silver nanoparticles at room temperature is synthesis through gel phases. Then the colloidal of silver nanoparticles was obtained by dissolving the corresponding alkaline gel to the chitosan solution.

In this work, we report the synthesis of silver-chitosan nanocomposites colloidal at room temperature by reducing $AgNO_3$ using glucose through gel phase, and chitosan as stabilizing agent and polymers matrix. The effect of amount of NaOH as accelerator, molar ratio of $AgNO_3$ to glucose and $AgNO_3$ concentration toward localized surface plasmon resonance (LSPR) bands of

Ag nanoparticle was studied. We also investigated stability of the nanocomposite colloidal at ambient temperature for 16 weeks storage.

EXPERIMENTAL SECTION

Materials

Chitosan with molecular weight (MW) of 1,077, 919.28 Da and degree of deacetylation (DD) of 73.44 % was purchased from Biotech Surindo Cirebon Indonesia. Silver nitrate ($AgNO_3$), acetic acid (CH_3COOH), sodium hydroxide (NaOH) and glucose ($C_6H_{12}O_6$) were purchased from Merck. All chemicals were used as received.

Instrumentation

UV–Vis spectra were performed with a Shimadzu UV3150 UV–Vis spectrophotometer operating in the absorbance mode. UV-Vis absorption spectra of the samples were recorded in the wavelength range of 300 to 600 nm. All measurements were performed after 10 times dilution. The size and distribution of particles were characterized by Transmission Electron Microscopy JEM-1400 at an accelerating voltage of 120 kV. The particle size distribution was determined using the "ImageJ 1.34u" free software. Employing "ImageJ 1.34u" the area of nanoparticles was determined in pixels. Pixels were converted to nanometers by applying scale on the picture. The diameter of nanoparticles was calculated from the average of height and width of particles.

Procedure

Preparation of silver-chitosan nanocomposites colloidal

A solution of chitosan (1% w/v) in acetic acid solution (1% v/v) was firstly prepared. Due to the poor solubility of chitosan, the mixture was stirred to achieve complete dissolution, and then kept overnight at room temperature. The solution was filtered to remove any impurity before use. Aqueous solution of $AgNO_3$ (0.012 g/mL) of 0.5 mL was added to 25 mL of chitosan (1% w/v) in a flask. This solution was stirred at room temperature for 10 min and then aqueous solution of glucose was added, so that the molar ratio of $AgNO_3$/glucose is 1:4. Then the solution was stirred for 10 min. After that various mole of aqueous solution of NaOH were added to prepare various samples (1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 mmole). They were denoted as S1 to S8. The gel was immediately formed in the solution and the color was turned brown. The reaction was continued for 25 min under stirring. Then,
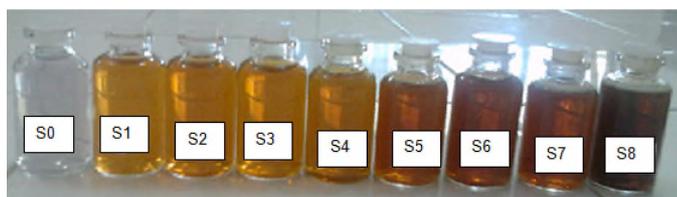
Fig 1. Photographs of silver-chitosan nanocomposites colloidal synthesized at different moles of NaOH
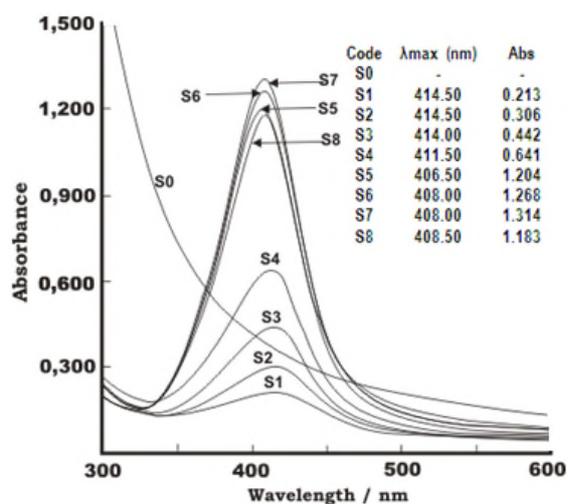
Fig 2. The UV-Vis spectra of silver-chitosan nanocomposites colloidal prepared at different mole of NaOH

the resulted gel was dissolved in 35 mL chitosan (1% v/v) and stirred to achieve complete dissolution to form silver/Chitosan nanocomposites colloidal. Similar procedure was done at various molar ratio of Ag/glucose are 1:1, 1:2, 1:4, 1:6, 1:8, 1:10, and 1:12, by using 5 mmole of NaOH and 2.0 % (w/w) ($AgNO_3$/chitosan) of $AgNO_3$. The samples were denoted as G1 to G7, respectively. Similar procedure was carried out again at various concentration of $AgNO_3$, namely 0.4% (A1) 0.8% (A2), 1.2% (A3), 1.6% (A4), 2.0% (A5), 2.4% (A6), 2.8% (A7), 3.2% (A8), 3.6% (A9), 4.0% (A10)), by using 5 mmole of NaOH and molar ratio of Ag/glucose of 1:4. Silver-Chitosan nanocomposites colloidal were characterized by UV-Vis Spectrophotometer and TEM.

RESULT AND DISCUSSION

Synthesis of Silver-Chitosan Nanocomposites Colloidal

Green synthesis of silver-chitosan nanocomposites colloidal have been performed by chemical reduction. It was employed by using non toxic chemicals, environmentally benign solvents, renewable materials and energy efficiency. This synthesis was carried out at

room temperature by using NaOH as an accelerator agent in the reduction reaction of silver ions, glucose as reducing agent and chitosan (Chit) as stabilizer agent. As containing aldehyde moieties, glucose can reduce silver ions to metallic silver and oxidize itself into glucolic acid. The possible step reactions during silver nanoparticles formation can be in the following:

$$Ag^+_{(aq)} + chit_{(aq)} \rightarrow \left[Ag(chit)\right]^+_{(aq)}$$

$$2\left[Ag(chit)\right]^+_{(Aq)} + 2OH^-_{(Aq)} + C_5H_{11}O_5 - CHO_{(Aq)} \rightarrow 2Ag_{(s)} + 2chit_{(aq)} +$$

$$H_2O_{(l)} + C_5H_{11}O_5 - COOH_{(aq)}$$
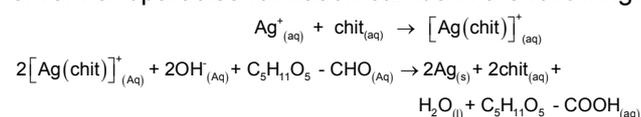
The color of the colloid resulted from different volumes of NaOH was rapidly changed from colorless to yellow, brown and dark brown, as shown in Fig. 1 indicating the formation of Ag nanoparticles [2]. The intense colors are suggested due to the localized surface plasmon resonance (LSPR) absorption of silver nanoparticles [26]. The surface of a metal is like a plasma, having free electrons in the conduction band and positively charged nuclei. LSPR is a collective excitation of the electrons in the conduction band near the surface of the nanoparticles [26]. The electrons are limited to specific vibrations modes by the particle's size and shape. Metallic nanoparticles have characteristic of optical absorption spectrums in the UV-Vis region [27]. Therefore LSPR phenomenon can be observed by UV-Vis spectroscopy.

UV-Vis spectroscopy is an important technique to ascertain the formation and stability of metal nanoparticles in solution based on an optical phenomenon of LSPR. The effect of NaOH amount, molar ratio of $AgNO_3$ to glucose and $AgNO_3$ concentration toward LSPR bands that represented by UV-Vis spectra were explained in the following.

Effect of NaOH Amount on LSPR Bands

The UV-Vis absorption spectra of colloidal prepared at various moles of NaOH are shown in Fig. 2. The obtained silver nanoparticles displayed absorption peaks, the characteristic LSPR band for silver, centered from 408 to 414 nm. The observed single SPR band indicates that the silver nanoparticles are spherical in shape [26]. It is confirmed by the TEM images (Fig. 3).

As indicated in Fig. 2, LSPR band could not be observed without addition of NaOH (sample S0). It is also observed that the LSPR peaks are getting narrow as the volume of NaOH increasing. The intensity of LSPR band at higher moles of NaOH (S1 to S7) also increased significantly indicating the presence of higher concentration of silver nanoparticles. However, further addition of NaOH decreases the absorbance (S8). This may be due to the formation of denser gel phase at higher amount of NaOH. It is assumed that there is a
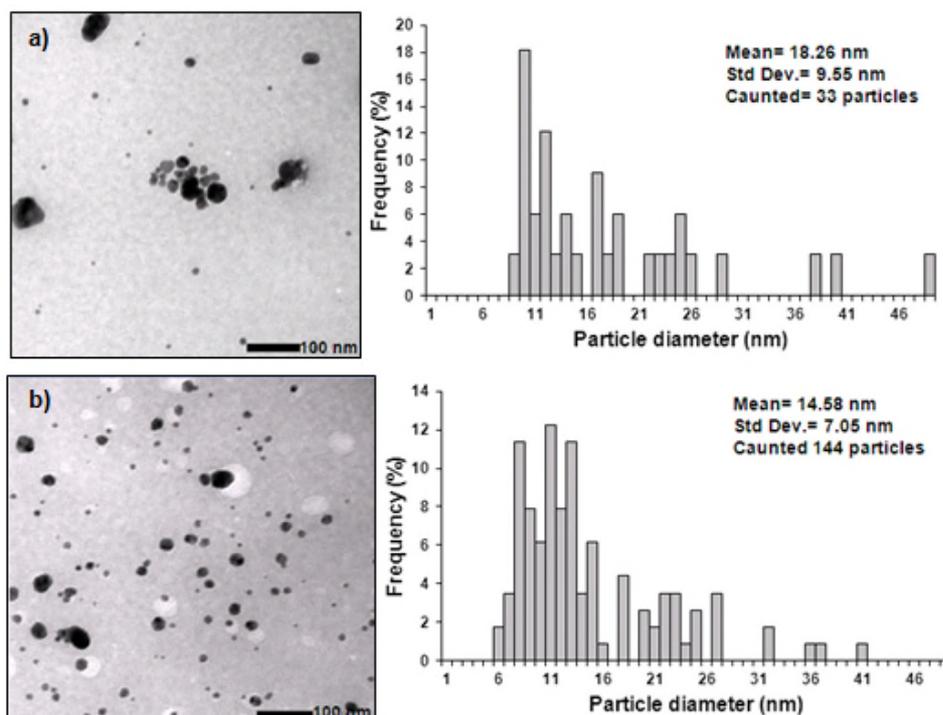
Fig 3. TEM images of silver nanoparticles and the corresponding particle size distribution of (a) sample S2 and (b) sample S5.
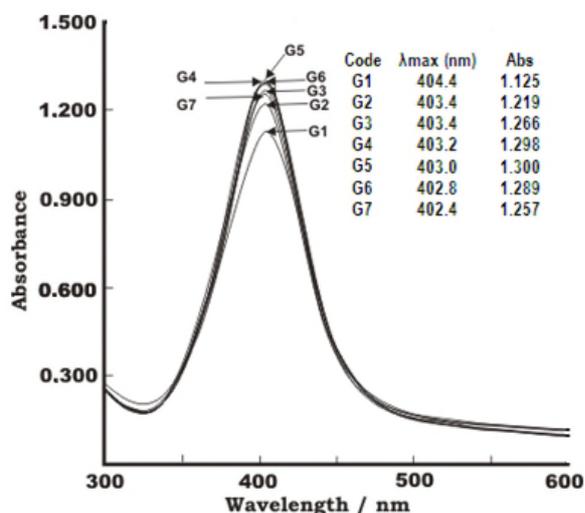
Fig 4. The UV-Vis spectra of silver-chitosan nanocomposites colloidal prepared at different molar ratio of AgNO₃/glucose
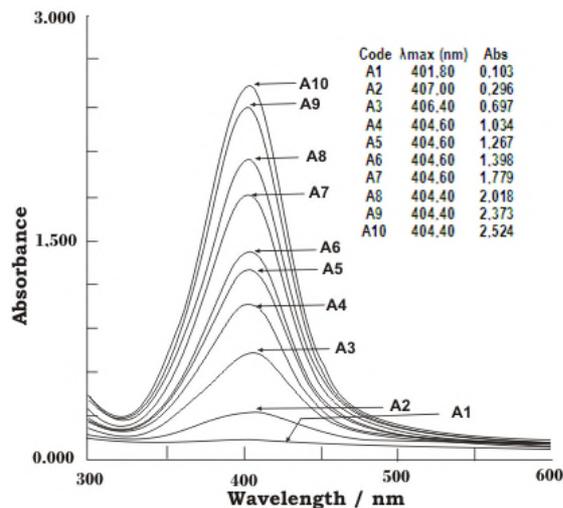
Fig 5. The UV-visible spectra of silver-chitosan nanocomposites colloidal prepared at different AgNO₃ concentration

resisted reduction of silver ions into silver nanoparticles.

A blue-shifting LSPR absorption bands at higher NaOH volume (S1 to S5) has also been observed. This phenomenon could be related to a decrease in the particle size of Ag nanoparticles as also observed by Heath [28]. While for S8, the LSPR absorption band is red-shifted to 408.5 nm due to the increased size of Ag nanoparticles.

Fig. 3 shows the representative TEM micrographs of the nanocomposites colloidal (sample S2 and S5). It is shown that all silver nanoparticles are spherical. These results demonstrate good agreement with the LSPR single peak results obtained in Fig. 2. The diameter particle of the particles in the sample S2 is 18.26±9.55 nm over the counted 33 particles and the sample S5 is 14.58±7.05 nm of 114 particles counted
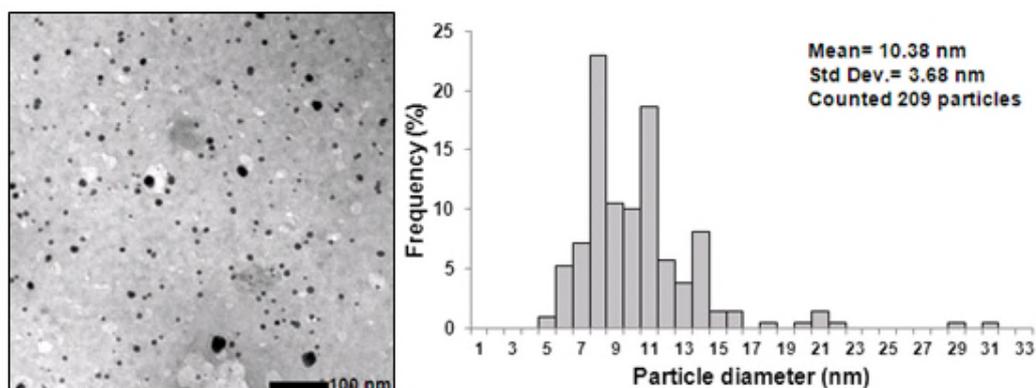
Fig 6. TEM image of silver nanoparticles and Particles size distribution of silver nanoparticles (Sample A9)
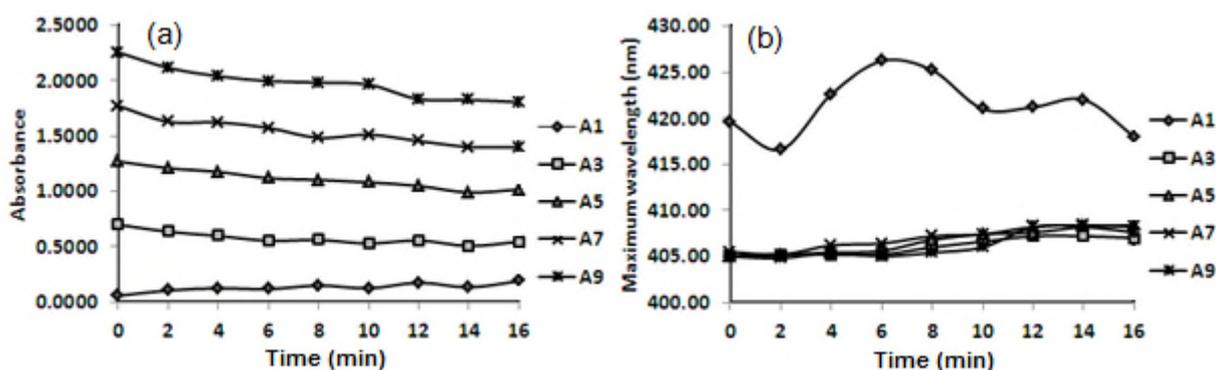
Fig 7. Changes of (a) absorbance and (b) maximum wavelength of silver-chitosan nanocomposites colloidal during time of storage at room temperature

shown in Fig. 3. The particle size of sample S2 is larger than the sample S5 in line with LSPR absorption band that showed blue-shifting from 414.50 to 406.50 nm. It is clear that the use of NaOH amount effecting particle size of the resulting Ag nanoparticles. It is also observed that more homogeneous particles (standard deviation of 7.05 nm) produced at higher amount of NaOH (sample S5), compared to the one at lower amount NaOH (sample S2) with standard deviation of 9.55 nm. This may be due to dense gel phase of chitosan produced at high amount of NaOH leading to inhibition of particle aggregation.

Effect of Molar Ratio of AgNO₃ to Glucose on LSPR Bands

Fig. 4 displays SPR bands at alternated glucose concentration. It is evident that, at initial increase of glucose concentration (G1 to G2), the LSPR band intensity also significantly increases. However, higher glucose concentration (G3 to G7) does not significantly increase the absorbance. While blue-shifting of LSPR absorption bands at higher glucose concentration (G1–G7) have been observed. This phenomenon could be related to a decrease in the particle size of silver nanoparticles at higher glucose concentration. Silver

nitrate was successfully reduced by glucose in the presence of chitosan as stabilizing agent. Based on the reaction of formation silver nanoparticles using glucose as reducing agent, it can be seen that the coefficient ratio of Ag^+ and glucose is 2:1. However in this experiment, optimal molar ratio is 1:8. This synthesis required high amount of glucose than the reaction stoichiometry possibly because some silver ions were so trapped in the chitosan linkages that hindering the contact with glucose [2].

Effect of AgNO₃ Concentration on LSPR Bands

The UV–Vis absorption spectra of silver-chitosan nanocomposites produced at various concentrations of silver nitrate are shown in Fig. 5 with the related value of λ_{max} (nm) and absorbance. The gradual increase of AgNO₃ concentration from A1 to A10 also increases the absorbances indicating the increase of silver nanoparticles concentration. The absorption peaks due to LSPR of silver nanoparticles are blue-shifted (A2–A10), indicating the decrease of the size of the silver nanoparticles [12].

Fig. 6 shows the representative of TEM micrographs of the silver-chitosan nanocomposites

colloidal corresponding to the sample A9. The TEM image shows that silver nanoparticles are spherical. Based on ImageJ analysis of 209 particles, the size of silver nanoparticles are in the range of 5 to 31 nm with mean of 9.362±3.68 nm, as showed in Fig. 6. The presence of some larger particles may be due to particle overlapping. Silver nanoparticles at this size range can be applied as a good antibacterial material. [4,6,26].

Stability of Silver-Chitosan Nanocomposites Colloidal

To investigate stability of nanocomposite colloidal, we have measured the absorption of the colloid prepared at various $AgNO_3$ concentrations for 16 weeks. The evolution of LSPR bands from UV-Vis spectra is shown in Fig. 7a and 7b. Absorptions for sample A3 to A9 tend to slightly decrease, but for A1, it slightly increases. The slight changes indicate that there was no obvious change toward amount of silver nanoparticles for 16 weeks. In terms of wavelength, for sample A3 to A9 tend to slightly increase. As the particles increase in size, the absorption peak usually shifts toward the red wavelengths [28]. The stable position of absorbance peak for sample A1 to A9 indicates that the storage for 16 weeks does not cause the aggregation of nanoparticles. This suggests that chitosan can be used as a good stabilizer agent for silver nanoparticles.

CONCLUSION

Synthesis of silver-chitosan nanocomposites colloidal were successfully carried out from $AgNO_3$/chitosan solution by using glucose as reducing agent and NaOH as accelerator without any heating treatment. The resulting nanocomposites colloidal show the formation of silver nanoparticles by exhibiting typical single LSPR band of silver nanoparticles at 402.4–414.5 nm. The LSPR bands were influenced by NaOH amount, ratio molar $AgNO_3$/glucose and concentration of $AgNO_3$ as the metal precursor. Based on the TEM image, silver nanoparticles was spherical with dominant size range of 6 to 18 nm. Based on the LSPR bands, silver-chitosan nanocomposites colloidal were stable during storage until 16 weeks. Compared to the other synthetic methods, this synthesis route is green and simple. The newly prepared silver-chitosan nanocomposites colloidal may have many potential applications in medical and biological industries.

ACKNOWLEDGEMENT

REFERENCES

1. Alexandre, M., and Dubois, P., 2000, *Mater. Sci. Eng., R*, 28(1-2), 1–63.
2. Shameli, K., Ahmad, M.B., Zargar, M., Yunus, W.M.Z.W., Ibrahim, N.A., Shabanzadeh, P., Ghaffari, M., and Moghaddam, 2011, *Int. J. Nanomed.*, 6, 271–284.
3. Honary, S., Ghajar, K., Khazaeli, P., and Shalchian, P., 2011, *Trop. J. Pharm. Res.*, 10(1), 69–74.
4. Wei, D., Sun, W., Qian, W., Ye, Y., and Ma, X., 2009, *Carbohydr. Res.*, 344(17), 2375–2382.
5. Vorobyova, S.A., Lesnikovich, A.I., and Sobal, N.S., 1999, *Colloids Surf., A*, 152(3), 375–379.
6. Shameli, K., Ahmad, M.B., Yunus, W.M.Z.W., Ibrahim, N.A., Gharayebi, Y., and Sedaghat, S., 2010, *Int. J. Nanomed.*, 5, 1067–1077.
7. Li, Z., Li, Y., Qian, X-F., Yin, J., and Zhu, Z-K., 2005, *Appl. Surf. Sci.*, 250(1-4), 109–116.
8. Tsuji, T., Watanabe, N., and Tsuji, M., 2003, *Appl. Surf. Sci.*, 211(1-4), 189–193.
9. Chudasama, B., Vala, A.K., Andhariya, N., Mehta, R.V., and Upadhyay, R.V., 2010, *J. Nanopart. Res.*, 12(5), 1677–1685.
10. Faraha, A.A., Alvarez-Puebla, R.A., and Fenniri, H., 2008, *J. Colloid Interface Sci.*, 319(2), 572–576.
11. Zheng, M., Gu, M., Jin, Y., and Jin, G., 2001, *Mater. Res. Bull.*, 36, 853–859.
12. Luo, C., Zhang, Y., Zeng, X., Zeng, Y., and Wang, Y., 2005, *J. Colloid Interface Sci.*, 288(2), 444–448.
13. Singh, N., and Khanna, P.K., 2007, *Mater. Chem. Phys.*, 104(2-3), 367–372.
14. Zhou, Y., Yu, S.H., Wang, C.Y., Li, X.G., Zhu, Y.R., and Chen, Z.Y., 1999, *Adv. Mater.*, 11(10), 850–852.
15. Cai, J., Kimura, S., Wada, M., and Kuga, S., 2009, *Biomacromolecules*, 10(1), 87–94.
16. Huang, H., Yuan, Q., and Yang, X., 2004, *Colloids Surf., B*, 39(1-2), 31–37.
17. Raveendran, P., Fu, J., and Wallen, S.L., 2003, *J. Am. Chem. Soc.*, 125(46), 13940–13941.
18. Pranoto, Y., Rakshit, S.K., and Salokhe, V.M., 2005, *LWT Food Sci. Technol.*, 38(8), 859–865.
19. Slawson, R.M., Van Dyke, M.I., Lee, H., and Trevors, J.T., 1992, *Plasmid*, 27(1), 72–79.
20. Zhao, G.J., and Stevens, S.E., 1998, *Biometals*, 11(1), 27–32.
21. Sharma, V.K., Yngard, R.A., and Lin, Y., 2008, *Adv. Colloid Interface Sci.,* 145(1-2), 83–96.

22. Darroudi, M., Ahmad, M.B., Abdullah, A.H., Ibrahim, N.A., and Shameli, K., 2010, *Int. J. Mol. Sci.*, 11(10), 3898–3905.

23. Kim, K.M., Son, J.H., Kim, S.K., Weller, C.L., and Hanna, M.A., 2006, *J. Food Sci.*, 71(3), 119–124.

24. Singh, N., Sinha, I., and Mandal, R.K., 2009, *Mater. Lett.*, 63(3-4), 425–427.

25. Božanić, D.K., Dimitrijević-Branković, S., Bibić, N., Luyt, A.S., and Djoković, V., 2011, *Carbohydr. Polym.*, 83(2), 883–890.

26. Guzmán, M.G., Dille, J., and Godet, S., 2009, *Int. J. Chem. Biol. Eng.*, 2(3), 104–111.

27. Šileikaitė, A., Puišo, J., Prosyčevas, I., and Tamulevičius, S., 2009, *Mater. Sci. (Medžiagotyra)*, 15(1), 21–27.

28. Heath, J.R., 1989, *Phys. Rev. B: Codens. Matter*, 40(14), 9982–9985.